CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020815

PHARMACOLOGY REVIEW(S)

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NDA 20,815 **November 21, 1997**

PHARMACOLOGY/TOXICOLOGY REVIEW OF NDA SUBMISSION

Sponsor: Eli Lilly and Company
Drug: Raloxifene hydrochloride (Evista™)
Category: Estrogen agonist/antagonist
Indication: Prevention of osteoporosis
Submission: June 8, 1997

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I Concur
Ronald W. Steigerwalt
11/21/97

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HFD-510/Steigerwalt/Kuijpers/Hedin
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Recommendation code: AP

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Raloxifene
Eli Lilly

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Meeting Minutes (Executive Carcinogenicity Assessment Committee)

Abbreviations

APR	Animal Pharmacology Report
NPR	Nonclinical Pharmacology Report
TR	Toxicology Report
RAL	raloxifene
EE(2)	17-alpha-ethinyl-estradiol, or 17-beta-estradiol
TAM	tamoxifen
DROL	droloxifene
CEE	conjugated equine estrogens
PTH	parathyroid hormone
ER	estrogen receptor
ERE	estrogen response element
RRE	raloxifene response element
HRT	hormone replacement therapy
OVX	ovariectomized, or ovariectomy
BMD	bone mineral density
HMM	histomorphometry
LDL	low density lipoprotein
HDL	high density lipoprotein
BP	blood pressure
BW	body weight
HD	high does
LD	low dose
mkd	mg/kg/day
dd	dose-dependent
sign	significant
ns	non-significant
R1	raloxifene (1 mg/kg/day)
R5	raloxifene (5 mg/kg/day)

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Raloxifene
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INTRODUCTION

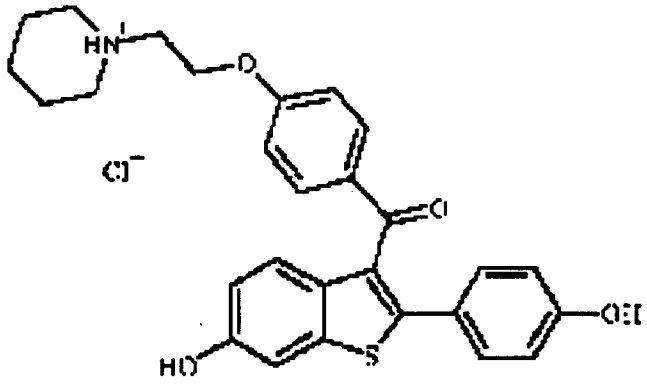
Raloxifene hydrochloride (LY 139481) is a Selective Estrogen Receptor Modulator (SERM) developed for use in prevention of osteoporosis in postmenopausal women. Raloxifene is a benzothiophene derivative, and has species- and tissue-selective estrogenic and anti-estrogenic properties. On bone and lipid metabolism it appears to have an estrogen-like effect and can increase bone mineral density, and decrease serum cholesterol. The clinical advantage of this compound as compared to hormone replacement therapy (HRT) is claimed to be a minimal estrogenic effect in uterus and breast, and thus a reduced risk for development of endometrial and breast cancer.

Raloxifene, like other SERMs, can interact with the estrogen receptor, which is a soluble ligand-induced transcription factor. The activated ligand-receptor complex binds, directly or indirectly, to a DNA sequence ("the response element") upstream of estrogen-responsive genes, and interacts with other transcription factors ("effectors") in the cell to modulate mRNA and protein synthesis. It can be considered as a multipartite system, i.e., ligand + receptor + response element + effector. The nature of the biological response to ER receptor occupation is the result of a complex interaction between the following 4 elements of this system:

1. Ligand
2. Receptor (ER-α or ER-β)
3. Response element (ERE, RRE, AP-1)
4. Intracellular effectors

Elements 2, 3 and 4 bestow the response its cell- and gene-specificity.

Chemical structure of raloxifene



CLINICAL INFORMATION

Indication: Prevention of osteoporosis
Target population: Postmenopausal women at risk for osteoporosis
Recommended dose: 60 mg/day
Dosage route: Oral
Dosage formulation: Tablet

Three pivotal Phase III randomized, placebo-controlled, double-blind, 36-month clinical trials (studies GGGF, GGGG, and GGGH) are currently being carried out in a total of 1764 postmenopausal, non-osteoporotic women. The doses of raloxifene studied are 30, 60 and 150 mg/day. In study GGGH Premarin (0.625 mg/day) is used as a comparator. All women receive 400-600 mg/day Ca supplements. Primary endpoints measured are BMD of lumbar spine and hip. Other endpoints are biochemical markers of bone metabolism, serum lipids and endometrial thickness and histology. For this NDA, 24-month interim analyses of the data have been submitted. Fracture incidences are not determined in studies GGGF, GGGG, or GGGH. A large Phase III study, GGGK, for the indication of treatment of osteoporosis, is being conducted in ca. 7000 postmenopausal, osteoporotic women. Fracture data are collected in this study.

The 24-month interim results of the Phase III prevention trials indicate that BMD of lumbar spine and hip are increased by in raloxifene-treated as compared to placebo-treated women. The efficacy of raloxifene appears to be considerably less than the efficacy of estrogen (0.3-0.4x). Bone marker data indicate an anti-resorptive effect of raloxifene. Total serum cholesterol and LDL-, but not HDL-cholesterol are decreased. The main adverse events of raloxifene therapy are venous thromboembolism (VTE), hot flashes and leg cramps. There is no significant evidence of endometrial proliferative activity.

NONCLINICAL PHARMACOLOGY

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Bone efficacy

Several short term studies on bone effects of raloxifene were carried out in rats. Two critical long term bone quality studies were done in ovariectomized rats (12 months) and monkeys (24 months). These were "prevention model" studies, i.e., animals were dosed immediately upon ovariectomy. The duration of animal dosing in the long term studies corresponded to 3-6 years of dosing in humans.

List of abbreviations

OVX	ovariectomy
RAL	raloxifene
EE2	ethinyl-estradiol
ALN	alendronate
TAM	tamoxifen
GH	growth hormone
BMD	bone mineral density (g/cm^2)
BMC	bone mineral content (g)
X-area	cross sectional area
SPA	single photon absorptiometry
QCT	quantitative computed tomography
DEXA	dual energy X-ray absorptiometry (=DXA)
HMM	histomorphometry
MAR	mineral apposition rate
BFR	bone formation rate
PYR	pyridinoline
FtF	force to failure
F_u	ultimate force (=FtF) (=ultimate load)
σ_u	ultimate strength, or ultimate stress
Su	ultimate strength ($=\sigma_u$)

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Bone Efficacy

RAT STUDIES
(Item 5, Vols. 1.1-1.2, 18 Studies/18Reports)

1. Report W53-01

(Published paper + 4 Appendices)
Raloxifene prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats

Methods

75-day old female Sprague-Dawley rats were OVX'ed and treated for 5 weeks by oral gavage with raloxifene (RAL), or EE2 (0.1 mkd). Diet contained 0.5% calcium, 0.4% phosphorus. After necropsy, BMD of femur and tibia was measured by SPA or DEXA.

Results

- At the *distal metaphysis of the femur*, RAL caused a dose-dependent blockade of BMD reduction, of maximal 55% at 1 mg/kg/day (*Figure 2*). Approximate ED_{50} was 0.03 mkd.
- In the *proximal tibial metaphysis* the blockade of BMD reduction was up to ca. 70% (*Figure 3*). Approximate ED_{50} was 0.3 mkd.
- Effect of RAL was maximal at 1 mkd. Effect of 10 mkd RAL tended to be less than that of 1 mkd (this may be secondary effect of body weight reduction)
- Ethynyl estradiol (0.1 mkd) blocked BMD loss at the two bone sites by 70% and 50% respectively *(Figs. 2, 3)*. The effect of EE2 was maximal at 0.1 mg/kg/day.

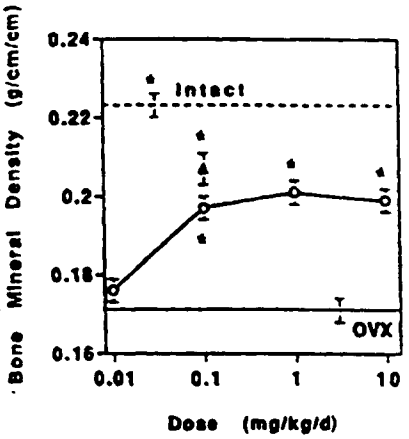

Figure 2. Effect of raloxifene on bone mineral density at the distal metaphysis of the femur in OVX rats as detected by single photon absorptiometry. Each point represents the mean BMD (±SEM) combined from six separate experiments for intact controls (*dashed line*: $n = 28$). OVX control (*dotted line*: $n = 29$). raloxifene groups (O—O; $n = 30$ for each dose level). and ethynyl estradiol (0.1 mg/kg; group: Δ: $n = 12$). ANOVA of the mean BMD indicated that there was a significant main effect ($F[6, 182] = 31.66$; $P = 0.0001$). asterisks indicate groups significantly distinct from the OVX control at $P \leq 0.05$.

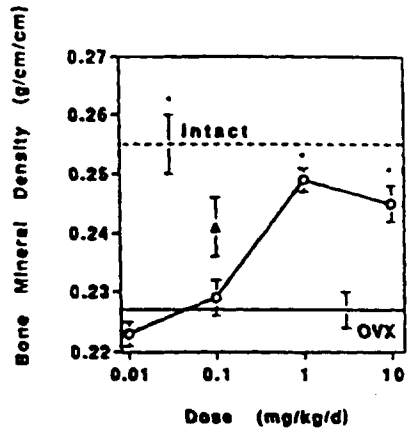

Figure 3. Effect of raloxifene on BMD at the proximal metaphysis of the tibia in OVX rats as detected by dual x-ray absorptiometry. Each point represents the mean BMD (±SEM) for intact controls (*dashed line*: $n = 5$). OVX control (*dotted line*: $n = 5$). raloxifene groups (O—O; $n = 10$–12). and ethynyl estradiol (0.1 mg/kg; group: Δ: $n = 6$). ANOVA of the mean BMD indicated that there was a significant main effect ($F[6, 53] = 15.98$; $P = 0.0001$); asterisks indicate groups significantly distinct from the OVX control at $P \leq 0.05$.

- Raloxifene caused a dose-related reduction in serum cholesterol of maximal 65% at 10 mkd. ED_{50} was ca. 0.2 mkd. Estradiol (0.1 mkd) caused a 65% reduction.
- In the uterus, raloxifene reversed the OVX-induced decrease in uterine wet weight by maximal 20%, estradiol (0.1 mkd) by 90%.
- Raloxifene (0.1 mkd) caused a non-significant 50% elevation of uterine epithelial cell height, a 25% increase of myometrial thickness, and a 25% increase of stromal expansion, but no stromal eosinophilia.
- OVX-treated animals gained more weight than controls (120 g vs. 77g). EE_2-treatment of OVX animals markedly reduced increase in BW gain so that the BW was less than in SHAM animals. RAL (0.01-10 mkd) also reduced BW gain in OVX animals in dose-dependent manner, but maximal effect was less than of 0.1 mkd EE2.

Appendix 1.

Bone efficacy as determined by quantitative computed tomography with raloxifene in 75-day old OVX rats

Results of study of 2 months duration rather than 5 weeks (femur: BMD (QCT), BMC, X-area)

- In distal femur, dosing with RAL for 2 months caused similar effect on BMD as dosing for 5 weeks (maximal 67% reversal of BMD reduction). RAL also partially prevented the loss of BMC (70% reversal). OVX or RAL+OVX did not affect cross-sectional (X-) area.
- In mid-diaphysis of femur, there were no effects of OVX or RAL on BMD, BMC, or X-area.

Appendix 2.

Bone efficacy trial with raloxifene in 75-day-old OVX rats using various dosing schedules

- Effect of dosing schedule: data not relevant

Appendix 3.

Bone efficacy studies with raloxifene in 75-day-old OVX rats using corn oil (po vs sc), polyethylene glycol, or minipumps

Results of 5-7 weeks studies of RAL, using different vehicles or dosing routes (sc, oral) on 3 parameters

- Effect of dosing vehicle/route: no appreciable differences between polyethylene glycol and corn oil (oral) vehicles with respect to body weight, uterine weight, BMD distal metaphysis
- Femur (RAL by s.c. implant, no sham control data):

Mid diaphysis: Cortical BMD not affected by RAL as compared to OVX
Distal metaphysis: Trabecular BMD increased as compared to OVX
Whole femur: wet weight (mg) decreased by RAL, ash weight (mg) unchanged.

Appendix 4. Quantitative computed tomography analysis of raloxifene effects on the fibula

Results from 6-month study on fibula (QCT, BMD, BMC, X-area)

- In the fibula (lower leg, back), treatment for 6 months with 3 mkd raloxifene (RAL) or 0.1 mkd EE_2 caused partial prevention of the 20% OVX-induced BMD decrease (RAL 35%,

EE_2 80%). The effect of OVX consisted of a decrease in BMC concomitant with a decrease in X-area. EE2, but not RAL, partially prevented the X-area reduction.

Conclusions

*1. **Main study**:*

- In OVX animals, RAL has similar body weight effect as EE2 (suppression of increase)
- RAL has a small uterotropic effect as compared to EE2.
- RAL and EE2 reduce serum cholesterol to similar extents.
- In distal femoral and proximal tibial metaphysis RAL (1 mkd) and EE2 (0.1 mkd) prevent OVX-induced loss of BMD to similar extents

*2. **Appendices**:*

- BMD:
  Femoral diaphysis: BMD not affected by OVX or OVX/RAL
  Fibula: After 6 months, BMD decreased by OVX, decrease reversed by EE2 and also but much less by RAL
- X-area:
  Femoral meta- or diaphysis: X-area not affected by OVX or RAL
  Fibula: X-area decreased by OVX, and reversed by EE2 but not RAL

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2. Report W53-13

12-Month bone efficacy study with raloxifene and ethynyl estradiol in ovariectomized rats

Methods

Adult female Sprague-Dawley rats (20/dose group), weight age weeks, were sham/OVX-ed and treated daily for 12 months by oral gavage. Groups were: SHAM + vehicle (20% cyclodextrin), OVX + vehicle, OVX + EE_2 (0.1 mkd), OVX + RAL (3 mg/kg/day). *[NOTE: Only one RAL dose was used].* After 4, 6, 10, 12 months 20 animals/group were killed and bones were removed and frozen. Assay of BMD of proximal tibia (lower leg, front), femoral (thigh) mid-shaft, and L-4 vertebrae was done by pQCT, and in distal femur relative bone mass was determined by X-ray (gray scale) image analysis. *(NOTE: Data are from 4-12 month period after OVX, baseline data not available)*

Purpose

Determine long term effects of raloxifene

Results

Body weight:

BW was reduced by EE2 , and by RAL as compared to OVX and SHAM controls. From 6 months on, BW in OVX was not significantly different from SHAM.

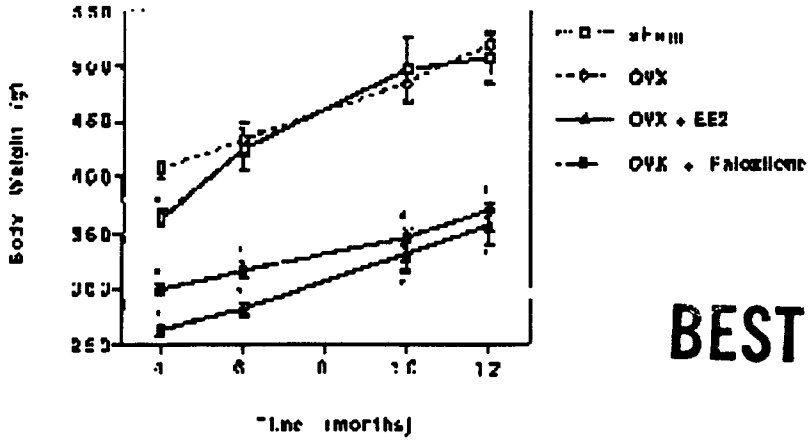


Figure 1. Oral raloxifene or ethynyl estradiol (EE_2) reduced body weight in ovariectomized (OVX) rats. Each point represents mean body weight ± SEM for 20 rats. * = p≤0.05 versus the OVX control.

Bone parameters:
The BMD and force to failure (FtF) at 4,6, 10 and 12 months after start of treatment are shown in the figures below for the following bone sites:

Vertebra L4 (BMD and FtF) (compression test)
Femoral Shaft (BMD and FtF) (bending test)
Femoral Neck (FtF only) (compression/bending)
Proximal Tibia (BMD only)

BMD = bone mineral density
FtF = force to failure = load to failure = ultimate force = breaking force or breaking strength (N)
S_u = ultimate stress or ultimate strength (N/mm^2)
In compression:
FtF (N) = S_u (N/m^2) x area (m^2)

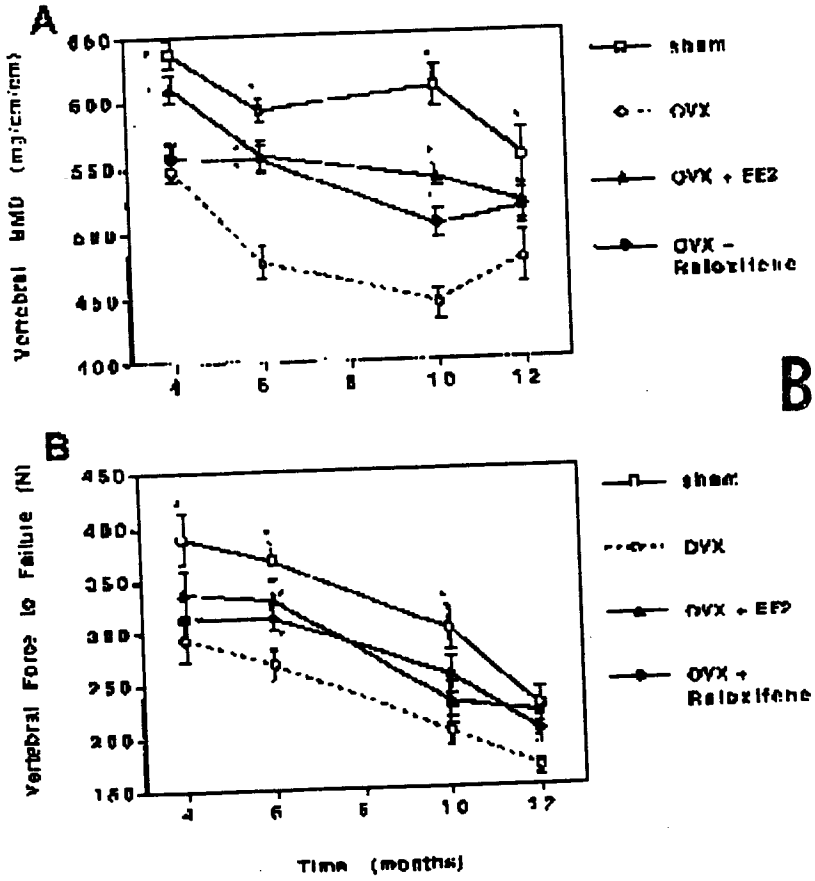


Figure 2. Oral raloxifene or ethynyl estradiol (EE_2) prevented loss of bone (Panel A) and strength (Panel B) in lumbar vertebrae of ovariectomized (OVX) rats. Each point represents the mean bone mineral density (in mg/cm²) or force to failure (in N) ± SEM for 20 rats. * = p≤0.05 versus the OVX control.

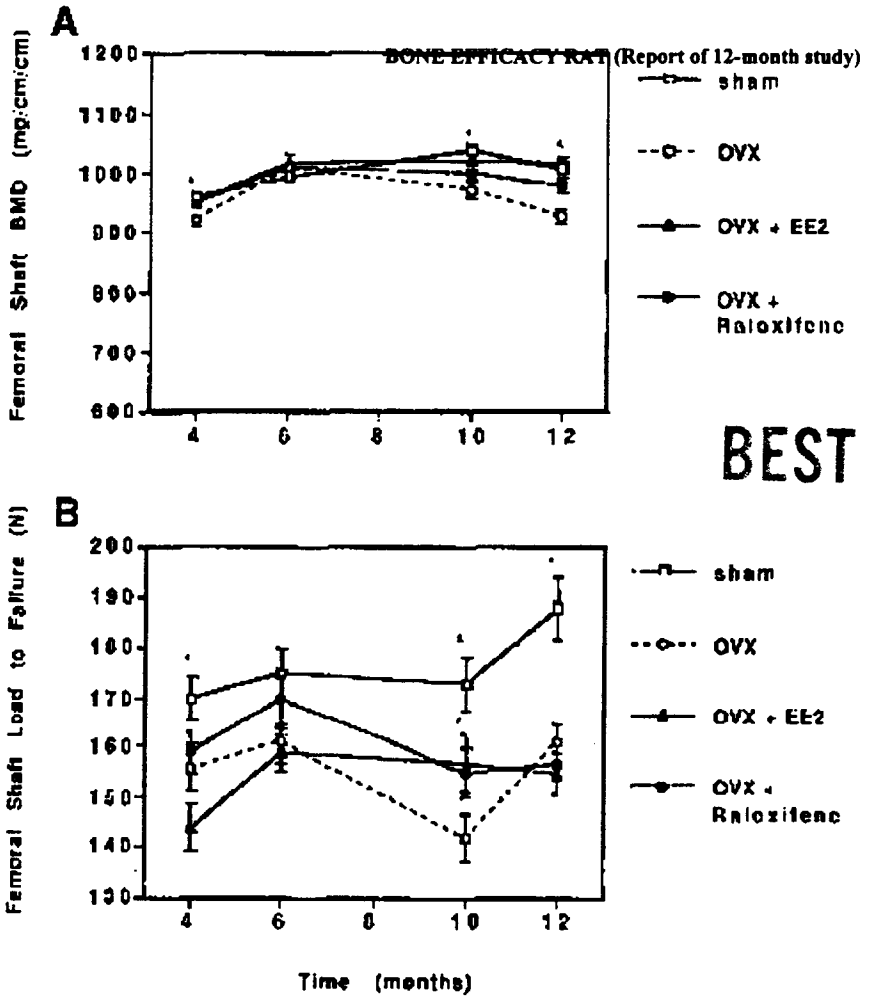
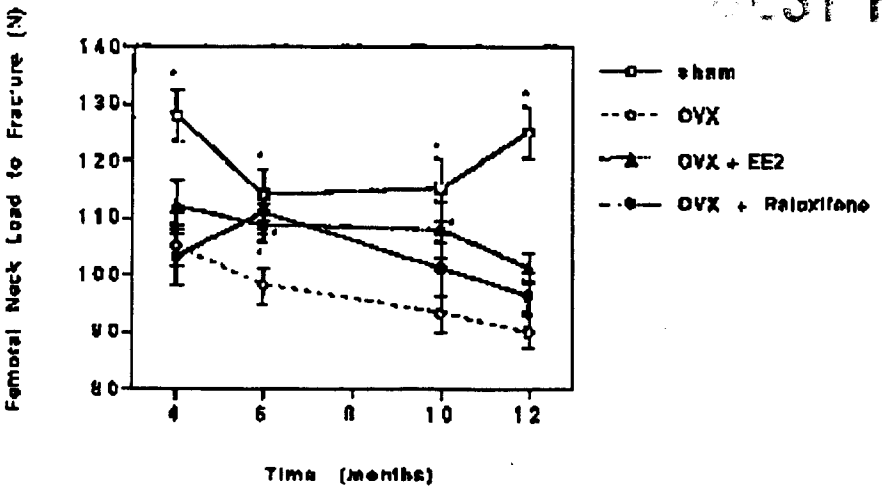


Figure 3. Oral raloxifene or ethynyl estradiol (EE_2) produced minimal effects on femur diaphyseal bone mass (Panel A) or strength (Panel B) of ovariectomized (OVX) rats. Each point represents the mean bone mineral density (in mg/cm^2) or load to failure (in N) ± SEM for 28 rats. * = p≤0.05 versus the OVX control.



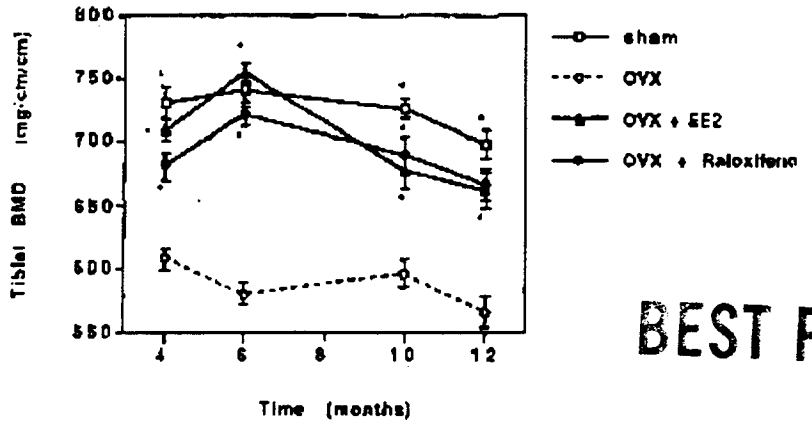


Figure 6. Oral raloxifene or ethynyl estradiol (EE_2) prevented bone loss in the proximal tibia of ovariectomized (OVX) rats. Each point represents the mean bone mineral density (in mg/cm²) ± SEM for 20 rats. * = p≤0.05 versus the OVX control.

Conclusions (See also *FIGURE A*, Appendix 1, below)

BMD and bone strength

- In the vertebrae, OVX caused a decrease in BMD (ca. 15%) and FtF (ca. 25%). The decrease was prevented significantly by EE2 and RAL (ca. 50%). Effect of EE2 was slightly larger than RAL.
  The largest effect of OVX occurred in the 0-4 mo period after OVX. Since there were no 0-mo baseline data this is based on extrapolation, assuming BMD is similar in all groups at baseline.
  There was an age-related decrease of vertebral BMD and FtF: in sham animals, over 12 month period, BMD and FtF decreases were 15% and 40%, respectively. At 12 months, the effects of OVX, raloxifene and EE2 on BMD and FtF were no longer significant.
- In the mid-femur (shaft), OVX caused a significant BMD reduction although much smaller and after a longer treatment period (> 6mo) than in vertebrae (ca. 5% at 10-12 mo), which was completely prevented by EE2 and for ca. 60% by RAL. The FtF (3-point bending), which was slightly decreased by OVX (by ca. 15%), was not significantly affected by either EE2 or RAL.
- In the femoral neck, FtF was decreased by OVX (by ca. 20%), and both EE2 and RAL partially prevented this effect to similar extents There were no data on BMD of femoral neck!.
- In the distal metaphysis, EE2 and RAL prevented the loss of relative bone mass caused by OVX, with EE2 slightly more efficacious than RAL (results not shown in review).
- In the proximal tibia, OVX caused a ca. 20% decrease in BMD, prevented to similar extents by EE2 and RAL (by ca. 75%) throughout 12-mo treatment period.
- At all cancellous bone sites, the effect of OVX, and of EE2 or RAL treatment appeared to

take place on the first 6 months of treatment. After that the difference in BMD betweensham, OVX and treated remained fairly constant.

Serum parameters:

- Serum cholesterol was not affected by OVX. RAL caused a larger reduction in cholesterol than EE2
- Serum osteocalcin decreased over time in both SHAM and OVX. Osteocalcin was increased slightly (ns) by OVX. EE2 and RAL had no effect on this increase. RAL but not EE2 slightly but significantly increased osteocalcin at 12 months.
- OVX caused a slight increase in serum IGF-1. EE2 reduced IGF-1 to below-sham levels, RAL did not affect IGF-1.
- EE2 reversed the decrease of serum GH caused by OVX. RAL did not.
- Serum Ca was minimally reduced in OVX as compared to SHAM. At 6-10 mo, EE2 reversed the decrease (by ca. 70%), RAL did not.
- No data on alkaline phosphatase or urinary crosslinks.

Uterine wet weight:

- Uterine wet weight was reduced markedly by OVX. EE2 reversed this decrease by ca. 60% and RAL by ca. 10% (both effects significant).

Plasma levels

- In this study, plasma levels were not determined. From a 90-day toxicity study (F344 rats, oral dietary route) we can make the following approximation: At a dose of 3 mkd, the Css value in rat plasma is ca. 4.5 ng/ml. This is 2-10-fold (average 4-fold) the expected therapeutic human plasma

Appendix 1.

Biomechanics and macrogeometry of vertebrae and femoral mid-shaft (diaphysis) from OVX rats treated with raloxifene or EE2 for 12 months

Methods

Load-deformation curve was translated into strain-stress curve, and following parameters were determined:

FtF (N), stiffness (N/mm), toughness (AUC of stress-strain curve in plastic region), ultimate stress or strength (MPa=N/m^2), Young's modulus (Mpa). Also: cross-sectional(X-) area (mm^2) (vertebrae only), and cortical moment of inertia (CSMI) (mm^4), density (g/cm^3) and thickness (mm) (femur only).

Results

Vertebrae (Table A and Figure A below):

Stiffness: not significantly affected.

Toughness: increased by RAL and EE2.

X-area: *(This parameter was calculated according to formula; X-area = ΠAB/4 where A and B are the width of the vertebral body minus processes, along anterior-posterior (A) and medial-lateral (B) axes. This determination is an approximation at best).*
X-area was decreased significantly by OVX at 4, 6 and 12 mo. Explanation of this finding was not provided.

Strength: *(More accurate term is ultimate stress or ultimate strength, S_u (or σ_u). This is also a calculated relatively inaccurate parameter (FtF/Area).*
S_u was decreased by OVX throughout 12 months (effect sign at 6 and 10 months). EE2 increased S_u at all times, sign at 6 mo. RAL increased S_u also, but less than EE2.

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Table A. **Vertebral load to failure and geometry analysis in ovariectomized rats.**

GROUP	Length of Treatment (months)	Vertebral Stiffness (N/mm)	(TOUGHNESS) Work to Failure (mJ)	Vertebral Cross-Sectional Area (centrum, mm^2)	Vertebral Strength (MPa)	Vertebral Young's Modulus (MPa)
Sham Control [a]	4	2637 ± 231*	1.2 ± 0.2	13.4 ± 0.5*	29.7 ± 1.9	468 ± 47
OVX Control [a]	4	1966 ± 204	1.1 ± 0.1	11.3 ± 0.5	26.1 ± 1.4	382 ± 38
Ethynyl Estradiol [a]	4	2048 ± 229	1.5 ± 0.2*	11.0 ± 0.4	31.1 ± 2.0	402 ± 42
Raloxifene [a]	4	1967 ± 196	1.6 ± 0.1*	11.0 ± 0.4	29.1 ± 2.0	359 ± 43
Sham Control	6	2370 ± 334	1.7 ± 0.2	12.4 ± 0.6*	31.2 ± 2.3*	457 ± 70
OVX Control	6	2026 ± 138	1.3 ± 0.1	11.0 ± 0.4	25.1 ± 1.5	461 ± 51
Ethynyl Estradiol	6	2362 ± 206	1.9 ± 0.2	10.2 ± 0.5	34.0 ± 2.5*	532 ± 51
Raloxifene	6	1961 ± 162	2.1 ± 0.4	11.1 ± 0.4	28.6 ± 1.4	388 ± 39
Sham Control	10	2290 ± 246	1.3 ± 0.2	11.0 ± 0.4	27.6 ± 2.1*	566 ± 63
OVX Control	10	1442 ± 177	1.2 ± 0.2	10.5 ± 0.4	19.6 ± 1.4	374 ± 51
Ethynyl Estradiol	10	1848 ± 281	1.4 ± 0.3	10.8 ± 0.5	21.5 ± 1.8	438 ± 79
Raloxifene	10	1698 ± 223	2.0 ± 0.2*	10.5 ± 0.6	24.8 ± 1.7*	403 ± 63
Sham Control	12	2065 ± 229	1.1 ± 0.2	10.3 ± 0.5*	22.4 ± 1.6	628 ± 70
OVX Control	12	1669 ± 172	1.3 ± 0.3	8.5 ± 0.5	20.9 ± 1.1	537 ± 58
Ethynyl Estradiol	12	1917 ± 242	1.1 ± 0.2	8.7 ± 0.4	25.6 ± 2.2	574 ± 73
Raloxifene	12	1534 ± 170	1.9 ± 0.4	8.9 ± 0.4	22.6 ± 1.4	388 ± 33

All values are mean ±SEM (n=20).

[a] Ethynyl estradiol (0.1 mg/kg), raloxifene (3 mg/kg) or vehicle (20% β-hydroxycyclodextrin) for the ovariectomized (OVX) and Sham controls were administered PO in a volume of 1 ml/kg.

* $p \leq$ versus appropriate (OVX) control as indicated by one-way ANOVA with post-hoc Fisher's PLSD.

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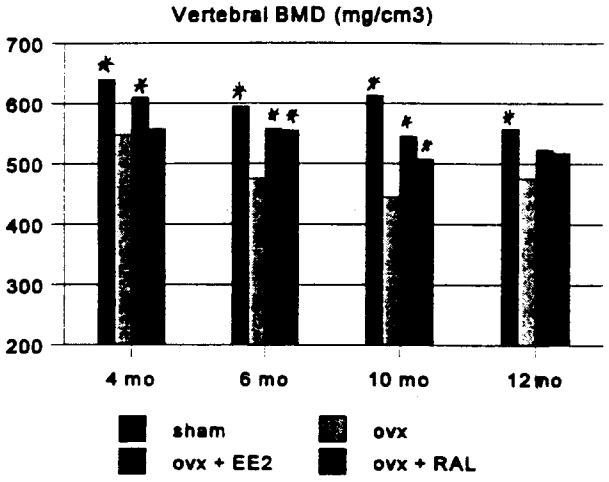
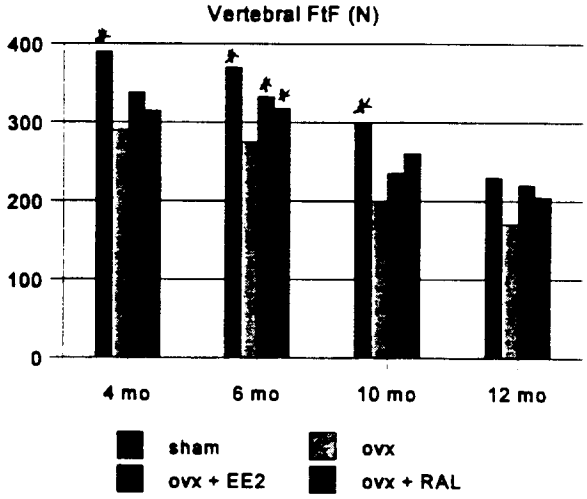
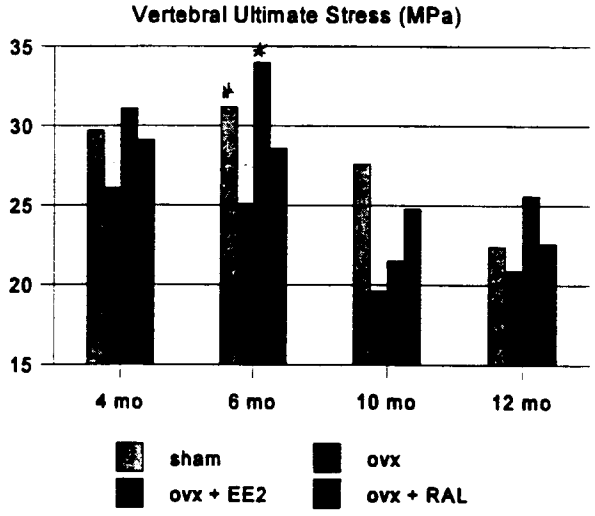
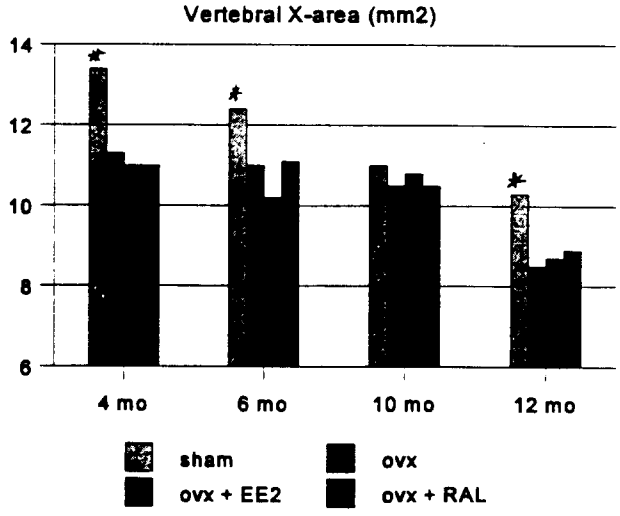
FIGURE A.
Effect of EE2 and RAL on BMD, Biomechanical Parameters (FtF, Ultimate stress, Toughness) and X-sectional Area of rat vertebrae (= significantly different from OVX)*









Femoral Diaphysis (Table B)
Parameters toughness, Young's modulus, moment of inertia, strength (Su) and density not significantly affected. Slight decrease of stiffness by OVX, no effects of EE2 and RAL on stiffness.

Table B. Femoral diaphyseal load to failure and geometry analysis in ovariectomized rats.

GROUP	Length of Treatment (months)	Femoral Stiffness (N/mm)	Femoral Work to Failure (mJ)	Femoral Cortical Thickness (mm)	Femoral Moment of Inertia (mm^4)	Femoral Strength (MPa)	Femoral Young's Modulus (MPa)	Femoral Cortical Density (g/cm^3)[a]
Sham Control[b]	4	485 ± 18	6.6 ± 0.3	0.88 ± 0.02	4.7 ± 0.2*	196 ± 4*	7373 ± 220	1.91 ± 0.03
OVX Control[b]	4	455 ± 27	6.7 ± 0.3	0.84 ± 0.02	3.6 ± 0.2	220 ± 5	9080 ± 429	1.89 ± 0.04
Ethynyl Estradiol[b]	4	430 ± 25	5.5 ± 0.2*	0.83 ± 0.01	3.5 ± 0.2	213 ± 5	8922 ± 502	1.93 ± 0.02
Raloxifene[b]	4	482 ± 18	6.6 ± 0.3	0.89 ± 0.02*	3.7 ± 0.2	222 ± 7	9591 ± 574	1.96 ± 0.01
Sham Control	6	481 ± 14	6.1 ± 0.4	0.91 ± 0.01	4.4 ± 0.2*	212 ± 6	7964 ± 272	1.97 ± 0.02
OVX Control	6	451 ± 12	6.2 ± 0.4	0.89 ± 0.01	3.5 ± 0.2	228 ± 5	9196 ± 320	1.96 ± 0.03
Ethynyl Estradiol	6	459 ± 13	5.3 ± 0.3	0.87 ± 0.02	3.5 ± 0.1	228 ± 6	9411 ± 416	1.99 ± 0.01
Raloxifene	6	477 ± 19	6.1 ± 0.2	0.88 ± 0.02	3.9 ± 0.3	229 ± 6	9077 ± 417	1.98 ± 0.02
Sham Control	10	454 ± 23	5.5 ± 0.4	0.92 ± 0.02	4.9 ± 0.2*	185 ± 7	6773 ± 399	2.05 ± 0.05
OVX Control	10	368 ± 19	5.3 ± 0.4	0.85 ± 0.02	3.8 ± 0.2	178 ± 6	6982 ± 342	2.00 ± 0.03
Ethynyl Estradiol	10	384 ± 14	5.6 ± 0.3	0.87 ± 0.02	4.3 ± 0.3	177 ± 8	6794 ± 442	2.05 ± 0.04
Raloxifene	10	396 ± 21	6.2 ± 0.4	0.84 ± 0.02	3.8 ± 0.2	193 ± 9	7510 ± 493	2.00 ± 0.03
Sham Control	12	542 ± 25	5.0 ± 0.2	0.88 ± 0.02*	5.2 ± 0.3*	205 ± 6	7764 ± 515	1.91 ± 0.03
OVX Control	12	505 ± 19	4.8 ± 0.3	0.78 ± 0.01	4.3 ± 0.2	202 ± 6	8551 ± 455	1.97 ± 0.03
Ethynyl Estradiol	12	453 ± 18	4.3 ± 0.2	0.80 ± 0.01	3.8 ± 0.2	214 ± 6	8684 ± 403	1.93 ± 0.02
Raloxifene	12	474 ± 17	4.8 ± 0.3	0.81 ± 0.01	3.4 ± 0.2*	234 ± 6*	10103 ± 421*	1.96 ± 0.02

All values are mean ±SEM (n=20).

[a] Femur wet density as determined by Archimedes' Principle (whole tissue measurement)

[b] Ethynyl estradiol (0.1 mg/kg), raloxifene (3 mg/kg) or vehicle (20% β-hydroxycyclodextrin) for the ovariectomized (OVX) and Sham controls were administered PO in a volume of 1 ml/kg.

* $p \leq$ versus appropriate (OVX) control as indicated by one-way ANOVA with post-hoc Fisher's PLSD.

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Appendix 2.

Raloxifene preserves bone strength and bone mass in OVX rats

(Published paper with 6-month data on BMD and bone "strength" of vertebrae, femur, proximal tibia)

Results

(Data from pooled treatment groups + control)

- Vertebral breaking force (L6) significantly correlated to vertebral BMD (L4) (r=0.39).
- Femoral neck breaking force significantly correlated to proximal tibial BMD (r=0.35).

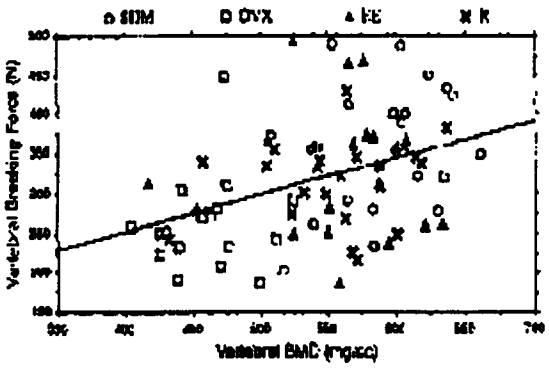


FIG. 1. BMD in a 1.0-mm section through the L4 vertebra measured using quantitative computed tomography was correlated with the force required to crush the L6 vertebra ($r = 0.39$, $P < 0.05$). Increased BMD observed after raloxifene or EE treatment of ovariectomized rats was associated with increased bone strength in the vertebrae.

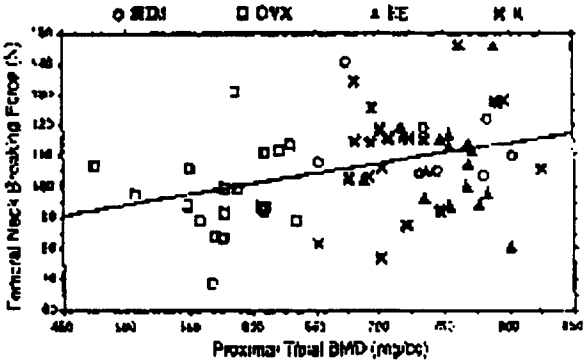


FIG. 2. BMD in a 1.0-mm section through the proximal tibia measured using quantitative computed tomography was correlated with the force required to break the femoral neck ($r = 0.35$, $P < 0.01$).

Appendix 3.

Raloxifene preserves bone mass with a smaller reduction in bone formation rate than estrogen

(Histomorphometry data from 6-mo and 10-mo time points, 12-month rat study)

Methods (See also Main Report 2, W53-13, p.10)

Report contains histomorphometric data on proximal tibia and L5 vertebrae, after 6 and 10 mo of treatment of 75-day ol' rats. Rats were given calcein at 10 and 3 days before sacrifice. The calcein label deposits at sites of bone formation and is a tool to study bone formation dynamics.

Results

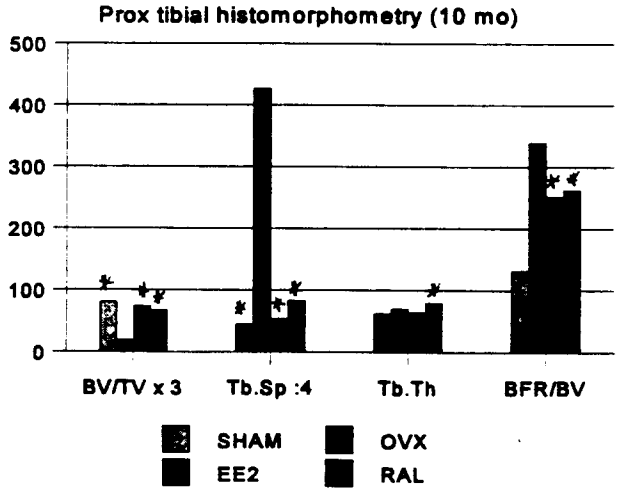
TABLE 1 and FIGURE 1

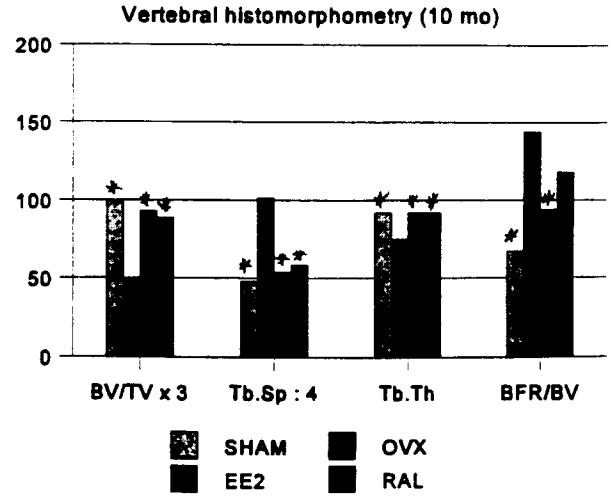
TABLE 1. Histomorphometry data in proximal tibia and lumbar vertebrae at 10 months: stati-stoically significant changes

	Proximal Tibia						L5 Vertebrae					
	OVX vs. SHAM	EE2 vs. SHAM	RAL vs. SHAM	EE vs. OVX	RAL vs. OVX	RAL vs. EE2	OVX vs. SHAM	EE2 vs. SHAM	RAL vs. SHAM	EE vs. OVX	RAL vs. OVX	RAL vs. EE2
BV/TV	--	nd	nd	+	+	nd	-	nd	nd	+	+	nd
Tb.Th	nd	nd	+	nd	+	+	-	nd	nd	+	+	nd
Tb.N	--	-	-	++	+	-	-	nd	-	+	+	nd
Tb.Sp	++	nd	nd	--	--	nd	++	nd	nd	--	--	nd
MAR	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
MS/BS at 6 mo	++	nd	++	--	nd	++	++	nd	nd	-	-	+
MS/BS at 10 mo	++	+	++	--	-	+	++	+	+	-	-	nd
BFR/BV at 6 mo	+	nd	+	-	nd	+	+	nd	nd	-	-	nd
BFR/BV at 10 mo	++	+	+	-	-	nd	++	+	+	-	-	nd

nd = no difference

FIGURE 1. Histomorphometry of rat vertebrae and proximal tibia





Conclusions

BV/TV: RAL (3 mkd) prevents OVX-induced bone loss in lumbar vertebrae and tibia to same extent as EE2.

Tb.Th and Tb.N.: OVX causes a decrease in vertebral and tibial Tb.N, and in vertebral Tb.Th. EE2 and RAL reverse the effects on Tb.N, and on vertebral Tb.Th. However, in RAL-treated tibia trabecular thickness is larger and trabecular number is smaller than in estrogen-treated bones: RAL increases trabecular thickness above sham level at 10 months. This difference between EE2 and RAL is not seen in vertebrae.

Tb.Sp.: OVX increases separation. Both EE2 and RAL reverse the effect at both bone sites.

MAR: In tibia, OVX increases mineral apposition rate. EE2 nor RAL affect this increase. In vertebrae, MAR is not affected.

MS/BS: OVX increases mineralizing surface (MS/BS) in tibia and vertebrae, at 6 and 10 mo; EE2 reverses MS/BS partially, at both sites, both times. In tibia, RAL reverses MS/BS partially at 10 mo. In vertebrae, RAL decreases MS/BS at 6 mo less than EE2, at 10 mo similarly as EE2.

BFR/BV: OVX increases bone formation rate (BFR/BV) at both bone sites, at both 6 and 10 mo; EE2 suppresses the OVX-increase in BFR/BV in tibia and vertebrae completely at 6 mo, partially at 10 mo. In tibia, RAL partially suppresses BFR after 10 mo. In vertebrae, RAL partially suppresses the increase in BFR/BV at both times.

Thus, RAL reverses the OVX-induced increases in MS/BS and BFR/BV in tibia and vertebrae to a lesser extent or with a lag time as compared to EE2. This means that the bone formation rate in bone treated with raloxifene is larger than in bone treated with EE2, even though bone mass (BV/TV or BMD) is the same. Thus, it seems reasonable to conclude that bone resorption is therefore also larger in raloxifene-treated, and that formation-resorption coupling is different in raloxifene- than in estrogen-treated.

Note 1: Parameters differently affected by EE2 and raloxifene: Tb.Th, Tb.N., MS/BS and BFR/BV.

Note 2: Increased BFR in OVX animals correlates with the small increases in serum osteocalcin seen in the 12-month study.

Conclusions from 12-month rat bone efficacy study

(Conclusions are valid for doses of 0.1 mkd EE2 and 3 mkd RAL)

- Ovariectomy reduces BMD of vertebrae, femur and tibia of 10-11 week old rats.
- The OVX-induced reduction of BMD was prevented by EE2 and RAL. The effect of EE2 was usually slightly larger than the effect of RAL.
- Cancellous bone loss occurred mainly in the first 4 month after OVX. Beyond that BMD changed in a parallel fashion in all groups, in other words, bone loss was no longer increased by OVX or reduced by RAL or EE2. Some cortical bone loss became evident only after 6 months of OVX.
- The OVX-induced BMD reduction was accompanied by a reduction in FtF, which was also partially prevented by EE2 and RAL. The effect of EE2 was slightly larger than of RAL.
- In vertebrae, the increase in FtF by EE2 and raloxifene, as compared to OVX controls, was accompanied by an increase in ultimate stress (S_u), which persisted throughout the 12-mo period. There were no biomechanical data from tibia.
- Biochemical marker assays showed a small increasing effect of OVX on serum osteocalcin, but no effect of EE2 or RAL. Other bone turnover markers were not measured.
- HMM analysis indicated that the BMD loss caused by OVX was due to a decrease in % bone volume resulting from an increase in trabecular separation. OVX increased BFR, and EE2 and RAL partially reversed the increase in BFR. The effect of EE2 was larger than of RAL. In tibia, but not vertebrae, RAL treatment resulted in fewer and thicker trabeculae than EE2-treatment.
- At the 6 months time point, there was a significant correlation (r 0.39, $P < 0.001$) between vertebral BMD and FtF, when data for all groups were pooled.

  Note: This correlation between BMD and FtF is interesting, but the regression analysis needs to be done for the separate groups and for all the different time points in order to judge whether the relationship remains the same for all groups throughout time.

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3. Report W53-11

(Published paper)

Histomorphometry studies with raloxifene in 75-day old ovariectomized rats

Methods

Adult female Sprague-Dawley rats (7-12/dose group), weight age (75 days), were sham/OVX-ed and, after 2 days, treated daily for 35 days (5 wks) by oral gavage. Groups were: SHAM + vehicle (20% cyclodextrin), OVX + vehicle, OVX + EE2 (0.1 mkd), OVX + raloxifene (3 mg/kg/day). Histomorphometric, primary and secondary (derived) measurements of cortical and cancellous (metaphyseal secondary spongiosa) bone of the *proximal tibia* was carried out after labelling with tetracycline 1 day post-surgery, and with calcein and alizarin 7 and 1 days before sacrifice. Data were given in two-dimensional format.

Results

Cortical bone:

- No change in X-sectional, medullary, cortical bone area of whole transverse tibia section.
- Periosteal bone formation rate (Ps.BFR) increased by OVX, reduced to below sham levels by EE2 and RAL. Endocortical BFR not affected.
- Endocortical labeled perimeter (Ec.L.Pm) (alazarin) reduced by OVX, reversed partially by EE2 and RAL. Thus, resorption increased by OVX, effect prevented by EE2 and RAL.

Cancellous bone:

- Bone surface (BS/TV) and volume (BV/TV) reduced by OVX, effect largely prevented by EE2 and RAL.
- MAR increased by OVX, reduced to below sham by EE2, and reduced to sham by RAL.
- Resorption of tetracycline increased by OVX, prevented completely by EE2 and RAL.
- Osteoclast number and eroded surface increased by OVX, prevented partially and similarly by EE2 and RAL.
- BFR (surface referent) increased by OVX, increase prevented completely by EE2 but not significantly by RAL
- Calcein labeled surface (surface referent) increased by OVX, effect prevented by EE2 but not by RAL.

Appendix 1.

Effects of raloxifene on tibia histomorphometry in OVX rats (2-month treatment period)

Methods

Treatment for 2 months after OVX by RAL (cancellous bone of proximal tibia metaphysis)

Results

- RAL prevents OVX-induced reduction in bone volume (BV/TV) and trabecular number (Tb.N), and prevents increase in trabecular separation (Tb.Sp.) in dose-dependent manner. Tb.Th not affected by OVX or RAL. At the 10 mkd dose, parameters were same as those of sham controls.

- OVX decreased connectivity (i.e., increased free trabeculi and reduced trabecular connections) and RAL prevented this effect in dd manner. At the 10 mkd dose, parameters were same as those of sham controls.

4. Report BN5-01

Raloxifene effects as evaluated by conventional and X-ray absorptiometry techniques

Methods

Treatment of 6-month old rats for 4-5 weeks with EE2 (0.1 mkd) or RAL (1 mkd) for 4 weeks. DXA analysis of femur and lumbar vertebrae, SPA of femur.

Results

- OVX decreased uterine wet weight by 75%, EE2 increased it above sham, RAL had 10% of the effect of EE2.
- OVX caused increase in projected area of femur, and a net decrease in femoral BMD. BMD was slightly increased by EE2 and RAL, area was not affected.
- Femoral diaphyseal moment of inertia (calculated from inner and outer diameters of cortical bone) increased by OVX, suggesting radially outward redistribution of bone. Increase prevented by EE2, but not by RAL.
- OVX caused increase in projected area of L1-4. Increase prevented by estrogen, but not by RAL. However, in another experiment the area change by OVX (or OVX+RAL) was not seen.

5. Report BN5-08

Same data as described in Report 4 (published paper)

6. Report BN5-10

Comparative X-ray densitometry of bones from OVX rats

QCT analysis more accurate than DXA for small bones. However, *in vivo* vertebrae could not accurately be analyzed by QCT (positioning problem).

7. Report BN5-09

Longitudinal and cross-sectional anlysis of raloxifene effects on the tibiae from OVX aged rats

Methods

Treatment of 6-month old OVX rats with EE2 or RAL for 35 days.
DXA and QCT of proximal tibia only.

Results

Proximal tibia: BMD decreased by OVX, decrease dd prevented by EE2 and RAL to same extent. Serum cholesterol decreased by EE2 more than by RAL. In OVX rats, uterine weight increased markedly by EE2, minimally by RAL.

8. Animal Pharmacology Report 27

Raloxifene bone density

Description of X-ray image analysis method for determining bone density (digitized radiographs)

9. Report R43-06

Effects of raloxifene HCl and ethynyl estradiol on biochemical bone markers and serum cholesterol in ovariectomized rats

Methods

Adult female Sprague-Dawley rats(5-10/dose group), weight , age , were sham/OVX-ed and treated daily, starting 1-17 days after OVX, by oral gavage for 4-49 days. Groups were: SHAM + vehicle (20% cyclodextrin), OVX + vehicle, OVX + EE2 (0.1 mkd), OVX + RAL (1-3 mg/kg/day). Assay: BMD (X-ray analysis), body weight, serum cholesterol and osteocalcin, urine pyridinoline (resorption marker).

Results

- In 35-49 day dosing study, starting 2 days post OVX, EE2 and RAL partially prevented the OVX-induced bone loss (RAL 60% as effective as EE2). RAL had a small, non-significant effect on uterine weight loss, while EE2 prevented it for ca. 65%. In OVX animals, there was a small increase in urinary pyridinoline, which was prevented by EE2 and RAL.
- In 5-day dosing experiment, starting 15 days post-OVX, RAL was as effective as EE2 in reducing serum cholesterol. EE2 and RAL slightly decreased PYR/deoxyPYR as compared to OVX controls. Effect of OVX not shown.
- In 21-day dosing study, starting 17 days post-OVX, the OVX-induced increase in body weight was reversed equally (by ca. 80%) by EE2 and RAL. RAL slightly but non-significantly reversed the OVX-induced loss in uterine weight by while EE2 reversed it by ca. 70%. OVX caused an increase in serum osteocalcin. Both EE2 and RAL prevented the effect. EE2 lowered the osteocalcin levels to below sham values, RAL to sham values. EE2 and RAL caused a moderate decrease in urinary pyridinoline as compared to sham or OVX-controls; OVX alone had no effect on this parameter.
- In 49-day dosing experiment, starting 2 day post-OVX, RAL and EE2 were equally effective in reducing serum cholesterol in the OVX animals (by ca. 50%). RAL (3 mkd) was slightly less effective than EE2 in preventing OVX-induced bone loss (55% vs. 70%). OVX slightly increased PYR, increase prevented equally by RAL (3mkd) and EE2.

10. Animal Pharmacology Report 33

The effects of raloxifene on rodent serum bone-related parameters and uterine epithelial height

Methods

12-week old animals were OVX'ed and treated with 0.1 mkd EE2 or 0.01-10 mkd RAL for 35 days.

Results

RAL decreased serum osteocalcin in OVX animals towards intact control values in dose-related manner. As compared to OVX controls, RAL increased uterine luminal epithelium thickness slightly, while EE2 increased it above intact control levels.

11. Report R43-07

(Published paper)

Time-dependent changes in biochemical bone markers and serum cholesterol in ovariectomized rats: Effects of raloxifene HCl, tamoxifen, estrogen, and alendronate

Methods

75-day old SD rats were ovariectomized, and after 2 weeks, treated with Ethinyl Estradiol (EE2) (0.1 mkd), Raloxifene (RAL) (3mkd), Tamoxifen (TAM) (3 mkd), or Alendronate (ALN) (3 mkd), for 21 days.

Results

- Serum cholesterol was by EE2, RAL, TAM, but not ALN.
- Effect on bone markers (Table 1):

Table 1. Effect of EE2, RAL, TAM, ALN on biochemical markers of bone turnover

	Days of treatment	Serum Osteocalcin (*formation or turnover marker*)	Urinary Pyridinoline (*resorption marker*)
OVX	4	1.4x (Sham ctrl)	1.4x
OVX + EE2	7	0.8x (Ovx ctrl)	-
	14	-	0.7x
	21	0.65x	-
OVX + RAL, TAM or ALN	7	0.9x	-
	14	-	0.7x
	21	0.8x	-

OVX	(vs SHAM control)	formation ↑↑ resorption ↑↑
EE2	(vs OVX control)	formation ↓↓ resorption ↓↓
RAL, TAM, ALN	(vs OVX control)	formation ↓ resorption ↓↓

Conclusion

Resorption is affected similarly by EE2 and RAL, formation is not. The data suggest that formation and resorption markers have only qualitative value.

12. Report BN5-02

(Published paper + 1 Appendix)

Advantages of raloxifene over alendronate or estrogen on nonreproductive and reproductive tissues in the long-term dosing of ovariectomized rats

Methods

10-11 week old SD rats were OVX'ed and treated for 10 months with RAL (3 mkd), EE2 (0.1 mkd), both orally, or with ALN (0.03 mg/kg s.c. 2x/wk). pQCT used for densitometry, calcein label (20 mg/kg, 2x) for dynamic histomorphometry of lumbar vertebrae and proximal tibiae. Biomechanical assessment of vertebral strength was carried out by compression test. (Same study as described in Report 2).

Results

- EE2 and RAL prevented bone loss in L4-5, and proximal tibia; RAL was less efficacious than EE2 in both sites x re: BMD, 0.9x re: BV/TV). ALN was less efficacious than RAL.
- In proximal tibia, RAL had different histomorphometric effects than EE2:

Table 1. Effects of EE2, RAL and ALN on tibia cancellous bone (10-month data)

	Ovx-Sham	EE2-Ovx	RAL-Ovx	ALN-Ovx
BV/TV	-75% *	+269% *	+238% *	+139% *
Tb.Th	+13%	-8%	+13% *	-15% *
Tb.N	-79% *	+318% *	+209% *	+187% *
Tb.Sp	+858% *	-87% *	-81% *	-79% *
BFR/BV	+161% *	-26% *	-23% *	-54% *

Note: Histomorphometry data are same as described in Report 2 (12-month study)
* significant difference vs. appropriate control ($P < 0.05$)

- L6 breaking force was reduced by OVX, reduction prevented partially by EE2 and RAL.

Table 2. L6 Breaking force

	Sham	Ovx	Ovx-EE2	Ovx-RAL	Ovx-ALN
Breaking Force	300	200	230	250	175

- L4 X-area not affected by OVX or treatment, L6 area decreased at 10 mo by OVX.
- Tibia X-area reduced: 10% by OVX (significant), and 5% by OVX+EE2, RAL or ALN (n.s.)
- Body weight reduced by EE2 and RAL, not ALN.
- OVX-induced reduced uterine wet weight increased by EE2, not by RAL or ALN
- Serum cholesterol decreased by ca: 30% by RAL, not significantly affected by EE2, n.s. increased by ALN.

Appendix 1.
QCT analysis of raloxifene efficacy in proximal tibiae, centrum of L-4 vertebra, and femora
Results *(10-month data)*

- Effect of OVX and of EE2 and RAL in vertebrae and tibia was similar as reported before. Proximal tibial X-area was decreased by OVX, decrease was prevented partially by RAL, not by EE2.
- In distal femoral metaphysis, RAL had 0.65x efficacy of EE2 in preventing the 30% BMD loss caused by OVX. In mid-femoral diaphysis OVX caused a 6% loss of BMD, prevented for 75% by EE2, but not significantly by RAL (0.55x effect of EE2) .
- Femoral X-area slightly decreased (15%) by OVX, not modulated by any treatment.

13. Report BN5-03
Comparison of raloxifene, tamoxifen, nafoxidene, or estrogen efficacy on nonreproductive and reproductive tissues from ovariectomized rats
Methods
Six-month old rats were OVX-ed and treated for 4-8 weeks. Measurements: Bone densitometry, uterine histology, body weight, serum cholesterol.

Results (BMD)

- ED_{50} of RAL to prevent Ovx-induced BMD loss: 0.3 mg/kg/day
- Maximal efficacy of RAL produced at: ng/kg/day
- Maximal efficacy and potency of RAL similar in distal femoral metaphysis, proximal tibia, and L1-4

14. Report BN5-11
Raloxifene, tamoxifen, nafoxidene, or estrogen effects on nonreproductive and reproductive tissues in ovariectomized rats
(Published paper)

Data as in previous report BN5-03 (Report 13).

15. Report W53-12
In vivo comparison of various selective estrogen receptor modulators to conjugated equine estrogens in ovariectomized rats
Methods
Effect were measured of of SERMs (raloxifene, tamoxifene, droloxifene, idoxifene, centrchroman) and Premarin for 2 months in OVX rats, on bone (BMD, X-ray grey scale), uterus (epithelial height, eosinophil peroxidase activity), serum cholesterol, serum osteocalcin

Results

All dose-response curves are different (different slopes and different maximal effects). Compared to other SERMs RAL looks very good in that it can effectively protect bone and reduce serum cholesterol, while it only minimally stimulates the uterus at a certain dose. All other SERMs have some disadvantage, or don't affect all parameters at the chosen doses. The efficacy of RAL on bone at the lower dose end is less than of PR, tamoxifene, droloxifene, centchroman. However, at the higher dose end (10 mkd) it is only less than the efficacy of PR. Over the whole dose range the efficacy is the same as that of idoxifene. However, other SERMs have disadvantage of uterine stimulation, and/or less cholesterol-lowering effect. E.g., for droloxifene you need a high enough dose (> 1mkd) to get good bone protection, but at this dose there is uterine stimulation. Centchroman is also relatively potent/efficacious in bone, but again, at doses that protect bone significantly, it stimulates the uterus.

Table 1. Selectivity Coefficients for SERM or Premarin Modulation of Serum Cholesterol or Bone Responses, Relative to Uterine Responses

SELECTIVITY COEFFICIENT: UTERINE EOSINOPHIL PEROXIDASE ACTIVITY REFERENT					
COMPOUND	Eosinophil Peroxidase [a] ED_{x}	Cholesterol [a] ED_{x}	Bone [a] ED_{x}	Cholesterol Selectivity Coefficient [b]	Bone Selectivity Coefficient [c]
raloxifene	> 10	0.60	0.75	> 16.7	> 13.3
tamoxifen	0.02	0.27	0.10	0.07	0.20
centchroman	0.07	0.47	0.10	0.16	0.70
droloxifene	0.93	3.00	2.27	0.31	0.41
idoxifene	0.01	0.26	0.57	0.04	0.02
Premarin	0.3	1.57	0.95	0.08	2.60

SELECTIVITY COEFFICIENT: UTERINE EPITHELIAL HEIGHT REFERENT					
COMPOUND	Epithelial Cell Height [a] ED_{x}	Cholesterol [a] ED_{x}	Bone [a] ED_{x}	Cholesterol Selectivity Coefficient [b]	Bone Selectivity Coefficient [c]
raloxifene	> 10	0.60	0.75	> 16.7	> 13.3
tamoxifen	0.01	0.27	0.10	0.04	0.10
centchroman	0.04	0.47	0.10	0.09	0.40
droloxifene	1.14	3.00	2.27	0.38	0.51
idoxifene	0.03	0.26	0.57	0.12	0.05
Premarin	0.07	1.57	0.95	0.04	1.40

Data for this table were calculated from experiments 9270 and 9288 which are summarized in Figures 3, 4, 5, and 7.

a ED_{x} values were calculated from the linear portion of the log dose-response curve by regression analysis as described in the methods section

b The cholesterol selectivity coefficient was calculated by dividing the respective uterine ED_{50} value by the cholesterol (percent control) ED_{20} value

c The bone selectivity coefficient was calculated by dividing the respective uterine ED_{50} value by the bone (quantitative computer-assisted analysis of distal femur) ED_{20} value

16. Report W53-07

Combination dosing studies using raloxifene, with estradiol, provera, alendronate or growth hormone in ovariectomized rats

Methods

75-day old OVX rats, treated for 35 days.
BMD (SPA or X-ray image grey scale analysis) of distal femoral metaphysis.

Results

- ALN has better bone-protective effect than RAL (X-ray method)
- Effects of RAL and ALN on bone mass are additive
- RAL does not antagonize EE2 effect on bone BMD, but does antagonize EE2 effect on uterine wet weight (!). The effects of raloxifene and EE2 were sometimes additive.
- RAL and Provera have additive protective effects on bone BMD. RAL does not antagonize the stimulatory effect of Provera on uterus.
- GH may shift RAL dose-response curve for bone to the left.

17. Report CG3-01

The anabolic effect of synthetic hPTH 1-34 given alone or in combination with estrogen, raloxifene or tamoxifen in young ovariectomized rats

Conclusion

In OVX rats, PTH alone is as effective as PTH + EE2 or PTH + RAL in increasing bone formation, bone mass, and bone strength.

18. Report W53-02

Variations of the ovariectomized rat model: Effect of raloxifene in hypophysectomized, hysterectomized, low calcium diet fed, and restricted diet fed rats

Methods

Six-month (or 75-day old) old SD rats were OVX'ed, HYPOX/OVX'ed or HYSTEX/OVX'ed, and, after 7 days, treated for 35 (or 4) days with RAL (dose?) or EE2 (dose?). GH (1 mg/kg) was given s.c. For diet calcium studies, rats were fed either normal calcium diet (0.5% Ca , 0.4% P), or low calcium diet (0.01% Ca, 0.4% P). In the restricted diet study, animals were given either *ad lib* food access (feed 0.5% Ca, 0.4% P), or restricted food access. Treatment was started 2 days after surgery, and lasted 35 days. Left femoral BMD assessed by SPA (Norland) or X-ray image analysis (grey scale) (Nicolet NXR-1200).

Results

- HYPOX by itself has more pronounced effect on BMD(SPA) and bone X-ray image grey scale (↓), and on uterine weight (↓) than OVX does. HYPOX has no effect on serum cholesterol, while OVX slightly increases it. HYPOX decreases body weight (BW), while OVX slightly increases it.
- In OVX, EE2 prevents uterine weight loss to a large extent, RAL to minor extent. In

HYPOX/OVX, effects of EE2 and RAL are as in OVX.

- In OVX, RAL and especially EE2 markedly increase the reduced bone mass. In HYPOX animals OVX only slightly reduces bone mass further. EE2 does not reverse the loss of bone (as measured by SPA) caused by OVX of HYPOX animals, while the effect of RAL on HYOX/OVX animals is unclear. RAL does not reverse the bone loss causedby HYPOX. Data errors are large.
- In OVX, RAL and especially EE2 markedly decrease the slightly increased serum cholesterol below Sham levels. In HYPOX/OVX, EE2 and RAL decrease serum cholesterol to much lesser extent than in OVX (ca. 50% of the effect).
- In OVX, EE2 and RAL both decrease the slightly increased body weight (BW). In HYPOX/OVX, EE2 and RAL both decrease BW to similar extent as in OVX. However, in 75-day old rats, 4-day treatment with RAL does not decrease BW in HYPOX/OVX, while it does in OVX.
- In HYPOX/OVX, GH slightly increases uterine weight, but does not reverse bone loss or BW loss. GH does not alter effect of EE2 or RAL on BMD, uterine weight and BW.
- Effects of hysterectomy inconclusive.

Conclusions

A. HYPOX studies

- Hypophysectomy by itself decreases body weight, and causes reductions in bone mass and uterine weight larger than the ones caused by ovariectomy.
- Hypophysectomy of OVX rats attenuates the cholesterol-lowering effects of EE2 and RAL.
- Hypophysectomy of OVX rats blocks the bone loss prevention by EE2 and RAL.
- Hypophysectomy of OVX rats has no effect on uterine weight loss reversal and body weight gain reversal by EE2 and RAL.

B. Diet studies

- Restricted diet by itself causes no bone loss, some BW loss, no cholesterol change.
- Restricted diet has no effect on prevention of OVX-induced bone loss by EE2 or RAL.
- Restricted diet prevents body weight increase due to OVX, and BW loss in OVX by EE2 or RAL.
- Restricted diet does not affect hypocholesteremic effect of EE2, but markedly attenuates the cholesterol-lowering effect of RAL (!)
- Low Ca diet for 35 days causes reduction in bone mass in 6-month old rats.
- Low Ca diet in OVX rats does not alter the effects of either EE2 or RAL on body weight, uterine weight, bone mass or cholesterol levels.

OTHER RAT STUDIES

In intact rats (4-35 day studies) raloxifene did not reduce bone mass, suggesting raloxifene does not antagonize estrogens action on bone. This is in contrast to tamoxifen, which increases bone mass in OVX rats, but can decrease it in intact rats (NPR W53-04).

SUMMARY OF RESULTS FROM RAT BONE EFFICACY STUDIES

Body Weight *(1wk -12mo data)*

- Ovariectomy causes a transient increase in body weight of rats
- Raloxifene and estrogen reduce the body weight of ovariectomized animals throughout treatment to levels below sham control values.

Bone Mineral density (BMD) *(5wk and 4-12mo data)*

- In the OVX rat model, raloxifene partially prevents the reduction in BMD caused by ovariectomy in both cancellous and cortical bone.
- The maximal effect of raloxifene was usually somewhat less than the maximal effect of estrogen.
- The effect of ovariectomy and the protective effects of raloxifene and estrogen on BMD of vertebrae, tibia and distal femur were most evident in the first 6 months after ovariectomy. The effect of ovariectomy and treatment on cortical bone was smaller and took longer to establish (>6 mo).

Serum bone markers *(3-7wk and 4-12mo data)*

- Ovariectomy caused a small increase in serum osteocalcin (bone formation marker). In the short term studies (3 wks) the increase was prevented by EE2 more than by raloxifene. In the long term study neither estrogen or raloxifene had a significant effect
- In short term studies (3-7wks), urinary pyrolidine and deoxypyrolidine (bone resorption markers) are slightly increased by ovariectomy. Both raloxifene and estrogen prevented the increase of these parameters. There were no long term data for this marker.

Histomorphometry *(5wk and 6-10mo data)*

- In vertebrae and proximal tibia, relative bone surface and bone volume (BS/TV and BV/TV) are reduced by ovariectomy. This effect is largely prevented by estrogen and raloxifene.
- Resorption surface and osteoclast number are increased by ovariectomy. The increase is partially prevented by estrogen and raloxifene to similar extents.
- In tibia, mineral apposition rate (MAR) is increased by ovariectomy. In a short term study (5 wks) estrogen prevented the increase more than raloxifene. In the long term (10 mo) estrogen nor raloxifene prevented the increase.
- In tibia and vertebrae, mineralizing surface (MS/BS) is increased by ovariectomy in both short and long term studies. Estrogen immediately suppresses this increase. Raloxifene prevents the increase in a delayed manner and to a lesser extent than estrogen.
- Both in short and long term experiments ovariectomy causes a large increase in bone formation rate (BFR), in vertebrae and proximal tibia. Estrogen prevents the increase in bone formation rate immediately upon dosing. In the proximal tibia, raloxifene prevents BFR like estrogen, only after a delay of at least 6 months. In vertebrae, this difference between estrogen and raloxifene was not as prominent.
- After 10 months of treatment, the tibial trabeculae of OVX animals treated with raloxifene

are thicker and less numerous than those of estrogen-treated animals. This difference is not observed in the vertebrae.

Biomechanics *(4-12mo data)*

- Ovariectomy causes a reduction in the force to failure (FtF) of vertebrae, femoral neck and diaphysis, and tibial proximal metaphysis. The reduction is partially prevented by estrogen and raloxifene. The efficacy of estrogen is generally somewhat larger than of raloxifene.
- The efficacy of both estrogen and raloxifene with regard to FtF preservation is most significant at 6-10 months.
- In vertebrae, the increase in bone strength (FtF) by estrogen and raloxifene, as compared to OVX, is accompanied by an increase in ultimate stress (S_u).
- There was a significant correlation ($r = 0.39$, $P < 0.001$) between vertebral BMD and FtF (6-month data).

Serum cholesterol *(5-7wk and 4-12mo data)*

- Raloxifene decreases serum total cholesterol level.
- The effect of raloxifene is similar to or larger than the effect of estrogen.

Uterine effects *(5wk and 4-12mo data)*

- Raloxifene has a slight stimulatory effect on uterine weight in OVX animals. The effects of estrogen on uterine weight and histological parameters are marked. (For more data see Uterine Effects)

Plasma levels

- From 90-day toxicity data we can assume that the Css value in rat plasma at 3 mg/kg/day (ca. 4.5 ng/ml) is 2-10-fold (average 4-fold) the expected therapeutic human plasma level of

Other data

- Compared to Premarin and the other SERMs, tamoxifen, droloxifene, idoxifene and centchroman, raloxifene has the most favorable bone efficacy-uterine safety profile.
- Raloxifene has similar efficacy as alendronate with regard to prevention of bone loss.
- A pituitary factor appears to be involved in the preservation of bone by EE2 and RAL.
- Diet restriction does not affect the bone protective effect of either estrogen or raloxifene, but does attenuate the cholesterol reducing effect of raloxifene.

Bone Efficacy

MONKEY STUDY
(Item 5, Vols. 1.1-1.4; 1 Study/8 Reports)

STUDY X-93-20

A study of the effect of raloxifene (LY139481) hydrochloride on bone and coronary artery atherosclerosis in ovariectomized female cynomolgus monkeys
(Includes 1 executive summary and 8 reports)

Introduction: The advantage of the monkey model is the Haversian modeling like humans, and the fact that bones are bigger and biomechanical testing easier to be done. The cholesterol-fed monkey develops atherosclerosis and this can be used as a model for human atherosclerosis. As human, the monkey has a 28-day cycle, and a proliferative response of uterus to estrogen.This experiment was partly done to test the monkey model since it is relatively inestablished. The group size was chosen to be large enough so as to provide enough statistical power. An other advantage of the monkey model is a similar metabolism and PK of raloxifene as in humans.

!. Report 1

A study of the effect of raloxifene (LY139481) hydrochloride on bone and coronary artery atherosclerosis in ovariectomized female cynomolgus monkeys (Study X-93-20).
(Final Report + 9 appendices, Author: C.P.Jerome)
Lot DPD 14732.

, Study period June 1994-June 1996.

NOTE: Sponsor stated that 1 report is to be submitted in a year containing following data:
-All histomorphometry (HMM) analyses of distal radius, prox femoral neck, femoral mid-shaft
-Surface histology measurements (W.Th, O.Th., OS/BS, ES/BS) of 2nd iliac biopsy and lumbar vertebrae.

Methods

Cynomolgus monkeys (n= 135), weight 3 kg, age > 9 years, were purchased from Indonesia and quarantined for at least 90 days 9 (13 wks). From 5 weeks after start of quarantine, animals were fed an experimental diet containing 0.41 mg/Cal cholesterol and 0.3% calcium, and 0.3% phosphorus. Animals (/dose group) were (1) sham-operated (***sham***), or OVX-ed, and dosed, from day after surgery, orally from a syringe using a liquid vehicle suspension (Crystal light fruit punch drink) with (2) placebo (***Ovx***), (3) RAL 1 mkd (***R1***), (4) RAL 5 mkd (***R5***), or (5) Premarin (0.04 mkd) (***PR***), for 24 months. The 1 mkd RAL dose was expected to give plasma levels approximating the ones in humans dosed with 60 mg (1 mkd). Treatment was for 2 years which is equivalent to 4-6 years in humans.

Determined were:

*Survival and clinical signs
*Body weight
*Serum hormones (estrone, estradiol, progesterone): every 3-6 months
*PK (RAL and T.RAL): at 1 mo, 10.5 mo, 22.5 mo
*Bone mass: at baseline and every 6 months: Lumbar spine (L2-L4) and whole body BMC by DXA, proximal tibia and distal radius BMC by QCT (sequential peel method: 4 concentric zones analyzed, each comprising 25% of total slice area: outer, middle, inner and central zones. Since area was the same, BMC was measured parameter.
*Biomarkers and bone metabolism markers: serum/urine calcium and phosphorus, urine creatinine, serum alkaline phosphatase (ALP), acid phosphatase (ACP) and tartrate-resistant ACP (TRAP), osteocalcin (bone Gla protein, BGP), urinary collagen degradation products (3-hydroxypyridinium collagen crosslinks: pyridinoline and deoxypyridinoline) (CrossLaps ELISA), all quarterly. Cardiovascular risk factors: plasma lipids/lipoproteins, every 3-6 mo.
*Histological and histomorphometry: Iliac biopsies taken at 0 and 16 months; Lumbar spine (LV2), femur neck and midshaft, and distal radius removed at 24 months.
Structural analysis (Optimas system), done on 4-6 concentric zones; histological and label histomorphometry (HMM) measurements done in endocortical (Ec) region (outer three zones combined), and cancellous (Cn) area (remaining zones). Fluorochromes used: tetracycline (0 mo), calcein (mo 16), xylenol orange (mo 24) on a 1-12-1-7 day schedule.

Month	0	16	24
Sample	Iliac biopsy	Iliac biopsy	LV2, femur (neck/shaft), radius (distal)
Structure (Optimas)	+	+	LV2
Histology	+	+	LV2
HMM	-	+	LV2, femur, radius

APPEARS THIS WAY
ON ORIGINAL

HMM parameters (primary and derived):

Variable	Abbreviation	Units
Bone volume	BV/TV	%
Bone surface/volume	BS/BV	mm^2/mm^3
Osteoid surface	OS/BS	%
Eroded surface	ES/BS	%
Osteoclastic surface	Oc.S/BS	%
Wall thickness	W.Th	µm
Osteoid thickness	O.Th	µm
Single-labeled surface	sLS/BS	%
Double-labeled surface	dLS/BS	%
Mineralizing surface	MS/BS	%
Mineral apposition rate	MAR	µm/day
Bone formation rate, surface referent	BFR/BS	$\mu m^3/\mu m^2$/year
Bone formation rate, bone volume referent	BFR/BV	%/year
Bone formation rate, tissue volume referent	BFR/TV	%/year
Baseline label retained	L.Le/B.Ar	mm/mm^2

*Biomechanical parameters: humeral diaphysis, proximal femoral neck, lumbar spine (L3 and L4): 24 months
*Histology and -morphometry of uterine epithelium and mammary gland: 24 months
*Quantitative coronary angiography: 24 months
*Radiography of spine, wrist, knee at baseline, and of spine also at 24 mo (check for vertebral deformity)
*Qualitative evaluation of bone structure (woven/laminar) by polarized light and epifluorescence microscopy.

Results

Survival -

Of a total of 117 animals that entered the study, 3 were removed, 11 died. Deaths were evenly distributed among dose groups and none of the deaths appeared treatment-related.

Clinical signs -

83 incidents, involving 58 animals, distributed evenly over treatment groups. Upon terminal labeling with xylenol orange, 3 monkeys had severe neurological signs and were terminated (1 Sham, 1 Ovx, 1 R1)

Body weight -

No significant effects

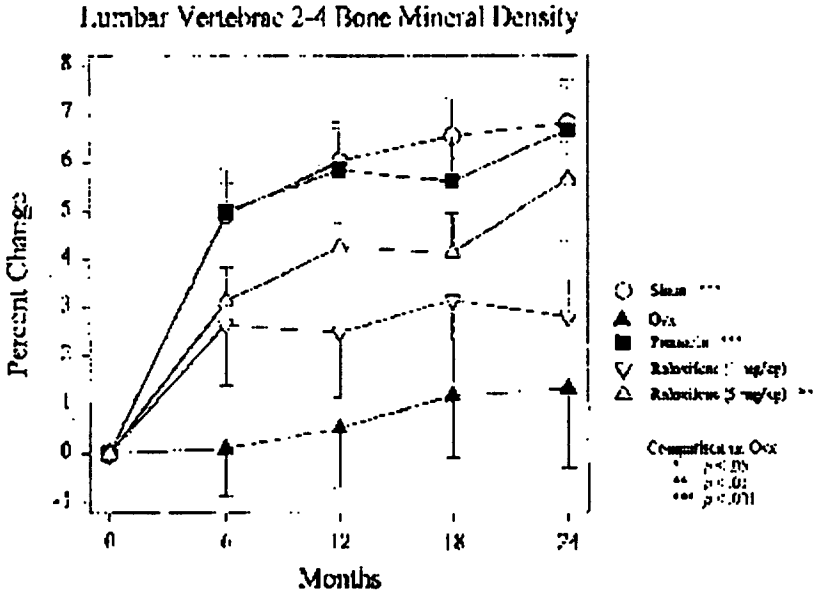
BMD -

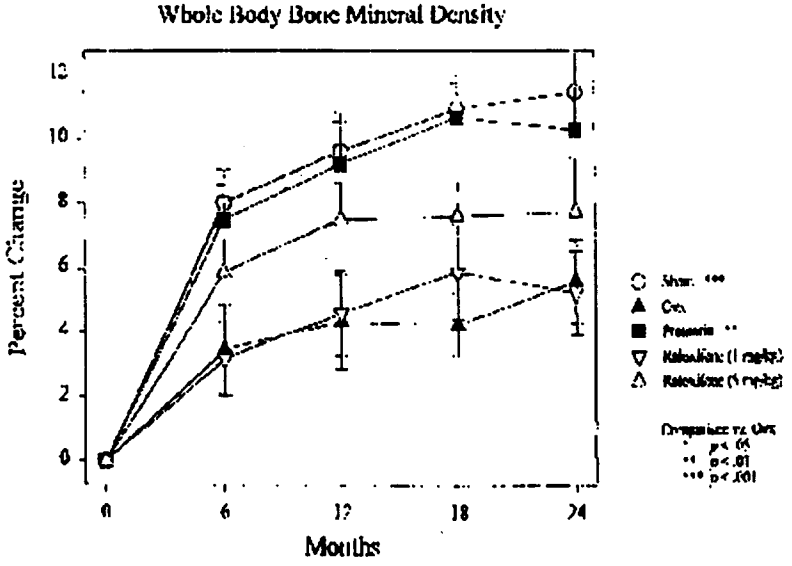
A.. Lumbar vertebrae, Whole body (DXA)

-All groups gained bone mass.

-BMD increase in OVX was decreased as compared to SHAM control

-Premarin and raloxifene prevented this "relative osteopenia"





B. Tibia, Radius (QCT)

- Tibia (4 zones): In inner and central zone, BMC minimally decreased in Ovx; Effect reversed by RAL and PR. In other zones: no consistent effects of either OVX or treatment.
- Radius (4 zones): In central zone, BMC decreased in Ovx vs. Sham; Effect partially reversed by RAL, and also but less by PR; no other consistent effects.

Figure 3. Change in Tibia BMC Across Zones

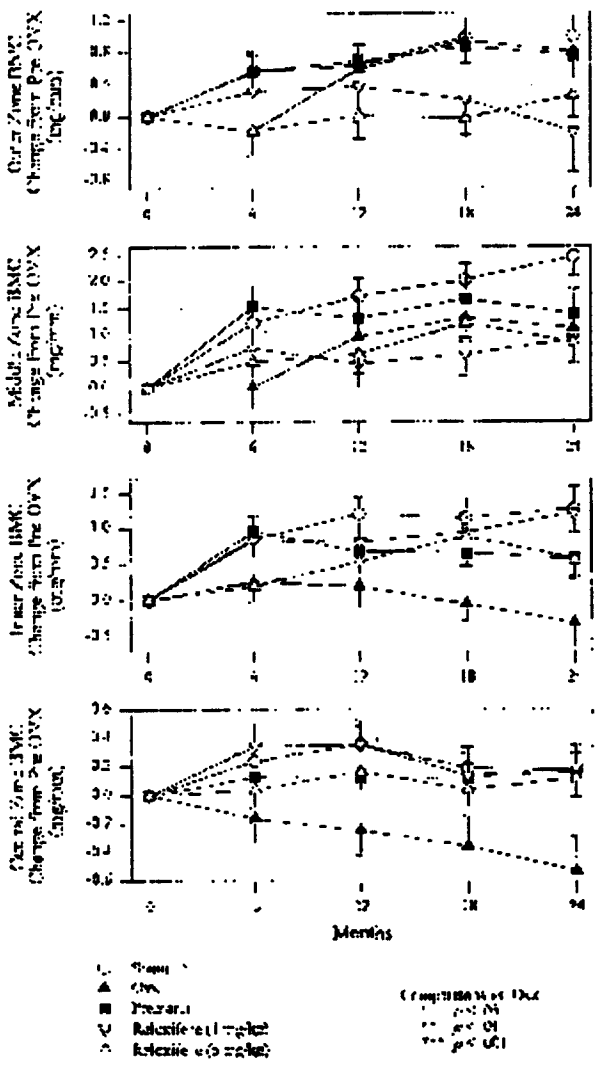
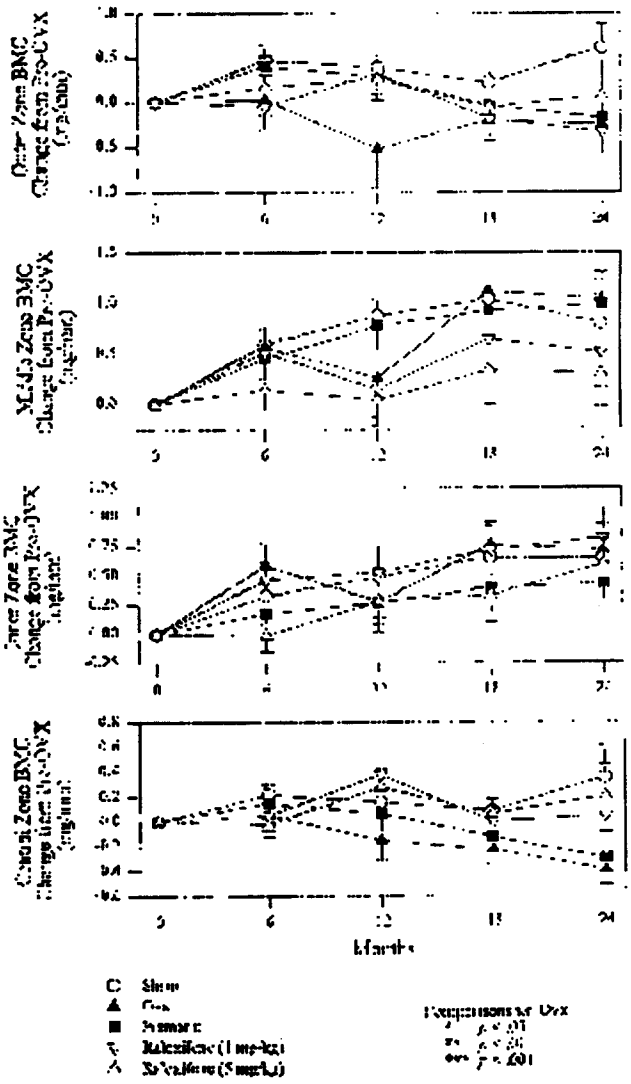


Figure 4. Change in Radius BMC Across Zones



Synopsis of results (BMD)

Vertebrae/Whole body:

- Premarin and raloxifene prevent the OVX-induced inhibition of BMD gain in lumbar vertebrae and whole body.
- In vertebrae, the effects of R5 and PR are significant, while the effect of R1 (which was planned to be optimal dose) is not significant. The effect of R5 is smaller than (0.6-0.8x) the effect of Premarin.
- In whole body, Premarin and R5 significantly prevent the relative bone loss, while R1 has no effect. Effect of R5 is ca. 0.5x effect of Premarin.
- The BMD effect of OVX and of RAL or Premarin occurs mainly in the first 6 months after ovariectomy. After that, the BMD of all groups changes in parallel.

Tibia/Radius:

- In proximal tibia and distal radius, the anticipated effect of OVX, PR and RAL on BMD is only observed in the central zone.
- In other tibial and radial zones there are no consistent and/or significant effects of OVX or treatment.

Bone biomarkers/ serum hormones/ serum lipids -

	Sham - change over time	Ovx vs. Sham	Ovx/PR vs. Ovx	Ovx/PR vs. Sham	Ovx/R1 vs. Ovx	Ovx/R1 vs. Sham	Ovx/R5 vs. Ovx	Ovx/R5 vs. Sham
Body weight	+	<	=	<	=	<	=	<
ALP	=	>	<<	<	<	=	<	=
Gla protein	=	>	<	=	<	>	<	>
ACP	=	=	=	=	=	=	=	=
TRAP	+	>	<	<	<	=	<	=
Serum Ca	+	>	<	<	<	>	<	>
Serum P	=	>	<	<	<	=	=	>
Urine Ca/Cr	=	<	>	=	>	=	>	=
Urine P/Cr	-	=	=	=	=	=	=	=
Urinary Crosslaps	-	>	<	<	<	=	<	=
Estrone	= (55-90 pg/ml)	<	>>	>>	=	<	=	<
Progesterone	= (1.5-2.5 ng/ml)	<<	=	<<	=	<<	=	<<

Estradiol	= (40-80 pg/ml)	<< (<0.3x)	>>	>> (3x !)	=	<< (<5 pg/ml	=	<< (<5 pg/ml
Total Cholesterol	=	>>	<<	>	<	>	<	>
HDL-chol	+	<	=	<	=	<	=	<
non-HDL-chol	=	>>	<	>	<	>	<	>
LDL-chol	=	>>	<	>	<	>	=	>>
LDL-Mw	=	>	<	=	<	>	=	>
Lipoprotein (a)	=	>	<	=	=	>	>	>>
Apo-B	=	>	<	=	<	=	=	>
Apo-E	=	>	<	=	<	>	<	>
Total triglyc	=	=	=	>	=	>	=	>
V + I LDL-chol	=	>>	<	=	<	>	<	>

SUMMARY TABLE. Effects on BMD, serum bone markers, serum cholesterol (significant unless noted otherwise)

	OVX (vs. Sham)	OVX + PR, R1 or R5 (vs. OVX control)		
		PR	R1	R5
L2-L4 BMD	↓	↑	↑ns	↑
Whole body BMD (DXA)	↓	↑	ns	↑ns
Tibia Radius BMC (QCT)	ns	ns	ns	ns
Alk Phos *(formation marker)*	↑	↓↓	↓ns	↓
Bone Gla *(turnover marker)*	↑	↓↓	↓ns	↓ns
Acid Phos	ns	ns	ns	ns
TRAP *(resorption marker)*	↑	↓↓	↓ns	↓
Crosslaps *(resorption marker)*	↑	↓↓	↓	↓
Cholesterol	↑	↓	↓	↓ns
HDL-Chol	↓	=	=	=
LDL-Chol	↑↑	↓	↓	=
V+I LDL-Chol	↑↑	↓	↓	↓
Triglycerides	=	=	=	=

ns = no significant change

Synopsis of results (bone markers and cholesterol)

- Bone formation and resorption markers (ALP, Gla, TRAP, Crosslaps) are increased by OVX. Premarin decreases all markers below Sham-level. R1 and R5 decrease markers much less than PR. Part of the effects of R1 and R5 were non-significant. For all markers, there was no appreciable difference between the effects of R1 and R5. The smaller effects of R1, R5 than of Premarin are consistent with their lesser effect on BMD.
- Cholesterol is increased by OVX, decreased by PR and R1, R5. Effect of PR similar as RAL.
- HDL-cholesterol is decreased by OVX, but unaffected by PR or R1,R5.
- LDL and V+I LDL-cholesterol are markedly increased by OVX, and decreased partially by PR and R1, R5.

Spine radiography -

Table 1. Incidence of spinal deformity at necropsy in various treatment groups

% Incidence of	Vertebrae affected	Animals affected
Vertebral compression	Sham > Ovx (?!) PR < Ovx R1, R5 = Ovx	Sham > Ovx (?!) PR < Ovx R1,R5 > Ovx (?!)
Disk Space Narrowing	Sham < Ovx PR < Ovx R1 < Ovx R5 > Ovx (?!)	Sham < Ovx PR < Ovx R1 < Ovx R5 > Ovx (?!)
Osteophytosis (presence of osseous outgrowths)	Sham = Ovx PR < Ovx R1, R5 = Ovx	Sham < Ovx PR < Ovx R1, R5 = Ovx

- Vertebral compression data were not reliable
- Disk space narrowed by OVX; PR and R1 prevent, R5 intensifies the effect (?!)
- Osteophytosis occurred more frequently in OVX; PR prevented, R1, R5 has no effect

Histomorphometry -

(A1) Iliac biopsy (16 mo) *(Data from Vol. 1.3, Tables 17b, 18b)*

Group comparison of histomorphometric data from iliac biopsy (Ec and Cn zone combined)

		Ovx vs. Sham	Ovx/PR vs. Ovx	Ovx/PR vs. Sham	Ovx/R1 vs. Ovx	Ovx/R1 vs. Sham	Ovx/R5 vs. Ovx	Ovx/R5 vs. Sham	Stat. Test
Structural/Optimas Data									
BV/TV (%)	no significant differences between treatment groups								ns
BS/BV (mm^2/mm^3)	no significant differences between treatment groups								ns
Label Histomorphometry Data									
BV/TV (%)	no significant differences between treatment groups								ns
BS/BV (mm^2/mm^3)	no significant differences between treatment groups								ns
sLS/BS (%)	no significant differences between treatment groups								ns
dLS/BS (%)		>>	<<	<	=	>	<	=	Sham, PR < OVX
MS/BS (%)		>>	<<	<	=	>	<	>	Sham, PR, R5 < OVX
MAR (um/day)		>	<<	<	=	>	<	=	Sham, PR < OVX
BFR/BS ($um/um^2/y$)		>>	<<	<	<	>	<	=	Sham, PR, R5 < OVX
BFR/BV (%/yr)		>>	<<	<	<	>	<	>	Sham, PR, R5 < OVX
BFR/TV (%/yr)		>>	<<	<	<	>	<	=	Sham, PR, R5 < OVX
L.Le/B.Ar		<	>	>	>	=	>	=	PR > OVX

Note: Changes in Ec and Cn zone were most of the time very similar

(B) Lumbar Vertebrae 2 (24 mo) *(Data from Vol. 1.3, Tables 19, 20)*

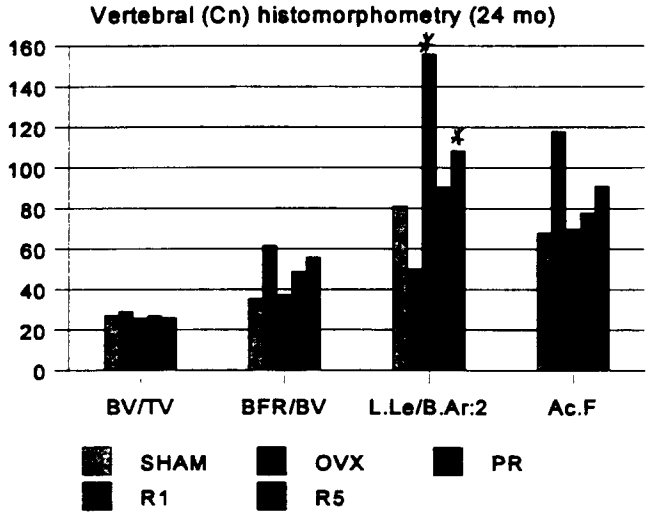
Group comparison of histomorphometric data from Lumbar Vertebrae 2

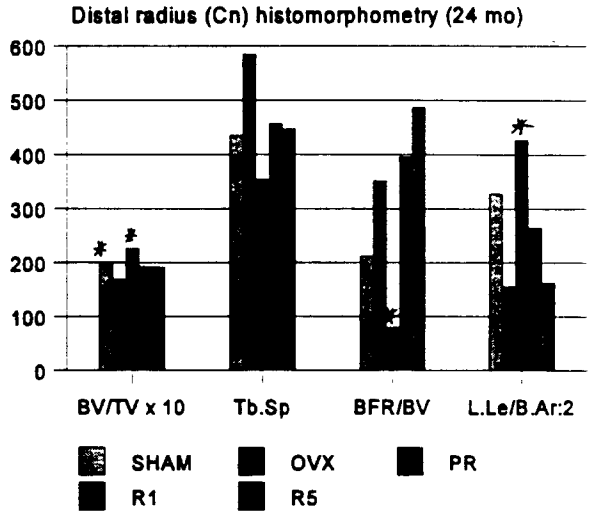
	Zone	Ovx vs. Sham	Ovx/PR vs. Ovx	Ovx/PR vs. Sham	Ovx/R1 vs. Ovx	Ovx/R1 vs. Sham	Ovx/R5 vs. Ovx	Ovx/R5 vs. Sham	Stat. Test
Structural/Optimas Data									
BV/TV (%)	Ec (zone 1-3)	> (?!)	<	<	=	>	<	>	ns
	Cn (zone 4-6)	no significant differences between treatment groups							
BS/BV (mm^2/mm^3)	Ec	<	>	>	=	<	>	<	ns
	Cn	no significant differences between treatment groups							
Label Histomorphometry Data									
BV/TV (%)	Ec	<	>	<	>	<	=	<	ns
	Cn	no significant differences between treatment groups							
BS/BV (mm^2/mm^3)	Ec	>	<	=	<	>	=	>	ns
	Cn	no significant differences between treatment groups							
sLS/BS (%)	Ec, Cn	no significant differences between treatment groups							
dLS/BS (%)	Ec	>>	<<	<	<	=	<	=	Sham, PR, R1, R5 < OVX
	Cn !!	>	<	=	>	>	>>	>>	ns
MS/BS (%)	Ec	>	<<	<	<	<	<	=	PR, R1 < OVX
	Cn !!	>	<	>	=	>	> !!	>	ns
MAR (um/day)	Ec	>	<<	<	<	>	<	=	PR< OVX
	Cn !!	>	<<	<	<	<	= !!	>	PR, R1< OVX
BFR/BS (um/um^2/yr)	Ec	>	<<	<	<	=	<	=	Sham, PR, R1, R5 < OVX
	Cn !!	>	<	=	<	>	= !!	>	ns
BFR/BV (%/yr)	Ec	>	<<	<	<	>	<	>	Sham, PR, R1, R5 < OVX
	Cn	>	<	=	<	>	= !!	>	ns
BFR/TV (%/yr)	Ec	>	<	<	<	=	<	=	Sham, PR, R1, R5 < OVX
	Cn	>	<	=	<	>	<	>	ns

L.Le/B.Ar	Ec	=	>>	>>	=	=	>	>	PR > OVX
	Cn	<	>>	>	>	=	>>	>	PR, R5 >OVX

Note: Zone numbers indicate distance from periostal surface

(C) Additional HMM data: Vertebrae and Distal Radius (NDA amendment, October 17, 1997)
FIGURE 1: Histomorphometry of vertebrae and distal radius





1. Data from vertebrae show that despite lack of effect on BV/TV, there appear to be effects of OVX, PR and RAL on BFR/BV, L.Le/B.Ar (baseline label retained, i.e., reverse of resorption). The lack of an effect on the HMM parameter BV/TV is unexplained and inconsistent with DXA data on BMD.
2. Data from distal radius show that unlike in the PR-group, resorption and formation in RAL-groups are not affected, and are in fact similar as in OVX. However, BV/TV (and BMD) in RAL-groups is slightly higher than in OVX, and raloxifene has a small reversal effect on Tb.Sp, although these effects were again smaller than of Premarin. A similar effect was seen in the rat.
3. Upon ovariectomy both formation and resorption are increased as compared to sham, and the balance between the two is altered in such a way that resorption surpasses formation resulting in net bone loss. These radius data are interesting and may suggest that the balance or coupling between bone formation and resorption is different in RAL-treated than in estrogen- or Premarin-treated, but also different than in OVX animals.

Synopsis of results (histomorphometry)

Ilium (16 mo biopsy)

SUMMARY TABLE. Effects on main HMM parameters of ilium

Effect		dLS/BS	MS/BS	MAR	BFR	L.Le/B.Ar
vs. SHAM	OVX	↑	↑	↑	↑	↓
vs. OVX	PR	↓↓	↓↓	↓↓	↓↓	↑↑
	R1	=	=	=	↓	↑
	R5	↓	↓	↓	↓	↑

- No effects on BV/TV and BS/BV by two microscopic technique (Optimas, Label HMM)
- However, dynamic HMM parameters indicated inhibition by RAL of OVX-induced increase in bone turnover, although less than by PR.

Vertebrae (L2) (24 months)

SUMMARY TABLE. Effects on main HMM parameters of vertebrae.

Effect in endocortical (Ec) region		BV/TV	BS/BV	dLS/BS	MS/BS	MAR	BFR	L.Le/B.Ar
vs.SHAM	OVX	↓	↑	↑↑	↑	↑	↑	=
vs.OVX	PR	↑	↓	↓↓	↓↓	↓↓	↓↓	↑↑
	R1	↑	↓	↓	↓	↓	↓	=
	R5	=	=	↓	↓	↓	↓	↑

Effect in cancellous (Cn) region		BV/TV	BS/BV	dLS/BS	MS/BS	MAR	BFR	L.Le/B.Ar
vs.SHAM	OVX	= (?!)	=	↑	↑	↑	↑	↓
vs.OVX	PR	=	=	↓	↓	↓↓	↓	↑↑
	R1	=	=	↑ (?!)	=	↓	↓	↑
	R5	=	=	↑↑ (?!)	↑ (?!)	=	=	↑↑

- Optimas data on effects of OVX, PR and RAL on BV/TV and BS/BV were opposite of expected
- There was a decrease in BV/TV in the Ec region, prevented by PR and RAL, but there was no effect on BV/TV in the cancellous (Cn) region (?)
- Bone formation and resorption were decreased by PR more than by RAL.

Remark:

HMM data from vertebrae (Cn region) were unexpected and inconsistent with BMD data. Sometimes the effect of R5 was less than the effect of R1 (MAR, BFR) and sometimes the effects of raloxifene were the opposite of the effect of PR (dLS/BS, MS/BS).

Qualitative bone evaluation -
Ilium and vertebrae: No abnormalities of bone lamellation, collagen lamellae obvious. Some abnormalities in fluorescent label extent or distribution, a few animals woth cartilage nodules. Effects not treatment-related.

2. Report 2

Plasma pharmacokinetics in OVX monkeys following multiple oral doses of 1 or 5 mg/kg of raloxifene for 2 years (Study X-93-20).

(ADME Report 36, Lilly Research Labs, IN)

Methods

Sampling: On Day 30, 315, 675 over 48-h period after dosing (one days' dose missed), N=8

Assay: HPLC/UV or LC/MS/MS

Total raloxifene = raloxifene in sample after incubation with B-glucuronidase

Results

Table 1. PK parameters

DAY	DOSE	1 mg/kg				5 mg/kg			
		$AUC_{(1-48h)}$ (ngxh/ml)	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{(1-48h)}$ (ngxh/ml)	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)
Day 30	Ral	86	2.0	11	NC	170	3.8	11	NC
	T.Ral	1009	31	3	46	3611	90	6	27
Day 315	Ral	53	1.5	7	18	132	3.8	4	36
	T.Ral	255	22	5	NC	2369	80	1	25
Day 675	Ral	105	2.1	16	NC	145	4.2	7	NC
	T.Ral	1514	36	6	37	2783	86	6	25
Average	Ral	81(n=3)	1.9	11	18 (n=1)	149 (n=3)	3.9	7.3	36 (n=1)
	T.Ral	926 (n=3) 1262 (n=2)	30	4.7	42 (n=2)	2921 (n=3)	85	4.3	26 (n=3)

NC = not calculabable

Ranges (for both 1 mkd and 5 mkd dose groups):

Ral/T.Ral (AUC basis) (value 0.21 at 1 mkd on Day 315 excluded)

(C_{max} basis)

T_{max}

$T_{1/2}$ (Ral)

(T.Ral)

Table 2. C_{ss} values for human and monkey

Css	Monkey (avg. of 3 sampling days)		Human	Ratio Monkey:Human	
Dose	1 mkd	5 mkd	60 mg/day	1 mkd	5 mkd
Ral (ng/ml)	1.9	3.9	(avg. 1.0)	2x	4x
T.Ral (ng/ml)	30	85	(avg. 170)	0.2x	0.5x

Conclusion

At the 1 mkd and 5 mkd dose respectively, the monkey plasma levels of unconjugated raloxifene were 2x and 4x, and the plasma levels of total raloxifene were 0.17x and 0.5x the predicted human plasma levels at the 60 mg/day dose.

3. Report 3

Results from biomechanical tests (Study X-93-20)

(Dept. Orthopaedic Surgery, Indiana University, IN) Report author Charles H. Turner.

Methods

Dose groups: Control, OVX, PR, R1, R2 (N = 19, 20, 23, 21, 20)

Tested: Excised bones: L3 and L4 (compression), proximal femur, midshaft femur (machined beam), midshaft humerus

Measured parameters:

Table 1. Variables reported for the humerus, proximal femoral neck, proximal femoral beam specimens, and third and fourth lumbar vertebrae (L3 and L4)

Humerus
average cortical thickness (t)
ultimate force is the maximum force the specimen can withstand, in N, (F_u)
slope of the linear portion of the force-displacement curve, in N/mm, (slope)
area under the load-displacement curve, in N-mm, (area)

Proximal Femoral Neck
ultimate force is the maximum force the specimen can withstand, in N, (F_u)

Lumbar Spine
cross-sectional area, in mm^2, (A)
yield force is the force at a 0.2% offset, in N, (F_y)
ultimate force is the maximum force the specimen can withstand, in N, (F_u)
slope of the linear portion of the force-displacement curve, in N/mm, (slope)
yield stress, MPa, (σ_y)
ultimate stress, MPa, (σ_u)
Young's modulus, MPa, (E)
toughness, MPa, (tough)

Proximal Femoral Beam Specimens
ultimate stress, MPa, (σ_u)
Young's modulus, GPa, (E)
toughness, MPa, (tough)
ultimate strain, %, (ε_u)

Results

(A) Biomechanical parameters: See Tables 2 and 3 (from Vol. 1.4)

Table 2. Summary of results. P-values for ANOVA and Fisher's posthoc comparisons are listed. (The number of specimens for each group is shown in parentheses.)

Measurement	Groups SHM	OVX	PR	R1	R2	ANOVA (p-value)	Fisher's PLSD
Humerus							
t (mm)	1.62±0.04 (19)	1.61±0.03 (20)	1.58±0.04 (23)	1.59±0.04 (21)	1.64±0.03 (20)	0.78	
F_U (N)	717±26 (19)	706±25 (20)	680±26 (23)	652±22 (21)	702±25 (20)	0.39	
slope (N/mm)	696±21 (19)	688±22 (20)	668±20 (23)	654±21 (21)	677±28 (20)	0.71	
area (N-mm)	1564±112 (19)	1752±136 (20)	1635±94 (23)	1596±81 (21)	1502±88 (20)	0.55	
Proximal Femoral Neck							
F_U (N)	1200±42 (16)	1144±54 (18)	1314±48 (20)	1199±48 (21)	1200±62 (17)	0.12	
Third Lumbar Vertebra (L3)							
A (mm^2)	87.1±2.0 (19)	81.4±2.3 (20)	91.6±2.3 (23)	88.3±2.7 (21)	91.3±2.7 (19)	0.02	R1 & R2 > OVX R1 & R2 > E
F_y (N)	1500±78 (19)	1474±75 (20)	1696±81 (23)	1516±82 (21)	1483±80 (19)	0.29	
F_U (N)	1952±91 (19)	1818±86 (20)	2125±101 (23)	1898±100 (21)	1920±104 (19)	0.21	
slope (N/mm)	4870±347 (19)	4577±316 (20)	5440±348 (23)	5513±286 (21)	4951±317 (19)	0.21	
σ_y (MPa)	17.5±1.0 (19)	18.2±0.9 (20)	20.9±1.0 (23)	17.3±1.2 (21)	18.4±1.0 (19)	0.03	E > SHM, R1, R2

Table 2. Continued.

Measurement	Groups SHM	OVX	PR	R1	R2	ANOVA (p-value)	Fisher's PLSD
Fourth Lumbar Vertebra (L4)							
A (mm^2)	86.8±2.2 (19)	89.6±2.6 (20)	91.7±2.3 (23)	96.1±3.1 (21)	90.7±3.1 (20)	0.06	
F_y (N)	1673±80 (19)	1532±76 (20)	1831±77 (23)	1600±101 (21)	1689±93 (20)	0.14	
F_U (N)	2133±84 (19)	1912±76 (20)	2208±93 (23)	1956±127 (21)	2066±113 (20)	0.21	
slope (N/mm)	6169±295 (19)	4737±297 (20)	6051±231 (23)	4980±365 (21)	5057±328 (20)	0.03	E > all groups
σ_y (MPa)	17.6±0.8 (19)	17.2±0.8 (20)	20.1±0.7 (23)	16.4±1.0 (21)	17.1±0.9 (20)	0.02	E > all groups
σ_u (MPa)	22.1±0.9 (19)	21.5±0.8 (20)	24.4±1.0 (23)	20.2±1.3 (21)	20.9±1.1 (20)	0.04	E > OVX, R1, R2
E (MPa)	433±27 (19)	422±23 (20)	516±21 (23)	408±30 (21)	410±21 (20)	0.01	E > all groups
tough (MPa)	1.94±0.14 (19)	1.81±0.15 (20)	1.84±0.13 (23)	1.43±0.14 (21)	1.59±0.17 (20)	0.15	
Third and Fourth Lumbar Vertebrae (L3+L4)/2							
A (mm^2)	22.0±2.2 (19)	85.5±2.3 (20)	88.7±2.6 (23)	93.6±2.7 (21)	96.7±2.8 (20)	0.02	R1 & R2 > OVX R2 > E
F_y (N)	1520±67 (19)	1504±70 (20)	1758±73 (23)	1558±92 (21)	1599±84 (20)	0.17	
F_U (N)	2042±84 (19)	1809±76 (20)	2174±89 (23)	1914±115 (21)	2008±107 (20)	0.10	
slope (N/mm)	5020±230 (19)	4697±237 (20)	5758±218 (23)	5249±263 (21)	5026±274 (20)	0.05	E > SHM, OVX, R2
σ_y (MPa)	17.3±0.8 (19)	17.7±0.8 (20)	20.5±0.8 (23)	16.8±1.0 (21)	16.8±0.9 (20)	0.01	E > all groups
σ_u (MPa)	22.4±1.0 (19)	22.0±0.9 (20)	25.4±1.1 (23)	20.8±1.3 (21)	21.3±1.1 (20)	0.02	E > OVX, R1, R2
E (MPa)	443±22 (19)	434±18 (20)	516±21 (23)	458±26 (21)	428±22 (20)	0.02	E > all groups

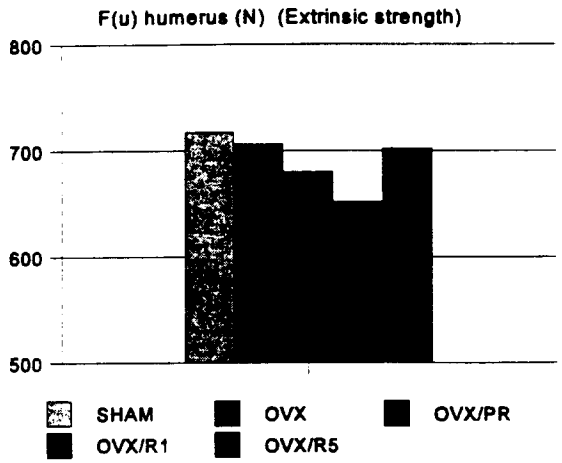
Table 2. Continued.

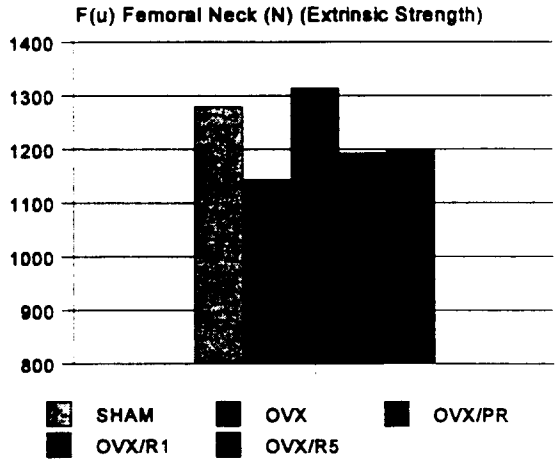
Measurement	Groups					ANOVA	Fisher's PLSD
	SHM	OVX	PR	R1	R2	(p-value)	
Proximal Femoral Beam Specimen							
σ_u (MPa)	325±9 (18)	315±9 (19)	337±6 (22)	331±7 (21)	310±8 (19)	0.09	
E (GPa)	18.7±0.4 (18)	17.5±0.5 (19)	18.8±0.3 (22)	18.9±0.4 (21)	17.8±0.4 (19)	0.03	SHM, E, R1 > OVX
tough (MPa)	10.4±0.8 (16)	10.1±0.7 (19)	10.1±0.6 (22)	8.6±0.5 (21)	8.6±0.7 (19)	0.20	
ε_u (%)	4.3±0.3 (18)	4.3±0.3 (19)	4.1±0.2 (22)	3.7±0.2 (21)	3.8±0.3 (19)	0.32	

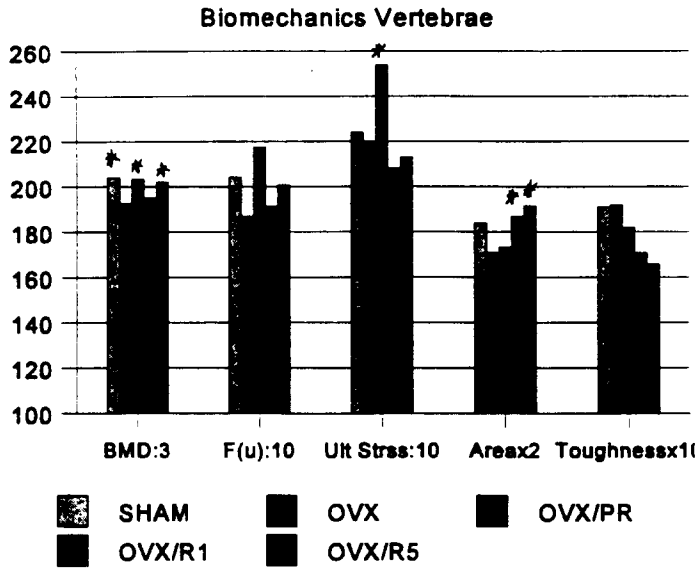
Table 3. Summary of adjusted results after omission of vertebral data due to questionable test results. P-values for ANOVA and Fisher's posthoc comparisons are listed. (The number of specimens for each group is shown in parentheses.)

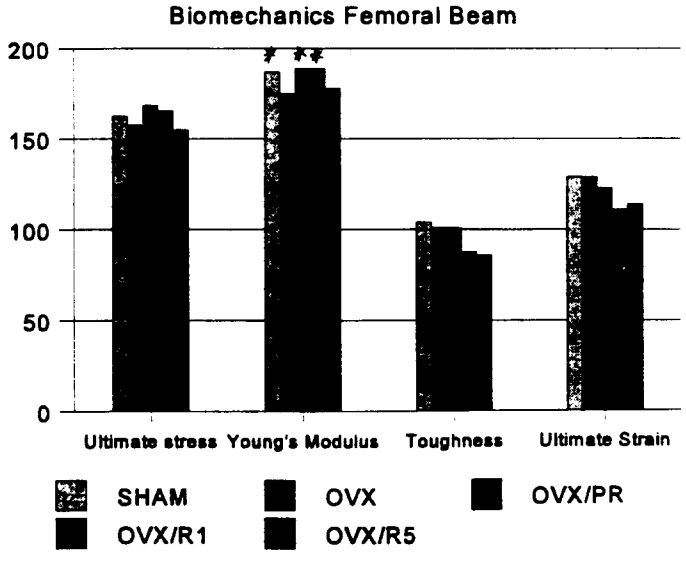
Measurement	Groups					ANOVA	Fisher's PLSD
	SHM	OVX	PR	R1	R2	(p-value)	
Third Lumbar Vertebra (L3)							
F_u (N)	1987±103 (15)	1848±70 (17)	2068±100 (19)	1942±136 (15)	1878±101 (18)	0.54	
σ_u (MPa)	23.7±1.3 (15)	22.3±0.9 (17)	25.5±1.3 (19)	22.7±1.6 (15)	20.9±1.3 (18)	0.15	
tough (MPa)	1.85±0.21 (14)	1.58±0.15 (16)	1.71±0.15 (17)	1.28±0.17 (12)	1.46±0.20 (14)	0.26	
Fourth Lumbar Vertebra (L4)							
F_u (N)	2124±98 (18)	1911±80 (18)	2210±96 (21)	1938±127 (19)	2056±126 (18)	0.25	
σ_u (MPa)	22.1±0.9 (18)	21.9±0.8 (18)	24.3±1.0 (21)	20.2±1.3 (19)	21.2±1.2 (18)	0.07	
tough (MPa)	1.75±0.17 (15)	1.35±0.07 (12)	1.58±0.14 (19)	1.38±0.13 (16)	1.37±0.17 (13)	0.13	
Third and Fourth Lumbar Vertebra (L3+L4)/2							
F_u (N)	2053±82 (19)	1858±73 (20)	2108±96 (21)	1938±117 (20)	2011±111 (20)	0.20	
σ_u (MPa)	22.7±0.9 (19)	22.1±0.8 (20)	25.2±1.1 (21)	20.9±1.4 (20)	21.3±1.1 (20)	0.05	E > OVX, R1, R2
tough (MPa)	1.78±0.15 (18)	1.42±0.13 (16)	1.66±0.13 (21)	1.38±0.14 (18)	1.50±0.17 (16)	0.42	

FIGURE: Graphical representation of data from Tables 2, 3
(* = significantly different from OVX)









(B) Correlation BMD and F_u (vertebrae): See Figure 1 (from Vol. 1.4)

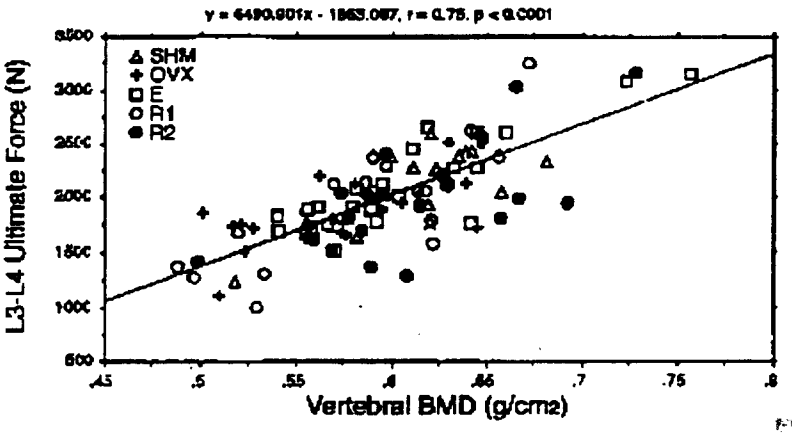


Figure 1. Ultimate force (strength) for the lumbar vertebrae correlated well with the lumbar spine bone mineral density.

Synopsis of results

Lumbar vertebrae (compression test)

- F_u decreased by OVX (n.s.). Decrease reversed completely to above-Sham level by PR, reversed partially by R1, R5 (n.s.)
- Area decreased by OVX (n.s.), decrease not prevented by PR. Area increased sign. above Sham level by R1, R5 (*Note: Area measurement is probably inaccurate, and does not agree with data on projected area from DEXA*).
- Ultimate stress σ_u unaffected by OVX, sign. increased by PR, and unaffected by R1, R5 *(Note: Ultimate stress is calculated from Fu and Area, and may thus be inaccurate)*
- Yield stress unaffected by OVX, increased by PR (sign.), and unaffected by R1, R5
- Young's modulus increased by PR (sign.), unaffected by R1, R5
- Toughness decreased by OVX (n.s.), decrease partially prevented by PR, not by R1, R5 (n.s.)
- Correlation between spinal BMD and ultimate load (L3 + L4/2) statistically significant (r = 0.75)

Humerus (3-pt bending test)

- No biomechanical effects

Proximal femoral neck (compression/bending)

- F_u decreased in OVX, reversed completely by PR, partially by R1 and R5 (results not statistically significant, n.s.)

Proximal femoral beam specimen (3-pt bending test)

- Ultimate stress not affected by OVX, Premarin or RAL.
- Young's modulus decreased by OVX (sign), decrease prevented by PR and R1, but not by R5.

Conclusions

1. Despite the expected trends, all effects on ultimate load (F_u) at all bone sites were not statistically significant.
2. In vertebrae, there was a significant positive correlation between BMD and F_u, similar for all treatment groups. This is indirect evidence that BMD is a dependable predictor of bone strength.

4. Report 4
Endometrial and mammary gland evaluations (Study X-93-20)
Original final report.
Report Author J. Mark Kline.

Methods
Histopathology/Histomorphometry of uterine/mammary tissues from 2-year bone quality study
Groups: Sham, OVX, Premarin (0.04 mg/kg/day), raloxifene (1 and 5 mg/kg/day)

Results
There were no neoplastic lesions in any group.

Uterine Histology and Histomorphometry
- Uterine weight was not affected by raloxifene (Communication with Sponsor).
- PR caused proliferative lesions (hyperplasia) in endometrium of OVX animals, RAL did not
- Detailed histologic examination showed some minimal Premarin-like effects of raloxifene: (A) epithelial proliferation score (R1 and R5), stromal expansion (R1 and R5); (B) glandular shape change (R1 and R5), N:C ratio decrease (R1 and R5); (C) stromal density change (R1 and R5). However, unlike effect of Premarin, glandular mitoses were decreased and stromal mitoses were unaffected by raloxifene.
- Raloxifene caused a minimal increase in estrogenicity scores in a few animals (3/43), while Premarin induced a slight to large increase in estrogenicity scores in all animals (24/24).
- There was no effect of raloxifene on endometrial thickness, % glands, % epithelium, % lumen and % stroma. All these morphometric parameters were increased by Premarin, only % stroma was decreased.
- Uterine artery atherosclerosis was increased by OVX. This effect was clearly inhibited by Premarin, and slightly inhibited by R1 and R5.

Mammary Gland Histology
Table 1. Incidence of lobular hyperplasia

		SHAM	OVX	PR	R1	R2
Number of animals evaluated		19	20	24	21	22
Diffuse (mild-moderate)		12	1	20	0	1
Focal or multifocal	mild	2	3	2	7	2
	minimal	1	0	0	2	5
	moderate	0	0	0	1	0

- Premarin group had a large incidence of diffuse lobular hyperplasia (20/24 animals)
- Raloxifene groups had a moderate incidence of focal or multifocal lobular hyperplasia (10/21 and 7/22 animals). The difference between diffuse and focal hyperplasia is not completely clear, but may be limited to the extent of the lesion.

- Ductal hyperplasia was seen in SHAM, Premarin- and R5-treated animals (incidences 1/19, 3/24, 1/22), not in OVX or R1-treated animals. Ductal hyperplasia may be indicative of a pre-neoplastic lesion.

Mammary Gland Histomorphometry

- No morphometric effect of OVX, PR or RAL on maximum glandular thickness.
- Area of biggest lobule decreased by OVX. Decrease prevented for 110% by PR and for 90% by R1 (!).
- Total lobular area decreased by OVX, reversed above sham by PR (sign) and reversed to sham levels by R1 (ns)
- Percent area occupied by epithelium decreased by OVX, increased to above-sham levels by PR (sign) and reversed to sham levels by R1 (ns).
- #Lobules per field not affected by OVX, increased to above-sham by PR (sign), but not changed by R1 or R5

Conclusions

1. Raloxifene causes a moderate incidence of (multi)focal lobular hyperplasia in the mammary gland of OVX monkeys. Premarin causes a large incidence of mostly diffuse hyperplasia.
2. Premarin may cause a small incidence of ductal hyperplasia.
3. It is unclear from the data whether raloxifene causes ductal hyperplasia..

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5. Report 5

Effects of raloxifene and conjugated equine estrogens on coronary artery atherosclerosis of female cynomolgus monkeys (Study X-93-20)

Report Authors

Th.B. Clarkson and M.S. Anthony.

Methods

Histomorphometry of coronary artery in Intact, OVX, OVX + R1, OVX + R5, OVX + CEE (Premarin 0.04 mkd) groups:

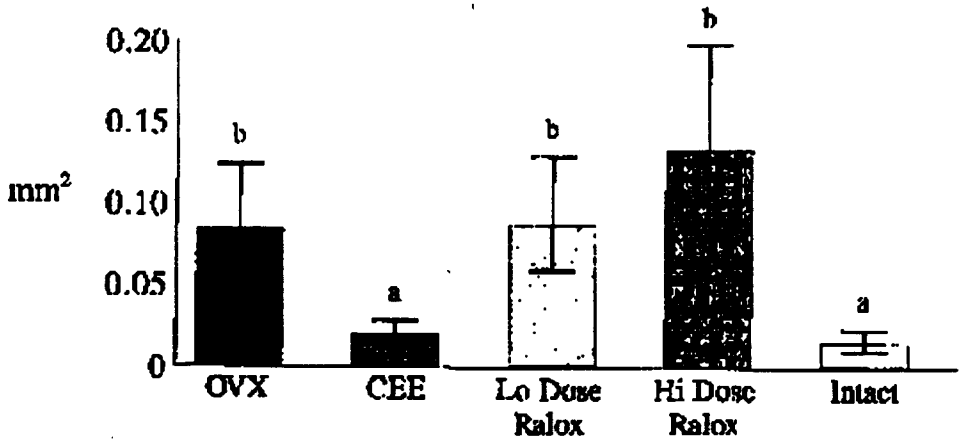
1. Intimal area (describes plaque size)
2. Intimal area per unit length of internal elastic lamina (avg. intimal thickness)
3. Area within internal elastic lamina (describes artery size)
4. Luminal area (area for blood flow)

Results

Intimal Area (Figure 3)

The extensiveness of coronary artery atherosclerosis is shown schematically in Figure 3.

Figure 3.
Coronary Artery Atherosclerosis: Intimal Area*



* Data are retransformed means + SEM, analyses done on log transformed data, adjusted for baseline LDLC+VLDLC and HDLC
Bars with different letters are significantly different ($p<0.01$)
Average of 3 coronary arteries, 5 blocks per artery

- OVX increases intimal area
- OVX + CEE prevents increase completely, and keeps area at level of intact animals
- R1 and R5 do not significantly affect OVX-induced increase

Luminal area was the same in all groups

Conclusions

- OVX increases plaque extent in OVX animals. Raloxifene had no significant effect on arterial plaque extent, while PR reversed extent to sham level. Thus, raloxifene does not protect against coronary artery atherosclerosis, while Premarin does.

APPEARS THIS WAY
ON ORIGINAL

6. Report 6

The effects of raloxifene on dilator responses of large epicardial coronary arteries of OVX atherosclerotic monkeys (Study X-93-20)

Report Author J. Koudy Williams.

Results

No significant effects.

7. Report 7

Test article and dose form assay report (Study X-93-20)

Report describes test article and dose preparation for this study.
Lilly Research Labs. Thomas O.York.

8. Report 8

Quality Assurance Activities (Study X-93-20)

List of inspections, audits, and training sessions pertaining to 2-year primate study assays and procedures.
Lilly Research Labs. Daniel R. Herman

APPEARS THIS WAY

SUMMARY OF RESULTS FROM MONKEY BONE EFFICACY STUDY

Body Weight

- No effects of ovariectomy or treatment

Bone Mineral density (BMD)

- In the cynomolgus monkey, raloxifene prevents the OVX-induced relative loss of BMD in vertebrae, whole body, and central tibia and radius. The effect of the highest dose of raloxifene used was smaller than the effect of Premarin. The maximal effect of raloxifene in this model is unknown.

Serum bone markers

- Bone turnover (resorption and formation), as indicated by serum biochemical markers is stimulated by OVX.
- Bone marker data indicate a large suppression of bone formation and resorption by Premarin to below sham-levels, and a variable suppression by raloxifene.

Histomorphometry

- BV/TV and BS/BV in ilium and vertebrae are not significantly affected by ovariectomy or treatment. This finding is unexplained. Trabecular parameters were not assessed. Recent data on bone volume and trabecular architecture from distal radius are more consistent with BMD results.
- OVX induces an increase in bone formation rate (BFR) and resorption. This increase is suppressed by raloxifene, usually to a lesser extent than by Premarin.

Biomechanics

- There are no clear biomechanical effects of OVX or treatment on humerus.
- Ultimate load (F_u) of femoral neck is n.s. decreased by OVX. This decrease appears to be prevented by Premarin, and to a small extent also by raloxifene.
- In vertebrae, OVX decreases F_u. Premarin appears to increase F_u, and raloxifene increases Fu also but to a lesser extent. F_u effects are not significant. Ultimate stress, S_u, is not significantly affected by OVX or raloxifene, but is significantly increased by Premarin. *Note*: X-area and therefore S_u may have been determined inaccurately by the manual technique applied. QCT data indeed do not suggest an effect of OVX or treatment on vertebral X- area.
- There is a significant positive correlation between vertebral BMD and F_u. This correlation (slope of regression line, and correlation coefficient) is very similar for Premarin and raloxifene groups.
- The latter result is the most dependable, although indirect indication that bone strength is changed in parallel with BMD in Premarin and raloxifene groups, as in the rat. From this we can conlude that BMD is a valid surrogate marker for bone strength.

Serum cholesterol

- Cholesterol and LDL-colesterol levels are increased by OVX. HDL-cholesterol is decreased by OVX. The changes in cholesterol and LDL-cholesterol are suppressed similarly by R1, R5 and Premarin. HDL is not affected by treatment. Triglycerides are unaffected by OVX or treatment.

Atherosclerosis

- OVX increases coronary artery plaque extent in OVX animals. Raloxifene (R1, R5) has no significant effect on arterial plaque extent, while PR reversed it to sham level.
- In the uterus, Premarin and raloxifene decrease uterine artery atherosclerosis. The effect of Premarin is more pronounced than of R1 and R5.

Uterine effects

- There is no effect of raloxifene on uterine weight in OVX monkeys.
- Raloxifene has no remarkable stimulatory effect on the uterine endometrium, while Premarin induces hyperplastic endometrial proliferation.

Mammary gland effects

- Raloxifene causes focal lobular hyperplasia in several OVX monkeys.
- Premarin causes diffuse lobular hyperplasia in almost all OVX animals.

Plasma levels

- In R1 and R5 groups, plasma levels of unconjugated raloxifene were 2x and 4x the predicted human levels at the 60 mg/day dose.

SUMMARY AND EVALUATION OF ANIMAL BONE STUDIES

RAT

The 1-year rat study showed that raloxifene can prevent the OVX-induced decrease of BMD with similar efficacy as estrogen. A 5-fold optimal dose of raloxifene was not tested.

Both estrogen and raloxifene appear to prevent cancellous bone loss by maintaining trabecular architecture.

OVX-induced bone loss is associated with an increase in bone turnover, which is prevented by estrogen and raloxifene.

In bones from raloxifene-treated animals having a similar mass as those from estrogen-treated animals, bone turnover was usually less suppressed by raloxifene than by estrogen.

Ovariectomy decreased ultimate bone strength, F_u, in vertebrae and femoral neck. Estrogen and raloxifene increased vertebral and femoral neck F_u in parallel with the increase in BMD.

The effect of estrogen and raloxifene on vertebral F_u was parallelled by an increase of ultimate stress (σ_u - F_u/mm^2).

MONKEY

The 2-year monkey study showed that Premarin and raloxifene prevent the relative osteopenia caused by ovariectomy.

The high dose of raloxifene (5 mg/kg/day) turned out to be an optimal dose for bone preservation, and was moderately less efficacious than Premarin. Thus, the effects of a 5-fold optimal dose were actually not assessed.

Histomorphometry data were variably consistent with BMD findings.

Bone formation and resorption were increased by ovariectomy. Bone turnover was significantly suppressed by Premarin. Turnover was also suppressed by raloxifene, but the extent and significance was dependent on the bone site evaluated.

Vertebral and femoral neck bone strength (F_u) appeared to be decreased in OVX animals. Premarin increased F_u. Raloxifene also appeared to increase vertebral F_u dose-dependently, but to a lesser extent than Premarin. Femoral neck F_u was virtually unaffected by raloxifene. Humeral F_u was unaffected by any treatment. None of the effects on Fu were statistically significant.

Vertebral ultimate stress, σ_u , was significantly increased by Premarin, but not significantly affected by ovariectomy or raloxifene. The relationship between F_u and σ_u in monkey vertebrae is

not clear from the data submitted.

There was a significant correlation between vertebral BMD and F_u, which was similar for the different groups (SHAM, OVX, Premarin, R1 and R5 combined).

REMARK
The 2-year monkey efficacy study showed that the model as used was not ideal. Both Sham and OVX monkeys gained bone mass (BMD) throughout the study, although OVX gained less than Sham.The BMD gain was probably due to the fact that the bone mass of the feral monkeys was not stabilized at the start of the study. Nutritional factors may have been involved in the increase observed. Another drawback was that a 5x optimal dose (as recommended in the Division's Guidelines for Evaluation of Agents for the Prevention and Treatment of Osteoporosis) was not tested.

CONCLUSIONS

- In both rats and monkeys, raloxifene and estrogen increased cancellous bone BMD as compared to ovariectomized controls.
- In rat lumbar vertebrae , raloxifene appeared to be associated with improved architecture and bone strength.
- In rats, raloxifene induced a suppression of bone turnover.
- Histomorphometry and biomechanical data from the monkey study lacked consistency and/or significance to prompt solid conclusions.
- There were no significant negative effects of raloxifene on bone quality.

Uterine Effects

Abbreviations

APR	Animal Pharmacology Report
NPR	Nonclinical Pharmacology Report
RAL	Raloxifene
TAM	Tamoxifen
DROL	Droloxifene
EE2	Estradiol
OVX	Ovariectomized
EI	Eosinophil Infiltration
ES	Estrogenicity Score

APPEARS THIS WAY
ON ORIGINAL

RESULTS

1. Studies in estrogen-deficient animals

APR 40

Methods

OVX rat, 35-day daily dosing, po or sc, histology study

Scoring system: Total estrogenicity score = myometrial thickness + endometrial stromal thickness + endometrial epithelial height + stromal eosinophilic infiltrate.

Results

OVX caused a decrease in uterine weight, and in all 4 histological parameters.

Decrease in uterine weight was ca. 75%. RAL increased uterine weight by ca. 40% (vs. OVX).

RAL minimally increased stromal eosinophilia (1.1-1.2x), while TAM and estradiol increased eosinophilia by maximally 2.5x and 5x, respectively.

RAL caused a 1.1x increase of epithelial height, estradiol caused a 3.5x increase, and TAM a 2.5x increase.

Both RAL and TAM increased myometrial thickness slightly (ca. 1.1x), estradiol markedly (2x).

Both RAL and TAM increased uterine stromal expansion slightly (ca. 1.3x), estradiol increased it ca. 2x. Effect of RAL was always less than of TAM.

Estradiol restored all uterine parameters and weight to intact levels.

Effect on total Estrogenicity Score: OVX vs. Intact: 0.45x. Treated vs. OVX: RAL 1.2x, TAM 1.4x (only!), estradiol 2.5x.

APR 37

Methods

OVX rat, sc, 4-day daily dosing

Results

Effects of OVX: Uterine weight 0.2x, EI 0.02x. In OVX rats, 17-β-estradiol and TAM caused eosinophil infiltration (EI), while RAL did not. All 3 treatments caused an increase in uterine wet weight (estradiol 4x, TAM 2x, RAL 1.3x). RAL antagonized the EE2-induced EI.

NPR W53-03

Methods

OVX rats, 75-day old, oral or sc, 4-day daily dosing, start of treatment 14 days after OVX.

Results

No data on effect of OVX. In OVX rats, RAL caused a slight to moderate increase in uterine weight (1.2x-1.4x, estradiol 2.5x). The effect of estradiol and RAL on uterine weight is reversible. RAL does not affect eosinophil content. EE2 and TAM increase weight and eosinophil peroxidase activity. RAL antagonizes EE2-stimulation of uterine weight and eosinophil peroxidase activity.

APR 36
Methods
OVX mouse, 2 sc injections in 24h, animals killed at 48h
Results
OVX effect: Uterine weight 0.15x. In OVX, RAL stimulates uterine weight moderately (1.4-2x), but less than 17-β-estradiol or TAM (3.7x or 3.8x)

APR 15
Methods
Immature rat (19-20 days old), sc, 3-day daily dosing
Results
RAL caused increase of uterine weight (wet wt and rel to body wt: 1.34 and 1.55x, resp) and uterine absolute RNA and protein content, not of uterine DNA or % water content. Estrogen increased all parameters, particularly RNA and protein content . RAL antagonizes estrogen effects.

2. Studies in estrogen-replete animals

NPR W53-08
Methods
Immature rat (21 day old), po or sc, 3-day daily dosing
Results
RAL could completely antagonize estrogen-induced increase in uterine relative to body weight, TAM/DROL/IDO partially antagonized. RAL alone caused some increase in rel (to BW) uterine weight (1.5x maximal), compared to estradiol 3.4x, TAM 2.3x (maximal effect).

APR 21,19,14
Methods
Immature rat or adult OVX rat, po or sc or dermal, various treatment durations
Results
RAL antagonizes uterotrophic weight effect of estradiol, not of PROG, TAM (?), or androstenediol.

APR 5
Methods
Rat, age not mentioned, sc, 3 day daily dosing, histology study
Results
RAL minimally stimulated epithelial cell growth, uterine size and uterine lumen size (effect on myometrium and/or stroma not mentioned). TAM simulated epithelium markedly (hypertrophy),

and also stimulated myometrium and stroma. Estradiol stimulated uterus markedly (hypertrophy and hyperplasia), but it was unspecified what regions.

APR 1

Methods

Rat or mouse, various ages, immature and adult, intact and OVX, sc or po, dosing 3 days or more.

Results

RAL blocked stimulatory action of estrogen on uterine weight to levels observed with raloxifene alone, when given in advance or with estrogen. TAM did not block. RAL by itself stimulated also but less (ca. 20-30%) than estrogen.

NPR CHO-03

Methods

OVX rat, 75-day old, p.o., 4-35-day daily dosing

Results (see 3 figures below)

RAL mildly increased uterine wet weight (LD 1.54x, HD 1.25x). RAL increased epithelial thickness (1.3x, TAM 2.7x), but had no significant effect on EI. Estradiol and TAM increased all 3 parameters. TAM increased uterine weight 1.8x, estradiol 4.1x. DROL increased uterine wet weight (1.2x) and EI. Idoxifene increased uterine wet weight 1.4x. RAL antagonized effects of estradiol, TAM and DROL on uterine weight, epithelial thickness and/or EI.

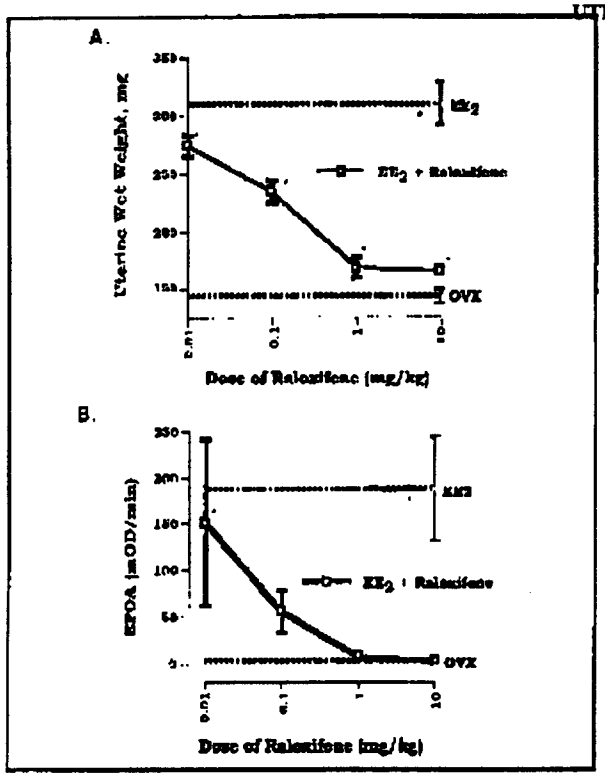
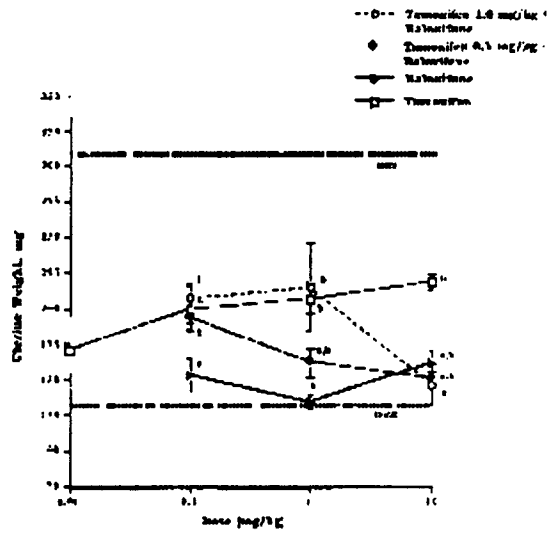


Figure 3. Raloxifene inhibits the uterotrophic effects of estradiol on uterine wet weight (panel A) and eosinophil peroxidase activity (EPOA; panel B). Ethynyl estradiol (EE2) was administered at 0.1 mg/kg with or without increasing doses of raloxifene as indicated by the x axis. ** p<0.05 versus



igure 4. The effect of raloxifene, tamoxifen and estrogen on uterine wet weight after 4 days of dosing. Tamoxifen was administered at 0.1 and 1.0 mg/kg combined with increasing doses of raloxifene as detailed on the x axis. Ethynyl estradiol at a single dose of 0.1 mg/kg was also included. a = p<0.05 versus tamoxifen at equivalent dose. b= p<0.05 versus OVX. Plotted values represent the mean of individual measurements from 5 different animals ± standard error of the mean

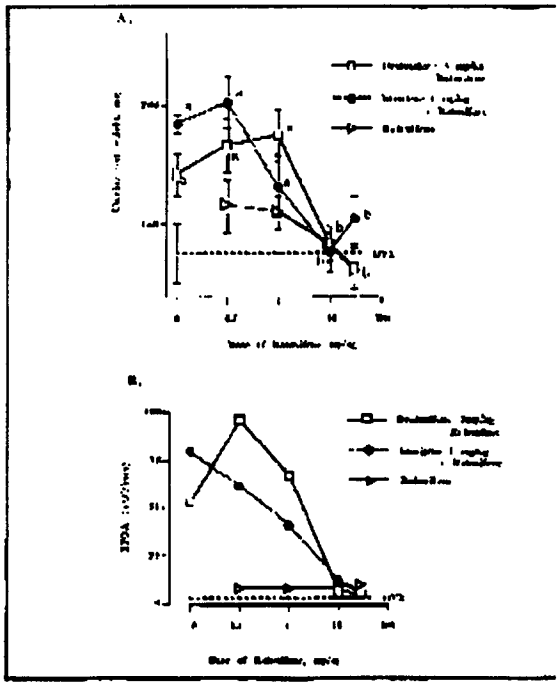


Figure 5 Effect of idoxifene and droloxifene on uterine wet weight (panel A) and eosinophil peroxidase activity (EPOA; panel B). Droloxifene was dosed at 2 mg/kg and idoxifene at 1 mg/kg

NPR W53-16

Methods

Rhesus macaque female monkeys with endometriosis, po, daily dosing

Results

RAL suppressed endometriosis. RAL increased estradiol levels ca. 3.5x in monkeys.

NPR CHO-02

Methods

Guinea pig, po, 1-30 day dosing

Results

Chronic RAL dosing caused regression of estrogen-induced uterine leiomyomas.

NPR P81-04

Methods

Rabbits, 37 week dosing; groups: SHAM, OVX, OVX +17-B-estradiol (4 mg/day), OVX + raloxifene (1 mkd for 10 wks, 10 mkd for remaining 27 wks).

Results

No effect of RAL on uterine weight of OVX rabbits.

Aortic cholesterol content:

SHAM	473
OVX	577
RAL	397 ($p<0.05$, vs OVX)
EE	177 ($p<0.001$, vs OVX)

SUMMARY TABLES (RAT)

- The effect of raloxifene, estradiol and tamoxifene on uterine weight(%) in OVX or immature rats and OVX mice is shown in Table 1(A,B).
- The effects shown are effects at a dose of raloxifene of (rat) and 30 mg/kg/day (mouse). In rats the RAL dose causing a maximal response was appr. 0.1 mg/kg/day (oral or s.c.), and higher doses caused the same or a lesser effect. In mice 30 mg/kg/day had a maximal effect.
- The estradiol dose was 0.1 mg/kg/day in the rat and 0.01 or 0.1 mg/kg/day in the mouse. The tamoxifen dose was usually 10 mg/kg/day; at this dose the maximal effect was obtained in both rat and mouse.

Table 1A. Effect on absolute uterine weight (% of intact control)

			OVX +		
	Intact	OVX	Raloxifene	Estradiol	Tamoxifen
RAT	100	20	28	62	42
MOUSE	100	15	26	51	54

Table 1B. Effect on absolute uterine weight (% of OVX control)

			OVX +		
	Intact	OVX	Raloxifene	Estradiol	Tamoxifen
RAT	465	100	141	310	210
MOUSE	667	100	170	340	360

Data are combined data from studies in mature and immature OVX rats (n = 7) and OVX mice (n = 2).
Effects shown are effects at doses causing maximal response raloxifene 0.1-1 mg/kg/day; estradiol 0.1 mg/kg/day; tamoxifen 10 mg/kg/day)

- The effect of raloxifene, estradiol, tamoxifen on body weight is shown in Table 2

Table 2. Effect on body weight in female rats (% of intact control)

			OVX +		
	Intact	OVX	RAL (0.1-10 mkd)	Estradiol (0.1 mkd)	Tamoxifen (0.1 mkd)
RAT	100	113	99	90	95

Data are from 2 experiments (Report W53-01 and Report CHO-03, Table 2A); dosing was for 35 (?) consecutive days

- Uterine effects, expressed as relative uterine-to-body weight, of OVX and of OVX + raloxifene, estradiol or tamoxifen in rats are shown in Table 3A,B.

Table 3A. Effect of raloxifene on absolute uterine weight in the rat

RAT			OVX +		
	OVX	Intact	Estradiol (0.1 mg/kg/day)	Raloxifene (0.1-1 mg/kg/day)	Tamoxifen (10 mg/kg/day)
Uterine weight (% of OVX control)	100	465	310	140	210
Δ% (vs. OVX control)	-	+365	+210	+40	+110

Data are from n = 7 (rat)
Data represent measurements of absolute uterine weights
Effects shown are effects at doses causing maximal response (raloxifene estradiol 0.1 mg/kg/day; tamoxifen 10 mg/kg/day)

Table 3B. Effect of raloxifene on relative uterine weight in the rat

RAT			OVX +		
	OVX	Intact	Estradiol (0.1 mg/kg/day)	Raloxifene (0.1-1 mg/kg/day)	Tamoxifen (10 mg/kg/day)
Uterine weight (% of OVX control)	100	525	390	160	250
Δ% (vs. OVX control)	-	+425	+290	+60	+150

Data are from n = 7 (rat)
Data represent measurements of relative (to body weight) uterine weights
Effects shown are effects at doses causing maximal response (raloxifene estradiol 0.1 mg/kg/day; tamoxifen 10 mg/kg/day)

OTHER REPORTS

Results

- In OVX rabbits, raloxifene did not affect uterine weight (VOL. 1.7, p6, p30).
- In intact rats (4-35 day studies), raloxifene had uterine antagonistic effects (NPR W53-04).
- In a 3-month toxicity study in the intact rat, uterine hypoplasia was seen in (almost) all animals of all dose groups, at raloxifene exposures of 1-250x the human intended exposure.
- In a 3-month toxicity study in the intact mouse, uterine hypoplasia and mammary gland hypoplasia occurred at 3-300x the intended human exposure.

MONKEY STUDIES

Results

- In a 1-year toxicity study in intact and OVX monkeys, using doses of (5-20 x human exposure in both intact and OVX animals) the following was seen:

Histopathology findings in 1-year toxicity studies in monkeys

		Control	Treated		
Dose (mg/kg/day)		0	15	30	100
Intact monkeys (N=4/group)					
Uterus	Slight atrophy	0	0	2	1
	Moderate atrophy	0	1	1	3
	Fibroma	0	1	0	0
Mammary gland	Adenoma	1	0	0	0
Ovary	Luteal cyst	0	0	1	0
OVX monkeys (N=4/group)		0	1	0	0
Uterus	Slight atrophy	0	1	0	0
	Moderate atrophy	4	3	4	4
Mammary gland	Fibroadenoma	0	1	0	0

Relative reproductive organ weights in 1-year toxicity study in monkeys

Intact females					
Ovary		13.6	19	30.8*	33.6*
Uterus		204	147	103*	126
Mammary gland		no data			
OVX females					
Mammary gland		no data			
Uterus		83	78	79	78

- In a 2-year efficacy study in OVX monkeys, using doses of raloxifene of 1 and 5 mg/kg/day (2-4x human exposure), and Premarin (0.04 mg/kg/day) as comparator (n=20-25/dose group), uterine weight was not affected. There was also no effect of raloxifene on endometrial thickness, % glands, % epithelium, % lumen and % stroma. All these morphometric parameters were increased by Premarin, only % stroma was decreased.

  Histologic examination of the uterus showed some minimal Premarin-like effects of raloxifene: (A) epithelial proliferation (R1 and R5), stromal expansion (R1 and R5), (B) glandular shape change (R1 and R5), N:C ratio decrease (R1 and R5), (C) stromal density change (R1 and R5). However, unlike effect of Premarin, glandular mitoses were decreased and stromal mitoses were unaffected by raloxifene.

  Raloxifene caused minimally increased estrogenicity scores in a few animals (3/43), while Premarin induced slight to large estrogenicity scores in all animals (24/24).

SUMMARY OF RESULTS FROM UTERINE STUDIES

The effect of raloxifene on various uterine parameters was tested in several studies in ovariectomized or immature rats, in which the serum estradiol levels are very low. A few studies were done in OVX mice, and there were some results from long-term studies in OVX monkeys. The effect of raloxifene was compared to that of 17-β-estradiol or Premarin, and other SERMs. In addition, the effect of raloxifene on the action of estradiol itself and some other estrogen agonists was investigated in the rat.

RAT

- Ovariectomy caused a large decrease in uterine weigth of ca. 80%.
- In all studies, raloxifene caused an increase in uterine weigth. The effect was dose-dependent and reached a maximum at doses ≤ 0.01 mg/kg/day.
- In the rat, the increase in absolute (*relative*) uterine weight by raloxifene was ca. **40%** (*60%*). The effect was **20%** (*22%*) of the effect of estradiol (range , and ca. **40%** (*40%*) of the effect of tamoxifen.
- Raloxifene had no or a minimal effect on eosinophilic infiltration of the uterine stroma. Estradiol had a marked effect on this parameter.
- Raloxifene had a small effect on uterine epithelial cell height. Both estradiol and tamoxifen caused a large increase of this parameter (raloxifene: 5% of estradiol effect, 20% of tamoxifen effect).
- Raloxifene slightly increased myometrial and stromal thickness (1.1-1.3x). The effect was similar to the effect of tamoxifen. Estradiol caused a marked increases in the thickness of these layers (raloxifene: ; of estradiol effect).
- Raloxifene antagonized the stimulatory effect of estradiol on uterine weight, epithelial thickness and eosinophilic infiltration in a dose-dependent manner.
- Tamoxifen increased uterine weight by ca. 150%, which is ca. half the effect of estrogen. Tamoxifen caused eosinophilic infiltration and markedly increased epithelial cell size. Tamoxifen increased myometrial and stromal thickness to the same extent as raloxifene. Tamoxifen only partially antagonized the uterine effects of estradiol. The uterine effect of tamoxifen could be antagonized by raloxifene.

MONKEY

- Ovariectomy caused a decrease in uterine weigth and uterine atrophy.
- Raloxifene did not reverse the decreased uterine weight or uterine atrophy caused by OVX.
- There were minimal histologic effects of raloxifene on the uterus in a long term study with doses causing 2-4x human expected exposure.

CONCLUSIONS

- Raloxifene has a small but significant estrogen-like effect in the rat uterus.
- The effect is manifested preferentially in uterine stroma and myometrium.
- Raloxifene has a negligible agonistic effect in the monkey uterus.
- Raloxifene antagonizes uterine stimulation by estradiol and triphenylethylene SERMs.
- The effects of raloxifene appear to be mediated through the estrogen receptor.

Mammary Gland Effects

RESULTS

1. *In vitro studies*

In MCF-7 cells, a human mammary tumor cell line (adenocarcinoma), raloxifene blocked cell proliferation in presence of 10 pM estradiol (IC50 0.4 nM). In absence of estrogen, raloxifene had no proliferative effect. Tamoxifen in these cells was a 1000-fold less potent estradiol antagonist than RAL (NPR Z77-03).

2. *In vivo studies*

A.

In the rat DMBA-induced mammary tumor model, RAL weakly antagonized tumor growth as compared to OVX. This represented weak antagonism of ovarian estrogen-induced pituitary prolactin release (APR 2). Raloxifene treatment of female rats for 140 days) prevented nitrosomethylurea (NMU)-induced breast cancer to some extent (NPR Z77-06).

B.

In long term rodent oncogenicity studies (Toxicology Reports 59, 60), raloxifene caused mammary gland hypoplasia in the intact female mouse, at doses causing 1-35x human exposure (parent drug referent). In the intact female rat raloxifene also caused hypoplasia, and a decreased incidence of mammary gland tumors (at 20-425x human exposure). In the male rats, there was a slight increase in mammary gland benign tumor incidence (at 10-50x human exposure).

C.

In a long term 1-year monkey toxicity study in intact and OVX animals (N=4/dose group) (doses 15-30-100 mkd, causing 5-10-20x human exposure), there were no significant effects of raloxifene on mammary gland histology (glands in all groups including intact controls were described as "inactive") or tumor incidence.

D.

In a long term monkey efficacy study (Study X-93-20), in which mammary gland histology was examined in detail, raloxifene (, equivalent to 2-4x human exposure) caused (multi)focal mammary gland lobular hyperplasia, in contrast to Premarin which caused diffuse hyperplasia.

Results

Histology

Incidence of lobular and ductal hyperplasia in monkey mammary gland

			SHAM	OVX	PR	R1	R5
Number of animals evaluated			19	20	24	21	22
Lobular hyperplasia	Diffuse	mild-moderate	12	1	20	0	1
	Focal or multifocal	minimal	1	0	0	2	5
		mild	2	3	2	7	2
		moderate	0	0	0	1	0
Ductal hyperplasia		minimal	0	0	2	0	0
		mild	1	0	0	0	1
		moderate	0	0	1	0	0

- Sham and Premarin group had a large incidence of diffuse lobular hyperplasia (5 out of 6 animals)
- Raloxifene groups had a moderate incidence of focal or multifocal lobular hyperplasia (2-3 out of 6 animals)
- Some ductal hyperplasia was seen in SHAM, Premarin- and R5-treated (incidences 1/19, 3/24, 1/22), not in OVX or R1-treated.

Morphometry:

- No morphometric effect of OVX, PR or RAL on maximum glandular thickness.
- Area of biggest lobule decreased by OVX. Decrease significantly prevented for 110% by PR and for 90% by R1 (!).
- Total lobular area decreased by OVX, reversed to above-sham level by PR (sign), and reversed to sham level by R1 (ns) ·
- Percent area occupied by epithelium decreased by OVX, reversed to above-sham level by PR (sign) and reversed to sham level by R1 (ns).
- #Lobules per field not affected by OVX, increased to above-sham by PR (sign), but not changed by R1 or R5.

CONCLUSIONS

- Raloxifene inhibits estrogen-stimulated mammary gland cell proliferation.
- In OVX monkeys, raloxifene causes (multi)focal lobular hyperplasia in the mammary gland, while estrogen causes diffuse hyperplasia.
- It is unclear whether Premarin or raloxifene cause ductal hyperplasia.

Other pharmacology

1. Cholesterol homeostasis and atherosclerosis

Cholesterol

Raloxifene lowered serum total cholesterol levels in OVX rats and monkeys, presumably by estrogen receptor-mediated induction of hepatic LDL receptors. Raloxifene lowered HDL cholesterol in OVX rats (APR 34, NPR P81-02, R43-06). HDL cholesterol was not affected in OVX monkeys, while LDL cholesterol was slightly reduced (NPR X-93-20). The latter also occurs in humans.

In intact rats (m, f) RAL lowered serum cholesterol (NPR W53-09). In rabbits, RAL reduced serum total cholesterol, but not HDL cholesterol (APR 35). The effect of raloxifene on diet- or mechanically induced arterial injury was inconsistent in various models and species (rats, rabbits).

Atherosclerosis

1. Report NPR P81-04

Methods

Rabbits, 37 week dosing; groups: SHAM, OVX, OVX +17-B-estradiol (4 mg/day), OVX + raloxifene (1 mkd for 10 wks, 10 mkd for remaining 27 wks). Measured were uterine weight and aortic cholesterol content.

Results

No effect of RAL on uterine weight of OVX rabbits.

Aortic cholesterol content:

SHAM	473
OVX	577
RAL	397 ($p<0.05$, vs OVX)
EE	177 ($p<0.001$, vs OVX)

2. Study X-93-20- Report 5

Results

In OVX monkeys, despite effects on serum cholesterol, raloxifene did not reduce coronary artery plaque size, while Premarin did (NPR X-93-20). This suggests that raloxifene does not protect against coronary artery atherosclerosis. However, uterine artery atherosclerosis was slightly reduced by raloxifene.

2. Tumor biology studies

In MCF-7 cells, a human mammary tumor cell line (adenocarcinoma), raloxifene blocked cell proliferation in presence of 10 pM estradiol (IC50 0.4 nM). In absence of estrogen, raloxifene had no proliferative effect. Tamoxifen in these cells was a 1000-fold less potent estradiol antagonist than RAL (NPR Z77-03).

In the rat DMBA-induced mammary tumor model, RAL weakly antagonized tumor growth as compared to OVX. This represented weak antagonism of ovarian estrogen-induced pituitary

prolactin release (APR 2). Raloxifene treatment of female rats for 140 days) prevented nitrosomethylurea (NMU)-induced breast cancer to some extent (NPR Z77-06).
In male Syrian hamsters estradiol causes kidney tumors. Raloxifene did not prevent these tumors in this model; in fact, raloxifene in combination with estradiol induced testicular tumors in addition to the kidney tumors already present (NPR W10-03).

3. Safety pharmacology studies

These were studies of raloxifene effects on CNS, neuroendocrine, CV, renal, GI systems.
In male mice, raloxifene at high doses (>800 mk) caused increased respiratory rate, and at >50 mg/kg it potentiated acid-induced writhing.
Raloxifene had some inhibitory effects on serotonin- and NE-induced contractile responses in in vitro smooth and cardiac muscle preparations, probably through calcium channel antagonism (NPR P67-03 and APR 9). However, in vivo, in OVX rats raloxifene nor estrogen had an effect on mean arterial pressure, heart rate or on serotonin- or NE-induced responses (NPR P67-01).
RAL has no effect on GI motility (GPR 3). In conscious male rats, raloxifene at did not affect arterial BP, systolic or diastolic BP, pulse pressure, heart rate (GPR 2). In anesthetized dogs, IV- raloxifene (4-20 mk) caused vasodilation, and increased respiratory rate (APR 8)
RAL had no effect on the immune response in mice (APR 10).

4. Cell biology studies (In vitro studies on mechanism of action)

A. Bone cell biology
Results suggest that estradiol and RAL may stimulate TGF-B1 and TGF-B3 secretion from mouse and human osteoblast-like cell lines (osteosarcomas) (NPR R43-02). TGF-B3 is thought to inhibit bone resorption through an effect on osteoclast activity and differentiation. In these cell systems a direct effect of RAL or estradiol on bone resorption by osteoclast was not demonstrated, but these agents did appear to cause inhibition of osteoclast formation (NPR BN5-06).
In neonatal mouse calvariae, bone resorption stimulated by PTH was inhibited by RAL but not by 17-B-estradiol (NPR ATI-01). The significance of this finding is unclear. It may be related to the lack of effective estrogen metabolites in the calvariae system.

B. Binding studies
Radioligand binding studies in MCF-7 cell lysates indicated binding of RAL to estrogen receptor (IC_{50} RAL=2.5x IC_{50} estradiol). Raloxifene also binds to a subset of tamoxifen binding sites, i.e., intracellular proteins other than the ER, with lower affinity than to ER. Tamoxifen does not clearly bind to ER in these cells (NPR Z77-02). In the human osteosarcoma MG-63 cell line RAL, unlike estradiol, does not appear to bind to ER (APR 39).
Rat uterine cell lysates contained 5 proteins that bound specifically to raloxifene: disulfide isomerase, three isoenzymes of glutathione S-transferase and macrophage migration inhibition factor. RAL inhibited disulfide isomerase and glutathione S-transferase activity. Disulfide isomerase but not glutathione S-transferase was also inhibited by estradiol (NPR W10-01). In the

osteoblast-like cell line MC3T3-E1 raloxifene binds to a high-affinity binding site that does not bind 17-B-estradiol. Equilin and 17-A-dihydroequilenin also bind to this site but with 100-fold lower affinity than raloxifene (NPR R43-01).

C. Gene regulation.

These studies focused on the regulation of gene promotor activity. In female rats, the OVX-induced ca. 20% reduction in BMD was associated with a 2-fold reduction of TGF-B3 mRNA in femur. In transfection assays, a promotor sequence of theTGFB3 gene lacking an ERE mediated RAL- or estradiol- induced reporter gene expression. RAL inhibited the ERE-containing vitellogenin promotor expression as an antagonist. TGF-B3 inhibits the differentiation and bone resorptive activities of mouse and avian osteoclasts (NPR CH3-01). Study report CH3-02 describes the identification of a novel ER-regulated element, the socalled RRE, in the TGF-B3 gene. An estradiol metabolite and epidermal growth factor also activate the RRE pathway (NPR CH3-02). In rat femora, raloxifene increases mRNA levels of genes encoding collagen, osteonectin, and decorin. These genes contain RRE-like sequences. In MC3T3 osteoblast-like cells RAL upregulates TGFB3 mRNA expression (NPR CH3-03).

In cultured human hepatoma Hep G2 cells, estradiol upregulated LDL-receptor promotor activity in ER-dependent manner. RAL upregulated this promotor in an ER-independent fashion (NPR CH3-04). In femoral RNA samples of RAL-treated OVX rats 48 cDNA fragments were present that were not seen in samples from vehicle-treated animals. In latter, 36 cDNA fragments were seen that were absent from samples of RAL-treated (NPR CH3-05).

Raloxifene had comparable binding affinity for ER-A and ER-B overexpressed in Cos-1 cells (NPR CH3-08). The final gene transcription effect of SERMS and estradiol depends on the response element (ERE, RRE, AP-1) and the type of receptor involved.

In yeast, S. cerevisiae, RAL and TAM did not antagonize estradiol/ER-mediated expression of reporter genes, and were in fact slightly agonistic themselves (NPR Z77-08).

D. Other in vitro cell biology studies

In osteosarcoma MG-63 and UMR-106 cells, and in rat osteoblast LROB46 cells, raloxifene (100nM) had no effect on alkaline phosphatase expression (NPR Z77-01). In bone or lymphoid cells of human or mouse origin estradiol inhibited (IC50>1 uM) proliferation stimulated by IL-2, IL-4 or IL-6, or leukocyte inhibitory factor (LIF). RAL was more potent than EE2 in inhibiting proliferation (IC50<1uM) stimulated by IL-6 or LIF, not IL-2 or IL-4 (NPR Z77-07). This result suggests that RAL and EE2 can inhibit the osteoclast differentiation activity of locally produced cytokines. Another finding is that OVX causes an increase of IL-6 levels in the serum, possibly increasing stromal cell activity, which appears to be inhibited by EE2 and RAL, but more so by EE2. These two distinct findings point to two levels of estrogenic effects on bone cell activity and differentiation.

CONCLUSIONS

- In vitro and in vivo data on molecular, cellular and biological effects of raloxifene indicate that there are several similarities but also several differences between the mechanisms of action of raloxifene and estrogen.

TOXICOLOGY

General Toxicology

Edited FROM: Raloxifene (LY139481) HCl Application Summary (Item 2, Vol. 2.1)

Raloxifene has been investigated in a variety of toxicology studies: a battery of in vivo and in vitro genetic toxicology assays; single-dose acute studies; subchronic studies in rats, mice, and monkeys; chronic studies in rats, monkeys, and dogs; and oncogenic studies in rats and mice. In addition, studies were conducted in rats and rabbits to evaluate the reproductive and developmental toxicity potential of raloxifene. The toxicology studies with raloxifene were conducted over a relatively long period of time. The earliest acute studies were done in 1981, while the chronic monkey and oncogenic studies were completed in 1996 and 1997, respectively. Dose calculations for studies conducted in the 1980s were based on the raloxifene hydrochloride salt, whereas calculations for studies performed in the 1990s were based on raloxifene free base. For comparison, a raloxifene dose of 100 mg/kg is equivalent to a raloxifene HCl dose of approximately 108 mg/kg.

Acute Toxicity

In acute toxicity studies, no mortality occurred in mice or rats administered single 5000-mg/kg oral doses of raloxifene HCl. An intraperitoneal dose of 2000 mg/kg given to rats produced 20% mortality. Clinical signs included leg weakness, soft stools, and compound-colored feces in rats given raloxifene orally, and leg weakness, hypoactivity, and poor grooming in rats given the compound parenterally. No effects were seen in dogs or monkeys given a single oral dose of 300 mg/kg. A single 1000-mg/kg dose of raloxifene was associated with abnormal stool in cynomolgus monkeys.

Repeated-Dose Toxicity

Studies at relatively low doses

B6C3F1 mice administered raloxifene HCl in the diet for 3 months at average daily doses up to approximately 120 mg/kg had decreases in body weight. The most notable treatment-related finding was the estrogen antagonist effect of decreased uterine weight. The 6-month and 1-year dietary studies in Fischer 344 rats at doses up to approximately 25 mg/kg produced similar findings. In males, there were treatment-related decreases in food consumption and body weight gain. In female rats, decreased uterine weights and moderate elevations in serum alkaline phosphatase occurred at all doses. Moderate increases in adrenal weights were also seen in rats that received raloxifene. Mineralization of the corticomedullary tubules of the kidneys occurred in both male and female rats of all dose groups. In a 6-month study in dogs at doses up to 30 mg/kg, treatment-related findings were decreased prostate weights in 2 of the 4 high-dose dogs, and aspermatogenesis and slight prostatic atrophy in 1 of those 2 dogs. The effects on the prostate are consistent with the pharmacologic activity of raloxifene. No effects were observed in female dogs. There were no proliferative changes and no ocular effects in the chronic studies in rats and dogs.

Recent studies at relatively high doses

Subchronic studies were conducted with CD-1 mice, Fischer 344 rats, and cynomolgus monkeys

using raloxifene doses up to approximately 1700, 700, and 1000 mg/kg, respectively. The primary findings in rodents included reduced food consumption and reduced body weight; decreased uterine and pituitary weights; and uterine hypoplasia, vaginal mucoid metaplasia, and ovarian changes. However, in female mice, body weight was increased at raloxifene doses ≥184 mg/kg.

In a 1-month study in monkeys effects observed were decreased food consumption, various stool abnormalities in high-dose animals, and reduced thymus weights in males. At all doses, reduced uterine weights and ovarian cysts were observed. The cause of abnormal stools monkeys given 1000 mg/kg was unknown.

A 1-year toxicity study was conducted in cynomolgus monkeys in intact females, OVX females, and juvenile males at daily raloxifene doses of 0, 15, 30, or 100 mg/kg. Increases (2- to 6-fold above control values) in serum alanine transaminase (ALT) were observed in all groups of raloxifene-treated OVX females, but only in the mid- and high-dose groups of intact females. Serum ALT values in males were unaffected. Other serum enzymes associated with impaired liver function were not similarly increased, and there were no significant morphologic hepatocellular changes in any treated animals. Because estrogen has been shown to induce elevations in serum transaminases in the absence of hepatocellular damage, the increased serum ALT values seen in this study were likely related to the estrogenic activity of raloxifene in the liver.

In the 1-year study, in intact females, reduced uterine weight and generalized atrophy of the uterus were observed. Atrophy was also seen in vagina and cervix. Ovarian weights were significantly increased in the mid- and high-dose groups. Ovaries in intact raloxifene-treated animals had developing follicles and/or corpora. One (1/4) animal had a luteal cyst. In OVX females, the reduced uterine weight and altered morphology was not affected by raloxifene. In males, pituitary weights were reduced at all dose levels and thymus weights were decreased in high-dose males. Pituitary morphology was not altered. There were no proliferative lesions in any tissues or organs and no ocular effects. The notable effects in this study were likely attributable to raloxifene's pharmacologic activity as a SERM.

Special Studies

Several special studies with raloxifene have also been conducted. Results from these studies have indicated the following: (1) raloxifene showed no antigenic potential in a guinea pig model; (2) the ER concentrations in the uteri of raloxifene-treated mice were elevated compared to controls, but testicular androgen receptors were not affected by raloxifene treatment; (3) raloxifene caused slight dermal and ocular irritation in rabbits; (4) inhalation of raloxifene HCl did not cause mortality in rats exposed to an aerosol concentration of 1.87 mg/L (1870 mg/m3) for 1 hour; and (5) inhalation exposure of monkeys for 8 hours to dry powder aerosols of raloxifene HCl did not affect pulmonary function at airborne concentrations up to 26.9 mg/m3.

Genetic Toxicology

Raloxifene was found to be non-genotoxic in the following tests (Table 5 below):
1. Ames bacterial mutagenesis assay with(out) metabolic activation 2. Unscheduled DNA synthesis assay in rat hepatocytes 3. Mouse lymphoma assay for mammalian cell mutation 4. CHO chromosomal aberration assay 5. In vivo sister chromatid exchange assay in Chinese hamsters 6. In vivo mouse micronucleus assay.

Tamoxifen, another SERM with related pharmacologic action, which - unlike raloxifene- caused liver tumors in rats, was also negative in a conventional battery of genotoxicity tests. However, the compound tested positive in two additional assays, the DNA adduct assay and an MCL-5 human lymphoblastoma cell clastogenicity assay.

Two meetings were held between the Division and the Executice CAC (Carcinogenicity Assessment Committee, CDER) to discuss the carcinogenicity findings (Meeting Minutes attached, ATTACHMENT As a result, the Sponsor met on November 5 with The Division and the full Carcinogenicity Assessment Committee to discuss whether and which additional genotoxicity tests would be appropriate. Possible tests included DNA adduct formation test and MCL-5 assay.

DESCRIPTION OF ASSAYS

DNA adduct formation
Sponsor submitted one report with the results of a rat study to investigate the formation of adducts in the liver. Female SD rats (n=4/control, 3/dose groups), age 8 weeks, were given i.p. injections of raloxifene HCl or RU 39,411 at doses of 5, 10, 20 mg/kg. Rats were killed 6 h after treatment, and livers were stored at -70°C. DNA adduct analysis was carried out by nuclease P_1-enhanced ^{32}P-postlabeling. As a positive control, the steroidal anti-estrogen RU-39,411 was used. The data were inconclusive since there was a large variability in number and intensity of ^{32}P-labeled spots in the autoradiograms within treatment groups. The target tissue was also inappropriate since it was a tissue with no tumor findings.

MCL-5 assay
The MCL-5 assay is a micronucleus test using human lymphoblastoid cells expressing high amounts of P450 isoenzymes.This assay was shown to be positive with tamoxifen, a SERM with unrelated chemical structure, but with a related ER-mediated pharmacologic mechanism. The micronucleus formation is thought to be due to adduct-mediated clastogenicity.The positive effect of tamoxifen in this assay is thought to be the result of conversion of tamoxifen to a reactive hydroxylated metabolite. It has been recommended previously to the Sponsor to carry

out the MCL-5 micronucleus assay with raloxifene.

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Table 5. Results of Mutagenicity Studies with Raloxifene HCl.

Report Number	Type of Study	Species, Cells	Route of Administration	Doses/Concentrations	Results
1, 15	Ames	*Escherichia coli*; *Salmonella typhimurium*	Not applicable	250 to 4000 µg/plate in activated and non-activated assays	Negative
20	Ames	*Escherichia coli*	Not applicable	late in nonactivated assay; with metabolic activation	Negative
2	Unscheduled DNA synthesis	Adult rat, hepatocytes	Not applicable		Negative
13	Forward mutation at thymidine kinase locus	L5178Y TK$^{+/-}$ mouse lymphoma	Not applicable	nonactivated; with metabolic activation	Negative
21	Chromosome aberration	Chinese Hamster ovary	Not applicable	nonactivated; with metabolic activation	Negative
17	Sister chromatid exchange, in vivo	Chinese hamster, bone marrow	Oral		Negative
19	Micronucleus, in vivo	Mouse, bone marrow	Oral		Negative

Carcinogenicity (see NDA Review Carcinogenicity, ATTACHMENT)

MOUSE STUDY

METHODS

CD-1 mice (60/sex/dose group) (Studies M00494 and M00594), initial weight 25g (m) and 21g (f), were treated with **0, 0.005, 0.03, 0.15%** raloxifene in the diet for 21 months. These dose levels provided average daily doses (mg/kg) of: **males 0, 6.5, 38, 195 mg/kg**, and **females 0, 8.7, 49, 225 mg/kg**. The HD was based on a 25x AUC ratio (mouse:human) of the parent compound raloxifene. In study M00694, 33/sex/group (M00694) initial weight 27g (m) and 22 g (f) were treated with same dietary %'s for 12 months. Groups included 3 replacement animals/sex. Plasma samples were collected after 3 and 12 months.

RESULTS

Survival

Survival is shown in **TABLE 1**. There was a statistically significant positive dose-mortality trend (p-value 0.03) in male mice. The increased mortality was partly due to prostatic and testicular neoplasms. There was no effect on survival in female mice.

TABLE 1. Survival of mice in 21-month carcinogenicity study with raloxifene

	# At start of study		# At treatment termination	
Dose group	male	female	male*	female
Control	60	60	48	47
LD	60	60	50	46
MD	60	60	48	48
HD	60	60	41	48

*significant positive dose-mortality trend

Body weight

Raloxifene caused a slight decrease in body weight (BW) in all dose groups of male mice. Raloxifene caused a minimal decrease in BW in LD and MD female mice, and a slight increase in HD female mice. BW decrease in males was parallelled by decreases in food consumption (FC) and efficiency of food utilization (EFU). In females, FC was decreased, while EFU was decreased in LD, increased in HD.

Toxicological findings

Main toxic effects excluding body weight and tumors:

Males

1. Decreased leukocyte subset counts in all male treated.
2. Increased relative testes weight in all treated.
3. High incidence of testicular brown pigmentation in all male treated. Hyperplasia of testes and prostate in MD and HD males.

Females

1. Hepatocellular cytomegaly in HD females
2. Increased ovary weights in all treated
3. Decreased incidence of ovarian cysts and markedly increased incidence of persistent follicular dilation in all treated. Ovarian brown pigmentation increased in all treated. Increased incidence of tubular hyperplasia in all treated.
4. Uterine atrophy in all treated. Decreased severity of cystic endometrial change. Occurrence of mucosal hyperplasia and deciduoma specifically in animals with ovarian tumors.
5. Decreased incidence of vaginal cornification and increased incidence of epithelial mucification in all treated.
6. Large incidence of mammary gland hypoplasia in all treated.
7. Marked incidence of bone hypertrophy in all dose groups.

Tumor findings

Tumor findings are shown in **TABLE 2.**

TABLE 2. Tumor incidence in mice treated with raloxifene, with survival-adjusted statistical evaluation (linear trend test)
(B) benign, (M) malignant
n.s. = non-significant

Males

Tissue	Tumor	Incidence (Number of animals in control-LD-MD-HD)	Historical control incidence (6 studies)	Statistical significance according to:	
				Sponsor	Div. Biometrics
Testis	Interstitial cell tumor (M)	0-0-2-10		SIGN (<0.001)	SIGN (<0.0001)
	Interstitial cell tumor (B)	2-1-13-17		SIGN (<0.001)	SIGN (<0.0001)
Prostate	Adenocarcinoma (M) + Adenoma (B)	0-0-4-4	0,1,0,0,0,0	no data	SIGN (0.023)
	Leiomyoblastoma (B)	0-0-0-4		SIGN (0.003)	SIGN (0.005)

Females

Tissue	Tumor	Incidence (nr. of animals)		Statistical significance (Trendtest) ccording to:	
				Sponsor	Div. Biometrics
Liver	Hepatocellular adenoma/carcinoma (B,M)	1-1-3-6	1,0,1,2,1,0	no data	n.s. (0.010) (Trendtest) n.s. (>0.01) (pairwise; ctrl vs. LD, MD or HD)
Ovary	Granulosa cell tumor (M)	0-2-4-8		SIGN (0.001)	SIGN (0.002)
	Granulosa cell tumor (B)	0-4-3-6		SIGN (0.017)	SIGN (0.025)
	Luteoma (B)	3-6-7-10		SIGN (0.025)	n.s. (0.031)
	Granulosa/theca/luteoma (B,M)	4-12-17-27	0,1,1,0,0,2	no data	SIGN (<0.001)
	Tubular/papillary adenoma (B)	1-9-7-4	1,0,0,1,2,1	no data	n.s. (0.66) (Trendtest) SIGN (<0.01) (pairwise; LD) n.s. (>0.01) (pairwise; ctrl-MD,HD)
	All ovarian tumors (M,B) combined	4-19-20-29	no hc data	SIGN (<0.001)	not tested

Tumors that were treatment-related in a statistically significant manner:
1. Ovarian sex cord/stromal cell tumors in females (benign and malignant)
2. Testicular interstitial cell tumors in males (benign and malignant)
3. Prostatic leiomyoblastoma in males (benign)
4. Prostatic adenoma and adenocarcinoma (pooled) in males (benign and malignant)

Plasma levels

Raloxifene is absorbed rapidly, undergoes extensive first pass metabolism in intestinal mucosa and liver, and is subject to enterohepatic circulation with hydrolyzation of glucuronide-conjugates in GI tract.

In mouse plasma, ca. is parent drug, the remaining part is mostly raloxifene-6-glucuronide, and to a lesser extent raloxifene-4'-glucuronide and raloxifene-4'-6-diglucuronide. No oxidative metabolites are found. In humans, of total raloxifene in hydrolyzed plasma (TRHP) is parent drug, and the major metabolite is the 4'-glucuronide.

TABLE 3 and **TABLE 4** below show AUC values for raloxifene (RAL), and for raloxifene plus all its glucuronides, or total raloxifene (T.RAL = TRHP = total raloxifene in plasma hydrolyzed by B-glucuronidase), after 3 and 12 months of oral dosing. In Table 4, calculated AUC multiples (mouse:human) are presented for raloxifene and total raloxifene. The multiples are based on plasma drug levels.

TABLE 3. AUC levels of raloxifene (RAL) and total raloxifene (T.RAL) in CD-1 mice on Day 91

	Dose (mg/kg)	AUC (RAL) (ngxh/ml)	AUC (T. RAL) (ngxh/ml)	AUC ratio RAL:T.RAL (%)
male (n=3)	6.9	24	552	4.3
	41	120	3624	3.3
	211	624	21456	2.9
female (n=3)	9.2	48	840	5.7
	51	240	6792	3.5
	235	960	44064	2.2

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TABLE 4. Multiples of expected human exposure in CD-1 mice carcinogenicity study on Day 365

	Dose (mg/kg)	AUC_{0-24h} (RAL) (ngxh/ml)	AUC_{0-24h} (T.RAL) (ngxh/ml)	AUC ratio RAL:T.RAL (%)	AUC multiple mouse/human	
					RAL	T.RAL
male	6	18 (20)**	151 (450)	11.9*	0.75	0.04 (0.13)**
	36	112	2718	4.1	4.7	0.78
	184	587	15089	3.9	24	4.3
female	7.7	7 (24)	532	1.3*	0.3 (1)**	0.15
	41	171	3849	4.4	7	1.1
	198	820	14151	5.8	34	4.0
Human	1	24	3500	0.7	-	-

* Values at these doses are relatively unreliable because many plasma samples were BLQ

** Values in parenthesis are hypothetical, assuming that only the value for T.RAL in LD f (532 ngxh/ml) is correct and that other measurements of RAL and T.RAL at LD are inaccurate, and assuming that AUC ratio Ral:T.Ral is constant (4.5%) over whole dose range,

Protein Binding

Method: Ultracentrifugation of plasma samples of rat, monkey, human with added raloxifene (conjugate)

TABLE 5. *In vitro* protein binding in rats, monkeys and humans

	Raloxifene		Raloxifene 6 and 4'-glucuronides	
	Concentration range (ng/ml)	Binding (%)	Concentration (ng/ml)	Binding (%)
RAT				no data
MONKEY			200	99
HUMAN			200	99
MOUSE	-	no data	-	no data

Although no data are available for mice (samples too small) it is reasonable to assume that protein binding is also in the range Thus, AUC values of parent raloxifene can be used for calculating the human exposure multiples of the doses used in the mouse carcinogenicity studies.

DISCUSSION

The tumors of most clinical concern found in this study are the ovarian tumors in females. Benign and malignant ovarian granulosa cell tumors and benign luteomas were seen in all dose groups including the LD in which the systemic exposure to parent drug is only 0.3x the expected exposure in postmenopausal women treated with 60 mg/day.

There are 5 main categories of ovarian tumors in rodents and other mammals, the three most important

ones being epithelial tumors, sex-cord/stromal tumors (granulosa/theca cell tumors), and germ cell tumors. In rodents, especially mice, ovarian tumors of the first two categories can develop as a result of a variety of experimental treatments such as exposure to radiation or carcinogenic toxins (dimethylbenzanthracene, DMBA, and nitrofurantoin). They can also occur in transgenic animals in which oocytes are absent or lost, in which the estrogen receptor is absent, or in which there is hypersecretion of LH.

Although until very recently (Risma et al, 1995) there was no direct proof for this hypothesis, most studies suggest that in all these experimental models the destruction of oocytes or the lack of estrogenic receptor stimulation abates the negative feedback of endogenous estrogen through the hypothalamo-pituitary-ovary (HPO) axis on the production of the anterior pituitary gonadotropins, LH and FSH. The resulting pituitary hypersecretion of LH and/or FSH is thought to be associated with ovarian stimulation and tumor induction.

In the various mouse models, in which there is germ cell (oocyte) loss or ovarian dysgenesis, the ovarian tumors found are mostly derived from epithelium (tubular adenomas) or from epithelial cells with varying contributions of stromal cells (tubulostromal adenomas), and occasionally from stromal or interstitial cells (granulosa/theca cell tumors). In estrogen receptor knockout (ERKO) mice the tumors that develop are granulosa cell tumors, and in LH-hypersecreting mice, tumors from granulosa/theca-interstitial cell origin appear. The benign tubulostromal tumor is one of the most common type of epithelial tumor in mice, while this tumor occurs only at very low frequency in rats or other species, and not in humans.

In humans, ca. 90% of the ovarian tumors are epithelial or germ cell origin, while less than 10% are derived from sex-cord/stromal cells. The most common type of ovarian cancer in humans is the epithelial serous cystadenocarcinoma, which occurs at low frequency in mice and rats.

Tamoxifen, a SERM of the triphenylethylene group, distinct in structure from the benzothiophene group, also induces a high incidence of ovarian benign granulosa cell tumors at doses of in female mice (1-10x human dose multiple on basis of body surface area) and a high incidence of testicular interstitial cell tumors at 50 mg/kg/day in male mice .These data suggests that the ovarian (and testicular) tumors elicited by SERMs are due to their specific pharmacologic action, most likely their modulating effect on estrogen receptor-mediated events.

The testicular Leydig cell tumors seen in male mice treated with raloxifene (or tamoxifen), are the result of either (1) an anti-estrogenic LH level increase and subsequent Leydig cell stimulation, or (2) a direct paracrine estrogenic effect on Leydig cell proliferation (Clegg et al, 1997). The mechanism of the prostate tumorigenesis by raloxifene is unclear.

Relevance of mouse ovarian tumors

Sponsor suggests that, in the intact mouse, raloxifene, due to its estrogen-antagonistic properties, causes overstimulation of pituitary LH/FSH secretion and that stimulation of the ovary by these hormones causes the ovarian tumors. In a recent study in mice, using doses similar to the LD and HD used in the carcinogencity study, a 2-fold (n.s.) and 5-fold (sign.) increase in serum LH levels was observed upon 1-month raloxifene treatment.This could explain the raloxifene-induced incidence of ovarian/testicular

tumors in mice.

Extensive clinical use of tamoxifen in pre- and postmenopausal women and HRT in postmenopausal women is not associated with an increased risk of developing ovarian cancer; and clinical trials with raloxifene in postmenopausal women have produced no evidence of ovarian abnormalities or increased ovarian cancer.

Reviewers comments

1. Apart from the ovarian and testicular tumors, the tumor profile of tamoxifen is different from that of raloxifene. Therefore, caution is warranted in equating tamoxifen with raloxifene with respect to their mechanisms of action.
2. Even though pituitary gonadotropin levels may be increased in the long term carcinogencity studies, , ovarian carcinogenesis induced by a mechanism other than pituitary hormone hypersecretion, e.g., an estrogenic mechanism, cannot be excluded. Ovarian granulosa cells contain the ER-β receptor, which can activate gene transcription at the AP1 response element when bound to raloxifene, but not when liganded with estradiol (Paech et al, Science, 277, p.1508, 1997).
3. Clinical data on LH and FSH in postmenopausal women treated with raloxifene:

(A). In a Phase II study (GGGB), serum estradiol, LH, and FSH were monitored in postmenopausal women taking either placebo, Premarin, raloxifene (200 mg/day) or raloxifene (600 mg/day). After Visit 5 at 2 months the following changes were observed in these parameters:

Study GGGB: Effect of PREM, RAL 200 and Ral600 on Estradiol, FSH, LH (8-wk study)

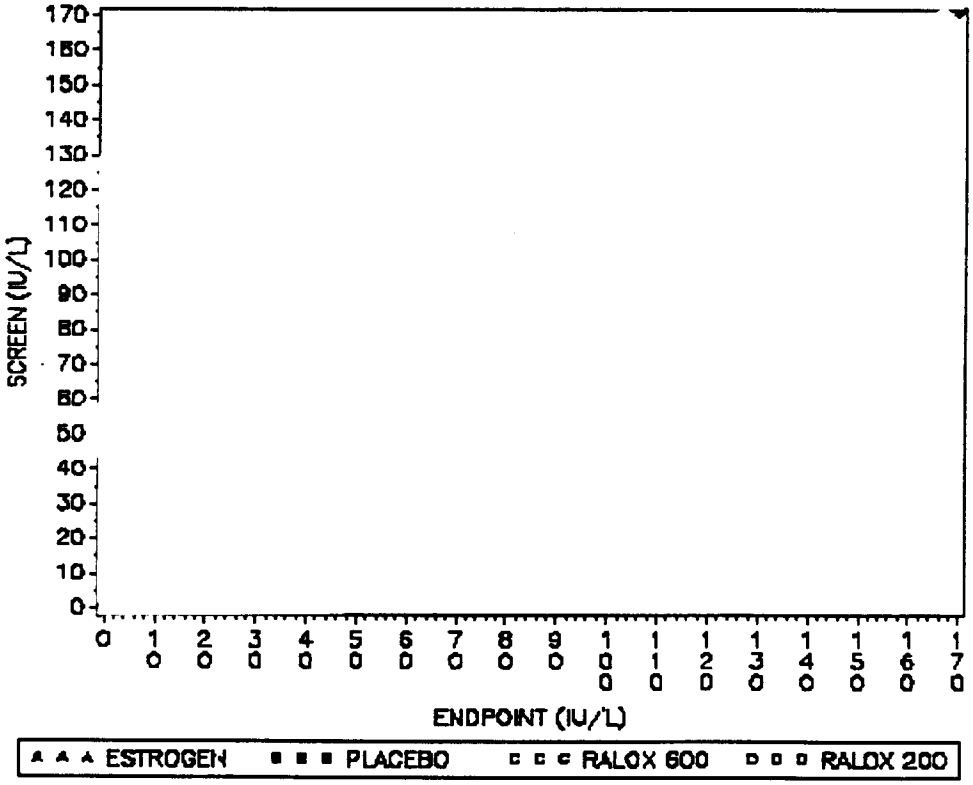
			Mean percent change from baseline (in parentheses: change vs. placebo)			
	Baseline value (mIU/ml; pg/ml)	#patients Visit1-Visit5	Placebo	PREM	Ral 200	Ral 600
FSH	105	64-58	-2.8	-38 (-35.2)	-8.6 (-5.8)	-9.9 (-7.1)
LH	29	64-57	-6.2	-8.6 (-2.4)	4.1 (+10.3)	1.6 (+7.8)
Estradiol	29	49-59	84.6	1752 (+1667)	246 (+161)	247 (+162)

These data show that there is a slight increase in LH levels in raloxifene-treated women, and a slight decrease in FSH. Although the effect on average LH levels seems minimal, in some women taking 200 or 600 mg raloxifene, LH levels were increased up to 4-fold (See figures below)

(B) The 2-year raloxifene safety database suggests that there are no TVU (transvaginal ultrasound)-identified ovarian abnormalities and no increased risk of ovarian cancer. However, the safety database is fairly small and the treatment duration too short to draw conclusive inferences at this point.

LILLY PROTOCOL H3S–MC–GGGB
Evaluation of the Short–term Effects of Raloxifene on Markers of Skeletal, Endocrine, and Cardiovascular Metabolism.

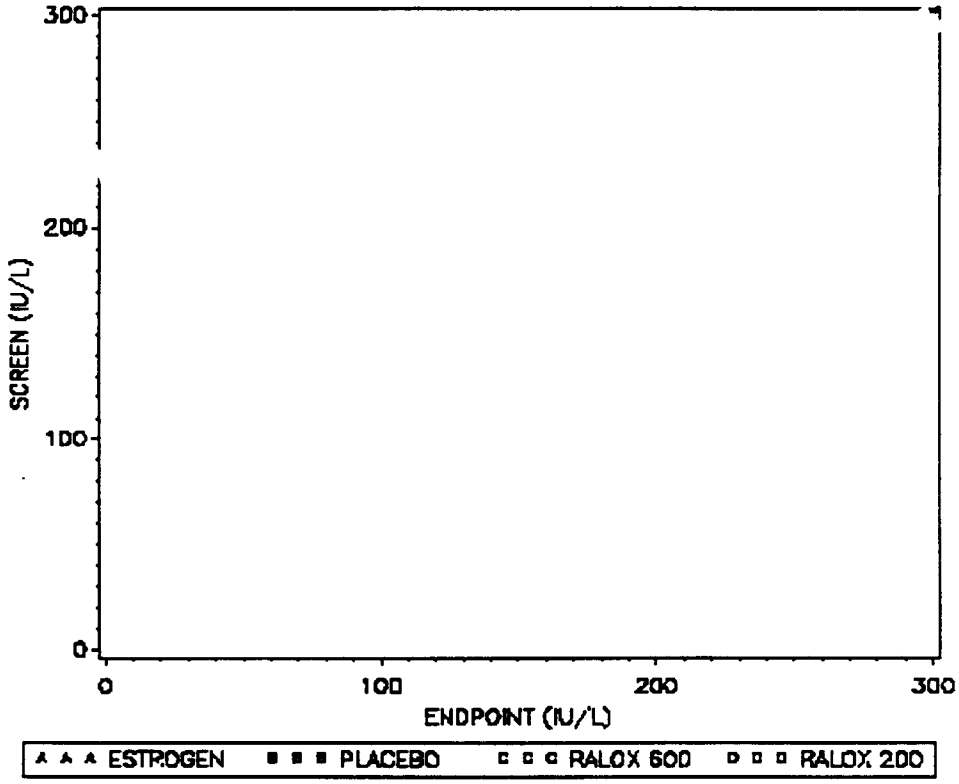
ATTACHMENT GGGB.14.1.2: Endocrine Laboratory Values.
Scatter Plot of Endpoint vs Screen by Analyte (LH).



NOTE: Diagonal line indicates no change.
See Section 7.5.3 for comments on extreme outlying values.

LILLY PROTOCOL H3S–MC–GGGB
Evaluation of the Short–term Effects of Raloxifene on
Markers of Skeletal, Endocrine, and Cardiovascular Metabolism.

ATTACHMENT GGGB.14.1.1: Endocrine Laboratory Values.
Scatter Plot of Endpoint vs Screen by Analyte (FSH).



NOTE: Diagonal line indicates no change.
See Section 7.5.3 for comments on extreme outlying values.

RAT STUDY (see Review, ATTACHMENT)

METHODS

F344 rats (60/sex/dose group), age 7-8 weeks, initial weight 146g (m) and 121g (f), were treated with **0, 0.005, 0.02, 0.1% (males)** or **0, 0.02, 0.1, 0.5% (females)** raloxifene in the diet for 24 months. These dose levels provided average daily doses (mg/kg) of: **males 0, 2.3, 9.3, 48 mg/kg**, and **females 0, 10.4, 51, 259 mg/kg**. The HD was based on a 25x AUC ratio (rat:human) of the parent drug raloxifene. For PK measurements, plasma samples were collected after 3, 12 and 18 months.

RESULTS

Survival

Survival is shown in **TABLE 1**. There was a statistically significant (at 0.05 level) negative dose-mortality trend (p-value 0.001) in male rats. There was no significant effect in female rats.

TABLE 1. Survival of rats in 24-month carcinogenicity study with raloxifene

	# At start of study		Survival at 18 mo		Survival at 21 mo		Survival at 24 mo (treatment termination)	
Dose group	male	female	male	female	male	female	male	female
Control								
LD								
MD								
HD								

Body weight

Raloxifene caused a dose-related slight-to-moderate decrease in body weight in all dose groups of male rats. Raloxifene caused a slight decrease in body weight in all dose groups of female rats from the 13th month of treatment. Body weight changes were roughly parallelled by changes in efficiency of food utilization.

Toxicological findings

The main toxic effects excluding tumors were:

Males

1. Increased mortality in LD males correlated with largely increased incidence of progressive glomerulonephrosis (PGN) and secondary renal hyperparathyroidism in this dose group. Sponsor believes PGN and associated mortality is suppressed in MD and HD males due to lesser food/protein intake.
2. Decreased lymphocyte and monocyte counts in males.
3. Decreased relative-to-body weights of prostate and spleen in males.
4. Increased incidence of testicular atrophy in all treated.

5. Decreased incidence and severity of prostate inflammation in all treated.
6. Increased incidence of thymus hyperplasia in MD, HD.

Females

1. Increased relative weight of ovaries and adrenal glands in all treated females.
2. Decreased severity of PGN, and increased incidence of tubular mineralization in all treated.
3. Increased incidence of thymus hyperplasia in all treated.
4. Increased incidence of cysts and of follicular prominence in all treated.
3. Uterine atrophy in all treated.
4. Decreased incidence of epithelial mucification in all treated.
5. Large incidence of mammary gland hypoplasia in all treated.

Tumor findings

Tumor findings are shown in **TABLE 2.**

TABLE 2. Tumor findings with raloxifene in F344 rats with survival-adjusted statistical evaluation (linear trend test)
(B) benign, (M) malignant
n.s. = non-significant

Males

Site	Tumor	Incidence (Number of animals in control-LD-MD-HD	Historical control incidence (6 studies)	Statistical significance (trendtest) according to:	
				Sponsor	Div. Biometrics
Kidney	Renal cell carcinoma (M)	0-0-0-3	0,2,0,0,0,1	n.s. (0.077)	SIGN (0.022)
Mammary gland	Fibroadenoma (B)	1-2-3-6	1,1,1,2,1,1	n.s. (0.082)	n.s. (0.02)

Females

Site	Tumor	Incidence in control-LD-MD-HD		Statistical significance according to:	
				Sponsor	Div. Biometrics
Ovary	Granulosa cell tumor (B)	0-0-0-4		SIGN (0.004)	SIGN (0.004)
	Granulosa-theca tumor (B)	0-0-1-3		SIGN (0.002)	SIGN (0.021)
	All ovarian tumors combined: Granulosa/theca/thecoma (B,M)	0-1-1-8		SIGN (<0.001)	SIGN (<0.025)

Note: The positive trend in the incidence of kidney renal cell carcinoma was evaluated to be non-significant by Sponsor. However, the Statistical Reviewer, who appropriately included the low-survival LD group in the analysis, showed this finding to be statistically significant.

Significant positive findings were:
1. Ovarian granulosa and granulosa-theca cell tumors in females (benign)
2. Kidney renal cell carcinoma in males (malignant)

Tumors with a clearly negative dose-related trend (no test by Statistical Reviewer) were:

Males	*Incidence*
Testicular interstitial cell tumor (B)	30-4-3-5
Pituitary adenoma (B)	36-8-14-14
Mononuclear cell leukemia (M)	17-3-5-5
Females	
Pituitary adenoma (B)	49-11-11-7
Mammary gland fibroadenoma (B)	12-1-3-6
Mammary gland adenocarcinoma (M)	3-0-0-0
Uterine endometrial stromal tumor (B)	5-0-1-0

Plasma levels

Raloxifene is absorbed rapidly, and undergoes extensive first pass metabolism and enterohepatic circulation, with hydrolyzation of glucuronide-conjugates in GI tract.
In the rat, 16-38% is parent drug, the remaining part is mostly raloxifene-6-glucuronide, and to lesser extent raloxifene-4'-glucuronide and raloxifene-4'-6-diglucuronide. The metabolites are found in plasma, bile and feces. Rat bile also contains a trace of raloxifene-6-monosulfate, and an unidentified oxidative metabolite. The major part of the drug is excreted in the faeces (96%).

TABLE 3 and **TABLE 4** below shows AUC values for raloxifene (RAL), and for raloxifene plus its combined glucuronides (T.RAL=TRHP=total raloxifene in hydrolyzed plasma) after 3, 12 and 18 months. In Table 4, AUC multiples are presented for the parent raloxifene and total raloxifene. As in mice, values are based on plasma drug levels.

TABLE 3. $AUC_{(0-24h)}$ values for raloxifene and Total Raloxifene on Day 90, 363, 545 in F344 rats

		AUC (0-24h) RAL (ng x h/ml)			AUC (0-24h) T. RAL (ng x h/ml)			AUC ratio RAL: T.RAL (%)		
	Dose (mg/kg/d) (Day 90- 363- 545)	Day 90	Day 363	Day 545	Day 90	Day 363	Day 545	D90	D363	D545
Males	2.6- 2.1- 2.2							90	0	12
	10- 8.3- 8.7							30	18	18
	54- 43- 44							39	16	21
Females	13- 9.2- 9.0							29	24	16
	65- 45- 44							38	26	19
	317- 227-229							35	25	17

TABLE 4. Multiples of expected human exposure in rat carcinogenicity study with raloxifene (rat study data from Day 545)

	Dose	$AUC_{(RAL)}$	$AUC_{(T,RAL)}$	$AUC_{(RAL)}$ multiple	$AUC_{(T,RAL)}$ multiple
Males	LD	33	269	1.4x	0.08x
	MD	209	1147	9x	0.32x
	HD	1242	6013	52x	1.7x
Females	LD	485	2957	20x	0.84x
	MD	2344	12719	98x	3.6x
	HD	10154	58805	423x	17x
Human	1 mg/kg/day	24	3500		

Protein binding

Raloxifene and its monoglucuronides are highly bound to plasma proteins (See mouse study, TABLE 5)

DISCUSSION

In rats, raloxifene causes tumors in ovary and kidney (in males). The ovarian granulosa/theca cell tumors occur at low incidences at multiples of ca. 100x and 400x the expected human exposure at 60 mg/day. Tamoxifen at doses up to 35 mg/kg did not cause ovarian tumors in females or renal tumors in males. However, early termination of the tamoxifen rat study may have accounted for this negative result.

Most ovarian tumors seen in rats are granulosa cell tumors. Some fifty years ago, Biskind and Biskind (1944) showed that transplantation of the ovaries to the spleen causes development of this kind of tumor within one year. As in the mice, the tumorigenic mechanism is thought to be hormonal, due to lowered levels of ovarian estrogen in plasma, and resulting gonadotropin (LH, FSH) hypersecretion by the pituitary. Similarly, raloxifene could induce the ovarian tumors by acting as an antiestrogen in the HPO axis. However, in short term studies in intact rats, RAL had no consistent effect on LH, FSH, or prolactin (APR 16).

Tamoxifen (5, 20, 35 mg/kg/day) causes a dose-related, up to 70% incidence of liver tumors in the rats A significant incidence of liver tumors was not seen in the raloxifene-treated rats.

As tamoxifen, raloxifene caused a large decrease in the incidence of pituitary adenoma in male and female rats, and of mammary gland tumors in female rats in all dose groups. The decreased incidence of mammary tumors in female rats may be related to the decreased incidence of pituitary adenomas, and may reflect and estrogenic effect of raloxifene. In ageing female rats, prolactinomas are common, and prolactin has a proliferative effect on mammary epithelial cells. Raloxifene, acting as an estrogen, may suppress the feedback stimulation of the pituitary caused by the fall in estrogen upon ageing, thus preventing the incidence of pituitary and mammary tumors. If this is so, this central estrogenic action of raloxifene seems to occur at much lower doses than the anti-estrogenic effect that may be responsible for the ovarian tumors. The increased incidence in mammary tumors in males may also represent an anti-

estrogenic effect of raloxifene.

The decreased incidence of testicular tumors in treated rats can be explained as follows. In the ageing control F344 rat serum estradiol levels increase and are correlated with the development of testicular interstitial cell tumors (Clegg et al, 1997). Raloxifene might act as a testicular anti-estrogen, thus suppressing the occurrence of these tumors. This effect is basically different from the mouse, in which raloxifene causes testicular tumors, and control animals do not develop these tumors (**TABLE 6**).

TABLE 6. Effect of raloxifene on tumors in CD-1 mice and F344 rats

	Testicular tumors	Ovarian tumors
MOUSE	↑	↑
RAT	↓	↑

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CONCLUSIONS FROM CARCINOGENICITY STUDIES

- Significant tumors findings (SUMMARY TABLES below)

OVARY	rat, mouse
TESTIS	mouse
PROSTATE	mouse
KIDNEY	rat (male)

- The most obvious and important finding is the increased incidence of ovarian tumors in female mice and rats. The finding causes concern particularly because in mice the tumors occur at plasma levels equivalent to the intended human therapeutic levels.

- It is plausible that the ovarian carcinogenesis caused in the intact animals is hormonally mediated by an anti-estrogenic action of the SERM raloxifene in the HPO axis, through increased gonadotropin levels and prolonged ovarian stimulation.

- We can not exclude an alternative carcinogenetic mechanism of action, be it either epigenetic or genotoxic.

- Additional genotoxicity tests are recommended.

- The clinical relevance of the ovarian tumor findings is unclear.

- Continued endocrinological, uterine and mammary gland screening in clinical studies is recommended.

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MOUSE TUMORS. Tumor incidence (# tumors/group of 60 animals) in mice treated with raloxifene for 21 months

						Statistical significance	
	Dose group	Control	LD	MD	HD	Trendtest	Pairwise test
FEMALES	Human multiple (T.RAL)	**0x**	**0.15x**	**1.1x**	**4.0x**		
	Human multiple (RAL)	**0x**	**0.3x**	**7x**	**34x**		
Ovary	Granulosa cell tumor (M)	0	2	4	8	*	
	Granulosa cell tumor (B)	0	4	3	6	*	
	Epithelial tumors, combined (B)	1	9	7	4	ns	* (LD)
	All tumors, combined (B,M)	4	19	20	29	*	
MALES	Human multiple (T.RAL)	**0x**	**0.04x**	**0.8x**	**4.3x**		
	Human multiple (RAL)	**0x**	**0.75x**	**4.7x**	**24x**		
Testis	Interstitial cell tumors, combined (B,M)	2	1	15	27	*	
Prostate	Adenoma (B), adenocarcinoma (M), combined	0	0	4	4	*	
	Leiomyoblastoma (B)	0	0	0	4	*	

RAT TUMORS. Tumor incidence (# tumors/group of 60 animals in rats treated with raloxifene for 24 months

						Statistical significance	
	Dose group	Control	LD	MD	HD	Trendtest	Pairwise test
FEMALES	Human multiple (T.RAL)	**0x**	**0.84x**	**3.6x**	**17x**		
	Human multiple (RAL)	**0x**	**20x**	**98x**	**423x**		
Ovary	Granulosa cell tumor (B)	0	0	0	4	*	
	Granulosa theca tumor (B)	0	0	1	3	*	
	All tumors combined (B,M)	0	1	1	8	*	
MALES	Human multiple (T.RAL)	**0x**	**0.08x**	**0.32x**	**1.7x**		
	Human multiple (RAL)	**0x**	**1.4x**	**9x**	**52x**		
Kidney	Renal cell carcinoma (B)	0	0	0	3	*	

ATTACHMENT - CARCINOGENICITY STUDY REVIEWS

AN ONCOGENIC STUDY AND COMPANION BLOOD LEVEL STUDY IN CD-1 MICE
Study nr. M00494 (males), M00594 (females), M00694 (PK study) Toxicology Report No. 59 (Lilly Research Labs, IN). Study period April 1994- January 1996. GLP quality assurance statement included.

INTRODUCTION - Raloxifene is being developed as an alternative agent for HRT for the prevention of postmenopausal osteoporosis. The adverse effects of raloxifene in the clinical situation are not completely elucidated. Adverse effect of estrogens include cardiovascular, hepatic and neoplastic disease. This study addresses the issue of possible carcinogenic potential of the compound

PROCEDURES - CD-1 mice (60/sex/dose group) (M00494 and M00594), initial weight 25g (m) and 21g (f), were treated with **0, 0.005, 0.03, 0.15%** raloxifene in the diet for 21 months. These dose levels provided average daily doses (mg/kg) of: **males 0, 6.5, 38, 195 mg/kg**, and **females 0, 8.7, 49, 225 mg/kg**. HD was based on 25x AUC ratio (mouse:human) of the parent drug raloxifene. In study M00694, 33/sex/group (M00694) initial weight 27g (m) and 22 g (f) were treated with same dietary %'s for 12 months. Groups included 3 replacement animals/sex. Plasma samples were collected after 3 and 12 months.

RESULTS -
Test article dosage -

Survival -
Percentage survival:
Studies M00494/00594:
Males: 80%-83%-80%-68%
Females: 78%-77%-80%-80%

Clinical signs -
Males: Increased incidence in HD of: abdominal distention, decreased muscle tone, rough coat, piloerection, seizure, testicular enlargement
Females: Increased incidence in MD, HD of: red or missing ears

Body weight -
Males - Decrease in BW and BW gain in LD, MD, HD
BW after 12 months: -9%, -11%, -10%
Females - Decrease in BW and BW gain in LD, MD. Increase in BW (gain) in HD
BW after 12 months: -2% non-significant (n.s.) decrease, -1% n.s. decrease, +9%

Food consumption -
Males -
Decrease in LD, MD, HD

After 12 months, average daily FC in LD, MD, HD significantly decreased by 4%, 6%, 5%.

Females -
Decrease in MD, HD
After 12 months, average daily FC in MD, HD significantly decreased by 6%, 5%.

Efficiency of food utilization -
Males -
During Months 3-21 cumulative EFU slightly and non-dose-dependently decreased in LD, MD, HD.
After 12 months, cumulative efficiency of food utilization (cEFU) decreased by 16%, 16%, 18%.
Females -
During Months 1-9 cEFU decreased in LD. No effects in MD. During Months 1-21 cEFU increased in HD (effect largest at start of treatment, then attenuating)
After 12 months cEFU increased in HD (by 34%).

Exposure -
Methods -
- Plasma assayed for raloxifene and total raloxifene (i.e. raloxifene in plasma after hydrolysis with β-glucuronidase)
- Samples taken on: Day 91 and Day 365
- Doses (mg/kg) based on average of daily food consumed during 1 week before sampling
- AUC determined by trapezoidal rule

-

- Plasma concentrations of both RAL and T.RAL in m and f dependent on time of day. Lowest values at 1400h, highest values at 0600hh, intermediate values at 2200h. This is probably due to feeding pattern in mice.
- AUC values of RAL and T.RAL approximately linear and proportional with dose.
- Ratio RAL:T.RAL appr. 4% in m, in f

Hematology - (All changes non-dose-dependent)
At 640 days: Slight increases in MCV, MCH in all male and female treated. Minimal 6-8% decrease in erythrocyte count in all male treated. Moderate decrease in all leukocyte subset (lymphocytes, neutrophils, monocytes, eosinophils, basophils) counts in all male treated.

Clinical Chemistry -
At 640 days: Slight to moderate non-dose-dependent (ndd) 24-78% increases in ALP in all male and female treated. Slight ndd decrease in BUN in all female treated. Slight 30% increase in BUN in HD m. Slight increases in total bilirubin in MD, HD f and HD m.

Organ Weights -
Relative-to-body-weight:
Kidney: Slight increase in HD m,f

Spleen: Decrease of 0.8x (control value) in HD f
Brain: Increase of ca. 1.1x in all treated m, and decrease of 0.9xin HD f
Testis*: Increase of 1.2x, 2.7x, 3.1x control in LD, MD, HD m (all significant)
Ovary*: Increase of 1.4x, 3.6x, 5.1x control in LD, MD, HD f (none statistically significant)
Uterus: Decrease of 0.27x, 0.28x, 0.2x in LD, MD, HD f (all significant)
Liver*: Increase of 1.01x in HD f
Heart: No changes
Note: Changes are *tumor-related

Pathology (Final diagnoses) -
MALES *(Numbers given are numbers of animals in control-LD-MD-HD groups)*
(A) Cause of death
Non-neoplastic:
Most frequent: Amyloidosis 2-0-3-2, mouse urologic syndrome 3-2-2-4. Total 7-5-7-9
Neoplastic:
Benign tumors (B)
Interstitial cell tumor (testicular origin) 0-0-0-1
Total 0-0-0-1
Malignant tumors (M)
Prostatic adenocarcinoma 0-0-0-1
Prostatic squamous cell carcinoma 0-0-0-1
Brain stem astrocytoma 0-0-1-0
Splenic hemangiosarcoma 0-0-1-0
Interstitial cell tumor (testicular origin) 0-0-1-2
Lymphosarcoma 0-2-0-4
Others (all apparently non-treatment-related) 4-2-4-4
Total 5-4-7-12

Total # deaths due to tumors	5-4-7-13
Total # deaths due to any cause	12-9-14-22

(B) Whole animal findings
Non-neoplastic:
Amyloidosis 2-0-4-2, mouse urologic syndrome 3-2-3-4
Neoplastic:
Lymphosarcoma (M) 4-5-0-5
Plasma cell myeloma (M) 0-0-0-1

(C) Organ findings
Urinary bladder: Dilation 2-2-3-5
Liver:
Hepatocellular adenoma (B) 5-4-7-4
Hepatocellular carcinoma (M) 2-9-4-3
Lung: Alveolar/bronchiolar carcinoma (M) 4-1-2-1
Testis:
Interstitial cell hyperplasia: slight 2-2-5-5, moderate 1-0-4-4, severe 0-0-0-1, total incidence 3-2-9-10.
Interstitial brown pigmentation:minimal/slight 0-10-42-38, moderate/severe 0-0-8-15,

total incidence 0-10-50-53.
Interstitial cell tumor (B) 2-1-13-17
Interstitial cell tumor (M) 0-0-2-10
Total incidence interstitial cell tumors (B/M) 2-1-15-27
Total incidence bilateral tumors (B/M) 1/2-0/1-5/15-13/27 (50-0-33-50%)
Epididymis: Sarcoma (undifferentiated) (M) 0-0-0-1
Prostate:
Inflammation: moderate/severe 1-1-2-3
Mucosal hyperplasia: slight, focal 2-3-9-11
Adenoma (B) 0-0-2-2
Adenocarcinoma (M) 0-0-2-2
Leiomyoblastoma (B) 0-0-0-4
Squamous cell carcinoma (M) 0-0-0-1
Seminal vesicle:
Inflammation: moderate/severe 0-0-2-2
Adenoma (B) 0-1-0-0
Skin: Inflammation: moderate/severe 0-1-2-4
Harderian gland: Inflammation, moderate 0-1-0-0, Adenoma (B) 4-3-3-3
Bone marrow: Myelofibrosis 0-1-0-2
Adrenal gland:
Cortical cyst: moderate 0-0-0-2
Cortical hyperplasia 1-2-4-2
Adrenocortical adenoma, subcapsular cells (B) 0-1-2-1
Pheochromocytoma (B) 0-0-1-0
Brain stem: Astrocytoma (M) 0-0-1-0
Eye: Hypopion 0-1-0-1

FEMALES

(A) Cause of death

Non-neoplastic:

Most frequent: Amyloidosis 1-2-0-0, mouse urologic syndrome 1-1-0-0, bacterial nephritis 0-1-0-1. Total 8-9-4-3

Neoplastic:

Benign tumors (B)
Granulosa cell tumor 0-0-1-0
Ovarian papillary adenoma 0-0-1-0
Others 0-0-1-0
Total 0-0-3-0

Malignant tumors (M)
Granulosa cell tumor 0-2-0-4
Lymphosarcoma 2-2-3-4
Uterine leiomyosarcoma 2-0-0-0
Others 1-2-3-2 (possibly treatment-related)
Total 5-6-6-10

Total # deaths due to tumors	5-6-9-10
Total # deaths due to any cause	13-15-13-13

(B) Whole animal findings
Non-neoplastic:
Amyloidosis 5-3-4-1, mouse urologic syndrome 1-1-0-0
Neoplastic:
Histiocytic sarcoma (M) 0-0-1-0
Lymphosarcoma (M) 15-10-14-7

(C) Organ findings
Kidney: Tubular nephrosis, minimal/ slight 0-1-1-3, moderate 0-1-0-0
Urinary bladder: Dilation 0-1-0-1
Liver:
Hepatocellular cytomegaly, minimal/slight 3-4-4-15, moderate 0-0-0-2, total incidence 3-4-4-17
Focal hepatocellular hyperplasia 1-1-1-2
Hepatocellular adenoma (B) 1-0-0-4
Hepatocellular carcinoma 0-1-3-2
Gallbladder: Adenoma (B) 0-0-0-1
Lung: Alveolar/bronchiolar carcinoma (M) 0-1-2-1
Mediastinum: Sarcoma, undifferentiated (M) 0-0-1-0
Peritoneum: Osteosarcoma (M) 0-0-0-1
Ovary:
Cyst, minimal/slight 26-23-27-17, moderate/severe 12-7-2-3. Total incidence 38-30-29-20.
Persistent (i.e., no ovulation occurred) hemorrhagic follicular dilation, minimal/slight 12-30-31-30, moderate 1-4-11-10. Total incidence 13-34-42-40.
Brown pigmentation, minimal/slight 23-10-5-7, moderate/severe 0-39-50-46, total incidence 23-49-55-53.
Cystic papillary hyperplasia 0-0-1-0
Tubular hyperplasia 1-14-7-9
Papillary adenoma (B) 1-8-5-4
Tubular adenoma (B) 0-1-2-0
Granulosa cell tumor (B) 0-0-0-1
Granulosa theca tumor (B) 0-4-3-6
Luteoma (B) 3-6-7-10
Thecoma (B) 0-1-1-1
Granulosa cell tumor (M) 0-2-4-8
Luteoma (M) 1-0-2-2
Total incidence of benign neoplasms 4-20-18-22
Total incidence of malignant neoplasms 1-2-6-10
Incidence of animals with ovarian neoplasms 4-19-20-29
Uterus:
Atrophy, slight 0-6-9-7, moderate 0-22-23-34, severe 0-23-20-13, total incidence 0-51-52-54
Cystic endometrial change, minimal/slight 35-51-47-41, moderate/severe 12-1-1-0, total incidence 47-52-48-41
Diffuse papillary mucosal hyperplasia 0-0-1-8
Deciduoma (non-neoplastic proliferation) 0-2-5-6

Leiomyoma (B) 0-1-1-0
Papillary cystadenoma (B) 1-0-2-0
Leiomyosarcoma (M) 3-0-0-0

Vagina:
Cornification, minimal/slight 23-3-1-2, moderate 9-0-0-0, total incidence 32-3-1-2
Epithelial mucification, minimal/slight 13-31-3-37, moderate 2-11-1-1, total incidence 15-42-34-38

Mammary gland: Hypoplasia, slight 1-4-8-8, moderate/severe 1-41-40-42, total incidence 2-48-45-50

Harderian gland:
Hyperplasia 0-0-0-1
Inflammation, moderate 0-1-0-2, severe 0-0-1-0
Adenoma (B) 2-4-4-6

Bone:
Hyperostosis, minimal/slight 2-18-13-16, moderate/severe 0-7-7-7, total incidence 2-25-20-23
Osteoma 0-0-1-0
Osteochondrosarcoma 0-1-0-0

Adrenal gland:
Cortical cyst, moderate 0-0-2-3, severe 0-1-1-0, total 0-1-3-3
Adrenocortical adenoma (B) 0-0-1-0
Adrenocortical adenoma, subcapsular cells (B) 1-0-2-1
Pheochromocytoma (B) 0-0-1-1
Adrenocortical adenocarcinoma (M) 0-0-2-0

Thyroid:
Follicular epithelial hyperplasia 0-1-0-0
Follicular cell adenoma 0-0-1-0

Eye: Hyphema 0-0-0-1, hypopyon 0-0-0-2, keratitis 1-0-2-5

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Statistical analysis (*Sponsor*)
Tumor incidence
Males

Site	Tumor	Incidence (nr. of animals)	Trend test one-sided p-value	Randomization trend test one-sided p-value	Groups
Testis	Interstitial cell tumor (M)	0-0-2-10	<0.001	<0.001	Ctrl-HD
				<0.108	Ctrl-MD
	Interstitial cell tumor (B)	2-1-13-17	<0.001	<0.001	Ctrl-HD
				<0.001	Ctrl-MD
Prostate	Adenocarcinoma (M)	0-0-2-2	0.031	0.050	Ctrl-HD
				0.110	Ctrl-MD
	Adenoma (B)	0-0-2-2	0.025	0.042	Ctrl-HD
				0.110	Ctrl-MD
	Leiomyoblastoma (B)	0-0-0-4	0.003	0.003	Ctrl-HD

Females

Site	Tumor	Incidence (nr. of animals)	Trend test one-sided p-value	Randomization trend test one-sided p-value (p-value exact permutation trend test)	Groups
Liver	Hepatocellular carcinoma (M)	0-1-3-2	0.077	- (0.055)	-
	Hepatocellular adenoma (B)	1-0-0-4	0.036	0.055 (0.11)	Ctrl-HD
Ovary	Granulosa cell tumor (M)	0-2-4-8	0.001	0.001	Ctrl-HD
				0.024	Ctrl-MD
				0.253	Ctrl-LD
	Granulosa cell tumor (B)	3-6-7-10	0.017	0.017	Ctrl-HD
				0.082	Ctrl-MD
	Thecoma	0-1-1-1	n.s.		
	Luteoma (B)	3-6-7-10	0.025	0.025	Ctrl-HD
				0.119	Ctrl-MD

	Luteoma (M)	1-0-2-2	n.s.		
	Tubular/papillary adenoma (B)	1-9-7-4	n.s.		
Uterus	Mucosal hyperplasia	0-0-1-8	no data		
	Deciduoma	0-2-5-6	no data		

SUMMARY AND EVALUATION -

FINDINGS

1. Minimal decrease in BW in all treated males, minimal increase in BW in HD females
2. Slight decrease in EFU in all treated males, slight increase in EFU in HD females
3. Decrease in leukocyte counts in all treated males
4. Increase in serum ALP in all treated males and females, and slight increase in serum bilirubin in HD males and MD, HD females.
5. Increase in relative-to-body weights of testes and ovaries in all dose groups.
6. Pathology -

Males:

- Increase in mortality due to tumors (benign or malignant) in HD m
- Testis: Brown pigmentation in LD, MD, HD. Increased incidence of Interstitial cell hyperplasia in MD, HD. Increased incidence of benign and malignant interstitial cell tumors in MD, HD.
- Prostate: Slightly increased incidence of inflammation in HD. Increased incidence of mucosal hyperplasia in MD, HD. Increased incidence of benign and malignant tumors in MD, HD.
- Adrenal gland: Increased incidence of cysts in HD. Slightly increased incidence of cortical hyperplasia and benign tumors in LD, MD, HD

Females:

- Decreased incidence of amyloidosis and of lymphosarcoma in HD.
- Liver: Increased incidence of hepatocytomegaly in HD.
- Ovary: Slightly decreased incidence of cysts in LD, MD, HD. Largely increased incidence of persistent follicular dilation in all treated. Increased incidence of brown pigmentation in all treated. Increased incidence of tubular hyperplasia in all treated. Increased incidence of benign and malignant tumors in LD, MD, HD.
- Uterus: Occurrence of atrophy in all treated. Decreased incidence of moderate/severe cystic endometrial change in all treated. Mucosal hyperplasia in MD, HD. Deciduoma in all treated. Decreased incidence of leiomyosarcoma in all treated.
- Vagina: Decreased incidence of cornification in all treated. Increased incidence of epithelial mucification in all treated.
- Mammary gland: Increased incidence of hypoplasia in all dose groups.
- Bone: Hyperostosis in LD, MD, HD.
- Adrenal gland: Cortical cyst in MD, HD.
- Thyroid: Occurrence of follicular cell hyperplasia and adenoma in LD, MD.
- Eye: Increased incidence of keratitis in HD.

COMMENTS

Whether a SERM, such as raloxifene, acts like an estrogen or an anti-estrogen depends on the tissue and the genes involved in the response. By binding to the estrogen-receptor (ER), raloxifene can inhibit estrogen from acting through its receptor and the ERE, or interact itself with an estrogen response element (ERE) or another specific response element (RRE) present in particular genes.

- Body weight and EFU effects are estrogenic actions of raloxifene. Estrogens are growth-inhibiting in rats and mice through decreased food and water intake, and through changes in adiposity and regulated body weight (Hart, 1990).

- The ALP increase and bilirubin increase, and the hepatocellular cytomegaly in females are likely to constitute an estrogenic effect of raloxifene on the mouse liver. Estrogen can cause elevations of serum liver enzymes and bilirubin, and cause hepatocyte swelling, necrosis and fatty changes in various species (Hart, 1990).

- The leukocyte decreases appear to be another estrogenic effect of raloxifene. Bone marrow hypoplasia, hypocellularity and anemia can be induced in rodents and other species by estrogens. In mice, but not rat, leukocyte counts can also be reduced (Hart, 1990)

- Hyperostotic effect on bone in females appears to be estrogenic. Estrogen can cause osteosclerosis in mice (Hart, 1990).

- Adrenal cortical hypertrophy and hyperplasia has been seen in response to estrogens in rodents, and are thus estrogenic effects.

- Unlike estrogens, raloxifene did not cause pituitary, liver, adrenal, thyroid weight increases.

- Changes in vaginal cornification and mucification are probably due to an estrogenic action of raloxifene (Hart, 1990).

- Mammary gland hypoplasia is probably an anti-estrogenic effect since estrogens induce mammary duct growth in rodents (Hart, 1990).

- Increased testis and ovary weights are related to tumors in the respective organs.

- Testis/prostate:
  The raloxifene effects on the testis (pigmentation, hyperplasia, tumors) are likely to be estrogenic. Estradiol and the estrogen agonist/antagonist tamoxifen induce interstitial (Leydig) cell tumors in mice (not in rats) via their ability to modulate LH levels or via a direct stimulation of interstitial cells (Table 5) (Tucker, 1984; Clegg et al, 1997). The tumors induced by tamoxifen are thus thought to be due to an estrogenic action of tamoxifen on mouse testis. The raloxifene effects on prostate (inflammation, hyperplasia, tumors) may also be directly estrogenic or indirectly anti-estrogenic Prostate

tumors were not seen with tamoxifen.

- Uterus:
  The effects of raloxifene on the uterus seen in this study (weight loss, atrophy, less cystic endometrial change) are clearly anti-estrogenic. Estrogens stimulate the uterus, and cause a uterine weight increase with epithelial hyperplasia, edema, and myometrial hypertrophy, and cystic endometrial hyperplasia and endometrial adenocarcinoma after prolonged administration.

  The uterine mucosal hyperplasia, as well as the occurrence of deciduomas, is most likely the secondary result of estradiol secretion by the ovarian tumors: 8/8 HD animals with mucosal hyperplasia and 6/6 HD animals with deciduomas had ovarian luteoma or granulosa cell tumors.

- Ovary:
  The follicular dilation and decreased cyst incidence indicate that raloxifene acts as an anti-estrogen on the ovary. Estrogens depress follicular and can induce ovarian cysts (Hart, 1990)

  The most prominent finding is the increased incidence of benign ovarian tumors in all dose groups, and malignant tumors in MD and HD groups. Since these tumors occur in LD and MD groups in which the exposure is merely a fraction (!) or a low multiple of the expected human exposure at the 60 mg therapeutic dose (Table 4, APPENDIX), this is of particular concern.

  Sponsor speculates that the induction of ovarian tumors is due to an indirect effect of raloxifene on the ovary, namely through interference with the negative feedback through the hypothalamo-pituitary axis. They suggest that raloxifene acts as a central estrogen-antagonist in the mouse causing an increased pituitary hormone secretion of LH and/or FSH which stimulate growth of ovarian sex cord-stromal cells.

  In a 15-month study, the SERM tamoxifen was also found to cause ovarian granulosa cell tumors, and a depression of uterine leiomyosarcoma in mice . A 13-month study showed granulosa cell hyperplasia due to estradiol treatment in 1/20 mice and a granulosa cell tumor in 1/20 animals treated with tamoxifen (20 mg/kg) (Tucker, 1984). These data suggest that the ovarian granulosa cell tumorigenesis might have an estrogenic nature.

  The ovarian tumors that are induced in various experimental mouse systems (irradiation, ovary transplantation, germ cell deletion) where extremely low estrogen levels are thought to be the cause of the tumors - probably via an increase in gonadotrophin release - are mostly so-called tubulostromal adenomas, which are tubular in nature and derived primarily from the ovarian epithelium. These tumors have been dismissed as irrelevant for the human situation since they do not have a histological human equivalent.

  However, the ovarian tumors induced by raloxifene are for a large part granulosa or

theca cell tumors (sex cord-stromal tumors) which are derived from the stromal cells. It is unclear whether the CD-1 mice strain is not prone to tubulostromal tumors or whether the raloxifene-induced tumors are caused by another mechanism than the tubulostromal ones.

If the ovarian tumors are due to an anti-estrogenic effect via the pituitary, the clinical relevance of this is unknown. The fact that so far no relation has been found between LH or FSH levels and the occurrence of ovarian tumors (Blaakaer, 1992) does not mean that a change in the levels could affect this occurrence.

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APPENDIX

TABLE 1. Test article dosage

	Oncogenic studies		Blood level study			
Study #	M00494	M00594	M00694 (@ 3mo)		M00694 (@ 12 mo)	
Sex	males	females	males	females	males	females
Dietary concentration (%)	Average daily dose of raloxifene (mg/kg)					
0.005	6.5 (7)*	8.7 (9)	6.9	9.1	6.0	7.7
0.03	38 (41)	49 (53)	41	51	36	41
0.15	195 (210)	225 (242)	211	235	184	198

*(in parentheses) raloxifene hydrochloride doses

TABLE 2. Number of animals

Study Nrs. M00494/00594	# At start of study	Died/killed moribund		Missing	# At treatment termination	
Dose group		male	female	male	male	female
Control	60	12	13	0	48	47
LD	60	9	14	1	50	46
MD	60	12	12	0	48	48
HD	60	19	12	0	41	48

Study Nr. M00694	# At start of study	Died/killed moribund	Missing
Dose group			male
Control	33	0	0
LD	33	4	0
MD	33	2	0
HD	33	4	3

TABLE 3. Day 91 (sample 0600h) plasma concentrations in CD-1 mice

	dose	RAL (ng/ml)	total RAL (ng/ml)	ratio RAL:T.RAL
male (n=3)	6.9	1	23	4.3
	41	5	151	3.3
	211	26	894	2.9
female (n=3)	9.2	2	35	5.7
	51	10	283	3.5
	235	40	1836	2.2

From previous submission:

Table 1. Plasma concentrations after 3 months (**Day 91**) in CD-1 mice

	Plasma concentration (ng/ml) In parentheses: (AUC in ng x h/ml)			
	Males		Females	
Daily dose (%)	Raloxifene	Total Raloxifene	Raloxifene	Total Raloxifene
0.005%	1 (24)	23 (552)	2 (48)	35 (840)
0.03%	5 (120)	151 (3624)	10 (240)	283 (6792)
0.15%	26 (624)	894 (21456)	40 (960)	1836 (44064)

Ratio (raloxifene:total raloxifene) in males in females

TABLE 4. Day 365 (samples at 0, 8, 16, 24 h after 0600h) plasma concentrations in CD-1 mice

	Dose (mg/kg)	RAL (ng/ml)				T.RAL (ng/ml)				$AUC_{0\text{-}24h}$ (RAL) (ngxh/ml)	$AUC_{0\text{-}24h}$ (T.RAL) (ngxh/ml)	AUC ratio RAL: T.RAL
h after 0600h		0	8	16	24	0	8	16	24	(in parentheses) mouse:human dose multiple		
male	6									18 (0.75x)	151 (0.04x)	11.9*
	36									112 (4.7x)	2718 (0.8x)	4.1
	184									587 (24x)	15089 (4.3x)	3.9
female	7.7									7 (0.3x)	532 (0.15x)	1.3*
	41									171 (7x)	3849 (1.1x)	4.4
	198									820 (34x)	14151 (4.0x)	5.8
Human	1									24	3500	0.7

*Values at these dose are relatively unreliable because many samples were BLQ

Table 5. Incidence (%) of tumors in AP mice treated with tamoxifen

	Dose (mg/kg/d)	0	5	50
Females	Survival (%)	60	68	48
	Ovary granulosa cell adenoma (B)	0	36	36
	Uterus leio-myosarcoma	4	0	0
	Pituitary adenoma	0	6	6
	Thymus lymphosarcoma	8	0	4
Males	Survival (%)	64	44	68
	Liver hepatoma (B)	8	4	0
	Testis interstitial cell tumor (B)	0	8	84

(AP-1 mice, 25/sex/dose group, route: stomach tube/diet, 15 months)

AN ONCOGENIC STUDY IN FISCHER 344 RATS
Study nr. R00594 (males), R00694 (females). Toxicology Report No. 60 (Lilly Research Labs, IN). Study period March 1994-March 1996. GLP quality assurance statement included.

PROCEDURES - F344 rats (60/sex/dose group), _ , initial weight 146g (m) and 121g (f), were treated with **0, 0.005, 0.02, 0.1% (males)** or **0, 0.02, 0.1, 0.5 % (females)** raloxifene in the diet for 24 months. These dose levels provided average daily doses (mg/kg) of: **males 0, 2.3, 9.3, 48 mg/kg**, and **females 0, 10.4, 51, 259 mg/kg.**
For PK measurements, plasma samples were collected after 3, 12 and 18 months.

RESULTS -
Test article dosage -
See Table 1 (Attachment)

Survival -
Percentage survival:
Males: 37%-10%-43%-63%
Females: 65%-80%-78%-78%

Clinical signs -
Males: Increased incidence in LD of: rough hair coat, thinness and pallor. Decreased incidence in HD of: rough hair coat, thinness and pallor.

Body weight -
Males -
(BW of controls increased to maximum of ca. 480g at ca. 12 mo, then decreased again to ca. 420g. at 24 mo)
Decrease in BW and BW gain in LD, MD, HD throughout treatment
After 11 mo: BW -17%, -24%, -29%, BW gain -25%, -35%, -42%
After 24 mo: BW -17%, -18%, -17%, BW gain -39%, -35%, -42%
Females -
(BW of controls increased to maximum of ca. 330g at 24 mo.)
Decrease in BW and BW gain in LD, MD, HD from 12 mo of treatment
After 11 mo BW: -3% n.s., +2% n.s., -0%, BW gain -5% n.s., +5% n.s., -0%.
After 24 mo BW: -16%, -12%, -15%, BW gain -24%, -18%, -24%

Food consumption -
Males -
FC: In LD, MD, HD, decrease throughout treatment. At 24 mo, average daily FC -20%, -19%, -18%.
Relative FC: In HD, increase of , throughout treatment, at 24 mo increase of 12%. In MD, increase of 3-10% through Month 21. In LD, increase of through Month 18.
Females -
FC: In LD, HD, decrease throughout treatment. In MD, decrease from 1-4 mo and from

10-24 mo. At 24 mo, average daily FC -10%, -7%, -7%.
Relative FC: In HD, minimal decrease at from 6-12 mo, minimal increase at 21 mo. In MD, slight decrease from 6-18 mo. In LD, minimal decrease at 9 mo and 15 mo.

Efficiency of food utilization -

Males -

During Months 1-23 or 1-24 cumulative efficiency of food utilization (cEFU) decreased slightly and dose-dependently in LD, MD, HD.
At 24 mo, cEFU decreased by ca. 10% in all dose groups (no longer significant).

Females -

During Month 1 and from Month 5-10 cEFU increased, and from Month 14-24 cEFU decreased in HD. From Month 5-12 cEFU increased, and from Month 15-24 decreased in MD. From Month 2-5 and Month 13-24 decreased in LD.
At 24 mo, cEFU -13%, -11%, -18%.

Toxicokinetics -

Methods -

- Plasma assayed for raloxifene and total raloxifene (i.e. raloxifene in plasma after hydrolysis with β-glucuronidase)
- Samples taken on: Day 90, Day 363, and Day 545
- Doses (mg/kg) based on average of daily food consumed during 1 week before sampling
- AUC determined by trapezoidal rule

Results: See Tables 3-4 (Attachment) -

- Plasma concentrations of both RAL and T.RAL in m and f dependent on time of day. Lowest values usually seen at 1400h, highest values at 2200h or 0600h. This is probably due to feeding pattern in rats (night-feeders).
- AUC values of RAL and T.RAL approximately linear and proportional with dose. However, in females, AUC (RAL) $_{(0\text{-}24h)}$ in MD-HD dose range less than proportional to dose.
- Ratio RAL:T.RAL

Day 90	males	females
Day 363	males	females
Day 545	males	females

Hematology

At 738 days (end of treatment):

Males: Minimal decreases in MCV, MCH, and minimal increase in erythrocyte count in all treated. Moderate non-dose-dependent decrease in leukocyte count in all treated. Moderate 40% decrease in lymphocyte count in LD, and 1.5-2x increase in MD, HD. Large non-dd 90% decrease in monocyte count in all treated. Slight 30% decrease in neutrophil count in HD. Small 13% increase in APTT in HD.

Females: Minimal decreases in MCV, MCH, and minimal increase in erythrocyte count, Hb concentration and PCV in all treated. Moderate 60% decrease in leukocyte count in LD only. Moderate 70% decrease in lymphocyte count in LD, and 1.7x increase in HD. No changes in monocyte count. Slight decrease in neutrophil count in LD, MD, and slight 30% increase in HD. Minimal increase in APTT and PT in all treated.

Clinical Chemistry -

At 738 days:

TABLE . Percentage change vs. controls of clinical chemistry parameters (statistically significant changes)

		glucose	total bilirubin	cholesterol	P	Na	total protein
males	LD	+50	-36	-48	+12	+1[ns]	-3[ns]
	MD	+34	-43	-51	+24	+2	-6[ns]
	HD	+56	-44	-56	+17	+3	-5[ns]

		triglycerides	total bilirubin	cholesterol	P	Na	ALT	AST	GGT	ALP
females	LD	-64	-68	-82	+14	+3	-7	-32	+75	+77
	MD	-57	+5	-70	+12	+3	+25	-3	+49	+120
	HD	-61	-49	-67	+15	+3	+43	+5	+62[ns]	+157
		total protein	albumin	globulin	A/G					
	LD	-11	-5	-17	+14					
	MD	-12	-7	-18	+13					
	HD	-12	-6	-19	+16					

ns = non-significant

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Organ Weights -

Relative-to-body-weight:

Kidney: Slight non-dd increase in LD, MD, HD m and minimal non-dd increase in all treated f

Liver: Slight ca. 20% decrease in LD, MD f

Heart: Slight non-dd increase in LD, MD, HD m, minimal increase in HD f

Spleen: Decrease of 60%, 50% in MD, HD m, but no statistically significant changes in f (large S.D. in all treated m and f)

Brain: Slight non-dd increase in all treated m and f

Testis: Slight increase of ca. 20% in HD m (S.D. ca. 30% in all dose groups)

Prostate: Non-dd decrease of ca. 25% in all treated m.

Ovary*: Increase of 1.2x, 1.1x, 3.0x (control value) in LD, MD, HD f (S.D. 20% in control, LD, MD, and S.D. 400% in HD due to 1 animal with granulosa cell tumor)

Adrenal: Non-dd ca. 20% increase in all treated m, non-dd ca. 40% increase in all treated f

Thyroid/parathyroid: Moderate 60% increase in HD m (due to 1 animal with C-cell adenoma), non-dd 30% decrease in LD, MD, HD f

Note: Changes are *tumor-related

Pathology (Final diagnoses) -

MALES

(A) Cause of death
Non-neoplastic:
Most frequent: Periarteritis 0-9-3-0, progressive glomerulonephrosis 15-49-25-6
Neoplastic:
Benign tumors (B)
Pituitary adenoma 17-5-2-4
Others 3-0-1-1
Total 20-5-3-5
Malignant tumors (M)
Lymphosarcoma 3-0-2-1
Mononuclear cell leukemia 17-3-5-5
Others (all apparently non-treatment-related) 7-3-4-2
Total 27-6-11-8

(B) Whole animal findings
Non-neoplastic:
Secondary renal hyperparathyroidism 3-39-19-3
Periarteritis 1-1-1-2
Neoplastic:
Lymphosarcoma (M) 6-1-3-4
Mononuclear cell leukemia (M) 24-3-5-8

(C) Organ findings
Kidney:
Cortical tubular mineralization, minimal/slight 0-5-8-4, marked 0-7-0-0, moderate 2-26-14-1. Total 2-38-22-5
Progressive glomerulonephrosis (PGN) minimal/slight 25-5-16-24, marked 9-5-10-6, moderate 23-4-11-19, severe 3-46-23-8. Total 60-60-60-57.
Renal cell carcinoma (M) 0-0-0-3
Liver:
Hepatocellular hyperplasia, focal 0-0-2-2
Biliary proliferation 3-0-0-0
Hepatocellular adenoma (B) 0-0-0-2
Hepatocellular carcinoma (M) 0-2-0-0
Heart:
Mineralization, minimal/slight 2-15-13-0, marked 0-5-2-0, moderate 0-15-1-1. Total 2-35-16-1
Atrial thrombosis 1-3-0-0 (4-6-1-3 p.96 ???)
Aorta: Mineralization 3-38-19-1
Vessel: Periarteritis, moderate 0-0-1-1, severe 0-5-1-0. Total 0-5-2-1
Lung: Hyperplasia, pneumocyte-tye II 1-3-2-0. Minimal/slight multifocal mineralization 1-8-0-0
Spleen: Increased hematopoiesis 15-18-14-3
Thymus: Epithelial hyperplasia 22-27-35-41. Involution 13-10-5-7
Pancreas:
Acinar atrophy, minimal/slight 23-19-18-20, moderate 1-1-4-2. Total 24-20-22-22.
Periarteritis, minimal/slight 1-15-10-11, marked 0-4-1-0, moderate 1-0-2-0, severe 0-2-

0-0. Total 2-21-13-11.
Islet cell adenoma (B) 4-0-1-0, islet cell carcinoma (M) 0-0-2-0
Stomach: Mucosal mineralization 3-37-20-6
Testis:
Atrophy, minimal/slight 18-38-46-43, marked 4-5-4-3, moderate 5-13-7-5, severe 3-3-3-0. Total 30-59-60-51
Interstitial cell hyperplasia 7-1-1-0
Interstitial cell tumor (B) 30-4-3-5
Prostate:
Inflammation: minimal/slight 33-36-42-40, moderate/severe 23-10-2-2. Total 56-46-44-42.
Skin: Fibroma (B) 3-0-1-0, keratoacanthoma (B) 2-1-0-0
Mammary gland: Fibroadenoma (B) 1-2-3-6
Bone: Hyperostosis 0-0-0-1. Fibrous osteodystrophy 2-40-21-6
Bone marrow: Hypercellularity 10-21-19-5
Adrenal gland:
Medullary hyperplasia 18-20-12-12
Adrenocortical adenocarcinoma (M) 0-0-0-1
Pheochromocytoma (M) 0-1-0-1
Thyroid:
C-cell carcinoma (M) 0-0-0-1
Follicular cell carcinoma (M) 0-2-1-0
Pituitary: Hyperplasia 9-12-16-12, adenoma (B) 36-8-14-14

FEMALES

(A) Cause of death

Non-neoplastic:
Most frequent: Renal necrosis 2-2-0-1
Neoplastic:
Benign tumors (B)
Pituitary adenoma 12-0-1-0
Others 0-0-0-1
Total 12-0-1-1
Malignant tumors (M)
Mononuclear cell leukemia 11-8-7-11
Others 2-0-2-0
Total 13-8-9-11

(B) Whole animal findings

Non-neoplastic:
Secondary renal hyperparathyroidism 1-0-0-0
Neoplastic:
Lymphosarcoma (M) 0-0-0-1
Mononuclear cell leukemia (M) 19-11-14-16

(C) Organ findings

Kidney: Cortical tubular mineralization, minimal/slight 13-59-57-54, total 13-59-60-57. Progressive glomerulonephrosis, minimal 6-39-37-36, slight 31-6-6-8, moderate 15-0-0-0, total 52-45-43-44. Cortical tubular necrosis, minimal/slight 1-1-0-0, marked 0-0-0-1, moderate 3-1-2-0, total 4-2-2-1.
Liver:
Hepatocellular atypia 9-9-2-3
Hepatocellular adenoma (B) 0-1-1-0
Hepatocellular carcinoma (M) 0-1-0-2
Thymus: Epithelial hyperplasia 20-35-40-39
Pancreas: Acinar atrophy, moderate 0-0-1-1.
Ovary:
Cyst 4-16-16-16
Follicular prominence 2-57-57-47
Granulosa cell tumor (B) 0-0-0-4
Granulosa-theca tumor (B) 0-0-1-3
Thecoma (B) 0-0-0-1
Granulosa-theca tumor, necrotizing (M) 0-1-0-0
Uterus:
Atrophy, diffuse 0-59-60-60
Endometrial stromal tumor (B) 5-0-1-0
Papillary adenoma (B) 1-0-0-0
Vagina: Mucification 44-20-19-13
Skin: Fibrosarcoma (M) 0-0-1-1, keratoacanthoma (B) 2-0-0-1
Mammary gland:
Hypoplasia 0-53-49-48
Fibroadenoma (B) 12-1-3-6
Adenocarcinoma (M) 3-0-0-0
Skeletal muscle: Myopathy, moderate 0-0-2-1
Bone: Hyperostosis 6-0-3-0. Sclerosis 0-0-2-0
Bone marrow: Hypercellularity 1-3-0-6
Adrenal gland:
Cortical hyperplasia 6-6-18-4
Medullary hyperplasia 2-2-1-2
Adrenocortical adenoma (B) 0-1-0-3
Pheochromocytoma (B) 1-7-3-5
Thyroid:
C-cell carcinoma (M) 2-0-0-0
Follicular cell adenoma (B) 0-1-0-0
Pituitary:
Cyst, moderate 0-1-1-1
Hyperplasia 7-7-10-4
Adenoma (B) 49-11-11-7

Main histopathological findings:

Incidence (%) of treatment-related changes in male rats, excluding tumors

MALES	Dose (mg/kg/d)	0	2.3	9.3	48
Kidney	Mineralization	3	63	37	8
	Progressive glomerulonephrosis (sev)	3	46	23	8
Prostate	Inflammation (moderate/severe)	38	17	3	3
Thymus	Epithelial hyperplasia	37	45	58	68

Incidence (%) of treatment-related changes in female rats, excluding tumors

FEMALES	Dose (mg/kg/d)	0	10.4	51	259
Kidney	Cortical tubule mineralization	22	98	100	95
	Progressive glomerulonephrosis minimal slight/moderate	 10 77	 65 10	 62 10	 60 13
Liver	Atypia	15	15	3	5
Thymus	Epithelial hyperplasia	33	58	67	65
Ovary	Cyst	7	27	27	27
	Follicular prominence	3	95	95	78
Uterus	Atrophy	0	97	100	100
Mammary gland	Hypoplasia	0	88	82	80

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Statistical analysis

Tumor incidence

Males

Site	Tumor	Incidence	Trend test one-sided p-value Grps 00, 02, 03 (Grp 01 excluded)
Kidney	Renal cell carcinoma (M)	0(-0)-0-3	0.077
Mammary gland	Fibroadenoma (B)	1(-2)-3-6	0.082

Females

Site	Tumor	Incidence	Trend test one-sided p-value
Liver	Hepatocellular carcinoma (M)	0-1-0-2	0.110
Adrenal	Adrenocortical adenoma	0-1-0-3	0.052
Ovary	Granulosa cell tumor (B)	0-0-0-4	0.004
	Granulosa-theca tumor (B)	0-0-1-3	0.002
	Granulosa-theca tmour (M)	0-1-0-0	no data
	Thecoma	0-0-0-1	no data
	Pooled benign granulosa/theca cell tumors	0-0-1-8	<0.001
	Pooled ovarian tumors	0-1-1-8	<0.001

Tumors with negative dose-related trend: (%)

Males

Testis	Interstitial cell tumor (B)	30	4	3	5
Pituitary	Adenoma	36	8	14	14

Females

Uterus	Endometrial stromal tumor	8	0	2	0
Mamm gland	Fibroadenoma	12	1	3	6
	Adenocarcinoma	3	0	0	0
Pituitary	Adenoma	49	11	11	7

SUMMARY AND EVALUATION

FINDINGS

1. Survival strongly reduced in LD males, and increased in HD males.
2. Body weight decreased dose-dependently (dd) in all male dose groups from start of treatment, and decreased non-dd in all female groups from ca. 12 months.
3. Cumulative EFU decreased dd in all male dose groups throughout treatment, and decreased non-dd in all female dose groups from ca. 13 months.
4. Leukocyte count decreased in all treated males and in LD females. Lymphocyte count decreased in LD m and f, and increased in MD m and HD m and f.
5. Total bilirubin decreased in males and females. ALP and GGT increased in HD f.
6. Serum total protein decreased in all female and male dose groups.
7. Relative weights: Decrease of spleen in MD, HD m. Decrease of prostate in all treated m. Increase of ovary in all treated f. Slight increase of adrenal, and decrease of thyroid/parathyroid in all treated f.
8. Pathology

   Main treatment-related pathology findings are shown in tabular form on pp.8-9

   Males:

   Whole body: Decreased incidence of fatal and non-fatal mononuclear cell leukemia in all dose groups. Increased incidence of secondary renal hyperparathyrodism with inverse dose relationship in LD, MD

   Kidney: Increased severity of glomerulonephrosis and associated increased incidence of mineralization in kidney cortex and other organs (heart, aorta, stomach) and of femoral osteodystrophy in LD and MD. Occurrence of renal cell carcinoma in HD.

   Liver: Slight incidence of adenoma and carcinoma in treated.

   Spleen: Reduced incidence of age-related increased hematopoiesis in HD.

   Bone marrow: Increased incidence of hypercellularity in LD, MD.

   Thymus: Dose-dependent increased incidence of epithelial hyperplasia. Incidence of involution decreased in MD, HD.

   Pancreas: Decreased incidence of pancreatic islet cell adenoma in all treated.

   Prostate: Decreased severity and incidence of inflammation at all doses.

   Testis: Largely decreased incidence of interstitial cell tumor in all dose groups

   Mammary gland: Increased incidence of fibroadenoma in all dose groups.

   Pituitary: Largely decreased incidence of adenoma in all dose groups.

   Females:

   Kidney: Decreased severity of glomerulonephrosis, and increased incidence of cortical tubule mineralization in all dose groups.

   Liver: Slight incidence of adenoma and carcinoma in treated.

   Bone marrow: Increased incidence of hypercellularity in HD.

   Thymus: Increased incidence of epithelial hyperplasia in all dose groups

   Ovary: Increased incidence of follicular prominence and of cysts in all dose groups. Occurrence of benign granulosa-theca cell tumor in MD and HD. Occurrence of malignant granulosa-theca cell tumor in LD.

   Uterus: Atrophy in all treated. Decreased incidence of endometrial stromal tumor in all dose groups

   Vagina: Decreased incidence of epithelial mucification in all treated.

Mammary gland: Large incidence of hypoplasia in all dose groups. Decreased incidence of fibroadenoma and adenocarcinoma in all dose groups.
Pituitary: Largely decreased incidence of adenoma in all dose groups.

COMMENTS

- Increased survival in HD males is probably due to decreased body weight.
- Decrease in body weight, mainly in males, reflects a central estrogenic effect of raloxifene on food and water intake, and metabolism. In the females the effect is delayed probably because of changing estradiol physiology in aging rat. The same body weight effect was seen in the male mouse oncogenicity study.
- The leukocyte and lymphocyte count reduction in both sexes might reflect an estrogenic effect of raloxifene. However, the lymphocyte count increase and hypercellularity of the bone marrow points to an anti-estrogenic effect. Effects on hematopoietic system are therefore complex. The repression of splenic hematopoiesis and decreased spleen weight in males is probably an anti-estrogenic effect: The aging F344 rat has increased estradiol levels (Clegg et al, 1997), which causes various effects, including splenic hematopoiesis, in the controls.
- ALP and GGT increase in females could reflect estrogen-like liver toxicity of raloxifene, as seen in the mouse. Total serum protein and bilirubin decreases could be due to hepatic toxicity or renal pathology.
- The increased severity of kidney glomerulonephrosis in males is associated with increased incidence of renal and other ectopic mineralizations (secondary renal hyperparathyroidism). The effect is most pronounced in the LD group, and causes high mortality in this group. In females, however, the severity of glomerulonephrosis is reduced. Nevertheless, as in males, there is an increased incidence of mineralization in the kidney itself, while, unlike in males, mineralization in other organs is not observed. The reason for this difference between sexes is unclear.
- The reduced PGN in males at higher doses of raloxifene as compared to low doses may be a secondary effect of the decreased food and protein intake in the higher dose groups. PGN is known to be exacerbated by high protein intake.
- Renal cell carcinoma is a rare tumor. In an 18-month female rat study with tamoxifen renal cell carcinoma was also observed. However, the tumor was only seen in animals treated after a single dose of the carcinogen NDEA (IARC, Vol. 66, 1996)
- Thyroid/parathyroid weight reduction may be an anti-estrogenic effect, since estrogens can cause thyroid hypertrophy (Hart, 1990)

- Reduction of leukemia incidence in male has unknown cause, and may be related to decrease in body weight.

- Mammary gland tumor incidence increase in males, and decrease in females may be the result of estrogenic and/or anti-estrogenic effects of raloxifene on pituitary and/or mammary gland itself, altering endocrine status of animal.

- Thymus hyperplasia reflects an action of raloxifene through ER in thymus gland.

- Testis interstitial tumor incidence reduction reflects an anti-estrogenic action of raloxifene in the aging rat, where increased estradiol causes the occurrence of these tumors.

- The ovarian follicle prominence points to an anti-estrogenic raloxifene action, while the cysts are more consistent with an estrogenic action.

- The ovarian tumors seen in MD and HD treated animals (granulosa-theca cell tumor, thecoma) are the kind of ovarian tumors that are usually seen in the rat. The same tumor species were seen in the treated mice, however at much lower drug exposure values, especially of the parent compound (TABLE 6, Appendix). In the rat, granulosa-theca cell tumors can be induced by transplantation of the ovary to e.g. the liver of kidney. It is thought that these tumors are caused indirectly, due to estrogen level decline, by increased gonadotrophin production by the pituitary gland. Raloxifene may act similarly through a central anti-estrogenic effect, as discussed for the mouse.

- Uterine atrophy and decreased endometrial tumor incidence, and suppression of vaginal mucification reflect anti-estrogenic actions of raloxifene.

- The pituitary tumor incidence decrease is likely due to an anti-estrogenic effect of raloxifene. The pituitary tumors are probably induced by prolonged endogenous estrogen stimulation.

- Comparison of the effects of raloxifene to those of tamoxifen in the rat (TABLE 5, Appendix) yields the following: Tamoxifen induces liver tumors, raloxifene dose not. Both agents cause pituitary tumor suppression, and both can cause renal cell carcinoma, although tamoxifen only after NDEA treatment. In the mammary gland, the anti-tumor effect of tamoxifen and raloxifene is the same in female rats.Ovarian tumors were not seen with the applied doses of tamoxifen. However, premature cessation of the tamoxifene MD and HD groups may account for this negative finding.

- Comparison of the tumorigenic effects of raloxifene between rats and mice shows: Ovarian tumors are induced in both species. Testicular tumors are induced in mouse, but suppressed in the aging rat. In the rat, but not in the mouse, the incidences of pituitary tumors, mammary gland tumors in the females, and uterine tumors are all reduced in the treated dose groups. Prostate tumors occur in treated mice, not rats.

- Exposure to unconjugated raloxifene is comparable in male rats and mice, but much higher (> 10x) in female rats than in female mice (TABLE 6, Appendix). Exposure to all raloxifene conjugates together in male rats is ca. ½ of that in male mice, and ca. 4x higher in female rats than mice.

REFERENCES

Clegg, E.D. et al (1997) Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reproductive Toxicology 11, 107

Hart, J.E. (1990) Endocrine pathology of estrogens: species differences. Pharmac.Ther. 47, 203

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APPENDIX

TABLE 1. Test article dosage

	Oncogenic studies	
Study #	R00594	R00694
Sex	males	females
Dietary concentration (%)	Average daily dose of raloxifene (mg/kg)	
0.005	2.3 (2.5)*	-
0.02	9.3 (10)	10.4 (11.2)
0.1	48 (52)	51 (55)
0.5	-	259 (279)

*(in parentheses) raloxifene hydrochloride doses

TABLE 2. Number of animals

Study Nrs. R00594/00694	# At start of study	Survival at 18 mo		Survival at 21 mo		Survival at treatment termination	
Dose group		male	female	male	female	male	female
Control	60						
LD	60						
MD	60						
HD	60						

TABLE 3. Day 363 (samples at 0, 8, 16, 24 h after 0600h) plasma concentrations in F344 rats *

	Dose (mg/kg)	RAL (ng/ml)				T.RAL (ng/ml)				AUC_{0-24h} (RAL) (ngxh/ml)	AUC_{0-24h} (T.RAL) (ngxh/ml)	AUC ratio RAL: T.RAL (%)
h after 0600h		0	8	16	24	0	8	16	24			
male	2.1[&]									0	410	0
	8.3									223	1262	18
	43									1481	9282	16
female	9.2									618	2562	24
	45									3289	12487	26
	227									12085	48417	25

* Values on Day 90 and Day 545 not mentioned in review

[&] Values at this dose are relatively unreliable because many samples were BLQ

Data from previous submission (Serial Nr. 041) :

Table 1. Plasma concentrations after 3 months (Day 91) in F344 rats

	Plasma concentration (ng/ml) In parentheses:(AUC in ng x h/ml)			
	Males		Females	
Group	Raloxifene	Total Raloxifene	Raloxifene	Total Raloxifene
LD	1 (30)	1 (30)	19 (433)	65 (1470)
MD	9 (190)	27 (627)	101 (2314)	287 (6145)
HD	48 (1179)	124 (3001)	383 (9171)	1115 (26095)

Ratio (raloxifene:total raloxifene) in males 30-40%

TABLE 4A. $AUC_{(0-24h)}$ values for Raloxifene and Total RALoxifene on Day 90, 363, 545 in F344 rats

		AUC (0-24h) RAL (ng x h/ml)			AUC (0-24h) Total RAL (ng x h/ml)		
	Dose (mg/kg/d) (Day 90- 363- 545)	Day 90	Day 363	Day 545	Day 90	Day 363	Day 545
Males	2.6- 2.1- 2.2	27			30		
	10- 8.3- 8.7	190			627		
	54- 43- 44	1179			3001		
Females	13- 9.2- 9.0	433			1470		
	65- 45- 44	2314			6145		
	317- 227-229	9171			26095		

TABLE 4B. Multiples of human exposure in rat oncogenicity study

	Dose	$AUC_{(RAL)}$	$AUC_{(T.RAL)}$	Human $AUC_{(RAL)}$ multiple	Human $AUC_{(T.RAL)}$ multiple
Males	LD	33	269	**1.4x**	**0.08x**
	MD	209	1147	**9x**	**0.32x**
	HD	1242	6013	**52x**	**1.7x**
Females	LD	485	2957	**20x**	**0.8x**
	MD	2344	12719	**98x**	**3.6x**
	HD	10154	58805	**423x**	**17x**
Human	1 mg/kg/day	24	3500		

TABLE 5. Incidence (%) of tumors in AP Wistar rats, treated with tamoxifen*

	Dose (mg/kg/day)	0	5	20	35
Females	Survival (%)	-	↑	↓	↓
	Mammary adenocarcinoma	9	0	0	0
	Pituitary adenoma	73	0	0	0
	Liver adenoma carcinoma	1 0	4 12	12 71	17 71
Males	Survival (%)	-	↑	↓	↓
	Pituitary adenoma	14	2	0	0
	Parathyroid adenoma	10	0	0	0
	Liver adenoma carcinoma	1 1	16 16	22 67	16 67

*Results from oncogenic study in rats (AP Wistar rats, 51 or 52/sex/dose group, gastric installation, 2 years; termination of MD at 87 wks, of HD at 71 wks) (Greaves et al, 1993)

TABLE 6. Comparison of raloxifene exposure in rat and mouse oncogenicity studies.

	Dose group	$AUC_{(RAL)}$	$AUC_{(T.RAL)}$	Multiple of human AUC

		males	females	males	females	males	females
RAT	LD					1.4x	20x
	MD					9x	98x
	HD					52x	423x
MOUSE	LD					0.75x	0.3x
	MD					4.7x	7x
	HD					24x	34x

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ATTACHMENT

Meeting Minutes Executive CAC

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Executive CAC
Sept. 30, 1997

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
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Author of Draft: Wendelyn Schmidt, edited by Gemma Kuijpers and Ron Steigerwalt

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 20-815
Raloxifene hydrochloride (Evista)
Eli Lilly and Co.

Mouse and Rat Carcinogenicity Studies:

This was the second presentation to the Exec CAC on the carcinogenicity studies for this compound. The committee had previously concurred that the study doses were adequate. A statistically significant incidence of tumors was found in the ovary (rat and mouse), testis and prostate (mouse) and kidney (male rat). Exposure levels at which ovarian tumors were noted in the mouse were similar to those in humans at the intended therapeutic dose.

The Committee previously suggested conducting additional genotoxicity tests, such as DNA adduct formation assays, similar to the ones used in investigating the animal carcinogenicity of the related SERM, tamoxifen. Raloxifene does not share the same chemistry as tamoxifen that appears to be involved in DNA adducting, and a report of a DNA adduct assay which the sponsor concluded to be negative was submitted to the NDA. However, for this DNA adducting study, there are questions regarding the adequacy of the study, the target tissue examined, the results and the interpretation of the assay suggesting that as conducted, it may have provided little useful information. It is not known whether raloxifene is genotoxic in the MCL-5 assay, which has been shown to be positive for tamoxifen.

A possible hormonal mechanism underlying the reproductive organ tumor formation is stimulation due to an increased release of the pituitary gonadotropins, LH and/or FSH, and Sponsor has put this mechanism forward as an explanation of the rat and mouse tumor findings. In a recent 1-month mouse study, using approximately the same LD and HD of raloxifene as in the carcinogenicity study, increases in serum LH of 2x-5x were noted (telecommunication with Sponsor). This study was not available for review by the committee. In an 8-week clinical study in postmenopausal women, raloxifene caused a minimal increase in serum LH levels of no more than 10%. If a hormonal mechanism is responsible for the tumor response in intact animals, it might not be relevant for the clinical situation.

Executive CAC Recommendations and Conclusions:

1. The Exec CAC felt that, considering the relative exposure in mice as compared to humans, the extent and multiple locations of positive tumor findings and the prevention indication, additional information is important to assess the relevance of the tumors seen in the mouse and rat with raloxifene. The committee believes that the sponsor has not fully evaluated genotoxic mechanisms as recommended in ICH guidances on genotoxicity testing and testing for carcinogenic potential. In these guidelines, it states that additional genetic toxicology studies may be required when there are positive findings in a carcinogenicity study. A strong recommendation was made for the performance of additional appropriately conducted tests that have been found useful for other products in this pharmacologic class (eg. an adequate DNA ^{32}P post-labeling study with appropriate controls and the MCL-5 assay).
2. It was recommended by the committee that the division consult genotoxicity experts at the NCTR (Dan Sheehan, Dan Casciano) regarding possible alternative genetic toxicity testing.

also given in Table 1. In the mouse, a significant positive trend in tumor incidence was seen in testis (interstitial cell tumor), prostate (leiomyoblastoma), and ovary (granulosa-theca-luteoma). Tumors in the prostate (adenoma and adenocarcinoma) and tumors in the liver of female mice (adenoma and adenocarcinoma) were increased but not with statistically significantly positive trend. The trend was not assessed for pooled adenoma and adenocarcinoma) but is likely to be significant based on the numbers observed. Epithelial ovarian tumors (papillary adenoma) were increased in all dose groups in a non-dose-related manner: incidence was decreased in HD as compared to LD and MD. There is no obvious explanation for this. Pairwise statistical comparison would be appropriate for this type of tumor.

General discussion

1. Pooled liver adenoma/carcinoma data need to be assessed statistically.
2. Since there are no appreciable amounts of active metabolites, but only glucuronide and sulfate conjugates, AUC multiples of parent drug are adequate for comparison with humans if the degree of protein binding is comparable across species. The comparative protein binding needs to be documented
3. A discussion on the relevance and nature of additional genotoxicity tests, particularly DNA adduct assays, was held. The issue was brought up because tamoxifen, another SERM although of unrelated chemical structure, caused the same reproductive tumors as raloxifene in mice. Tamoxifen also causes liver tumors in rats and formation of DNA adducts in rat liver cells. In addition, tamoxifen has been evaluated by IARC to be a carcinogen in humans and its use is associated with increased observation of endometrial cancer. A DNA adduct assay was done by Sponsor with raloxifene, but test results were inconclusive. The Committee felt that, considering the prevention indication, it would be valuable to get results from tests that were positive for tamoxifen, and, if done, that the labeling should make mention of the results of the test for raloxifene.

Executive CAC Recommendations and Conclusions:

The dosing was considered adequate. The following parameters should be re-examined: AUC, considering protein binding effects, genotoxicity, particularly in comparison with assays tamoxifen has been observed to be positive in, and statistical analysis with appropriate pooling of types of tumors (e.g. liver adenomas/adenocarcinomas).

9/30/97

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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/Division File, HFD-510, NDA 20-815
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Executive CAC
August 19, 1997

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Author of Draft: Wendelyn Schmidt, edited by Gemma Kuijpers

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 20,815
Raloxifene HCl, (Evista™)
Eli Lilly & Co.

Rodent Carcinogenicity Studies:

Raloxifene is a selective estrogen receptor modulator (SERM) for use in the prevention of osteoporosis in post-menopausal women. Raloxifene was negative in the mutagenic/clastogenic assays employed.

Rat carcinogenicity study

Survival was significantly decreased in the rat LD males (Table 1). Body weight was decreased in all rat male and female dose groups. AUC multiples of expected human exposure are also given in Table 1.

TABLE 1. Results on survival and body weight, and AUC multiples

	RAT		MOUSE	
	male	female	male	female
Survival (control-LD-MD-HD) (n)	22-6-26-38	39-48-47-47	48-50-48-41	47-46-48-48
Body weight (LD-MD-HD) (%) mouse @ 12 mo	-17, -18, -17%	-16, -12, -15%	-9, -11, -10%	-2, -1, +9%
human AUC multiple (LD-MD-HD)	1.4-9-50x	20-100-425x	0.75-5-25x	0.3-7-35x

In the rat, a significant positive trend in tumor incidence was seen in male kidney (renal cell tumor) and female ovary (granulosa and granulosa-theca cell tumor). Tumors with clearly negative trend (testis, male and female pituitary, female mammary gland) were noted. Some of these tumors appeared to have a lower incidence in LD and/or MD groups than in HD groups, e.g. male pituitary adenoma and female mammary fibroadenoma. This was probably not due to body weight, since body weight was decreased similarly at all doses.

Mouse carcinogenicity study:

Survival was decreased in the mouse HD males (Table 1). Body weight was decreased in all mouse male dose groups, and increased in HD females. AUC multiples of expected human exposure are

10/30/97

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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