

**REVIEW OF REPORT #J-146363:**  
**52-WEEK I.V. TOXICITY STUDY OF Ro21-5535 IN BEAGLE DOGS**  
**WITH INTERMITTENT ADMINISTRATION**

**NOTE:** Study performed by Dept. of Toxicology and Pathology Nippon Roche Research Center. Kamakura JAPAN. Study dates 1/91-6/92. Study dated 2/2/95. Signed QA and GLP (Japanese) statements provided. Lot # G006091

**PURPOSE:** To examine the toxicity of rocaltrol administered i.v. to dogs for 52 weeks on an intermittent schedule.

**EXPERIMENTAL DESIGN:** 5/sex/group 6 month-old dogs were administered 0, 0.01, 0.02 and 0.04/0.06 µg/kg/day for 52 weeks 3 times/week (Monday, Wednesday and Friday). Injection volume: 0.1 ml/kg. 1 animal/group was maintained for a further 4 week recovery period. Note: The original high dose selected was 0.04 µg/kg/day. During an interim sampling on day 91, it was determined that the calcium levels and expected findings on bodyweight were not markedly changed. Therefore, the dose was increased to 0.06 µg/kg for the remainder of the study.

**FORMULATION:** A formulation for parenteral administration was used. Composition was identical to the formulation used in the 13-week study.

**RESULTS**

**OBSERVED EFFECTS:** Erythema in ears, limbs around rim of eyes and/or muzzle accompanied with edema around the rim of eyes was sporadically observed in animals within a minute after dosing and subsided within 1 h. This suggested a "histamine-like" response. The sponsor attributed this to the presence of Tween-20 or other agent in the vehicle, but this was not documented (no individual data for observed effects was provided).

Severe or medium grade emaciation observed in the high dose group.

No irritation at injection site.

**MORTALITY:** None.

**BODY WEIGHT:** High dose animals began to lose weight when 0.06 µg/kg dose was initiated. During recovery, a rapid increase in body weight was apparent. Weights were nearly completely restored to day 91 levels by the end of the 4 week recovery period for males. Data in females is unclear because it appeared that the recovery animals were not on the same growth curves as the other group. Therefore, the growth curve of recovery females was disjointed from the animals sacrificed in week 52. Weight gain in the recovery females was apparent, however.

**FOOD CONSUMPTION:** Severe and progressive anorexia was noted in the high dose group after the 0.06 µg/kg dose was initiated. Immediate increase in food consumption occurred during recovery and accompanied by body weight regain.

**VITAL SIGNS:** No treatment-related changes in ECG or neurological examinations.

OPHTHALMIC EXAMINATION: No treatment-related changes.

HEMATOLOGY: No treatment-related changes were noted.

COAGULATION: No treatment-related changes.

BONE MARROW: No data.

BLOOD CHEMISTRY: Increase in BUN (3-4X controls), Ca, inorganic phosphorus (for phosphorus, this is compared to controls. When compared to prestudy, there was actually a decrease in IP). Decreased Mg and Cl. All in the high dose group after the increase to 0.06 µg/kg.

URINALYSIS: Slight decrease of specific gravity in the 0.06 µg/kg group.

ORGAN WEIGHTS: Slight increase of absolute and relative lung and kidney weights were observed in high dose animals. These were similar to controls after the recovery period for the male but not for the female.

GROSS PATHOLOGY: Severe emaciation. Fading or whitish change and rough surface of kidneys and "whitish points of the spleen" were noted in high dose animals of both sexes. There was also a whitish change in heart and lungs noted as well as atrophy of the thymus in HD animals.

HISTOPATHOLOGY:

HEART: Calcification in endocardium, myocardium, coronary arterial wall, aortic wall and pulmonary arterial wall of high dose dogs. Thickening of endocardium and tunica intima of the coronary artery and vasculitis of the coronary were observed and suggested as secondary to previously mentioned calcifications. Calcification was still noted in the coronary arterial wall and aortic wall in a high dose female after the recovery period.

KIDNEYS: In the high dose group: calcification in both the cortex and medulla mainly in the proximal tubules and Bowman's capsules and epithelium of proximal tubules in the high dose group. Chronic nephropathy-like changes including basophilic change of proximal tubules, dilatation of distal tubules, fibrosis in the interstitium, small round cell infiltration in interstitium and degeneration of glomeruli. These did not improve during the recovery period.

LUNG AND TRACHEA: Calcification in the alveolar wall, bronchial cartilage and bronchial/bronchiolar epithelium of the lung and the epithelium and cartilage of the trachea in the high dose group. Little improvement after recovery period.

SALIVARY GLANDS: Calcification in submandibular glands in all groups which remained in the high dose animals after the recovery period.

THYROID AND PARATHYROID: Hyperplasia of parafollicular cells in high dose group. Atrophy of parathyroid cells and cystic change of parathyroid at high dose. Kursteiner's cyst of parathyroid was observed in the mid dose male and high dose male and female.

BONE: Proliferation of bone, osteoid and fibrous tissue in marrow and an increase in osteoclasts and proliferation of hematopoietic cells all in the high dose group. Little change at recovery.

THYMUS: Atrophy in high dose group. Increase of lymphocytes in cortex and medulla in the high dose group noted at the end of the recovery.

STOMACH: Calcification in the glandular stomach mucosa and muscular layer in the high dose animals. Fibrosis in the glandular stomach mucosa was also noted. No improvement after recovery period.

LIVER: Brown pigment deposits in Kupffer cells in the high dose group with no improvement after recovery period.

SPLEEN: Brown pigment deposits in red pulp and enlargement of splenic nodules in the high dose group. This did not improve after recovery period for the high dose group. Brown pigment deposits were noted after recovery in the 0.02  $\mu\text{g}/\text{kg}$  group.

NOTE: Estimated to be \_\_\_\_\_ . The NOTE represents an equivalent exposure to the maximum proposed human dose based on surface area comparisons.

### COMBINED SUMMARY OF DOG TOXICOLOGY

1. Toxic effects noted in both the 13- and 52-week I.V. toxicity studies in dogs were related primarily to the expected physiological effects of the agent. These included: increase in serum calcium and calcification of many tissues (heart, kidney, lung, salivary glands) and ossification of bone.
2. Mortality was observed in the 0.1  $\mu\text{g}/\text{kg}/\text{day}$  group in the 13-week study between days 50-60. Treatment was discontinued in this group on day 56 due to excessive toxicity.
3. Treatment at 0.05 and 0.1  $\mu\text{g}/\text{kg}$  in the 13-week study and at 0.06  $\mu\text{g}/\text{kg}$  (but not 0.4  $\mu\text{g}/\text{kg}$ ) in the 52-week study resulted in severe anorexia and loss of body weight. When treatment was discontinued, appetite was regained immediately and body weight was restored to near prestudy levels by 4 weeks of recovery. There was a tendency for 0.025  $\mu\text{g}/\text{kg}/\text{day}$  (low dose) females in the 13-week study to lose weight as well.
4. "Histamine-like" effect was noted in both tox studies which lasted ~1 h (Erythema in ears, limbs, rim of eyes and/or muzzle). This was attributed to an agent in the vehicle, but specific data were not provided to support this.
5. Consistent decrease in Mg, Cl and increase in BUN, Ca and inorganic phosphate were noted in both toxicology studies (0.05 and 0.1  $\mu\text{g}/\text{kg}/\text{day}$  in the 13-week study and 0.06  $\mu\text{g}/\text{kg}/\text{day}$  in the 52-week study). Slight increase in Ca at 0.025  $\mu\text{g}/\text{kg}$  in the 13 week study was also noted. There was an increase in total cholesterol in the 13 week study consistent with the findings in the rat studies.
6. Increased Hb, Hct, RBC, platelets and monocytes were noted at 0.05  $\mu\text{g}/\text{kg}$  and 0.1  $\mu\text{g}/\text{kg}$  in the 13-week study but not at 0.06  $\mu\text{g}/\text{kg}$  in the 52-week study.
7. Slight decrease of specific gravity of urine was noted in both studies.
8. Absolute and relative lung and kidney weights were increased at 0.06  $\mu\text{g}/\text{kg}$  in the 52-week study. There were no clear changes in organ weights in the 13-week study which could not be attributed to the weight loss observed. However, changes in the relative weights of thymus (decrease), lung and liver (both increased) in the high dose group of the 13 week study are consistent with changes observed in the 52-week study.

9. Histopathology: The following were noted in both studies: Increase in proliferation of bone and osteoclasts, atrophy of thymus, and brown pigment deposits in Kupffer cells in the liver. Chronic nephropathy-like changes were noted at 0.06  $\mu\text{g}/\text{kg}$  in the 52-week study and in all treated groups of the 13-week study. A whitish or fading change of the kidney was noted in the high dose of the 52 week study. Hyperplasia of the parafollicular cells of the parathyroid were noted in the 0.05 and 0.1  $\mu\text{g}/\text{kg}$  in the 13-week study and the 0.06  $\mu\text{g}/\text{kg}$  group in the 52-week study. Atrophy of the parathyroid cells were noted in the 0.01, 0.05 (13-week) and 0.06 (52-week)  $\mu\text{g}/\text{kg}$  groups. All of these changes were slow to recover and many were not improved after 4-5 weeks of recovery.
10. Strictly speaking, a NOEL was not established for the 6 month dog study. However, since the only treatment-related effect noted in the low dose group was increased serum Ca, and this is an expected effect of the test agent, a no adverse effect level could be assumed to be 0.025  $\mu\text{g}/\text{kg}/\text{day}$ . In the 52-week study, no adverse events were noted at 0.04  $\mu\text{g}/\text{kg}/\text{day}$ , but this treatment only lasted for 91 days. No effects were noted at 0.02  $\mu\text{g}/\text{kg}$  which was administered for the entire 52 weeks. In relationship to the doses recommended in the NDA and currently being used in clinical trials (0.5  $\mu\text{g}$  or 0.01  $\mu\text{g}/\text{kg}$ ) this provides a safety margin of only 1-2-fold on a  $\text{mg}/\text{m}^2$  basis.

**REVIEW OF STUDY #GCR J-146116:**  
**REPRODUCTION SEGMENT II STUDY OF ROCALTROL (INJECTABLE) IN RATS**

**NOTE:** The following study was performed at Nippon Roche Research Center, Department of Toxicology and Pathology, JAPAN. Study dates 4/92-1/93. Study report dated 4/19/93. Signed QA and GLP statements provided. Lot# G006191.

**PURPOSE:** Reproduction segment II: developmental toxicology evaluation of injectable Rocaltrol.

**EXPERIMENTAL DESIGN:**

Animals: Male and female \_\_\_\_\_ strain rat "specific pathogen-free" rats were used from \_\_\_\_\_  
Food and water were available *ad libitum*. F<sub>0</sub> generation females with successful copulation were randomized by a computerized program. N=36 for the control group. N=38 for drug-treated groups. Approximately 20 females were sacrificed on day 21 of gestation for teratological examinations (Caesarean Group), and the remaining females were allowed to litter and rear the F<sub>1</sub> pups until weaning (Natural Delivery Group). Pups were examined for external deformities at birth. Litters were culled to 8/litter (4/sex, if possible) on day 4 after delivery. Pups were scored for developmental events such as auricular opening, incisor eruption, eye opening etc. Upon weaning, two male and two female F<sub>1</sub> pups were selected from each litter for further study of learning and mating behavior. The remaining pups were examined for pathological and histological properties.

Drug administration: Based on Reproduction segment I studies, the following doses were chosen: 0.05  $\mu\text{g}/\text{kg}/\text{day}$  (no effect level), 0.15  $\mu\text{g}/\text{kg}/\text{day}$  (middose level), and 0.45  $\mu\text{g}/\text{kg}/\text{day}$  (definitive toxic level). Test and control articles were administered to females with successful copulation day 7 to 21 of the gestation period. Dosing volumes were determined based on body

weight on day 6 or 7 of gestation. Intravenous administration was chosen to correspond to proposed clinical use.

Mating studies: F<sub>1</sub> generation: After 77 days growth, one male was mated with one non-sibling female of the same dose group (first mating). Mating success was checked daily by vaginal smear. A male without successful copulation during a 14 day period was allowed to mate with a nontreated female for a second period of 14 days (2<sup>nd</sup> mating). A female without successful copulation during the initial 14 day period was allowed to mate with a proven fertile male to confirm fertility (3<sup>rd</sup> mating).

## RESULTS

### CAESAREAN GROUP:

Females were sacrificed on gestation day 21. 22 females were selected for the control group and 24 females were selected for each of the drug-treated groups. One control female delivered early and was eliminated from the study and one female in the 0.15 and 0.45 µg/kg dose groups had no implants. Total number of females for each group was therefore 21, 24, 23 and 23 for 0, 0.05, 0.15, and 0.45 µg/kg/day groups, respectively. As shown by the following table, there were no significant drug-related effects on number of corpora lutea, implantations, live fetuses, or fetal deaths. (Data are means calculated/litter).

DOSE (µg/kg/day) -	0 n=21	0.05 n=24	0.15 n=23	0.45 n=23
CORPORA LUTEA	14.9	15.0	15.0	15.1
IMPLANTATION	13.9	14.1	13.5	13.4
ALIVE FETUSES/LITTER	13.3	13.0	12.9	12.8
EARLY FETAL DEATH <sup>1</sup> (% to implantations)	4.2 ±7.6	7.8 ±8.0	6.3 ±9.7	4.3 ±6.4
LATE FETAL DEATH <sup>2</sup> (% to implantations)	0.3	0	0	0
ALIVE FETUS BODY WEIGHT (MALE)	5.3	5.4	5.3	5.0
ALIVE FETUS BODY WEIGHT (FEMALE)	5.0	5.2	5.1	4.8

<sup>1</sup> Measured as implantation site or placenta remnant

<sup>2</sup> Measured as macerated fetus, dead fetus

Some dams exhibited alopecia and had slightly decreased body weights in the 0.15 and 0.45 µg/kg/day groups. No statistically significant increased occurrence of dose-related external, visceral or skeletal anomalies were observed in pups of any group. The high dose pups (males and females), however, did have a slight statistically significant decrease in birth weight ( $p < 0.05$ ).

### NATURAL DELIVERY GROUP:

Two dams in control group and one in the 0.15 µg/kg/day group were not pregnant. N= 12, 14, 13, and 14 for the 0, 0.05, 0.15, and 0.45 µg/kg/day groups.

Clinical Signs: No mortality occurred during study. A few dams in the 0.15 and 0.45 µg/kg/day group showed alopecia. This occurred sporadically in the toxicology studies reviewed previously. No gross necropsy findings were detected in dams on the day of weaning.

Body weight: Dose-dependent decrease in body weight gain and food consumption was observed at 0.15 and 0.45 µg/kg/day groups. Dams recovered after treatment cessation during lactation. Food consumption of 0.15 and 0.45 µg/kg/day groups increased to control levels between day 17-19 of gestation.

Effects of treatment on gestation and pups: No drug-related changes were observed in implantations, live births, dead births, viability index (lactation day 0 to day 4) or lactation index (lactation day 4 to day 21).

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ON ORIGINAL

DOSE(µg/kg/day) -	0 n=12	0.05 n=14	0.15 n=13	0.45 n=14
IMPLANTATION	14.5	13.6	13.1	13.1
LIVE NEONATES	13.4	12.3	11.8	11.7
DEAD NEONATES	1.6	1.0	0.0	1.0
VIABILITY INDEX	99.5	99.4	99.5	93.7
LACTATION INDEX	100	100	100	98.6

Effects during lactation (post-treatment period):

**CLINICAL:** No drug-related effects were detected as external or internal anomalies, clinical signs or body weight development. No significant increase in skeletal anomalies although there was a statically significant increase in rudimentary cervical ribs in the 0.05 and 0.15 µg/kg groups which was not found in the 0.45 µg/kg group. Mean%±SD for 0, 0.5, 0.15 and 0.45 µg/kg were 0.4±2.0, 4.2±7.3, 5.9±11.3 and 2.5±5.0, respectively.

**DEVELOPMENTAL:** No changes were detected in auricular detachment, eruption of incisors or eyelid opening. No changes were observed in faculty test of sensation (sight, hearing and equilibrium) on lactation day 21. No drug-related changes were observed in skeletal examination by x-ray visceral examination and ossification (number of vertebrae).

Observations of F<sub>1</sub> rats from weaning to 11 weeks old:

No drug-related changes in clinical signs, weight gain or food consumption.

No drug-related changes in testis descent or vaginal opening.

No drug-related changes in open field test and learning tests.

APPEARS THIS WAY  
ON ORIGINAL

Reproductive performance:

DOSE $\mu\text{g}/\text{kg}/\text{day}$	0		0.05		0.15		0.45	
	MALE n=12	FEMALE n=12	MALE n=14	FEMALE n=14	MALE n=13	FEMALE n=12	MALE n=13	FEMALE n=12
MATING ABILITY	100%	100%	100%	100%	100%	100%	100%	100%
F <sub>2</sub> BODY WEIGHTS	5.1	4.8	5.2	5.0	5.4	5.2	5.2	4.9
FERTILITY RATE (male)	100%		93%		100%		100%	
PREGNANCY RATE (female)	67%		93%		92%		92%	
IMPLANTATION OF F <sub>2</sub> (MEAN)	13.5		15.1		13.5		14.2	
LIVING NEONATES	12.9		13.9		12.4		13.3	
CORPORA LUTEA	15.3		16.2		15.0		15.5	

No anomalies were found in the F<sub>2</sub> generation. F<sub>1</sub> rats that did not show reproductive capability were examined by necropsy. No gross pathology was found that related to reproductive performance. No gross necropsy findings were detected in the F<sub>1</sub> generation.

**SUMMARY**

Reproduction segment II study of Rocaltrol was performed intravenously in rats at dose levels of 0.05, 0.15, and 0.45  $\mu\text{g}/\text{kg}/\text{day}$  (approximately 0.75, 2.25, 6.75 times the maximum proposed human dose based on surface area). Dams in the 0.15 and 0.45  $\mu\text{g}/\text{kg}/\text{day}$  levels showed some toxicological changes such as slight inhibition of body weight gain and food consumption as well as some alopecia. This trend reversed after treatment was stopped during lactation. F<sub>1</sub> fetuses showed slight decrease of body weight in the 0.45  $\mu\text{g}/\text{kg}/\text{day}$  group, but showed no drug-related teratological or reprotoxicological effects in any group. The non-effective dose was estimated to be 0.05  $\mu\text{g}/\text{kg}/\text{day}$  in dams, and 0.15  $\mu\text{g}/\text{kg}/\text{day}$  in F<sub>1</sub> fetuses which represent multiples of the maximum proposed human dose of approximately 0.75 and 2.25, for dams and fetuses, respectively, based on body surface area comparisons.

**REVIEW OF REPORT #N-161895:**  
**MICRONUCLEUS TEST IN MICE TREATED WITH Ro21-5535**

**Note:** Study dates: 12/94-2/95. Performed at F.Hoffmann-La Roche Ltd, Basel, Switzerland. GLP statement provided. Lot #2010025

**Purpose:** *In Vivo* assessment of clastogenic potential (including chromosomal breakage and/or spindle damage) of Ro21-553 using a mouse micronucleus assay.

**Experimental Design:** Calcitriol was administered to male and female Füllinsdorf Moro Albino mice orally at single doses of 1, 2, and 4 mg/kg. Bone marrow smears from 24 and 48 h were evaluated for the high dose group. All groups were evaluated at 24 h. 5/sex/treatment group were evaluated. 500-1000 PCE were evaluated per group. A preliminary study determined that 1/2 from a 3 mg/kg and 2/4 from a 4 mg/kg group died at the third day after administration. It was determined that 4 mg/kg would be a feasible dose since animals were killed after 48h. Additional animals were included in this group to make up for any lost.

**Vehicle control:** (per ml): 5 mg Carboxymethylcellulose, 4 µl tween-80, 5µl benzylalcohol, 9µg NaCl. (agent dissolved in EtOH prior to addition to vehicle: 1% final EtOH concentration maximum.)

**Positive control:** Procarbazine hydrochloride. (6 mice were treated; 5 were evaluated at 24 h)

**Criteria for positive result:** Statistically significant increase in micronucleated PCE's compared to vehicle control.

**Results:** There was no significant increase of micronucleated polychromatic erythrocytes. There was no significant change in PCE/NCE ratios, but the high dose is justified by the mortality determined in the preliminary study. Sensitivity of the assay was demonstrated by the positive result in the positive control group.

**Evaluation:** The results constitute a negative response for calcitriol in the mouse micronucleus assay under these conditions.

**REVIEW OF REPORT #N138443:**  
**THE EFFECT OF CALCITRIOL AND RELATED COMPOUNDS ON TUMOR PROMOTION AND CELL PROLIFERATION AND THE POTENTIAL FOR HUMAN CANCER RISK**

**PURPOSE:** The contents of this document comprise a summary of data available relating the the potential for carcinogenic/promoter effects of Calcitriol. No original data are provided Dated 8/2/95.

**SUMMARY:** Calcitriol, an essential endogenous substance, is to be used in physiologic amounts for essentially replacement therapy. The current understanding of this Division is that such substances do not necessarily require carcinogenicity bioassays for approval of chronic use indications. However, exploration of alternative models (e.g., tumor promotion, cell proliferation assays, etc) are commonly suggested to determine if there is a cause for concern regarding potential carcinogenicity. This is not the procedure for non-endogenous

analogs of such substances. Rodent carcinogenicity studies with Calcitriol were initiated, but then discontinued after 4 months of treatment.

#### INITIATION PROMOTION MODELS

Calcitriol applied topically twice a week to DMBA-treated mice was found to stimulate tumor promotion. However, when administered in combination with TPA, there was an inhibition of tumor promotion in DMBA initiated CD-1 and Sencar mice.

In MNU treated rats,  $1\alpha$ -(OH) $D_3$  suppressed lithocholic acid promotion of colon tumors

Vitamin  $D_3$  suppressed the colon tumor incidence in DMN treated rats on a high, but not a low fat diet.

Vitamin  $D_3$  had no effect on dimethylhydrazine-induced colon carcinogenesis in F344 rats.

In DMBA-treated rats, higher dietary levels of Vitamin D inhibited mammary tumorigenesis in the presence of low amounts of calcium and phosphate. When dietary calcium, phosphate and vitamin D were decreased, mammary tumor yields increased.

#### TUMOR CELL LINE GROWTH *IN VIVO*

Calcitriol or  $1\alpha$ -(OH) $D_3$  prolonged survival of mice inoculated with M1 leukemia cells.

$1\alpha$ -(OH) $D_3$  reduced the size of transplanted sarcoma 180 cells in mice and reduced the number of lung metastases resulting from implantation of Lewis lung adenoma cells in mice.

Calcitriol stimulated the growth of the  $1\alpha,25$ -(OH) $_2D_3$  receptor-rich osteogenic sarcoma cells inoculated into athymic nude mice.

No consistent effect was noted on human melanoma cells with low levels of receptors or receptor deficient rat sarcoma cells.

#### *IN VITRO* STUDIES

Proliferation of melanoma tumor cells containing receptors for  $1\alpha,25$ -(OH) $_2D_3$  was inhibited by calcitriol.

Human breast cancer cell line T-47-DF proliferation was inhibited by calcitriol at physiologic doses.

CHO clonal cell growth was inhibited by  $1\alpha,25$ -(OH) $_2D_3$

ROS 17/2.8 cell lines (rat sarcoma) colony formation in soft agar was inhibited by Calcitriol.

In T-47D breast cancer cells and melanoma MM96 cells, stimulation of growth occurred at low concentrations of calcitriol while an inhibitory effect was observed at high

concentrations. The two metabolites, 1, 24,25-(OH)<sub>2</sub>D<sub>3</sub> 1, 24,26-(OH)<sub>2</sub>D<sub>3</sub> did not stimulate growth, but were as potent as calcitriol at inhibition of growth. This same effect was noted for calcitriol in human promyelocytic cell line (HL-60) where 10<sup>-9</sup> M stimulated growth and 10<sup>-8</sup> M inhibited growth.

Calcitriol reduced the proliferation of JB6 mouse epidermal cells *in vitro*, it enhanced TPA induced anchorage independent growth without increasing cell proliferation. Calcitriol induced anchorage independent growth in JB6 cells which was inhibited by retinoic acid.

Calcitriol suppressed growth and induced HL-60 cells to differentiate into mature myeloid cells.

Calcitriol induced mouse myeloid leukemia cells to differentiate into macrophages *in vitro* and induced terminal differentiation of mouse epidermal cells in primary cell culture to cornified cells.

Calcitriol did not induce cell transformation of the SHE cell assay when tested alone, but enhanced cell transformation in cells pretreated with a number of known chemical carcinogens.

Calcitriol enhanced methylcholanthrene-induced transformation of BALB 3T3 cells *in vitro*.

#### HUMAN CANCER RISK EVALUATION

The sponsor indicates that it is difficult to extrapolate human cancer risk from the experimental data. As evident above, there are both potential anti-cancer activities (inhibition of cell proliferation and tumor growth and differentiation-inducing properties) as well as promoter-like and enhancement of promoter effects. There is a suggestion in the studies where biphasic effects were noted that these opposite activities might be dissociated. Since calcitriol is used essentially as a replacement therapy, any potential cancer risk would not be expected to exceed that of a population with normal vitamin D status. This is why rodent carcinogenicity studies are not generally required for endogenous substances used as replacement therapy. However, given the mixed findings in the studies described, the requirement for carcinogenicity bioassays for non-endogenous analogs and high doses of calcitriol will have to be considered on a case-by-case basis. The use of alternative models as described above should take into account relevant human exposure levels when possible.

**REVIEW OF ROCHE STUDY F-92-17:**  
**EFFECT OF DIETARY SUPPLEMENTATION OF 1,25(OH)<sub>2</sub>D<sub>3</sub> (RO21-5535) TO CONTROL**  
**OR TIBIAL DYSCHONDROPLASIA-INDUCING DIETS ON PERFORMANCE AND**  
**INCIDENCE OF TIBIAL DYSCHONDROPLASIA IN 3-WEEK-OLD AND 6-WEEK-OLD MALE**  
**BROILER CHICKENS**

**NOTE:** Performed by sponsor. No GLP statement provided. Study dates: 9/92-11/92. Ro21-5535 crystalline 0.0120% solution (w/v) Lot #CDG-20113-36.

**PURPOSE:** To evaluate the effect of Ro21-5535 in two diets on the performance and incidence of tibial dyschondroplasia at 3 and 6 weeks in male broiler chickens. The two diets were 1) \_\_\_\_\_ Control diet and 2) \_\_\_\_\_ D. diet (The latter diet was adjusted to the same calcium,

phosphorus, sodium and chloride in the University of Georgia-Tibial Dyschondroplasia-inducing diet).

**EXPERIMENTAL DESIGN:** Day-old sexed male broiler chickens (\_\_\_\_\_ 30 chicks/pen) were randomized to one of two of the above listed diets and to treatment groups of 0, 5 or 10 µg Rocaltrol/kg of feed for each diet. Food and water were available *ad libitum*. On day 22, 10 birds were killed for examination of the tibia. On day 43, the remaining 20 birds from each pen were sacrificed for examination of tibias.

## RESULTS

1. Compared to control diet, the T.D. diet resulted in lower body weight gains (8% by day 42), and reduced feed intake (10% by day 21 and 5% by day 42). Feed efficiency was improved during 0-21 only. The T.D. diet also resulted in significant decreases in values for tibia ash, tibia calcium, tibia phosphorus and significant increase in the incidence of T.D. and average T.D. score by both day 22 and 43.
2. CONTROL DIET: The 5 µg/kg levels of Rocaltrol in the control diet caused depressed weight gains and poorer feed efficiency during the 0-21 day and 0-42 day periods. The T.D. incidence was reduced and the average T.D. score improved (from 0.43 to 0.23) by day 22. At the 10µg/kg level, both the body weight gains and feed intake were significantly depressed by day 42 and the incidence of T.D. and average T.D. scores were reduced by 40 and 33%, respectively by day 43.
3. T.D. DIET: Supplementation of the T.D. diet with rocaltrol at 5 and 10 µg/kg resulted by 21 days in incremental increases in body weight gains, feed intake, tibia ash, tibia calcium and tibia phosphorus and a significant decrease in the incidence of T.D. from 73% to 53% at 5 µg/kg and to 35% at 10 µg/kg with a significant dose dependent decrease in the average T.D. score by day 22. These values were similar to those animals on the control diet.
4. By day 42, the T.D. birds supplemented with rocaltrol had higher body weight gains, feed intake, tibia ash and tibia calcium. A significant reduction in both the incidence of T. D. and average T.D. lesion score was also noted.

In summary, under conditions of deficiency, treatment with rocaltrol resulted in improvement in weight gains, feed efficiency and feed intake as well as bone quality. Under control diet conditions, improvement of bone quality occurred, but there was a comparative decrease in weight gain and feed efficiency.

**REVIEW OF ROCHE STUDIES F-92-18 AND F-93-01:**  
**EFFECT OF DIETARY SUPPLEMENTATION OF 1,25(OH)<sub>2</sub>D<sub>3</sub> (RO21-5535)**  
**TO CONTROL, INTERMEDIATE OR TIBIAL DYSCHONDROPLASIA-INDUCING DIETS ON**  
**PERFORMANCE AND INCIDENCE OF TIBIAL DYSCHONDROPLASIA**  
**IN 3-WEEK-OLD MALE BROILER CHICKENS**

**NOTE:** Performed by sponsor. No GLP statement provided. Study dates: 10/92-11/92 (study #F-92-18) and 1/93-2/93 (study #F-93-01). Ro21-5535 crystalline 0.0120% solution (w/v) Lot #CDG-20113-36.

**PURPOSE:** To evaluate the effect of Ro21-5535 (5 µg/kg diet) on the performance and incidence of tibial dyschondroplasia at 3 weeks in male broiler chickens. The diets were 1) Control diet, 2) I, 3) II, 4) III and 5) D. These diets are designed as ranging from control to "grossly imbalanced" which lead to tibial dysplasia. A table of dietary treatments follows:

**Calculated Dietary Concentration (As Fed)**

TREATMENT	Na %	Cl%	Ca%	AVAILABLE P %	1,25(OH) <sub>2</sub> D <sub>3</sub> (µg/kg feed)	LASALOCID* PPM
control	0.14	0.24	1.00	0.42	0 or 5	100
I	0.19	0.31	1.00	0.42	0 or 5	100
II	0.19	0.31	0.84	0.46	0 or 5	100
III	0.19	0.31	0.68	0.50	0 or 5	100
D.	0.19	0.31	0.51	0.53	0 or 5	100

**\*PREVENTATIVE FOR COCCIDIOSIS**

**EXPERIMENTAL DESIGN:** Day-old sexed male broiler chickens were randomized to one of the above listed diets. 10 chicks /pen were fed the appropriate diet from day 0 to 21. Food and water were available *ad libitum*. On day 21, the surviving birds from each of the 60 pens (6 replicate pens/treatment) were killed and examined for rickets and tibial dyschondroplasia lesions. Data from the two studies were pooled.

**SUMMARY OF RESULTS**

TREATMENT	1,25(OH) <sub>2</sub> D <sub>3</sub> (µg/kg feed)	0-21 DAYS				ON DAY 21	ON DAY 21
		AV. ST GAIN/BIRD (g)	ADJ. FEED INTAKE/BIRD (g)	ADJ. FEED/GAIN	AV. MORTALITY (#)	AV. T.D. SCORE (#)	INCIDENCE OF T.D. (%)
CONTROL	0	596.72 <sup>bc</sup>	974.71 <sup>ab</sup>	1.65 <sup>ab</sup>	0.068 <sup>a</sup>	0.41 <sup>bc</sup>	17.50 <sup>bc</sup>
CONTROL	5	548.67 <sup>d</sup>	963.59 <sup>ab</sup>	1.77 <sup>a</sup>	0.017 <sup>a</sup>	0.21 <sup>c</sup>	11.67 <sup>c</sup>
I	0	625.50 <sup>ab</sup>	1009.61 <sup>ab</sup>	1.62 <sup>ab</sup>	0.034 <sup>a</sup>	0.41 <sup>bc</sup>	17.50 <sup>bc</sup>
I	5	604.28 <sup>bc</sup>	1020.83 <sup>ab</sup>	1.70 <sup>ab</sup>	0.074 <sup>a</sup>	0.50 <sup>bc</sup>	22.73 <sup>bc</sup>
II	0	663.96 <sup>a</sup>	1030.53 <sup>ab</sup>	1.57 <sup>b</sup>	0.076 <sup>a</sup>	0.73 <sup>bc</sup>	30.00 <sup>bc</sup>
II	5	661.62 <sup>a</sup>	1039.67 <sup>a</sup>	1.58 <sup>b</sup>	0.110 <sup>a</sup>	0.50 <sup>bc</sup>	20.00 <sup>bc</sup>
III	0	614.94 <sup>abc</sup>	1004.84 <sup>ab</sup>	1.64 <sup>ab</sup>	0.085 <sup>a</sup>	1.01 <sup>b</sup>	40.83 <sup>b</sup>
III	5	658.77 <sup>a</sup>	1016.38 <sup>ab</sup>	1.56 <sup>b</sup>	0.085 <sup>a</sup>	0.52 <sup>bc</sup>	25.83 <sup>bc</sup>
D.	0	562.41 <sup>cd</sup>	933.25 <sup>b</sup>	1.66 <sup>ab</sup>	0.034 <sup>a</sup>	1.89 <sup>a</sup>	70.00 <sup>a</sup>
D.	5	604.40 <sup>bc</sup>	981.58 <sup>ab</sup>	1.64 <sup>ab</sup>	0.082 <sup>a</sup>	0.68 <sup>bc</sup>	27.50 <sup>bc</sup>

<sup>a,b,c,d</sup> Means within a column with different superscripts are different (P 0.05)

## DISCUSSION

### EFFECT OF DIET ALONE:

1. Increasing the Na from \_\_\_\_\_ and Cl from \_\_\_\_\_ (cf control and \_\_\_\_\_ diets) resulted in numerical increase in body weight gains, feed intake and feed efficiency at all Ca:P ratios except 1:1 (i.e. in all diets except the \_\_\_\_\_ D.).
2. Lowering calcium and increasing available phosphate (cf \_\_\_\_\_ I to \_\_\_\_\_ II and III) did not affect bird weight gain, feed intake or feed efficiency. However, there were higher \_\_\_\_\_ D. incidences and average \_\_\_\_\_ D. scores.
3. Comparing the \_\_\_\_\_ D. diet to control diet, there was a 6% reduction in average body weight gains, 4% reduction in feed intake and significant increase in incidence of \_\_\_\_\_ D. \_\_\_\_\_ and average \_\_\_\_\_ D. scores \_\_\_\_\_.

### EFFECT OF 1,25 (OH)<sub>2</sub>D<sub>3</sub> SUPPLEMENTATION:

4. Significant reduction in body weight gains and numerical reduction in feed intake for control diet and \_\_\_\_\_ I diet.
5. \_\_\_\_\_ II diet +1,25 (OH)<sub>2</sub>D<sub>3</sub> did not influence the body weight gains or feed intake.
6. \_\_\_\_\_ III diets +1,25 (OH)<sub>2</sub>D<sub>3</sub> caused numerically higher body weight gains, feed intake and improved efficiency.
7. A decrease in incidence of \_\_\_\_\_ D. and average \_\_\_\_\_ D. scores occurred in all groups (including controls) when treated with 1,25 (OH)<sub>2</sub>D<sub>3</sub>.

### SUMMARY OF ABSTRACTS AND MANUSCRIPTS OF ROCHE-SPONSORED STUDIES

#### **L-Ascorbic Acid Is Required for Vitamin D Metabolite-Induced Osteocalcin Secretion in Primary Rat Osteoblasts R.Goralczyk, Hoffmann-La Roche LTD, Basel, Switzerland (abstract only)**

It is known that both Vitamins D and C are necessary for normal bone growth. The author investigated the effects of combinations of 25(OH)D<sub>3</sub> or 1,25(OH)<sub>2</sub>D<sub>3</sub> with L-ascorbic acid on the secretion of osteocalcin by primary rat osteoblasts obtained from day 21 fetal rat calvaria. Precultured cells (3 weeks) were exposed to each agent alone and in combinations. 25(OH)D<sub>3</sub> (10<sup>-9</sup>-10<sup>-6</sup>M) or 1,25(OH)<sub>2</sub>D<sub>3</sub> (10<sup>-12</sup>-10<sup>-6</sup>M) induced low or non-detectable levels of osteocalcin (>1ng/mgDNA). 25-200 μM ascorbic acid alone stimulated OC to 1-5 ng/mg DNA. 200 μM ascorbic acid and 10<sup>-9</sup>-10<sup>-6</sup>M 25(OH)D<sub>3</sub> or 10<sup>-10</sup>-10<sup>-6</sup> M 1,25(OH)<sub>2</sub>D<sub>3</sub> increased OC release to 6-25 ng/mg DNA. The effect was detectable 24 h after exposure and increased over 48 h to over 40 ng/mg DNA. This was not blocked by indomethacin (a prostaglandin synthesis inhibitor). Inhibitors of collagen synthesis (3,4-dehydroproline, DHP) or collagen crosslinking (β-aminopropionitril, BAPN) both inhibited the synthesis induced by combinations of these agents by approximately \_\_\_\_\_. Since these did not block the response completely, the author proposed that other mechanisms may contribute to the synergism between these agents.

**1,25-Dihydroxyvitamin D<sub>3</sub> inhibits the production of IL-12 by human monocytes and B-cells** J. Lemire, L. Beck, D.Faherty, M. Gately and H. Spiegelberg. (abstract only)

1,25-(OH)<sub>2</sub>D<sub>3</sub> inhibited Th<sub>1</sub>-mediated murine autoimmune disease and suppressed IL-2 and IFN-g secretion by murine and human Th<sub>1</sub> cells. Half-maximal inhibition of IL-12 production was achieved at 10<sup>-10</sup>M and 10<sup>-9</sup>M of 1,25(OH)<sub>2</sub>D<sub>3</sub> in monocytes and B cells, respectively. This was enhanced in monocytes that were preincubated for 24h with 1,25(OH)<sub>2</sub>D<sub>3</sub> prior to activation with *S. aureus* Cowan type II. The authors suggest inhibition of Th<sub>1</sub> cell function by 1,25(OH)<sub>2</sub>D<sub>3</sub> is mediated by the suppression of IL-12 secretion by antigen presenting cells.

**Effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> and Vitamin D Analogs on Developmental Control of Cell Growth and Tissue-Specific Gene Expression During Osteoblast Differentiation.** Stein-GS, Lian-JB, Uskokovic-M, Aronow-M, Shalhoub-V, Owen-T, and Barone-L. For publication in *Bioorganic and Medicinal Chemistry Letters*

Sequential expression of genes associated with progressive osteoblast phenotype development was examined in cultured rat calvarial osteoblasts. Three segments of differentiation were described according to specific genes expressed: 1) proliferation (increased *cfos*, Histone 4, type I collagen), 2) matrix maturation (alkaline phosphatase, matrix Gla protein) and 3) mineralization (osteocalcin, osteopontin, collagenase). Activation or deactivation of various genes occurs at one of 2 transition points. These transition points are defined as restriction points during osteoblast differentiation to which developmental expression of genes can proceed but cannot pass without additional signaling mechanisms. Vitamin D has been identified as one of the agents which permits crossing of the transition points. Competency for responsiveness to vitamin D through the stages of differentiation are reflected at the tissue level by preferential effects on expression of vitamin D-responsive genes. Vitamin D-treated osteoblasts during the proliferation period alters levels of gene expression to reflect a profile characteristic of post-proliferative, mature bone cells while at later stages can promote expression of genes that reinitiate osteoblast activity. Thus, the effects of vitamin D are highly dependent on the differentiated stage of the bone cell. Vitamin D acts principally as an enhancer of osteocalcin gene transcription and the magnitude of the enhancement is dependent on basal activity. A heterogenous series of tissue-specific and developmental stage-specific factors interact with primary DNA binding proteins contributing to regulatory specificity. Combinations or modification of accessory factors with the Vitamin D receptor may contribute to pleiotropic transcriptional control where either positive or negative activity is observed.

**Effect of Ascorbic Acid and 1,25 (OH)<sub>2</sub>D<sub>3</sub> on Bone Cell Metabolism in Relation to the Development of Tibial Dyschondroplasia.** L. Völker. Roche Manuscript #B-160'749.

Note: Supported by sponsor, but there was no Roche author. Dr. Völker apparently wrote the summary provided with the manuscript. The authors listed on the actual manuscript (no reference to publication) were: C-Farquharson<sup>1</sup>, JS-Rennie<sup>1</sup>, N-Loveridge<sup>2</sup>, CC-Whitehead<sup>1</sup>, from \_\_\_\_\_

Ascorbic acid has been shown to stimulate the endogenous synthesis of 1,25 (OH)<sub>2</sub>D<sub>3</sub> (Weiser et al *Vitamin D, Molecular, Cellular and Clinical Endocrinology*, Walter de Gruyter & Co, Berlin). Therefore, the effects of the combination of these two agents on the development of TD in young fast-growing broilers was examined. Two experiments were performed which

investigated the effects of dietary supplementation of ascorbic acid (200 mg/kg) and 1,25 (OH)<sub>2</sub>D<sub>3</sub> (5µg/kg) alone or in combination on bone and plasma characteristics of birds fed a balanced (Ca:P; 2:1) or imbalanced diet (Ca:P; 1:1). The imbalanced diet increased the incidence of TD compared to the balanced diet. The diets were fed to day-old chicks (20/group) and blood samples were taken for Ca, P and alkaline phosphatase prior to terminal killing.

The results of this experiment confirmed previous observations that supplementary dietary 1,25 (OH)<sub>2</sub>D<sub>3</sub> was effective in preventing TD with diets that were balanced or imbalanced in calcium and phosphorus. Supplementary dietary ascorbic acid may also have a role in the prevention of TD and the preventative role of ascorbic acid is influenced by the calcium:phosphorus ratio. *In situ* biochemistry showed that there was no alteration in ALP activity of the hypertrophic chondrocytes in TD in response to dietary supplementation which suggests that the metabolites may exert their effects on chondrocyte maturation in the less differentiated prehypertrophic chondrocytes.

**Metabolism of 1,25 (OH)<sub>2</sub>-16-ene-D<sub>3</sub> in Kidney: Influence of Structural Modification of D-Ring on Side Chain Metabolism. G-Satyanarayana Reddy, JW-Clark, K-Y-Tserng, MR-Uskokovic and JA-McLane. (the latter 2 authors are from Roche) To be published in Bioorganic and Medicinal Chemistry Letters.**

Vitamin D produces various biological effects via both genomic and nongenomic pathways. Structural alteration of 1,25(OH)<sub>2</sub>D<sub>3</sub> can result in dissociation of its calcemic action from its actions on cell growth and differentiation. e.g., 25(OH)-16,23-diene D<sub>3</sub> binds to the vitamin D receptor 1000X less than 1,25(OH)<sub>2</sub>D<sub>3</sub>, but stimulated intestinal calcium absorption 2.5X more than 1,25(OH)<sub>2</sub>D<sub>3</sub>. In contrast, 1,25(OH)<sub>2</sub>-16-eneD<sub>3</sub> binds to the vitamin D receptor 2.5X better than 1,25(OH)<sub>2</sub>D<sub>3</sub>, but causes no stimulation of intestinal calcium absorption. The various unique biological activities of 16-ene analogs of 1,25(OH)<sub>2</sub>D<sub>3</sub> can be due to several factors which include 1). changes in binding to the intracellular vitamin D receptor and extracellular serum vitamin D binding protein (DBP) and 2). changes in their cellular metabolism and final inactivation. The metabolism of 1,25(OH)<sub>2</sub>D<sub>3</sub> was compared with 1,25(OH)<sub>2</sub>-16-eneD<sub>3</sub> to determine the contribution of the second aspect to the unique properties of the analogs.

HPLC and mass spectra were measured to determine the metabolic products resulting from a rat kidney perfusion model for 8h.

These comparative studies examined 1,25 (OH)<sub>2</sub>D<sub>3</sub> and 1,25 (OH)<sub>2</sub>-16-eneD<sub>3</sub> metabolism in rat kidney. Both compounds were metabolized into their respective 24 hydroxy and 24-oxo metabolites. While 1,25 (OH)<sub>2</sub>-24-oxo-D<sub>3</sub> was further metabolized into 1,23,25 (OH)<sub>3</sub>-24-oxo-D<sub>3</sub>, 1,25(OH)<sub>2</sub>-24-oxo-16-eneD<sub>3</sub> resisted C-23 hydroxylation. The minor change in D-ring structure appeared to cause major changes in the side chain metabolism.

**Cancer Combination Chemotherapy with Retinoids: Experimental Rationale. W-Bollag, S-Majewski and S-Jablonska PRCO and University of Warsaw, Dept of Dermatology For publication in "Leukemia" from Workshop: Retinoids in Oncology, London, 1/21-22. 1994. Roche Manuscript #B-159 460.**

This review paper explores the interactions of retinoids, cytokines and 1,25(OH)<sub>2</sub>D<sub>3</sub> and potential application of combinations of these agents to chemotherapy. Effects on cell differentiation, proliferation and angiogenesis were examined. Although this is a survey of a

variety of agents which focused primarily on retinoids, several specific effects of  $1,25(\text{OH})_2\text{D}_3$  were described:

1. Alone,  $1,25(\text{OH})_2\text{D}_3$  induced differentiation in the transformed hemopoietic cell lines such as HL-60 and U937. However, in sufficiently high doses to cause differentiation, side-effects including increased intestinal calcium absorption, bone calcium mobilization, hypercalcemia and calcium tissue deposition would limit its utility. In cell lines (HL-60) the effect of retinoids and  $1,25(\text{OH})_2\text{D}_3$  analogs were enhanced in combination (although it was not clear if this was a synergistic effect or even additive effect). A series of analogs with less influence on calcium metabolism but strong differentiation-inducing effects have also been designed.

2. Similar effects on proliferation of transformed human cell types as for differentiation were also noted. Again, it was expected that the "calcium liabilities" of  $1,25(\text{OH})_2\text{D}_3$  limit the unmodified agent utility in this effect, but modified  $1,25(\text{OH})_2\text{D}_3$  agents or combination with retinoids could prove useful therapeutically.

3. Effects on angiogenesis were also similar to above.

Overall, the data presented are limited to demonstration of specific *in vitro* effects of  $1,25(\text{OH})_2\text{D}_3$  and the main thrust of the paper is largely speculative.

**Effect of Dietary Supplementation of  $1,25(\text{OH})_2\text{D}_3$  (Ro21-5535) to Control or Tibial Dyschondroplasia-Inducing Diets on Performance and Incidence of Tibial Dyschondroplasia in 3-Week-Old and 6-Week-Old Male Broiler Chickens (Roche Study F-92-17) G.G. Untawale, H.G. Eisenbeis and R.W. Miller.**

**NOTE:** Performed by sponsor. No GLP statement provided. Study dates: 9/92-11/92. Ro21-5535 crystalline 0.0120% solution (w/v) Lot #CDG-20113-36.

**PURPOSE:** To evaluate the effect of Ro21-5535 in two diets on the performance and incidence of tibial dyschondroplasia at 3 and 6 weeks in male broiler chickens. The two diets were 1) \_\_\_\_\_ Control diet and 2) \_\_\_\_\_ D. diet- the diet above adjusted to same calcium, phosphorus, sodium and chloride in the University of Georgia-T.D.-inducing diet)

**EXPERIMENTAL DESIGN:** Day-old sexed male broiler chickens \_\_\_\_\_ were randomized to one of two of the above listed diets and to treatment groups of 0, 5 or 10  $\mu\text{g}$  Rocaltrol/kg of feed for each diet. 30 chicks /pen were utilized. Food and water were available *ad libitum*. On day 22, 10 birds were killed for examination of the tibia. On day 43, the remaining 20 birds from each pen were sacrificed for examination of tibias.

## RESULTS

1. Compared to control diet, the T.D. diet resulted in lower body weight gains (8%) by day 42), reduced feed intake (10% by day 21 and 5% by day 42). Feed efficiency was improved during 0-21 only. The T.D. diet also resulted in significant decreases in values for tibia ash, tibia calcium, tibia phosphorus, and significant increase in the incidence of T.D. and average T.D. score by both day 22 and 43.

2. The 5 µg/kg levels of Rocaltrol in the control diet caused depressed weight gains and poorer feed efficiency during the 0-21 day and 0-42 day periods while the T.D. incidence was reduced and the average T.D. score (from 0.43 to 0.23) by day 22. At the 10µg/kg level, both the body weight gains and feed intake were significantly depressed by day 42 and the incidence of T.D. and average T.D. scores were reduced by 40 and 33%, respectively by day 43.

3. Supplementation of the T.D. diet with rocaltrol at 5 and 10 µg/kg resulted by 21 days in incremental increases in body weight gains, feed intake, tibia ash, tibia calcium and tibia phosphorus and a significant decrease in the incidence of T.D. from 73% to 53% at 5 µg/kg and to 35% at 10 µg/kg with a significant dose dependent decrease in the average T.D. score by day 22. These values were similar to those animals on the control diet.

4. By day 42, the T.D. birds supplemented with rocaltrol had higher body weight gains, feed intake, tibia ash and tibia calcium. A significant reduction in both the incidence of T. D. and average T.D. lesion score was also noted.

**Renal Calcification in Suckling Rats After High Doses of Calcitriol (1,25-Dihydroxy cholecalciferol) Dostal, Toverud, Peach Arch. Pathol Lab Mec 108: 410-415. 1984**

This paper was presented in conjunction with the sponsor's indication that testing in young animals was not necessary to provide coverage for pediatric use. Briefly, 2 and 3 week old rats were treated with four oral daily doses of 2 ng/g calcitriol beginning on days 9 or 16 of age. Blood was collected by heart puncture and kidneys processed for histology and calcium and magnesium determinations 24 h after the fourth dose. Both light and electron microscopic evaluations were performed. The authors concluded that the locations and types of renal calcifications caused by high doses of calcitriol in suckling rats were similar to those observed in vitamin D toxicity in adult rats. The calcification was more extensive and involved glomeruli and the medulla in the younger rats, possibly due to the relatively greater hypercalcemia observed in these rats. The authors suggest that the renal calcification appears to be a direct effect of elevated calcium levels. Thus, the sponsor concludes, one could assume that if hypercalcemia is avoided in both adult and pediatric populations, there should be no significant differences in toxicities when using this in pediatric populations. They use this as justification for not doing studies in juvenile animals. However, it is important to point out that the authors of this paper could not exclude the possibility of a direct toxic effect of Vitamin D. If this is the case, these results could be interpreted as indicating a potential need for such studies. However, the sponsor also notes that there is extensive experience in children which indicates relative safety. If this is the case, no studies in young animals are necessary.

**Expert Report Update on the Pharmaco-Toxicological Documentation for Rocaltrol. Manuscript No. N-135085 Dated January 3, 1994. Dr. Marvin Cohen**

This summary was previously submitted under this NDA. Although it is a summary and not an original report, some of the points made in this document were deemed significant in understanding the safety of chronic use of this agent.

**Effects in animal models of osteoporosis:**

Several published studies in ovariectomized rats indicate that calcitriol significantly decreased the calcium loss from bone that occurred after ovariectomy. One study measured effects on "fracture strength" (Matsumoto et al, J. Nutr. Sci. Vitaminol 31 (suppl): S61-S65, 1985). In this study, calcitriol had no significant effect on fracture strength. Data from dogs was discussed. In a one year study, calcitriol at 25 ng/kg/day reversed the effects of ovariectomy on bone mass

without producing adverse effects on plasma calcium or plasma creatinine levels. Other animal models of osteoporosis (e.g., lactating rats, immobilization-induced osteoporosis and inflammation-induced osteoporosis) were briefly mentioned, but the author seemed to suggest that while there were some positive findings, results were not consistently positive and were more variable than with ovariectomized models.

## OVERALL PHARMACOLOGY/TOXICOLOGY SUMMARY

### ACUTE ORAL TOXICOLOGY

Trans-calcitriol at 1.0, 2.0, and 4.0 mg/kg in rats did not elicit any clinical effects after a single oral dose followed by 14 days of observation. Calcitriol, on the other hand, caused transient (<6h) respiratory depression at 1.0 and 2.0 mg/kg in approximately 50% of the animals. Respiratory depression, tremors, decreased motor activity, ptosis and abnormal gait were exhibited at the high dose. The single high dose resulted in 100% mortality between days 4-9 after dosing. Thus, trans-calcitriol, the penultimate intermediate of a new synthetic procedure, exhibits considerably less acute oral toxicity in mice compared to calcitriol.

The impurity (Cis-methyl, ethyl derivative) at the 0.2% level did not affect the acute oral toxicity of rocalcrol in mice.

### MULTIPLE DOSE TOXICOLOGY

**13 AND 26 WEEKS IN RATS:** Major toxicological changes from the daily administration of Rocaltrol in the thirteen week study resulted from hypercalcemia. Calcium levels were increased in all dose groups. Some parathyroid atrophy (approx. 50% of animals) and calcium deposits were noted in the lung and kidney at 0.05 µg/kg/day dose. A generalized tissue deposition of calcium occurred at doses of 0.15 µg/kg/day and higher. A sporadic "focal whitish change of eyes" was noted in the 0.15 and 0.45 µg/kg/day groups. A decrease in body weight was noted in the 0.15 and 0.45 µg/kg/day groups. Most organ weight changes could be attributed to change in body weight. However, an increase in the absolute and relative weights in kidneys in females and decrease in relative and absolute weights in reproductive organs in 0.15 and 0.45 µg/kg/day males and females (prostate, seminal vesicles, uterus and ovaries) appeared to be drug-related. There was an increase in total cholesterol, and decrease in total protein, potassium, MG, BUN for both sexes. Cholinesterase was decreased for females in both studies while it was increased for males in the 13 week study.

The NOAEL level was estimated to be at or below 0.05 µg/kg/day with intravenous administration in this experiment. Toxic responses noted at higher doses showed signs of improvement after the recovery period, although calcium deposits were relatively unchanged in kidneys or eyes. The NOAEL represents an equivalent exposure to the maximum proposed human dose based on surface area comparisons.

Major toxicological changes from the administration of Rocaltrol in the twenty-six week intermittent dosing study resulted from hypercalcemia. Some parathyroid atrophy (approx. 70-80% of animals) was noted at 0.15 µg/kg/day dose and above. As in the 13 week study, the kidney and eyes seem particularly prone to calcium deposits even at the lowest dose.

The NOAEL level was estimated to be at or below 0.05 µg/kg/day with intravenous administration in this experiment. Toxic responses were similar to, but in most cases, milder than the 13 week continuous administration study. This could be due to the intermittent dose schedule in this study. The NOAEL represents an equivalent or slightly lower exposure to the maximum proposed human dose based on surface area comparisons.

**13 AND 26 WEEKS IN DOGS:** Toxic effects noted in both the 13- and 52-week I.V. toxicity studies were related primarily to the expected physiological effects of the agent. These included: increase in serum calcium and calcification of many tissues (heart, kidney, lung, salivary glands) and ossification of bone. Mortality was observed in the 0.1 µg/kg/day group in the 13-week study between days 50-60. Treatment was discontinued in this group on day 56 due to excessive toxicity.

Treatment at 0.05 and 0.1 µg/kg in the 13-week study and at 0.06 µg/kg (but not 0.4 µg/kg) in the 52-week study resulted in severe anorexia and loss of body weight. When treatment was discontinued, appetite was regained immediately and body weight was restored to near prestudy levels by 4 weeks of recovery. There was a tendency for 0.025 g/kg/day (low dose) females in the 13-week study to lose weight as well.

"Histamine-like" effect was noted in both tox studies which lasted 1 h (Erythema in ears, limbs, rim of eyes and/or muzzle). This was attributed to an agent in the vehicle, but specific data were not provided to support this.

There was a consistent decrease in Mg, Cl and increase in BUN, Ca and inorganic phosphate were noted in both toxicology studies (0.05 and 0/1 µg/kg/day in the 13-week study and 0.06 µg/kg/day in the 52-week study). Slight increase in Ca at 0.025 µg/kg in the 13 week study was also noted. In rats, BUN was decreased.

Increased Hb, Hct, RBC, platelets and monocytes were noted at 0.05 µg/kg and 0.1 µg/kg in the 13-week study but not at 0.06 µg/kg in the 52-week study.

Slight decrease of specific gravity of urine was noted in both studies.

Absolute and relative lung and kidney weights were increased at 0.06 µg/kg in the 52-week study. There were no clear changes in organ weights in the 13-week study which could not be attributed to the weight loss observed. However, changes in the relative weights of thymus (decreased), lung and liver (both increased) in the high dose group of the 13 week study are consistent with changes observed in the 52-week study.

**Histopathology:** The following were noted in both studies: Increase in proliferation of bone and osteoclasts, atrophy of thymus, and brown pigment deposits in Kupffer cells in the liver. Chronic nephropathy-like changes were noted at 0.06 µg/kg in the 52-week study and in all treated groups of the 13-week study. A whitish or fading change of the kidney was noted in the high dose of the 52 week study. Hyperplasia of the parafollicular cells of the parathyroid were noted in the 0.05 and 0.1 µg/kg in the 13-week study and the 0.06 µg/kg group in the 52-week study. Atrophy of the parathyroid cells were noted in the 0.01, 0.05 (13-week) and 0.06 (52-week) µg/kg groups. **All of these changes were slow to recover and many were not improved after 4-5 weeks of recovery.**

#### TISSUE DISTRIBUTION:

A rapid and wide distribution into most tissues, reaching maximal levels by 0.5 h after dosing. In most organs, the levels were lower than blood. No major sex differences were observed. Relatively high levels were present in the small intestine contents, lung, liver, kidney, adrenal and parathyroid. Relatively low levels were found in brain, spinal cord and thymus. Rapid elimination was observed from all organs so that by 24 h after dosing, low levels of radioactivity were detectable only in the liver, kidney, skin, lung and the Harderian gland. By 72 h, the drug-related material was found only in the kidney at very low levels. The only sex difference observed was the distribution in the kidney after 6 h (higher in the cortex in males and higher in the medulla in females).

High levels observed in the intestine are consistent with the known biliary excretion of the test agent. Tissue concentrations determined by \_\_\_\_\_ were similar to the qualitative observations obtained by autoradiography.

Ro 21-5535 was excreted predominantly by the fecal route with 59.3 and 47.2% of the dose recovered in feces of male and female rats, respectively, by 72 h. Urinary excretion was higher in females compared to male rats (11.8 and 5.5% of dose for females and males, respectively). (Incomplete recovery was attributed to the formation of tritiated water which is retained in tissues for a half-life of approximately 3.6 days in rats. This is lost during the lyophilization of sections and is thus not observed in the autoradiographs).

#### REPRODUCTIVE TOXICOLOGY

Reproduction segment II study of Rocaltrol was performed intravenously in rats at dose levels of 0.05, 0.15, and 0.45  $\mu\text{g}/\text{kg}/\text{day}$ . Dams in the 0.15 and 0.45  $\mu\text{g}/\text{kg}/\text{day}$  levels showed some toxicological changes such as slight inhibition of body weight gain and food consumption as well as some alopecia. This trend reversed after treatment was stopped during lactation. F<sub>1</sub> fetuses showed slight decrease of body weight in the 0.45  $\mu\text{g}/\text{kg}/\text{day}$  group, but showed no drug-related teratological or reprotoxicological effects in any group. The non-effective dose was estimated to be 0.05  $\mu\text{g}/\text{kg}/\text{day}$  in dams, and 0.15  $\mu\text{g}/\text{kg}/\text{day}$  in F<sub>1</sub> fetuses. This represents a multiple of approximately equal the exposure at the proposed human maximum dose for dams and approximately twice the proposed maximal human dose for fetuses.

#### GENETIC TOXICOLOGY

The results constitute a negative response for calcitriol in the mouse micronucleus assay under these conditions

#### ASSESSMENT OF CARCINOGENIC POTENTIAL

The sponsor indicates that it is difficult to extrapolate human cancer risk from the experimental data. As evident above, there are both potential anti-cancer activities (inhibition of cell proliferation and tumor growth and differentiation-inducing properties) as well as promoter-like and enhancement of promoter effects. There is a suggestion in the studies where biphasic effects were noted that these opposite activities might be dissociated. Since calcitriol is used essentially as a replacement therapy, any potential cancer risk would not be expected to exceed that of a population with normal vitamin D status. This is why rodent carcinogenicity studies are not generally required for endogenous

substances used as replacement therapy. However, given the mixed findings in the studies described, the requirement for carcinogenicity bioassays for non-endogenous analogs and high doses of calcitriol will have to be considered on a case-by-case basis. The use of alternative models as described above should take into account relevant human exposure levels when possible.

### RECOMMENDATIONS

The toxicology studies provided in this supplement were performed by the intravenous route which are not easily translated to multiples of human oral exposure. Toxicity appears to be related primarily to the hypercalcemic effect of the test agent. The studies presented do not alter the basic safety evaluation of this agent. However, the results from the reproductive (Segment II) study and genetic toxicity (mouse micronucleus test) should be included in the label. The label should be updated to current standards regarding multiples of human exposure based on AUC or mg/m<sup>2</sup>. Bioavailability will have to be taken into account to estimate safety margins.

From the pharmacology/toxicology standpoint, this supplement is approved pending labeling changes.

### LABELING REVIEW (TO BE COMMUNICATED TO SPONSOR)

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Ronald W. Steigerwalt, Ph.D.  
Pharmacology team leader

**APPEARS THIS WAY  
ON ORIGINAL**