

ratios of exposure/dose based on PK estimates described above are compared to the ratios generated by mg/m² data:

Comparison of ratios of exposure levels:

Method:	mg/m ²	AUC
Rat	6	6
Dog	2	6
Human	1	1

It is clear that the averaged results of the PK estimates generate similar ratios of exposure levels to the estimates based on mg/m². Instead of, requesting a complete description of how each data point was selected for the calculations used in the PK experiments, and then requesting a reevaluation of the data using more sophisticated mathematical models and computations, we decided to base cross-species dose comparisons on mg/m².

These ratios will be used to compare exposure levels that caused reproductive toxicity in rats to the exposure levels associated with recommended human doses (see label review).

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SUMMARY
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Toxicity Studies:

In the acute IV studies conducted in rats and mice (reviewed previously by G.K.) no adverse effects were detected up to doses of 16 ug/kg.

In long term IV studies (reviewed here) conducted in dogs and rats the typical spectrum of toxicities began to appear in rats at doses of 3 ug/kg. Dogs were much more sensitive and demonstrated significant toxicity at 0.6 ug/kg. These toxicities result directly or secondarily from the expected pharmacological activity of a Vitamin D; decreases in serum PTH, hypercalcemia, and mineralization of soft tissues, especially kidneys, aorta, GI tissues and heart. Dogs and rats that became hypercalcemic showed hypercalcemia induced weight loss. Dogs also had increased levels of liver enzymes in some studies but these changes were not physiologically important. 6-month and 1-year studies in dogs uncovered some longer term effects of Paracalcin. Parathyroid atrophy was noted, but this would be considered a therapeutic effect in patients. Other effects; aciduria, increased BUN, renal nephropathy and anemia, were secondary to long term hypercalcemia and weight loss

In 6-month studies the rat NOAEL was 0.1 ug/kg/dose. In dogs the NOAEL was 0.02 ug/kg/dose in 6-month and 1-year studies. These doses are lower (on a mg/kg basis) than the highest recommended human dose (0.24 ug/kg). In other words, the highest recommended human dose would have toxic effects (on a mg/kg basis) in rats and dogs over six months. This is acceptable because human patients will be hypocalcemic, with renal failure, and will be less sensitive to the toxic effects of this drug, namely hypoparathyroidism and hypercalcemia, due to increased calcium absorption in the gut and reabsorption in kidneys. This indicates the need to closely monitor patients for hypercalcemia since hypercalcemia is the expected and most sensitive clinical indicator of toxicity. In addition dogs seem to be extraordinarily sensitive to the hypercalcemic effects of Vitamin D.

Comparison of NOAEL across species:			
		ug/kg	ug/m ²
NOAEL in 6-Mo Rat Study		0.10 ug/kg	0.6 ug/m ²
NOAEL in 6-Mo Dog Study		0.06 ug/kg	1.2 ug/m ²
NOAEL in 12-Mo Dog Study		0.02 ug/kg	0.4 ug/m ²
Maximum Recommended Human Dose:		0.24 ug/kg	8.9 ug/m ²

In all three studies the lowest effective dose (the dose which suppressed PTH levels - the desired clinical endpoint) also caused hypercalcemia. This clearly demonstrates that there is little "margin of safety" between the dose needed for efficacy (suppression of PTH) and the dose where toxicity (hypercalcemia) develops in healthy animals. This emphasizes the need to titrate the dose carefully to achieve efficacy without hypercalcemia. None of these experiments was designed to (or fortuitously demonstrated) a "therapeutic index" for this drug or compared the "margin of safety" for this drug to any other drug's "margin of safety" However, this question was addressed in a clinical trial.

None of the toxicity studies specifically examined bone histology. There were no significant reports of adverse effects on bones in any of the studies.

- 0.02 ug of Paracalcin / kg, 3 times per week IV can be considered a NOAEL in dogs for one year.
- 0.1 ug of Paracalcin / kg, 3 times per week IV can be considered a NOAEL in rats for six months.
- All of the significant toxicities seen in these experiments were secondary to hypercalcemia.
- The toxic effects of hypercalcemia can be quite severe over time, including; nephropathy, testicular degeneration, soft tissue mineralization (including heart and aorta), weight loss and death.
- The hypercalcemic effect of Paracalcin occurs at slightly higher doses than required to suppress PTH levels. Serum calcium levels should be carefully monitored to obtain a therapeutic dose while avoiding hypercalcemia.

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Genotoxicity / Carcinogenicity:

- The Ames Assay does not indicate a mutagenic potential for Paracalcin.
- The Mouse Lymphoma Assay does not indicate a mutagenic potential for Paracalcin.
- The Human Cultured Lymphocyte Assay indicates that Paracalcin does not induce chromosomal damage in the presence or absence of metabolic activation with S-9.
- Paracalcin was not clastogenic *in vivo* in the Rat Micronucleus Assay.

Reproductive toxicity:

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The Segment I study revealed no significant toxicity in rats reproduction or fertility when given up to 20 ug/kg, three times per week, prior to mating and during early gestation.

In Segment 2 studies:

When Paracalcin was administered to gravid female rats on Gestation days 6 through 17 there were no adverse effects on fetal growth or survival at the maximum dose tested (3.0 ug/kg/day). In the dams, although increased serum calcium was noted at the LD (0.3 ug/kg), toxic maternal effects (decreased weight gain) were noted only at (and above) the MD (1 ug/kg/day) leaving 0.3 ug/kg/day as the maternal NOAEL.

When Paracalcin was administered to gravid female rabbits on Gestation days 6 through 18 there were no adverse effects on fetal growth or survival at the LD or MD (0.03 or 0.1 ug/kg/day, respectively). In the dams, increased serum calcium and phosphorous and decreased PTH was noted at the MD (0.1 ug/kg) and HD. Toxic maternal effects (decreased food consumption and weight gain, and calcification of the kidneys) were noted only at the HD (0.3 ug/kg/day) leaving the LD 0.03 ug/kg/day as the maternal NOAEL.

In both Segment 2 studies the high dose fetuses appeared subject to a possible increased rate of resorption; One of 19 total litters in the rabbit study and one entire litter of 24 litters in the rat study was resorbed.

In the Segment 3 study; when Paracalcin was administered to gravid female rats on Gestation day 6 through Lactation day 20, 3 times per week, there were no adverse effects on maternal or fetal growth, survival, behavior or reproductive capability at doses up to the MD (3 ug/kg/day). As in the segment 2 studies, the high dose (in this case 20 ug/kg) fetuses appeared subject to a possible increased rate of resorption; one of 24 total litters was resorbed. Infant mortality of the F1 pups was significantly higher in the HD group (4.6%) than in the control group (1.1%) as measured on day 4 after birth. The HD dams were subject to transiently decreased maternal weight gain. Increased serum calcium and phosphorous and decreased PTH would have been noted in all treated dams had it been examined. Necropsy of F0 generation seems to have been quite superficial because no tissue calcification was noted. Equal doses had caused considerable soft tissue mineralization in similar rat studies. It is unknown whether the increased demand for calcium reduced this effect or whether the effect was not noted because the tissues were not microscopically examined. Necropsy of the F1 generation also reflected a very superficial examination and no adverse effects were detected.

Overall the most sensitive threshold of maternal and fetal effects was the rabbit Segment II study which showed maternal effects at the 0.1 ug/kg MD and increased resorptions at the 0.3 ug/kg/day HD.

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Pharmacokinetics:

Paracalcin is rapidly distributed in the central compartment. (In separate in vitro assays the drug was found to be 100% protein bound in the plasma and distributed to the plasma fraction of whole blood from several species.) The only organs where the drug was found to be concentrated were the liver and the gut (the predominant route of excretion). The half life of the parent compound in plasma was considerably longer in dogs than in rats. There was little difference between male and female groups in single dose studies.

In repeated dose studies T1/2 was ~3h in male rats and ~1.5h in female rats. AUC values were correspondingly larger in males. This is likely due to gender related differences in glucuronidation in rats. No gender related differences in PK data were noted in dogs. Both male and female dogs demonstrated T1/2 of ~3h. Healthy human subjects had T1/2 ~5h with no gender related differences noted. Because dosing was every-other-day there was no appreciable drug accumulation in 5 days. AUCs were linear with dose in rats, dogs and humans.

In patients, a dose of 0.24 ug/kg resulted in an AUC of 27,000 pg*h/ml. Comparable (AUC) exposure would require a dose 2-fold higher in dogs than in patients and 4-fold (1 ug/kg, males) or 8-fold (2 ug/kg, females) higher in rats than in patients. This is similar to the ratios of doses predicted from mg/m² corrections which would require 2-fold higher doses in dogs and 6-fold in rats.

Comparison of ratios of exposure levels:

Method:	mg/m ²	AUC
Rat	6	
Dog	2	6
Human	1	1

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Metabolism, tissue distribution and elimination:

No significant levels of metabolites were detected in the plasma of rats, dogs or humans in any of the ADME studies. In rats, of the radioactivity was recovered in the feces. Gender differences were slight and would be expected to be smaller in humans. Females excreted 8.6 % in the urine (during the first day). 78% of the labeled dose was found in the bile in 24 h and 30% of the dose was excreted between. Four major metabolites (M2- M5) were identified in the bile and feces, but the parent compound and M-1 (3.6 and 9.2 % respectively) were found only in the bile. Since there was no evidence for resorption they must have been further metabolized in the gut.

Parent drug and metabolites (%) in the bile of rats 0-24 h after IV dose:

N= 2m + 2f	M-1	M-2	M-3	M-4	M-5	M-6	other
Male rats	3.6	9.2	10.4	50.8	5.6	4.9	15.5
Female rats	2.8	7.2	8.2	39.8	4.4	3.8	12.2

Tissue concentrations were generally lower than plasma except the GI tract, and liver, suggesting biliary excretion. Kidneys, lung, thyroid, pituitary and adrenals also had levels similar to plasma. Tissue levels remained relatively constant for two hours and Almost all radioactivity was cleared by 72 h. Similar results were obtained with the pigmented rats and there was no evidence of concentration of Paracalcin in melanin containing tissues.

In dogs 95% of the radioactivity was cleared during the course of the experiment (72 h), 85% in the feces and 11% in the urine and cagewash. This suggests biliary excretion as the major route of elimination. of the labeled dose was found in the bile in the first 6 h and ~6% of the dose was found in the bile. This low level was expected since only 8.25% of the dose was recovered in the feces in the first 24 h, and the half-life is 17 h.

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Seven major metabolites (M1 - M4 and M6 - M8) were identified in the bile. M2, M4, M6, and M8 appear to be glucuronidated metabolites. M3 corresponds to the major biliary metabolite (M4) in rats. Relative contributions of metabolites to various secretions were:

Parent and Metabolites (% of total recovered) in secretions of dogs after an IV dose:

	Parent	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	Other
Bile (6 h)	1	5.3	15.6	26.6	4.6	x	28.7	1.4	13.5	3.4
Feces (120 h)	24.3	x	x	39.6	x	18	x	x	x	18
Urine (24 h)	x	18	x	46.1	2	x	x	x	13.6	20

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Evaluation of PK:

I questioned the sponsor about the 6-fold difference in the half life estimates in two dog experiments. They responded that the estimates may have been based on data points that were in error due to the fact that they were influenced by the distribution phase of the drug or that they were near the limit of detection of the assay resulting in low or high estimates of half-life respectively.

These confounding factors could have influenced the PK estimates described in all of the above studies. For this reason, all of the PK estimates may contain several fold errors due to technical limitations. Considering the fact that the dose of this drug used clinically must be titrated to the patients individual response, the exact PK parameters in various species are only important for cross species comparisons of toxicity. These ratios can often be reasonably estimated based on mg/m² ratios. The ratios of exposure/dose based on PK estimates described above are compared to the ratios generated by mg/m² data above. It is clear that the averaged results of the PK estimates generate similar ratios of exposure levels to the estimates based on mg/m². Instead of, requesting a complete description of how each data point was selected for the calculations used in the PK experiments, and then requesting a reevaluation of the data using more sophisticated mathematical models and computations, we decided to base cross-species comparisons on mg/m² ratios

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Initial Review of Pharmacology Sections of the Label

The following sections of the label should be revised as shown below:

- Original text:
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5. **Carcinogenesis, Mutagenesis, Impairment of Fertility:** Long-term studies in animals to evaluate the carcinogenic potential of paracalcin have not been completed. Paracalcin was not genotoxic in the in vitro microbial mutagenesis assay with and without metabolic activation, in an in vitro mammalian cell mutagenesis assay with and without metabolic activation, in an in vivo mouse micronucleus assay, and in an in vitro human lymphocyte cell chromosomal aberration assay with and without metabolic activation. CaPTHrol had no effect on fertility (male or female) in rats at intravenous doses up to 20 mcg/kg/dose (equivalent to 10 to 125 times the 0.04 to 0.24 mcg/kg human dose based on pharmacologic and pharmacokinetic studies).
 6. **Use in Pregnancy: Pregnancy Category C.** Fertility, general reproduction, embryotoxicity, and peri- and postnatal toxicity studies were conducted in rats and rabbits. Paracalcin had no effect on fertility or early general reproduction indices in rats. Minimal decreases in offspring viability were observed in pregnant rats and rabbits treated with paracalcin, but only at dosages which were maternally toxic. No other effects on offspring development were observed. Paracalcin was not teratogenic.
There are no studies in pregnant women. CaPTHrol should be used during pregnancy only if the benefit justifies the potential risk to the mother and fetus.
 7. **Nursing Mothers:** It is not known whether paracalcin is excreted in human milk. CaPTHrol has not been studied in nursing mothers and should only be given if the benefits to the mother outweigh the potential risks to the infant.

- Revised text:
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5. **Carcinogenesis, Mutagenesis, Impairment of Fertility:** Long-term studies in animals to evaluate the carcinogenic potential of paracalcin have not been completed. Paracalcin did not exhibit genetic toxicity in vitro with or without metabolic activation in the microbial mutagenesis assay (Ames Assay), mouse lymphoma mutagenesis assay (L5178Y), or a human lymphocyte cell chromosomal aberration assay. There was also no evidence of genetic toxicity in an in vivo mouse micronucleus assay. CaPTHrol had no effect on fertility (male or female) in rats at intravenous doses up to 20 mcg/kg/dose _____ human dose based on surface area, mg/m².
 6. **Use in Pregnancy: Pregnancy Category C.** CaPTHrol has been shown to cause minimal decreases in fetal viability in rats and rabbits at doses 2- and 0.4-times the human dose. Fertility, general reproduction, embryotoxicity, and peri- and postnatal toxicity studies were conducted in rats and rabbits at up to 2- and 0.4-times the maximum recommended human dose respectively, based on comparisons of dose/surface area (mg/m²). Paracalcin had no effect on fertility or early general reproduction indices in rats and rabbits. At the highest doses tested, maternal toxicity and minimal decreases in offspring viability were observed in pregnant rats and rabbits. No other effects on offspring development were observed. Paracalcin was not teratogenic.
There are no adequate and well controlled studies in pregnant women. CaPTHrol should be used during pregnancy only if the potential benefit justifies the potential risk to the mother and fetus.
 7. **Nursing Mothers:** It is not known whether paracalcin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when CaPTHrol is administered to a nursing woman.

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Pharmacology Recommendation:

Pharmacology recommends approval of Paracalcin for the treatment of renal osteodystrophy in patients with renal failure providing appropriate revisions are made in the label.

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Daniel T. Coleman, Ph.D.
Pharmacologist.

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Kuijpers

May 4, 1995

Sponsor: Abbott Laboratories, Abbott Park, IL 60064-3537
Date submitted: March 31, 1995

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PHARMACOLOGY REVIEW OF ORIGINAL IND

Drug: 19-nor-1,25-dihydroxyvitamin D₂ Analog Injection
Category: Hormone
Indication: Hypocalcemia and elevated PTH levels in end-stage renal disease

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HFD-510
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Sponsor: Abbott Laboratories
Drug: 19-nor-1,25-dihydroxyvitamin D₂ Analog Injection
Category: Hormone
Indication: Hypocalcemia and elevated PTH levels in end-stage renal disease
Dosage formulation: 1 ml ampul containing 5 µg 19-nor-vitD₂ analog, 20%(v/v) ethanol, and 30%(v/v) propylene glycol in water.
Dosage route: Intravenous
Clinical Status: Phase I

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A. CLINICAL PLANS

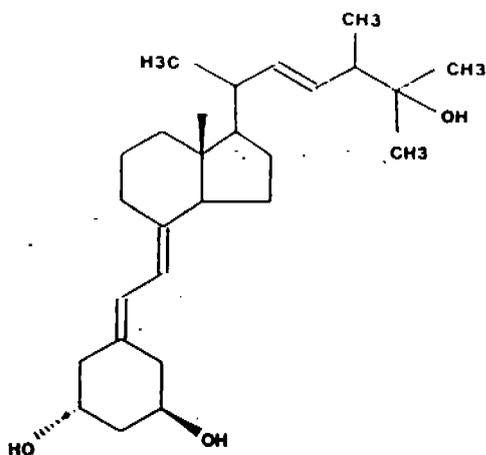
A Phase I study is planned to assess the safety of the 19-nor-vitamin D₂ analog. The study is a placebo-controlled, randomized, escalating dose trial, with 2 groups of 6 healthy volunteers each, in which the dose in each group will be double the previous dose in the alternate group. First, 4 individuals in group A will be given an i.v. dose of 0.005 µg/kg, and 2 will receive placebo. If, within 48 h post dose, the serum Ca levels remain below 11 mg/dl, and the Ca x PO₄ product ≤ 70, the next dose (0.01 µg/kg) will be given to the other group. Similarly, after assessment of Ca and Ca x PO₄, post- and pre-dosing, the groups will receive, alternatingly, doses of 0.02, 0.04, 0.08, 0.16 µg/kg. Volunteers will be on standard diet and will be fasted for ≥ 8 hours prior to dosing. Vital signs will be recorded, and blood and urine samples will be collected up to 48 h post dose, for hematological, clinical chemical and pharmacokinetic measurements. Samples will be frozen and shipped to Abbott Laboratories, IL, for assay of the 19-nor-vitamin D₂ analog.

Calcitriol (Calcijex[®]) is the currently approved vitamin D treatment for the same indication as the present submission, and is given three times a week at i.v. doses between _____ . As with calcitriol, dosing with 19-nor-vitD₂ analog is intended to be done intravenously, three times a week, with an intended maximum dose of 0.3 µg/kg, i.e., ca. 18 µg (6 x maximum dose calcitriol).

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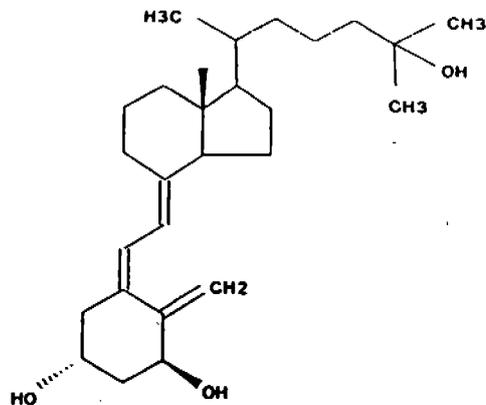
B. CHEMISTRY

The 19-nor-vitamin D₂ analog (19-nor-1 α ,25-dihydroxy-Vitamin D₂, or 19-nor-1,25-(OH)₂D₂) is a synthetic analog of calcitriol (1,25-(OH)₂D₃). It lacks the exocyclic methylene group at the 19th position, and has the steroid side chain of naturally occurring vitamin D₂:



19-nor-1- α , 25-dihydroxyvitamin D₂

(alternatively, 19-nor-1 alpha, 3 beta, 25-trihydroxy-9,10-secoergosta-5(Z), 7(E), 22(E)-triene)



Calcitriol

Molecular formula: C₂₇H₄₄O₃

Molecular weight: 417

Solubility: insoluble in H₂O, soluble in ethanol

C. PHARMACOLOGY

The sponsor presents the following evidence for the pharmacologic action of the 19-nor-vitamin D₂ analog (Investigator's Brochure, Vol.1) :

1. 19-nor-1,25-(OH)₂D₂, just as 1,25-(OH)₂D₃ (calcitriol), has a dose-dependent suppressive effect on PTH secretion in cultured bovine parathyroid cells. The two vitamin D analogs are equipotent in this assay.
2. In nephrectomized, uremic rats, 19-nor-(OH)₂D₂ analog increases total serum Ca. The effect of the analog at 100 ng/rat is similar as the effect of 1,25-(OH)₂D₃ at 10 ng/rat, i.e., an increase in serum Ca from 10 mg/dl to ca. 12.5 mg/dl. Further, 19-nor-(OH)₂D₂ (8-25-75 ng/rat) dose-dependently suppresses uremic rat PTH levels (down to 25% at 75 ng), while the concentration of ionized Ca in serum is not significantly increased. By contrast, 1,25-(OH)₂D₃ at 8 ng/rat (0.03 μ g/kg) suppresses PTH levels similarly as 75 ng (0.3 μ g/kg) 19-nor-(OH)₂D₂, while inducing a small but significant increase in ICa. Also, the 8 ng dose of 1,25-(OH)₂D₃ causes significant hyperphosphatemia (from 5.6 to 8.6 mg/dl), while 8, 25 and 75 ng doses 19-nor-1,25-(OH)₂D₂ do not.
3. 19-nor-(OH)₂D₂ analog _____ suppresses pre-pro PTH mRNA to the same extent as 1,25-(OH)₂D₃ _____.

In conclusion, there is evidence that 19-nor-(OH)₂D₂ has a similar pharmacodynamic effect as 1,25-(OH)₂D₃, namely increasing Ca absorption and serum Ca levels, and, directly and indirectly, suppressing PTH secretion. The 19-nor-vitamin D₂ analog appears to be less potent (2-10x in rats) with respect to both effects. Also, the data show that 19-nor-(OH)₂D₂ causes less hypercalcemia and hyperphosphatemia than 1,25-(OH)₂D₃ in uremic rats.

E. TOXICOLOGY

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ACUTE I.V. TOXICITY STUDY IN RATS

Study Nr. TA93-113 (Vol:4). Study period March 1993 (Abbott Laboratories, IL) Lot Nrs. 93-0083 (vit D₂), 93-0084 (vit D₃).

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PURPOSE

To evaluate the acute i.v. toxicity of 19-nor-1,25-dihydroxyvitamin D₃ and 19-nor-1,25-dihydroxyvitamin D₂ in rats

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PROCEDURES

VAF Crl:CD[®]BR rats (1/sex/dose group), age 6 weeks, weight _____ were treated with a single i.v. dose of 12, 16 µg/kg vit D₂ analog, or 15, 20 µg/kg vit D₃ analog in 30% (v/v) ethanol. As the drug concentration in ethanol was set, the HD level was dictated by the ANOEL (approximate no observed effect level) of the 30% ethanol vehicle. Rats were observed for two weeks after dosing.

RESULTS

Clinical signs - None

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Mortality - None

Body Weight - No effects

Gross Pathology - No drug-related effects

Histopathology - Liver: Mild diffuse congestion and centrilobular vacuolar degeneration in HD m treated with vitamin D₃ analog.

ACUTE TOXICITY

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ANOEL/MLD levels -

		ANOEL	MLD (minimal lethal dose)
Vit D ₂ analog	M	> 16 µg/kg	> 16 µg/kg
	F	> 16 µg/kg	> 16 µg/kg
Vit D ₃ analog	M	15 µg/kg	> 20 µg/kg
	F	> 20 µg/kg	> 20 µg/kg

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ACUTE I.V. TOXICITY STUDY IN MICE

Study Nr. TD93-114 (Vol.4) Study period March 1993 (Abbott Laboratories, IL) Lot Nrs. 93-0083 (vit D₂), 93-0084 (vit D₃).

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PURPOSE -

To evaluate the acute i.v. toxicity of 19-nor-1,25-dihydroxyvitamin D₃ and 19-nor-1,25-dihydroxyvitamin D₂ in mice

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PROCEDURES

VAF CrI:CD-1^R(ICR)BR mice (1/sex/dose group), were treated with a single i.v. dose of 20, 24 µg/kg vitamin D₂ analog, or 25, 30 µg/kg vit D₃ analog. The HD level was dictated by the ANOEL of the 30% ethanol vehicle. Mice were observed for two weeks after dosing.

RESULTS

Clinical signs - None

Mortality - None

Body Weight - No effects

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Gross Pathology - No drug-related effects

Histopathology - No drug-related effects

ANOEL/MLD levels -

	ANOEL	MLD (minimal lethal dose)
Vit D ₂ analog	> 24 µg/kg	> 24 µg/kg
Vit D ₃ analog	> 30 µg/kg	> 30 µg/kg

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ONE-MONTH I.V. TOXICITY STUDY IN RATS (TWO-WEEK RECOVERY)

Study Nr. TA94-051 (Vol.3). Study period July - September 1994 (Abbott Laboratories, IL)
Lot Nr. 94-0584 (85-552-JE).

PURPOSE - To evaluate the i.v. toxicity, and its reversibility, of the 19-nor-vitamin D₂ analog in rats.

PROCEDURES - Cri:CD(SD)BR rats (10 or 15/sex/dose group), _____, _____ were treated three times a week, 2 or 3 days apart, with 0.3, 3, 20 µg/kg in propylene glycol/ethanol/water (1ml/kg). Control group received vehicle alone. 5/sex/group (control, HD) were used for a 2-week recovery study. Food and water were offered ad libitum. Animals were fasted o/n before blood sampling at necropsy.

RESULTS -

Clinical Signs - Purple discoloration of tail during dosing in m and f with incidence 16/30, 17/20, 20/20, 30/30, on 2,3,4,4 days average.

Mortality - None

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Body Weight (*All changes expressed as factor x control value*) - Slight reduction in body weight gain of HD (m) (0.9x, not significant, n.s.)

Food Consumption - No effects

Vital Signs - No data

Ophthalmoscopy - No effects

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Hematology - No effects

Coagulation - Slight dose-related, reversible decrease in aPTT in all dose groups in males (HD 0.8x) and in HD females (0.9x n.s.)

Serum PTH - Dose-related, reversible decrease in all dose groups, in males (0.5x, 0.1x, 0.02x), and females (0.9x n.s., 0.5x, 0.2x)

Clinical Chemistry (*Quantitated changes statistically significant, unless stated otherwise*) -

Males: Drug-dose-related increase in serum Ca in all groups (MD, HD 10%, 20%); Slight increase in P in MD (20%); After recovery, Ca and P back to normal in all dose groups.

Females: No increase in serum Ca; Slight increase in P (HD 1.2x). After recovery, P returned to normal.

Urinalysis - Amber-colored urine and presence of blood in all groups, including control. Incidence: **Males:** ca. 2/3 animals per group; **Females:** ca. 1/2 per group. Both effects were reversible. Likely cause is hemolysis due to propylene glycol vehicle.

ONE MONTH I.V. TOXICITY STUDY IN RATS (0.3-3-20 µg/kg)

Organ Weights - Males: Reversible increase in relative liver weight (1.1x); After recovery, increase in absolute and relative spleen weight (1.3x both).

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Gross Pathology - After recovery, kidney discoloration in 1/5 HD f.

Histopathology (*N/sex = 10-10-10-10-10 in main study, 5-5 in recovery groups*) -

Kidney: tubular basophilia (m 1-1-1-3, f 1-0-0-0), mineralization in corticomedullary areas or distal collecting tubules (nephrocalcinosis) (m 1-1-10-9, f 5-5-9-9), microlith (m 0-0-1-1, f 0-0-1-0). After recovery persistent tubular basophilia (m 1-3, f 0-2), mineralization (m 0-4, f 3-4), microlith (m 0-1, f 0-1).

Aorta: mineralization in 1 HD m, and in 1 HD m after recovery.

Heart: mineralization of arteries in 1 HD m, and in 1 HD m after recovery.

Stomach: mineralization of fundic mucosa in 1 HD m, and in 2 HD m after recovery.

Mesentery: mineralization and periarteritis in 1 HD f.

Lung: osseous metaplasia in 1 HD m.

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ONE-MONTH I.V. TOXICITY STUDY IN DOGS (TWO WEEK RECOVERY)

Study nr. TB94-131 (Vol.4). Study period July - September 1994 (Abbott Laboratories, IL) Lot Nr. 94-0584 (85-552-JE).

PURPOSE - To evaluate the i.v. toxicity, and its reversibility, of the 19-nor-vitamin D analog in dogs.

PROCEDURES - Beagle dogs (3 or 5/sex/dose group), age _____ weight _____ were treated three times a week, 2 or 3 days apart, with 0, 0.1, 0.3, 0.6, 1 µg/kg (control, LD, MLD, MHD, HD) in 30% (v/v) propylene glycol / 20% (v/v) ethanol / water (1 ml/kg). 2/sex/group (control, HD) were used for a 2 week recovery study. From 12 days before dosing, dogs were fed a diet containing 0.2% calcium, 0.2% phosphorus and no added vitamin D.

RESULTS (*N_{total} = 10-6-6-6-10 in main study, 4-4 in recovery study*) -

Clinical signs - Vocalization in 1 MHD, 6 HD, and aggressive behavior in 2 HD. Emaciation with absence of or tarry stools in 1 MHD, 4 HD. Decreased activity in 1 HD, and cold to touch in 1 MHD, 1 HD. Red or dark-colored urine in 1-1-3-3-4, indicating hemolysis. All signs observed on 1-5 days average.

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Mortality - None

Body Weight - Dose-dependent reduction in all but LD groups, more pronounced in males than in females. BW change (as % of BW at start of treatment) reduced from +5% (control) to +1%, -9%, -16% (MLD, MHD, HD*) (m+f avg, *significant). Upon recovery, BW (HD) increased more than BW (control).

ONE-MONTH I.V. TOXICITY STUDY IN DOGS (0.1-0.3-0.6-1 µg/kg)

Food Consumption - Males: Slight, moderate, marked decreases in MLD, MHD, HD.
Females: Moderate decrease in HD. Changes reversed upon recovery.

Vital Signs - Electrocardiograms normal in all dose groups.

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Ophthalmoscopy - No effects.

Hematology - Hemoconcentration in 1 HD m. Mild neutropenia in 1 MLD m, 1 MHD m, 1 HD m and 2 HD f.

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Bone marrow - No abnormalities

Coagulation - aPTT increased 1.2x in MHD, HD (m and f). Partial reversal upon recovery.

Serum PTH (*Changes expressed as factor x control*) -

Pronounced and significant reduction in all dose groups, similar after 2 and 4 wks, in males (0.1x, 0.2x, 0.15x, 0.1x) and in females (0.3x, 0.1x, 0.1x, 0.1x). After recovery, PTH reversed to above control value (1.8x)

Clinical chemistry (*N/sex = 5-3-3-3-5 in main study, N/sex = 2-2 in recovery study*) -

Males -

BUN (Blood urea nitrogen) reversibly increased in 2 MHD and 2 HD; Creatinine reversibly increased in 1 MHD, 2 HD

Ca increased dose-dependently (dd) in all groups, up to 1.5x in HD. Increase at 4 wks slightly less than at 2 wks. Hypercalcemia (Ca > 13 mg/dl) at 2 wks in 0-0-0-2-5, at 4 wks in 0-0-1-3-4 animals. Hypercalcemia completely reversed in 2 weeks

P moderately decreased in MHD, HD, not reversed after recovery

Bilirubin increased in MLD, MHD, HD (2x, 2x, 1.5x, all n.s.), not reversed after recovery;

ALT increased in 1 MHD (3x); AST increased in 1 HD (3x); after recovery, ALT, AST and GGT increased in 1 HD (10x, 3x, 6x) (Group means of ALT, AST, GGT not significantly affected) Latter recovery dog had hepatocellular degeneration.

Females -

BUN reversibly increased in 4 HD; Creatinine increased reversibly in 1 HD

Ca increased dd in all groups, up to 1.5x in HD. Effect at 4 wks slightly less than at 2 wks. Hypercalcemia at 2 wks in 0-0-0-3-5, at 4 wks in 0-0-0-2-3. Hypercalcemia completely reversed after recovery

P slightly decreased in MHD, HD, partially reversed after recovery

Bilirubin increased in 1 MHD, 2 HD (1.3x, 1.2x, n.s. both), reversed after recovery;

ALKPH, ALT and AST increased in 1 HD (2x, 15x, 6x); after recovery, ALKPH, ALT, AST increased in 1 HD (3x, 6x, 2x) (Group means of ALKPH, ALT, AST not significantly affected). Latter recovery dog had hepatocellular damage.

Fractional Ca excretion (*Quantitated changes statistically significant, unless stated otherwise*) -

Males - Dose-dependent increase at 2 wks in all dose groups (4x n.s., 13x n.s., 13x, 55x).

ONE-MONTH I.V. TOXICITY STUDY IN DOGS (0.1-0.3-0.6-1 µg/kg)

At 4 wks, effect enhanced in MLD and MHD, and reduced in HD. Accordingly, hypercalciuria (Ca excretion >0.4%) at 2 wks in 0-0-1-2-5, and at 4 wks in 0-0-^{0.3}2-3. After 2 wks recovery, reversal of effect in 1/2 HD (2x control), but not in other 1/2 (30x control).

Females - Dose-dependent increase at 2 wks in all dose groups (3x n.s., 3x n.s., 17x, 20x). At 4 wks, effect greatly enhanced in MLD, and slightly reduced in MHD and HD. Hypercalciuria at 2 wks in 0-0-0-3-4, at 4 wks in 0-0-2-3. After recovery, reversal in 2/2 HD (2x control).

Urinalysis - At end of treatment, low specific gravity in 2-1-1-3-5, after recovery in 0-1. Glucosuria in 1 HD m, not seen after recovery. Granular casts in 1 HD m, no longer seen after 2 weeks recovery.

Organ Weights - Males: Thymus: Moderate or marked decrease in absolute and relative weight in 1/3 MLD, 3/3 MHD, 3/3 HD, no longer seen after recovery.

Gross Pathology - *Numbers given in parentheses indicate:*

(Incidence in main study, N = 6-6-6-6-6) (Incidence after 2 wks recovery, N = 4-4)

Whole animal thin (0-0-0-2-2) (0-1)

Heart plaque (firm foci above aorta valve) (0-1-4-6-6) (0-4)

Spleen focal discoloration (0-0-0-1-1), not seen after recovery (0-0)

Thymus discoloration (0-0-0-1-0) (0-2)

Kidney focal discoloration in cortico-medullary junction (0-0-0-1-2) (0-2)

Specifically in *males*:

Thymus thin (0-0-0-0-1)(0-0)

Prostate small, especially so at higher doses (1-1-1-1-2) (1-1).

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Histopathology - *Numbers given in parentheses indicate:*

(Incidence in main study, N = 6-6-6-6-6) (Incidence in recovery study, N = 4-4)

Aorta: Inflammation (0-0-1-4-3)(0-4) and mineralization (0-0-3-6-6)(0-4). Effect in males more pronounced than in females.

Kidney: Cortical tubule degeneration (0-0-5-6-6)(0-3), dilatation (0-1-4-4-5)(0-2) and regeneration (0-1-6-6-6)(0-4). De- and regeneration more pronounced in males than in females. Cortical inflammation (0-0-4-5-6)(0-4) and mineralization (0-0-4-6-6)(0-3). After recovery, corticomedullary mineralization (0-2).

Spleen: Mineralization (0-0-0-2-0)(no recovery data)

Liver: Hydropic degeneration (0-0-0-1-0)(0-2). In females, after recovery, bile and hemosiderin pigmentation (0-1)

Lung: Inflammation (0-0-0-1-5)(no recovery data)

Parathyroid: Atrophy, reversed after recovery (0-0-0-1-6)(0-0)

Testis: hypocellularity (0-1-0-2-3)(1-1)

Thymus: Atrophy (0-0-2-3-4)(0-1). In females cysts (0-0-0-1-1)(0-1), and, after recovery, hemorrhage (0-2).

Prostate: Hypoplasia (1-1-0-1-3)(0-1)

Vein injection site: Pigmentation (1-3-3-2-4)(2-2), fibroplasia (1-2-4-5-6)(2-2).

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F. GENETIC TOXICOLOGY

BACTERIAL REVERSE MUTATION ASSAY

Study Nr. TX94-310 (Vol. 4). (Abott Laboratories, IL). Abstract only.

PURPOSE - Evaluation of mutagenic activity of 19-nor vitamin D₂ analog using "Ames" test

METHODS - Pre-incubation test method ± metabolic activation system. Dose range: 1-3000 µg/plate. Three plates/dose. Salmonella strains: TA-1535, TA-1537, TA-98, TA-100. Escherichia coli strain: WP2uvrA-

RESULT - Test compound precipitated at three highest concentrations. 19-nor-vitamin D₂ analog was non-mutagenic in this assay.

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G. SUMMARY AND EVALUATION

The current IND is for the parenteral use of 19-nor-1,25-dihydroxy-vitamin D₂ for the treatment of hypocalcemia and elevated parathyroid hormone levels in end-stage renal disease. Pharmacological studies indicate efficacy to increase serum Ca levels and suppress PTH levels in the nephrectomized rat. The sponsor argues that use of the 19-nor-vitamin D₂ analog, in contrast to the currently approved vitamin D₃ analog, calcitriol (Calcijex[®]), might be associated with a lesser risk of hypercalcemia and hyperphosphatemia resulting from vitamin D treatment. Some evidence for this, in the rat model, is given in the submission. However, clinical trials will have to clarify this issue in humans.

TOXICITY

Toxicity studies reported are two non-conclusive, acute i.v. studies in rats and mice, and two one-month i.v. studies, one in rats (LD,MD,HD) and one in dogs (LD,MLD,MHD,HD). Males are more sensitive to the toxic effects than females. Dogs are much more sensitive to toxicity than rats. Toxicities found are:

1. Agression, emaciation, reduction in body weight and food consumption in MHD and HD dogs.
2. Hypercalcemia in MD and HD male rats, and hypercalcemia and hypercalciuria in MLD, MHD and HD dogs. Marked reversible reduction in serum PTH levels at all doses in rats and dogs. Reversible parathyroid atrophy in MHD and HD dogs.
3. Soft tissue mineralization in kidney, aorta, heart (rat and dog), spleen (dog), stomach and mesentery (rat) at medium and high doses.
4. Increases in BUN and creatinine in some MHD and HD dogs.
5. Increase in bilirubin in some MLD, MHD and HD dogs. Increases in AST, ALT, GGT, ALKPH in a few MHD and HD dogs.
6. Inflammation of kidney cortex, aorta and lung in MLD, MHD and HD dogs. Kidney tubule damage in MLD, MHD, and HD dogs, and liver degeneration, particularly after recovery, in MHD and HD dogs. Liver damage also seen in acute rat study (HD).
7. Decrease in thymus weight associated with thymus atrophy in MLD, MHD and HD dogs.
8. Hemolysis in control and all dose groups in rats, and in MLD, MHD, HD groups in dogs.
9. Local effect: increased or accelerated incidence of injection site discoloration (rats) or pigmentation and fibroplasia (dogs) at all doses.

No mutagenic potential was observed in the Ames bacterial assay, with or without metabolic activation.

COMMENTS:

- A. The reversible hypercalcemia, hypercalciuria, PTH level reduction, and parathyroid atrophy are the expected and desired pharmacological effects of the test substance in normocalcemic animals with normal PTH levels.
- B. The BUN and creatinine level disturbances may be the result of a hypercalcemia-induced decrease in renal perfusion, perhaps due to vasoconstriction. The soft tissue mineralizations, kidney damage, liver enzyme elevations and liver damage, and the inflammatory changes are probably caused by the hypercalcemia and ensuing cytotoxic extra- or intracellular Ca concentrations. The behavioral, body weight and food consumption effects are also most likely the result of hypercalcemia. This means that all toxic effects, except 7, 8, 9, are probably secondary to the hypercalcemia.
- C. The cause and significance of the changes in the thymus are unclear.
- D. The hemolysis is most likely due to the propylene glycol vehicle, which is administered at a dose of 1ml/kg. It is probably of no clinical concern, since the vehicle dose in humans will be only _____ the animal dose.
- E. Locally, the test substance may cause some histological changes.

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CONCLUSIONS:

- 1. Sponsor concludes that the ANOEL (approximate no observed effect level) is **0.3 µg/kg (LD)** in rats, and **0.1 µg/kg (LD)** in dogs. Although, as noted by the sponsor, 1 LD dog had kidney tubule dilatation and regeneration, this reviewer agrees with this conclusion.
- 2. The intended maximal human dose is **0.3 µg/kg**. On a weight basis, this is approximately equal to the ANOEL in rats and 3 x the ANOEL in dogs. However, expressed per unit surface area, this dose would correspond to an approximate **2 µg/kg (MD)** dose in rats, and a **0.6 µg/kg (MHD)** dose in dogs (both 6 x ANOEL). At these doses, many toxic effects do occur, the majority of which are the likely result of the hypercalcemia induced in normocalcemic animals. In the clinical situation, however, the test substance will be used to restore normocalcemia in hypocalcemic patients, and serum Ca and P concentrations will be monitored continuously, so that hypercalcemia can be readily reversed and its associated toxicity prevented by adjustment of dose. Thus, the hypercalcemia-related toxicities observed in the animal studies are of minor relevance for the present indication.

LD

Considering the above, pharmacology has no objection to the proposed clinical studies.

H. TO BE COMMUNICATED TO SPONSOR

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No action indicated at this time.

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Gemma A. Kuijpers, Ph.D.

MAR 7 1997

February 25, 1997

Sponsor: Abbott Laboratories, Abbott Park, IL 60064-3537
 Drug: 19-nor-1,25-dihydroxyvitamin D₂ Analog Injection
 Category: Hormone
 Indication: Hypocalcemia and elevated PTH levels in end-stage renal disease

REVIEW OF PHARMACOLOGY/TOXICOLOGY INFORMATION AMENDMENTS

Submission date	Serial Number	Volume	Studies
October 31, 1995	009	2	-Genotoxicity (HPBL) -PK/Metabolism
December 1, 1995	010	3	-Toxicity dog, IV, 3 mo -Toxicity rat, MTD, SC -Carcinogenicity dose selection rats and mice
January 19, 1996	012	5	-PK/Distribution -PK/Protein Binding -Toxicity rat, IV, 3 mo -Toxicity mice, MTD, SC

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Gemma A. Kuijpers, Ph.D.

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 HFD-510/Steigerwalt/Hedin/Lutwak/Kuijpers
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PHARMACOKINETICS

Sponsor: Abbott Laboratories
Drug: 19-nor-1,25-dihydroxyvitamin D₂ Analog Injection
Category: Hormone
Indication: Hypocalcemia and elevated PTH levels in end-stage renal disease
Dosage formulation: 1 ml ampul containing 5 µg 19-nor-vitD₂ analog, 20%(v/v) ethanol, and 30%(v/v) propylene glycol in water.
Dosage route: Intravenous
Clinical Status: Phase III

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A. INTRODUCTION

Calcitriol (Calcijex[®]) (1,25-(OH)₂D₃) is the currently approved vitamin D treatment for the indication of hypocalcemia and elevated PTH-levels in end-stage renal disease (ESRD). It is given 3x/week at i.v. doses between _____. As with calcitriol, dosing with 19-nor-vitD₂ analog is intended to be done I.V., 3x/week, with an intended maximum dose of 0.32 µg/kg, i.e., ca. 18 µg (6 x maximum dose calcitriol).

Sponsor has presented evidence that 19-nor-(OH)₂D₂ has a similar pharmacodynamic effect as 1,25-(OH)₂D₃, namely increasing Ca absorption and serum Ca levels, and, directly and indirectly, suppressing PTH secretion. The 19-nor-vitamin D₂ analog appears to be less (2-10x in rats) potent with respect to both effects. Also, the data show that 19-nor-(OH)₂D₂ causes less hypercalcemia and hyperphosphatemia than 1,25-(OH)₂D₃ in uremic rats.

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B. CLINICAL STATUS

Phase III studies:

- 95027 A twelve to thirty-two week comparison between 19-nor-1α,25-dihydroxyvitamin D₂ and oral calcitriol
- 95028 A twelve to thirty-two week comparison between 19-nor-1α,25-dihydroxyvitamin D₂ and oral calcitriol
- 95034 A twenty-four week comparison between 19-nor-1α,25-dihydroxyvitamin D₂ and intravenous calcitriol.
- 95035 Multidose evaluation of 19-nor-1α,25-dihydroxyvitamin D₂ in ESRD patients undergoing hemodialysis
- 95036 Multidose evaluation of 19-nor-1α,25-dihydroxyvitamin D₂ in ESRD patients undergoing hemodialysis

PHARMACOKINETICS

95037 Multidose evaluation of 19-nor-1 α ,25-dihydroxyvitamin D2 in ESRD patients undergoing hemodialysis

95022 Multidose evaluation of 19-nor-1 α ,25-dihydroxyvitamin D2 in ESRD patients undergoing hemodialysis

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C. PHARMACOKINETICS

SUMMARY OF THE METABOLISM OF ABBOTT-122358 IN RATS (Vol. 2.1)

No Study Nr., Study period, or Lot Nr. given. Summary presented as Drug Metabolism Clinical Brochure No.1.

METHODS

RPT #12
Sprague-Dawley rats (4/sex) were dosed intravenously with 1.7 μ g/kg [3 H]-labelled compound, in 20% ethanol, 20% propylene glycol and water. Fecal, urinary and biliary excretion were assessed over a 72-h post dosing period. Plasma levels of parent drug were measured over a 12-h period. Metabolites were measured in all excretions.

RESULTS

Fecal excretion over 72 h was 99.5% (m) and 89.5% (f) of dose, urinary excretion 0.7% (m) and 8.6% (f). Biliary excretion was 78% over 24 h in males. Excreted radioactivity in both bile, feces and urine consisted mostly of metabolites (>90%), of which "M3" was major component. Plasma T_x was 7.5 ± 1.3 h (mean, sd), and AUC_{0-12h} was 25 ± 13 ng x h/ml; these were similar in m and f.

IN VITRO DISTRIBUTION OF [3 H]ABBOTT-122358 BETWEEN ERYTHROCYTES AND PLASMA IN HUMAN BLOOD (Vol. 5.1)

Drug Metabolism Report No. 5 (Abbott Labs). Study Period November - December 1995. Lot Nr. 47946-SS-183B. Protocol No. V95-036.

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METHODS

19-nor-vitD₂ analog was labelled with 3 H in position 9 and 11 (48.7 Ci/mmol). Stock solutions were prepared in 40% ethanol with final concentrations of 1, 10, 100, 1000 ng/ml. Purity of solution was checked. Blood samples were taken from 4 adults (2m, 2f), and test substance was added up to concentrations of 0.01, 0.1, 1.0, 10 ng/ml. Samples were incubated for 1 h at 37°C. Whole blood and plasma samples were assayed for radioactivity by ____ Drug was stable in plasma as checked ____ Determined were C_{blood} (dpm/ml), C_{plasma} (C_p)(dpm/ml) and H (hematocrit). C_{rbc} (dpm/ml in RBC) was calculated as $(C_{blood} - [C_p \times (1-H)]) / H$. Fraction of test substance in whole blood bound to RBC was defined as f_{rbc} .

RESULTS

PHARMACOKINETICS

Results given in Table 1. Drug resides mainly in plasma, and fraction bound to RBC was $\leq 2\%$ over whole concentration range. No clear difference between m and f.

Table 1. Concentrations of 19-nor-vitD₂ analog in blood, plasma, and red blood cells.

19-nor-vitD analog (ng/ml)	C _{blood} /C _p (SD in %)	C _{red} /C _p	f _{red}
0.01	0.53 (26%)	0.04	0.02
0.1	0.54 (15%)	0.02	0.02
1	0.56 (11%)	<0.01	<0.01
10	0.55 (9%)	<0.01	<0.01

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CONCLUSION

19 nor-vitD₂ analog does not significantly bind to blood cells.

IN VITRO PROTEIN BINDING OF [³H]ABBOTT-122358 IN MOUSE, RAT, DOG, MONKEY AND HUMAN PLASMA (Vol. 5.1)

Drug Metabolism Report No. 3 (Abbott Labs). Study period October - November 1995. Lot Nr. 47946-SS-183B). Protocol Nr. V95-032.

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METHODS

19-nor-vitD₂ analog was labelled with ³H in position 9 and 11 (48.7 Ci/mmol). Stock solutions were prepared in 100% ethanol with final concentrations of 0.1, 0.5, 2, 10 μ g/ml. Purity of solution was checked by _____. Heparinized blood samples were obtained from mice, rats, beagle dogs, cynomolgus monkeys and man (n \geq 2/sex), and spiked with 1, 5, 20, 100 ng/ml of test substance. Equilibrium dialysis was carried out in dialysis cells with plasma on one side and buffer on the other, and membranes in between with a M_w cut off of _____, for 2 h at 37°C. Samples of plasma and buffer were assayed by _____ and % bound was calculated as [(dpm/ml plasma - dpm/ml buffer)/dpm/ml plasma] x 100%.

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RESULTS

At 100 ng/ml, test substance was 95% stable in plasma. Protein binding was 99.9%-100% in all five species over the whole concentration range _____, in both sexes. Sponsor suggests that test substance may be bound to the globulin vitamin-D-binding-protein (DBP), which is the major binding protein for circulating vitD-metabolites.

CONCLUSION

Plasma protein binding of 19-nor-vitD₂ is highly extensive in all species investigated.

PHARMACOKINETICS

PHARMACOKINETIC PARAMETERS IN RATS AND HUMANS

Submission: Facsimile from Sponsor, February 28, 1996

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Table 1. Pharmacokinetic parameters for 19-nor-vitaminD2 in rats and humans.

	Dose (µg/kg)	C _{max} (ng/mL)*	AUC _(x-y) (ng.h/ml)*	x-y (h)	note
Rat (N=8)	3.0, i.v.	11.7	43	0-24	values: females<males
Rat (N=8)	3.0, s.c.	6.5	71	0-24	females<males
Rat (extrapolated)	1.5, s.c. (HD carcino study)	3.3	21.5	0-24	
Human (N=4, males)	0.16, i.v.	1.15	5.4	0-∞	
Human (extrapolated)	0.3, i.v.	2.15	10	0-∞	

* Standard deviations of C_{max} and AUC values were between 50 and 80% in rats (N=4/sex), and ca. 15% in humans (N=4 males).

C. GENERAL TOXICOLOGY

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THREE-MONTH I.V. TOXICITY STUDY IN DOGS (Vol. 3.1).

Study Nr. TB94-345 (Abbott Laboratories, IL). Study period January - September 1995.
Lot Nr. #94-0584 (19-nor-vitD2), and Lot Nr. #95-0053 (calcitriol).

PURPOSE - To determine toxicity of 19-nor-vitD2 analog in three-month chronic dog study

PROCEDURES- Beagle dogs (4/sex/dose group), age _____, weight _____ were treated three times a week, 2 or 3 days apart, with 0, 0.02, 0.1, 0.3 µg/kg/day (control, LD, MD, HD) of the 19-nor-vitD2 analog in 30% (v/v) propylene glycol and 20% (v/v) ethanol in water (0.2 ml/kg), or with 0.3 µg/kg/day (XD) of calcitriol (Calcijex[®]) (0.15 ml/kg) for 13 weeks. Compound was administered intravenously, via lateral saphenous or cephalic vein (5 ml/min _____). From 3 weeks before dosing, dogs were fed a diet containing ca. 0.5% Ca, 0.4% P, and 0.8 IU/g vitamin D.

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RESULTS - (N_{total} = 8-8-8-8-8)

Mortality - All 8/8 XD terminated due to poor condition on Day 29. 1 HD m each killed moribund on Days 42 and 50. Remaining 6/8 HD terminated on Day 57.

Clinical signs (N_{total} = 8-8-8-8-8 in first, 8-8-8-8 in second, 8-8-8 in third month) -
Abnormal stool in 1-1-0-6-8 in first, in 2-1-2-7 in second, and in 0-0-1 in third month.

THREE-MONTH I.V. TOXICITY STUDY IN DOGS

Dehydration in 0-0-0-1-7 in first, and in 0-0-0-6 in second month. Emaciation in 0-0-0-1-8 in first, and in 0-0-0-6 in second month. Swollen injection site in 5-4-5-6-0 in first, in 1-1-1-0 in second, and in 1-2-2 in third month.

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Body Weight (significant effects) -

Males: BW reduced in HD, XD from 2nd week on, and in MD from 7th week on. BW reduced in XD after 1 mo (0.7x initial weight), in HD after 1 mo and 2 mo (0.85x, 0.7x), in MD after 2 mo and 3 mo (0.95x, 0.9x).

Females: BW reduced in XD from 2nd week on, in HD from 3rd week on. BW reduced in XD after 1 mo (0.65x), in HD after 1 mo and 2 mo (0.85x, 0.7x).

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Food Consumption -

Males: FC reduced in XD from 2nd week on, in HD from 3rd week on. FC non-significantly (n.s.) reduced in XD after 1 mo (0.2x), in HD after 1 mo and 2 mo (0.5x, 0.5x).

Females: FC reduced in XD from 2nd week on, in HD from 3rd week on. FC n.s. reduced in XD after 1 mo (0.15x), in HD after 1 mo and 2 mo (0.4x, 0.15x).

Vital Signs - Electrocardiograms unchanged in LD, MD after three months. HD and XD not examined.

Ophthalmoscopy - No changes in LD, MD after three mo. HD, XD not examined.

Hematology - After 2 months, mild neutropenia in 2/4 HD m and 3/4 HD f.

Bone marrow - No data.

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Coagulation (significant effects) -

Males and females: aPTT increased 1.2x, 1.35x (HD, XD) after 1 month, and 1.3x (HD) after 2 months.

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Serum PTH -

Males and females: Dose- and time-dependent, pronounced and significant reductions in males and females, after 1 month in MD, HD, XD (0.2x, 0.2x, 0.15x), after 2 months in LD, MD, HD (0.4x, 0.05x, 0.05x), and after 3 months in LD, MD (0.45x, 0.1x).

Note: PTH was suppressed in animals with both hyper- and normocalcemia, indicating (also) a direct effect of vitD on PTH secretion.

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Clinical Chemistry -

Males -

Blood urea nitrogen (BUN) increased dose-dependently (dd) and time-dependently (td) in MD, HD, XD, up to 2x in XD @ 1 month (mo), and 2x in MD @ 3 mo. Creatinine increased dd and td in MD, HD, XD, up to 1.3x in XD @ 1 mo. Changes become significant as time progresses and dose increases.

Ca increased dd and td in all dose groups, up to 1.5x in XD @ 1 mo. Changes significant in MD, HD, XD at all times. 1 LD dog had hypercalcemia (Ca > 13 mg/dl) @ 3 mo.