CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 021068 and 18044/S025

PHARMACOLOGY REVIEW(S)

NDA 18-044 S025 & NDA 21-068 **Review Completed: July 21, 1998**

Sponsor: Hoffmann-La Roche Inc, Nutley, NJ 07110-1199
Date Submitted: November 18, 1998
Date Received: November 20, 1998

DRUG: Rocaltrol; Calcitriol; Ro 21-5535; 1 ,25 dihydroxycholecalciferol, 1 ,25-dihydroxyvitamin D_3, (5Z,7E)-9,10-seco-5,7,10(19)-cholestatriene-1 alpha, 3 beta, 25-triol.
CATEGORY: Vitamin D metabolite
CLINICAL INDICATION: Supplement to cover Secondary Hyperparathyroidism in Patients with Chronic Renal Failure.

	TABLE OF CONTENTS	Page #

/S/

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

cc: NDA Arch
HFD510
HFD510/Steigerwalt/Lutwak/Weber
Recommendation code: AP

NDA 18-044 S-025 **Review completed: July 21, 1998**

Sponsor: Hoffmann-La Roche Inc, Nutley, NJ 07110-1199

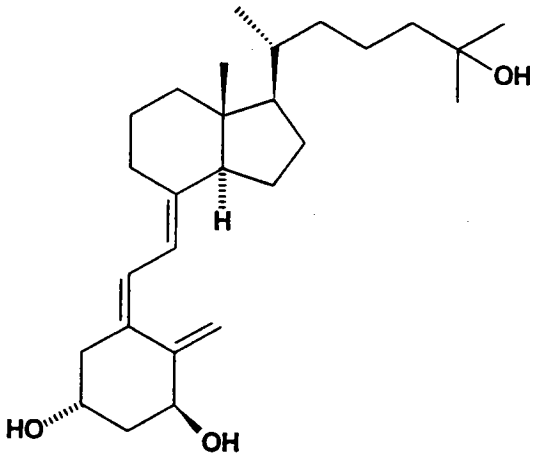
Date Submitted: November 18, 1998 **Date Received:** November 20, 1998

PHARMACOLOGY REVIEW OF NDA SUPPLEMENT
SUPPLEMENT TO NDA 18-044 serial #025 (NOVEMBER 18, 1998)

DRUG: Rocaltrol; Calcitriol; Ro 21-5535; 1 ,25 dihydroxycholecalciferol, 1 ,25-dihydroxyvitamin D_3, (5Z,7E)-9,10-seco-5,7,10(19)-cholestatriene-1 alpha, 3 beta, 25-triol.

CATEGORY: Vitamin D metabolite

STRUCTURAL FORMULA: MW 416.65



CLINICAL INDICATION: Supplement to cover Secondary Hyperparathyroidism in Patients with Chronic Renal Failure. Proposed maximum dose is 0.5 µg/day ~0.01 µg/kg/day ~0.4 µg/m^2. Recommended initial dose is 0.25 µg/kg/day. Dose may be titrated as necessary.

RELATED IND/NDA: Approved NDA 18,044

INTRODUCTION

The current submission seeks approval of Rocaltrol® for treatment of secondary hyperparathyroidism in adult and pediatric predialysis patients with moderate to severe renal failure. Rocaltrol® is a synthetic Vitamin D analog currently labeled for use in management of hypocalcemia and resultant metabolic bone disease in patients undergoing chronic renal dialysis. It is also indicated in the management of hypocalcemia in patients with postsurgical hypoparathyroidism, idiopathic hypoparathyroidism and pseudohypoparathyroidism. Currently, it is not labeled for use in the United States for secondary hyperparathyroidism in patients with chronic renal failure, postmenopausal osteoporosis or vitamin D-dependent rickets although the latter two indications are approved in other countries.

The sponsor indicates that two-year carcinogenicity studies were not conducted to support chronic use since Rocaltrol® is an essential endogenous substance being used in physiologic amounts for replacement therapy. Thus, any potential cancer risk would not be expected to exceed that of a population with normal vitamin D and calcium homeostasis. There are some alternative studies that have been done and submitted that address this issue and are reviewed herein.

The sponsor also indicates that studies in young animals were not conducted with Rocaltrol® because the same formulation will be used in adult and pediatric patients and because extensive clinical data in pediatric patients demonstrate the safety of Rocaltrol® in the pediatric patient population.

REVIEW OF STUDY GCR No. N-127783: COMPARISON OF ACUTE ORAL TOXICITY OF CALCITRIOL AND TRANS-CALCITRIOL IN MICE

NOTE: This report was included in a supplement to NDA 18,044 as a _____ is the _____ of the new synthesis. There are no plans to use calcitriol produced using this _____ in clinical studies until the NDA supplement is approved and the _____ submitted to this IND.

PURPOSE: To determine the acute oral toxicity in mice of the _____ in a _____ of calcitriol _____ compared to the acute oral toxicity of calcitriol _____ prepared using the new _____

EXPERIMENTAL DESIGN: Six CD-1 Charles River mice/sex/group (6-8 weeks old) received a single oral dose of test article in neobee oil (0.5 mg/ml). Mice were observed 14 days for clinical signs and mortality. Doses were 1.0, 2.0 and 4.0 mg/kg for both _____ in volumes of 2, 4, and 8 ml/kg, respectively.

RESULTS

OBSERVED EFFECTS: No clinical signs were observed in Ro22-3790-treated rats. The most prevalent signs resulting from Ro21-5535 treatment were respiratory depression, decreased motor activity, tremors, ptosis and abnormal gait. Except for respiratory depression in the 1.0

and 2.0 mg/kg Ro21-5535 groups which was observed only within the first 6 hours after administration (5/12 and 7/12, respectively), the clinical signs were limited to the high dose Ro21-5535 group.

MORTALITY: 12/12 animals died at the 4.0 mg/kg dose of Ro21-5535. Death occurred between days 4-9 after dosing. No mortality was seen in 1.0 and 2.0 mg/kg groups for Ro22-5535 or at any of the doses of Ro22-3790.

BODY WEIGHT: No significant drug-related changes in body weight gain were seen in survivors of any group during the study period.

SUMMARY

_____ is the _____ in a new _____ ; procedure being submitted to NDA 18,044 as a _____ The purpose of this study was to examine the relative toxicity of the two compounds.

Trans-calcitriol at 1.0, 2.0, and 4.0 mg/kg 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.

REVIEW OF REPORT #N-138376:
A COMPARATIVE ACUTE ORAL TOXICITY STUDY IN MICE OF Ro21-5535 (CALCITRIOL) WITH AND WITHOUT THE IMPURITY Ro26-5787 (THE CIS-METHYL, ETHYL DERIVATIVE)

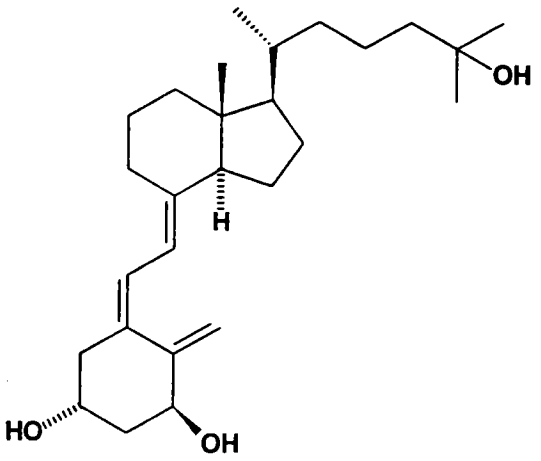
NOTE: Study performed by Dept. of Toxicology and Pathology Nutley, NJ 1/95-2/95. Study report dated 4/25/95. Signed QA and GLP (FDA) statements provided. Calcitriol lot#19862-270A, Calcitriol +0.2%impurity lot #EXP399094.

PURPOSE: To determine the acute oral toxicity in mice _____

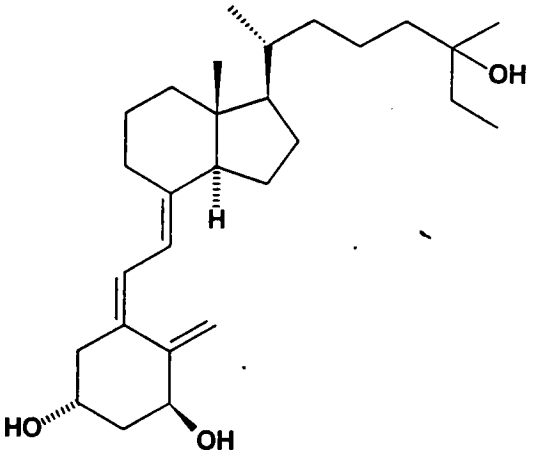
_____

EXPERIMENTAL DESIGN: A single oral administration of either calcitriol or calcitriol + impurity was administered to 6-weel-old mice (6/sex/group) by gavage at doses of 1.0, 2.0 or 3.0 mg/kg and volumes of 2, 4, and 6 ml, respectively. Vehicle was Miglyol 812. Animals were observed for 14 days postdosing.

STRUCTURES OF ROCALTROL AND THE METHYL, ETHYL IMPURITY



Calcitriol



Cis-Methyl, Ethyl Impurity

RESULTS

OBSERVED EFFECTS: Ataxia, decreased motor activity, respiratory depression and tremors were noted predominantly at the 3 mg/kg level in females and 2 mg/kg in males. Effects were similar for both agents.

MORTALITY: 1/12 (females in each case) died in each of the two groups at 1.0 mg/kg; 4/12 (all males for calcitriol, 3 males, 1 female for calcitriol + impurity) died at 2 mg/kg and more than 50% died (11/12 and 8/12) in the two groups at the high dose (6 males, 5 females for calcitriol; 5 males, 3 females for calcitriol + impurity). All deaths occurred 3-6 days after dosing.

BODY WEIGHT: Weight gains occurred similarly in both groups throughout the study.

CONCLUSION:

The impurity at the 0.2% level does not affect the acute oral toxicity of rocaltrol in mice.

There was an amendment to this research report presented as a separate document. It dealt with the stability of the drug substance since it was noted that some samples were allowed to sit at room temperature overnight. The testing laboratory provided a statement that based on previous stability data, these samples were not likely to have been affected by such storage.

REVIEW OF STUDY GCR No.J-146087:
13-WEEK INTRAVENOUS TOXICITY STUDY OF ROCALTROL IN RATS

The following study was performed at Nippon Roche Research Center, Department of Toxicology and Pathology, JAPAN. Study dates 7/90-7/91. Report date 4/19/93. Lot # G006191. Signed GLP and QA statements provided.

PURPOSE: To assess the toxicity of Rocaltrol administered to rats daily for 13 weeks by an intravenous route.

EXPERIMENTAL DESIGN: 4-week-old SD rats (Nippon SLC) were divided into 16 rats/group/sex. Rats were treated for 13 weeks i.v. with Rocaltrol at 0, 0.05, 0.15, and 0.45 µg/kg/day through the tail vein. Volume was 0.2ml/100g body weight. Vehicle contained (per ml) Sodium Chloride (1.5 mg), Sodium phosphate monobasic (9.2 mg), Edetate disodium (1.0 mg), isoascorbic acid (10.0 mg), Polysorbate 20 (4.0 mg), and sodium hydroxide (q.s., pH 7.0 ± 0.1) (H_20 q.s. ad. 1.0 ml). At the end of 13 weeks, 10 animals from each group were sacrificed and examined. The remaining 6 in each group were sacrificed after a 4 week recovery period. Blood samples were collected by cardiac puncture from three hour fasted ether anesthetized rats at both the end of dosing and the end of the 4 week recovery period.

RESULTS

OBSERVED EFFECTS: Local hair rarefaction or loss in a few rats in the 0.15 and 0.45 µg/kg/day groups after one week. Numbers not provided to determine actual incidence.

MORTALITY: No drug-related deaths. One control male died due to a "kidney mass" on day 111 (recovery period).

BODY WEIGHT: Averaged data presented graphically only. No individual data or errors were reported. **Males**: Dose-related decrease in weight gain apparent throughout dosing period in the 0.45 µg/kg/day group. Weight gain resumed during recovery period but did not reach control levels. A similar, but smaller effect on weight gain was seen in the 0.15 µg/kg/day group starting at week 4. Changes in body weights estimated from the graphs at 13 weeks were approximately 30 and 70% decreases for MD and HD respectively compared to controls. **Females**: Similar to males, but overall weight increase in controls was lower than in males, thus there was an overall smaller apparent effect on weight gain. Changes in body weights estimated from the graphs at 13 weeks were approximately 30 and 45% decreases for MD and HD respectively compared to controls. **Food consumption:** Decrease (approximately 1/3) in food consumption in both males and females of high dose group. Slight decrease in the 0.15 µg/kg/day group in both sexes. No water consumption data. No weight loss in any group was reported.

OPHTHALMIC EXAMINATION: At end of dosing period: "focal whitish change of eyes" was observed sporadically in 0.15 and 0.45 µg/kg/day groups. This was also noted at the end of the recovery period. In addition, a whitish opacity of the lens was observed in a female control rat and a female from the 0.05 µg/kg/day group. No changes in eye fundus exam or electroretinograms were observed.

HEMATOLOGY: No individual data presented. Averaged data indicate no obvious dose-related effects. At the end of the dosing period, the only changes considered out of the normal limits were an increase in MCV and MCH in males at 0.45µg/kg/day and a decrease in platelets in females at 0.45 µg/kg/day. (-13% compared to controls). There was a statistically significant increase in lymphocytes and a decrease in eosinophils in females, but these did not appear to be toxicologically significant. At the end of the recovery period, a decrease in RBC and increase in BMC, MCV and MCH in males at 0.15 and 0.45 µg/kg/day and the decrease in RBC and increase in reticulocytes in females at 0.45 µg/kg/day were the only measurements considered outside of normal limits.

COAGULATION: Not presented.

APPEARS THIS WAY ON ORIGINAL

BONE MARROW: Not presented.

BLOOD CHEMISTRY: No individual data presented. Averaged data indicate that serum calcium was increased in both sexes in a dose dependent manner. Other changes considered out of the normal range at the end of dosing were as follows:

	VEHICLE	0.05 µg/kg/day	0.15 µg/kg/day	0.45 µg/kg/day
MALES				
Ca (mg/dl)	10.13	12.08**	13.61**	14.08**
CHOLINESTERASE (IU/L)	77.4	91.1	105.2*	142.5**
TOTAL CHOLESTEROL (mg/dl)	80.8	81.5	99.9**	111.6**
K (mEq/L)	4.25	4.03	3.91*	3.80*
Mg (mg/dl)	2.44	2.30	2.17*	2.08*
BUN (mg/dl)	19.1	18.0	15.6**	13.1**
TOTAL PROTEIN (g/dl)	6.81	6.71	6.61	6.11**
TRIGLYCERIDES (mg/dl)	69.1	78.4	50.8	36.3**
FEMALES				
Ca (mg/dl)	10.51	11.99**	13.33**	13.73**
CHOLINESTERASE (IU/L)	908.6	808.3	446.3**	332.9**
TOTAL CHOLESTEROL (mg/dl)	87.3	94.2	91.7	103.0*
K (mEq/L)	4.28	4.36	4.03	3.61**
Mg (mg/dl)	2.82	2.57	2.53	2.28**
BUN (mg/dl)	16.1	16.2	14.4	12.8**
TOTAL PROTEIN (g/dl)	7.10	6.65	6.37**	6.14**
TRIGLYCERIDES (mg/dl)	41.7	44.9	36.1	33.0

*p≤0.05; **p≤0.01

APPEARS THIS WAY ON ORIGINAL

No changes outside of normal ranges were observed in blood chemistry after the recovery period.

URINALYSIS: At the end of dosing, changes outside normal ranges included: hyaline casts in both sexes at 0.15 and 0.45 µg/kg/day and calcium oxalate crystals, calcium bicarbonate crystals and sodium urate crystals in all drug treated groups. There was not a clear dose relationship in the severity of these occurrences. No significant drug-related changes outside of normal ranges were observed after the recovery period.

ORGAN WEIGHTS: A decrease in relative and absolute weights of reproductive organs (prostate, seminal vesicle, uterus and ovary) was observed at 0.15 and 0.45 µg/kg/day. In addition, an increase in absolute and relative weights of kidney in the females at both of these

doses was observed (a slight decrease in absolute weights of kidneys in males was observed at the 0.15 and 0.45 µg/kg/day dose while there was an increase in relative weights).

A decrease in the relative and absolute weights of the female pituitary was also noted (significant at the 0.15 and 0.45 µg/kg/day dose for absolute weights and at 0.45 µg/kg/day for relative weights)

By the end of the dosing period, a generalized statistically significant decrease in absolute organ weights was found in the following organ systems at 0.15 and 0.45 µg/kg/day in both sexes: brain, pituitary, salivary gland, thymus, heart, lung, liver, and spleen. An increase in absolute adrenal weight was found in male 0.05 and 0.15 µg/kg/day groups. Absolute thyroid weights were decreased in males at all doses. Females exhibited no change in thyroid or adrenal weights. Increases in relative weights were seen for both sexes at 0.15 and 0.45 µg/kg/day doses for the above listed organ systems. These changes were attributed to the decreased body weights of the rats.

During the recovery period, these differences (including the changes in the reproductive organs) returned to near normal or showed significant signs of improvement.

GROSS PATHOLOGY: Presented only in summary. No frequencies were provided for events occurring more than once. At the end of dosing period: "fading/focal whitish change of kidney" and smallness of thymus and reproductive organs noted at 0.15 and 0.45 µg/kg/day (both sexes). At 0.45 µg/kg/day, the following were noted: cystic change of parathyroid, focal whitish change of eyes and osteosclerosis-like change in bone marrow. Also, a focal yellowish change of liver in a male at 0.45 µg/kg/day male was noted. At the end of the recovery period, the whitish change in the eyes was observed sporadically in all dose groups. A whitish opacity of the lens was observed in a control and 0.05 µg/kg/ml female. Also, a kidney mass and a mass in the right epididymis of a male in the 0.15 µg/kg/day group was noted.

HISTOPATHOLOGY: Major organs affected by Rocaltrol administration were kidneys, parathyroids, eyes, bone and reproductive organs. There was a widespread deposition of calcium in a number of tissues including: coronary artery, aorta, thymus, bronchial epithelium (males only), trachea, tongue, stomach, kidney and eyes. Single occurrences of calcifications in the high dose group were also found in the lymphnodes and the alveolar walls of the lung. Interestingly, calcification of the pulmonary artery in controls as well as treated animals of both sexes were observed. This may indicate a predilection of this strain of rat for calcifications. Some thymus atrophy was noted, particularly in high dose males. In addition to calcification, kidneys exhibited a basophilic change in the tubules, a dilatation of distal tubules and some fibrosis and inflammation in the 0.15 and 0.45 µg/kg/day groups. Parathyroid atrophy was obvious at 0.05 µg/kg/day and above in both sexes. This was evident in almost all of the animals in the mid and high dose groups. "Cystic change" was also noted in parathyroids of both sexes at the high dose level. Information is provided on bone (increases in osteoclasts, osteoid and "proliferation of bone") but these are better dealt with in efficacy studies. Lack of information on how the bone changes were quantified limits the usefulness of these observations. A table of dose related occurrences follows:

HISTOPATHOLOGY FROM 13-WEEK I.V. STUDY OF TOXICITY OF ROCALTROL IN RATS.

n=10, end of dosing period.

DOSE µg/kg/day→		0		0.05		0.15		0.45	
FINDING↓	GRADE↓	♂	♀	♂	♀	♂	♀	♂	♀
HEART									
Calcium deposits coronary artery wall	1	0	0	0	0	3	1	5	2
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Aorta: calcium deposits in tunica media									
	1	0	0	0	0	4	1	5	1
	2	0	0	0	0	0	0	1	1
	3	0	0	0	0	0	0	0	0
THYMUS									
Atrophy	1	0	0	0	0	2	1	4	1
	2	0	0	0	0	0	0	2	0
	3	0	0	0	0	0	0	0	0
Medullary Ca deposits	1	0	0	0	0	0	1	1	1
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
LUNG									
Calcification in pulmonary artery wall	1	4	3	4	1	2	3	5	3
	2	0	0	0	0	0	0	0	0
Ca deposits in bronchial epithelium	3	0	0	0	0	0	0	0	0
	1	0		0		3		2	
	2	0		0		0		0	
	3	0		0		0		0	
TRACHEA									
Ca deposits in cartilage	1	0	0	1	0	6	3	3	3
	2	0	0	0	0	2	2	4	5
	3	0	0	0	0	1	0	1	0
Ca deposits in epithelium									
	1	0	0	1	0	2	1	2	1
	2	0	0	0	0	1	1	1	0
	3	0	0	0	0	0	0	0	0
TONGUE									
Ca deposits in arterial wall	1	0	0	0	0	2	2	0	2
	2	0	0	0	0	2	1	1	2
	3	0	0	0	0	2	0	8	2
STOMACH									
Ca deposits in glandular mucosa	1	0	0	1	0	5	2	6	6
	2	0	0	0	0	2	1	2	2
	3	0	0	0	0	0	0	1	0

KIDNEY									
Basophilic change in tubules	1	2	0	5	0	2	3	4	5
	2	0	0	0	0	5	2	5	3
	3	0	0	0	0	2	1	1	2
Ca deposit in cortex	1	0	0	1	0	4	3	5	1
	2	0	0	0	0	4	0	3	3
	3	0	0	0	0	0	1	2	3
Ca deposit in medulla	1	0	0	3	5	3	0	1	2
	2	0	0	3	2	6	4	4	3
	3	0	0	0	1	1	5	5	5
Ca deposit in papilla	1	0	0	1	2	7	6	6	6
	2	0	0	0	1	0	1	2	4
	3	0	0	0	0	0	0	0	0
Dilatation of distal tubules	1	0	0	0	1	5	4	3	6
	2	0	0	0	0	3	2	7	4
	3	0	0	0	0	0	0	0	0
Medullary fibrosis	1	0	0	0	0	0	0	1	2
	2	0	0	0	0	0	0	1	0
	3	0	0	0	0	0	0	0	0
Interstitial fibrosis	1	0	0	0	0	1	0	4	2
	2	0	0	0	0	1	1	2	1
	3	0	0	0	0	0	0	0	1
Small round cell infiltration of interstitium	1	0	0	0	0	5	2	3	5
	2	0	0	0	0	1	0	1	1
	3	0	0	0	0	0	0	0	0
ADRENAL									
Lipid droplets in zona fasciculata	1	0	0	0	1	3	0	2	2
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
THYROID									
Large follicles	1	0	0	0	2	1	2	2	2
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
PARATHYROID									
Cystic change	1	0	0	0	0	0	1	3	2
	2	0	0	0	0	0	0	3	1
	3	0	0	0	0	0	0	1	2
Atrophy	1	0	0	4	5	10	9	4	7
	2	0	0	0	0	0	0	2	1
	3	0	0	0	0	0	0	2	2

EYE									
Ca deposit conjunctiva	1	1	0	4	2	1	3	0	1
	2	0	0	0	0	0	0	1	1
	3	0	0	0	0	0	0	0	0
Ca deposit corneal epithelium	1	0	0	2	2	3	4	3	1
	2	0	0	1	0	5	1	2	3
	3	0	0	0	0	0	0	4	2
Inflammatory cell infiltration in conjunctiva	1	1	0	1	0	1	2	3	1
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
BONE (femur marrow)									
Osteoclasts	1	0	0	0	Q	6	6	4	5
	2	0	0	0	0	1	2	2	2
	3	0	0	0	0	0	0	0	1
Proliferation of osteoid	1	0	0	0	0	1	1	4	3
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Proliferation of bone	1	0	0	2	0	3	4	2	5
	2	0	0	0	0	6	5	5	2
	3	0	0	0	0	1	1	3	3
BONE (sternum)									
Osteoclasts	1	0	0	0	0	7	7	4	7
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Proliferation of osteoid	1	0	0	0	0	2	1	4	2
	2	0	0	0	0	0	0	3	1
	3	0	0	0	0	0	0	0	0
Proliferation of bone	1	0	0	5	3	4	6	0	1
	2	0	0	0	0	5	4	5	7
	3	0	0	0	0	1	0	5	2
SEMINAL VESICLE								5	
Immaturity	1	0		0		2		0	
	2	0		0		1		0	
	3	0		0		0			
PROSTATE									
Immaturity	1	0		0		1		2	
	2	0		0		0		0	
	3	0		0		0		0	

OVARY								
Corpus luteum size	1		0		0		3	6
	2		0		0		0	0
	3		0		0		0	0
Proliferation of interstitial gland cells	1		0		0		6	9
	2		0		0		0	0
	3		0		0		0	0
UTERUS								
Hypofunctional change	1		0		0		4	6
	2		0		0		0	0
	3		0		0		0	0
VAGINA								
Hypofunctional change	1		0		0		4	6
	2		0		0		0	0
	3		0		0		0	0

After the recovery period, most drug related histopathological changes showed considerable improvement, most notably the parathyroid atrophy. Some decrease in number of occurrences were found for calcium deposition in the tongue and stomach, but were only slightly improved in kidney or eyes.

SUMMARY

Major toxicological changes from the daily administration of Rocaltrol in the thirteen week study in rats 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 gain 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. In both sexes, there was a statistically significant increase in total cholesterol. Cholinesterase was increased in males and decreased in females. In both sexes, there was decreased potassium, magnesium, BUN and total protein. Triglycerides were decreased significantly in males and there was a nonsignificant trend for decrease in this parameter for females.

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.

REVIEW OF STUDY GCR No. J-146088:
26-WEEK INTRAVENOUS TOXICITY STUDY OF ROCALTROL WITH INTERMITTENT ADMINISTRATION IN RATS

The following study was performed at Nippon Roche Research Center, Department of Toxicology and Pathology, JAPAN. Dated 4/9/93. Study dates 5/91-11/92. Lot #G006191 Signed QA and GLP statements provided.

PURPOSE: To assess the toxicity of Rocaltrol administered intravenously in rats for 26 weeks on an intermittent schedule.

EXPERIMENTAL DESIGN: 4-week-old SD Rats (Nippon SLC) were divided into 10 rats/group/sex. These were treated i.v. for 26 weeks on an intermittent schedule (Mon./Wed./Fri.) at doses of 0.05, 0.15, 0.45, and 0.9 µg/kg/dose. Volume was 0.2ml/100g body weight. Vehicle contained (per ml) Sodium Chloride (1.5 mg), Sodium phosphate monobasic (9.2 mg), Edetate disodium (1.0 mg), isoascorbic acid (10.0 mg), Polysorbate 20 (4.0 mg), and sodium hydroxide (q.s., pH 7.0 ± 0.1) (H_20 q.s. ad. 1.0 ml). All animals were sacrificed at the end of 26 weeks.

RESULTS

OBSERVED EFFECTS: Local hair rarefaction or loss in all groups, including controls. Incidence was said to increase in a dose-dependent fashion, but no numbers were given.

MORTALITY: No drug-related deaths. A 0.45 µg/kg/dose male died due to an accident.

BODY WEIGHT: Averaged data (no individual values or errors) presented graphically only. There was a trend in both sexes for a decrease in weight gain with increasing dose (decrease in body weights of 25% in males and 20% in females estimated from graph compared to controls). This was most evident at 0.45 and 0.9 µg/kg/dose. Weight gain reached a plateau in all groups by 13 weeks into the study. High dose groups remained at a lower plateau weight than the control or low dose groups. It was noted that a decrease in weight gain was noted on the day after each dose. However, there was some regaining of the loss over the extended time between dosings between a Friday dose and a Monday dose. Overall decrease in weight gain was less severe than the 13 week daily administration toxicity study. Beyond these fluctuations, there was no apparent weight loss when groups were averaged. It was noted that food consumption was decreased the day after dosing. This recovered over the weekend and usually resulted in an increase in food consumption on Monday. This fluctuation was noted each week in both sexes, particularly at the 0.45 and 0.9 µg/kg/dose groups. Water consumption was not reported.

OPHTHALMIC EXAMINATION: A "focal whitish change" of the eyes was observed sporadically in both sexes at 0.15 µg/kg/dose and higher. A corneal erosion was noted in a male of 0.45 µg/kg/dose. A superficial diffuse keratitis of the cornea was noted in all groups (including controls). This was attributed to the use of an animal holder during dosing. No drug-related changes were noted in either the eye fundus examination or the electroretinograms.

HEMATOLOGY: Only averaged data presented. Most changes were determined to be within normal limits except for an increase of BMC in females at the 0.45 and 0.9 µg/kg/dose groups. There was an insignificant trend for increased BMC in males.

COAGULATION: Not presented.

BONE MARROW: Not presented.

BLOOD CHEMISTRY: Changes outside of normal limits included an increase in calcium in all animals treated with rocaltrol and the total cholesterol in males at 0.45 and 0.9 µg/kg/dose. A slight decrease in total protein in males at 0.9 µg/kg/dose was noted as well as a decrease in total protein and albumin in females at 0.45 and 0.9 µg/kg/dose in females. A decrease in cholinesterase in females at 0.9 µg/kg/dose was also reported. These correlate well with the findings in the 13-week study.

URINALYSIS: Changes outside of normal limits included hyaline cast in both sexes at 0.15 and higher µg/kg/dose, calcium oxalate crystals, calcium bicarbonate crystals and sodium urate crystals in all drug treated groups. There was not a clear dose relationship in the severity of these occurrences.

ORGAN WEIGHTS: Most of the changes in organ weights can be attributed to the decrease in weight gain, although the lack of individual data make this difficult to determine. For changes in organ weights that cannot be attributed to decrease in weight gain, most notably, an increase in the relative kidney weight in females of 0.15 µg/kg/dose and above and the decrease in uterine absolute and relative weight in the 0.9 µg/kg/dose group, there is good correlation with the results obtained in the 13 week toxicity study. Data for male reproductive organs do not correlate as well although absolute weights of epididymis and seminal vesicles do decrease. Relative weight of kidneys also increased in the males in a dose-dependent manner. The overall lack of effect compared to the 13 week study even at the higher dose levels suggests that the intermittent schedule may allow enough recovery to counter the many effects seen in the shorter study.

GROSS PATHOLOGY: Presented as summary only. A "focal fading whitish change" of kidney and smallness of uterus were observed in female rats of 0.45µg/kg/dose and over. Small testes and epididymis were noted in a male at 0.9µg/kg/dose.

HISTOPATHOLOGY: Calcium deposits were noted in both sexes as follows:

0.05 µg/kg/dose and over:
- kidney medulla and papilla
- trachea (cartilage)
- eye

APPEARS THIS WAY
ON ORIGINAL

0.15 µg/kg/dose and over:
- kidney cortex
- stomach mucosa (male only)
- tongue (arterial wall)

0.45 µg/kg/dose and over:

heart
lung (bronchial/bronchiolar epithelium)
stomach muscular layer (male only)
aorta (tunica media)

Also noted were: basophilic change of kidney tubules and small round cell infiltration in the interstitium in males at 0.05µg/kg/dose and above and females at 0.45µg/kg/dose and above, dilatation of distal tubules in males at 0.15µg/kg/dose and females at 0.45µg/kg/dose and above, fibrosis of the interstitial space in males at 0.45µg/kg/dose and females at 0.9µg/kg/dose, cystic change in parathyroid in a single males and females at 0.9µg/kg/dose and parathyroid atrophy in males and females at 0.15µg/kg/dose and above. In females at doses of 0.45 µg/kg/dose and over there was a hypofunctional change in uterus and vagina as well as a decrease in corpus luteum size. Both sexes exhibited an increase of lipid droplets in the zona fasciculata of the adrenal glands at 0.9 µg/kg/dose. Information is provided on bone (increases in osteoclasts, osteoid and "proliferation of bone") but these are better dealt with in efficacy studies. Lack of information on how the bone changes were quantified limits the usefulness of these observations. Dose related occurrences of brown pigment deposits in the red pulp of the spleen were observed in males to a greater extent than in females. Eosinophil leukocyte infiltration of the iris and cornea were also observed in males of the 0.45 and 0.9 µg/kg/dose groups.

SUMMARY

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 exposure to the maximum proposed human dose based on surface area comparisons.

COMBINED SUMMARY OF RAT TOXICOLOGY STUDIES

Toxicity of Rocaltrol was tested by intravenous administration to rats in a 13-week study at 0.05, 0.15 and 0.45 µg/kg/day. This study included a four week recovery period. Another study tested the toxicity of Rocaltrol when administered intravenously on an intermittent (3X/week) schedule at doses of 0.05, 0.15, 0.45 and 0.9 µg/kg/dose for 26 weeks. Results were similar between the two studies:

a. No drug-related mortalities occurred in either study.

b. Local hair rarefaction was noted in all groups. This may have been dose related, but individual data were not provided.

c. A decrease in body weight gain and food consumption was noted in both studies and was dose related (30 and 70% for 0.15 and 0.45 in the 13 week study). This appeared to be less severe in the 26 week study (~20-25% at doses >0.45µg/kg).

d. In both studies, the primary toxic effects appeared to be due to hypercalcemia. Elevated serum calcium was observed in all drug-treated groups. There was a dose-related incidence in calcium deposition in soft tissues. The kidney and eye appeared to be particularly sensitive. Parathyroid atrophy was noted at 0.05 µg/kg in the 13 week study (0.15 µg/kg in the 26 week study) and occurred in nearly all animals at the higher doses. A "cystic change" in the parathyroid was noted at high doses in both studies.

e. In both studies, there was a dose-related increase in total cholesterol, a decrease in total protein (males and females) and a decrease in cholinesterase in females only in the 26 week study. In the 13 week study, cholinesterase was increased in males and decreased in females. In males and females, there were decreases in potassium, Mg, and BUN.

f. Primary changes in urinalysis in both studies included hyaline casts, calcium oxalate crystals, calcium bicarbonate crystals and sodium urate crystals, although there was not a clear relationship between dose and severity of occurrence.

g. In both studies, an increase in relative kidney weight was observed in females at 0.15 µg/kg and above. In the 13 week study, there was a decrease in absolute and relative weights of prostate, seminal vesicle, uterus and ovary at 0.15 µg/kg and above. This was not seen as clearly in the 26 week study.

h. In addition to calcium deposits, there was a dose related basophilic change in the kidney tubules, a dilatation of distal tubules and some fibrosis and inflammation of the kidney.

i. Most of the effects listed above improved considerably during the four week recovery period after the 13 week study. The toxic responses appeared to be less severe in the 26 week study, perhaps because of the intermittent drug administration protocol.

j. NOAEL is <0.5µg/kg/day in the 13 week study and =0.5 µg/kg/dose in the 26 week study

REVIEW OF REPORT #B-162489: WHOLE BODY AUTORADIOGRAPHIC STUDY WITH Ro21-5535 IN RATS AFTER A SINGLE INTRAVENOUS ADMINISTRATION

NOTE: Study dates: 12/93-4/94. Report dated 6/28/94. Signed GLP and QA statement provided.

PURPOSE: To assess qualitatively the disposition of ^{3}H-Ro21-5535 in rats.

EXPERIMENTAL DESIGN: Single i.v. dose of 12 µg (1.2ml)/kg of ^{3}H-Ro21-5535 to 4 Sprague-Dawley (Füllinsdorf) rats/sex. 48.7 µCi/µg). Distribution was assessed at 0.5, 6, 24, and 72 h postdosing (1 animals/sex/timepoint). The left half of each animal was submitted for autoradiography, the other half was dissected and the total radioactivity was analyzed by tissue combustion and subsequent _____. Urine and feces were collected to assess extent of excretion.

RESULTS: 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).

REVIEW OF REPORT #J-146362:
13-WEEK I.V. TOXICITY STUDY OF Ro21-5535 IN BEAGLE DOGS

NOTE: Study performed by Dept. of Toxicology and Pathology Nippon Roche Research Center. Kamakura JAPAN. Study dated 6/90-6/91. Signed QA and GLP (Japanese) statements provided. Lot # G006091

PURPOSE: To examine the toxicity of rocaltrol administered i.v. to dogs for 13 weeks.

EXPERIMENTAL DESIGN: 5/sex/group 6 month-old beagle dogs were administered 0, 0.025, 0.05 and 0.1 µg/kg/day for 13 weeks. Injection volume: 0.1 ml/kg. Note: Due to excessive toxicity, the high dose group was discontinued on day 56 and permitted a further 5-week recovery.

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 emaciation was observed in the high dose group and in most of the animals of the mid dose group.

There was no irritation at the injection site.

MORTALITY: Three high dose animals died. One male died on day 50, one female died on day 55 and one male died on day 58. Gross observations revealed hemorrhage of stomach and congestion of intestines. Histopathologically, calcification was observed in the elastica of the aorta and pulmonary artery. On day 56, treatment was discontinued in the high dose group due to excessive toxicity. These animals were still followed through the treatment and recovery periods.

BODY WEIGHT: Clear reduction of body weight in mid- and high-dose groups. For the high dose group, this was 47 and 42% for males and females, respectively, on week 8 (body weight loss). By the end of the administration period (after being off drug for 5 weeks, since treatment of the high dose group was discontinued early) the loss was 32 and 35% for males and females, respectively, of their initial weights. Weights returned to _____ of initial weights after the 4-5 weeks scheduled recovery period. No significant change in weights were noted in the low dose males, although low dose females tended to lose weight after week 5-6

FOOD CONSUMPTION: Severe anorexia was observed in mid and high dose groups. An immediate and striking increase in food consumption was apparent in parallel with body weight regain.

VITAL SIGNS: No treatment-related change in ECG or neurological tests.

OPHTHALMIC EXAMINATION: No treatment-related effects.

HEMATOLOGY: Increased Hb, Hct, RBC, platelets, monocytes in mid and high dose groups (these were not all clearly progressive or dose-related). Decreased eosinophils were observed in mid and high dose groups. All tended to recovery after treatment withdrawal.

COAGULATION: No treatment-related changes in PT or APTT.

BONE MARROW: No data.

BLOOD CHEMISTRY: Increase in BUN, Ca, and total cholesterol in the mid and high dose groups. Decrease in Mg, Cl and A/G ratio in mid and high dose groups. Slight increase of calcium level in the low dose group. In addition, there was an increase in mean ALP in high dose group after day 63 which returned to normal for the males, but not for females at the end of the recovery period. Slightly decreased ALB and increased TG were also noted in high dose males and females.

URINALYSIS: Slight decrease of pH and specific gravity in mid dose group.

ORGAN WEIGHTS: Absolute weights: Decreased thymus and prostate in the mid dose group. Decreased heart, testis, epididymides, prostate and pituitaries in high dose and especially the mid-dose groups. Increased relative organ weights of pituitary, adrenal, lung, liver, kidney, spleen and ovaries were observed in the mid dose group. Increased thymus, lung and liver weights were observed in the high dose group. These increases are likely due to the weight loss observed in these groups. There were no clear dose-related changes that were not due to weight loss.

GROSS PATHOLOGY: Calcification of aorta and/or pulmonary artery in the mid and high dose groups. Involution of thymus, testes and prostate were observed in the mid dose group. Ossification of femur was observed in the middose group.

HISTOPATHOLOGY: Most significant findings relate to calcification of tissues in high and mid dose groups. These included:
HEART: (endocardium, myocardium, coronary arterial wall, aortic wall and pulmonary arterial wall) with secondary change of thickening of endocardium and tunica intima of coronary artery. Thickening of tunica intima of the coronary artery was not recovered at the end of the recovery period.
KIDNEY: Calcification in cortex and medulla in all treated groups. Chronic nephropathy (basophilic change of proximal tubules, dilatation of distal tubules, fibrosis in interstitium and small round cell infiltration in the interstitium were noted in all treated groups. In recovery groups, calcification was noted in the low and mid dose groups. The nephropathy showed partial recovery, but fibrosis in interstitium and small round cell infiltration were not recovered.
LUNG AND TRACHEA: Calcification in alveolar wall, bronchial cartilage and bronchial/bronchiolar epithelium of lung and epithelium and cartilage of trachea in the mid dose group.
SALIVARY GLANDS: Calcification noted in submandibular glands in all groups, including control, although there was a dose-dependent aspect to this. Atrophy of acini in females of low and mid dose groups. Partial recovery of atrophy occurred during recovery period.
THYROID AND PARATHYROID: Hyperplasia of parafollicular cells in mid and high dose groups. Atrophy of parathyroid cells in low and mid dose groups. Hyperplasia of parafollicular cells did not recover in recovery groups.
BONE: Proliferation of bone (osteoid and fibrous tissue in marrow in mid and high dose groups measured in femur and sternum. Increase in osteoclasts in high dose group. Partial recovery of proliferation and osteoid during recovery period, however, the increase of osteoclasts did not recover in a mid dose female.
REPRODUCTIVE ORGANS: Hypofunctional changes in testes, epididymides, prostate, ovary and uterus in mid and high dose groups. These changes improved during the recovery period.
THYMUS: atrophy in low and mid dose groups. increase of lymphocytes in cortex and medulla in the high dose groups. These changes did not recover.
LIVER: Brown pigment deposits in Kupffer cells in all treated groups. This improved during the recovery period.
EYE: Basophilic granules in retina was dose-dependently shown in control and treated groups.
OTHER: Calcification in choroid plexus of cerebellum and medulla oblongata and glandular stomach mucosa. The calcification of the glandular stomach did not recover in the mid dose group during the recovery period.

NOAEL is estimated at _____. The NOAEL represents a slightly lower exposure to the maximum proposed human dose based on surface area comparisons.