

**Reviewers Overall Summary and Conclusions of
Genotoxicity and Carcinogenicity Studies:**

- 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.
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**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
Fertility and General Reproduction Study of Paracalcin Male and
Female Rats: Study No. TA 95-206**

Purpose:

To evaluate the effects of Paracalcin on fertility and early embryonic development in rats dosed IV three times per week, prior to mating and through early gestation. Males, females and embryos were examined after Day 13 of Gestation.

Experimental design:

Testing Facility: Drug Safety Evaluation Division, Abbott Labs, Abbott Park, IL.
Study #: TA 95-206
Study Initiated: August, 95.
Study Completed: Jan. 26, 96.

Dose & Formulation: 0, 0.3, 3 & 20 ug/kg/dose, IV, in 20% EtOH, 30% Propylene Glycol/Water. Three times per week. Males: 4 weeks prior to and 4 weeks after mating. Females: 2 weeks prior to mating, while mating, and until gestation day 7.

Batch of drug: lot 96-016-IE

GLP statement: Included.

Animals: 280 CrI:CD(SD)BR rats

| <u>Group:</u> | <u>Dose (ug/kg):</u> | <u># of Animals:</u> |
|---------------|----------------------|---------------------------------|
| 1 | 0 | 24 + 5 Males and 24 + 5 Females |
| 2 | 0.3 | 24 + 5 Males and 24 + 5 Females |
| 3 | 3.0 | 24 + 5 Males and 24 + 5 Females |
| 4 | 20.0 | 24 + 5 Males and 24 + 5 Females |

All females were killed on day 13 and mothers and fetuses examined. All males were killed after the last female was killed (4 weeks after mating).

Satellite rats (5) from each group were used for blood collection to measure serum calcium and PTH levels.

Pharmacokinetic measurements were not made.

Dose Selection:

No rationale provided. However, the doses are the same as in the 1 month rat toxicity study in which the low dose was NOAEL and the high dose yielded kidney and soft tissue mineralization and diminished weight gain after one month. This high dose (20 ug/kg) was _____ the expected _____ human dose (on a mg/kg basis) at the time the study was conducted. Since then, the recommended initial human dose (in the proposed label) has been reduced to _____. The high dose (120 ug/m²) is 13.5 times the highest recommended initial human dose (8.88 ug/m²), on a mg/m² basis.

Results and discussion:

Observed effects:

There was no significant drug related effect. There were some injection site reactions, matted fur and reddening of the urine (an effect of hemolysis due to the propylene glycol vehicle) in all animals including controls.

Mortality:

No deaths reported. No animals were sacrificed prematurely.

Body Weight:

In males the low and mid dose group gained slightly (but statistically significantly) more weight than the controls. The high dose group did not gain significantly more than the controls and none of the differences in weight were toxicologically meaningful. There was no effect on body weight or weight gain in female rats.

Food Consumption:

In all groups there was no effect on food consumption.

Blood Chemistry:

Mid and high dose animals had slight but statistically significantly elevated serum calcium (11 mg/dl → 13 mg/dl). All drug treated animals had dramatically reduced serum PTH levels. These are expected pharmacological actions of the drug.

Estrous Cycles:

There were no effects on estrogen levels or estrus.

Reproductive Evaluation:

No drug related changes were noted in any reproductive indices: Copulation, Fertility, Fecundity and Copulatory Interval.

Uterine observations:

Number of corpora lutea, implantation sites, viable fetuses, dead fetuses, resorptions and pre and post implantation losses were unaffected by exposure to drug.

Summary and Conclusions:

All doses of Paracalcin tested dramatically reduce PTH levels in rats, consistent with the dose being _____ human dose. No significant toxicity was observed in these animals during the course of this study. No toxic or adverse effect on reproduction or fertility was observed in this study.

**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
Evaluation of the effects of IV Paracalcin on the embryonic and fetal
development of the rat. Segment II (DART). TA 95-258**

Purpose:

To evaluate the effects of Paracalcin exposure during Gestation Days 6-17 on fetal development in rats. Dams and fetuses were examined on Day 20 of Gestation.

Experimental design:

Testing Facility: Drug Safety Evaluation Division, Abbott Labs, Abbott Park, IL.

Study #: TA 95-258

Study Initiated: December, 1995.
Study Completed: 7/18/96.

Dose & Formulation: 0, 0.3, 1, & 3 ug/kg/day, IV, in 20% EtOH, 30% Propylene Glycol/Water. Gestation Days 6-17.

Batch of drug: lot 95-0520

GLP statement: Included.

Animals: 112 sperm positive CrI:CD(SD)BR rats

| <u>Group:</u> | <u>Dose (ug/kg):</u> | <u># of Animals:</u> |
|---------------|----------------------|----------------------|
| 1 | 0 | 24 + 4 Females |
| 2 | 0.3 | 24 + 4 Females |
| 3 | 1.0 | 24 + 4 Females |
| 4 | 3.0 | 24 + 4 Females |

All Dams were killed on day 20 and mothers and fetuses examined.

Satellite rats (4) from each group were used for blood collection to measure serum calcium and phosphorous levels. On day 17 blood samples were collected at 0, 0.5, 2, 6, 12, and 24 h for drug levels.

Pharmacokinetic measurements were not made.

Dose Selection:

A dose range finding study was conducted (TA 95-201) (not reviewed) in pregnant rats exposed to 0, 0.3, 3, 10, and 20 ug/kg/day on Gestation Days 6-17. Dehydration was noted in 3, 10 and 20 ug/kg/day animals. One 10 ug rat died prematurely and marked weight loss in the 10 and 20 ug/kg/day groups lead to premature euthanasia. As a result the 3 ug/kg/day dose was selected as the HD group for this study.

Observed effects:

There was no significant drug related effect. There were some injection site reactions, matted fur, alopecia and reddening of the urine (an effect of hemolysis due to the propylene glycol vehicle) in all animals including controls.

Mortality:

No deaths reported. No animals were sacrificed prematurely.

Body Weight:

Weight gain was slightly reduced during dosing (GD 9-12) in the 1 ug/kg/day group. Weight gain was virtually halted during dosing (GD 6-12) in the 3 ug/kg/day group, but then paralleled the gains in the other groups during the final days of dosing and after dosing. As a result of these differences in weight gain, the HD (3 ug/kg/day group) weighed significantly less than the other groups from GD 12 onward. No treatment related differences were noted in the LD (0.3 ug/kg/day).

Food Consumption:

There was a significant reduction in food consumption in the HD group during the interval of drug exposure. In both the MD and HD groups food consumption increased dramatically and was greater than in controls during the post treatment interval. No treatment related differences were noted in the LD group (0.3 ug/kg/day).

Blood Chemistry:

All drug exposed groups had slight but statistically significantly elevated serum calcium (11-13 ug/dl). There were no effects on serum phosphorous levels. The effect on calcium is an expected pharmacological action of the drug and not a toxic effect, however it could affect the development of the fetus.

Gross Pathology (maternal):

No drug related changes were noted.

Litter observations:

No treatment related findings although 1 dam in the HD group had complete resorption of her litter. Losses of the remaining dams were not significantly different in any group. Average number, weight and sex of fetuses was unaffected.

| Observation: | Control | 0.3 ug/kg | 1.0 ug/kg | 3.0 ug/kg |
|--------------------|---------|-----------|-----------|-----------|
| # Pregnant to term | 21/21 | 24/24 | 22/22 | 24/24 |
| # implantations | 13 | 14 | 14 | 14 |
| # tot. resorptions | 0/21 | 0/24 | 0/22 | 1/24 |
| # viable fetuses | 13 | 14 | 14 | 14 |
| mean weight (g): | 3.9 | 4.1 | 4.2 | 4.0 |

Fetal Alterations:

External:

No aberrations seen more than once:

Visceral:
 No treatment related findings. Aberrations seen:

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| Abnormality: Group (n) | # of occurrences: | | | |
|-----------------------------|-------------------|---------|---------|---------|
| | C (143) | L (164) | M (155) | H (163) |
| Lung, small lobes | 2 | 3 | 2 | 2 |
| Cryptorchidism | 5 | 6 | 7 | 7 |
| Renal ectopia | 0 | 1 | 1 | 0 |
| Kidney, undeveloped papilla | 0 | 1 | 0 | 0 |
| Kidney, delayed papilla | 0 | 1 | 3 | 2 |
| Kidney, reduced size | 0 | 1 | 0 | 0 |
| Ovarian ectopia | 0 | 1 | 0 | 0 |
| No variations | 136 | 152 | 143 | 152 |

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Skeletal:

No treatment related findings. Aberrations seen more in any drug treated group than control:

| Abnormality: Group (n) | # of occurrences: | | | |
|--|-------------------|---------|---------|---------|
| | C (131) | L (156) | M (146) | H (153) |
| Bent ribs | 0 | 0 | 6 | 0 |
| Pelvic girdle incomplete ossification | 1 | 2 | 0 | 2 |
| Skull incomplete ossification | 19 | 34 | 11 | 5 |
| Sternum incompletely ossified | 57 | 59 | 56 | 67 |
| Vertebral column incompletely ossified | 0 | 0 | 0 | 2 |

Individual incidence of several additional malformations were reported. The distribution and incidence did not suggest a relationship to treatment. There was also no effect on the total incidence of all malformations.

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Summary and conclusions:

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). Although increased serum calcium was noted at the LD (0.3 ug/kg), toxic maternal effects (decreased weight gain) were noted only at the MD (1 ug/kg/day) leaving 0.3 ug/kg/day as the maternal NOEL. The high dose fetuses appeared subject to a possible increased rate of resorption (One entire litter of a total of 24 litters was resorbed. This is consistent with the rabbit Segment II study, where one of 19 litters was resorbed in the HD group).

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**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
Evaluation of the effects of IV Paracalcin on the embryonic and fetal
development of the rabbit. (Seg. II DART) TE 95-267**

Purpose:

To evaluate the effects of Paracalcin exposure during Gestation Days 6-18 on fetal development in rabbits. Dams and fetuses were examined on Day 29 of Gestation.

Experimental design:

Testing Facility: Drug Safety Evaluation Division. Abbott Labs, Abbott Park, IL.

Study #: TE 95-267

Study Initiated: December, 1995.

Study Completed: 7/29/96.

Dose & Formulation: 0, 0.03, 0.1, & 0.3 ug/kg/day, IV,
in 20% EtOH, 20% Propylene Glycol/Water.
Gestation Days 6-18.

Batch of drug: lot 95-0539

GLP statement: Included.

Animals: 96 mated New Zealand White does

| <u>Group:</u> | <u>1</u> | <u>Dose (ug/kg):</u> | <u>0</u> | <u># of Animals:</u> | <u>20 + 4 Females</u> |
|---------------|----------|----------------------|----------|----------------------|-----------------------|
| | 2 | | 0.03 | | 20 + 4 Females |
| | 3 | | 0.1 | | 20 + 4 Females |
| | 4 | | 0.3 | | 20 + 4 Females |

All Does were killed on day 29 and mothers and fetuses examined.

Satellite rabbits (4) from each group were used for blood collection to measure serum calcium and phosphorous levels. On day 19 blood samples were collected at 0, 0.5, 2, 6, 12, and 24 h for drug levels.

Pharmacokinetic measurements were not reported here.

Dose Selection:

A dose range finding study was conducted (TA 95-225) (not reviewed) in pregnant rats exposed to 0, 0.1, 0.5, 2.5, and 10 ug/kg/day on Gestation Days 6-18. Maternal death and soft tissue mineralization was noted in the 2.5 and 10 ug/kg/day animals. All 0.5 ug/kg/day animals had decreased food consumption and weight loss. In this group 1 aborted and 1 resorbed, and all had increased resorptions. As a result 0.3 ug/kg/day was selected as the HD group for this study and 0.03 ug/kg/day is the LD used. The range of doses selected overlaps the intended clinical dose and therefor is actually (about 3-fold) below the intended exposure level based on mg/m² or AUC.

Observed effects:

There was no significant drug related toxicity. There were some injection site reactions, (bruising and discoloration possibly due to the vehicle) in all animals including controls.

Mortality:

No deaths reported. No animals were sacrificed prematurely.

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Body Weight:

Weight gain was similar in all but the HD group. The HD (0.3 ug/kg/day) rabbits lost weight from GD 6→12 and consumed significantly less food than the other groups. After GD 12 food consumption gradually increased in the HD group until it was normal at GD 24. During this time the HD does gained weight at the same rate as the other groups but maintained a consistently lower average weight. No treatment related differences were noted in the LD or MD groups.

Food Consumption:

There was a significant reduction in food consumption in the HD group during the interval of drug exposure. No treatment related differences were noted in the MD or LD group (0.1 and 0.03 ug/kg/day).

Blood Chemistry:

MD and HD rabbits had slight dose dependent increases in serum calcium _____ and phosphorous _____ and decreases in _____. These effects are an expected pharmacological action of the drug and not a toxic effect, however it could affect the development of the fetus.

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Gross Pathology (maternal):

The only potential drug related changes noted were multiple white pinpoint lesions in the kidneys of 1/20 MD and 5/20 HD rabbits. These lesions are consistent with mineralization often encountered with this class of compounds.

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Litter observations:

No treatment related findings except that 1 dam in the HD group had complete resorption of her litter as a result the average number of resorptions in the HD group was significantly greater than in the control group and greater than the highest historical control group. Losses of the remaining dams were not significantly different in any group. Average number, weight and sex of fetuses was unaffected.

| Observation: | Control | 0.03 mg/kg | 0.1 mg/kg | 0.3 mg/kg |
|-------------------------|---------|------------|-----------|-----------|
| # Pregnant to term | 20/20 | 20/20 | 19/19 | 19/19 |
| # implantations | 9 | 10 | 9 | 9 |
| # tot. resorptions | 0/20 | 0/20 | 0/19 | 1/19 |
| # viable fetuses/litter | 9 | 9 | 8 | 8 |
| mean weight (g): | 41 | 42 | 40 | 41 |

Fetal Alterations:

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External:

No aberrations seen more than once:

Visceral:

No treatment related findings. Aberrations seen more than once or more in treated than controls:

| Abnormality: Group (n) | # of occurrences: | | | |
|----------------------------|-------------------|---------|---------|---------|
| | C (178) | L (181) | M (161) | H (144) |
| bulbous vessel | 0 | 0 | 0 | 2 |
| gall bladder agenesis | 3 | 0 | 1 | 2 |
| partial cerebrum agenesis | 0 | 0 | 2 | 2 |
| Pituitary cleft | 0 | 1 | 1 | 0 |
| Lung Azygous lobe agenesis | 10 | 15 | 32 | 16 |
| No variations | 172 | 180 | 157 | 139 |

Skeletal:

No treatment related findings. Aberrations seen more in any drug treated group than control:

| Abnormality: Group (n) | # of occurrences: | | | |
|--|-------------------|---------|---------|---------|
| | C (131) | L (156) | M (146) | H (153) |
| Fused ribs | 1 | 0 | 0 | 1 |
| Hemivertebra | 1 | 0 | 0 | 1 |
| Sternebra fused | 0 | 0 | 1 | 4 |
| Pelvic girdle, pubis incompletely ossified | 0 | 1 | 1 | 2 |
| no ossification delay | 62 | 81 | 55 | 49 |

Individual incidence of several additional malformations were reported. The distribution and incidence did not suggest a relationship to treatment. There was also no effect on the total incidence of all malformations.

Summary and conclusions:

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 MD (0.1 ug/kg/day). The high dose fetuses appeared subject to a possible increased rate of resorption (One of 19 total litters was resorbed. This is consistent with the rat Segment II study, where one litter of 24 litters was resorbed in the HD group). 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 0.03 ug/kg/day as the maternal NOEL.

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**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
A Perinatal and Postnatal Study in Rats with Paracalcin.
(TA 95-277)**

Purpose:

To evaluate the effects of Paracalcin exposure from Gestation Day 6 - Lactation Day 20 on Maternal, fetal and neonatal development as well as the reproductive capacity of the F1 generation in rats. Dams and pups were sacrificed on Day 21 of Lactation except 20 F1 pups/group that were allowed to continue as parental animals. These F1 pups were mated and subsequently examined on Day 13 of their subsequent gestation.

Experimental design:

Testing Facility:

Study #: TA 95-277

Study Initiated: Feb., 1996.

Study Completed: Oct., 1996.

Dose & Formulation: 0, 0.3, 3.0, & 20 ug/kg/day, IV,
in 20% EtOH, 30% Propylene Glycol/Water.
Gestation Day 6 - Lactation Day 20.

Batch of drug: lot 95-0520

GLP statement: Included.

Animals: 125 Sprague Dawley CrI:CD-BR VAF/Plus Females
cohabited individually in nesting boxes with 1 of 125 males (5 Mo. old)

| <u>Group:</u> | <u>Dose (ug/kg):</u> | <u># of Animals:</u> |
|---------------|----------------------|----------------------|
| 1 | 0 | 25 Females |
| 2 | 0.3 | 25 Females |
| 3 | 3.0 | 25 Females |
| 4 | 20 | 25 Females |

All females were allowed to deliver in individual nesting cages.

Nursing and nesting behavior and retrieval were monitored.

On Lactation Day 4 the litters were culled to a maximum size of 8 (4 of each sex when possible).

Pups development was carefully examined.

Dams were killed on Lactation Day 21 and examined.

1 male and 1 female from each litter were selected for breeding.

Nonselected pups were necropsied.

At 12 weeks of age F1 pairs were mated (avoiding siblings).

Weight and behavior was closely monitored.

All F1 animals were necropsied on Gestation Day 13.

Pharmacokinetic measurements were not made and there were no satellite groups.

Dose Selection:

No rationale provided. However, the doses are the same as in the 1 month rat toxicity study in which the low dose was NOAEL and the high dose yielded kidney and soft tissue mineralization and diminished weight gain after one month. This high dose (20 ug/kg) was _____ times the expected _____) human dose (on a mg/kg basis) at the time the study was conducted. Since then, the recommended initial human dose (in the proposed label) has been reduced to _____. The high dose (120 ug/m²) is 13.5 times the highest recommended initial human dose (8.88 ug/m²), on a mg/m² basis.

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Observed effects:

There was no significant drug related toxicity. There was some transient brownish urine in the HD group only.

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Mortality:

No deaths reported. One HD female was killed on Lactation Day 1 due to total litter loss.

Body Weight and Food Consumption:

Weight gain was similar in all but the HD group. The HD (0.3 ug/kg/day) rabbits lost weight on GD 18 and LD 4 and 10. On subsequent days their food consumption and weight gain was increased

Blood Chemistry:

Not examined.

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Gestation, Parturition and Lactation:

No effect on gestation lengths, delivery duration, nesting behavior or lactation.

Litter Retrieval:

Slightly lower in controls. This is not considered meaningful.

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Gross Pathology (maternal):

No remarkable findings in any group or the one euthanized rabbit. From previous studies I would have expected them to find some soft tissue mineralization in the HD dams, but they did not report such a finding. This may have been due to increased maternal need for calcium for the fetus and lactation. The effect may have been missed since the tissue was not examined microscopically.

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F1 Generation:

Litter observations:

No treatment related findings except that 1 dam in the HD group had complete loss of her litter. As a result the HD group had significantly greater percent of losses than controls. This level of losses was not greater than the historical record of losses for this strain of rats however, this does not rule out the possibility that there is a slight drug related effect on pups viability. There were several pups in the HD group that were gasping, cool or purple on day one. These pups were all dead or cannibalized by day 4. Losses of the remaining dams were not significantly different in any group. Average number, weight and sex of fetuses was unaffected.

| Observation: | Control | 0.3 ug/kg | 3 ug/kg | 20 ug/kg |
|------------------------|---------|-----------|---------|----------|
| # Pregnant to term | 25/25 | 24/24 | 25/25 | 24/24 |
| # implantations (avg.) | 16 | 17 | 17 | 16 |
| # tot. resorptions | 0/20 | 0/20 | 0/19 | 1/19 |
| # viable births/litter | 15 | 15 | 15 | 15 |
| losses by day 4 (%) | 1.1 | 2.1 | 2.4 | 4.6* |

Pup Necropsy Observations:

No treatment related findings were reported. Examination seems to have been very superficial.

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Pup Development and Functional Tests:

Pinnae detachment, surface righting response, cliff aversion, eye and vaginal opening, startle and auditory responses were all comparable among all groups.

Pup Behavioral Tests:

No differences in; open field parameters, swimming trials and T-maze tests.

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F1 Survival and Body Weight Changes:

Growth and survival of all groups was similar.

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F1 Copulation, Fertility and Precoital Intervals:

Copulation and fertility indices were comparable among groups. The LD group had a long precoital interval and the HD and MD groups had slightly lower pregnancy rates but these differences are not biologically meaningful.

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Summary and conclusions:

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 the MD (3 ug/kg/day). The high dose fetuses appeared subject to a possible increased rate of resorption; one entire litter of 24 total litters was completely 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 showed transiently decreased maternal weight gain. Increased serum calcium and phosphorous and decreased PTH would probably have been noted, as a direct effect of even the LD, in all treated dams (had it been examined). It would have been interesting to see the effect of increased maternal calcium requirements on this hypercalcemia. Necropsy of F0 generation seems to have been quite superficial because no tissue calcification was noted. Necropsy of the F1 generation also reflected a very superficial examination and adverse effects were found.

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PHARMACOLOGY AND TOXICOLOGY REVIEW
Reviewers Overall Summary and Conclusions of
Reproductive Toxicity Studies:

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 NOEL.

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 NOEL.

In both Segment 2 studies the high dose fetuses appeared subject to a possible increased rate of resorption; One litter of 19 total litters in the rabbit study and one of 24 litters in the rat study was entirely 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 entire litter of 24 total litters was completely 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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PHARMACOLOGY AND TOXICOLOGY REVIEW
Metabolism and distribution of ³H-AB-122358 in Rats:
Drug Metabolism Report No. 2

PURPOSE:

To determine the metabolism, disposition and excretion of a single IV dose of ³H-Ab-122358 in rats over 72 hours.

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EXPERIMENTAL DESIGN:

Testing Facility:

Drug Metabolism Department
Abbott Laboratories, Abbott Park, IL 60064.

Date of experiment:

5/3/95 and 6/6/95

Study Report Written:

5/9/96

GLP statement, Q/A:

GLP statement included,

Dose & Formulation:

1.7 ug/kg, (1 ml/kg) IV.
1.7 ug/ml. Lot 47946-SS-124, (48.7 Ci/mmol)
Dissolved in 20% EtOH, 20% propylene glycol, 60% water.

Dose Selection:

No rationale provided.

Animals:

4 M and 4 F Sprague-Dawley Rats
2 M and 2 F S-D Rats w jugular cannulas.
2 M and 2 F S-D rats w bile duct cannulas.
All 8-12 wks old, 209-274 g.

Protocol:

After injection of ³H-Ab-122358 into the femoral vein; plasma, fecal, urinary, and biliary levels of parent compound and metabolites were measured by
Biliary metabolites were further analyzed for glucuronidation by enzymatic hydrolysis.

RESULTS:

Plasma levels declined exponentially from an initial mean level of 11.72 ng eq/ml. 99.7% (M) to 88.5 % (F) of the radioactivity was recovered in the feces. No metabolites were discovered in the blood. 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 2-4 h. 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.

PK of Paracalcin (no metabolites in blood)

| N= 2m + 2f | T 1/2 (h) | AUC (ng/h/ml) |
|--------------|-----------|---------------|
| Male rats: | 3.3 | 38.3 |
| Female rats: | 3.6 | 27.7 |

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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 |

Conclusions:

In the rat ³H-Ab-122358 is rapidly cleared (T1/2 ~3.5 h) by liver metabolism and biliary secretion. of the radioactivity was recovered in the feces.

PHARMACOLOGY AND TOXICOLOGY REVIEW
The in vitro protein binding of ³H-AB-122358
in Mouse, Rat, Dog, Monkey & Human plasma:
Drug Metabolism Report No. 3

PURPOSE:

To determine the percentage of ³H-Ab-122358 bound to protein in plasma from mouse, rat, dog, monkey & humans using equilibrium dialysis in vitro.

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EXPERIMENTAL DESIGN:

Testing Facility: Drug Metabolism Department
Abbott Laboratories, Abbott Park, IL 60064.

Date of experiment: 6/95

Study Report Written: 12/19/95

GLP statement, Q/A: GLP statement included,

Dose & Formulation: Lot 47946-SS-124, (48.7 Ci/mmol)
Dissolved in 100% EtOH, 10 ug/ml.

Dose Selection: Determined by specific activity.

Animals: Plasma was prepared from fasted lab animals and normal human volunteers.

Protocol: Heparinized blood was separated into plasma.
3-5 ml samples were prepared with 1, 5, 20 and 100 ng/ml ³H-Ab-122358 added.
2 ml samples were dialyzed (MW cut off, 12,000-14,000) at 37 degrees C for 2h.

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RESULTS AND DISCUSSION:

In all species tested 1-100 ng/ml ³H-Ab-122358 bound to plasma proteins. This may be accounted for by binding to circulating Vitamin D Binding Protein (VBP). Vitamin D usually circulates bound to VBP and other proteins. This binding is known to be only 3% saturated by vitamin D and its metabolites, leaving plenty of capacity to bind Paracalcin.

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CONCLUSIONS:

In the rat ³H-Ab-122358 is extensively protein bound and is likely to bind to the VBP. This would help to explain why it remains largely in the central compartment.

However, for a highly bound protein, a small % change in % bound can result in a significant change in free (active) drug.

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PHARMACOLOGY AND TOXICOLOGY REVIEW
In vitro distribution of ³H-AB-122358
between erythrocytes and plasma in human blood:
Drug Metabolism Report No. 5.

PURPOSE:

To determine the distribution of ³H-Ab-122358 between plasma and red blood cells in human blood in vitro.

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EXPERIMENTAL DESIGN:

| | | |
|--------------------------------|--|------------------|
| Testing Facility: | Drug Metabolism Department Abbott Laboratories, Abbott Park, IL 60064. | |
| Date of experiment: | 11/95 | |
| Study Report Written: | 12/19/95 | |
| GLP statement, Q/A: | GLP statement included, | APPEARS THIS WAY |
| Dose & Formulation: | Lot 47946-SS-183B, (48.7 Ci/mmol) Dissolved in 40% EtOH, 10 ug/ml. | ON ORIGINAL |
| Dose Selection: | Determined by specific activity. | |
| Animals: | Plasma was prepared from fasted normal human volunteers. | |
| Protocol: | Heparinized blood was spiked with 0.01, 0.1, 1 and 10 ng/ml ³ H-Ab-122358 for 1h at 37 degrees C. 3 ml samples were centrifuged to separate RBCs. | |

RESULTS AND DISCUSSION:

In human blood in vitro _____ of ³H-Ab-122358 was found in the plasma with only trace percentages in the RBCs. This indicates that the drug is not taken up into blood cells.

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CONCLUSIONS:

Circulating ³H-Ab-122358 is not expected to be associated with blood cells and is primarily found in the plasma.

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PHARMACOLOGY AND TOXICOLOGY REVIEW
PK of Paracalcin after single and multiple doses
in normal healthy human volunteers:
Drug Metabolism Report No 6

PURPOSE:

To determine the safety and PK/PD profiles of single and multiple IV doses of ³H-Ab-122358 in normal human volunteers.

EXPERIMENTAL DESIGN:

Study No. M95-18, Report No. 6.
Testing Facility: Abbott Clinical Pharmacology Research Unit
Victory Memorial Hospital, Waukegan IL..
Date of experiment: Between 6/5/95 and 6/23/95
Study Report Written: 4/12/96
GLP statement, Q/A: GLP statement included,
Dose & Formulation: 0.04, 0.08 or 0.16 ug/kg IV, three times in six days.
5 ug/ml. 1 ml glass ampules
Lot 96-383-DK
Formulation not described in this report.
Dose Selection: No rationale provided.

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Subjects:

| | All: | Males: n=13 | Females: n=5 |
|-------------|---------------|--------------|--------------|
| Age (yrs.) | 27 +/- 7.8 | 26.7 +/- 6.9 | 28 +/- 10.8 |
| Weight (kg) | 72.7 +/- 11.4 | 77.5 +/- 8.1 | 60 +/- 9.3 |
| Height (cm) | 175 +/- 8.6 | 179 +/- 5.9 | 165 +/- 6 |

Protocol:

This was a phase I double blind, placebo controlled, escalating dose study. Patients were randomized into three dose groups (0.04, 0.08 or 0.16 ug/kg). Four subjects within each group received drug and two received placebo. Subjects received a dose every other day for a total of three doses. PK was determined after the first and last dose. PTH levels were measured.

Analysis:

Samples were analyzed _____, with a validated receptor assay. Lower limit, 40 pg/ml.

RESULTS:

Plasma levels declined exponentially and rapidly. They were below the limit of detection before the second dose. All doses therefore were essentially single doses administered after a washout period.

| Parameter (n=4) | 0.04 mg/kg | 0.08 mg/kg | 0.18 mg/kg |
|-----------------|-------------|--------------|--------------|
| C max (pg/ml) | 256 +/- 44 | 664 +/- 146 | 1242 +/- 226 |
| AUC (pg*hr/ml) | 683 +/- 165 | 2221 +/- 401 | 5247 +/- 893 |
| CL (L/h) | 4.2 +/- 2.1 | 2.7 +/- 0.6 | 2.4 +/- 0.4 |
| t 1/2 (h) | 2.7 +/- 0.4 | 5.3 +/- 1.3 | 7.3 +/- 1.0 |
| Vd (L) | 17 +/- 10 | 20 +/- 6 | 23 +/- 2 |

Results after the last dose were not significantly different from these results.

Conclusions:

In normal humans Ab-122358 is rapidly cleared (T1/2 ~5-7 h). PK is linear over the dose range tested. T1/2 for low dose is inaccurate due to effect of distribution phase. PTH values were not significantly altered, but baseline values were much lower than expected in the patient population.

PHARMACOLOGY AND TOXICOLOGY REVIEW
Plasma drug concentration tabulation
from 3-month SC rat MTD study:
Drug Metabolism Report No 7

PURPOSE:

To assess the exposure levels of rats in a study (TA94-370) designed to determine the pathogenic effects of Ab-122358 administered SC 3 times a week over a period of three months.

EXPERIMENTAL DESIGN:

Testing Facility:

Division of Drug Safety Evaluation
Abbott Laboratories, Abbott Park, IL 60064.

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Date of experiment:

Between 10/30/95 and 12/8/95

Study Report Written:

8/15/96

GLP statement, Q/A:

GLP statement included,

Dose & Formulation:

0, 0.1, 0.5, 3.0 ug/kg/dose, SC 3 times/week.

1 ml/kg/dose Lot (See tox study, only in IND)

Dissolved in 20% EtOH, 30% propylene glycol, 50% water.

Dose Selection:

No rationale provided here.

Animals:

80 M and 80 F Cr:CD(SD)BR Rats

Protocol:

Rats were dosed 3 times per week for three months. 5 satellite rats/group had blood collected for PK on days 0 and 77 at 0.5, 2, 6, 12 & 24 h.

Analysis:

Samples were analyzed with a validated receptor assay. Lower limit, 40 pg/250 ul.

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RESULTS:

Plasma levels declined exponentially to 0 within 24 h in all but the HD males.
AUC (24h) values were calculated and tabulated.

| Dose: | Males, AUC (24h) (pg*h/ml) | Females, AUC (24h) (pg*h/ml) |
|-----------|----------------------------|------------------------------|
| 0 | 0 | 0 |
| 0.1 ug/kg | 4956 | 1649 |
| 0.5 ug/kg | 13337 | 8063 |
| 3 ug/kg | 90969 | 50212 |

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Conclusions:

In the rat, Paracalcin is rapidly cleared.
AUCs for males are nearly double those in females for equivalent doses.
AUCs increased roughly linearly over this dose range

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PHARMACOLOGY AND TOXICOLOGY REVIEW
Plasma drug concentration & PK tabulation
from a 3-month IV rat tox study:
Drug Metabolism Report No. 8

PURPOSE:

To assess the exposure levels of rats in a study (TA94-344) designed to determine the pathogenic effects of Ab-122358 administered IV 3 times a week over a period of three months.

EXPERIMENTAL DESIGN:

Testing Facility: Division of Drug Safety Evaluation
Abbott Laboratories, Abbott Park, IL 60064. **APPEARS THIS WAY
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Date of experiment: Between 10/30/95 and 12/8/95

Study Report Written: 11/20/96

GLP statement, Q/A: **GLP statement included, analysis QAd.**

Dose & Formulation: 0, 0.1, 0.5, 3.0 ug/kg/dose, IV 3 times/week.
A fifth group received 3 ug/kg Calcitriol but was not analyzed for PK.
1 ml/kg/dose Lot: See tox study, only in IND.
Dissolved in 20% EtOH, 30% propylene glycol, 50% water.

Dose Selection: No rationale provided here.

Animals: 75 M and 75 F Cr:CD(SD)BR Rats

Protocol: Rats were dosed 3 times per week for three months. 5 satellite rats/group had blood collected for PK on day 83 at 0.5, 2, 6, 12 & 24 h.

Analysis: Samples were analyzed with a validated receptor assay. Lower limit, 40 pg/250 ul. **APPEARS THIS WAY
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RESULTS:

Plasma levels declined exponentially to 0 within 24 h in all but one HD male.
T1/2 and AUC (infinity) values were calculated and tabulated.

| Dose: | T1/2 Males (h) | T1/2 Females (h) | Males, AUC (pg*h/ml) | Females, AUC (pg*h/ml) |
|-----------|----------------|------------------|----------------------|------------------------|
| 0 | 0 | 0 | 0 | 0 |
| 0.1 ug/kg | 2.1 +/- 1.9 | 1.1 +/- 0.1 | 3015 +/- 633 | 702 +/- 339 |
| 0.5 ug/kg | 3.5 +/- 1.1 | 1.6 +/- 0.1 | 14598 +/- 1532 | 6887 +/- 1117 |
| 3 ug/kg | 3.2 +/- 0.7 | 1.5 +/- 0.1 | 84332 +/- 8271 | 40206 +/- 12984 |

Conclusions:

In the rat, Paracalcin is rapidly cleared.
AUCs for males are nearly double those in females for equivalent doses.
AUCs increased roughly linearly over this dose range

In patients, 0.24 ug/kg (the maximum recommended dose) resulted in an AUC of 27,000 pg*h/ml. Estimating from the table above, it would require a dose of 1 ug/kg in male rats (or 2 ug/kg in females) to achieve the same exposure level in rats on an AUC basis (27,000 pg*h/ml) as the exposure level from the maximum recommended human dose. These doses are 4-fold and 8-fold higher than the human dose (respectively). This estimate is for parent compound alone, but, there are no metabolites in blood.

PHARMACOLOGY AND TOXICOLOGY REVIEW
Tissue distribution of radioactivity following IV ³H-AB-122358 in Rats:
Drug Metabolism Report No. 10

PURPOSE:

To determine the distribution of total radioactivity in plasma, blood and various tissues, over time, of a single IV dose of 3 ug/kg ³H-Ab-122358 in male & female albino Sprague Dawley Rats and to determine the propensity to bind to melanin containing tissues in male pigmented Lister Hooded rats.

EXPERIMENTAL DESIGN:

Testing Facility:

| | | |
|--------------------------------|--|---------------------------------|
| Date of experiment: | 10/95 - 12/95 | APPEARS THIS WAY ON ORIGINAL |
| Study Report Written: | 7/23/96 | |
| GLP statement, Q/A: | GLP statement and QA Included. | |
| Dose & Formulation: | 3 ug/kg, (1 ml/kg) IV. 3 ug/ml. Lot 47946-SS-183C, (48.7 Ci/mmol) Dissolved in 20% EtOH, 20% propylene glycol, 60% water. | |
| Dose Selection: | No rationale provided. | |
| Animals: | 9 groups each of: 3 M and 3 F Sprague-Dawley Rats and 3 male pigmented Lister Hooded rats. All 7-8 wks old. Animals were housed in individual metabolism cages and fed ad lib. | |
| Protocol: | After injection of ³ H-Ab-122358 into the tail vein; groups of three male and 3 female SD rats were sacrificed by CO ₂ narcosis at 0.25, 0.5, 1, 2, 4, 6, 12, 24 and 72 h and pigmented rats were sacrificed at 0.25, 1, 24 and 72 h. Plasma, blood (by cardiac puncture) and the following tissues were removed and counted from the SD rats: adrenals, bone mineral, bone marrow, brain, caecum, eyes, fat, heart, kidneys, large intestine, liver, lungs, lymph nodes, muscle, pancreas, plasma, pituitary, prostate/ovaries, salivary gland, skin small intestine, spleen, stomach, testes/uterus, thymus, thyroid, and remaining carcass. From the pigmented rats, the eyes, kidney, liver, plasma, pigmented skin and non pigmented skin were collected and counted. | APPEARS THIS WAY ON ORIGINAL |

RESULTS:

Plasma levels declined exponentially from an initial mean level of _____ Gender differences were insignificant. Only low levels of radioactivity were recovered in reproductive tissue. T1/2 and AUC were not presented but plasma concentration declined rapidly to _____ of maximum by 2 h (consistent with previous studies). 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 (4-6h for GI) and then declined rapidly. (See attached table) 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.

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Conclusions:

1. In the rat ³H-Ab-122358 is similarly distributed in males and females.
2. It is rapidly cleared.
3. It was not preferentially distributed to any tissue except liver and GI tract (it's route of elimination).
4. Excretion was essentially complete by 72 h.
5. There was no retention of Paracalcin by melanin containing tissues.

PHARMACOLOGY AND TOXICOLOGY REVIEW
Metabolism and disposition of IV ³H-AB-122358 in Dogs:
Drug Metabolism Report No. 12

PURPOSE:

To determine the metabolism, disposition and excretion of a single IV dose of ³H-Ab-122358 over 72 hours.

EXPERIMENTAL DESIGN:

Testing Facility:

Drug Metabolism Department
Abbott Laboratories, Abbott Park, IL 60064.

Date of experiment:

1/31/96 and 2/6/96

Study Report Written:

10/9/96

GLP statement, Q/A:

GLP statement included,

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Dose & Formulation:

0.1 ug/kg, (0.5 ml/kg) IV .
0.2 ug/ml. Lot 47946-SS-124, (48.7 Ci/mmol)
Dissolved in 20% EtOH, 30% propylene glycol, 50% water.
"Within the anticipated toxicological range".

Dose Selection:

Animals:

3 M and 3 F beagle dogs
All dogs were _____ And fasted over night.

Protocol:

³H-Ab-122358 was administered by slow bolus into the cephalic vein.
3M and 3F dogs were housed in individual metabolism cages. The first 24 hrs, urine was collected over dry ice. Blood samples were taken from the jugular vein using heparinized Vacutainers after 0, 0.1, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, and 72 hours. Samples were centrifuged to separate plasma. For the second protocol a male and a female dog were maintained under anesthesia so that the bile duct could be surgically cannulated. Bile was collected during 0-2, 2-4, and 4-6 h after dosing via the cephalic vein. Blood was collected at 0.25, 1 and 4 h., and urine was collected at 6 h. Plasma, fecal, urinary, and biliary levels of parent compound and metabolites were measured
Metabolites were further analyzed for glucuronidation by enzymatic hydrolysis.

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RESULTS:

Plasma levels declined exponentially from an initial (6 minute) mean level of .45 ng eq/ml. AUC was 3.29 +/- 0.26 ng*h/ml, with a t1/2 of 17 +/- 1.4 h. Comparison of levels of radioactivity in plasma vs. whole blood indicate that _____ of the tracer is in the plasma. 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 between _____. 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.

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. The reappearance of parent compound in the feces may be due to microbial action on metabolites.

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 |

Conclusions:

In the dog ³H-Ab-122358 is rapidly (although slower than rats) cleared (T1/2 ~17 h) by liver metabolism and biliary secretion. 85% of the radioactivity was recovered (in at least 8 metabolites) in the feces and appears to be eliminated by biliary secretion. No circulating metabolites were identified.

PHARMACOLOGY AND TOXICOLOGY REVIEW

Plasma drug concentration & PK tabulation from an IV dog tox study: Drug Metabolism Report No. 18

PURPOSE:

To assess the exposure levels of dogs in a study (TA94-345) designed to determine the pathogenic effects of Ab-122358 administered IV 3 times a week over a period of three months.

EXPERIMENTAL DESIGN:

Testing Facility:

Division of Drug Safety Evaluation
Abbott Laboratories, Abbott Park, IL 60064.

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Date of experiment:

Between 9/12/96 and 10/4/96

Study Report Written:

12/12/96

GLP statement, Q/A:

GLP statement included, analysis QAd.

Dose & Formulation:

0, 0.02, 0.1, 0.3 ug/kg/dose, IV 3 times/week.

A fifth group received 0.3 ug/kg Calcitriol but was not analyzed for PK.
1 ml/kg/dose Lot: 94-0584

Dissolved in 20% EtOH, 30% propylene glycol, 50% water.

Dose Selection:

No rationale provided here.

Animals:

20 M and 20 F purebred beagle dogs

Protocol:

Dogs (4/group) were dosed 3 times per week for three months.

Blood was collected from the jugular vein for PK on day 0 and 80
at 0.5, 1, 2, 4, 8, 12 & 24 h. in heparinized Vacutainers.

Analysis:

Samples were analyzed _____ with a validated receptor
assay. Lower limit, 40 pg/250 ul.

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RESULTS:

Plasma levels declined exponentially to 0 within 24 h in all but two HD males.
Cmax, T1/2 and AUC (24h) values were calculated and tabulated. There were no significant gender
related differences or differences between the results on day 0 and day 80.

| Dose: | Cmax (pg/ml) | T1/2 (h) | AUC (pg*h/ml) |
|------------|--------------|----------|---------------|
| 0 | 0 | 0 | 0 |
| 0.02 ug/kg | 82 | ND | 629 |
| 0.1 ug/kg | 317 | ND | 1393 |
| 0.3 ug/kg | 1081 | 3.2 | 6003 |

Conclusions:

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As in the rat, Paracalcin is rapidly cleared in the dog.
AUCs increased roughly linearly over this dose range _____

In patients, a dose of 0.24 ug/kg resulted in an AUC of 27,000 pg*h/ml.
From the table above, comparable (AUC) exposure in dogs would require a (1.5 ug/kg) dose,
6-fold higher (on a mg/kg basis) in dogs than in patients. This estimate is for parent compound alone.

PHARMACOLOGY AND TOXICOLOGY REVIEW
Assay of AB-122358 in human plasma using a combination of
Extraction, Binding and RRA:
Drug Metabolism Report No. 19

This report describes the assay employed by Unilabs Clinical Research to measure Paracalcin levels. They employed a standard technique of separating the sample on HPLC and analyzing the fractions with a radio-receptor assay using calf thymus vitamin D receptor. ³H-AB-122358 was used as a standard to validate the method. ³H-1,25-Dihydroxyvitamin D3 was used as the tracer in the assay. Assays were conducted according to GLP and were QAd.

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PHARMACOLOGY AND TOXICOLOGY REVIEW
Reviewers Overall Summary and Conclusions of ADME Studies:

Summary Table of PK results for Paracalcin.

| Species: | Study: | Sex: | Dose: (ug/kg) | [C]: (ng/ml) | normalized [C]: (ng/ml/mg/ml) | t 1/2: (h) | AUC: (ng*h/ml) | normalized AUC: (ng*h/ml/mg/ml) |
|----------|--------|------|------------------|-----------------|----------------------------------|---------------|-------------------|------------------------------------|
| RAT | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| DOG | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| HUMAN | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | | F | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |
| | R | M | 0.1 | 1.2 | 1.2 | 2.5 | 1.2 | 1.2 |

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Pharmacokinetics:

Following administration of a single IV dose of ³H-Ab-122358 in rats (1.7 ug/kg) and dogs (0.1 ug/kg) the drug was 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 6-fold higher in dogs than in patients and 4-fold (1 ug/kg in 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.

Metabolism tissue distribution and elimination:

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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 _____ our 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 between 4-6 h. 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.

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 |

Evaluation:

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I questioned the sponsor about the 6-fold difference in the half life estimates in the 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.

I believe that 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