CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-688

PHARMACOLOGY REVIEW

PHARMACOLOGY AND TOXICOLOGY REVIEW OF NDA

NDA #:	**21-688**
Product Name :	**Sensipar™ (Cinacalcet HCl)**
Sponsor:	**Amgen Inc, CA**
Indication:	**Treatment of primary and secondary hyperparathyroidism**
Division:	**HFD-510 (DMEDP)**
Reviewer:	**Gemma Kuijpers**
Date:	**February 12, 2004**

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability
Pending the proposed labeling changes, Pharmacology/Toxicology recommends approval of this NDA (AP)

1.2 Recommendation for nonclinical studies
None

1.3 Recommendations on labeling
See Team Leader Memo (K.Davis-Bruno, February 10, 2004) (Appendix)

2. Summary of nonclinical findings

2.1 Overview of nonclinical findings

The current NDA is for the use of cinacalcet in the [redacted] treatment of secondary hyperparathyroidism (HPT) in patients with chronic kidney disease, and the treatment of hypercalcemia in patients with parathyroid carcinoma or patients with primary HPT. In chronic kidney disease hypocalcemia results from a disturbance in renal phosphorus handling and decreased formation of $1,25(OH)_2$-VitD. Hypocalcemia causes increased parathyroid gland secretion of PTH as primary defense of the system against lowered serum calcium. Primary HPT is a hypercalcemic disorder that results from excessive secretion of PTH and is usually caused by parathyroid adenoma or primary parathyroid hyperplasia.

A dose titration regimen is proposed with oral doses of 30 mg up to 180 mg, once daily, in secondary HPT, [redacted] The dose is titrated in each individual patient based on a target level of PTH and/or serum calcium.

The calcium sensing receptor (CaR) is a G-protein coupled receptor and plays an important role in calcium homeostasis. It regulates the release of parathyroid hormone (PTH) from the parathyroid gland in response to changes in extracellular calcium. Cinacalcet is a calcimimetic and acts at the CaR to increase its sensitivity to extracellular calcium, thereby suppressing the secretion of PTH from the parathyroid gland. Cinacalcet can also stimulate calcitonin secretion through interaction with the CaR on thyroid C-cells.

Pharmacology
In monkey, rat and mouse tissues CaR mRNA was detected primarily in the parathyroid gland but also in kidney, GI tract, thyroid, CNS, pancreatic islets, adrenal gland, thymus, testis, bone and/or bone marrow. *In vitro* pharmacology studies demonstrated a potent and concentration-dependent stimulation of the CaR by cinacalcet. Modulation of the CaR by cinacalcet led to inhibition of PTH secretion from [redacted] cells, and stimulation of calcitonin release from rat thyroid C-cells.

In intact rats, cinacalcet induced inhibition of PTH secretion resulting in a rapid and reversible reduction in serum calcium levels with half maximal effect (ED_{50}) at an oral dose of 3 mg/kg (Cmax: ———). Using *in vivo* models of secondary hyperparathyroidism, such as the partially (5/6) ———— rat, it was demonstrated that cinacalcet causes a dose-dependent and transient reduction in serum PTH and reduces blood ionized calcium. Upon repeat dosing, cinacalcet prevented or attenuated parathyroid gland hyperplasia in the Nx rat. In one study in Nx rats, cinacalcet (15 mg/kg) suppressed bone turnover, reduced bone fibrosis and cortical porosity and increased cortical BMD and toughness. These effects were most likely mediated by the reduction in serum PTH. In parathyroidectomized (PTX) rats, cinacalcet reduced blood ionized calcium through activation of CaR-mediated thyroid calcitonin secretion. The studies identified the parathyroid and thyroid as target organs for the pharmacologic action of cinacalcet in the rat. Cinacalcet reduced hypercalcemia but had no effect on vascular mineralization in VitD-treated Nx rats. Effective oral doses (ED_{50-100}) in the *in vivo* rat studies were generally in the range of 10-30 mg/kg (Cmax ———).
In vitro receptor studies suggested that the transmembrane and/or intracellular domains of the CaR are required for sensitivity of the receptor to cinacalcet.
An animal model for primary hyperparathyroidism was not available.

Safety pharmacology

Note: Calculation of exposure multiples in nonclinical studies are based on the maximum 180 mg/day dose proposed for secondary hyperparathyroidism. Clinical PK data indicated maximal exposure (Cmax, AUC) at the 180 mg/day dose, and further exposure was not observed at doses >180 mg/day. Exposure at the maximum dose of 360 mg/day (90 mg QID) recommended for primary hyperparathyroidism is not known.

In safety pharmacology studies, single oral doses of cinacalcet had no effects on neuropharmacologic signs or body temperature, and no analgesic, anticonvulsant or proconvulsant effects in mice, at doses up to 200 mg/kg (equivalent to 6 times the human dose of 180 mg/day, based on mg/m^2). In mice, a decrease in spontaneous motor activity and an increase in gastric motility was observed at an oral dose of 200 mg/kg. In the guinea pig, an IV dose of 20 mg/kg caused a transient increase in airway resistance and bronchoconstriction. These effects may have been due to hypocalcemia or interaction of cinacalcet with central or peripheral ion channels/receptors. There were no significant cardiovascular or EKG effect in the dog at single oral doses up to 50 mg/kg (~ human Cmax @ 180 mg/day). EKG effects were also not observed in a 1-month dog toxicity study at doses up to 100 mg/kg/day (0.8x human AUC @ 180 mg/day). However, in repeat dose toxicity studies in the monkey QT and QTc interval prolongation was observed (see General Toxicity).

An *in vitro* cardiac ion channel study showed that cinacalcet at high concentrations blocked K_{ATP} channels, Kv4.3, Kv1.5 and hcNa channels. hERG channel activity were minimally affected. K_{ATP} channels are believed to be involved in the protective response of the body, e.g. the heart and the vasculature, to stress. In the heart, they mediate preconditioning in response to ischemic stress, and in blood vessels they may be involved in vasoconstriction. K_{ATP} channels may also have cardioprotecive effects through shortening of action potential and QT duration. K_{ATP} channels are known to mediate the insulin secretory response of pancreatic —

cells to glucose. Despite the *in vitro* effect on K_{ATP} channels, *in vivo* treatment of rats with cinacalcet did not affect blood glucose levels (i.e. insulin secretion) after an oral glucose challenge. However, in repeat dose studies in the rat and the monkey serum glucose was decreased. Also, in (sub)chronic rat toxicity studies myocardial damage was observed. These findings were possibly related to K_{ATP} channel blockage, or to hypocalcemia. Drug-induced blockage of K_{ATP} and other ion channels by cinacalcet (or its metabolites) constitutes a clinical concern, since it could affect CNS and cardiac function.

ADME

Cinacalcet is well absorbed upon oral administration (95%), and the major part of a dose is recovered in urine and bile in all species. T_{max} of parent and total drug-related radioactivity is <6h (dose range 1-10 mg/kg) in animals and humans. $T_{1/2}$ of parent drug is 2-9h in animals, and 10-35h in humans. Metabolites are cleared much slower than parent in animals, but at similar rate as parent in humans. Oral bioavailability (BA) in rats is <10%, in humans 20%. The low bioavailability is probably the result of extensive first pass metabolism in liver and possibly GI tract. Cinacalcet is highly protein bound (93% to 99%) in humans and animals. In rats, radioactivity representing parent or metabolite is widely distributed over numerous tissues. Plasma:tissue ratios exceeded 1 in liver, Harderian gland, kidney, adrenal, lung.

Cinacalcet is extensively metabolized by oxidative and conjugative pathways. The two main pathways are N-dealkylation resulting in carboxylic acid metabolites (M5-M8) and oxidation of the naphthalene ring producing dihydrodiols (M2-M3). CYP3A4, CYP1A2 are the major contributors to cinacalcet metabolism in humans. Parent drug accounts for a minor fraction of circulating radioactivity in animals and humans. There are no unique human metabolites. Metabolite profiles were qualitatively similar but quantitatively different across species. In humans, the major circulating plasma metabolite is M5, while minor plasma metabolites are M6 and M2-Glu. Major plasma metabolites in monkeys are M5, M7 and M2-Glu, and in rats M7 and M5. *In vitro* metabolite studies indicated that the carboxylic acid metabolites M5 and M7 and the glucuronated dihydrodiol metabolites (M2a-Glu, M2b-Glu) were inactive.

Excretion in mice and monkeys is mainly hepato-biliary (fecal) (20-40%), and urinary (50%). In rats, excretion is fecal (ca. 60%) and urinary (ca. 15%). In humans, excretion is mainly urinary (95%). Cinacalcet and related compounds are excreted in milk in lactating rats, with relatively high parent drug levels in milk. Cinacalcet crosses the placental barrier in rabbits, and fetal plasma levels are approximately 1/10 times maternal levels. Data on liver P450 content from a 1-year monkey toxicity study suggested the potential for microsomal enzyme induction.

General toxicity

Acute oral toxicity studies were carried out in mice and rats, and chronic toxicity studies were conducted up to one month in dogs, six months in rats and one year in monkeys. Cinacalcet administration in animals was dose-limited by the calcium-lowering effect of the drug, and by GI toxicity. The hypocalcemia was due to the reduction in serum PTH and confounded the interpretation of the results. As a result of the hypocalcemia, potential hypercalcemia-like toxicity of the calcimimetic may have gone undetected in the animal studies. Part of the toxicities may have been mediated by cinacalcet metabolites.

On average, parent drug exposure (AUC) in the long term toxicity studies in rats and monkeys, respectively, was 7.5 and 1.8 times the human AUC (648 ng·h/mL) at the 180 mg/day dose. Low AUC multiples in the monkey limited the predictive power of the toxicity studies. Intrinsic qualification of the major human metabolite M5 was performed in the rat and monkey studies. Of the minor human metabolites, the M2-glucuronide was well qualified in the monkey study, but M6 was poorly qualified in the animal studies.

In both acute and repeat dose toxicity studies in rats, dogs and monkeys, signs of hypocalcemia included hypoactivity, neuromuscular and respiratory effects, tremors, convulsions, and excessive salivation. In a 2-week rat study, convulsions were observed at 500 mg/kg/day (23x human AUC @ 180 mg/day), in conjunction with hypocalcemia. CNS toxicity including convulsions was also seen with the degradation product and putative metabolite, [redacted], at 100 mg/kg/day, with unknown relationship to serum calcium. This compound was, however, not detected in mouse plasma or excreta and is believed to be metabolically labile.

In the 3-month and 12-month monkey studies, prolongation of QT and QTc intervals was observed at 3- and 6-month time points. The increase was dose-dependent and occurred at all doses of 5, 50 and 100 mg/kg/day (0.1-2x human AUC @ 180 mg/day). The increase was maximally 60 msec (0.26sec in controls→0.32sec @ 100 mg/kg/day). The increase was due to ST segment prolongation and was significantly correlated to the dose-dependent reduction in serum ionized calcium of 10-40% (1.35, 1.2, 1.0, 0.9 mM @ 0, 5, 50, 100 mg/kg/day). QT prolongation was not observed in the 1-month dog study at similar doses (5, 50, 100 mg/kg/day) even though serum calcium was reduced by 20% at the highest dose (1.45→1.15 mM).

The relationship between calcium and QT(c) in monkeys treated with cinacalcet was similar as has been described for patients with hypoparathyroidism and hypocalcemia (Bronsky et al, 1961) and for volunteers given citrate to lower serum calcium (Davis et al, 1995). In these cases, a decrease in serum ionized calcium of 0.5 mmol/L was associated with an increase in QT(c) of approximately 60 msec, and a decrease of 0.2 mM with an increase in QaT(c) of 34 msec, respectively. This compares to an increase in QT(c) of 60 msec in the monkey with a decrease in serum ionized calcium of 0.5 mmol/L. Data on monkey QT(c) with endogenous or induced hypocalcemia are not available.

It is likely that the EKG findings (QT prolongation) and CNS toxicity (convulsions) are related to hypocalcemia. However, it can not be excluded that these adverse events are partially mediated by effects of cinacalcet or its metabolites on cardiac or CNS ion channels/receptors.

In the rat, cataracts were observed in all repeat dose toxicity studies at doses ≥ 2x human AUC @ 180 mg/day and were possibly associated with hypocalcemia. Cataracts were not seen in monkey or dog studies even though similar reductions in serum calcium levels were attained. Cataracts have been observed in rabbits when exposed to hypocalcemia and in individuals

with hypoparathyroidism, and may be the result of low calcium-induced derangement in lens ion composition.

Other toxicities of potential clinical concern because of unlikely or unclear relatedness to serum calcium included GI toxicity (abnormal feces, poor appetite, emesis, intestinal mucosa hyperplasia/inflammation; *monkey, dog, rat*), hematologic effects (decreased red blood cell parameters; *monkey, rat*), liver toxicity (increased enzymes, decreased serum protein, vacuolation, necrosis; *monkey, rat*), renal toxicity (BUN/creatinine increase, mineralization; *rat*), cardiac toxicity (myocardial degeneration/necrosis, left ventricular arterial hypertrophy; *rat, juvenile dog*), endocrine hormone changes (testicular atrophy and reduced testosterone, T3 decrease, T4 increase, Vitamin D reduction; *monkey*), and muscle toxicity (CPK increase, degeneration; *monkey*).

Urine volume was increased in dogs and monkeys and urinary calcium excretion was increased in rats and dogs. These effects were probably due to pharmacologic effects on the kidney CaR affecting calcium reabsorption and urine concentration. Kidney pelvis mineralization was observed in rats at 1.5x human AUC @ 180 mg/day, and kidney weight increase and slight tubular changes were observed in monkeys at 2x human AUC @ 180 mg/day. Renal toxicity may be particularly relevant for patients with primary hyperparathyroidism.

Serum testosterone levels were decreased at all doses of 5-100 mg/kg/day (0.1-2x human AUC @ 180 mg/day) in the 1-year monkey study. This was accompanied by testicular weight decrease at the high dose of 100 mg/kg/day. Testicular tubular atrophy or degeneration was also observed in the 1-and 6-month rat studies at 3x-7.5x human AUC @ 180 mg/day, and the 1-month dog study at 0.8x human AUC @ 180 mg/day.

Genotoxicity

Cinacalcet had no genotoxic potential, as demonstrated by negative results in three *in vitro* assays (Ames bacterial test, HGPRT mutation assay in CHO cells and chromosome aberration assay in CHO cells, all with and without metabolic activation), and one *in vivo* genotoxicity assay (mouse micronucleus assay).

Carcinogenicity

Two-year dietary carcinogenicity studies were conducted in rats and mice. Doses used in the rat study were 5, 15, 35 mg/kg/day in males, and 5, 20, 50→35 mg/kg/day in females. In mice doses used were 15, 50, 125 mg/kg/day in males and 15, 70, 200 mg/kg/day in females. In both species, there were decreases in body weight and effects related to the calcimimetic effects of the drug (hypocalcemia, hyperphosphatemia, soft tissue/vascular mineralization, particularly in kidney). In female mice, slightly but not statistically significant increases (above historical control) in the incidence of intermediate pituitary hyperplasia and adenoma and of erythroid leukemia were observed at the high dose of 200 mg/kg/day (1.6x human AUC @ 180 mg/day). In female rats, there was a slight, statistically significant increase in the incidence of lymphoma at the high dose of 50-35 mg/kg/day (1.4x human AUC @ 180 mg/day). However, based on historical control rates, the effect did not appear biologically significant. In male rats, a slight but not statistically significant increase in the incidence of

lung bronchio-alveolar adenoacarcinoma was observed at the high dose of 35 mg/kg/day (2.5x human AUC @ 180 mg/day). A decrease in parathyroid and thyroid C-cell hyperplasia and adenoma was noted in male and female rats at all doses from 5-50mg/kg/day (0.2x-2.5x human AUC @ 180 mg/day). There were no tumor findings that warranted mentioning in the label.

Reprotoxicity

A full battery of reproductive toxicity studies was performed in rats and rabbits. High doses were limited by maternal body weight effects and hypocalcemia. In an oral Segment I fertility study in rats (5, 25, 75 mg/kg/day), there were no effects on male or female fertility at doses up to 25 mg/kg/day (3x human AUC @ 180 mg/day). At 75 mg/kg/day (5.6x human AUC) there were slight decreases in the number of corpora lutea, implantation sites and live fetuses concurrent with maternal toxicity of decreased body weight and clinical signs.

In the oral Segment 2 study in rats (0, 2, 25, 50 mg/kg/day), there were no effects on fetal external, visceral or skeletal malformations or variations at doses up to 50 mg/kg/day (4.4x human AUC @ 180 mg/day). Fetal body weight was slightly reduced at 2, 25, 50 mg/kg/day in parallel with decreases in maternal food consumption and body weight gain. Maternal toxicity was also evident as clinical signs at 25 and 50 mg/kg/day.

In the oral Segment 2 study in rabbits (0, 2, 12, 25 mg/kg/day), there was no fetal toxicity (mortality, fetal weight), and there were no effects on fetal external, visceral, skeletal malformations (i.e., no teratogenicity) or variations at doses up to 25 mg/kg/day (0.4x human AUC @ 180 mg/day). Maternal toxicity was evident as clinical signs, decreased body weight gain and food consumption at 12 and 25 mg/kg/day. In the dose-range finding Segment 2 study in rabbits (0, 1, 5, 25, 100, 200 mg/kg/day), there were no external fetal anomalies at doses up to 100 mg/kg/day (2.8x human AUC @ 180 mg/day). Reductions in maternal food consumption and body weight were seen at doses ≥25 mg/kg/day, clinical signs at ≥100 mg/kg/day, and maternal mortality at 200 mg/kg/day.

In the oral Segment 3 study in rats (0, 5, 15, 25 mg/kg/day), one dam dosed with 15 mg/kg/day was found dead with a prolapsed uterus and delivery complications on the day of delivery. Reductions in maternal food consumption/body weight and F1 pup body weight were observed on PPD 10-17 at 25 mg/kg/day. A minimal reduction in F1 pup body weight gain unaccompanied by maternal effects was observed at 15 mg/kg/day on PPD 10-17. There were no effects on F1 pre- or postweaning development. In F1 male parental animals, there was an increased incidence of incisor abnormalities at 25 mg/kg/day.

Other studies

Toxicology studies were conducted to evaluate impurities, industrial toxicology, and other routes (IV) of administration. The studies demonstrated adequate qualification of impurities, and did not raise additional concern for the proposed oral use of cinacalcet in patients with primary or secondary HPT.

2.2 Nonclinical safety issues relevant to clinical use

In long term monkey studies, QT(c) interval was increased. Most likely, this effect was at least partially mediated by reductions in serum calcium. In rats, dogs and monkeys, CNS toxicity including convulsions in rats occurred in conjunction with hypocalcemia. There are potential effects of cinacalcet -and possibly its metabolites- on cardiac ion (K^+) channels and conduction. In particular, blockage of K_{ATP} channels could impair cardiac preconditioning in response to ischemic stress, or impair the defense against QT prolongation. The nonclinical data do not exclude a potential for cardiac conduction abnormalities and CNS toxicity independent of reductions in serum calcium. Nonclinical data also indicate a potential for GI and testicular toxicity. The CNS, GI and testicular toxicities may be related to the presence of CaR in those tissues.

Based on *in vitro* and *in vivo* nonclinical data, thorough evaluation of clinical trial data for any events related to cardiac conduction abnormalies under resting or stress conditions (EKG), myocardial and coronary artery disease, and CNS excitation (seizures) is recommended.

APPEARS THIS WAY
ON ORIGINAL

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

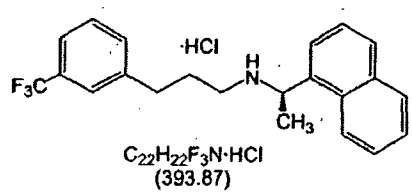
NDA number:	21-688
Submission date/type of submission:	September 5, 2003/ 505(b)1
Information to sponsor:	Yes (X) No ( )
Sponsor and/or agent:	Amgen Inc.
Manufacturer for drug substance:	

Reviewer name:	Gemma Kuijpers
Division name:	Division of Metabolic and Endocrine Drug Products
HFD #:	510
Review number:	1
Review completion date:	February 12, 2004

Drug:

Drug substance:	Cinacalcet hydrochloride (AMG-073 HCl)
Trade name:	Sensipar™
Generic name:	n/a
USAN name:	Cinacalcet hydrochloride (cinacalcet HCl)
INN name:	Cinacalcet hydrochloride
CAS registry number:	[364782-34-3]
Molecular formula/molecular weight:	$C_{22}H_{22}F_3N{\cdot}HCl$ / 393.87
Chirality:	The molecule contains a chiral center; cinacalcet is the active R-enantiomer.

Structure:



Relevant INDs/NDAs/DMFs:	IND 56,010
Drug class:	Calcimimetic
Mechanism of action:	AMG-073 HCl mimics the action of calcium at the parathyroid gland calcium receptor (CaR) and suppresses the release of PTH

Clinical formulation: Tablet (30 mg, 60 mg, 90 mg)

Route of administration: Oral (to be taken with food or shortly after a meal)

Indication and Usage (proposed label):
SENSIPAR™ is indicated for the ——— treatment of secondary hyperparathyroidism in patients with Chronic Kidney Disease, receiving or not receiving dialysis. SENSIPAR™ controls parathyroid hormone, serum calcium x phosphorus, phosphorus, and calcium levels in patients with Chronic Kidney Disease.

SENSIPAR™ is indicated for the treatment of hypercalcemia in patients with parathyroid carcinoma, or in patients with primary hyperparathyroidism for whom parathyroidectomy is not a treatment option.

Proposed dosage: In patients with secondary hyperparathyrodism and end-stage renal disease on dialysis, cinacalcet is to be administered orally starting at 30 mg, once daily. The dose should be titrated every 2 to 4 weeks up to 180 mg daily, to achieve a target PTH level. [redacted] In this review, exposure multiples for nonclinical studies have been based on the maximum 180 mg/day dose recommended for secondary hyperparathyroidism.

Disclaimer: Graphs and Tables were copied from the NDA submission.

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ON ORIGINAL

3.2 PHARMACOLOGY

3.2.1 Summary

Primary pharmacology

In monkey, rat and mouse tissues CaR mRNA was detected primarily in the parathyroid gland but also in kidney, GI tract, thyroid, CNS, pancreatic islets, adrenal gland, thymus, testis, bone and/or bone marrow. In vitro pharmacology studies demonstrated a potent and concentration-dependent stimulation of the CaR by cinacalcet. CaR modulation by cinacalcet lead to inhibition of PTH secretion from bovine parathroid cells, and stimulation of calcitonin release from rat thyroid C-cells. In intact rats, cinacalcet induced inhibition of PTH secretion resulting in a rapid and reversible reduction in serum calcium levels with half maximal effect (ED_{50}) at an oral dose of 3 mg/kg (Cmax: ———).

Studies in *in vivo* models of secondary hyperparathyroidism, such as the partially (5/6) nephrectomized (Nx) rat, demonstrated that cinacalcet caused a dose-dependent and transient reduction in serum PTH and reduced blood ionized calcium. Upon repeat dosing, cinacalcet also prevented or attenuated parathyroid gland hyperplasia in the Nx rat. In one study in Nx rats, cinacalcet (15 mg/kg) suppressed bone turnover, reduced bone fibrosis and cortical porosity and increased cortical BMD and toughness. These effects were most likely mediated by the reduction in serum PTH. In parathyroidectomized (PTX) rats, cinacalcet reduced blood ionized calcium through activation of CaR-mediated thyroid calcitonin secretion. The studies identified the parathyroid and thyroid as target organs for the pharmacologic action of cinacalcet in the rat. Cinacalcet reduced hypercalcemia but had no effect on vascular mineralization in VitD-treated Nx rats. Effective oral doses (ED_{50-100}) in the in vivo rat studies were generally in the range of 10-30 mg/kg (Cmax ———).

An animal model for primary hyperparathyroidism was not available.

Safety pharmacology

In safety pharmacology studies, single oral doses of cinacalcet had no effects on neuropharmacologic signs or body temperature, and no analgesic, anticonvulsant or proconvulsant effects in mice, at doses up to 200 mg/kg (6 times the human dose of 180 mg, based on mg/m2). A decrease in spontaneous motor activity and an increase in gastric motility was observed in mice at an oral dose of 200 mg/kg. The latter effects may have been due to hypocalcemia. In the guinea pig, 20 mg/kg (IV) caused a transient increase in airway resistance and bronchoconstriction, also possibly due to hypocalcemia. There were no significant cardiovascular or EKG effect in the dog at single oral doses up to 50 mg/kg (Cmax ———).

An *in vitro* cardiac ion channel study showed that cinacalcet at 500 ng/mL (1.27 uM) significantly blocked KATP channels by 96%. It also blocked Kv4.3, Kv1.5 and hcNa channels by 20-50%. hERG channel activity was blocked by 12%. KATP channels are believed to be involved in the protective response of the body, e.g. the heart and the vasculature, to stress. In the heart, they mediate preconditioning in response to ischemic stress, and in blood vessels they may be involved in vasoconstriction. KATP channels are also

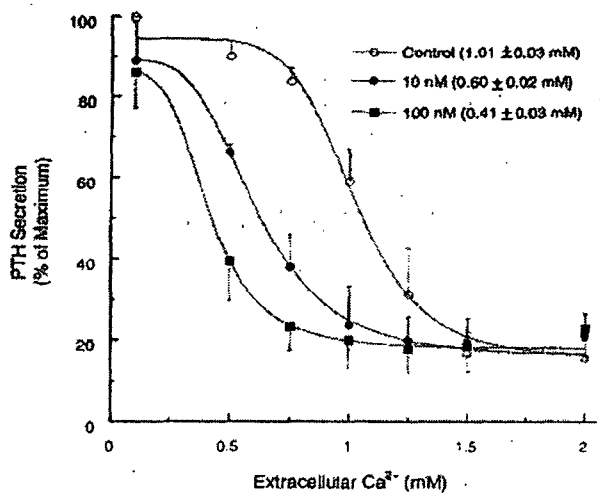
known to mediate the insulin secretory response of pancreatic B-cells to glucose. Despite the in vitro effect on KATP channels, in vivo treatment of rats with cinacalcet for 5 days (Cmax total . ———————————) did not affect blood glucose levels (i.e. insulin secretion) after an oral glucose challenge. However, in repeat dose studies in the rat and the monkey serum glucose was decreased. Also, in (sub)chronic rat toxicity studies myocardial damage was observed. These findings were possibly related to KATP channel blockage. Although the in vitro block of KATP currents occurred at much higher free drug concentrations than achieved in vivo in humans (ca. 1 ng/mL), the IC_{50} of the effect is not known. There is a theoretical concern for CNS hyperexcitation (seizures) mediated by KATP channel closure, since KATP channels may be involved in GABA-ergic neurotransmission. Also, metabolites were not tested and may have similar effects on ion channels.

Drug-induced blockage of KATP and other ion channels constitutes a clinical concern, since it could affect CNS and cardiac function. In particular, KATP blockage could impair the ability of the body to respond appropriately to hypoxic stress. Thus, thorough evaluation of clinical trial data for events related to cardiac conduction abnormalities in rest or stress (EKG: QT, ST), myocardial and coronary artery disease, vasoconstriction, CNS excitation and glucose homeostasis is recommended.

3.2.2 Primary pharmacodynamics

Mechanism of action: AMG-073 (cinacalcet) is a type II calcimimetic that acts as an allosteric modulator of the calcium-sensing receptor (CaR) on the parathyroid C-cell. It increases the sensitivity of the CaR to calcium. The receptor is activated by extracellular calcium via a negative coupling mechanism, with increased levels of Ca causing an inhibition of PTH secretion. Cinacalcet itself does not activate the receptor, but shifts the downward curve relating extracellular calcium concentration to PTH secretion to the left. Thus, in the presence of extracellular calcium, it reduces PTH secretion and lowers circulating PTH levels.

Figure 3. Cinacalcet Potentiates the Inhibitory Effects of Extracellular Ca^{2+} on PTH Secretion From Bovine Parathyroid Cells (Study PH99-007)



The calcium sensing receptor: The CaR is a "family C" G-protein coupled receptor (GPCR) and was cloned from bovine parathyroid gland in 1993 (Brown et al, 1993). The receptor has a very large NH2-terminal extracellular domain (ECD), a central core of ca. 250 amino acids with 7 predicted transmembrane domains (TMDs) and a large intracellular COOH-terminal tail of ca. 200 amino acids. Extracellular Ca (Ca_o) is believed binds to the ECD and perhaps the TMD's.

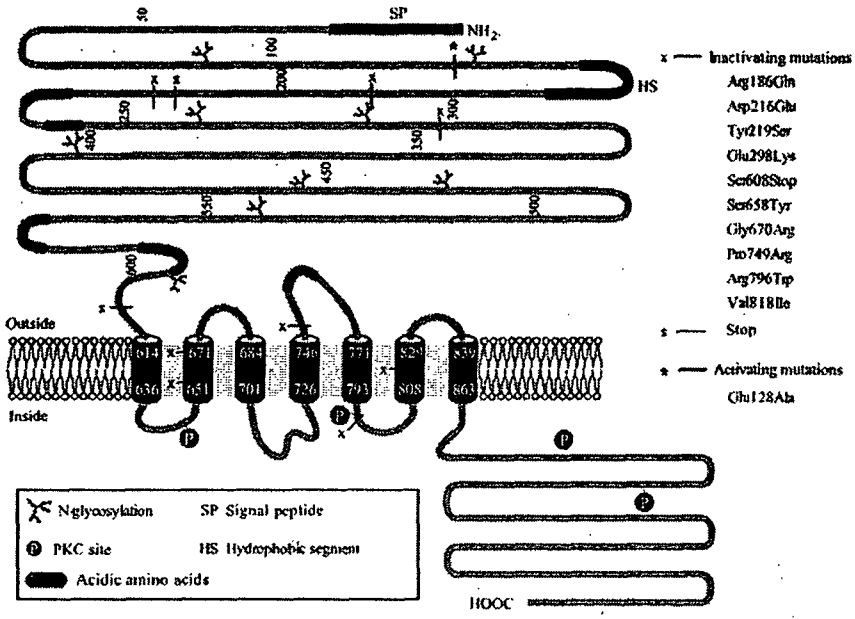


Fig. 2. Schematic representation of the principal structural features of the predicted BoPCaSR1 protein. Symbols are given in the key. Locations of known 'inactivating' and 'activating' mutations are indicated. See text for discussion. PKC, protein kinase C.

Members of the family C (or 3) GPCR's include Group I, II and III receptors. These 3 groups of receptors share at least 20% amino acid identity over their 7 TMD's. Group I contains the metabotropic glutamate receptors, mGluRs 1-8 (excitatory glutamate receptors in CNS), group II contains the CaR and a subfamily of "vomeronasal" pheromone receptors (VR's), and group III contains the $GABA_B$ receptors (inhibitory GABA receptors in CNS).

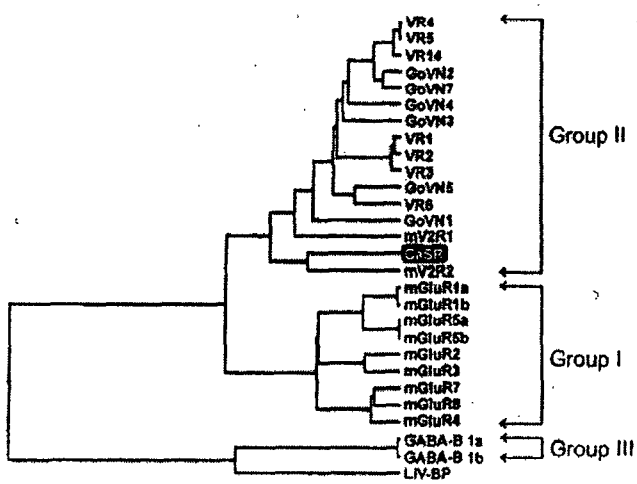
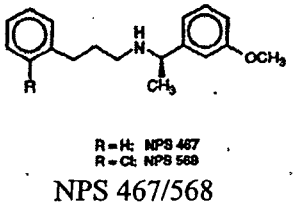


FIG. 2. "Tree" diagram showing the degrees of homology and proposed evolutionary relationships among the various members of the family C G protein-coupled receptors (GPCRs) described to date. The farther to the left that a given receptor branches off, the less related it is to the other receptors. For details see text. [From Brown et al. (61).]

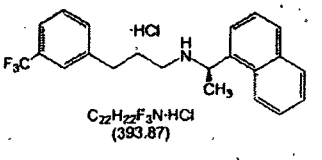
The CaR regulates a number of intracellular signaling mechanisms, i.e., phospholipases, MAPS and tyrosine kinases, and adenylate cyclase. Ligand binding to the receptor activates G-proteins which activate phospholipase C (PLC) and inhibit adenylate cyclase. Activation of the receptor also causes activation of phospholipase D and A2, probably through PLC-mediated activation of PKC. The receptor stimulates cell proliferation through activation of MAPK signaling cascades. The latter process is mediated by Ras-activation through tyrosine kinases. Another receptor-mediated event is an increase in intracellular calcium. The signaling mechanisms interact to regulate numerous cellular processes including (a) secretion (e.g. PTH and calcitonin), (b) cell proliferation, differentiation and apoptosis, and (c) gene expression.

The CaR is believed to have a central role in the regulation of PTH secretion. However, other secretory processes that are also under control of the CaR are the secretion of calcitonin (CT) from C cells, ACTH from the pituitary, gastrin from G-cells, insulin from B-cells and glucagon from A-cells, growth hormone from the pituitary, and PTHrP secretion (Brown and McLeod, 2001). CaR mRNA has been detected in a variety of tissues, but it is most abundantly present in parathyroid and kidney, tissues involved in mineral ion homeostasis. In the kidney, CaR is found along the entire nephron, and appears to play a role in tubular Ca, Mg, Na, Cl and water excretion/reabsorption. CaR has also been found in osteoclast lineage cells, osteoblast-like cells, chondrocyte-like cells, intestinal cells, placenta, rat striatum.

Calcimimetics: Type I calcimimetics are divalent cations other than calcium that mimic the effect of calcium in the absence of extracellular calcium. Type II calcimimetics are allosteric modulators of the CaR (Nemeth, 1998). The prototype molecule fendiline, a phenylalkylamine derivative, was the first type II calcimimetic. Modification of the prototype molecule lead to first generational calcimimetics, NPS R-467 and NPS R-568, and then to cinacalcet (AMG-073).



NPS 467/568



AMG-073

Type II calcimimetics interact with the transmembrane domain (TMD) of the CaR resulting in conformational changes that reduce the threshold for activation of the receptor by endogenous calcium. Thus, the parathyroid cell is led to believe that extracellular calcium levels are higher than they really are. The exact mechanism by which the activated CaR inhibits hormone secretion and the mechanism by which calcimimetics potentiate this effect remain unresolved.

Drug activity related to proposed indication: Hyperparathyroidism is characterized by elevated PTH levels and parathyroid gland hyperplasia. The disease is a consequence of chronic renal failure (CRF) and/or end stage renal disease (ESRD), and develops as a result of increased phosphate retention, decreased renal vitD production and hypocalcemia. HPT in renal patients can lead to secondary clinical complications in a number of tissues. Renal

osteodystrophy is a multifactorial disease of bone remodeling in end stage renal disease with HPT, and is classified as osteitis fibrosa, osteomalacia, or adynamic bone disease according to histologic features. The bone of HPT patients has a fibrotic marrow space, and there is a predisposition to fractures and impaired erythropoiesis. Increased bone resorption occurs mainly in the appendicular cortical skeleton. Excess PTH may also affect various other tissues (heart, neurons, muscle, vessels). Hypercalcemia can develop in severe cases. Together with hyperphosphatemia this can lead to soft tissue calcification as a result of increased CaxP product. Primary HPT is associated with persistently elevated serum calcium and PTH levels. Most cases are due to parathyroid gland adenoma. It is frequently asymptomatic, but can lead to high bone turnover, osteolysis, marrow fibrosis and soft tissue calcification.

Current therapy for secondary HPT includes oral phosphate binders, dietary P restriction, Ca supplementation and vitamin D therapy. Calcimimetic compounds mimic or potentiate the effects of extracellular calcium, and offer an alternative therapeutic approach for the treatment of HPT. AMG acts in an agonistic manner on the parathyroid gland CaR to increase the sensitivity of parathyroid cells to extracellular ionized calcium. Calcimimetics also act on parafollicular cells of the thyroid gland to increase calcitonin secretion. AMG-073 suppresses PTH secretion and increases calcitonin release. A reduction in serum PTH is expected to lead to improvement of renal osteodystrophy and bone pathology.

Studies related to primary PD

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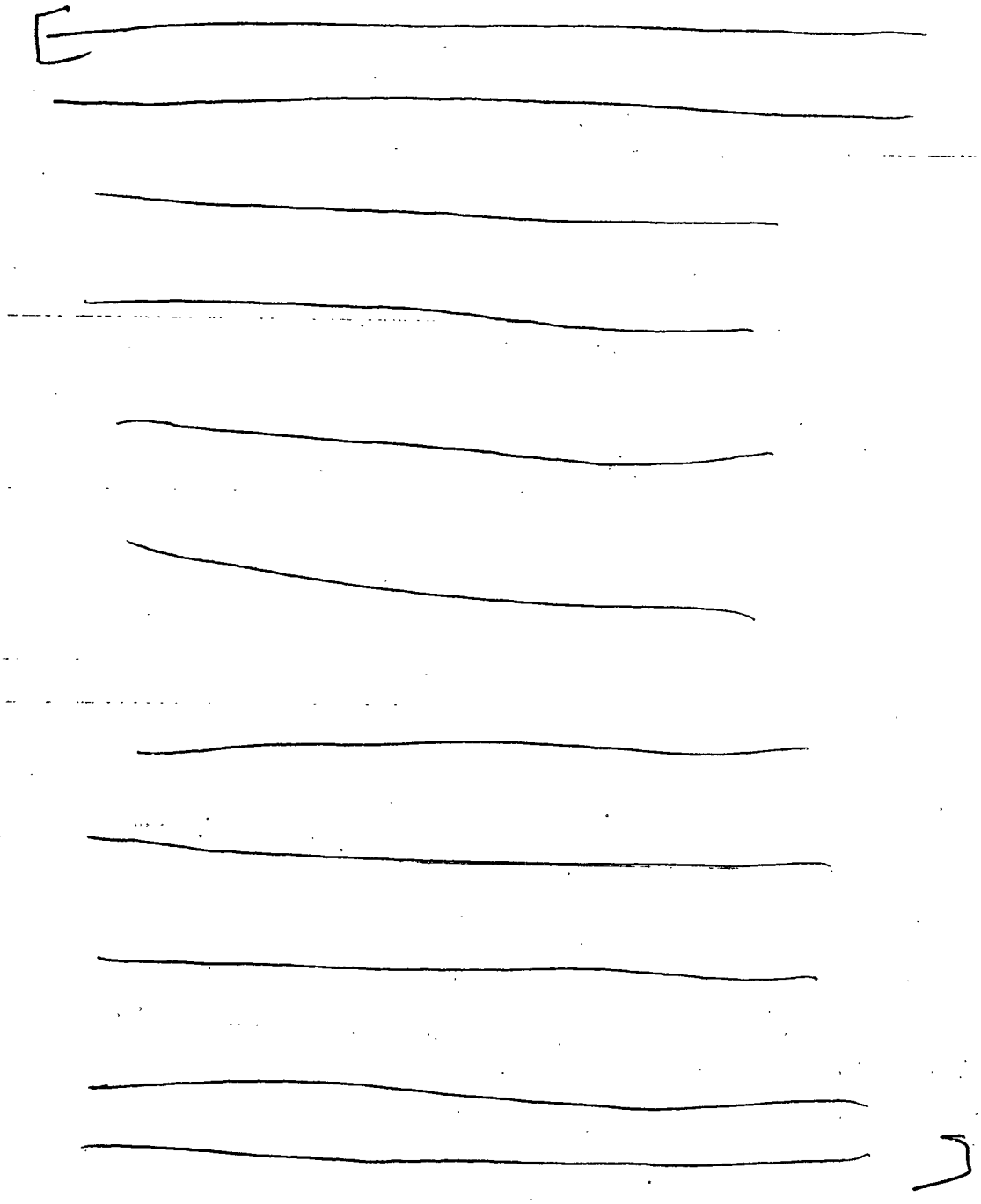


3.2.3 Secondary pharmacodynamics

Secondary pharmacology studies were conducted to investigate potential effects of cinacalcet in hypercalcemia of malignancy. Studies in two mouse tumor models (

testicular tumor, ——— colon tumor) suggested that the calcium lowering effect of cinacalet in these models is mediated through a reduction in PTH or stimulation of calcitonin release.

Vitamin D sterols are used in the management of secondary HPT to reduce PTH levels. Vitamin D, in conjunction with oral calcium used as phosphate binding agents, often leads to hypercalcemia, and soft tissue and vascular calcification. Cinacalcet (1, 3, 10 mg/kg) reduced blood ionized calcium levels in rats made hypercalcemic via vitamin D3 (1 ug, s.c.) administration (Study R2002076). This effect was not seen when ——— animals given vitamin D3 were also treated with cinacalcet. This confirms that cinacalcet reduces blood ionized calcium via the parathyroid and/or thyroid glands and can attenuate VitD-induced hypercalcemia.

Studies were conducted to investigate cinacalcet's effect on mineralization of vascular tissues such as associated with ESRD. The administration of vitamin D3 to Nx rats created a model where calcification of the aorta and its primary branches could be detected. Cinacalcet (1, 10 mg/kg) for 26 days had no effect on vascular mineralization but did reduce hypercalcemia caused by VitD (Study R2003071). The reason for the lack of effect on calcification is unclear.

Metabolite Studies
The major circulating metabolite in humans (M5) and metabolite M7 had no effect on intracellular calcium concentrations in CaR-transfected HEK cells. M2a-Glu and M2b-Glu were at least 333-fold less potent than cinacalcet in eliciting CaR-mediated calcitonin release from cultured bovine thyroid cells (for detailed review, see ADME section).

Other Studies
Study 960128: ——— Assay (non-GLP)
Cinacalcet at 10 uM (3937 ng/mL=70xC_{max} @ 180 mg/day) produced 50% or higher inhibition of specific ligand binding to the following receptors: alpha-1-adrenergic, central muscarinic, dopamine, and sigma receptors (all non-selective receptors), sodium site 2 (ion channel),and NK2 (neurokinine) receptors. It also inhibited binding of dopamine and serotonin to their transporter proteins. There was no significant inhibition of agonist binding at the GABA-B receptor, or inhibition of NMDA, AMPA, kainate or glycine binding at the ionotropic glutamate receptor. Ki values of reference compounds were between mostly 10^{-8} and 10^{-10}M.
The interactions identified may be at the basis of some of the CNS and GI effects observed in safety pharmacology, toxicity and clinical studies.

Proteins with inhibition of binding >50% by cinacalcet

Neurotransmitter receptor	Ion channel	Uptake/transporter	Brain/gut peptides
alpha-1 adrenergic non-selective	sodium site 2	dopamine	neurokinin, NK2
dopamine non-selective		serotonine	
central muscarinic non-selective			
serotonin non-selective			
sigma non-selective			

In vitro human blood assays

Intravenous formulations caused hemolysis in vitro in assays with rat, primate and human blood at 1.33-5 mg/mL (see local tolerance, in vitro)

Potential for cross reactivity with related G-protein coupled receptors

The G-protein coupled metabotropic glutamate receptors and GABA-B receptors share significant homology with the CaR. The calcimimetic NPS R-568 is a selective allosteric modulator at the CaR, and does not appear to interact with the mGluR, receptors with significant homology to the CaR (Nemeth et al, 1998; Hu et al, 2002). However, the effect of AMG-073 on mGluR or GABA-B receptor-mediated events has not been investigated. Modulators or agonists at the metabotropic glutamate and GABA-B receptors affect excitability in the CNS, and the GABA-B receptor is involved in insulin secretion from the pancreas. A potential interaction of cinacalcet and/or its metabolite(s) with the mGlu or GABA-B receptor(s) may lead to CNS effects, e.g., seizure activity, or interference with pancreatic insulin secretion. The absence of an interaction of cinacalcet with the ligand/agonist binding sites of the GABA-B and ionotropic glutamate receptors in the Novascreen assay does not exclude such a potential interaction.

3.2.4 Safety pharmacology

Safety pharmacology studies were conducted before the current ICH S7A and B documents were drafted. Some of the studies performed reflect previous guidelines (Japanese Guidelines for Nonclinical Studies of Drugs Manual 1995). A cardiovascular dog study, a respiratory guinea pig study, a neuropharmacological profile study, and a spontaneous motor activity study were performed as part of the ICH battery. A seven cardiac membrane channel assay study included evaluation of the hERG channel. Safety pharmacology studies were carried out using the oral route.

Exposure multiples (mg/m2) in safety pharmacology studies

	Dose	Mg/m2 multiple
Mouse	200 mg/kg, oral	5.56x
Rat	200 mg/kg, oral	11.1x
Guinea pig	20 mg/kg, IV ≈200 mg/kg, oral*	11.1x

Human dose: 180 mg dose, or 3 mg/kg (AUC 648 ngxh/mL)

*assuming 10% oral bioavailability

Neurological effects:

Studies 970091, 970095: In male CD-1 mice (10/grp), at oral doses of 20, 60, 200 mg/kg, cinacalcet did not produce neuropharmacologic signs or body temperature effects. An oral dose of 200 mg/kg had no anticonvulsant effects, defined as the potential to inhibit pentylenetetrazol- or strychnine-induced seizures or death.

Study 970096: In male mice, at oral doses of 200 mg/kg, cinacalcet neither potentiated nor inhibited the effect of submaximal proconvulsant electroshock of —— (i.e., a shock causing 10%-30% tonic extension of hindlimbs). Thus, cinacalcet did not show proconvulsant activity in this model.

Studies 970092, 970099, 970100: In male CD-1 mice (10/grp), at oral doses of 20, 60, 200 mg/kg, cinacalcet had no effect on phenylquinone-induced writhing (analgesic effects), and did not produce increases in barbiturate-induced sleep time.

Study 970100: In male CD-1 mice (10/grp), at oral doses of 20, 60, 200 mg/kg, cinacalcet decreased spontaneous activity (mean total beam breaks) at 200 mg/kg (oral) by 33% (10-15 min post dose) and 48% (15-20 min post dose), probably due to hypocalcemia-induced CNS effects. Activity was not affected at 20 or 60 mg/kg.

Study 970097: In SD rats (N=10/grp), 20, 60, 200 mg/kg (oral) had no antipyretic effects.

Cardiovascular effects:
Study 970127: Male Beagle dogs (N=3/grp) were given 2, 10, 50 mg/kg single oral doses. Reflux/regurgitation was observed in 1LD and 1HD animal. Serum ionized calcium was reduced post dosing. There were no effects on EKG (3 recording pre-dose, and @ 5, 15, 30 min, and 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24h post dose) or hemodynamic parameters (continuous recording up to 24h post dose of heart rate, systemic blood pressure, left ventricular pressure, maximum rate of contraction and relaxation, contractile index, pulmonary artery pressure, and cardiac output), at all doses. It was unclear if QT interval was assessed. EKG's were reported to be within normal limits with no evidence of treatment effect
Serum levels at 2h post dose: 21, 28, 91 ng/mL (Cmax), equivalent to up to —— human Cmax of 57 ng/mL @ 180 mg/day.

Effect on serum ionized Ca (pH 7.4 normalized) and serum parent drug (single dose dog study)

	Ca predose (mg/dL)	Ca post dose (2h) (mg/dL)	Cmax (ng/mL)
LD	5.87	5.56 (-5%)	——
MD	5.78	5.15 (-11%)	——
HD	5.89	5.04 (-14%)	——

Cmax human (180 mg/d)= ——

Thus, there were no significant cardiovascular or EKG effects in a single dose study in the dog.

Respiratory effects:

Study 970090: In anesthetized Hartley guinea pigs, 20 mg/kg (IV) produced a transient significant increase in airway resistance, probably due to bronchoconstriction, leading to death of 1 animal at 6 min post dose. This lethal effect may have been due to hypocalcemia and smooth muscle tetany in the lung. Study was performed using the IV route to reach a higher Cmax. TK evaluation was not performed.

Renal effects:
Study 970093: In male SD rats (N=10/grp), 20, 60, 200 mg/kg (oral) had no effect on urine volume output, pH, or urine Na, K, Cl concentrations. Increases in urine Ca were seen at all doses. This was probably due to the reduction in serum PTH and blockage of renal Ca-reabsorption. The effect was also observed in rat toxicity studies (1-mo and 6-mo studies).

Gastrointestinal effects:
Study 970098: In male CD mice (N=10/grp), doses of 20, 60, 200 mg/kg (oral) produced 12% increase, 4% decrease, 34% increase in gastric motility. The increase in the HD group was statistically significant. The result may reflect the findings of soft feces seen in general toxicity studies.

Study 970094: In Hartley guinea pig ileum *in vitro*, 100 ng/mL (___ multiple of Cmax) had no effects on contractile responses to Ach (0.01 ug/mL), histamine (0.1 ug/mL), BaCl (0.1 mg/mL).

These studies suggest a potential effect of cinacalcet on gastrointestinal motility/peristalsis. This is possibly secondary to hypocalcemia, which is known to increase gastric motility (Stewart et al, 1987).

Other effects:

Study 970035: Effects of AMG 099073-01 on Seven Cardiac Membrane Channels: L-type Ca2+, Kv4.3, KATP, Na+, Kv1.5, hKir2.2 and HERG

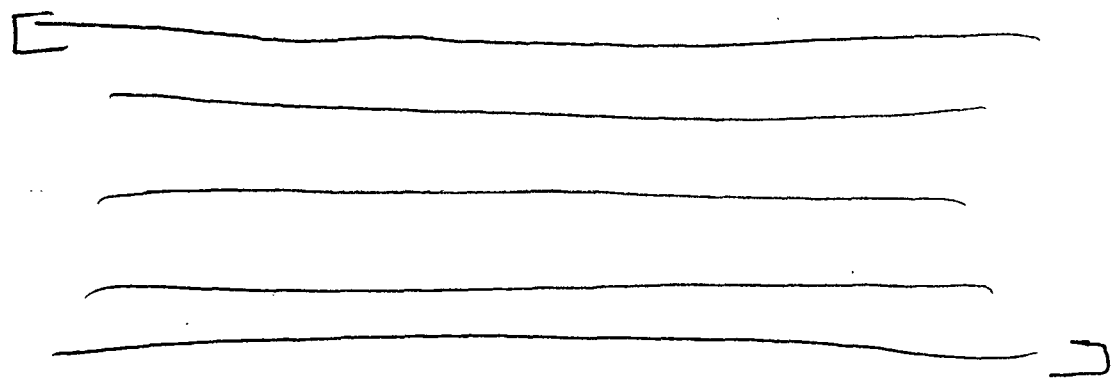
Introduction
The KATP channel is a potassium channel that is closed by intracellular ATP. The channel is present in metabolically active tissues such as the heart, vasculature, skeletal muscle, and pancreas, and is involved in the response of the body to stress. Also, the channel plays a role in the stimulation of insulin from pancreatic B-cells. In the absence of glucose, these channels are open, while in the presence of glucose they are closed as a result of an increase in intracellular ATP. Channel closing causes cell membrane depolarization and influx of extracellular Ca which stimulates insulin release. Thus, glucose taken up by the B-cell and blockers of this channel such as sulfonylureas cause (potentiation of) insulin release and hypoglycemia. Blockage of KATP channels has also been associated with CNS hyperexcitation due to GABA-ergic nerve cell depolarization. K_{ATP} channels may also have cardioprotecive effects through shortening of action potential and QT duration.

The Kv4.3 channel mediates an outward K current and is important for the early phase of repolarization (in contrast to the IKr channels derived from the hERG gene which are delayed rectifier channels important for late repolarization).

The Kv1.5 channel (IKur) is a delayed rectifier K+-channel and it has been suggested that inhibition of this channel by H1 receptor antagonists (e.g. loratidine) can contribute to drug-induced cardiac arrhythmia.

The Na channel is mainly involved in the upstroke of the action potential, and cardiac Na current determines QRS interval time.

Methods



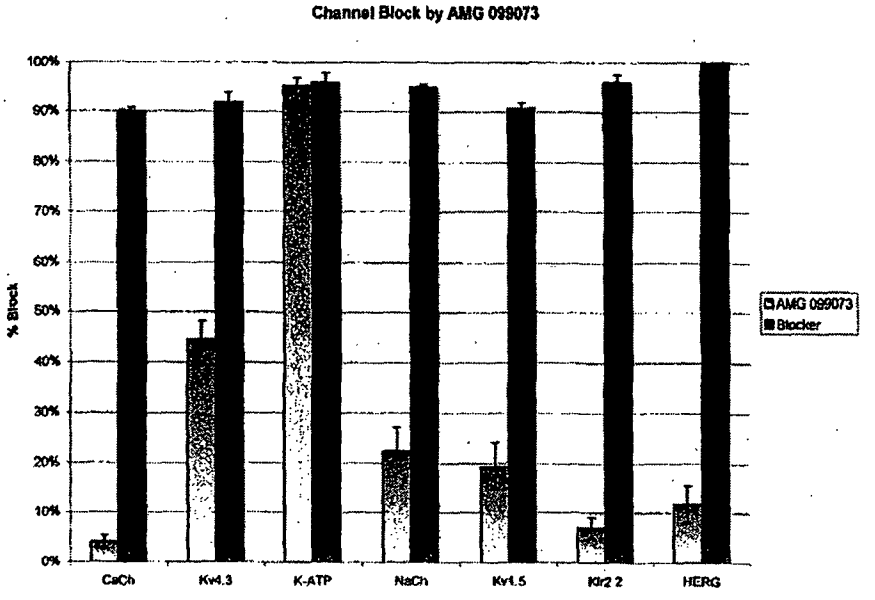
Results

The K_{ATP} channel showed significant block (95%) at 500 ng/mL. The positive control 300 uM glyburide (sulfonylurea) produced 96% inhibition. The blockage was time-independent. Based on this effect, a study was conducted to evaluate the hypoglycemic potential of cinacalcet in rats (Study 970145). In the rat study, cinacalcet (5-day oral doses of 10-50 mg/kg,) did not cause hypoglycemia. However, in the 6-month rat toxicity study (5, 25, 100 mkd), serum glucose was slightly decreased at 100 mg/kg/day (males and females, Wk 13 and/or 27). It should be noted that an effect of cinacalcet on Ca-dependent insulin release in vivo may be modulated by concomitant effects on serum calcium.

At 500 ng/mL, two K channels (Kv4.3 and Kv1.5) and the Na channel (hcNa) were inhibited by cinacalcet by 20-50%. Blockage was time-dependent.

Cinacalcet produced a 11.7% block of the hERG channel at 500 ng/mL (>10 times the median Cmax, ——— in humans @ 180 mg/day), as compared to 100% block with dofetilide (10 uM). HERG/IKr has the most important role in determining QT interval hence the pivotal role of *in vitro* hERG channel testing. The IC_{50} was not determined, but Sponsor stated it is > 500 ng/mL (1.27 uM). An IC_{50} of <10 uM raises a moderate clinical concern.

There was no effect of 500 ng/mL on L-type Ca or on hKir2.2 channels.



Conclusions

The in vitro data on ion channels suggest a potential effect of cinacalcet on cardiac electrophysiology through effects on a number of channels (KATP, Kv4.3, Kv1.5, Na, hERG).

The most prominent effect at 500 ng/mL was inhibition of the rat cardiac myocyte-derived KATP channel. The exact role of the channel in the heart remains unclear (Marban 2002). It is believed that this channel is a critical component in maintaining the body's homeostasis during the adaptive response to stress. Thus, opening of the KATP channels confers resistance of the heart to the stress of ischemia or hypoxia, and blockers of the channel are believed to prevent the development of such preconditioning (Suzuki et al, 2002). It has also been found that KATP knockout mice have coronary vasospasm and sometimes fatal ST elevation, a syndrome similar to human Prinzmetal angina. K_{ATP} channel opening may also have cardioprotecive effects through shortening of action potential and QT duration. Blockage of the KATP channels by cinacalcet may thus impair the ability to protect against stress or ischemic injury in the vasculature (vasocostriction) or cardiovascular system (Marban, 2002) or impair the defense against QT prolongation. The lack of effect in the single dose cardiovascular study in the dog may be due to lower free drug plasma concentrations than used in the in vitro study, or indicate a lack of effect in this species in vivo under the experimental conditions.

The KATP channel in the pancreas mediates B-cell excitation and insulin secretion. Data from the rat hypoglycemia study (#970145) suggest that at clinical dose levels there is no effect of cinacalcet on pancreatic KATP channels and insulin secretion. However, in the 6-month rat study at 100 mg/kg/day, and in the 12-month monkey study at 50 and 100 mg/kg/day (1-2x human AUC), serum glucose levels were decreased after 3 months of dosing. Also, in rats, in

2-week, 1-month and 6-month toxicity studies, histopathologic findings of myocardial necrosis/degeneration were observed at doses of 25-100 mg/kg/day (1.5x-7.5x human AUC). These effects may have been related to suppression of protective KATP potassium currents, resulting in membrane depolarization, Ca influx/overload and insulin release/myocardial damage. The effects may have been attenuated due to the hypocalcemia that occurred in these studies.

In conclusion, because the data on KATP channel mediated events are not consistent, a concern for KATP channel blockage by cinacalcet in the heart and/or other body systems remains. There is also a theoretical concern for seizures based on KATP channel closure-mediated CNS excitation.

In 3-month and 12-month oral monkey toxicity studies, QT(c) interval was increased after 3 to 6 months of dosing. The effect was dose-dependent and was seen at doses of 5-100 mkd (0.1-2x human AUC @ 180 mg/day). Most likely, the finding of increased QT and QTc in monkey toxicity studies was at least partially due to hypocalcemia. An effect through hERG channels appears unlikely judging from the ion channel data. However, it can not be excluded that effects of cinacalcet on other cardiac ion channels (KATP, Kv4.3, Kv1.5, hNa) are partially underlying the QT (ie ST) prolongation. QRS interval appeared unaffected in the 12-month monkey toxicity study suggesting lack of an *in vivo* effect on Na-current.

Study 970145: In vivo pharmacological evaluation of hypoglycemic potential of AMG099073 administered orally to rats. This study was carried out since 500 ng/mL caused significant *in vitro* inhibition of the KATP channel (present in pancreatic B-cells), which plays an important role in insulin secretion
Methods: In male SD rats (N=10/grp) oral doses of 0 (neg ctrl), 0 (pos ctrl), 10 or 50 mg/kg were given for 5 days. Rats were fasted O/N before Day 5. On the fifth day positive control rats were dosed with tolbutamide (30 mg/kg), a hypoglycemic. On day 5, rats were given an oral dose of glucose (1g/kg dextrose), at 1h post dose (tolbutamide), or 2 h post dose (cinacalcet). Serum glucose was analyzed at 0, 0.5, 1, 1.5, 2h post glucose load.
Results: Glucose levels in control, cinacalcet 10 mg/kg, 50 mg/kg, tolbutamide (30 mg/kg) groups: 98.9, 97.7, 97.6, 82.9 mg/dL. There was no significant effect of cinacalcet on fasting blood glucose in response to an oral glucose challenge. Cinacalcet exposure (AUC) in this study was approximately 0.3x-2x human AUC @ 180 mg/day dose, at 10-50 mg/kg. Free parent drug serum level (Cmax) was about [redacted] 250-fold lower than the concentrations tested in the KATP assay. Serum calcium levels were not reported, but it is expected there was a reduction in calcium due to PTH suppression.
Conclusions: The data suggest that cinacalcet at clinical dose levels has no pharmacologic effect on the KATP channel/sulfonylurea receptor in the pancreatic B-cell. However, lowered serum calcium levels may have interfered with an effect of cinacalcet on events mediated by KATP currents since these are triggered by calcium influx.

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Summary

ADME studies were conducted with radiolabeled or unlabeled compound. Studies were performed in rats, mice, monkeys, dogs mostly using the oral route (doses 1-100 mg/kg). Multiple labeled compounds, each labeled at a different position, were used to obtain information on cinacalcet and its metabolites (Fig. 1).
Validated analytical methods were used to quantitate plasma cinacalcet. All analytical methods used [redacted]. Liquid chromatography was used to separate the samples and cinacalcet was detected using [redacted]
The lower limit of quantitation was as low as [redacted] in human plasma and [redacted] in most animal species.

[redacted]

Cinacalcet is well absorbed upon oral administration (95%), and the major part of a dose is recovered in urine and bile in all species. T_{max} of parent and total drug-related radioactivity is <6h (dose range 1-10 mg/kg) in animals and humans. $T_{1/2}$ of parent drug is 2-9h in animals, and 10-35h in humans. Metabolites are cleared much slower than parent in animals, but at similar rate in humans. Oral bioavailability (BA) in rats is <10%, in humans 20%. The low bioavailability is probably the result of extensive first pass metabolism in liver and possibly GI tract.
Cinacalcet is highly protein bound (93% to 99%) in humans and animals. In rats, radioactivity representing parent or metabolite is widely distributed over numerous tissues. Plasma:tissue ratios exceeded [redacted] in liver, Harderian gland, kidney, adrenal, lung.
Cinacalcet is extensively metabolized by oxidative and conjugative pathways. The two main pathways are N-dealkylation resulting in carboxylic acid metabolites and oxidation of the naphthalene ring producing dihydrodiols. Parent drug accounts for a minor fraction of circulating radioactivity in animals and humans. There are no unique human metabolites. Metabolite profiles were qualitatively similar but quantitatively different across species. Limited information was available on the activity of the main human metabolites (M5, M2-Glu).
Excretion in mice and monkeys is mainly hepato-biliary (fecal) (20-40%), and urinary (50%). In rats, excretion is fecal (ca. 60%) and urinary (ca. 15%). In humans, excretion is mainly urinary (95%). Cinacalcet and related compounds are excreted in milk in lactating rats, with

relatively high parent drug levels in milk. Cinacalet crosses the placental barrier in rabbits, wich fetal plasma levels approximately 1/10x maternal levels.

3.3.2 Absorption

Cinacalcet is well absorbed and <5% of an oral radioactive dose is recovered in the feces of bile duct cannulated rats and dogs. Consistent with this, in vitro studies with Caco-2 cells demonstrated high permeability. In vitro, cinacalcet is not a substrate for efflux transporters e.g. P-glycoprotein.

In vivo studies showed a T_{max} of both parent and total radioactivity (parent + metabolites) of 1.5h-6h, depending on species and study (Table 2.6.5.3). In humans oral T max was 3-6h, in monkeys 4-5h. Based on data obtained in excretion studies with orally administered radioactive doses, absorption was at least 95% of dose in rats and dogs and at least 70% in monkeys (Table 2.6.5.13, below). In humans absorption was >83% based on urinary excretion of radioactivity.

Table 2.6.5.3. Pharmacokinetics: Absorption After a Single Dose
Test Article: Cinacalcet Hydrochloride (AMG 073)

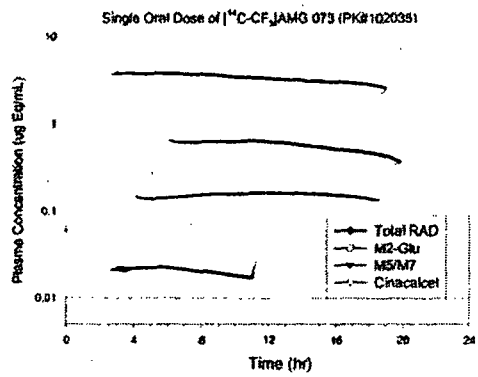
					Plasma Total Radioactivity				Plasma Cinacalcet[a,b]					
				Dose	T_{max}	C_{max}	$t_{1/2}$	AUC	T_{max}	C_{max}	$t_{1/2}$	AUC	CL	Vss
Study No.	Species	Sex (N)	Label	mg/kg	h	µg Eq/mL	h	µg Eq•h/mL	h	ng/mL	h	ng•h/mL	L/h/kg	L/kg
100229	Rat	M (3)		3.0 IV[c]					0		1.7	761	3.95	5.81
PK0012	Rat	M (3)	^{14}C-Naph	1 IV[d]			17.1	5.15						
	Rat	M (3)	^{14}C-Naph	1 PO[e]	6.0		20.5	1.74						
PK0007	Rat	M (5)		1 IV[d]							5.5	1570	1.00	
	Rat	M (5)		1 PO[e]					1.9		3.1	21.5		
PK97151	Dog	M (2)	^{14}C-2-CH$_2$	10 PO[e]	1.5		88.4	108						
	Dog	F (2)		10 PO[e]	4.0		137	135						
102035	Monkey	M(3)	^{14}C-CF$_3$	10 PO[e]	4.7		65.5	135	4.7		6.6	209.8		
101117	Monkey	M (5)		10 PO[e]					2.8		9.1	237.1		
980233	Human[f]	M (5)	^{14}C-CF$_3$	~1 PO	2.8		15.7	54.0	3.0		34.3	116.0		
990751	Human[f]	M (4)		~0.29 IV					0.33		19.9	270	~1.10	~17.6
20010105	Human[f]	M(30), F(12)		~1.3 PO[g]					5.5		10.3	245		

[a] Additional PK parameter data are presented in the Toxicology section.
[b] LC/MS/MS assay used for analysis of cinacalcet in all studies.
[c] PK independent of dose over the range of .
[d] Vehicle: pH 4.5 acetate buffer with 50 mg/mL mannitol.
[e] Vehicle: 0.5% methylcellulose..
[f] mg/kg dose and dose normalized PK parameters in humans calculated assuming a weight of 70 kg.
[g] Phase III formulation.

Sponsor concluded that oral bioavailability (BA, i.e. AUC_{oral}/AUC_{iv}) is less than 10% in rats and approximately 20% in humans. The low BA is likely due to first-pass metabolism, because IV plasma clearance is high (>75% of liver blood flow) in rats and humans. Oral clearance (CL/BA) is high in rats (>20 L/h/kg), dogs (200 L/h/kg) and monkeys (40 L/h/kg). No consistent sex differences in absorption and PK parameters were observed in nonclinical studies.

Parent vs. total drug levels and AUC multiples after single dose

Figure 5. Total Plasma Radioactivity, Cinacalcet and its Metabolites in Male Cynomolgus Monkey Plasma Following a Single Oral Dose of [^{14}C-CF$_3$]cinacalcet (Study 102035)



AUC

		Dose	Parent AUC (ngxh/mL)	Total AUC (ngeqxh/mL)	Parent:Total
Rat	14C-Naph	1 mg/kg, oral	21.5	1740	12%
Monkey	14C-CF3	10 mg/kg, oral	210	135000	0.16%
		1 mg/kg, oral (extrapolated)	(21)	(13500)	(0.16%)
Human	14C-CF3	1 mg/kg, oral	116	54000	0.21%

Cmax (Tmax = 2-5h)

		Dose	Parent Cmax (ngxh/mL)	Total Cmax (ngeqxh/mL)	Parent:Total
Rat	14C-Naph	1 mg/kg, oral			
Monkey	14C-CF3	10 mg/kg, oral			
Human	14C-CF3	1 mg/kg, oral			

The above tables show that after a single oral dose, the AUC ratio of parent drug to total drug-related material (parent+metabolites) is very low in humans (0.21%) and monkeys (0.16%), and much higher in rat (12%). Also, AUC ratio of parent:total is larger than Cmax ratio parent:total. This is to be expected based on larger T1/2 for total radioactivity than for parent. The different half lives for parent and metabolites, and species differences in these half lives, will lead to changes in these parent:total drug ratios as well as animal:human ratio of total drug levels upon multiple dosing.

Calculated (animal:human) AUC and dose ratios (rat, monkey)

	Dose	Parent AUC (ngxh/mL)	Total AUC (ngeqxh/mL)	Animal: Human (Parent AUC)	Animal: Human (Total AUC)	Animal: Human (mg/m2 ratio)
Rat	1 mg/kg	21.5	1740	19%	3.2%	17%
Monkey	1 mg/kg (*extrap'd*)	21	13500	18%	25%	33%
Human	1 mg/kg (single dose)	116	54000	100%	100%	100%
	3 (7-day multiple dose)	648	-	-	-	-

This shows that parent AUC in rat and monkey are as expected based on mg/m2 ratio. Also, total (parent + metabolite) AUC levels in monkeys as compared to humans is as expected based on mg/m2 ratio. However, total AUC in rats it is much lower than expected based on mg/m2 comparison.

Data on (animal:human) AUC ratios from toxicity studies

	Dose (mg/kg)	Parent AUC (ngxh/mL)	Animal: Human (Parent AUC)	Animal:Human (Mg/m2 ratio)
Rat	5	50-100	0.08-0.15x	0.28x
	100	3500-7000	5.4 -10.8x	5.6x
Monkey	5	50-100	0.08-0.15x	0.6x
	100	1180	1.8x	11x
Human	180mg (3mg/kg)	648	-	-

Based on actual TK data, the parent AUC multiple (rat:human) in the rat is 0.5-1x the expected AUC based on mg/m2 considerations, while in monkeys the parent AUC multiple (monkey:human) is a fraction (0.2x) of the expected level.

Taken together, the data show that in monkey parent levels and parent multiples (monkey:human) as observed in the repeat dose monkey toxicity studies are lower than expected based on mg/m2. This is probably due to extensive/rapid metabolism of parent drug in monkey. There may also be some limitation in absorption at higher doses since TK is non-linear. Due to a long T1/2 metabolites in monkey may accumulate to a larger extent than in human. In the rat, parent levels and multiples (rat:human) are as expected based on mg/m2 comparison. However, total parent + (naphthalene-group containing) metabolite levels in rats are relatively low, possibly due to enhanced elimination of (part of the) naphthalene-group containing metabolites. The levels of the cinnamic acid metabolites (M5-M8) as compared to parent in the rat are not clear, since there were no studies in rat with ^{14}C-labeled CF3-compound. However, data from the rat carcinogenicity study indicate that levels of M7 (AMG102664, main metabolite in rat) are 50-100x parent levels and metabolite studies have also shown low parent levels. This would indicate that parent:total drug levels in rat are <1-2%.

3.3.3 Distribution

In vitro studies showed that cinacalcet is highly protein bound (93% to 99%) in mouse, rat, dog, monkey and human plasma. Binding is similar in the concentration range of [redacted] ng/mL [redacted] in all species. This indicates that a direct comparison of total parent drug plasma concentrations between animals and humans is appropriate. In rats and humans, cinacalcet does not markedly partition into red blood cells.
Three studies on tissue distribution were performed in rats. In the first two studies with ^{14}C-2-CH_2-cinacalcet (single dose, oral, 10 mg/kg) tissue levels were analyzed by LSC (Study 97221) or whole-body autoradiography (Study 100142). Results showed that cinacalcet is widely distributed into tissues.

Tissue distribution (LSC, Study PK97221)
(male rat, 24h post dose, oral, 10 mg/kg, samples @ 4h, 8h, 24h)

Table 2.6.5.5a. Pharmacokinetics: Organ Distribution
Test Article: Cinacalcet Hydrochloride (AMG 073); Study Number: PK97221

Study No.	Study Design	Tissue	Radioactivity		AUC(0-t)	T/P[a]	Tissue	Radioactivity		AUC(0-t)	T/P
PK97221	Rat – M (4) F (4)		ng Eq/g					ng Eq/g			
	Label - ^{14}C-2-CH$_2$	Male[b]	4h	24h	ng Eq•h/g		Female[c]	4 h	24 h	ng Eq•h/g	
	Route – PO - fed	Liver	10800	6470	206960	3.01	Liver	9020	5120	183040	2.58
	Dose – 10 mg/kg	Adrenals	3420	4670	94740	1.38	Lung	6840	1520	95320	1.34
	Vehicle:	Lung	5710	1730	80980	1.18	Adrenals	3290	4000	85860	1.21
	Alkamuls EL-620/L	Kidney	3330	2480	78860	1.15	Kidney	4000	2310	82680	1.17
	Method: liquid	Adipose	1480	4970	73480	1.07	Pancreas	5170	1360	74160	1.05
	scintillation counting	Pancreas	3980	2360	71600	1.04	Plasma	3630	1450	70920	1.00
		Bladder	3220	1060	68960	1.00					
		Plasma	2780	1360	68800	1.00					

[a] T/P = ratio of AUC in tissue to AUC in plasma
[b] In males, T/P < 1 for adipose, spleen, prostate, thyroid, stomach, heart, skin, muscle, testes and brain.
[c] In Females, T/P < 1 for ovaries, adipose, spleen, urinary bladder, uterus, thyroid, heart, skin, stomach, muscle and brain.

Study 97221: % of dose in tissues in male rat after 10 mg/kg single dose

Tissue	% of dose in tissue @4h	% of dose in tissue @24h
Liver	4.7	2.81
Adrenal	0.006	0.009
Lung	0.24	0.08
Kidney	0.33	0.22
Pancreas	0.20	0.18
Plasma	0.41	0.22
Carcass	55	8.7
Carcass and tissues	ca. 88%	ca. 20%

Tissue distribution (Autoradiography, Study PK100142)
(male rat, 24h post dose, oral, 10 mg/kg, samples at 4h, 12h, 24h, 48h)

Table 2.6.5.5b. Pharmacokinetics: Organ Distribution
Test Article: Cinacalcet Hydrochloride (AMG 073); Study Number: 100142

Study No.	Study Design	Tissue	Radioactivity		AUC(0-t)	T/P[a]	Tissue	Radioactivity		AUC(0-t)	T/P	Vol. Page
100142	Rat – M (4) F (4)		µg Eq/g					µg Eq/g				
	Label - ^{14}C-2-CH$_2$	Male[b]	4 h	48 h	µg Eq•h/g		Female[c]	4 h	48 h	µg Eq•h/g		
	Route – PO - fed	Harderian	6.73	6.60	576	6.19	Harderian	3.49	7.69	492	5.66	
	Dose – 10 mg/kg	Liver	7.62	2.47	225	2.43	Liver	4.71	2.46	203	2.35	
	Vehicle:	Pancreas	6.79	1.15	135	1.46	Adrenals	2.15	2.40	117	1.35	
	Alkamuls EL-620/L	Kidney	3.74	1.79	130	1.41	Kidney	2.65	1.68	113	1.31	
	Method:	B. marrow	2.25	1.81	120	1.30	Pancreas	3.76	0.55	105	1.21	
	autoradiography	Adrenals	2.82	2.08	116	1.25	B. marrow	1.27	1.65	90	1.04	
		Spleen	3.39	1.42	98	1.06	Plasma	1.74	0.88	87	1.00	
		Plasma	3.38	0.89	93	1.00						

[a] T/P = ratio of AUC in tissue to AUC in plasma
[b] In males, T/P < 1 for lung, skin and thyroid.
[c] In females, T/P < 1 for lung, spleen and thyroid.

Both studies gave similar results and tissue:plasma levels were >1 in Harderian gland, liver, kidney, lung, pancreas, adrenal, bone marrow, spleen, brown fat. After oral dosing, radioactivity was also observed in esophagus, intestine, aorta, epididymis, lacrimal gland, skin, stomach, thymus, thyroid, trachea, urinary bladder, lymph nodes, pituitary, salivary gland, and low levels in cerebrum and cerebellum (PK 100142). Compound appears to cross the blood brain barrier.

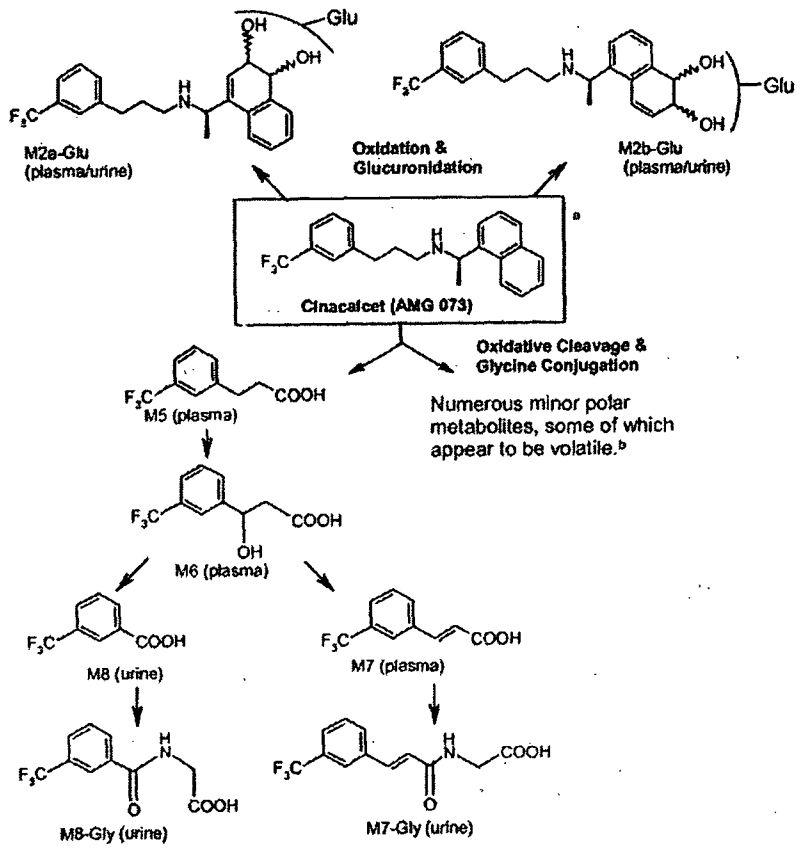
Radioactivity was maximal for most tissues at 4-12h and for some at 24-48h after dosing, and decreased slowly with different kinetics for different tissues. Plasma activity was maximal at 4h-12h. The data suggest accumulation with repeat daily dosing in plasma and tissues to different extents. For example, accumulation of parent and/or metabolites is expected in kidney, liver, bone marrow and adrenals, to a larger extent than in plasma.

Based on data from the second study (PK100142) with both IV and oral dosing (10 mg/kg) bioavailability of cinacalcet-associated radioactivity in the rat was approximately 85% to 90%. This compares to BA of parent of <10% and confirms that metabolism upon oral dosing reduces BA of parent drug.

The third study (PK0012, single dose, 1 mg/kg, IV or oral) was performed with ^{14}C-naphthalene-cinacalcet in rats. Tissue levels (measured at 1h-72h) after single oral dose were highest at 1-6h after dosing in liver (7.7), Harderian gland (3.4), kidney (3.4), lung (3), pancreas (1.5), hypophysis (1.3), adrenals (1.3) (tissue/plasma AUC ratio). Rate of absorption at 1 mg/kg, calculated as AUC (oral)/AUC(IV), was 33%.

3.3.4 Metabolism

Figure 2. Major Metabolic Pathways for Cinacalcet (AMG 073)



[a] Several minor metabolites were observed in animal models but not in humans: M3 (hydroxy-dihydrodiol) and its glucuronide M3-Glu, M1 (mono-hydroxy cinacalcet) and its glucuronide M1-Glu, M8 (m-CF_3-benzoic acid) and M9-Glu (dihydroxy-cinacalcet glucuronide).

[b] Based on Study 100655, where ^{14}C-methine cinacalcet (ie, drug labeled on the right side) was administered to mice. In the human ADME study, where ^{14}C-CF_3 cinacalcet (ie, drug labeled on the left side) was administered these metabolites are not radioactive and could not be detected.

Metabolite	Chemical name/code name
M1	Mono-hydroxy cinacalcet
M2	Dihydrodiol
M3	Hydroxy-dihydrodiol
M5	m-CF3-hydrocinnamic acid / AMG 501345
M6	m-CF3-hydroxy-hydrocinnamic acid
M7	m-CF3-cinnamic acid / AMG 102664
M8	m-CF3-benzoic acid
M9	Dihydroxy-cinacalcet

Cinacalcet is extensively metabolized by oxidative and conjugative pathways before excretion in bile or urine. Parent drug accounts for only a minor fraction of circulating radioactivity in both animals and humans.

In vitro data using microsomes (Study 100155) showed that cinacalcet undergoes NADPH-dependent oxidative metabolism in mice, rats, dogs, monkeys, and human microsomes. Metabolites were all more polar than parent compound. The *in vitro* metabolic profiles were qualitatively similar across species, except dog in which the major metabolite was a minor metabolite in other specie, and vice versa. The major metabolites produced by human microsomes are dihydrodiols (compounds obtained through oxidation of naphthalene ring).

Multiple CYP enzymes are involved in cinacalcet metabolism. In humans, CYP3A4 and CYP1A2 are the primary enzymes responsible for metabolism. The CYP3A4 inhibitor ketoconazole caused an approximate 2-fold increase in cinacalcet exposure in humans confirming a role of CYP3A4. Other enzymes involved in metabolism appear to be CYP2D6 and CYP2C9. By contrast, CYP2A6 and CYP2E1 are not involved.

In vivo, the primary routes of metabolism are:
(1) N-dealkylation to produce carboxylic acid derivatives
(2) oxidation of the naphthalene ring to form dihydrodiols.

The two main metabolic pathways appear to exist in all species examined *in vivo* (mice, rat, monkey, and human) and there appears to be no major unique human metabolite.

Excretion of metabolites

The dihydrodiol oxidative metabolites (M2a,b) are conjugated to glucuronides (M2a-Glu also called U12, and M2b-Glu also called U6) and excreted in urine. Some unconjugated M2 (and M1, M3) is also excreted in feces, as determined in mouse and monkey. These aglycones are probably hydrolyzed bile excretion products. Excreted in feces was also some unchanged parent compound, partly due to non-absorption. The cleavage product (M5) is further metabolized (M6, M7, M8) and M7 and M8 are conjugated to glycine before excretion in urine. The N-dealkylation also results in polar metabolites containing the naphthalene ring that could be detected only in the mouse excretion study (100655) (Table 2.6.5.9a). ——— was not detected in the mouse plasma or excreta (Study 100655).

Table 2.6.5.9a. Pharmacokinetics: Metabolism In Vivo
Test Article: Cinacalcet Hydrochloride (AMG 073)
Excreted Metabolites

						% Dose Excreted				% Radioactive Dose								
										Feces					Bile	Urine		
Study	Species	Sex (N)	Radio- label	Dose mg/kg	Study Days	Total[a]	Urine	Feces	Bile	Parent	M1	M2[b]	M3[b]	M1Glu	M2-Glu[b]	M3-Glu[b]	M7-Gly	M8-Gly
100524	Mouse	M (6)	^{14}C-CF_3	10 (PO)	4	88.6	50.6	37.2	NA	3.2	ND	16.9	1.9	NA	2.9	ND	39.6	2.5
		F (6)	^{14}C-CF_3	10 (PO)	4	97.2	69.0	27.3	NA	0.6	ND	10.1	1.3	NA	4.7	ND	52.1	5.2
100655	Mouse	M (6)	^{14}C-methine	10 (PO)	4	61.1	39.4	21.7	NA	0.7	ND	8.4	5.7	NA	4.4	1.8	NA	NA
		F (6)	^{14}C-methine	10 (PO)	4	59.1	35.0	24.2	NA	1.2	ND	7.2	4.5	NA	3.2	1.0	NA	NA
100677	Rat	BDC-M (3)	^{14}C-2-CH_2	10 (PO)	2	75.0	15.5	4.4	55.1	NA	NA	NA	NA	ND	34.3	ND	4.2	ND[c]
		BDC-F (3)	^{14}C-2-CH_2	10 (PO)	2	83.7	10.6	2.9	70.2	NA	NA	NA	NA	ND	43.8	ND	8.1	ND[c]
152035	Monkey	M (3)	^{14}C-CF_3	10 (PO)	5	80.4	56.6	19.7	NA	15.0	0.3	2.0	ND	NA	18.3	1.0	14.6	12.7
		M (3)	^{14}C-CF_3	100 (PO)	5	86.4	51.1	29.4	NA	11.1	14.2	0.6	ND	NA	16.0	2.5	13.9	11.1
		BDC-M (3)	^{14}C-CF_3	100 (PO)	5	95.8	35.9	19.1	38.4	5.1	8.0	5.4	ND	11.5	34.0	1.2	12.6	6.8
980233	Human	M (5)	^{14}C-CF_3	~1 (PO)[d]	8	96.3	83.2	13.1	NA	NA	NA	NA	NA	NA	32.1	ND	20.4	14.1
		M (5)[e]	^{14}C-CF_3	~1 (PO)[d]	8	97.4	80.3	16.9	NA	NA	NA	NA	NA	NA	38.4	ND	13.3	13.8

BDC = bile duct cannulated.
ND = not detected
NA = not assessed.
[a] Total recovery includes excrete and cage wash/wipes.
[b] M2/M2-Glu and M3/M3-Glu are presented as sum of regioisomers.
[c] due to position of the radiolabel, M8-Gly would not contain the radiolabel
[d] Human mg/kg dose calculated assuming a body weight of 70 kg
[e] Results from smokers.

Quantification of plasma metabolites

Circulating metabolite levels (expressed as % of total circulating radioactivity) are summarized in Table 2.6.5.9b. Circulating metabolites are similar in all species but there are quantitative differences.

In humans, M5 was the major circulating metabolite (73%), and minor ones were M2-Glu (11.2%) and M6 (9.1%). In the rat, M5 and M7 were major circulating metabolites (M7>M5), while M2-Glu and M6 were not detected. In the monkey, M2-Glu was the major circulating compound, but there were also significant and similar amounts of M5 and M7. In all species (mouse, rat, monkey, human) parent compound was present as trace amount in plasma. The metabolism data suggest that M6 is formed in monkeys, rats and mice, but rapidly converted to M7 and M8, while in humans conversion is slower resulting in measurable plasma levels of M6.

Table 2.6.5.9b. Pharmacokinetics: Metabolism In Vivo
Test Article: Cinacalcet Hydrochloride (AMG 073)
Circulating Metabolites

							Composition of plasma radioactivity			
Study	Species	Sex (N)	Radio-label	Dose	Time	Total plasma radioactivity	Cinacalcet	M2-Glu[a]	M5/M7[b]	M6[c]
						µg Eq/mL or g				
100524	Mouse	M (4)	^{14}C-CF$_3$	10 (PO)	8 h	0.65 ± 0.11	Trace[d]	Trace[d]	78.4%	ND
		F (4)	^{14}C-CF$_3$	10 (PO)	8 h	0.86 ± 0.18	Trace[d]	Trace[d]	95.4%	ND
100655	Mouse	M (6)	^{14}C-methine	10 (PO)	8 h	0.488 ± 0.104	Trace[d]	75.0%	ND[e]	ND[e]
		F (6)	^{14}C-methine	10 (PO)	8 h	0.546 ± 0.102	Trace[d]	88.0%	ND[e]	ND[e]
100677	Rat	M (3)	None	50 (PO)	4 h	Not applicable	Minor	ND	Major	ND
102035	Monkey	M (3)	^{14}C-CF$_3$	10 (PO)	8 h	5.27 ± 0.568	Trace[d]	58.7%	32.1%	Trace[d]
980233	Human[f]	M (5)	^{14}C-CF$_3$	~1 (PO)	3 h	2.65 ± 0.223	Trace[g]	11.2%[f]	73.0%[g]	9.1%[g]

ND = not detected.
NA = not applicable.
[a] Sum of regioisomeric dihydrodiol glucuronides
[b] M5/M7; CF_3-hydrocinnamic acid and CF_3-cinnamic acid, respectively - in humans only M5 was observed, whereas in mice, rats and monkeys both M5 and M7 were observed as co-eluting peaks.
[c] M6; CF_3-hydroxy-hydrocinnamic acid.
[d] Due to low levels, accurate measurement of these components could not be made.
[e] Due to lack of label in this portion of the molecule, these metabolites would not contain radioactivity.
[f] Results from non-smokers; comparable results observed in smokers.
[g] Calculated by dividing the amount reported for each metabolite by the total plasma radioactivity.

Table 2. Summary of the Metabolites of Cinacalcet in Humans and Animal Models

	Mouse		Rat		Monkey		Human	
Metabolite	Plasma	Excreta	Plasma	Excreta	Plasma	Excreta	Plasma	Excreta
M2-Glu	Major	Major	ND	Major	Major	Major	Minor	Major
M5	Major	ND	Minor	ND	Major	ND	Major	ND
M6	ND	ND	ND	ND	Minor	ND	Minor	ND
M7	Major	ND	Major	ND	Major	ND	ND	ND
M7-Gly	ND	Major	ND	Major	ND	Major	ND	Major
M8-Gly	ND	Major	ND	ND[a]	ND	Major	ND	Major

ND = not detected.
See Figure 2 for metabolite structures.
[a] Due to position of radiolabel, this metabolite is not expected to be radioactive.
Major in excreta defined as greater than 4% of dose. Major in plasma defined as greater than 25% of circulating radioactivity. Minor in plasma defined as less than 25% of circulating radioactivity.

Observations Table 2: The primary routes of metabolism were N- dealkylation leading to carboxylic acid derivatives and oxidation of naphthalene ring system to form dihydrodiols. The oxidative metabolites were further conjugated before elimination. Urinary radioactivity was predominantly composed of the glycine conjugates of carboxylic acid metabolites (M7- Gly and M8- Gly) and dihydrodiol glucuronides (M2- Glu). Fecal radioactivity was

composed of unchanged cinacalcet (< 4% of dose in mice and <15% dose in monkeys) and corresponding aglycones of urinary metabolites (eg, M2). The carboxylic acid metabolites (M5/ M6/ M7) and dihydrodiol glucuronide (M2- Glu) were the predominant components of plasma radioactivity. In vitro, the major and selected minor circulating metabolites of cinacalcet were found to be inactive or markedly less active than cinacalcet. There was no evidence for human specific metabolic pathways. Several minor metabolites were observed in animal models but not in humans: M3 (hydroxy- dihydrodiol) and its glucuronide M3- Glu, M1 (mono- hydroxy cinacalcet) and its glucuronide M1- Glu, M8 (m- CF_3-benzoic acid) and M9- Glu (dihydroxy- cinacalcet glucuronide). Overall, the circulating and excreted metabolite profile of cinacalcet in humans was similar to that observed in nonclinical models.

NOTE: Biotransformation of the naphthyl moiety and formation of dihydrodiol with subsequent glucuronidation leads to the M2 metabolites. The other transformation pathway (N-dealkylation) leads to the production of M5/M6/M7 metabolites. N-dealkylation also results in equivalent levels of naphthalene-containing portions of the molecule. One of these metabolites may be [redacted] which was tested in an acute rat toxicity study.
In studies with ^{14}C-CF3- or ^{14}C-CH_2-labeled compound all radioactivity represents cinnamic or benzoic acid metabolites (M5,6,7,8) but the naphthalene-group containing metabolites are undetected. Thus, apart from M2-Glu and M5/M7, naphthalene-group containing metabolites or their degradation products are present in the circulation and excreta at unknown levels. There were no data on metabolites using ^{14}C-naphthalene labeled compound. In studies with ^{14}C-methine label (e.g. Study 100655, mouse), part of the naphthalene-metabolites are detected. The methine group is thought to be metabolically labile in rodents yielding volatile components recoverable in breath. [redacted] was not a metabolite detected in mouse plasma or excreta (Study 100655).

Plasma parent and metabolite levels

The main toxicity studies were carried out in rats and monkeys. In these studies, TK data on plasma parent levels were collected. Metabolites were not measured, except for M7 in the rat carcinogenicity study. To aid in the interpretation of observed toxicities, Reviewer attempted to estimate plasma and metabolite levels in rats and monkeys and multiples of human levels, for parent, M2-glu, M5 and M7, based on all available PK data. Metabolite data were only from single dose studies, so it should be kept in mind that after multiple dosing these multiple estimates may be different. Although data on plasma metabolites are only for one time point (at approximate Tmax), it was assumed that calculations could be applied to AUC.

Plasma parent and metabolites (% of total at approx. Tmax) (single dose data)

	Study #	Parent (Cmax)	M2-Glu	M5 (501345)	M6	M7 (102664)
Rat	100677	1%*	-	20%*	-	80%*
Monkey	102035	0.27%	59%	16%	trace	16%
Human	980233	0.37%	11.2%	73%	9.1%	-

*estimate

Estimate of plasma parent and metabolite AUC (ngEqxh/mL) at 100 mg/kg/day HD in chronic rat and monkey studies

	Dose (mg/kg/day)	AUC (ngxh/mL)				
		Parent	M2-Glu	M5 (501345)	M6	M7 (102664)
Rat	100	3970*	(4000)**	79,400	(4000)**	317,600

Monkey	100	1180*	257,851	69,926	(4370)**	69,926
Human	3 (180 mg)	648	19,615	127,849	15,937	(1750)**

*data from toxicity studies, as calculated by Sponsor (6-mo rat, 1-year monkey)
** assuming this metabolite constitutes 1% of plasma drug-related material

Estimate of plasma metabolite:parent ratio (ngEqxh/mL) at 100 mg/kg/day HD in chronic rat and monkey studies

	Dose (mg/kg/day)	M2-Glu	M5 (501345)	M6	M7 (102664)
Rat	100	1x	20x	1x	80x
Monkey	100	220x	60x	3x	60x
Human	3 (180 mg)	30x	200x	25x	3x

Estimate of plasma parent and metabolite AUC multiples at 100 mg/kg/day HD in chronic rat and monkey toxicity studies

	Dose (mg/kg/day)	AUC (ngxh/mL)					
		Parent	M2-Glu	M5 (501345)	M6	M7 (102664)	M5+M7
Rat	100 mg/kg/day	**6.1x**	(0.2x)	**0.62x**	(0.25x)	(181x)	**3.1x**
Monkey	100 mg/kg/day	**1.8x**	**13x**	**0.55x**	(0.27x)	(40x)	**1.1x**
Human	3 mg/kg/day (180 mg)	1x	1x	1x	1x	1x	1x

Bolded are calculated multiples based on actual data, data in parentheses are based on assumption of low levels of 1%.

These calculations indicate that the M2-Glu metabolite was adequately qualified in monkey studies, with multiples attained ca. 6x higher than for parent. The M5 metabolite was also qualified in rat and monkey studies, but the multiples attained for this metabolite were not as large (>0.5x for both species). The M7 metabolite was adequately qualified in both species. When M5 and M7 were pooled, qualification was adequate mainly in rats. The M6 metabolite was present in human at much higher levels than monkeys and rats, and the multiples attained were low. Thus, qualification of M6 was relatively poor. However, note that plasma metabolite data were from single dose studies, and metabolite multiples attained in repeat dose studies may be different (i.e. higher) than calculated. An increase in multiple after 6 months or 12 months in the 50 and 100 mkd groups as compared to Day 1 was confirmed by actual data on metabolite M7 in the 12-month monkey toxicity study. Also, similarity in the M5, M6 and M7 metabolites may indicate similar pharmacologic/ toxicologic effects, which makes qualification of M6 less critical.
The calculations are confirmed by data on M7 plasma levels from the rat carcinogenicity study and 1-year monkey toxicity study. In those two studies, exposure (AUC, ngxh/mL) to M7 was 50x exposure to parent drug. For the metabolite M7, plasma exposure in ngEqxh/mL units is about 2x the AUC in ngxh/mL (1 ngxh/mL M7≈2ngxh/mL parent). The ngEqxh/mL multiple was thus 100x for M7 for rat and monkey. This agrees reasonably well with 60x and 80x calculated.

Activity of metabolites

An *in vitro* pharmacology study in HEK cells expressing the human calcium receptor was carried out with the major metabolites M5 (AMG 501345; human, monkey, mouse) and M7 (AMG 102664; monkey, rat, mouse) (Study #R2003214) . Both compounds (10 uM) did not

increase cytoplasmic calcium concentrations in fluo-3 loaded hCaR_HEK[redacted] cells. Positive control was NPS-467 (calcimimetic) (10 uM) which induced a large fluorescent Ca_i response. The relevance of these data is unclear since cinacalcet was not included in the experiments, there were no dose-response data, and the response measured was an intermediate effect with unknown quantitative relation to CaR-mediated biological response such as PTH release.

In an other study (Study #R2002079), the effect of AMG-073 and its M2-Glu metabolites (M2a, M2b) was measured on calcitonin release from rat medullary thyroid carcinoma cell line [redacted] These cells have low CaR levels (1/100x parathyroid levels). Extracellular calcium causes calcitonin release from these [redacted] cells, and AMG-073 shifted the dose-response curve to the left (potentiation) with EC_{50} of 34nM at 1.5 mM calcium. This is similar to the IC_{50} for AMG-073 to enhance the inhibitory effect of extracellular calcium on PTH release from cultured bovine parathyroid cells (A000273). In the [redacted] cells, M2a and M2b at 1000 nM had slight effects in terms of decreasing the calcium concentrations that induce half-maximal stimulation of calcitonin release (Ca-EC_{50}). AMG-073 had an effect in the range of 3-1000 nM. Based on Ca-EC_{50} values, M2a and M2b were >333 times less potent than the parent compound.

Table 1. Pharmacological Properties of Calcium-Stimulated Calcitonin Release from [redacted] Cells

	No Drug	R-AMG 073 (nM)					S-AMG 073 (nM)		U6 (nM)	U12 (nM)
Concentration	NA	3	30	100	300	1000	1000	3000	1000	1000
EC_{50} (mM)										

[redacted]

The pharmacologic activity of M6 on CaR-mediated or other events was not characterized. However, since this compound is structurally closely related to M5 (M6=hydroxylated M5) it is likely that, like M5 and M7, it has relatively little effect on CaR-mediated responses.
The pharmacologic or toxicologic effects of the naphthalene-group containing metabolites that are cleaved off after N-dealkylation were not investigated. Their cumulative levels are expected to be similar to the cumulative M5-8 levels.
Naphthalene is the active ingredient in moth balls and has been associated with various toxicities in humans and rats. Data from an acute toxicity study (#100413) with [redacted], a potential metabolite/cleavage product containing the naphthalene-moiety, suggest that this compound has CaR and hypocalcemic activity. This compound was tested because it is also an impurity and degradation product. However, it was not detected in mouse plasma or excreta and is probably rapidly metabolized.

3.3.5 Excretion

Fecal (hepato-biliary) and urinary elimination are the major routes of elimination for the nonclinical species. Labile metabolites (CO_2) were also eliminated via expired air. Urinary excretion consists of mainly glycine conjugates of carboxylic acid metabolites and dihydrodiol glucuronides in mice, rats, monkeys, and humans. Small amounts (< 15%) of unchanged drug were found in feces of mice and monkeys. There is no evidence that enterohepatic recirculation plays a role in cinacalcet disposition. In humans, greater than 96% of radioactivity was recovered in urine and feces, primarily via urinary excretion (83%).

Table 2.6.5.13. Pharmacokinetics: Excretion
Test Article: Cinacalcet Hydrochloride (AMG 073)

							Percent of Radioactive Dose Excreted[b]					
Study No.	Species	Sex (N)	Radio-Label	Dose mg/kg	Route[a]	Study Days	Urine	Feces	Bile	Breath	Tissues[c]	Total[d]
PK97220	Rat	M (5)	^{14}C-2-CH$_2$	10[e]	PO	4	19.9	37.9	NA	24.8	7.0	90.2
	Rat	F (5)	^{14}C-2-CH$_2$	10[e]	PO	4	23.4	41.2	NA	22.8	4.7	92.5
100147	Rat	M (3)	^{14}C-2-CH$_2$	10[e]	PO	2	15.5	4.4	55.1	NA	NA	75.0
	Rat	F (3)	^{14}C-2-CH$_2$	10[e]	PO	2	10.8	2.9	70.2	NA	NA	83.7
PK97151	Dog	M (2)	^{14}C-2-CH$_2$	10[f]	PO	7	16.2	64.3	NA	NA	NA	81.9
	Dog	F (2)	^{14}C-2-CH$_2$	10[f]	PO	7	28.6	49.0	NA	NA	NA	78.4
100170	Dog	M (2)	^{14}C-2-CH$_2$	10[f]	PO	7	10.1	4.4	74.2	NA	NA	89.1
100236	Rat	M (3)	^{3}H-naphthalene	10[e]	PO	4	22.4	36.9	NA	7.4	21.6[i]	88.3
	Rat	F (3)	^{3}H-naphthalene	10[e]	PO	4	24.2	40.7	NA	7.5	16.9[i]	89.4
PK0012	Rat	M (3)	[^{14}C]naphthalene	1[f]	PO	7	25.9	77.6	NA	ND	NA	103.8

							Percent of Radioactive Dose Excreted[b]					
Study No.	Species	Sex (N)	Radio-Label	Dose mg/kg	Route[a]	Study Days	Urine	Feces	Bile	Breath	Tissues[c]	Total[d]
100779	Rat	M (4)	^{14}C-methine	10[e]	PO	4	19.7	49.3	NA	17.5	2.7	89.6
	Rat	M (4)	^{14}C-methine	1[g]	IV	4	17.3	46.6	NA	19.4	3.4	87.2
100706	Monkey	M (3)	^{14}C-methine	10[f]	PO	9	47.9	28.0	NA	NA	NA	86.8
	Monkey	M (3)	^{14}C-methine	100[f]	PO	9	57.5	28.0	NA	NA	NA	91.3
	Monkey	M (3)	^{14}C-methine	100[f]	PO	5	44.1	11.9	40.3	NA	NA	98.2
100655	Mouse	M (9)	^{14}C-methine	10[f]	PO	7	38.8	24.3	NA	NA	<0.1	63.1
	Mouse	M (6)	^{14}C-methine	10[f]	PO	4	39.4	21.7	NA	NA	NA	61.7
	Mouse	F (6)	^{14}C-methine	10[f]	PO	4	35.0	24.2	NA	NA	NA	60.0

							Percent of Radioactive Dose Excreted[b]					
Study No.	Species	Sex (N)	Radio-Label	Dose mg/kg	Route[a]	Study Days	Urine	Feces	Bile	Breath	Tissues[c]	Total[d]
100577	Monkey	M (3)	^{14}C-CF$_3$	10[f]	PO	5	56.6	19.7	NA	NA	NA	80.4
	Monkey	M (3)	^{14}C-CF$_3$	100[f]	PO	5	51.1	29.4	NA	NA	NA	86.4
	Monkey	M (3)	^{14}C-CF$_3$	100[f]	PO	5	35.9	19.1	38.4	NA	NA	95.8
980233	Human[h]	M (5)	^{14}C-CF$_3$	~1[h]	PO	8	83.2	13.1	NA	NA	NA	96.3
100524	Mouse	M (6)	^{14}C-CF$_3$	10[f]	PO	4	50.8	37.2	NA	NA	NA	87.8
	Mouse	F (6)	^{14}C-CF$_3$	10[f]	PO	4	69.0	27.3	NA	NA	NA	96.3
100305	Rat	M (4)	^{14}C-CF$_3$	10[e]	PO	4	46.5	44.5	NA	0.4	0.3	91.2
100477	Rat	M (6)	^{14}C-CF$_3$	50[e,g]	PO	6[g]	45.1	40.1	NA	NA	5.8	91.0

NA = Not assessed.
[a] Dose administered fasted in studies 100170, 980233andPK0012.Doses given without regard to food in all other studies.
[b] All radioactivity determinations were done via liquid scintillation counting.
[c] Tissues include residual carcass.
[d] Total includes cage wash/wipes.
[e] Vehicle was Alkamuls EL-620/L.
[f] Vehicle was 0.5% methylcellulose.
[g] 50 mg/kg/day for 6 days with collection up to 24-h after last dose.
[h] Total dose in humans was 75 mg/kg administered as a soft gelatin capsule.
[i] Recovery of radioactivity in tissue presumably due to exchange of tritium into body water.

Excretion in milk

In rats, cinacalcet and related compounds are excreted into the milk, with relatively high levels of parent drug in milk as compared to plasma. Milk:plasma AUC ratio of total radioactivity was low at 0.46, while milk:plasma AUC ratio of parent drug was much higher at 9.6, indicating relatively high lacteal excretion of parent drug.

Table 2.6.5.7b. Pharmacokinetics: Study in Nursing Animals – Excretion in Milk of Rats
Test Article: Cinacalcet Hydrochloride (AMG 073); Study 102291

Excretion into Milk		Plasma	Milk	Milk/plasma ratio
Species: SD rat (N=18; 3/per time point)		Radioactivity		
Lactation day: Day 12 postpartum	T_{max} (h)	5	8	---
Radiolabel: [^{14}C-CF_3] cinacalcet	C_{max} (µg Eq/g)			
Feeding condition: Fed	AUC(0-24) (µg Eq•h/g)	55.3	25.4	0.459
Vehicle/Formulation: 0.5% CMC				
Method of Administration: Oral gavage		Cinacalcet		
Dose (mg/kg) : 10	T_{max} (h)	1	3	---
Dose Regimen: single dose	C_{max} (ng/mL)			
Analyte: Total radioactivity and cinacalcet	AUC(0-24) (ng Eq•h/g)	302.24	2895.04	9.58
Assay: LSC and LC/MS/MS				

Transplacental transfer

In rabbits cinacalcet was found to cross the placental barrier and the fetuses were exposed to the drug, although lower concentrations were measured in fetal blood than in maternal blood.

Table 2.6.5.7a. Pharmacokinetics: Study in Pregnant or Nursing Animals – Placental Transfer
Test Article: Cinacalcet Hydrochloride (AMG 073); Study 100021

Placental Transfer	
Species	New Zealand White Rabbit
Gestation day / Number of animals	Day 18, for maternal blood (n=3/dose); Day 21 for fetal blood (n=3/dose)
Vehicle/Formulation	0.5% Methyl cellulose suspension
Method of Administration	Oral
Dose (mg/kg)	0, 1, 5, 25, and 100 mg/kg
Dose Regimen	Once daily
Analyte	Cinacalcet
Assay	LC/MS/MS
Time (h)	Maternal: Over 24 hours post dose; Fetal and amniotic fluid: 2 hours post dose
Concentration	
Dam	
Fetus	Conc. At 2 h: 6.27 ng/mL at 25 mg/kg/day and 21.4 ng/mL at 100 mg/kg/day

3.3.6 Pharmacokinetic drug interactions

Inhibition of P450 enzymes by cinacalcet was evaluated in vitro in pooled human liver microsomes (Study 100157). IC50 values for cinacalcet to inhibit CYP1A2, CYP2C9, CYP2C19, and CY3A4 were higher than plasma drug concentrations (>10->300 uM). However, cinacalcet is a potent inhibitor of CYP2D6 (IC_{50} = 70 nM, compared to Cmax of [redacted]). Thus, cinacalcet is expected to inhibit the metabolism of CYP2D6 substrates in vivo.

In the 1-year monkey toxicology study there was a small increase (<2-fold) in hepatic CYP450 content at the highest dose of 100 mg/kg/day, after 6 or 12 months of dosing, suggesting a potential for slight P450 induction. Clinically there is no evidence for induction.

The effects of co-administration [redacted] injection (vitamin D, 0.1 ug/kg) on the PK of cinacalcet was investigated in monkeys. There was no significant effect on cinacalcet plasma concentrations over a 24h period. Conversely, since vitamin D is metabolized by specific hydroxylases (CYP24 and CYP27) it is unlikely its pharmacokinetics would be altered by cinacalcet.

3.3.7 Other studies

In a calcium homeostasis study in female monkeys (Study 100961), vehicle or cinacalcet (15 mg/kg) was given, orally, 2x daily, for 28 days. On Day 15, single doses (oral or IV) of $45CaCl_2$ were given and calcium excretion in urine and feces and calcium content of femur and ribs were determined. There was no effect on fecal or urinary calcium excretion. Bone calcium content was similar in both groups. However, data were highly variable. Calcium excretion was also unaffected in a pharmacology study in 5/6 Nx rats (Study R2002078).

3.4 TOXICOLOGY

3.4.1 Toxicity study summary

A summary and evaluation of the animal toxicity study findings is presented at the end of section 3.4.2

3.4.2. Single and repeat dose toxicity

- Studies were designed based on ICH, FDA, CPMP, and OECD guidelines
- Studies relevant for evaluation of oral cinacalcet toxicity were single and repeat dose toxicity studies, safety pharmacology studies, juvenile rat and dog studies, impurity studies, genotoxicity studies, carcinogenicity studies, and reprotoxicity studies.
- Single dose and subchronic toxicity studies in mice, rats, dogs and monkeys, of up to 1-month duration were described in the initial review of IND 56,010 (APPENDIX).
- A summary review of up to 1-week dose range finding studies in dog and monkey, a 1-month dog toxicity study, 28-day juvenile rat and dog studies and some other toxicity assays is included in this NDA review.
- Reviews of chronic rat study (6-month) and (sub)chronic monkey studies (3-month, 12-month) are included in this NDA review.
- Toxicokinetic evaluation was performed in all pivotal toxicity studies, and in reproductive dose-range finding studies and carcinogenicity studies.
- Tabular summaries of all pivotal single and repeat dose toxicity data and exposure multiples are provided, followed by evaluation and interpretation of animal toxicity findings.

Single and repeat dose studies in mice, rats, dogs, monkeys

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg[a])	GLP	Testing Facility	Study No.
Single-dose Toxicity							
	CD-1 Mouse	Oral Gavage	Single Dose	0, 10, 100, 500	Yes	[redacted]	970153
	CD-1 Mouse	Intraperitoneal Injection	Single Dose	0, 1, 5, 20	Yes	[redacted]	970154
	Sprague-Dawley Rat	Oral Gavage	Single Dose	1000 and 2000	No	Amgen	970012
	Sprague-Dawley Rat	Oral Gavage	Single Dose	0, 10, 100, or 500	Yes	[redacted]	970151
	Sprague-Dawley Rat	Oral Gavage	Single Dose	1000, 1500	Yes	[redacted]	100326
	Sprague-Dawley Rat	Intraperitoneal Injection	Single Dose	0, 1, 5, 20	Yes	[redacted]	970152
	Beagle Dogs	Oral gavage	Dosing on days 1 and 8	50, 100, and 200 of each of two different AMG 073 salts	No	[redacted]	100080

Type of Study	Species and Strain	Method of Administration	Duration of Dosing[a]	Doses (mg/kg[b])	GLP	Testing Facility	Study No.
Repeat-dose Toxicity (non-pivotal)							
	Beagle Dog	Oral Gavage	4 days[c]	16, 32, 64, 96, 128, 160, 200	No	[redacted]	970060
	Cynomolgus Monkey	Nasal Gastric Intubation	Dosing on days 1, 3, and 5	16 (day 1), 32 (day 3), 48 (day 5),	No	[redacted]	970142
	Cynomolgus Monkey	Nasal Gastric Intubation	1 week	0, 24, 48, 96	No	[redacted]	970147

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg[a])	GLP	Testing Facility	Study No.
Repeat-dose Toxicity (pivotal)							
	Sprague-Dawley Rat	Oral Gavage	14 days	0, 50, 250, 500	Yes	[redacted]	970018
	Sprague-Dawley Rat	Oral Gavage	28 days	0, 5, 50, 125	Yes	[redacted]	970070
	Sprague-Dawley Rat	Oral Gavage	6 months	0, 6, 26, 100	Yes	[redacted]	100082
	Beagle Dog	Oral Gavage	28 days	0, 5, 50, 100	Yes	[redacted]	970078
	Cynomolgus Monkey	Nasal Gastric Intubation	13 weeks	0, 5, 50, 100, 150[b]	Yes	[redacted]	100020
	Cynomolgus Monkey	Nasal Gastric Intubation	1 year	0, 5, 50, 100	Yes	[redacted]	100188

(Continued)

[a] Unless otherwise specified. If a NOAEL was determined for a study, it has been underlined.
[b] On days 1-15 animals received 150 mg/kg/day (75 mg/kg/bid), on days 16-83 animals received 100 mg/kg/day (50 mg/kg bid)

DOG AND MONKEY TOXICITY STUDIES OF ≤1 MONTH DURATION

NOTE: Data from acute oral and i.p. studies in mice and rats have been included in the tabular summary at the end of the toxicology section. An acute rat study with [redacted] is described in the special toxicology subsection.

Acute oral gavage dog toxicity study (#100080) in beagle dogs (3/sex/grp) with doses 0, 50, 100, 200 mg/kg AMG-073HCl or mesylate.

Results:

☐ Vomiting, discolored or watery stools, excessive salivation @ LD, MD, HD.
☐ Absorption was the same for both salts.

4-day oral gavage dog toxicity study (#970060) in beagle dogs (4/sex/grp) with escalating doses of 16, 32, 64, 96, 128, 160, 200 mg/kg AMG-073

Results:

☐ Vomiting, non-formed or liquid or mucoid feces at all doses
☐ Excessive salivation @ 32 mg/kg and higher
- Trembling @ 64 mg/kg and higher;

☐ Plasma calcium reduced @ all doses.
☐ TK analysis: Less than proportional increase in Cmax and AUC with dose, suggesting saturation of absorption

28-day toxicity oral (gavage) study (#970078) in dogs (N= 6 or 4 /sex/grp; N=2 recovery ctrl and HD) with doses 0, 5, 50, 100 mg/kg/day

- Excessive salivation in LD (1/4f), MD (all), HD (all); Emesis in LD (1/4), MD (all), HD (all), decreasing over course of study; Hypoactivity and tremors in MD, HD
- No effects on BW, FC
- Transient decrease in RBC, Hb, Hct in HDf
- No ophthalmoscopy abnormalities

- EKG's (pretreatment, and @ 4wks prior to sacrifice) were within normal limits. There were no effects on PRS, ST, QT, or QTc interval (Report Amendment 2)
- Minimal (<10%) decrease in serum total calcium in MDm and HDm,f
- Dose-dependent decrease in serum ionized calcium of maximally 8%, 19%, 20% in LD, MD, HD
- Decrease in serum PTH (control levels 25-53 pg/mL), @ 2h post-dose, in all dose groups. Effect was not clearly dose-related. Levels @2h post dose varied from 0 (BLQ) to 34 pg/mL.
- Urine volume increased in LDf, MDm,f, HDm,f (up to 2x in HD). Decreased urine specific gravity in MD, HD. Decreased urine Na, Cl, K concentrations (meg/L) at all doses. Increased urine Ca (mg/dL) and increased urine Ca excretion rate (mg/time) in MD, HD. Urine changes were NOT reversed in HD group after 2 weeks recovery.
- Lung weight (rel, abs) minimally increased in treated
- Pathology: Lung, mucoid exudate in 1/4HDm and 1/4Hdf, liver fibrosis in 1/4HDf, testis tubule degeneration in 1/4HDm. No heart abnormalities.
- TK analysis: Variable serum levels, low bioavailability. Average Cmax ranged from 12 ng/mL in LD to 99 ng/mL in HD, and AUC from 21 ngxh/mL in LD to 500 ngxh/mL in HD (Day 28 values). AUC in dog was about 10-fold lower than in rats at similar mg/kg doses. Dog was considered not an appropriate animal species for chronic toxicity testing. Dog appears very sensitive to hypocalcemia.

Acute oral (nasogastric intubation) toxicity study (# 970142) in monkeys (1/sex/grp) with doses 16, 32, 48 mg/kg (escalating dose study)

Results:

☐ Appetite suppression and decreased fecal output @ 32 and 48 mg/kg
☐ No effect on BW
☐ Reduced plasma ionized calcium @ all doses

1-week oral (nasogastric intubation) toxicity study (# 970147) in monkeys (2/sex/grp) with doses 0, 24, 48, 96 mg/kg/day

Results:

- Appetite suppression and decreased fecal output @ all doses
- Emesis @ 96 mg/kg/day
- Reduction in plasma ionized calcium @ all doses; maximal effect 22% to 27% in HD (4-6h post dose)
- Non-linear TK (increase less than proportional suggesting absorption saturation as seen in dogs)

JUVENILE RAT AND DOG 1-MONTH TOXICITY STUDIES

28-day oral toxicity study in juvenile rats with a 28-day recovery period (Study Nr. 101939)

Methods:

Juvenile rats (3 weeks, N=17/s/g) were dosed by oral gavage at 0, 0.5, 1.5, 5 mg/kg/day for 28 days, or for 28 days + 28 days recovery. The HD of 5 mkd was the NOAEL in the 28-day adult rat study. Satellite animals were used for plasma concentrations, pH, ionized Ca, and PTH. Bone biomarkers were measured in serum and urine. Femur was analyzed by pQCT.