CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-838

PHARMACOLOGY REVIEW(S)

K. Bongiovanni
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REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

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2/17/98

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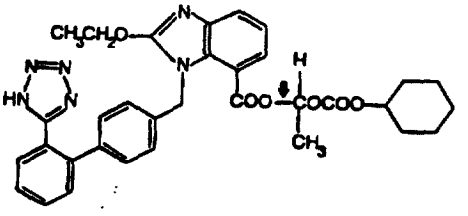
PRODUCT: ATACAND™ Tablets (Candesartan cilexetil, TCV-116, H212/91)

SPONSOR: Astra Merck
725 Chesterbrook Blvd.
Wayne, PA 19087-5677
(610) 695-1370

CHEMISTRY: Candesartan cilexetil (CAS No. 145040-37-5) is described as (±)-1-(cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]1H-benzimidazole-7-carboxylate. Its molecular weight is 610.67 and its empirical formula is $C_{33}H_{34}N_6O_6$. Candesartan cilexetil has a chiral center on the ester portion of the molecule and exists as two enantiomers; however, the active drug (candesartan) produced upon hydrolysis is achiral.



↓ site of ester hydrolysis.

PHARMACOLOGICAL CLASS: Angiotensin II Receptor Antagonist

PROPOSED INDICATION: Treatment of hypertension.

FORMULATION AND ROUTE OF ADMINISTRATION: ATACAND is formulated in tablets for oral use containing 4, 8 or 16 mg of candesartan cilexetil/tablet; excipients include hydroxypropyl cellulose NF, polyethylene glycol-8000 NF, lactose NF, corn starch NF, carboxymethylcellulose calcium NF, and magnesium stearate NF. Ferric oxide NF is added to the 8 and 16 mg tablets as a colorant.

PROPOSED DOSAGE REGIMEN: The recommended starting dose is 16 mg once daily and may be increased to 32 mg once daily.

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:

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INTRODUCTION

The renin-angiotensin system plays an important role in the regulation of blood pressure and fluid and electrolyte balance. The primary active hormone of this system is angiotensin II (AII). It is one of the most potent vasoconstrictor agents known. Blockade of the renin-angiotensin system, as observed with angiotensin converting enzyme (ACE) inhibitors, has proved to be effective in the treatment of hypertension and congestive heart failure.

The ACE inhibitors, though efficacious, are not specific for inhibition of the conversion of angiotensin I to angiotensin II. These drugs also interfere with the inactivation of bradykinin, enkephalins and other biologically active peptides. Angiotensin II receptor antagonists evolved from attempts to identify agents which possessed greater specificity against the functional effects of angiotensin II.

Angiotensin II binding sites are present in various tissues including rat and rabbit adrenal cortex and medulla, rat and human uterus, rat brain and aorta, bovine cerebellum and human renal artery. Recent studies indicate that AII receptors are not homogeneous but exist in two interconvertible forms. The receptor subtypes (AT-1 and AT-2) have differential tissue distribution and functional responses. Physiologically important actions of AII, such as vascular smooth muscle contraction and aldosterone biosynthesis are mediated through activation of AT-1 receptors. The AT-2 receptor is widely distributed in fetal tissues. In adult animals, some tissues contain primarily either AT-1 receptors (e.g. vascular tissue) or AT-2 receptors (e.g. brain), whereas other tissues contain the receptor subtypes in similar amounts. No functional role for AT-2 receptors has been unequivocally defined.

Candesartan cilexetil is a non-peptide prodrug that is hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan selectively antagonizes angiotensin II at AT-1 receptors *in vitro* and antagonizes the functional effects of angiotensin II *in vivo*.

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PHARMACODYNAMICS

Effects Related to Proposed Therapeutic Indication

Antihypertensive Effects in Hypertensive Rats

Candesartan cilexetil was administered orally by gavage to conscious male spontaneously hypertensive rats (SHR), 2-kidney 1-clip renal hypertensive rats (2K-1C-RHR), 1-kidney 1-clip renal hypertensive rats (1K-1C-RHR) and deoxycorticosterone acetate (DOCA)/salt hypertensive rats at doses of 0.01 to 10 mg/kg. Blood pressures (measured directly from implanted aortic catheters or indirectly by the tail cuff method) and heart rates were measured up to 24 hours following dosing.

In conscious SHR, single oral doses of 0.01 to 10 mg/kg of candesartan cilexetil reduced blood pressure in a dose-related manner (Fig. 1). The antihypertensive effects with 0.1 and 1.0 mg/kg (25 and 40 mmHg peak reductions in mean blood pressures, respectively) lasted over 10 hours. The peak antihypertensive effect elicited by the 10 mg/kg dose was comparable to that produced by 1 mg/kg candesartan cilexetil; the duration of antihypertensive effect by the higher dose was prolonged (30 mmHg reduction in blood pressure evident at 24 hours post dose). These doses of candesartan cilexetil had no significant effect on heart rate.

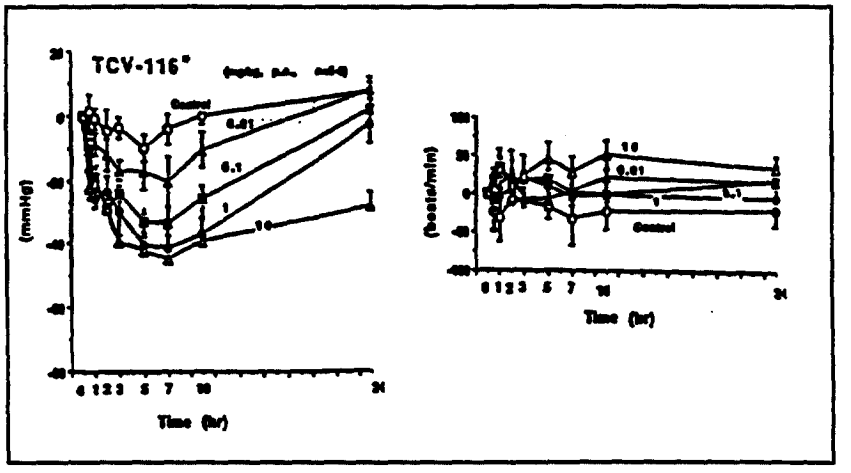

Figure 1. Blood pressure and heart rate effects of oral candesartan cilexetil in SHR.

Candesartan cilexetil was administered orally to SHR at doses of 0.1, 1 or 10 mg/kg/day for 2 weeks. Systolic blood pressure was measured by the tail cuff method just before and at 5 and 24 hours after drug administration on days 1, 3, 7 and 14; measurement of blood pressures was continued for 2 weeks after termination of drug. The 0.1 mg/kg/day dose

produced a lowering of systolic blood pressure in SHR that was consistent throughout the 2-week dosing period. The magnitude of the blood pressure lowering effects by the 1 and 10 mg/kg/day doses increased progressively during the 2-week dosing period (Fig. 2). The antihypertensive effects of candesartan cilexetil disappeared gradually after drug termination. Heart rate was not affected by candesartan cilexetil treatment.

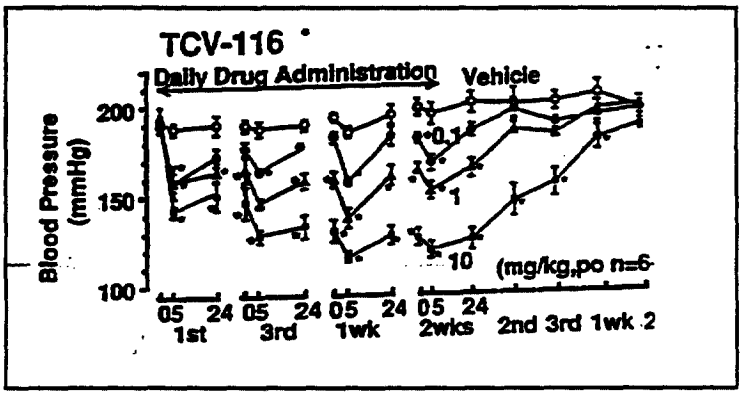

Figure 2. Blood pressure effect of repeated oral administration of candesartan cilexetil to SHR.

In 2K-1C-RHR, candesartan cilexetil lowered arterial pressure in a dose-related manner. Oral doses of 0.1 to 10 mg/kg produced maximum blood pressure reductions of 25 to 70 mmHg (Fig 3). Heart rates were not significantly changed by these doses of candesartan cilexetil. The antihypertensive effects produced by doses of 0.1, 1 and 10 mg/kg persisted beyond 24 hours after dosing.

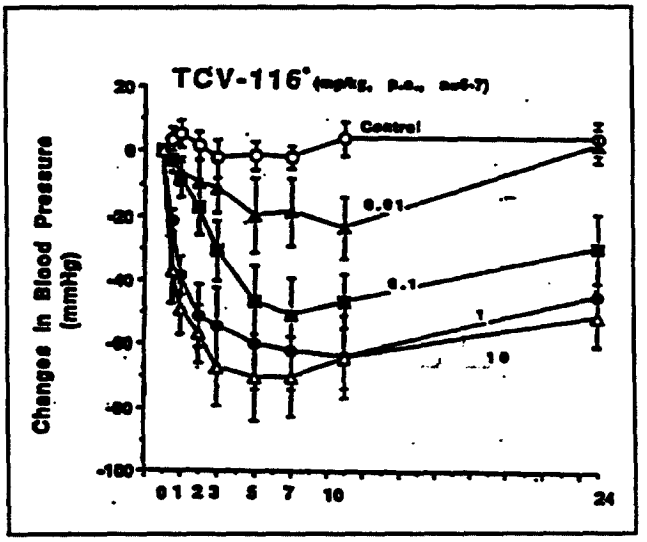

Figure 3. Blood pressure effects of oral candesartan cilexetil in 2K-1C-RHR.

Oral doses of 0.1 to 10 mg/kg of candesartan cilexetil reduced blood pressure in 1K-1C-RHR in a dose-related manner. Blood pressures were reduced by about 30 and 50 mmHg by doses of 1 and 10 mg/kg, respectively; the antihypertensive effect persisted for more than 24 hours. Heart rate was unaffected within this dosage range of candesartan cilexetil.

In DOCA/salt hypertensive rats, an oral dose of 10 mg/kg of candesartan cilexetil had no significant blood pressure lowering effect. Thus, the antihypertensive effect of candesartan cilexetil depends on the pretreatment level of plasma renin activity (and presumably elevated plasma angiotensin II activity) which appears to be suppressed in this animal model of hypertension.

An oral dose of 1mg/kg of candesartan cilexetil had no effect on blood pressure in normotensive rats; higher doses of 10 or 100 mg/kg caused a modest lowering (~10 mmHg) of blood pressure without affecting heart rate.

Antihypertensive Effects in Hypertensive Dogs

Candesartan cilexetil was evaluated for antihypertensive activity in 2-kidney, 1-clip renal hypertensive male Beagle dogs. Blood pressure was measured indirectly by the cuff method placed on the antebrachial artery and heart rate was calculated from the blood pressure pulses. Candesartan cilexetil was administered to the dogs when plasma renin activity (PRA) was elevated (3 days after surgical placement of clip to restrict renal blood flow) and when plasma renin activity had returned to normal levels (4 to 5 weeks after surgery). Under conditions of high PRA (mean PRA=6 ng AI/ml/hr), an oral dose of 0.3 mg/kg reduced blood pressure by 25 mmHg and the effect disappeared 7 hours after drug administration. The 1 mg/kg dose reduced blood pressure by 35 to 40 mmHg and the effect lasted over 10 hours. Heart rate was not affected by candesartan cilexetil. Under conditions of normal PRA (mean PRA=1-2 ng AI/ml/hr), the magnitude and duration of antihypertensive effects by oral doses of 0.3 and 1 mg/kg were similar to those produced under high PRA conditions. Heart rate was not significantly affected by candesartan cilexetil (Fig. 4).

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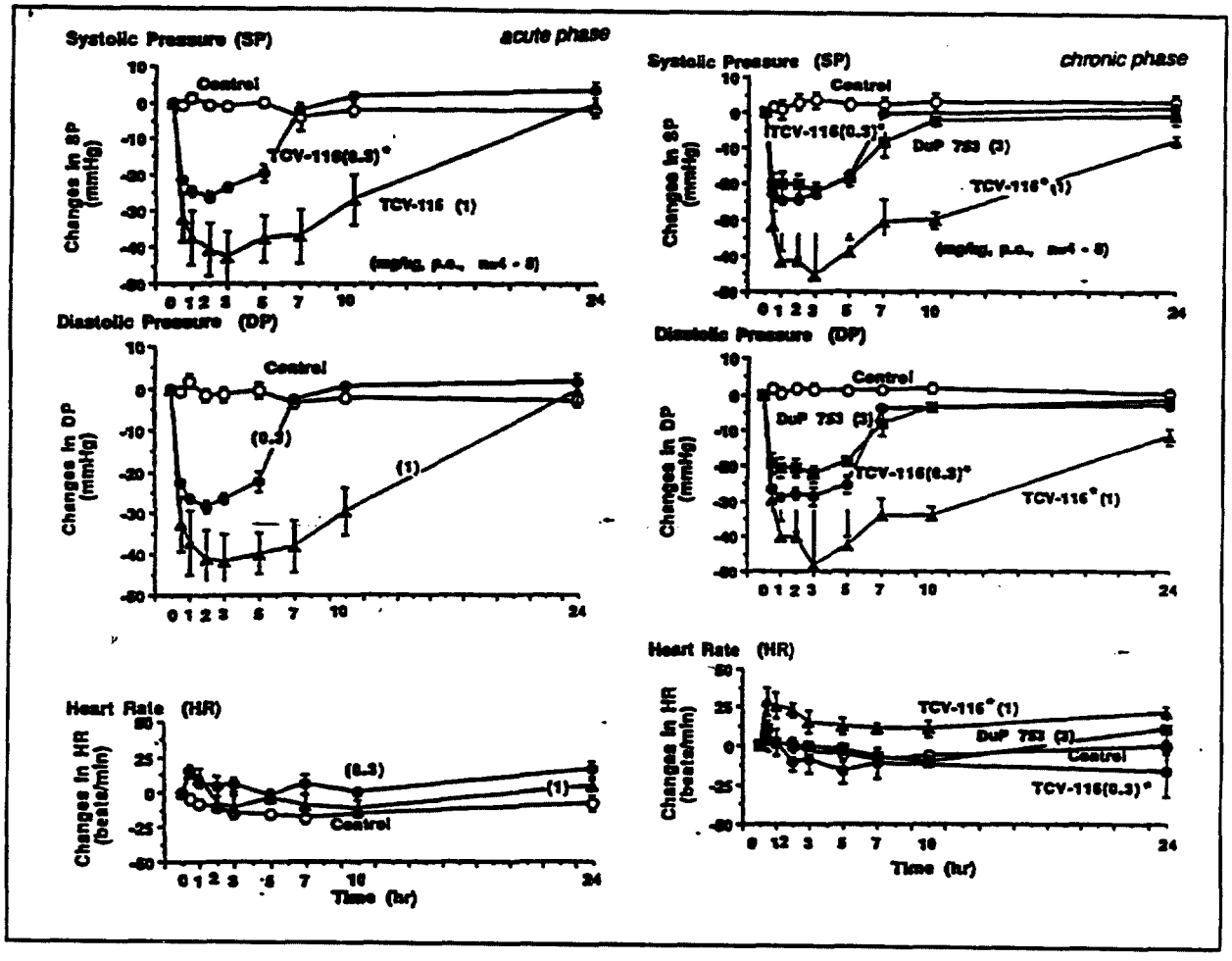

Figure 4. Blood pressure and heart rate effects of oral candesartan cilexetil in renal hypertensive dogs.

Mechanism of Antihypertensive Action/Drug-Receptor Interactions

Candesartan was tested for its ability to inhibit specific binding of radiolabelled angiotensin II (AII) to angiotensin AT_1 or AT_2 receptors. Microsomal fractions from homogenized freshly isolated bovine adrenal cortex, rabbit aorta or bovine cerebellum were used as sources of angiotensin II receptors. In rabbit aorta membrane fractions, candesartan inhibited the specific binding of the radioligand, [^{125}I]AII, at AT_1 receptor sites with an IC_{50}value of 2.86×10^{-8}M. Similarly, candesartan inhibited the specific binding of [^{125}I]AII to the AT_1 receptors to membrane fractions of bovine adrenal cortex with an IC_{50} value of 1.12×10^{-7}M. Candesartan was not effective (IC_{50} >10^{-5}M) in inhibiting the specific binding of [^{125}I]AII to the angiotensin AT_2 receptor of bovine cerebellum membrane fractions.

In conscious normotensive rats, candesartan given intravenously or candesartan cilexetil given orally produced dose-dependent inhibition of AII-induced pressor responses; the

ID_{50} values were 0.069 mg/kg PO for candesartan cilexetil and 0.033 mg/kg IV for candesartan. In conscious dogs, candesartan cilexetil inhibited the AII-induced pressor responses dose-dependently; the ID_{50} value for this effect was 0.06 mg/kg PO (Fig. 5).

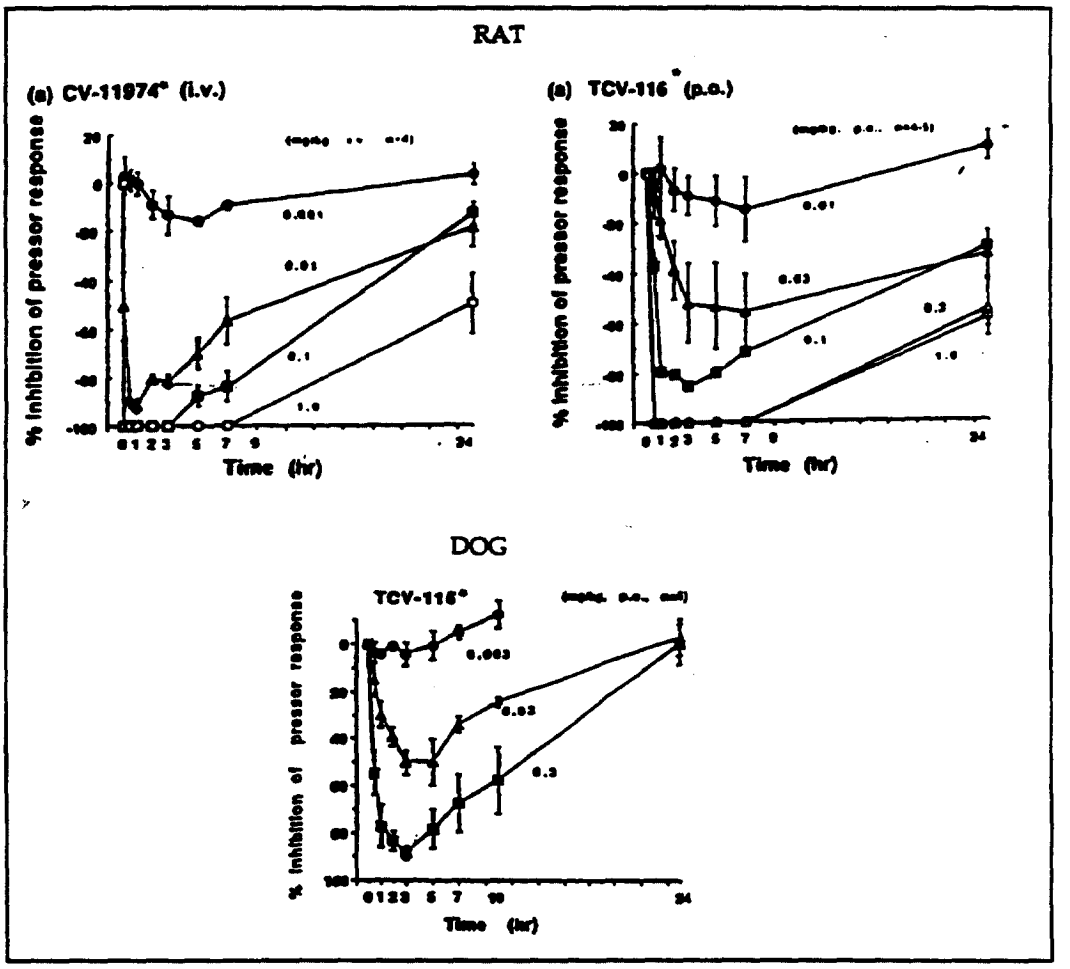

Figure 5. Inhibition of A-II-induced pressor responses by candesartan cilexetil or candesartan (CV 11974).

In isolated rabbit aortic strips, candesartan displayed potent inhibition of AII-induced vascular contractions (Fig. 6). Candesartan shifted the AII-induced contractile response curve to the right and reduced the maximal contractile response, suggesting noncompetitive AII antagonism. The pD'_2 value for this inhibitory effect was 9.97. Candesartan is an angiotensin-specific antagonist as, at tissue bath concentrations of 10^{-5}M, it did not inhibit the contractions induced by KCL, norepinephrine, serotonin, endothelin or prostaglandin F_{2a}.

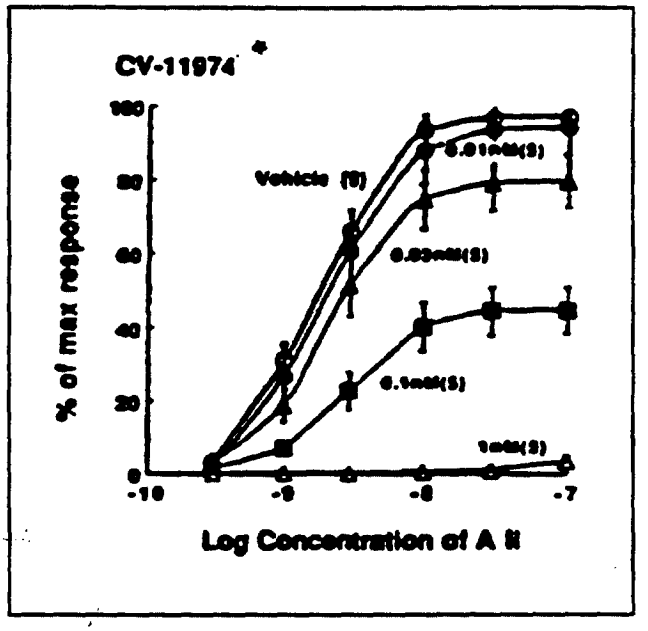

Figure 6. Inhibition by candesartan of A-II-induced vascular contractions in isolated rabbit aorta.

Effects on Plasma Renin and Aldosterone

In conscious normotensive rats, candesartan cilexetil induced marked increases in plasma renin activity at 1 and 5 hours after a single oral dose of 0.1 mg/kg; a lower dose of 0.01 mg/kg had no effect on plasma renin concentrations (Fig. 7).

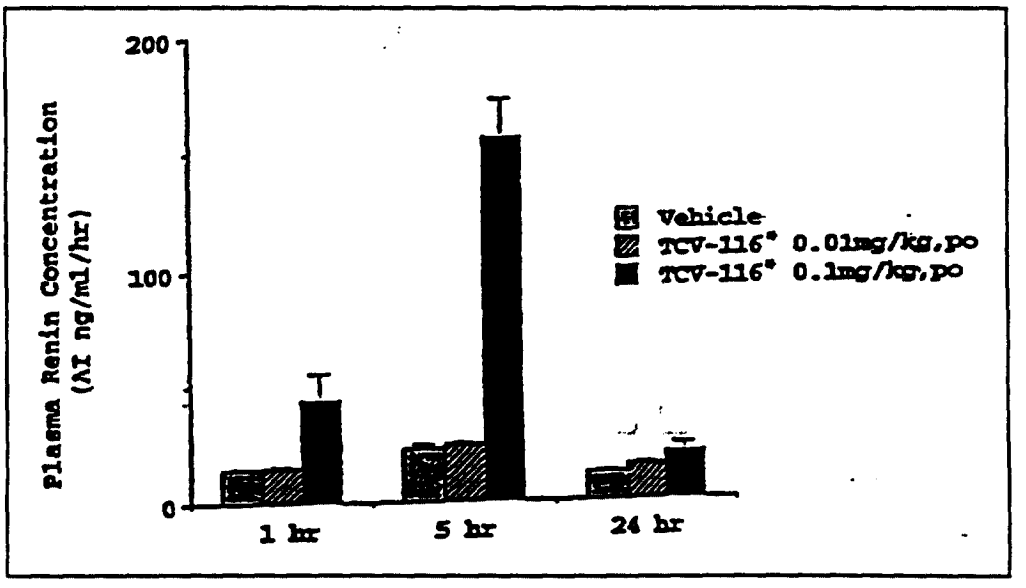

Figure 7. Plasma renin levels after oral candesartan cilexetil in rats.

In conscious SHR, candesartan cilexetil (1 mg/kg/day, PO, for 2 weeks) increased plasma renin, plasma AI and AII and reduced plasma aldosterone concentrations. This treatment had no effect on serum angiotensin converting enzyme (ACE) activity (Fig. 8)..

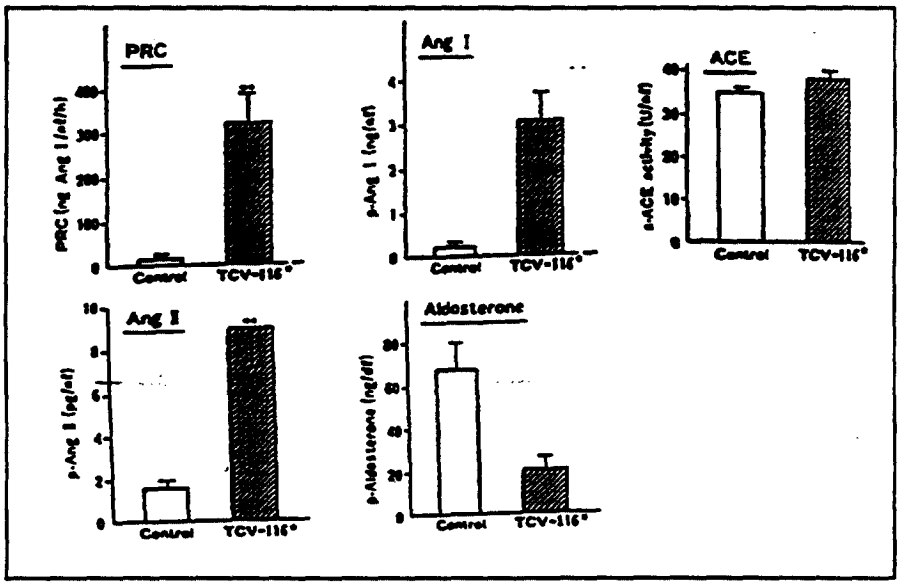

Figure 8. Effect of oral candesartan cilexetil on plasma renin, A-I, A-II and aldosterone levels in SHR.

Other Pharmacologic Effects/Safety Pharmacology

Candesartan cilexetil was examined in a variety of functional and behavioral test procedures for detection of other pharmacologic properties (Tables 1-5). Candesartan cilexetil was generally devoid of actions on the central, somatic and autonomic nervous systems, gastrointestinal system and pulmonary system. Effects on the cardiovascular system were primarily associated with actions on arterial vasculature and blood pressure; no drug effect on cardiac function was observed.

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Table 1. Candesartan Cilexetil Activity on Central Nervous and Somatic Nervous Systems.

Test System	Animals (#/group)	Dose (mg/kg) & Route	Results
Behavioural Observations	Mice (4)	1000 PO	Slight decrease in body tone 90 min after drug in 1/4 mice
Spontaneous Locomotor Activity	Mice (10)	30, 100, 300 PO	No Effect
Skeletal Muscle Coordination	Mice (10)	30. 100, 300 PO	No Effect
Anticonvulsant Action	Mice (10)	30, 100, 300 PO	No Effect
Pentobarbital Sleeping Time	Mice (10) Rats (6)	30, 100, 300 PO	No Effect
Analgesic Action	Mice (10)	30, 100, 300 PO	No Effect
Normal Body Temperature	Rats (6)	30, 100, 300 PO	No Effect
Spontaneous EEG and Behavior	Cats (3)	300 PO	No Effect
Spinal Reflex	Cats (3)	100 ID	No Effect
Neuromuscular Transmission (phrenic nerve diaphragm)	Rats (4)	10^{-6}, 10^{-5}, 10^{-4}M in vitro	No Effect

Table 2. Candesartan Cilexetil Activity on the Cardiovascular System.

Test System	Animals (#/group)	Dose (mg/kg) & Route	Results
Blood Pressure (BP), Heart Rate (HR), Blood Flow, ECG	Dogs (3)	3. 30, 100 ID	Slight decrease in BP and increase in renal blood flow at doses ≥3 mg/kg. No effect on HR or ECG
Blood Pressure (BP), Heart Rate (HR), LV Syst. Pressure Cardiac Output (CO), Peripheral Resistance (TPR)	Dogs (3)	30, 100 ID	Slight decreases in BP and TPR at 30 and 100 mg/kg. No effect on HR or LV Syst. Pressure.
Cardiac Rate and Contractile Force (isolated atria)	Guinea pigs (4)	10^{-6}, 10^{-5}, 10^{-4}M in vitro	No Effect

Table 3. Candesartan Cilexetil Activity on the Autonomic Nervous System.

Test System	Animals (#/group)	Dose (mg/kg) & Route	Results
Bradycardic response to right vagal stimulation, pressor response to bilateral carotid occlusion, nictitating membrane contraction to cervical nerve stimulation, BP responses to Ach, histamine and norepinephrine	Cats (3)	30, 100, 300 ID	Slight inhibitory effect on the pressor response to carotic occlusion at doses ≥100 mg/kg. No effect on other responses measured
Spasmogen-induced contraction of isolated ileum	Guinea pigs (4)	10^{-10} to 10^{-4}M in vitro	No Effect

Table 4. Candesartan Cilexetil Activity on Gastrointestinal and Smooth Muscle Function.

Test System	Animals (#/group)	Dose (mg/kg) & Route	Results
Gastric Emptying	Rats (6)	30, 100, 300 PO	Slight decrease in gastric emptying with 300mg/kg
Gastric Secretion	Rats (6)	30, 100, 300 ID	No Effect
Intestinal Transit	Rats (6)	30, 100, 300 PO	No Effect
Isolated Trachea	Guinea pigs (4)	10^{-5}, 10^{-4}M in vitro	No Effect
Ileum Motility	Rabbits (3)	10^{-7} to 10^{-4}M in vitro	No Effect
Uterine Motility	Rats (3)	10^{-7} to 10^{-4}M in vitro	No Effect

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Table 5. Other Pharmacologic Actions of Candesartan Cilexetil.

Test System	Animals (#/group)	Dose (mg/kg) & Route	Results
Urine and electrolyte (Na^+, K^+) excretion	Rats (5)	30, 100, 300 PO	Slight decrease in urine volume and sodium excretion with 300 mg/kg
Carrageenin-induced paw edema	Rats (5)	100, 300 PO	Slight inhibition of paw edema with 100 and 300mg/kg
Citric acid-induced cough	Guinea pigs (16-17)	10 PO	No potentiation of cough response
Capsacain or bradykinin-induced vascular permeability	Guinea pigs (8)	10 PO	No Effect

DRUG DISPOSITION

Absorption and Pharmacokinetics/Toxicokinetics

Radiolabelled candesartan cilexetil was used to assess the site and extent of drug absorption in rats and dogs (Takeda Study Report #C-42-753). Pylorus-ligated rats were used to determine extent of gastric absorption; to study the site of intestinal absorption, a loop of 6 cm length was selected in the small intestine or large intestine and ligated at both ends. [^{14}C]-candesartan cilexetil was administered directly into the stomach or intestinal loops at a dose of 1 mg/kg. Venous blood samples (via tail vein) collected at various post drug intervals were assayed for radioactivity. Plasma radioactivity was highest following administration of drug into the small intestine with decreasing concentrations after instillation in to the loop of the large intestine; the lowest concentration of radioactivity was noted after intragastric administration (Figure 9).

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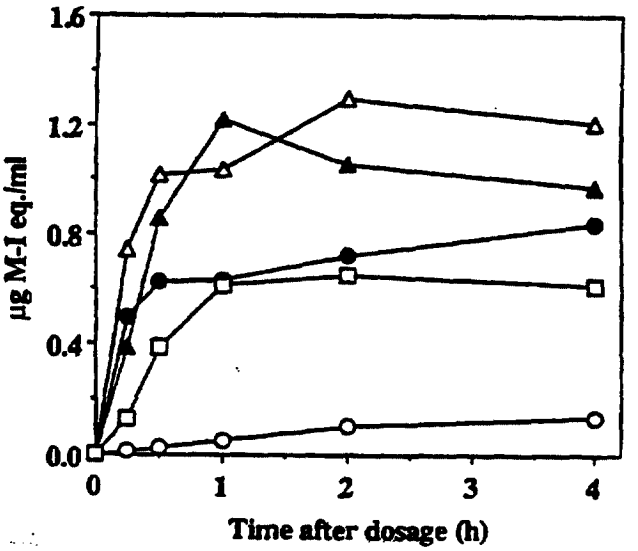

Figure 9. Absorption of radioactivity (expressed as M-I, candesartan, equivalent) in plasma after administration of radiolabelled 1 mg candesartan cilexetil/kg into the GI loops of rats. Stomach=open circles, duodenum=dark circles, jejunum=open triangles, ileum=dark triangles, large intestine=open squares.

Within 2 hours after intrajejunal administration of 1 mg [^{14}C]-candesartan cilexetil/kg, approximately 11% of the dosed radioactivity appeared in portal blood and about 76% of the total absorbed radioactivity was accounted for by the primary metabolite, candesartan, with only 2.5% appearing as unchanged drug. This suggests that candesartan cilexetil is hydrolyzed to candesartan in the intestinal mucosa prior to reaching the liver and the trace amount of parent compound is hydrolyzed hepatically before reaching the systemic circulation.

In rats and dogs, the Cmax and $AUC_{0\text{-}24}$ of candesartan after single oral doses of candesartan cilexetil increased nearly dose-proportionally (Table 6).

Table 6. Pharmacokinetics of Candesartan in Rats and Dogs after Oral Administration of Candesartan Cilexetil

Species	Dose (mg/kg, PO)	Candesartan Pharmacokinetic Parameters		
		Tmax, hr	Cmax, µg/ml	$AUC_{0\text{-}24}$, µg.h/ml
Rat	1	2	0.25	2.0
	10	1.3	3.3	18.3
	100	1.8	26.1	169
Dog	1	0.8	0.02	0.09
	10	0.5	0.28	1.1
	100	0.5	5.1	13.0

Values are the means from 3 animals.

The rate and extent of absorption of candesartan were investigated in fasted and fed male rats (Takeda Study # C-42-594) and dogs (Takeda Study # C-42-595) after a single oral dose of 1 mg candesartan cilexetil/kg suspended in 5% gum arabic solution. Maximum plasma concentrations of candesartan were observed about 2 hr after administration to rats and approximately 1 hr after administration to dogs (Tables 7 and 8). The oral bioavailability, calculated from the AUC for candesartan after oral administration of candesartan cilexetil and after intravenous administration of candesartan, was 19% in fasted rats and 18% in fasted dogs, respectively. A decrease in bioavailability was observed in fed dogs (reduced to ~5%), but not in fed rats (19% & 28%; values from 2 experiments).

Table 7. Pharmacokinetic Parameters of Candesartan in Rats Administered Candesartan Cilexetil PO or Candesartan IV.

Parameter	Candesartan Cilexetil 1 mg/kg PO			Candesartan 0.2 mg/kg IV
	Fed		Fasted	
	Exp. 1	Exp. 2		
Tmax (hr)	2.3	1.7	2.0	-
Cmax (ug/ml)	0.28	0.25	0.31	-
$t_{1/2}$ (hr)	3.8	~ 3.0	~ 3.0	1.3
$AUC_{0-\infty}$ (ug.hr/ml)	2.20	1.51	1.52	1.58

Table 8. Pharmacokinetic Parameters of Candesartan in Dogs Administered Candesartan Cilexetil PO or Candesartan IV.

Parameter	Candesartan Cilexetil 1 mg/kg PO		Candesartan 0.2 mg/kg IV
	Fed	Fasted	
Tmax (hr)	1.3	1.0	-
Cmax (ug/ml)	0.012	0.06	-
$t_{1/2}$ (hr)	4.3	~ 4.0	0.6
$AUC_{0-\infty}$ (ug.hr/ml)	0.07	0.25	0.28

Toxicokinetics

When candesartan cilexetil was given orally to rats in the 4 and 26 week toxicity studies, the Cmax and AUC for the primary metabolite, candesartan, increased with increasing doses (Tables 9 and 10). However in the 26-week toxicity study, at doses of 100 mg/kg/day and above, the increase in AUC for candesartan was less than dose-proportional. The AUC increased slightly with repeated administration in the higher dosage groups.

Table 9. Toxicokinetics of Candesartan after Oral Candesartan Cilexetil in Rats (4-Wk Toxicity Study).

Dose (mg/kg/day)*	Dosing Day	Cmax* (ug/ml)	Tmax* (hr)	AUC_{0-24}* (ug.hr/ml)
30	0	4.2	0.5	25.2
	13	2.3	2.0	14.4
	27	1.0	2.0	8.7
100	0	9.9	0.5	74.5
	13	4.8	0.5	48.4
	27	5.6	2.0	45.2
300	0	26.8	0.5	166.7
	13	11.7	2.0	90.3
	27	12.3	0.25	107.0

*Determined on the basis of mean plasma level of 6 rats (3/sex)

Table 10. Toxicokinetics of Candesartan after Oral Candesartan Cilexetil in Rats (26-Wk Toxicity Study).

Dose Group (mg/kg/day)	Sex	Cmax (ug/ml)*			AUC_{0-24} (ug.hr/ml)*		
		Week 1	Week 13	Week 26	Week 1	Week 13	Week 26
1	M	0.11	0.05	0.10	0.4	0.3	0.4
	F	0.25	0.15	0.17	2.5	0.5	1.0
10	M	1.7	1.3	1.5	12.0	11.0	6.5
	F	1.8	2.7	1.2	17.0	15.0	6.3
100	M	12.2	6.1	24.4	89.0	66.0	81
	F	12.2	15.2	22.5	86.0	84.0	117
1000	M	50.6	38.7	83.5	429.0	492.0	1068
	F	37.3	38.1	67.8	429.0	496.0	727

* Mean values based on 3/sex/group

When candesartan cilexetil was administered orally to dogs over 4, 26 or 52 weeks at doses of 2.4 to 300 mg/kg/day, the AUC of candesartan increased with increasing dose. At doses ≥ 60 mg/kg/day, the AUC, at a given dose, increased with repeated administration (Tables 11, 12 and 13)

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Table 11. Toxicokinetic Parameters of Candesartan in Dogs Administered Candesartan Cilexetil* (4-Wk Toxicity Study)

Dose Group (mg/kg/day PO)	Cmax (ng/ml)		AUC_{0-24} (ng.hr/ml)	
	Day 1	Day 28	Day 1	Day 28
2.4	41	42	288	241
12	195	163	996	1361
60	455	765	4194	7121
300	1391	2361	9193	14735

* Mean values based on 6 dogs/dose group (3M, 3F)

Table 12. Toxicokinetic Parameters of Candesartan in Dogs Administered Candesartan Cilexetil* (26-Wk Toxicity Study)

Dose Group (mg/kg/day PO)	Sex	Cmax (ng/ml)			AUC_{0-t} (ng.hr/ml)		
		Week 1	Week 13	Week 26	Week 1	Week 13	Week 26
4	M	47	52	47	230	194	263
	F	70	80	82	369	341	374
20	M	93	177	117	481	827	606
	F	87	136	111	402	768	417
100	M	404	507	459	1552	2302	1736
	F	394	648	648	2310	3856	3103

*Mean values based on 4 dogs/sex/group

Table 13. Toxicokinetics Parameters of Candesartan in Dogs Administered Candesartan Cilexetil* (52-Wk Toxicity Study)

Dose Group (mg/kg/day PO)	Sex	Cmax (ng/ml)			AUC_{0-24} (ng.hr/ml)		
		Week 1	Week 26	Week 52	Week 1	Week 26	Week 52
4	M	27	ND	38	92	ND	354
	F	64	52	40	522	322	329
20	M	73	34	57	779	307	821
	F	124	104	134	789	845	1819
100	M	208	272	196	2527	2183	2036
	F	222	307	406	3221	4811	4107
300	M	554	300	643	4314	4194	5875
	F	535	720	1280	3776	7482	10466

*Mean values based on 4 dogs/sex/group ND=Not determined; below level of detection.

In the mouse, Cmax and AUCs of candesartan increased with increasing dose following oral (gavage) doses of 10 to 300 mg candesartan cilexetil/kg/day for 13 weeks or 3 to 100 mg candesartan cilexetil/kg/day for 104 weeks (Tables 14 & 15). Similarly, Cmax and AUCs of candesartan increased, non-dose-proportionately, with increasing doses of candesartan cilexetil in pregnant mice when measured on days 6 and 15 of gestation (Table 16). No accumulation of drug with repeated dosing was observed in the mouse developmental toxicity and carcinogenicity studies.

Table 14. Toxicokinetics of Candesartan and Metabolite ,M-II, after Oral Administration of Candesartan Cilexetil in Mice (13-Wk Dose Rangefinding Toxicity Study)

Measured Compound	Dose Group (mg/kg/d)	Sex	Tmax (hr)	Cmax (ug/ml)	AUC_{0-24} (ug.hr/ml)
Unchanged Drug	300	M	0.25	0.006	0.005
		F	0.25	0.009	0.003
Metabolite M-I (Candesartan)	10	M	0.25	1.69	2.9
		F	0.50	3.09	6.2
	30	M	0.25	5.35	6.6
		F	0.25	9.75	17.3
	100	M	0.25	20.45	26.4
		F	0.25	31.44	54.8
	300	M	0.25	52.14	68.5
		F	0.25	84.33	120.3
Metabolite M-II	10	M	0.5	0.055	0.053
		F	0.25	0.091	0.127
	30	M	0.5	0.222	0.289
		F	0.5	0.399	0.665
	100	M	0.5	1.012	1.139
		F	0.5	1.108	1.865
	300	M	0.25	1.471	1.372
		F	0.25	2.390	2.648

Values are the means from 3 animals determined at the end of 13-week dosing period.

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Table 15. Toxicokinetics of Candesartan after Oral Administration of Candesartan Cilexetil in Mice. (104-Wk Carcinogenicity Study)

Candesartan Cilexetil Dose Group (mg/kg/day)	Dosing Week	Male (n=3)		Female (n=3)	
		Cmax, µg/ml	AUC_{0-24}, µg.hr.ml	Cmax, µg/ml	AUC_{0-24}, µg.hr.ml
3	Wk 26	0.27	0.5	0.63	0.9
	Wk 52	0.27	0.4	0.36	0.7
	Wk 78	0.27	0.6	0.40	1.3
	Wk 103[a]	0.19	-	0.43	-
10	Wk 26	0.96	1.9	1.4	3
	Wk 52[b]	0.66	-	1.7	-
	Wk 78[b]	0.72	-	1.0	-
	Wk 103[a]	0.63	-	2.2	-
30	Wk 26	2.7	5	4.0	7
	Wk 52	2.1	5	4.1	8
	Wk 78	2.7	9	4.9	15
	Wk 103[a]	3.0	-	5.6	-
100	Wk 26	4.4	9	9.3	16
	Wk 52	1.5	17	8.8	24
	Wk 78	7.4	22	9.9	26
	Wk 103[a]	6.6	-	11.0	-

[a] Single measurement only (1 hour postdose)
[b] Determination made from 2 points (0.5 and 1 hr post dose).
- Insufficient data points for determination.

Table 16. Candesartan Pharmacokinetic Results after Oral Administration of Candesartan Cilexetil to Pregnant Mice

Dose Group, mg/kg/day	Dosing Day	Pharmacokinetic Parameter		
		Tmax, hr	Cmax, µg/ml	AUC_{0-24}, µg.hr/ml
10	GD6	1	2.20	5.8
	GD15	1	2.35	8.0
100	GD6	0.5	10.5	35.4
	GD15	1	10.1	39.9
1000	GD6	0.25	26.8	124.3
	GD15	1	26.7	123.8

Distribution

Whole Body: The tissue distribution of radioactivity was assessed after single oral doses of 1 mg [^{14}C]-candesartan cilexetil/kg to Wistar (albino) and Long Evans (pigmented) rats. In Wistar rats (Takeda Study # C-42-594) the concentration of [^{14}C] in tissues was measured at 0.5, 3, 24 and 72 hours after oral administration of 1 mg [14C]-candesartan cilexetil/kg. At 3 hours after dosing, the

concentration of radioactivity was highest in the intestines followed in decreasing order by plasma, stomach, liver, kidney, lung and pituitary; radioactivity concentration was lowest in the brain. By 72 hours after dosing, the radioactivity concentrations throughout most tissues had decreased to very low levels (Table 17). In Long Evans rats (Takeda Study # C-42-519), the concentration of radioactivity in tissues at 3 hours after oral dosing with 1 mg [^{14}C]-candesartan cilexetil/kg was highest in the intestines, followed by liver, plasma, kidney, lung, skin, pituitary, heart and adrenal gland (Table 18). The concentration of radioactivity in black skin was comparable to that seen in white skin. Although radioactivity was detected in the ocular tissues, retina, lens, sclera and vitreous body, the concentrations detected in these tissues were only a fraction of that observed in plasma at 3 hours postdose. These results suggest that the radioactive products had no affinity for melanin.

Table 17. Distribution of Radioactivity in Wistar Rats

Tissue	Concentration of ^{14}C (μg N-1 eq./ml or g)			
	0.5h	3h	24h	72h
Plasma	0.239±0.023	0.389±0.046	0.045±0.005	0.021±0.002
Brain	0.002±0.000	0.004±0.001	< 0.001	< 0.001
Spinal cord	0.002±0.001	0.005±0.001	0.001±0.000	< 0.001
Pituitary	0.048±0.011	0.065±0.008	0.013±0.001	0.002±0.000
Eye ball	0.003±0.001	0.011±0.001	0.002±0.000	0.001±0.000
Harder's gland	0.013±0.002	0.028±0.007	0.005±0.001	0.002±0.000
Subsaxillary gland	0.023±0.002	0.039±0.007	0.005±0.001	0.002±0.001
Thyroid	0.025±0.008	0.054±0.011	0.011±0.002	0.001±0.000
Thymus	0.005±0.000	0.014±0.002	0.003±0.001	0.001±0.000
Heart	0.026±0.003	0.053±0.007	0.006±0.000	0.003±0.000
Lung	0.045±0.009	0.083±0.014	0.017±0.002	0.008±0.002
Liver	0.137±0.014	0.248±0.049	0.013±0.003	0.004±0.001
Spleen	0.021±0.001	0.031±0.004	0.006±0.001	0.002±0.000
Pancreas	0.019±0.004	0.031±0.004	0.004±0.000	0.002±0.000
Adrenal gland	0.039±0.004	0.055±0.007	0.013±0.001	0.004±0.001
Kidney	0.162±0.018	0.220±0.026	0.017±0.003	0.008±0.000
Testis	0.006±0.001	0.042±0.009	0.006±0.001	0.003±0.000
Skeletal muscle	0.004±0.001	0.013±0.003	0.002±0.001	0.001±0.001
Skin	0.009±0.001	0.040±0.007	0.010±0.001	0.004±0.001
Epididymal fat	0.003±0.001	0.014±0.004	0.002±0.001	0.001±0.001
Abdominal aorta	0.010±0.002	0.028±0.008	0.004±0.001	0.001±0.001
Inferior cava vein	0.021±0.004	0.036±0.011	0.007±0.004	< 0.001
Stomach	0.180±0.013	0.256±0.004	0.020±0.006	0.002±0.001
Intestine	0.371±0.140	0.552±0.168	0.028±0.023	0.003±0.001

Data are the mean values ± S.D. (N=3).

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Table 18. Distribution of Radioactivity in Long Evans Rats

Tissue	Level of ^{14}C (μg N-1 equivalents/g)			
	0.5h	3h	24h	72h
Plasma	0.318±0.141	0.405±0.014	0.020±0.006	0.013±0.002
Brain	0.003±0.001	0.003±0.000	<0.001	<0.001
Spinal cord	0.003±0.001	0.004±0.001	<0.001	<0.001
Pituitary	0.057±0.016	0.066†	0.007±0.002	0.005±0.001
Retina	0.004±0.001	0.006±0.001	<0.001	0.001±0.001
Lens	0.001±0.000	0.001±0.000	<0.001	<0.001
Sclera	0.009±0.002	0.033±0.006	0.002±0.001	0.002±0.001
Vitreous body	0.002±0.001	0.008±0.002	<0.001	0.001±0.001
Harder's gland	0.017±0.010	0.038±0.010	0.002±0.000	0.001±0.001
Submaxillary gland	0.039±0.027	0.044±0.001	0.002±0.001	0.002±0.000
Thyroid	0.047±0.014	0.049±0.004	0.006±0.001	0.003±0.001
Thymus	0.007±0.003	0.016±0.002	0.001±0.001	0.001±0.000
Heart	0.036±0.018	0.056±0.001	0.003±0.001	0.002±0.000
Lung	0.051±0.020	0.116±0.012	0.012±0.003	0.006±0.001
Liver	0.246±0.082	0.575±0.191	0.019±0.002	0.004±0.001
Spleen	0.026±0.010	0.032±0.002	0.003±0.001	0.001±0.001
Pancreas	0.035±0.013	0.038±0.005	0.003±0.001	0.001±0.000
Adrenal gland	0.045±0.013	0.055±0.006	0.010±0.002	0.003±0.001
Kidney	0.204±0.089	0.281±0.038	0.015±0.002	0.006±0.000
Testis	0.007±0.004	0.042±0.006	0.002±0.001	0.001±0.001
Abdominal aorta	0.016±0.005	0.032±0.009	0.002±0.001	0.002±0.001
Inferior cava vein	0.027±0.012	0.043±0.014	0.004±0.003	0.003±0.000
Skeletal muscle	0.005±0.003	0.017±0.002	0.001±0.000	0.001±0.000
White skin	0.014±0.007	0.066±0.008	0.004±0.001	0.002±0.001
Black skin	0.011±0.006	0.069±0.018	0.004±0.001	0.003±0.001
Epididymal fat	0.005±0.002	0.017±0.006	0.001±0.001	0.001±0.000
Bone marrow	0.025±0.014	0.033±0.004	0.002±0.000	0.002±0.001
Stomach	0.269±0.083	0.264±0.077	0.022±0.015	0.003±0.001
Intestine	0.466±0.166	0.588±0.057	0.016±0.006	0.002±0.000

Mean values ± S.D. (N=3). Plasma, μg/ml. † Mean values (N=2).

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Distribution into Erythrocytes: Studies (Takeda Studies # C-42-594 and # C-42-595) conducted *in vitro* investigated the extent of radioactivity that entered into rat and dog erythrocytes. When [^{14}C]-candesartan was added to whole blood at concentrations of 0.01, 0.1, 1.0 and 10 µg/ml, <2% of the radioactivity in blood entered into rat or dog erythrocytes (Table 19).

Table XX. Erythrocyte Radioactivity Levels After Addition of [^{14}C]-Candesartan into Whole Blood in Vitro.

Species	[^{14}C]-Candesartan Conc.., µg/ml	% of [^{14}C] Distributed in Erythrocytes
Rat	0.01	0.9
	0.1	0.7
	1.0	<0.1
	10	0.4
Dog	0.01	0.9
	0.1	<0.1
	1.0	<0.1
	10	1.5

Data are the mean values from 3 determinations. The radioactivity from whole blood and in plasma from the same sample were determined; the % of radioactivity distributed in erythrocytes was calculated using the hematocrit value.

Protein Binding: [^{14}C]- Candesartan was added *in vitro* to rat and dog plasma and human serum at final concentrations of 0.01, 0.1, 1 and 10 µg/ml to determine the extent of protein binding (Takeda Study # C-42-594 and C-42-595). The binding of candesartan to plasma or serum proteins was concentration-independent within this concentration range (Table 20).

Table 20. Binding of Candesartan to Plasma or Serum Proteins

Candesartan Conc.. µg/ml	% Protein Binding		
	Rat Plasma	Dog Plasma	Human Serum
0.01	99.6	97.5	99.5
0.1	99.8	96.8	99.4
1	99.8	96.7	99.6
10	99.8	96.7	99.5

Placental Transfer and Secretion into Milk: A dose of 1mg [^{14}C]-candesartan cilexetil/kg was administered orally to pregnant Wistar rats on day 19 of gestation to determine the extent of placental transfer of radioactivity into the fetuses (Takeda Study #C-42-740). At 0.5, 3, 8, 24, 32 and 48 hours after oral administration of candesartan cilexetil, fetuses and placentae were delivered by cesarean section (3 pregnant rats/time point). At the same time, maternal blood, amniotic fluid and fetal blood were collected. Radioactivity was detected in both fetal plasma and tissues; the concentrations of radioactivity at 0.5 to 8 hours postdose were higher in the maternal plasma than in the fetal plasma and tissues (Table 21). However, at 24 hours postdose, the radioactivity (comprised predominately of candesartan with no detectable amount of parent drug) in fetal plasma was higher than that seen in maternal plasma.

Table 21. Fetal Distribution of Radioactivity in Rats Given 1 mg [^{14}C] Candesartan Cilexetil/kg PO.

Tissue	Compound	Concentration of ^{14}C (μg M-I eq./ml or g)					
		0.5 h	3 h	8 h	24 h	32 h	48 h
Maternal plasma	Total ^{14}C	0.447±0.149	0.511±0.092	0.291±0.062	0.063±0.004	0.038±0.009	0.027±0.007
	M-I	0.391±0.156	0.328±0.065	0.146±0.035	0.005±0.006	0.002±0.001	<0.001
	Others	0.056±0.016	0.183±0.038	0.145±0.034	0.058±0.003	0.034±0.010	0.026±0.008
Placenta	Total ^{14}C	0.071±0.028	0.116±0.023	0.095±0.021	0.039±0.004	0.032±0.005	0.026±0.004
Amniotic fluid	Total ^{14}C	<0.001	<0.001	0.002±0.001	0.023±0.003	0.024±0.005	0.045±0.012
Fetal plasma	Total ^{14}C	0.001±0.000	0.053±0.007	0.126±0.009	0.159±0.023	0.185±0.053	0.133±0.022
	M-I	0.001±0.000	0.045±0.002	0.117±0.008	0.142±0.021	0.169±0.050	0.121±0.020
	Others	<0.001	0.008±0.006	0.009±0.001	0.017±0.003	0.016±0.004	0.012±0.002
Fetal tissue	Total ^{14}C	<0.001	0.006±0.001	0.023±0.002	0.051±0.005	0.062±0.017	0.054±0.009

Values; mean ± S.D. (N=3). Pregnant rats were used on day 19 of gestation.

The secretion of radioactivity into milk was studied using lactating Wistar rats (Takeda Study # C-42-740). A dose of 1mg [^{14}C]-candesartan cilexetil/kg was administered orally to lactating rats on day 14 after delivery to determine the levels of radioactivity appearing in milk. At 0.5, 3, 8 and 24 hours after dosing, milk was aspirated from the mammary glands of rats under ether anesthesia for determination of radioactivity. After collection of milk, the rats were killed and whole blood from the abdominal aorta and mammary gland tissue were obtained for measurement of radioactivity. Radioactivity was detected in the milk and mammary gland at each measurement interval. (Table 22). In the milk, and mammary gland, parent compound was not detected and the major component was candesartan. The concentrations of candesartan and other metabolites in the milk and mammary gland were lower than those in plasma at each time point.

Table 22. Levels of Radioactive Products in Milk of Lactating Rats Given a Single Oral Dose of 1 mg [^{14}C] Candesartan Cilexetil/kg.

Tissue	Compound	Concentration of ^{14}C (μg M-I eq./ml or g)			
		0.5 h	3 h	8 h	24 h
Plasma	Total ^{14}C	0.440±0.267	0.714±0.109	0.232±0.031	0.093±0.009
	M-I	0.354±0.213	0.451±0.137	0.061±0.014	0.001±0.000
	Others	0.086±0.066	0.264±0.079	0.171±0.018	0.092±0.009
Milk	Total ^{14}C	0.003±0.002	0.151±0.061	0.080±0.004	0.021±0.001
	M-I	0.003±0.002	0.124±0.059	0.038±0.005	<0.001
	Others	0.001±0.001	0.027±0.011	0.042±0.006	0.021±0.001
Mammary gland	Total ^{14}C	0.055±0.039	0.118±0.041	0.052±0.007	0.016±0.005
	M-I	0.047±0.032	0.083±0.032	0.018±0.002	0.001±0.000
	Others	0.008±0.007	0.035±0.020	0.034±0.006	0.015±0.005

Values; mean ± S.D. (N=3). Female rats were used on day 14 after delivery.

Metabolism

The metabolic profile of candesartan cilexetil in animals was assessed in male Wistar rats (210-326 gm; Takeda Study # C-42-594 and C-42-754) and Beagle dogs (8-11 kg; Takeda Study # C-42-595); four metabolites of candesartan cilexetil were identified.

After an oral dose of 1 mg [^{14}C]-candesartan cilexetil/kg to rats, the total radioactivity detected in plasma was accounted for by candesartan and candesartan-related glucuronides (carboxylic acid glucuronide and tetrazole-N-glucuronide. Unchanged candesartan cilexetil was not detectable in the urine and bile of rats. Only a small amount of parent compound was detected in the feces of rats. The major drug component found in feces was candesartan. A fourth metabolite, formed by o-deethylation of candesartan, was identified in rat feces. In biliary-cannulated Wistar rats given 1 mg [^{14}C]-candesartan cilexetil/kg intraduodenally, approximately 34% of the radioactive dose was obtained in the bile over 24 hours. The major drug component in bile was candesartan along with appreciable levels of conjugated metabolites of candesartan (Table 23).

Table 23. Composition of Radiolabeled Materials in Urine, Feces and Bile of Rats Given 1 mg [^{14}C]-Candesartan Cilexetil/kg PO or ID.

Route	Sample	Total [^{14}C] (% of Dose)	Radioactivity, % of Total Dose			
			Parent Drug	Metabolite M-I	Metabolite, MG	Others
Oral	$Urine_{0-24h}$	0.8	ND	0.6	0.1	0.1
	$Feces_{0-48h}$	94.4	1.3	80.5	0.8	11.8
ID	$Bile_{0-24h}$	33.5	ND	24.3	7.9	1.3

Metabolite M-I= Candesartan; Metabolite MG= Candesartan glucuronides (carboxylic acid glucuronide and/or N-tetrazole glucuronide). ND= Not detected.

Candesartan cilexetil administered orally to male Wistar rats at doses of 1, 10 or 100 mg/kg/day for 7 days did not induce or inhibit rat hepatic microsomal drug metabolism enzyme activity (aminopyrine-N-demethylase, alanine-4-hydroxylase, p-nitroanisole-O-demethylase and p-nitrophenyl glucuronosyl transferase; Takeda Study # C-42-863).

In beagle dogs given 1 mg [^{14}C]-candesartan cilexetil/kg orally, the parent compound was not detected in the plasma. Approximately 78% of the plasma radioactivity was accounted for by candesartan. Small amounts of glucuronides of candesartan, as well as other unknown metabolites, were also detected in dog plasma. A small amount of parent drug was detected in dog feces but not in urine. The major excretory product detected in urine and feces was candesartan (Tables 24 & 25).

Table 24. Composition of Radiolabeled Products in Plasma of Dogs Given 1 mg [^{14}C]-Candesartan Cilexetil/kg PO.

Compound	AUC_{0-8h}, ug.hr/ml*
Candesartan cilexetil	ND
Candesartan	0.046
Candesartan Glucuronides	0.005
Others	0.008

* AUC values calculated as candesartan equivalents.
ND= Not detected.

Table 25. Composition of Radiolabeled Products in Urine and Feces of Dogs Given 1 mg [^{14}C]-Candesartan Cilexetil/kg PO.

Sample	Total [^{14}C] (% of Dose)	Radioactivity, % of Total Dose			
		Parent Drug	Metabolite, M-I	Metabolite(s), MG	Others
$Urine_{0-24h}$	0.4	ND	0.3	0.1	<0.1
$Feces_{0-48h}$	99.7	4.4	90.1	1.1	4.1

Metabolite M-I= Candesartan; Metabolite MG= Candesartan glucuronides (carboxylic acid glucuronide and/or N-tetrazole glucuronide). ND= Not detected.

In rats and dogs given 1 mg/kg PO dose of candesartan [^{14}C]-cilexetil, the ester side chain of the parent drug was absorbed mainly as cyclohexanol and excreted predominately in the urine after being metabolized to cyclohexanediol and cyclohexanetriol.

The human metabolism of candesartan cilexetil was assessed *in vivo* in healthy volunteers (Takeda Study # C-42-958) and *in vitro* using human liver microsomes (Takeda Study # C-42-955). In human volunteers, the major drug component detected in plasma was candesartan (Cmax=233 ng/ml at 1.25 hr) following an oral solution of 8 mg [^{14}C]-candesartan cilexetil; very low plasma concentrations of candesartan cilexetil were detected up to 2 hours after oral dosing (Cmax=8.6 ng/ml occurring 0.5 hrs after dose). A second metabolite, formed by O-deethylation of candesartan, was also detected (Cmax=24 ng/ml at 4 hours after dosing) after oral candesartan cilexetil solution. The O-deethylated metabolite of candesartan was also detected after intravenous administration of 4 mg [^{14}C]-candesartan to the same subjects. The ratios of $AUC_{0-\infty}$ of the O-deethylated metabolite to $AUC_{0-\infty}$ of candesartan were about 0.16 after IV and 0.27 after oral administration. After both oral [^{14}C]-candesartan cilexetil and IV [^{14}C]-candesartan administration, approximately 70-80% of the radioactivity in urine and feces was identified as candesartan. Metabolism of candesartan by human liver microsomes yielded only the O-deethylated metabolite, a reaction catalyzed by isoenzymes within the cytochrome P-450 CYP2C family.

In summary, candesartan cilexetil given orally to animals and man is almost completely hydrolyzed during absorption and reaches the systemic circulation as the pharmacologically active metabolite, candesartan (M-I). In rats and dogs, candesartan is further conjugated to glucuronide metabolites, or undergoes, to a lesser extent, O-deethylation. In humans, approximately 20-30% of administered drug has been recoved as the O-deethylated metabolite of candesartan (Figure 10).

Figure 10. Proposed Metabolic Pathway of Candesartan Cilexetil

Excretion

In male Wistar rats given a single oral dose of 1 mg [^{14}C]-candesartan cilexetil/kg, almost all of the radioactivity was eliminated from the body within 72 hours, predominately via fecal elimination. Only a small fraction of the administered dose was detected in the urine (Table 26) and only a small amount of unmetabolized drug was detected in the feces of rats. The major component in the excreta was candesartan. In bile cannulated rats, approximately 34% of the dose was excreted in the bile over 24 hours (Takeda Study # C-42-594).

Table 26. Composition of Radiolabelled Materials in Urine, Feces or Bile of Rats Given 1 mg/kg PO Dose of Candesartan Cilexetil.

Species	Sample	Total ^{14}C (% of dose)	^{14}C-Labeled Composition (% of dose)			
			Parent	M-1[a]	M-2[b]	Others
Rat	Urine 0-24h	0.8	ND	0.6	0.1	0.1
	Feces 0-48h	94.4	1.3	80.5	0.8	11.8
Rat*	Bile 0-24h	33.5	ND	24.3	7.9	1.3

ND=Not detected *Intraduodenal administration in bile-cannulated rats.

[a] Pharmacologically active carboxylic acid metabolite, candesartan.

[b] Glucuronide of acid metabolite

In Beagle dogs given a single oral dose of 1 mg [^{14}C]-candesartan cilexetil/kg (Takeda Study # C-42-595), elimination of radioactivity was essentially complete within 48 hours (Table 27).

Table 27. Composition of Radiolabelled Materials in Urine and Feces of Dogs Given 1 mg/kg PO Dose of Candesartan Cilexetil.

Species	Sample	Total ^{14}C (% of dose)	^{14}C-Labeled Composition (% of dose)			
			Parent	M-1[a]	M-2[b]	Others
Dog	Urine 0-24h	0.4	ND	0.3	0.1	<0.1
	Feces 0-48h	99.7	4.4	90.1	1.1	4.1

ND=Not detected

[a] Pharmacologically active carboxylic acid metabolite, candesartan

[b] Glucuronide of acid metabolite

Within 72 hours after both oral (8 mg [^{14}C]-candesartan cilexetil solution) and intravenous (4 mg [^{14}C]-candesartan cilexetil solution) dosing in human subjects, >90% of the radioactivity was excreted (Figure 11). After oral administration, about 33% was excreted in urine and 67% in feces. After intravenous dosing, about 59 % was excreted in urine and 36% excreted in feces (Takeda Study # C-42-958,). The half-life for candesartan after oral [^{14}C]-candesartan cilexetil (8mg) administration was 9.3 hours; after intravenous administration of 4 mg [^{14}C]-candesartan cilexetil solution, the half life for candesartan was 9.7 hours.

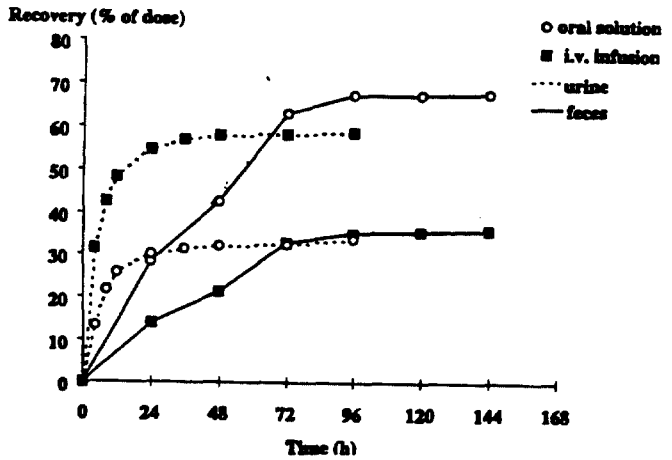

Figure 11. Recovery of Radioactivity (% of Dose) in Urine and Feces of Dogs After 8 mg [^{14}C]-Candesartan Cilexetil PO or 4 mg [^{14}C]-Candesartan Cilexetil IV.

ACUTE TOXICITY

Acute Oral Toxicity Study in Mice and Rats

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: T2912
Study Dates: 12/5/90 - 12/19/90

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Jcl:ICR mice (19-27 gm) and Jcl:Wistar rats (105-132 gm).

Drug Administration: Candesartan cilexetil (Lot #M464-005) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 500, 1000 and 2000 mg/kg (5/sex/dose group)

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity 5 to 6 times on the day of dosing and then once or twice daily during the 14 day post-dosing period. Body weights were recorded prior to dosing and 1, 2, 7 and 14 days after dosing. On Day 14, animals were killed and the visceral organs examined grossly.

Results: No deaths occurred and no clinical signs of toxicity were observed in mice or rats. No adverse effects on body weight or visceral tissues were detected. Thus, single oral doses up to 2000 mg/kg PO of candesartan cilexetil produced no toxic effects in mice or rats.

Acute Oral Toxicity Study in Dogs

Study Facility: Takeda Chemical Industries, Inc.; Japan
Study No: T2914
Study Dates: 3/22/93 - 4/06/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male Beagle dogs (8.5-9.9 Kg)

Drug Administration: Candesartan cilexetil (Lot #M464-021) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 0 (vehicle control) and 2000 mg/kg (2/dose group)

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity predose and daily during the 14 day post-dosing period. Body weights were recorded prior to dosing and 7 and 14 days after dosing. Food consumption was measured daily for 14 days after dosing. Hematology and clinical chemistry analyses were conducted predose and on days 1, 7, and 14 after dosing. Necropsy was conducted only when animals showed signs of toxicity.

Results: No deaths occurred and no clinical signs of toxicity were observed in control or treated animals. No treatment-related effects were noted in body weights, food consumption, hematology or in clinical chemistry parameters. Thus, a single oral dose of 2000 mg/kg PO of candesartan cilexetil produced no toxic effects in dogs.

Other Acute Single-Dose Toxicity Studies

Acute single-dose toxicity studies with candesartan cilexetil metabolites, impurities and a metabolic product of hydrolysis (1,2-cyclohexanediol), administered parenterally, were also conducted in mice and rats. Results of all acute single-dose toxicity studies are summarized in Table 28.

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Table 28. Summary of Single Dose Toxicity Studies

Species/ Strain	Study #	Test Article	Route	Group Size	Dose Levels, mg/kg	Lethal Dose, mg/kg (LD_{50}, MLD)	Signs of Toxicity, mg/kg dose
Mouse, Jcl:ICR	T2912	Candesartan cilexetil	p.o.	5M/5F	500, 1000, 2000	-	No signs of toxicity were observed
	T3175	Candesartan cilexetil	i.p.	5M/5F	250, 500, 1000,2000	Males LD_{50}= 807 Females LD_{50}=891	Decreased locomotor activity, respiratory depression, stupor at @≥500
	T2998	Candesartan & impurities (U-1, U-2, U-3, U-4, U-5, U-6)	p.o.	5M/5F	500, 1000, 2000	Candesartan or Impurities MLD ≥ 2000	Decreased locomotor activity, ataxia with all compounds @ 2000
	T2913	Candesartan	i.v.	5M/5F	910, 1180, 1540, 2000	Males LD_{50}= 1120 Females LD_{50}=1170	Decreased locomotor activity, ataxia, respiratory depression @ ≥910
	T3126	Candesartan metabolite, M-II	i.v.	5M/5F	500, 1000 2000	Males MLD=1000 Females MLD=2000	Decreased locomotor activity, ataxia, respiratory depression , convulsions @ ≥1000
	T3351	1,2 Cyclohexanediol	i.v.	5M/5F	1210,1450, 1740, 2080, 2500	Males MLD=1740 Females MLD=1450	Decreased locomotor activity, respiratory depression, ataxia, muscle hypotonia @≥1240
	T3351	1,2 Cyclohexanediol	i.p	5M/5F	3760, 4130, 4170, 4550, 5000	Males or females MLD=4130	Decreased locomotor activity, respiratory depression, ataxia, muscle hypotonia @≥3760
Rat, Jcl:Wistar	T3175	Candesartan cilexetil	i.p.	5M/5F	250, 500, 1000, 2000	Males LD_{50}= 940 Females LD_{50}=1210	Decreased locomotor activity, ataxia, respiratory depression @≥910
	T2912	Candesartan cilexetil	p.o	5M/5F	500, 1000, 2000	-	No signs of toxicity were observed
	T2913	Candesartan	i.v.	5M/5F	910, 1180, 1540, 2000	Males MLD=1180 Females MLD=1540	Decreased locomotor activity, ataxia, respiratory depression @≥910
	T3268	Candesartan metabolite, M-II	i.v.	5M/5F	1000, 2000	Males or Females MLD= 2000	Decreased locomotor activity, dyspnea, convulsions @≥2000
	T3351	1,2 Cyclohexanediol	i.v.	5M/5F	2410, 2890, 3740, 4170	Males or females MLD=2890	Decreased locomotor activity, respiratory depression, ataxia, muscle hypotonia @≥2410
	T3351	1,2 Cyclohexanediol	i.p	5M/5F	2890, 3470, 3790, 4170, 4550, 5000	Males or females MLD=3470	Decreased locomotor activity, respiratory depression, ataxia, muscle hypotonia @≥2890
Dog, Beagle	T2914	Candesartan cilexetil	p.o.	5M/5F	2000	-	No signs of toxicity were observed.

CHRONIC TOXICITY

Four-Week Oral Toxicity Study in Rats

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1110/SU
Study Dates: Beginning date 12/25/90. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female F344/Jcl rats (M=90-108 gm; F=76-88 gm at initiation of dosing); housed 1/cage.

Drug Administration: Candesartan cilexetil (Lot #M464-005) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 0 (vehicle control), 30, 100 and 300 mg/kg (10/sex/dose group, additional 4/sex/dose group for satellite toxicokinetic study)

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity predose and daily during the 4-week dosing period. Body weights were recorded prior to dosing, on treatment days 3 and 7 and then twice weekly thereafter. Food consumption was measured weekly. Ophthalmoscopic exams were performed on 5/sex/dose group prior to dosing and in week 4 of treatment. Urine output and water intake was measured in 5/sex/dose group during week 3 of treatment; urinalysis was performed on these animals on days 2 and 27 of the dosing period. Hematology and clinical chemistry analyses were conducted for all animals after the last dose; blood samples were obtained from the abdominal aorta prior to necropsy. Histopathology examination was conducted on sections from major organs and tissues (listed in Appendix A, pg.1) from control and high dose animals. Blood samples were obtained via a jugular vein from ether-anesthetized animals from the toxicokinetic satellite groups at 0, 0.25, 0.5 1, 2, 4, 8 and 24 hours after dosing on days 0, 13 and 27 for measurement of plasma candesartan levels the same animals were used for all time points and blood sampling days.

Results: No deaths occurred and no clinical signs of toxicity were observed in any main study group; 2 males in the toxicokinetic high-dose group died from excessive anesthesia during blood sampling. No treatment-related effects were noted on body weights. Food consumption was significantly lower than control in mid-dose males during treatment week 2 (-10%) and treatment week 3 (-8%), in high dose males during treatment week 2 (-10%) and treatment week 3 (-8%) and in all drug-treated females during treatment weeks

1 through 4 (-7% to -10%, non-dose related). Water intake was significantly above control in mid- (25%) and high-dose (30%) males; urine output was significantly above control in all treated male groups (37%, 57% and 55% for LD, MD and HD, respectively). Decreases (5%-8%) from control erythrocyte, hematocrit and hemoglobin values were seen in males and females in each treated group. Plasma urea nitrogen was, dose-relatedly, above control (18%-120%) in all treated male and female groups. Gross examination revealed no treatment-related pathology. Non-dose-related decreases from control absolute (11%-16%) and relative (11%-13%) heart weights were noted in males and females in all treated groups. Histopathologic examination revealed no treatment-related effects.

Cmax and AUC of candesartan increased dose-dependently; however, these values tended to decrease with repeated administration (Table 29). The half-lives of plasma candesartan could not be determined due to large variations of plasma drug concentrations.

Table 29. Toxicokinetic Results

Dose (mg/kg/day)	Dosing Day	Cmax* (ug/ml)	Tmax* (hr)	AUC_{0-24}* ug.hr/ml)
30	0	4.2	0.5	25.2
	13	2.3	2.0	14.4
	27	1.0	2.0	8.7
100	0	9.9	0.5	74.5
	13	4.8	0.5	48.4
	27	5.6	2.0	45.2
300	0	26.8	0.5	166.7
	13	11.7	2.0	90.3
	27	12.3	0.25	107.0

*Determined on the basis of mean plasma level of 6 rats (3/sex)

Twenty-Six-Week Oral Toxicity Study in Rats

Study Facility: Takeda Chemical Industries, Inc.; Japan
Study No: 1210/CH
Study Dates: Beginning date 4/18/91. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female F344/Jcl rats (M=82-100 gm; F=72-84 gm at initiation of dosing); housed 1/cage.

Drug Administration: Candesartan cilexetil (Lot #M464-008) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 0 (vehicle control), 1, 10, 100 and 1000 mg/kg (10/sex/dose group, additional 3/sex/dose group for satellite toxicokinetic study)

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity predose and twice daily during the 26-week treatment period. Body weights were recorded prior to dosing, on the day of dosing, twice weekly from week 1 to 5 and then once weekly thereafter. Food consumption was measured weekly. Ophthalmoscopic exams were performed on 5/sex/dose group prior to dosing and in week 26 of treatment. Urine output and water intake were measured in 5/sex/dose group during weeks 12 and 26 of treatment; urinalysis was performed on these animals in weeks 12 and 25 of the dosing period. Hematology and clinical chemistry analyses were conducted for all animals after the last dose; blood samples were obtained from the abdominal aorta prior to necropsy. Histopathology examination was conducted on sections from major organs and tissues (listed in Appendix A, pg. 2) from control and high dose (1000 mg/kg/day) animals. Blood samples were obtained via a jugular vein from ether-anesthetized animals from the toxicokinetic satellite groups at 0.25, 0.5, 1, 2, 4, 8 and 24 hours after dosing on treatment days 1, 24, 86, 136 and 184 (the same animals used for all time points and blood sampling days) for measurement of plasma candesartan levels.

Results: No deaths occurred and no clinical signs of toxicity were observed in any of the main study groups; one male from the low dose satellite toxicokinetic group died due to excessive anesthesia during blood sampling. Body weights of 1000 mg/kg/day males were significantly lower (10%) than control at weeks 25-26; body weights of females were not significantly affected by treatment. In the 1000 mg/kg/day group, food consumption was below (4%-7%) control in males and females from week 25. No treatment-related ocular abnormalities were observed. Increases in water intake (~30%) and urine output (~33%) from control levels were noted in males in the 100 and 1000 mg/kg/day groups. Erythrocyte counts, hematocrit values and hemoglobin concentrations were lower than control in males in the 10 mg/kg/day group and in males and females in the 100 and 1000 mg/kg/day groups (range of dose-related decreases in RBC=4-10%, Hct=4-9% and Hb=4-9%). Blood urea nitrogen was increased dose-dependently from control at doses ≥ 10 mg/kg/day in males (50-134%) and females (17-32%). Treatment-related decreases in absolute and relative heart weights and increases in absolute and relative kidney weights were observed at study termination. Significant differences in relative liver and absolute and relative adrenal weight were observed but they were neither consistent nor apparent in both sexes (table 30).

Table 30. Significant Effects on Relative Organ Weights

Parameter	Dose Group	% Difference From Control	
		Males	Females
Relative Heart Weight	10	-11	-11
	100	-7	-9
	1000	-11	-14
Relative Kidney Weight	10	+18	NS
	100	+21	+9
	1000	+22	+8
Relative Adrenal Weight	100	NS	-10
	1000	+79	NS
Relative Liver Weight	1000	NS	+6

NS= Not Significant

A dose-related increased incidence of red foci on the glandular stomach mucosa was seen in males (0/10, 1/10/, 3/10/ and 6/10 in control, 10, 100 and 1000 mg/kg/day groups, respectively) on gross examination; this effect was observed in females treated with 1000 mg/kg/day (5/10 vs. 1/10 for control). Microscopic examination showed treatment-related effects in kidneys and stomach (Table 31)

Table 31 Treatment-Related Histopathology (n=10/sex/group)

Tissue/Effect	Sex	Percent Incidence in Dose Group (mg/kg/day)				
		0 (Cont)	10	100	1000	
Kidney						
Basophilic Renal Tubule	M	30	20	80	100	100
	F	20	0	20	30	20
Renal Tubule Hypertrophy	M	10	10	60	100	100
	F	0	0	0	0	0
Cellular Infiltration	M	0	0	30	60	90
	F	0	0	0	0	0
Stomach						
Glandular Mucosal Erosion	M	10	0	10	30	70
	F	10	20	20	10	40

Cmax and AUC_{0-24} of the active component, candesartan, were increased dose-dependently (Table 32). In Week 26, a very small amount (0.1 to 0.44 ug/ml;

of unchanged candesartan cilexetil was detected in animals receiving 1000 mg/kg/day, but not in animals receiving lower doses of the prodrug. Cmax and AUC

values from high dose animals in treatment week 26 were higher than those observed during treatment weeks 1 and 13, indicating drug accumulation over time associated with the high dose. No obvious and consistent sex differences in Cmax and AUC values were noted. Toxicokinetic values obtained for Weeks 4 and 20, while not not given in the table below, were not significantly different from corresponding values obtained in prior or subsequent measurement periods.

Table 32. Toxicokinetics Candesartan in Rats Given Oral Candesartan Cilexetil

Dose Group	Sex	Cmax (ug/ml)*			AUC_{0-24} (ug.hr/ml)*		
		Week 1	Week 13	Week 26	Week 1	Week 13	Week 26
1	M	0.11	0.05	0.10	0.4	0.3	0.4
	F	0.25	0.15	0.17	2.5	0.5	1.0
10	M	1.7	1.3	1.5	12.0	11.0	6.5
	F	1.8	2.7	1.2	17.0	15.0	6.3
100	M	12.2	6.1	24.4	89.0	66.0	81
	F	12.2	15.2	22.5	86.0	84.0	117
1000	M	50.6	38.7	83.5	429.0	492.0	1068
	F	37.3	38.1	67.8	429.0	496.0	727

* Mean values based on 3/sex/group

Four-Week Oral Toxicity Study in Dogs

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1139/SU
Study Dates: Beginning date 12/28/90. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Beagle dogs (6-mo old; M=8.1-10.3 Kg, F=6.5-8.8 Kg); housed individually.

Drug Administration: Candesartan cilexetil (Lot #M464-006 & M464-007) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 0 (vehicle control), 2.4, 12, 60 and 300 mg/kg (3/sex/dose group).

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity predose and three times daily during the 4-week treatment period. Body weights

were recorded prior to dosing, on the day of dosing and then once weekly during the treatment period. Food consumption was measured daily. Body temperature and heart rate were measured pretreatment and on days 6, 13 and 24 of treatment. Electrocardiograms were recorded prior to treatment and on days 16 or 17 of treatment. Ophthalmoscopic exams were performed on all animals during the pretreatment period and in week 3 of the dosing period. Urine output and water intake were measured in all animals during the pretreatment period and on days 2, 9 and 22 of treatment; urinalysis was performed on these animals pretreatment and on days 3, 10 and 23 of the dosing period. Hematology and clinical chemistry analyses were conducted for all animals once during the pretreatment period and on days 7, 15 and 27 during the dosing period from blood collected from a cephalic vein. Blood samples were also taken from a cephalic vein from all animals at 0.5, 1, 2, 4, 8 and 24 hours after dosing on days 1 and 28 of the treatment period for measurement of plasma concentrations of parent drug and active metabolite. The dogs were necropsied after the dosing period. Histopathology examination was conducted on sections from major organs and tissues (listed in Appendix A, pg. 3) from all animals.

Results: No dogs died during the treatment period. Diarrhea was sporadically seen in 4/6 dogs (2M, 2F) in the 300 mg/kg/day group; colored evidence of drug compound was noted in the feces of dogs treated with 60 and 300 mg/kg/day of candesartan cilexetil. Body weight, food consumption, body temperature, heart rate and the elctrocardiograms were unaffected by treatment. Ophthalmoscopic exams revealed no treatment-related ocular abnormalities. Water intake was significantly (non-dose-related) increased above control during week 1 of treatment in male dogs treated with 2.4, 12 and 300 mg/kg/day; urine output and urinalysis parameters were not significantly affected. Erythrocyte, hematocrit and hemoglobin values were lower than control for one male and one female in the 300 mg/kg/day group on days 15 and 27. Increases in blood urea nitrogen were noted occasionally in one male and one female in the 300 mg/kg/day group; all other blood chemistry values were within normal ranges. Organ weights were unaffected by treatment. Gross pathology revealed slight pale discoloration of the kidneys in two males in the 60 mg/kg/day group and in one male and two females in the 300 mg/kg/day group. Microscopic examination showed regeneration in the renal tubular epithelium in one male in the 60 mg/kg/day group and in one male and two females in the 300 mg/kg/day group. Furthermore, dilatation of the renal tubules was observed in two males and one female in the 300 mg/kg/day group. A decrease in the number or loss of germ cells was observed in some of the seminiferous tubules of both testes in all males in the 300 mg/kg/day group. No other treatment-related histopathology was seen in any organ.

Cmax and AUC_{0-24} of the active metabolite were dose-dependent after the 1st and 28th doses; peak plasma concentrations occurred approximately 2 hours after dosing (Table 33). Cmax and AUC values for the 60 and 300 mg/kg/day groups were higher on treatment day 28 than on treatment day 1 indicating drug accumulation across time. No sex

differences in Cmax or AUCs for candesartan were observed. Plasma levels of the prodrug parent compound were detected at low levels (20-132 ng/ml; detection limit=20 ng/ml) in the 300 mg/kg/day group.

Table 33. Toxicokinetic Parameters for Candesartan in Dogs Administered Candesartan Cilexetil*

Dose Group (mg/kg/day PO)	Cmax (ng/ml)		AUC_{0-24} (ng.hr/ml)	
	Day 1	Day 28	Day 1	Day 28
2.4	41	42	288	241
12	195	163	996	1361
60	455	765	4194	7121
300	1391	2361	9193	14735

* Mean values based on 6 dogs/dose group (3M, 3F)

Note: A second four-week oral toxicity study (#1456/SU) in Beagle dogs was conducted by Takeda Chemical Industries, Inc. The sponsor states that, because the previous 4-week oral study was conducted in immature dogs (6-month old), it was of interest to assess the toxicity (particularly, drug-induced histopathology of the kidney and testes) in mature (10-month old) dogs. The oral doses of candesartan cilexetil (Lot #M464-016) evaluated were 0, 20, 100 and 300 mg/kg/day (3/sex/group). The nature of the treatment-related findings in this second study were similar to that of the prior study and included decreased erythrocyte parameters (RBCs, Hct and Hb) with 100 and 300 mg/kg/day, increased blood urea nitrogen with 100 and 300 mg/kg/day, erosion of the stomach mucosa in one female after 300 mg/kg/day, regeneration of renal tubular epithelium, dilatation of renal tubules and mononuclear cell infiltration in the kidneys after 100 mg/kg/day (2/6 dogs) and 300 mg/kg/day (2/6 dogs). However, no histopathology of testicular germ cells was detected. The non-toxic dosage level for oral candesartan cilexetil in mature dogs in this study was 20 mg/kg/day.

Twenty-Six-Week Oral Toxicity Study in Beagle Dogs

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1192/CH
Study Dates: Beginning date 4/22/91. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Beagle dogs (9-mo old; M=9.0-12.4 Kg, F=6.7-11.6 Kg at initiation of dosing); housed individually.

Drug Administration: Candesartan cilexetil (Lot #M464-009) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 0 (vehicle control), 4, 20 and 100 mg/kg/day (4/sex/dose group).

Note: High dose based on results of previous 4-week oral toxicity studies which showed renal and gastric toxicity with a dose of 300 mg/kg/day.

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity predose and three times daily during the dosing period. Body weights were recorded prior to dosing, on the first day of dosing, once weekly during weeks 1-14 and then every 4 weeks thereafter. Food consumption was measured weekly for 14 weeks and then every 4 weeks thereafter. Body temperature and heart rate were measured pretreatment and during weeks 14 and 26 of treatment. Electrocardiograms were recorded prior to treatment and during weeks 14 and 25 of the treatment period. Ophthalmoscopic exams were performed on all animals during the pretreatment period and during weeks 14 and 26 of the dosing period. Urine output and water intake were measured in all animals during the pretreatment period and in weeks 13 and 26 of treatment; urinalysis was performed on these animals pretreatment and during weeks 13 and 26 of the dosing period. Hematology and clinical chemistry analyses were conducted for all animals once during the pretreatment period and during weeks 4, 13 and 26 of the dosing period from blood collected from a cephalic vein. Blood samples were also taken from a cephalic vein from all animals 0.5, 1, 4 and 8 hours after dosing during weeks 1, 13 and 26 of the treatment period for measurement of plasma concentrations of parent drug and active metabolite. The dogs were necropsied after the dosing period. Histopathology examination was conducted on sections from major organs and tissues (listed in Appendix A, pg. 4) from all animals.

Results: One female in the 20 mg/kg/day group died on the last day of week 26 from suspected pulmonary aspiration of gastic contents. Prior to death the animal showed rapid breathing, decreased locomotor activity, decreased food consumption, mucous diarrhea and vomiting. At necropsy, multiple red foci were observed in the stomach. Histological examination revealed erosion and hemorrhage in the stomach, erosion of the esophagus, acute hemorrhage of the right lung and cellular infiltration of the left lung. Based on the course of clinical signs it was concluded that this animal died of suspected aspiration pneumonia. No other animals died during the study. In males and females treated with 100 mg/kg/day, a compound-like material was seen in the feces. Body weights of 3/4 females treated with 100 mg/kg/day during week 26 were slightly less (1-12%) than those before dosing commenced. Food consumption, body temperature, heart rate and electrocardiogram, urine output, water intake and urinalysis parameters were unaffected by treatment. No treatment-related ocular effects were observed. Decreases from control erythrocyte (-24%), hematocrit (-22%), and hemoglobin (-24%) values were observed in females treated with 100 mg/kg/day during weeks 13 and 26. Higher than control levels of blood urea nitrogen were noted in one female in the high dose group during weeks 4, 13 and 26. No treatment-related pathology was noted on gross examination. Relative, but

not absolute, kidney weights were (16%) above control in high dose females. Histological examination revealed mild regeneration of the tubular epithelium in one high-dose female. No other treatment-related histopathology was detected.

Plasma levels of parent compound were not detected in any dose-group. The plasma concentration of the active metabolite, candesartan, increased dose-dependently; peak levels were observed one hour following dosing (Table 34).

Table 34. Toxicokinetic Parameters of Candesartan in Dogs Administered Candesartan Cilexetil*

Dose Group (mg/kg/day)	Sex	Cmax (ng/ml)			AUC_{0-8} (ng.hr/ml)		
		Week 1	Week 13	Week 26	Week 1	Week 13	Week 26
4	M	47	52	47	230	194	263
	F	70	80	82	369	341	374
20	M	93	177	117	481	827	606
	F	87	136	111	402	768	417
100	M	404	507	459	1552	2302	1736
	F	394	648	648	2310	3856	3103

*Mean values based on 4 dogs/sex/group

Fifty-Two Week Oral Toxicity Study in Beagle Dogs

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1285/CH
Study Dates: Beginning date 3/18/92. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Beagle dogs (10-13 mo old; M=7.5-11.6 Kg, F=6.1-11.1 Kg at initiation of dosing); housed individually.

Drug Administration: Candesartan cilexetil (Lot #M464-012, M464-016 & M464-017) was suspended in 5% gum arabic aqueous solution and administered orally by gavage.

Dosage Levels: 0 (vehicle control), 4, 20, 100 and 300 mg/kg/day (4/sex/dose group).

Note: High dose based on results of previous 4-week and 26-week oral toxicity studies. In the 4-week study, renal and gastric toxicity were observed with a dose of 300 mg/kg/day. In the 26-week study, a high dose of 100 mg/kg/day was well tolerated with minimal gastric and renal toxicities.

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity predose and once daily during the dosing period. Body weights were recorded prior to dosing, on the day of dosing, once weekly during weeks 1-13 and then every 4 weeks during the treatment period thereafter. Food consumption was measured weekly for 13 weeks and then every 4 weeks thereafter. Body temperature and heart rate were measured pretreatment and during weeks 25 and 51 of treatment. Electrocardiograms were recorded prior to treatment and during weeks 25 and 51 of the treatment period. Ophthalmoscopic exams were performed on all animals during the pretreatment period and during weeks 25 and 51 of the dosing period. Urine output and water intake were measured in all animals during the pretreatment period and in weeks 26 and 52 of treatment; urinalysis was performed on these animals pretreatment and during weeks 26 and 52 of the dosing period. Hematology and clinical chemistry analyses were conducted for all animals once during the pretreatment period and during weeks 26 and 52 of the dosing period from blood collected from the cephalic vein. Blood samples were also taken from the cephalic vein from all animals at 0.5, 1, 4, 8 and 24 hours after dosing during weeks 1, 26 and 52 of the treatment period for measurement of plasma concentrations of parent drug and active metabolite. The dogs were necropsied after the dosing period. Histopathology examination was conducted on sections from major organs and tissues (listed in Appendix A, pg. 5) from all animals.

Results: No dogs died and no clinical sign of toxicity were observed during the study. No treatment-related effects on body weight, food consumption, body temperature, heart rate and the electrocardiogram were observed. No treatment-related ocular abnormalities or effects on urine output and water intake were seen in any group. An increase in urinary casts above control was observed during week 52 in one male in the 300 mg/kg/day group. Non-dose-related decreases (23-29%) from control platelet counts were observed in the 4, 20 and 100 mg/kg/day males during treatment week 26. A decrease from control neutrophil count in the 300 mg/kg/day males was observed during treatment week 52. Higher than control levels of urea nitrogen were noted in 1/4 males and 1/4 females in the 100 mg/kg/day group and in 2/4 males and 1/4 females in the 300 mg/kg/day dose group during treatment week 52. No treatment-related pathologies were noted during gross examination; organ weights were unaffected by treatment. Regeneration of renal tubular epithelium was observed in all groups including control; however, the severity of regeneration of the tubular epithelium was greater in one female in the 100 mg/kg/day group and in 2 males and 1 female in the 300 mg/kg/day group. Hypertrophy of the juxtaglomerular cells was observed in one female and all males in the 4 mg/kg/day group and in all animals receiving 20 mg/kg/day or more.

The Cmax and AUC_{0-24} of the active metabolite were dose-dependent following treatment. In the 20, 100 and 300 mg/kg/day females, the Cmax and AUC_{0-24} values during weeks 26 and 52 tended to be higher than those in corresponding males and showed a tendency towards increase with increase in treatment duration (Table 35).

Table 35. Toxicokinetics Parameters of Candesartan in Dogs Administered Candesartan Cilexetil*

Dose Group (mg/kg/day PO)	Sex	Cmax (ng/ml)			AUC_{0-24} (ng.hr/ml)		
		Week 1	Week 26	Week 52	Week 1	Week 26	Week 52
4	M	27	ND	38	92	ND	354
	F	64	52	40	522	322	329
20	M	73	34	57	779	307	821
	F	124	104	134	789	845	1819
100	M	208	272	196	2527	2183	2036
	F	222	307	406	3221	4811	4107
300	M	554	300	643	4314	4194	5875
	F	535	720	1280	3776	7482	10466

*Mean values based on 4 dogs/sex/group ND=Not determined; below level of detection.

Four-Week Oral Toxicity Study in Cynomolgus Monkeys

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: T3269
Study Dates: Not stated

GLP Compliance: Not stated

Animals: Male and female cynomolgus monkeys (3 years of age or older)

Drug Administration: Candasartan cilexetil (Lot # not stated) was suspended in aqueous 5% gum arabic solution and administered orally by gavage.

Dose Levels: 0 (vehicle), 2.4, 12, 60 and 300 mg/kg/day (2/sex/group)

Observations/Measurements: Animals were observed daily for mortality and clinical signs of toxicity. Body weights were measured weekly and food consumption was measured daily. Body temperature and heart rate were measured prior to dosing and on days 7 and 14 of the dosing period. Water intake and urine output were measured prior to dosing and during weeks 1 and 4 of the dosing period. Venous blood was obtained for hematology and clinical chemistry analyses predose and on days 16 and 28 of the dosing period. Blood was also obtained at various intervals on treatment days 1 and 28 for toxicokinetic analysis. At the end of the treatment period, animals were sacrificed and examined for gross pathology. Major organs were weighed and sections of major organs and tissues (listed in Appendix A, pg. 6) were examined microscopically.

Results

Mortality and Clinical Signs of Toxicity

One female in the 300 mg/kg/day group was sacrificed in a moribund state on day 18 of treatment. Vomiting and decreased locomotor activity were observed in this animal on dosing days 15 and 18, respectively. Wasting was noted in each of the females in the 300 mg/kg/day group beginning on day 15 of treatment. Other effects seen in treated animals were comparable to those seen in controls.

Body Weight and Food Consumption

Lower than pretreatment body weights were noted after 3 or 4 weeks of treatment for both females and one male in the 300 mg/kg/day group and, to a lesser extent, for one of two females in the 12 and 60 mg/kg/day groups (Table 36). Food consumption by the above noted male and one of the above noted females in the 300 mg/kg/day group and the above noted female in the 12 mg/kg/day group was appreciably lower (≥ 20%) than pretreatment levels.

Table 36. Body Weights (kg) in Cynomolgus Monkeys

Dose Group (mg/kg/day PO)	Sex/#	Dosing Period				
		Predose	Day 7	Day 14	Day 21	Day 28
0 (Vehicle)	M/#1	3.27	3.35	3.32	3.41	3.41
	M/#2	4.06	3.99	3.97	4.09	4.07
2.4	M/#1	3.17	3.17	3.14	3.20	3.16
	M/#2	4.24	4.44	4.19	4.37	4.47
12	M/#1	3.00	2.92	2.83	2.81	2.86
	M/#2	4.05	4.08	3.97	3.97	3.99
60	M/#1	3.14	3.16	3.19	3.33	3.35
	M/#2	3.32	3.32	3.32	3.26	3.40
300	M/#1	3.37	3.43	3.40	3.42	3.53
	M/#2	3.45	3.37	3.26	3.22	**3.11**
0 (Vehicle)	F/#1	3.44	3.41	3.29	3.34	3.43
	F/#2	2.43	2.51	2.41	2.43	2.47
2.4	F/#1	3.05	3.10	3.05	3.11	3.11
	F/#2	3.34	3.25	3.26	3.33	3.43
12	F/#1	2.96	2.96	2.96	2.93	2.94
	F/#2	2.33	2.27	2.16	**2.05**	**1.95**
60	F/#1	3.10	3.00	2.91	2.87	**2.75**
	F/#2	2.60	2.53	2.41	2.53	2.47
300	F/#1	2.75	2.64	**2.35**	Sacrificed	Sacrificed
	F/#2	2.21	2.19	2.04	**1.97**	**1.94**

Values in bold-face type indicate ≥ 10% reductions from predose levels.

Body Temperature, Heart Rate, Ophthalmoscopy, Water Intake and Urine Output

Body temperature was lower (≥ 2°C) than pretreatment level in one female in the 300 mg/kg/day group on day 10 of dosing and in one female in the 12 mg/kg/day group on day 27 of dosing. Heart rates were not affected by candesartan cilexetil treatment. No treatment-related ophthalmoscopic effects were observed. Candesartan cilexetil treatment had no effect on water intake or urine output.

Hematology and Blood Chemistry

Erythrocyte counts, hematocrit and hemoglobin concentration were lower than pretreatment and concurrent control levels in one male in the 300 mg/kg/day group at the end of the 4 week treatment period. Higher than predose and concurrent control levels of urea nitrogen and serum creatinine were observed in animals receiving ≥12 mg candesartan cilexetil/kg/day at 4 weeks after treatment; these effects were noted to a lesser degree and frequency at the end of the 2 week dosing period. Other effects on blood chemistry (higher than predose and concurrent control levels of serum potassium, serum calcium, ALT and AST) were observed sporadically with no obvious trend noted.

Gross Pathology, Organ Weights and Microscopic Pathology

No treatment related gross pathology was noted. Organ weights among candesartan cilexetil-treated monkeys did not differ appreciably from that of control. Microscopic examination revealed hypertrophy of the renal JG cells in 1/2 males and 2/2 females in the 12 mg/kg/day groups, 2/2 males and 2/2 females in the 60 mg/kg/day groups and 1/2 males and 2/2 females in the 300 mg/kg/day groups.

Toxicokinetics

The parent compound was detected in plasma in trace amounts after oral administration of candesartan cilexetil. The Cmax and AUC of the primary metabolite, candesartan, increased, non-dose-proportionally, with increasing dose (Table 37). The Cmax and AUC values for parent drug and metabolites increased as duration of dosing was lengthened.

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Table 37. Toxicokinetics Values after Oral Candesartan Cilexetil in Monkeys

Agent Measured	PK Parameter	Time of Sampling	Dose Group (mg/kg/day)			
			2.4	12	60	300
Candesartan Cilexetil	Cmax, ug/ml	Day 1 Week 4	BQL BQL	BQL 0.005	0.009 0.018	0.049 0.114
	AUC_{0-24}, ug.h/ml	Day 1 Week 4	BQL BQL	BQL 0.010	0.024 0.040	0.313 0.488
Candesartan	Cmax, ug/ml	Day 1 Week 4	0.07 0.15	0.50 0.98	2.25 3.19	6.76 17.89
	AUC_{0-24}, ug.h/ml	Day 1 Week 4	0.28 0.78	4.11 12.09	17.37 40.25	61.84 148.98
Metabolite M-II	Cmax, ug/ml	Day 1 Week 4	BQL BQL	0.004 0.012	0.020 0.024	0.092 0.160
	AUC_{0-24}, ug.h/ml	Day 1 Week 4	BQL BQL	0.036 0.148	0.306 0.537	1.107 2.564

BQL=Below quantifiable level

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REPRODUCTIVE TOXICOLOGY

Fertility Study in Male Rats

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1134/FE
Study Dates: Beginning date 11/20/90. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Jcl:Wistar rats (M=6 wks old, 140-164 gm; F=12 wks old, 190-215 gm at initiation of dosing).

Drug Administration: Candesartan cilexetil (Lot #A07730-07718) was suspended in 5% gum arabic aqueous solution and administered orally by gavage to males for 9 weeks before mating and throughout the mating period until the day when the pregnancies of most mated, non-treated females were confirmed (a total period of about 13 weeks).

Dosage Levels: 0 (vehicle control), 30, 100 and 300 mg/kg/day (10M/dose group).

Observations/Measurements: Males were observed for survival and clinical signs of toxicity twice daily during drug treatment; females were observed once daily. Body weights of males were obtained once a week throughout the study period. Food consumption was measured only for males once weekly before mating. Males and non-treated females were mated for 3 weeks on a one-to-one basis and mating was confirmed by presence of a copulation plug. The copulatory index (# of animals copulated x 100/# of animals cohabitated) and fertility index (# of pregnant animals x 100/# animals that copulated) were calculated for each group. Males were killed and necropsied after 13 weeks of dosing. Their reproductive and main organs were examined grossly. The testes, epididymides, seminal vesicles, ventral prostate, kidneys and liver were weighed and relative organ weights were calculated. Non-treated females were necropsied on day 20 of gestation. The uterus, ovaries and other main organs were examined grossly. The numbers of corpora lutea, implants, dead embryos/fetuses and live fetuses were counted. The sex ratios (# males x 100/# males and females) were determined. The live fetuses were weighed and examined for external abnormalities.

Results: No deaths occurred in any group and no general signs of toxicity were noted. Body weight gain was suppressed in the 300 mg/kg dose group compared to control; mean body weight in the 300 mg/kg/day group at the end of the dosing period was 7% lower than control. Food consumption in the 300 mg/kg/day group was slightly below control (~10%) during weeks 2, 4 and 13 of treatment. No differences were noted in the copulatory

index (100% for control and for each treated group) or fertility index (100% for control and for each treated group). There were no treatment-related differences from control in the numbers of corpora lutea, implants, percent implantation losses, number of live fetuses, fetal weights and sex ratios (Table 39). External examination of the fetuses revealed no treatment-related abnormalities. Gross examination of males at scheduled sacrifice showed no treatment-related pathology or effect on organ weight.

Table 39. C-Section Data (Gestation Day 20)

Parameter	Dose Group (mg candesartan cilexetil/kg/day)			
	0 (control)	30	100	300
Corpora Lutea (mean #)	15.7	16.3	16.4	16.1
Implants (mean #)	15.3	15.2	16.1	15.7
Pre-implantation loss (%)	2.5	6.9	2.1	2.7
Post-implantation loss (%)	5.8	5.8	6.8	5.5
Live Fetuses (mean #)	14.4	14.3	15.0	14.8
Fetal Weight (mean gm)				
Male	2.95	2.98	2.90	2.86
Female	2.73	2.76	2.71	2.67
Sex Ratio (% males)	49.7	49.4	49.2	57.7

Fertility Study in Male and Female Rats

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1516/FE
Study Dates: Beginning date 12/24/92. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Jcl:Wistar rats (M=6 wks old, 135-167gm; F=11 wks old, 191- 208 gm at initiation of dosing).

Drug Administration: Candesartan cilexetil (Lot #M464-018) was suspended in 5% gum arabic aqueous solution and administered orally by gavage to males for 9 weeks before mating, for 3 weeks during the mating period and for 3 weeks after the mating period; females were dosed for 2 weeks before mating, throughout the mating period and to day 7 of gestation..