The potential effect of Olmesartan on platelet aggregation was studied in platelet-rich plasma (PRP) prepared by centrifugation from blood samples obtained from rabbits (3 to 3.4 kg bw). Olmesartan was mixed at concentrations of 1, 10 or 100 µg/ml (5 samples/concentration) with PRP; 1 minute later, platelet aggregation was induced by the addition of adenosine diphosphate to a final concentration of 0.7 to 1.5 µM. The addition of olmesartan did not affect platelet aggregation.

2. DRUG DISPOSITION (ADME)

2.1. Pharmacokinetics of Olmesartan after Oral and Intravenous Administration of Olmesartan Medoxomil and Olmesartan to Rats (Report #RAM 139-055). Vol. 6.

This non GLP study was conducted by the Analytical and Metabolic Research Laboratories, Sankyo Co. Ltd., Fukuroi, Shizuoka 437, Japan, between February and October 1992.

Male Wistar- [redacted] rats (6 weeks old and weighing 129 to 144 gm) were fasted from the evening prior to dosing and were given single oral doses of 5 or 10 mg/kg (suspension) olmesartan medoxomil (OM), 5 mg/kg (suspension) olmesartan (4 males/group), or a single i.v. administration of 1 mg/kg olmesartan. OM was also administered orally in solution (5 mg/kg, n=4). OM (lot #5) and olmesartan (lot #4) were suspended in 0.5% CMC for oral dosing. Solutions containing either OM or olmesartan were prepared by dissolving the test substance in dimethylacetamide by warming and then by diluting with polyethylene glycol 400 and physiological saline. Blood samples were collected at 15 and 30 minutes and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 10 hours after oral administration; blood samples were collected at 1, 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, and 8 hours after i.v. administration. Blood was collected by exsanguination from a carotid artery (under anesthesia) from 4 animals/sampling point/route (n=44 rats for each oral study and 52 rats for the i.v. study). Plasma concentrations of olmesartan were determined by [redacted] assay with a limit of quantitation of [redacted] µg/ml.

Results

The plasma concentrations of olmesartan were determined after oral administration of the suspension of OM in CMC at 5 and 10 mg/kg and after the oral administration of the suspension of olmesartan in CMC at 5 mg/kg. After oral administration of OM at 5 mg/kg, the plasma concentration of olmesartan reached the maximum level (Cmax, 1.02 µg/ml) at 2.5 hr and decreased slowly thereafter monoexponentially. At 10 mg/kg, the time to reach maximum concentration (Cmax, 1.43 µg/ml) was slightly less, 2.0 hr. Thus, the Cmax value increased as the dose increased, while the Tmax showed a tendency to be prolonged at the higher dose and there was no or little change in the half-life value (Fig. 2.1.1). The AUC values at 5 (4.92 µg.h/ml) and 10 (11.10 µg.h/ml) mg/kg increased almost in proportion to the dose, and the dose-response curve for AUC was linear. On the other hand, after oral administration of 5 mg/kg olmesartan, plasma concentration of olmesartan reached a maximum level (Cmax, 0.32 µg/ml) far less than that observed after oral administration of parent compound at the same dose (Cmax, 1.02 µg/ml). There were no differences either in the Tmax or half-life (Table 2.1.1). The AUC value for olmesartan after oral administration of OM was approximately 3.3-fold higher than that after oral administration of olmesartan alone, demonstrating an improved oral absorption of the pro-drug, OM.

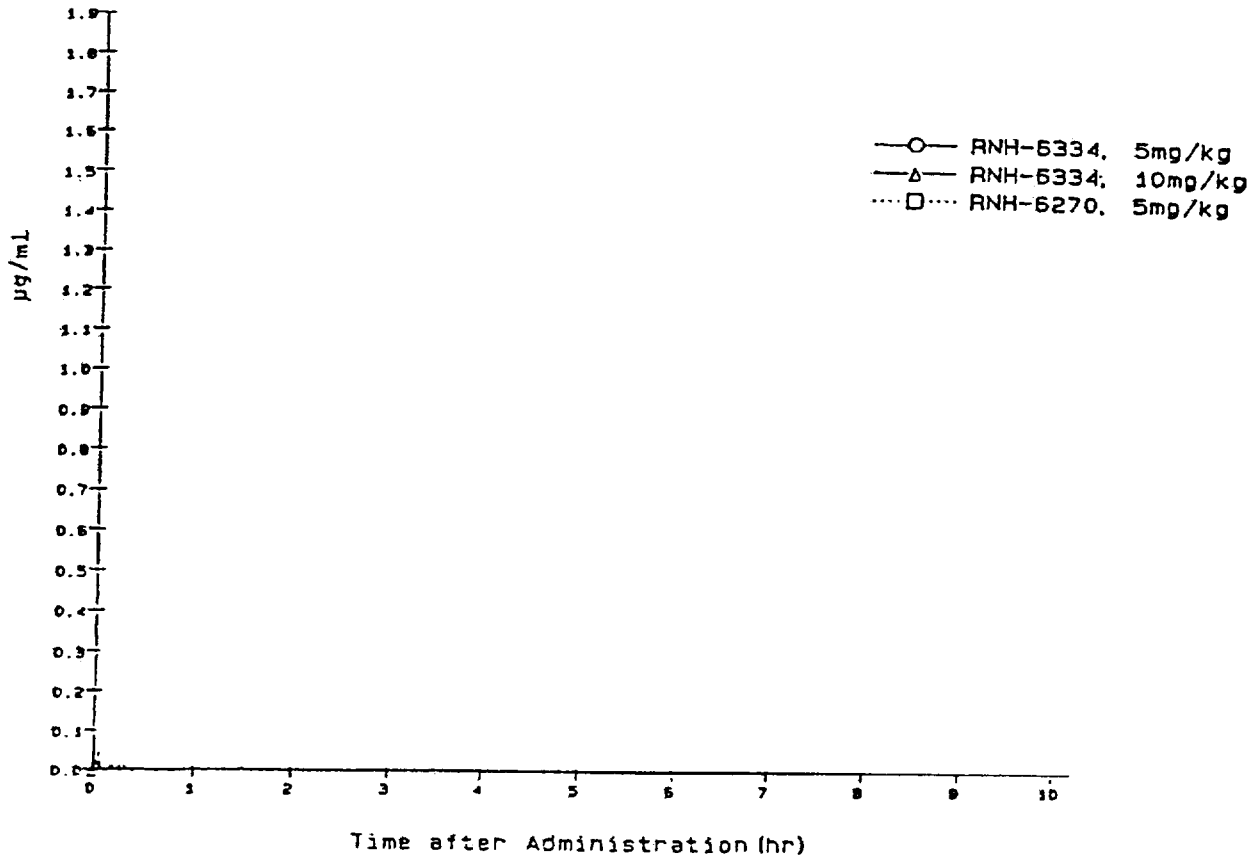


Fig. 2.1.1.: Plasma concentration of Olmesartan after oral administration of OM (RNH-6334) and olmesartan (RNH-6270) in rats (n=4)

TABLE 2.1.1

PHARMACOKINETIC PARAMETERS OF OLMESARTAN AFTER ORAL (CMC SUSPENSION) AND I.V. ADMINISTRATION OF OLMESARTAN MEDOXOMIL AND OLMESARTAN TO MALE RATS (MEAN VALUES)

Parameter	Olmesartan Medoxomil		Olmesartan	
	Oral (5 mg/kg)	Oral (10 mg/kg)	Oral (5 mg/kg)	I.V (1 mg/kg)
C_{max} [µg/ml]	1.02	1.43	0.32	-
T_{max} [hour]	2.50	2.00	2.50	-
$t_{½}$ [hour]	2.09	2.34	1.99	0.80
$AUC_{0-\infty}$ [µg·h/ml]	4.92	11.10	1.47	4.43
F (BA)[a], %	27.8	31.3	6.6	-

Note there are 4 mg equivalents of olmesartan in 5 mg OM.

[a]: BA (bioavailability) is calculated based on the formula AUC_{oral}/ $AUC_{i.v.}$ BA for the dose 5 mg OM is calculated as: 4.92/4.43 x 4 = 27.8.

The plasma concentration of olmesartan was determined after i.v. administration of olmesartan at 1 mg/kg. Peak blood levels (10.69 µg/ml) were reached at the first time point (1 min), and decreased rapidly with a half- life of 0.80 hr (ß-phase). A high AUC value of 4.43 µg.h/ml was noted (Table 2.1.1.1). The absolute bioavailability based on the ratios of AUC for olmesartan after oral to i.v. administration was 27.8% and 31.3% following administration of 5 and 10 mg/kg OM, respectively. However, the bioavailability was only 6.6% following administration of 5 mg/kg olmesartan.

Additionally, the study compared the plasma concentrations of olmesartan after oral administration of 5 mg/kg OM either in suspension or in solution. The AUC value for olmesartan after oral administration of OM in suspension was 6.20 µg.h/ml (BA, 35%) and after administration of OM in solution was 13.18 µg.h/ml (BA, 74%). Thus, the solution showed about 2 times greater AUC than the suspension indicating that the formulation affected the absorption of OM.

2.2. Pharmacokinetics of Olmesartan after Oral Administration of [^{14}C]Olmesartan Medoxomil to Rats (Report #GR 143-162). Vol. 6.

This non GLP study was conducted by the Analytical and Metabolic Research Laboratories, Sankyo Co. Ltd., Fukuroi, Shizuoka 437, Japan, between February 1994 and April 1997.

Male and female Wistar- [redacted] rats (7 and 8 weeks old and weighing 218.5 to 233 and 197 to 200 gm, respectively) were fasted from the evening prior to dosing. The dosage was 1 mg/kg [^{14}C]olmesartan medoxomil (lot #D-921006) administered orally by a stomach tube. [^{14}C]OM was dissolved in dimethylacetamide by warming and then diluted with polyethylene glycol 400 and physiological saline to a concentration of 1 mg/ml. Blood samples were collected from the jugular vein at 15, 30 and 45 minutes and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 10 hours after dosing (4 rats/sex/sampling point). Radioactivity was determined by [redacted]. The amount of radioactivity associated with OM, olmesartan and olmesartan glucuronide was quantified by [redacted]

Results

After oral administration of 1 mg/kg [^{14}C]olmesartan medoxomil to male rats, peak plasma level of total radioactivity (Cmax, 0.38 µg eq/ml) was attained at 0.88 hr, and decreased thereafter with a half-life of 1.81 hr. In females, Cmax, Tmax and $T_{1/2}$ were 0.31 mg eq/ml, 0.56 hr and 1.64 hr, respectively (Fig. 2.2.2.1). There were no statistically significant differences in any pharmacokinetics parameters between male and female animals (Table 2.2.1).

TABLE 2.2.1
PHARMACOKINETIC PARAMETERS OF TOTAL RADIOACTIVITY AND OLMESARTAN AFTER ORAL ADMINISTRATION OF 1 MG/KG OF [^{14}C]OLMESARTAN MEDOXOMIL TO MALE AND FEMALE RATS (MEANS OF INDIVIDUAL ANIMALS)

Parameter	Total Radioactivity		Olmesartan	
	Male	Female	Male	Female
$AUC_{0\text{-}10\ hr}$ (µg equiv.hr/ml)	0.99	0.95	0.63	0.71
$AUC_{0\text{-}\infty}$ (µg equiv.hr/ml)	1.07	1.03	0.65	0.74
$T_{1/2}$ (hr)	1.81	1.64	1.12	1.44
Cmax (µg equiv/ml)	0.38	0.31	0.30	0.28
Tmax (hr)	0.88	0.56	0.56	0.44
$MRT_{0\text{-}10\ hr}$ (hr)[1]	2.64	3.02	1.97	2.42
Cl total/F (ml/hr/kg)[2]	959.25	1123.70	1583.76	1585.44

1: $MRT_{0\text{-}10\ hr}$: Mean residence time
2: Cl total/F : apparent total clearance was calculated according to the equation, dose/$AUC_{(0\text{-}\infty)}$

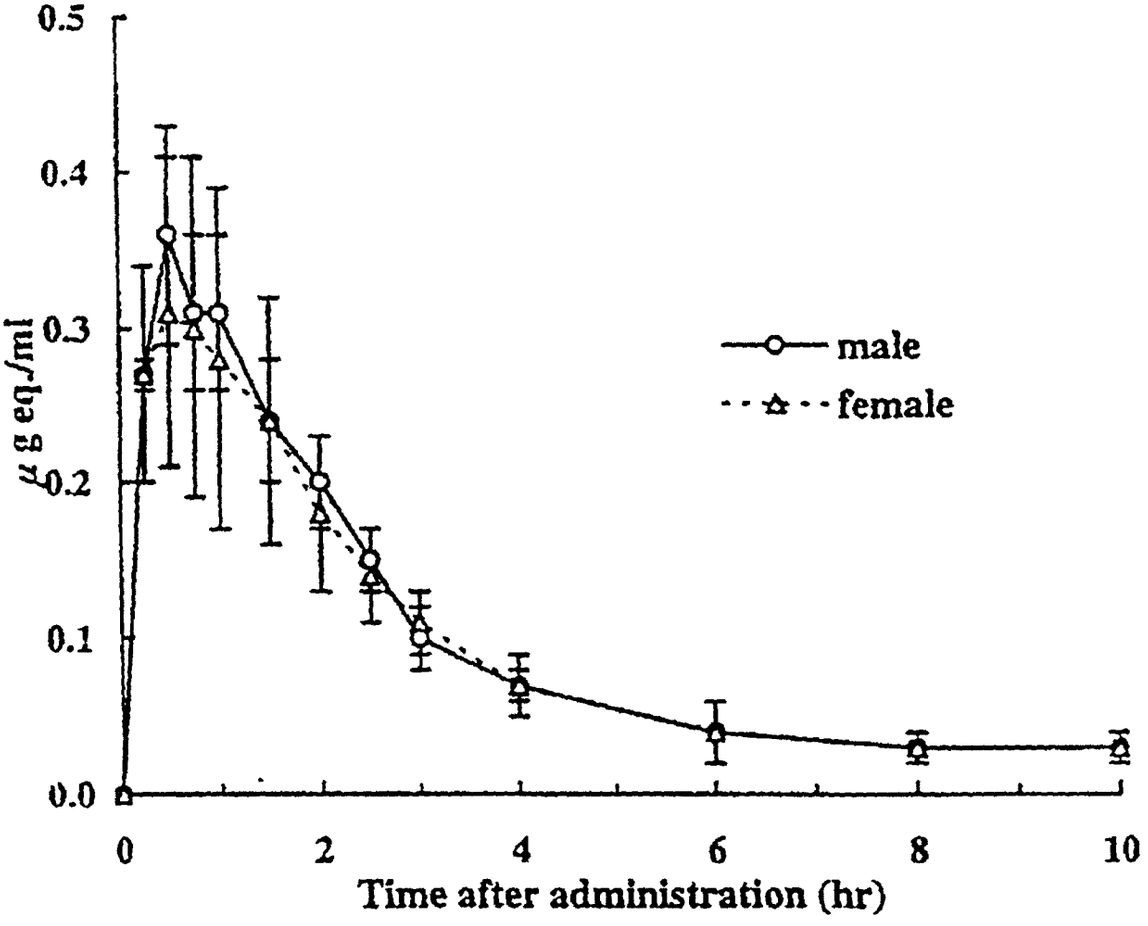
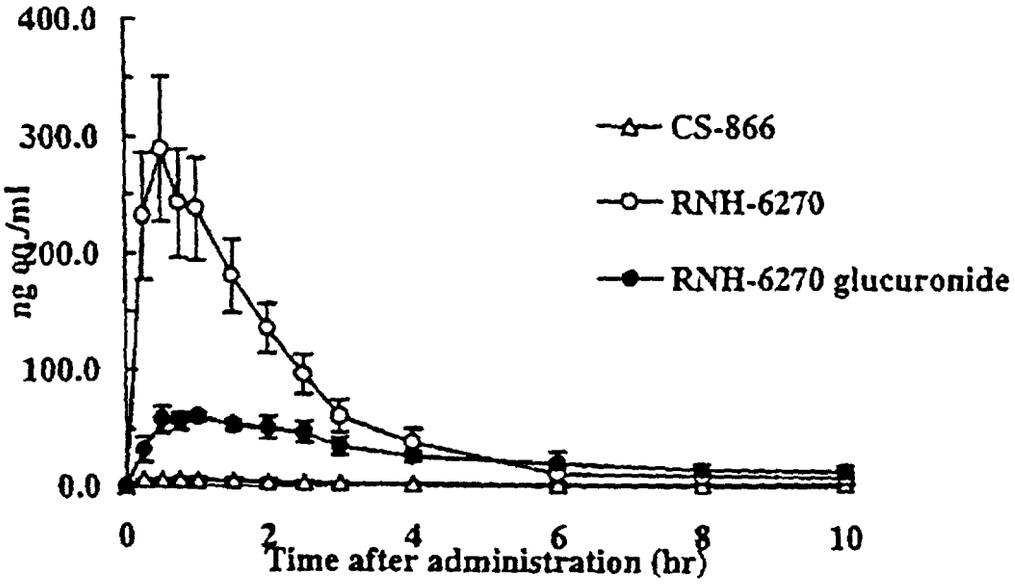


Fig.2.2.1.: Plasma concentrations of radioactivity after oral administration of 1mg/kg of [^{14}C]olmesartan medoxomil to male and female rats. Data shown as mean ± SEM (n=4).

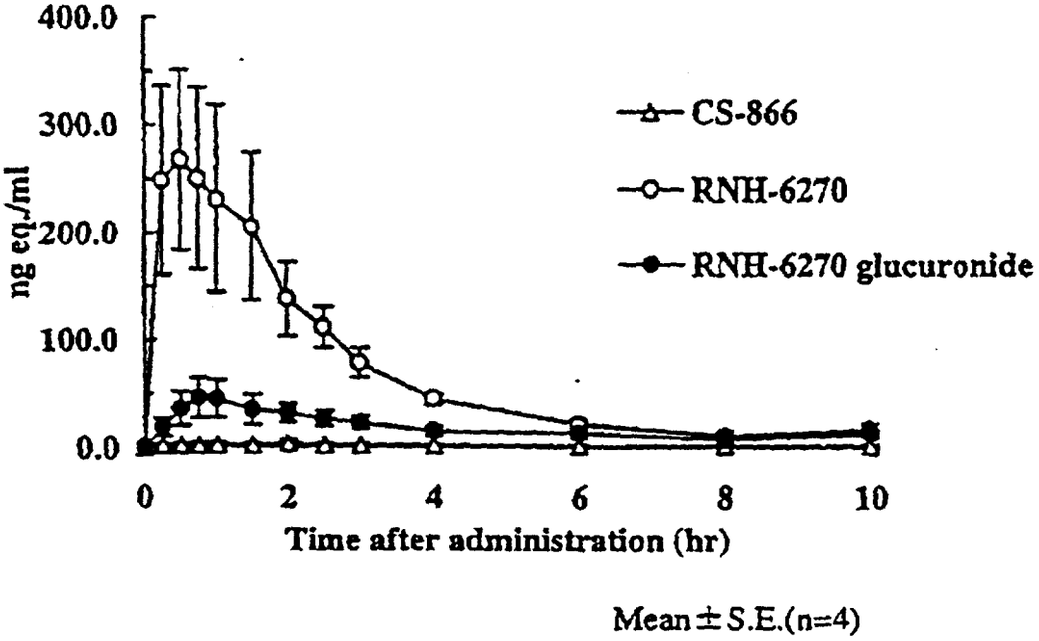
The ratios of the plasma radioactivity to the whole blood radioactivity at 1 and 2 hr after administration were 102.8 and 99.5%, respectively, in male rats and 101.5 and 99.2%, respectively, in female rats. This demonstrates that almost all the radioactivity is present in the plasma.

Olmesartan medoxomil accounted for only a small percentage of the total radioactivity in the plasma (approximately 1%) even at early time points (Fig. 2..2.2). At the 15-minute time point, olmesartan accounted for 89.11% of the radioactivity and olmesartan glucuronide accounted for 8.65%. Over the sampling time, the proportion of radioactivity accounted for by olmesartan decreased as the proportion of olmesartan glucuronide increased. At 10 hours, olmesartan accounted for 35.44% of the total radioactivity and olmesartan glucuronide accounted for 38.56% (Table 2.2.2 and 2.2.3).

Male



Female



Mean ± S.E.(n=4)

Fig.: 2.2.2.: Plasma concentrations of metabolites after oral administration of [^{14}C]olmesartan medoxomil (CS-866, 1 mg/kg) to male and female Wistar rats.

TABLE 2.2.2
PLASMA METABOLITES OF [^{14}C]OLMESARTAN MEDOXOMIL (CS-866, 1 MG/KG) AFTER ORAL ADMINISTRATION TO MALE WISTAR RATS

Time(hr)	% of radioactivity RNH-6270 glucuronide	RNH-6270	CS-866
0.25	11.66 ± 1.71	84.86 ± 1.72	2.37 ± 0.73
0.5	17.11 ± 2.02	80.35 ± 2.18	1.45 ± 0.24
0.75	19.13 ± 1.94	77.04 ± 2.78	1.83 ± 0.58
1.0	20.62 ± 2.94	75.92 ± 2.77	1.55 ± 0.77
1.5	22.81 ± 2.27	73.41 ± 2.40	2.06 ± 0.80
2.0	25.93 ± 2.38	68.71 ± 2.63	2.10 ± 0.91
2.5	31.31 ± 4.36	61.95 ± 5.70	2.26 ± 0.61
3.0	34.25 ± 3.47	58.32 ± 4.18	3.26 ± 0.73
4.0	38.61 ± 2.56	52.56 ± 5.84	4.37 ± 1.98
6.0	52.51 ± 6.53	24.87 ± 8.54	6.81 ± 3.32
8.0	42.47 ± 5.82	29.49 ± 1.05	8.40 ± 3.26
10.0	42.19 ± 3.83	25.19 ± 3.44	10.96 ± 1.99

Mean±S.E. (n=4)

TABLE 2.2.3
PLASMA METABOLITES OF [^{14}C]OLMESARTAN MEDOXOMIL (CS-866, 1 MG/KG) AFTER ORAL ADMINISTRATION TO FEMALE WISTAR RATS

Time(hr)	% of radioactivity RNH-6270 glucuronide	RNH-6270	CS-866
0.25	5.64 ± 2.38	93.46 ± 2.79	0.42 ± 0.29
0.5	6.66 ± 2.91	90.41 ± 3.27	0.36 ± 0.36
0.75	12.50 ± 4.20	86.89 ± 4.41	0.41 ± 0.37
1.0	13.89 ± 4.85	85.59 ± 4.92	0.42 ± 0.42
1.5	11.97 ± 4.02	87.34 ± 4.25	0.69 ± 0.40
2.0	17.97 ± 2.02	79.95 ± 2.01	1.66 ± 0.98
2.5	18.20 ± 3.35	78.99 ± 3.20	1.38 ± 0.80
3.0	21.24 ± 3.34	75.27 ± 3.24	1.88 ± 1.09
4.0	22.65 ± 3.94	71.54 ± 4.57	3.40 ± 1.96
6.0	31.37 ± 7.01	56.61 ± 3.98	4.00 ± 2.34
8.0	32.97 ± 2.40	45.51 ± 10.03	11.90 ± 6.26
10.0	19.93 ± 9.95	45.69 ± 8.47	5.59 ± 5.59

Mean±S.E. (n=4)

2.3. Pharmacokinetics of Olmesartan after Oral Administration of [^{14}C]Olmesartan Medoxomil to Five-Sixth Nephrectomized Male Rats (Report #GR 143-078). Vol. 5.

This non-GLP study was conducted by the Analytical and Metabolic Research Laboratories, Sankyo Co. Ltd., Fukuroi, Shizuoka 437, Japan, between September 1994 and October 1996. The objective of the study was to determine the pharmacokinetics and metabolism of the administered radioactivity in an animal model of renal failure, the 5/6-nephrectomized rat.

Male Wistar [redacted] rats (5 weeks old) had 2/3 (upper and lower parts) of one kidney removed. A week later, the second kidney was removed (resulting in 5/6-nephrectomy) leaving the renal artery untouched. The animals were used 4 weeks after the surgery. Five normal animals (333 to 342 gm) and six 5/6-nephrectomized rats (270 to 316 gm) were used in the study. The animals were fasted from the evening prior to dosing. The dosage was 1 mg/kg [^{14}C]olmesartan medoxomil (lot #D-930123) administered orally by a stomach tube. [^{14}C]OM was dissolved in dimethylacetamide by warming and then diluted with polyethylene glycol 400 and physiological saline to a concentration of 1 mg/ml. Blood samples were collected from the jugular vein at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 24 hr after dosing. Plasma levels of creatinine and BUN were determined at 24 hr after administration as indices of renal failure. Radioactivity was determined by [redacted]. The amount of radioactivity associated with OM, olmesartan and olmesartan glucuronide was quantified by [redacted]

Results

The creatinine and BUN levels in normal rats were 0.99 and 16.73 mg/dl. In contrast, the levels were 1.4 and 66.32 mg/dl, respectively, in the nephrectomized rats, indicating that renal failure had been induced. When total radioactivity was analyzed, the nephrectomized rats had a significantly prolonged mean residence time (MRT), approximately 38% greater than for the normal rats ($p < 0.01$); there were no significant changes in other pharmacokinetic parameters. (Table 2.3.1). Based on the results of this study, it was concluded that decreased renal function would not adversely affect plasma concentrations of the active metabolite, olmesartan, after administration of OM.

TABLE 2.3.1
PHARMACOKINETIC PARAMETERS OF TOTAL RADIOACTIVITY AND OLMESARTAN AFTER ORAL ADMINISTRATION OF 1 MG/KG OF [^{14}C]OLMESARTAN MEDOXOMIL TO NORMAL AND NEPHRECTOMIZED MALE RATS (MEANS OF INDIVIDUAL ANIMALS)

Parameter	Total Radioactivity		Olmesartan	
	Normal	Nephrectomized	Normal	Nephrectomized
AUC[a] (µg equiv•hr/mL)	3.02	2.59	2.04	1.50
T½ (hr)	1.12	1.30	0.97	1.50
MRT[a] (hr)	3.57	4.91	2.41	2.77
Tmax (hr)	1.40	1.46	2.00	0.5
Cmax (µg equiv/mL)	0.95	0.71	0.64	0.37
Cltot (mL/hr/kg)	312.50	386.10	490.20	666.67

[a]: 0-24 hr for total radioactivity and 0-10 hr for olmesartan

OM accounted for only a small percentage of the total radioactivity in the plasma in both normal (10.24%) and nephrectomized (4.97%) rats at very early time points. Olmesartan was detected as the main metabolite, with the glucuronic acid conjugate of olmesartan comprising about 20% of the total radioactivity (Table 2.3.2). Thus, the nephrectomized rats showed the same metabolic profile as that in the normal rats.

TABLE 2.3.2
PLASMA METABOLITES AFTER ORAL ADMINISTRATION OF 1 MG/KG [^{14}C]OLMESARTAN MEDOXOMIL TO NORMAL RATS OR 5/6-NEPHRECTOMIZED RATS (MEAN VALUES)

	% of Total Radioactivity									
	Normal					Nephrectomized				
	0.25 hr	1 hr	3 hr	6 hr	10 hr	0.25 hr	1 hr	3 hr	6 hr	10 hr
OM	10.24	1.68	2.71	12.22	19.75	4.97	2.59	2.11	5.25	15.06
Olm	56.43	71.63	74.89	45.84	25.98	77.54	72.40	72.00	69.54	52.02
Olm glu	19.59	24.36	20.45	30.48	21.18	9.67	23.63	23.39	18.59	13.58

2.4. Absorption, Distribution and Excretion After Repeated Oral Administration of [^{14}C]Olmesartan Medoxomil to Male Rats (Report # [redacted] Vol. 5.

This GLP study was conducted by [redacted] between May 27, 1998 and February 26, 1999.

Male Wistar strain SPF rats were 6 weeks of age and weighed 152.8 to 185.6 gm at initiation of drug administration. The dosage in all experiments was 5 mg/kg [^{14}C]olmesartan medoxomil (lot #D-970715) administered orally by a stomach tube once a day for a maximum of 21 days to non-fasted animals. [redacted] The study was divided into four experiments, the details of which are listed below.

TABLE 2.4.1.
EXPERIMENTAL DESIGN

Expt	Objective	Methods
1.	Determination of radioactivity concentrations in blood and plasma	Blood samples were taken at 0.25, 0.5 1, 2, 4, 6, 8, and 24 hours after the first dose; at 24 hours after the 2nd through the 20th dose; and at 0.25, 0.5 1, 2, 4, 6, 8, and 24 hour and thereafter every 24 hours up to 168 hours after the last dose (n=3/point). Same animals were used at all time points.
2	Determination of radioactivity concentrations in tissues	Rats were sacrificed at 1 and 24 h after the first dose, 24 h after the 7th and 15th dose, and 1, 24, 72, and 168 h after the 21st dose (n=3 /time point). The following tissues were examined for determination of tissue concentrations of radioactivity: plasma, blood, cerebrum, cerebellum, pituitary gland, eyeball, Harderian gland, thyroid gland, trachea, mandibular gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, pancreas, fat, brown fat, skeletal muscle, skin, bone marrow, aorta, mesenteric lymph node, testis, epididymis, prostate gland, stomach, jejunum, ileum, cecum, colon, and urinary bladder.
3.	Whole body autoradiography	One rat each was sacrificed at 1, 24, 72, and 168 h after the 21st dosing. [redacted] were made and [redacted] were prepared. Radioactivity was analyzed using a [redacted]
4.	Determination of excretion of radioactivity in urine, feces and expired air, and residual radioactivity in carcass	Urine and feces were collected 0 to 24 h after daily dosing and every 24 h up to 168 h after the 21st dose. Expired air was collected only 0 to 24 hours after the first dose. Total radioactivity was determined in urine, feces, expired air and carcass (n=3).

Radioactivity in blood, plasma, urine, feces, expired air, carcass and tissues was measured by [redacted]. The amount of radioactivity associated with OM, olmesartan and olmesartan glucuronide was quantified by [redacted]

Results

The radioactivity concentration in the plasma reached maximum at 2 hr and declined with a half-life of 3.5 h after the first dosing. The concentration of radioactivity in blood and plasma, 24 hours after dosing, ranged from [redacted] µg equiv/ml and [redacted] µg equiv/ml, respectively. After the last dose, the radioactivity concentration in the plasma reached maximum at 1 hr and declined with a half-life of 3.4 h and was below the detection limit after 48 hr (Table

2.4.1). No significant difference was observed between the $AUC_{(0-\infty)}$ after the first dosing and the $AUC_{(0-24h)}$ after the last dosing. So was the case with C_{max} and elimination half-life. This suggests that there was no accumulation of drug-related substances with repeated dosing.

TABLE 2.4.1
RADIOACTIVITY CONCENTRATION IN PLASMA AFTER A SINGLE OR A 21 DAY ORAL ADMINISTRATION OF 5 MG/KG OF [^{14}C]OLMESARTAN MEDOXOMIL TO MALE RATS

	Radioactivity concentration (μg eq. of CS-866/mL)	
Time	Single dose	21 doses
15 min	1.670 ± 0.485 (0.840)	3.457 ± 0.720 (1.247)
30	2.870 ± 0.500 (0.866)	4.895 ± 0.962 (1.666)
1 hr	4.238 ± 0.374 (0.648)	5.655 ± 0.973 (1.685)
2	5.183 ± 0.126 (0.218)	4.040 ± 0.454 (0.787)
4	3.215 ± 0.216 (0.374)	2.020 ± 0.375 (0.649)
6	1.427 ± 0.064 (0.111)	1.092 ± 0.177 (0.307)
8	0.621 ± 0.107 (0.185)	0.616 ± 0.038 (0.067)
24	0.034 ± 0.006 (0.010)	0.028 ± 0.005 (0.009)
48	—	N.D.
72	—	N.D.
96	—	N.D.
120	—	N.D.
144	—	N.D.
168	—	N.D.
Detection limit	0.022	0.022
tmax (hr)	2 ± 0 (0)	1 ± 0 (0)
Cmax (μg/mL)	5.183 ± 0.126 (0.218)	5.655 ± 0.973 (1.685)
t1/2 (hr)	3.5 ± 0.1 (0.2) (6-24hr)	3.4 ± 0.1 (0.2) (6-24hr)
AUC (μg eq.·hr·mL^{-1})	27.6 ± 1.0 (1.7) (0-24hr)	25.0 ± 3.2 (5.6) (0-24hr)
	27.8 ± 1.0 (1.7) (0-∞)	—
R	1.01 ± 0.00 (0.00)	—

Data are expressed as the mean ± SE (SD) of 3 animals
- : Not determined
ND : Not detected

Measurements of the concentrations of the radioactivity in the tissues showed that after oral administration the radioactivity was distributed to the entire organism. One hr after the first or 21st dose, the highest concentration of radioactivity was found in the liver with the next highest concentrations in plasma, GI tract, and blood. The concentrations of radioactivity in the tissues were generally comparable after a single dose and after 21 days of dosing (Table 2.4.2). Although radioactivity was increased in the lung, kidney, ileum, cecum, colon, and urinary bladder after 21 days of dosing, only the increase in the colon was statistically significant.

TABLE 2.4.2
TISSUE CONCENTRATIONS OF RADIOACTIVITY (μG EQUIV OF OM/G OR ML) 1 AND 24 HOURS AFTER DAILY ORAL ADMINISTRATION OF 5 MG [^{14}C]OLMESARTAN MEDOXOMIL/KG/DAY TO MALE RATS

Tissue	Single Dose		21 Doses	
	1 hr	24 hr	1 hr	24 hr
Plasma	4.537	0.037	4.526	0.042
Blood	2.811	0.022	2.719	0.029
Pituitary gland	0.743	ND	0.499	ND
Thyroid gland	0.694	ND	0.581	ND
Trachea	0.556	ND	0.479	ND
Mandibular gland	0.534	0.008	0.507	0.011
Heart	0.644	0.005	0.590	0.008
Lung	0.661	0.023	0.924	0.026
Liver	6.052	0.036	6.901	0.050
Kidney	3.899	0.025	5.280	0.050*
Brown fat	0	ND	.501	0.018
Skin	0.406	0.009	0.521	0.016
Bone marrow	0.452	ND	0.526	ND
Mesenteric lymph node	0.957	0.010	0.854	0.012
Stomach	3.740	0.125	3.850	0.089
Jejunum	3	0.009	.550	0.024
Ileum	1.417	0.075	4.402	0.212
Cecum	0.459	0.154	0.894	0.760
Colon	0.292	0.175	0.699*	0.151
Urinary bladder	0.566	ND	0.737	ND

Data are expressed as the mean of three animals
* : significantly different from the value of single dose (24 hr) at 5% level.
ND: Not detected

The radioactivity concentrations 24 hours after administration on days 7, 15, and 21 did not differ significantly from those after the first dose except for the Harderian gland and the kidney. The radioactivity concentrations in the Harderian gland on day 15 and in the kidney on days 15 and 21 were significantly higher than after the first dose. At 24 hours after the last dose, the radioactivity concentrations in the cecum and colon were 85% and 22% of their maximum levels, respectively. The radioactivity concentrations in the other tissues were not more than 8% of their maximum levels. The radioactivity in the cecum was the highest among the tissues and 18 times that in plasma; the radioactivity in ileum, colon, and stomach were the next highest and 2.1 to 5 times that in the plasma. At 72 hours after the last dose, the radioactivity concentration in the fat was 11% of the maximum; the radioactivity in all other tissues decreased to no more than 4% of their maximal levels. By 168 hours after the last dose, radioactivity was detectable only in fat (9.2% of maximum levels) and lung, kidney, brown fat, skin, and colon (0.2% to 2.3% of maximum levels).

The whole-body autoradiograms analyzed 1 hr after the 21st dosing showed high levels of radioactivity in the gastric and intestinal contents. The levels of radioactivity in the bile, kidney, liver, pancreas, stomach, and intestine were higher than that in blood. The levels of radioactivity in the mesenteric lymph node, lung, adrenal gland, and urinary bladder were comparable to the levels in blood. Levels of radioactivity in the heart, spleen, mandibular gland, pituitary gland,

thyroid gland, bone marrow, Harderian gland, epididymis, urine in bladder, prostate gland, skeletal muscle, testis, and thymus were lower than that in blood. The lowest levels of radioactivity were observed in the fat, eyeball, and brain. The overall distribution of radioactivity decreased at 24 hours after the last dose except for the intestinal and gastric contents, where still high levels were found Trace levels of radioactivity were found only in the intestine, kidney, and liver. The overall distribution of radioactivity was further decreased at 72 and 168 hours after the last dose and trace levels of radioactivity were found only in the intestinal contents at both times.

After oral administration, the radioactivity was excreted predominantly by the fecal route. The excretion in the urine and feces for 24 hours after the first dose was 0.2% and 90.7 ± 7.3%, respectively. The excretion of radioactivity in the urine and feces up to 24 hours after daily dosing was 0.2% to 0.3% and 95.5% to 99.9% of the cumulative dose, respectively, after the 2nd to the 21st doses. The excretion of radioactivity in urine and feces up to 168 hours after the 21st dose was 0.3% and 100.9% of the cumulative dose, respectively. There was no residual radioactivity in the carcass at 168 hours after the final dose. No radioactivity was observed in the expired air.

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2.5. Tissue Distribution of Radioactivity and Metabolites After Oral Administration of [^{14}C]Olmesartan Medoxomil to Male Rats (Report #GR 143-079). Vol. 5.

This non GLP study was conducted by the Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between April and October 1996. The objective of the study was to determine the concentrations of radioactivity in the plasma, tissues and organs after oral administration of [^{14}C]olmesartan medoxomil to male rats.

Male Wistar- [redacted] rats (6 weeks old and weighing between 161 and 194 gm) were fasted from the evening prior to dosing and were fed 6 hr after administration of test substance. [^{14}C]olmesartan medoxomil (25.108 µCi/mg, lot No. D-930123) was mixed with unlabeled OM (lot No. 4), and dissolved in dimethylacetamide to which was added PEG400 and physiological saline to achieve a concentration of 5 mg/ml. Test substance was administered orally (by stomach tube) to each rat at a dose of 5 mg/kg. The animals were sacrificed by anesthesia (diethyl ether) at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 24 hours after dosing (n = 4 rats/time point). The blood sample was collected by exsanguination from a carotid artery and the tissues and organs (total 18) were excised immediately. Tissues, organs and plasma samples were solubilized and total radioactivity was determined by [redacted]
Qualitative and quantitative analysis of metabolites was made from the liver and kidney samples collected at 60 min after dosing. These tissues were homogenized and the supernatant was spotted to [redacted] along with non-labeled OM and olmesartan.

Protein binding was determined from plasma samples collected at 1 and 3 hours after dosing using [redacted]

Results

After oral administration of 5 mg/kg [^{14}C]olmesartan medoxomil, peak plasma levels of radioactivity (4.72 µg equiv/ml) were attained at 1 hour. High concentrations of radioactivity were observed in the liver within 15 minutes after administration and exceeded the plasma concentration by 2- to 5-fold for up to 8 hours after administration. The maximum concentration observed in the liver was 12.69 µg equiv/gm at 1.5 hours after dosing. There were no other tissues or organs that showed concentrations greater than those observed in plasma. Radioactivity in whole blood and kidney were approximately one-half that observed in plasma. The concentrations in the lung, heart, adrenal, seminal vesicle, submaxillary gland, pancreas, testis, brown fat, lymph node, and thyroid were approximately one-tenth that observed in plasma. Levels of radioactivity were even lower in the thymus, eyeball and brain.

The pharmacokinetic parameters were calculated for each tissue and organ in addition to plasma and blood (Table 2.5.1). The plasma concentration-time curve showed a monophasic profile with a half-life of 1.68 hr. Similarly, the liver displayed a monophasic profile with a half-life of 1.55 hr. The elimination half-lives in other tissues and organs were 1.5 hr to 2.7 hr, showing decreases in parallel to the decrease of the plasma concentration. There was no accumulating tendency in any tissue or organ. The distribution of radioactivity observed in this study was similar to that observed after whole body autoradiography in rats.

TABLE 2.5.1
PHARMACOKINETIC PARAMETERS FOR PLASMA/TISSUE RADIOACTIVITY AFTER ORAL ADMINISTRATION OF 5 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL TO RATS (MEAN VALUES)

Tissue/Organ	Cmax (μg equiv/ml or g)	Tmax (hr)	t½ (hr)	$AUC_{0\text{-}24\ hr}$ (μg equiv.hr/ml or g)	$MRT_{0\text{-}24\ hr}$ (hr)	Tissue/Plasma AUC ratio
Plasma	4.72	1.00	1.68	15.84	3.68	1.00
Blood	2.94	1.00	1.69	9.45	3.86	0.60
Liver	12.69	1.50	1.55	48.35	3.48	3.05
Kidney	2.45	1.00	1.68	7.61	3.35	0.48
Pancreas	0.42	1.00	2.18	1.77	4.46	0.11
Heart	0.71	1.00	1.73	2.39	3.66	0.15
Lung	0.79	1.50	2.26	3.65	4.90	0.23
Thymus	0.19	1.50	2.46	0.97	4.97	0.06
Brain	0.05	1.00	2.77	0.25	5.02	0.02
White fat	0.36	0.50	1.87	1.02	3.79	0.06
Testis	0.43	1.50	2.22	1.92	4.67	0.12
Skeletal muscle	0.22	1.00	1.82	1.06	4.53	0.07
Spleen	0.32	1.00	2.11	1.37	4.84	0.09
Adrenal	1.44	0.25	2.14	2.62	4.32	0.17
Brown fat	0.49	1.50	1.97	2.11	4.46	0.13
Seminal vesicle	0.91	1.00	2.21	2.80	4.23	0.18
Submaxillary gland	0.75	1.00	1.81	2.50	4.02	0.16
Thyroid gland	0.75	1.00	1.95	2.80	3.99	0.18
Lymph node	0.50	1.50	2.24	2.33	5.11	0.15
Eyeball	0.18	1.50	2.58	0.87	5.53	0.05

The concentrations of radioactivity in whole blood and plasma as well as the ratio of the plasma radioactivity to the whole blood radioactivity was calculated based on the concentration data and hematocrit values. The ratios of plasma radioactivity to whole blood radioactivity were 99.26 and 95% at 0.25 and 8 hr, respectively, and demonstrate a low distribution of the radioactivity to the blood cells.

Plasma protein binding ratios determined by an ultrafiltration method were 98.84 % and 99.32% at 1 and 3 hr after dosing. Since only olmesartan was identified in these plasma samples, these values were considered to reflect the binding of olmesartan to plasma proteins.

The [redacted] revealed almost no parent compound. The metabolite, olmesartan, accounted for 88.29% of the radioactivity in the liver and 76.26% of the radioactivity in the kidney. Olmesartan glucuronide conjugate accounted for 1.65% and 5.23% of the radioactivity in the liver and kidney, respectively.

2.6. Placental Penetration and Transfer into Milk After Oral Administration of ^{14}C-Olmesartan Medoxomil to Pregnant/Lactating Rats. (Study #AE-2565-2G, Report # [redacted] Vol. 5.

[redacted] conducted this GLP study between May 27, 1998 and February 26, 1999.

Methods

Mated (at 9 weeks of age) and presumed pregnant female Sprague-Dawley rats from [redacted] were treated with a single oral dose of 5 mg/kg ^{14}C-olmesartan medoxomil (Lot #D-970715) on day 13 of gestation (271.6 to 312.9 g bw), on day 18 of gestation (313.5 to 353.9 g bw), or during lactation (postpartum day10; 334.3 to 353.9 g bw). The dosing solution, which was prepared in [redacted] was administered orally into the stomach using a syringe. The rats were not fasted prior to administration of the drug. There were no control animals in this study.

Whole body autoradiograms were prepared and the concentration of radioactivity in tissues was determined at designated times after dosing on day 13 and day 18 of gestation. Pregnant rats were sacrificed at 1, 24 and 48 hours after administration (1 rat/time point for whole body autoradiography and 3 rats/time point for tissue concentrations of radioactivity). Autoradiograms were analyzed using a [redacted] and radioactivity in tissues by [redacted] The tissues examined included plasma, blood, cerebrum, heart, lung, liver, kidney, adrenal gland, uterus, ovary, mammary gland, placenta, fetal membrane, fetus, yolk sac fluid (day 13 of gestation) and amniotic fluid (day 18 of gestation). Fetal blood, brain, heart, lung, liver, kidney, and digestive tract were examined on day 18 of gestation. After a single oral administration of ^{14}C-olmesartan medoxomil to lactating rats, milk and blood were collected at 0.5, 1, 2, 4, 8, and 24 hr after administration (same three rats were used at all time points). The sucklings were separated from their dam 1 hr before sampling and returned after sampling. Radioactivity in plasma and milk were determined by [redacted]

Results

Whole-body autoradiogram: Autoradiograms demonstrated that levels of radioactivity in the uterus, ovary, placenta and mammary gland were lower than that of maternal blood 1 hour after dosing; radioactivity in the yolk sac fluid, amniotic fluid, and fetus were barely detectable. For rats at day 13 of gestation, at 24 and 48 hour after dosing, the overall distribution of radioactivity decreased compared to the 1 hour time point and no radioactivity was observed in the uterus, ovary, placenta, mammary gland, yolk sac fluid, or fetus. For rats at day 18 of gestation, 24 hours after administration, trace levels of radioactivity were observed in the placenta and no radioactivity was observed in the ovary, uterus or mammary gland. However, relatively high levels of radioactivity were found in the yolk sac and fetal membrane; a low level of radioactivity was observed in the fetus, which was higher than that observed at 1 hour after dosing. At 48 hours after administration, the level of radioactivity in the fetal digestive tract was higher than that observed at 24 hours. The levels of radioactivity in the fetal organs, except the yolk sac, fetal membrane and digestive tract, were lower than at 24 hours after administration.

Concentration of radioactivity in tissues: After single oral administration of test substance on day 13 of pregnancy, the radioactivity concentrations in the uterus, ovary, mammary gland, fetal membrane and placenta peaked at the first sampling time, 1 hr. The concentrations in these tissues were 10% to 22% of maternal plasma concentration. The concentrations of radioactivity in the liver, kidney and blood at 1 hr were 0.6 to 1.5 times that in the maternal plasma (Table 2.6.1). At 24 hr after administration, the radioactivity concentrations in the uterus, placenta and fetal membrane were 1 to 1.4 times the maternal plasma concentration. The radioactivity was below the detection limit in the ovary and mammary gland. At 48 hr after dosing, the radioactivity was still detectable in both uterus and fetal membrane but not in plasma. Radioactivity was not detectable at this time in the yolk sac fluid (Table 2.6.1).

TABLE 2.6.1
CONCENTRATION OF RADIOACTIVITY IN TISSUES AFTER A SINGLE ORAL DOSE OF 5 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL TO RATS ON DAYS 13 OR 18 OF GESTATION (MEAN VALUES)

	Radioactivity (µg equiv/g or ml)					
	Day 13			Day 18		
Tissue	**1 hr**	**24 hr**	**48 hr**	**1 hr**	**24 hr**	**48 hr**
Plasma	3.406	0.014	ND[a]	4.283	0.029	ND
Blood	2.168	ND	ND	2.763	0.020	ND
Cerebrum	0.029	ND	ND	0.036	ND	ND
Heart	0.415	ND	ND	0.459	0.005	ND
Lung	0.526	0.015	0.009	0.715	0.032	0.012
Liver	5.014	0.041	0.029	5.985	0.070	0.016
Kidney	2.330	0.020	0.015	3.332	0.053	0.013
Adrenal gland	0.471	ND	ND	0.420	0.014	ND
Uterus	0.752	0.014	0.006	0.916	0.046	0.015
Ovary	0.683	ND	ND	0.668	0.012	ND
Mammary gland	0.360	ND	ND	0.381	0.007	ND
Placenta	0.507	0.015	ND	0.735	0.084	0.037
Fetal membrane	0.335	0.020	0.010	0.060	0.095	0.120
Yolk sac fluid	ND[a]	ND	ND	---[b]	---	---
Amniotic fluid	---	---	---	0.018	0.045	0.171
Fetus	0.008	ND	ND	0.008	0.150	0.141
Blood	---	---	---	0.015	0.210	0.148
Brain	---	---	---	ND	0.016	0.012
Heart	---	---	---	ND	0.072	0.048
Lung	---	---	---	0.006	0.082	0.063
Liver	---	---	---	0.013	0.094	0.102
Kidney	---	---	---	0.011	0.114	0.076
Digestive tract	---	---	---	0.006	0.141	0.574

[a]: ND, not detected
[b]: ---, not determined

For animals receiving the test substance on day 18 of pregnancy, the radioactivity concentration for tissues other than amniotic fluid, fetal membrane, fetus and fetal organs, reached peak 1 hr after administration. At 24 hours after dosing, radioactivity concentrations in the uterus, amniotic fluid, placenta, fetus, fetal blood, fetal digestive tract, fetal kidney, fetal membrane, fetal liver, fetal lung, and fetal heart exceeded the concentration in maternal plasma. At 48 hours after dosing, radioactivity concentrations in the fetal digestive tract and amniotic fluid were 4.1 and 3.8 times that at 24 hour, respectively. Concentrations of radioactivity in fetal membrane and fetal liver were 1.3 and 1.1 times that at 24 hours, respectively. The concentrations of radioactivity in other fetal tissues at this time were generally less those that observed at 24 hours. In contrast, at 48 hours radioactivity was below the level of detection in most of the maternal tissues (Table 2.6.1). Thus, on day 18 of pregnancy, the percentage distribution of radioactivity per fetus was 0% of the dose at 1 hour, 0.02% at 24 hours and 0.03% at 48 hours.

Transfer into Milk: After a single oral administration of ^{14}C-OM to lactating rats on the tenth day after delivery, the radioactivity concentration in the milk (as µg equivalents of OM) increased slowly and reached a maximum at 4 hr, decreased to 53% of the maximum at 8 hours after administration, and was below the detection limit at 24 hours. The radioactivity in the plasma reached a maximum at 30 min after oral administration and declined with a half-life of 8.2 hr (Fig. 2.6.1). The plasma $AUC_{0-\infty}$ was 4-fold higher than that of milk (Table 2.6.2).

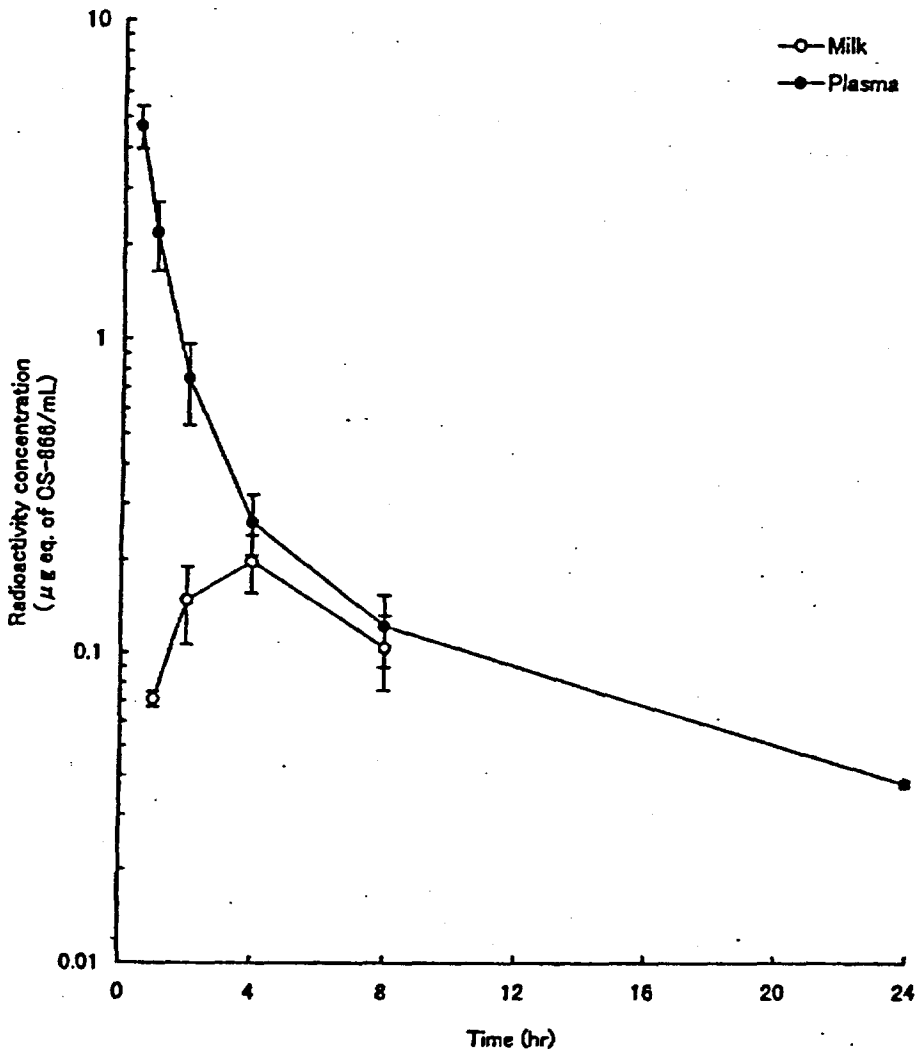


Fig. 2.6.1.: Radioactivity concentration in milk and plasma after a single oral administration of ^{14}C-OM to lactating rats. Data are expressed as the mean ± SEM of three animals.

TABLE 2.6.2.
RADIOACTIVITY CONCENTRATION IN MILK AND PLASMA AFTER A SINGLE ORAL ADMINISTRATION OF ^{14}C-OM TO NON-FASTING LACTATING RATS.

	Radioactivity concentration (μg eq. of CS-866/mL)	
Time	Milk	Plasma
30 min	N.D.	4.683 ± 0.714 (1.237)
1 hr	0.071 ± 0.004 (0.007)	2.174 ± 0.527 (0.914)
2	0.148 ± 0.042 (0.074)	0.749 ± 0.217 (0.377)
4	0.198 ± 0.042 (0.073)	0.264 ± 0.057 (0.099)
8	0.104 ± 0.028 (0.049)	0.122 ± 0.032 (0.056)
24	N.D.	0.038 ± 0.001 (0.002)
Detection limit	0.023	0.021
tmax	4 ± 0 (0) hr	30 ± 0 (0) min
Cmax (μg/mL)	0.198 ± 0.042 (0.073)	4.683 ± 0.714 (1.237)
t1/2 (hr) 4-24hr	—	8.2 ± 0.9 (1.6)
AUC (0-24hr)	—	7.41 ± 1.21 (2.09)
(μg eq.·hr·mL^{-1}) (0-∞)	1.78 ± 0.47 (0.81)	7.83 ± 1.16 (2.01)

Data are expressed as the mean ± S.E.(S.D.) of three animals.

N.D. : Not detected

— : Not determined

In summary, the study demonstrated that placental penetration of ^{14}C-OM was low during the period of organogenesis and that the transferred radioactivity was eliminated rapidly. But, when ^{14}C-OM was administered on day 18 of gestation, the placental penetration of radioactivity was higher and the elimination of the radioactivity transferred into fetus was slower than when administered on day 13 of gestation. Low concentrations of radioactivity transferred into milk at a rate slower than plasma and transferred out of milk at a rate faster than plasma.

2.7. Effects of Repeated Oral Administration of Olmesartan Medoxomil on Hepatic Drug-Metabolizing Enzymes in Rats (Report #GR 145-101). Vol. 6.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between March 1997 and June 1999.

Male Wistar —— rats were 7 weeks old and weighed 203 to 210 gm at the start of the study. Olmesartan medoxomil (batch #NH004C1) was given at doses of 1, 50 or 100 mg/kg/day (6/group) each day for 7 days (by stomach tube) as a suspension in 0.5% CMC. The control animals (6/group) received the vehicle. Animals were weighed daily. On day 8, the animals were exsanguinated by decapitation under diethyl ether anesthesia and the liver was quickly removed. Liver microsomes were prepared and analyzed for cytochrome P450 content and the activities of aniline hydroxylase, aminopyrine demethylase, 7-methoxycoumarin demethylase, 7-ethoxycoumarin deethylase, 7-propoxycoumarin depropylase, and olmesartan medoxomil – hydrolyzing enzyme (in order to determine the amount of olmesartan produced). The amounts of cytochrome P450 isozymes CYP1A1, CYP2B1, CYP2C6, CYP2C11, CYP2C13, CYP2E1, CYP3A2, and CYP4A1 were determined by Western blot analysis.

Results

Mean body weights of rats receiving 50 and 100 mg/kg/day were significantly decreased ($p < 0.05$) relative to the control group, beginning on day 4 of the study (5%) and continuing through termination of the study (12%). The decrease was dose-dependent but decrements did increase with the duration of treatment. Both absolute and relative liver weights were significantly decreased ($p < 0.05$) relative to control in these two groups (Table 2.7.1).

TABLE 2.7.1
BODY AND LIVER WEIGHTS BEFORE AND AFTER REPEATED ADMINISTRATION OF OLMESARTAN MEDOXOMIL (CS-866)

	Body weight		Liver weight	
	(Day 1)	(Day 8)	(g)	(% of Body weight)
Control	206.3 ± 1.6	259.8 ± 3.3	13.04 ± 0.35	5.02 ± 0.12
CS-866 1mg/kg	204.7 ± 1.2	250.2 ± 2.8	12.29 ± 0.25	4.91 ± 0.09
CS-866 50mg/kg	208.2 ± 1.7	232.3 ± 2.5*	9.56 ± 0.43*	4.11 ± 0.15*
CS-866 100mg/kg	207.7 ± 2.4	228.7 ± 4.8*	9.95 ± 0.65*	4.33 ± 0.21*

*: $p<0.05$ (Mean±S.E, n=6.)

Cytochrome P450 content was significantly decreased ($p < 0.05$) in all treated groups with values of 0.54, 0.45, 0.44, and 0.39 nmol/mg protein for the control, 1, 50, and 100 mg/kg/day groups. The activity of aniline hydroxylase was significantly increased ($p < 0.05$) in the 50 and 100 mg/kg/day groups; the activity of the other hepatic drug-metabolizing enzymes was unaffected. The olmesartan medoxomil -hydrolyzing enzyme activities were 20.31 to 20.79 nmol/min/mg protein in the OM-treated groups and were approximately 1.4-fold higher than the value of 15.16 nmol/min/mg protein in the control group; the differences, however, were not statistically significant. The Western blot analysis showed no marked differences between the OM-treated groups and the control group in the amount of each enzyme isoform, demonstrating no enzyme induction. The decrease in total cytochrome P450, according to the sponsor, may be due to a decrease in unknown isoforms and may be related to the decrease in body weight gain. The study concludes that repeated oral administration of OM would have only very minor effects on hepatic drug-metabolizing enzymes.

2.8. *In Vitro* Hydrolysis of Olmesartan Medoxomil and RNH-8097 in Liver and Intestine of Rat (Report #GR 146-072). Vol. 6.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between December 22, 1998 and September 9, 1999.

Male Wistar- [redacted] rats were 7 weeks old and weighed 185.5-203.7 gm. The six males were fasted from the evening prior to the day of experiment. The liver and small intestine were removed, washed, homogenized, and 9,000g supernatant (S9) fractions were prepared from individual animals. Two hydrolysis experiments (OM and RNH-8097) were conducted and for each, three rats were used.

OM (lot No. NH208C) was dissolved in DMSO and added to the S9 fraction of the liver and small intestine at a final concentration of 0.5 mM to investigate the production of olmesartan and RNH-8097. Aliquots of the reaction mixture were collected at 0, 5, 15, 30, and 60 minutes after the addition of OM, and acetonitrile was added to stop the reaction. The mixture was centrifuged and the supernatant fraction was used as the sample for measurement of olmesartan by [redacted]. The remaining reaction mixture was used for determination of RNH-8097 (the ester side chain of OM) by [redacted] after ethyl acetate extraction.

The hydrolysis of RNH-8097 was also determined in S9 fractions of the liver and small intestine. RNH-8097 (lot No. A20-31-165) was dissolved in distilled water and added to the S9 fractions at a final concentration of 0.5 mM. At 0, 5, 15, 30, and 60 minutes after starting the reaction, ethyl acetate was added to each tube and the RNH-8097 was extracted. The disappearance of RNH-8097 in the reaction mixture was determined by [redacted]

Results

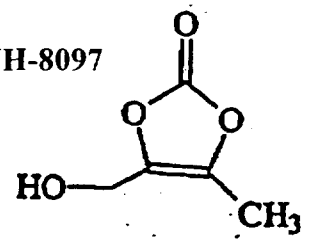
In the hepatic S9 fraction, OM was hydrolyzed to olmesartan almost completely within 60 minutes of starting the reaction; the production of olmesartan was approximately linear up to 15 minutes. In the small intestinal S9 fraction, production of olmesartan was almost linear with time until 60 min. The concentration of olmesartan in intestine fraction was approximately 7-fold lower than that observed in the hepatic S9 fraction (Table 2.8.1). Production of RNH-8097 was not observed in either liver or small intestine S9 fractions after addition of OM for up to 60 min.

TABLE 2.8.1
HYDROLYSIS OF OM IN S9 FRACTIONS OF RAT LIVER AND SMALL INTESTINE (MEAN VALUES)

	Production of olmesartan (nmol/mg protein)	
Incubation Time (min)	Small Intestine	Liver
0	2.16	5.74
5	14.60	95.77
15	34.83	226.12
30	60.42	296.59
60	84.80	315.44

Addition of RNH-8097 to the hepatic S9 fraction resulted in its decomposition, almost linearly with the incubation time, and the activity at 60 minutes was 18.14 nmol/mg protein. This activity was 1/63 of the activity for hydrolysis of OM in the liver, calculated by extrapolating the initial activity (5 minutes) to 60 minutes. In contrast, in the small intestinal S9 fraction, almost no decomposition of the unchanged RNH-8097 was observed.

RNH-8097



The results thus demonstrated that OM is rapidly hydrolyzed to olmesartan in hepatic and small intestinal S9 fractions, with the activity in the hepatic S9 fraction approximately 7-fold higher than that observed in the small intestinal fraction. RNH-8097 is not produced as the metabolic intermediate during hydrolysis of OM. OM possesses 3 ester bonds which can be hydrolyzed. One ester bond is between the carboxyl group of olmesartan and the hydroxyl group of RNH-8097. The other 2 ester bonds are on the diester structure of carbonic acid present in RNH-8097. Hydrolysis of the ester bond between olmesartan and RNH-8097 appears unlikely, as no RNH-8097 was detected in the incubation mixtures after addition of OM, and the hydrolysis of either of the diester bonds in RNH-8097 is quite likely the initial metabolic reaction. Based on this view, the metabolism of OM was predicted to proceed *via* the metabolic intermediate to olmesartan and diacetyl (Fig. 2.8.1).

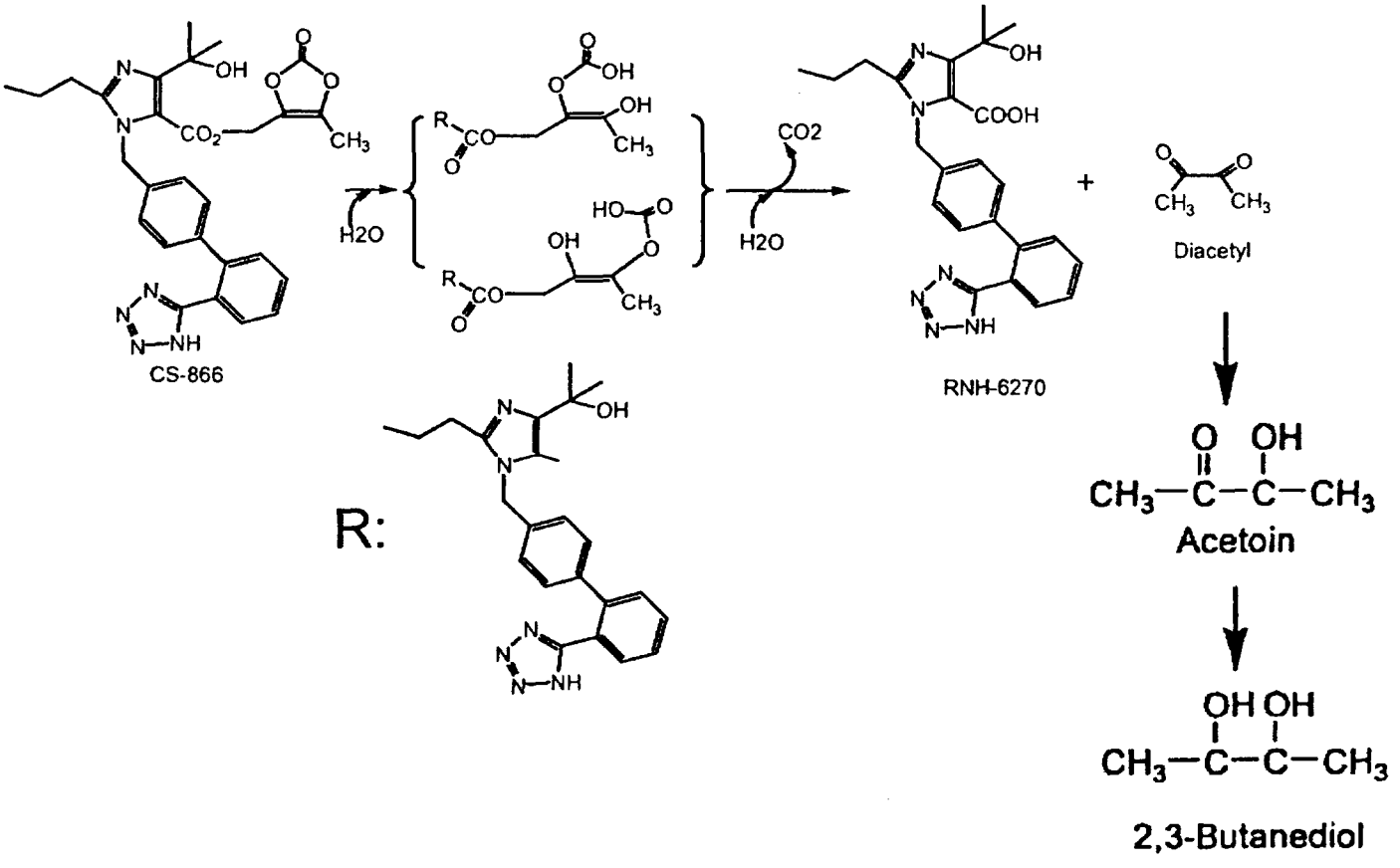


Fig. 2.8.1.: Proposed mechanism for the hydrolysis of olmesartan medoxomil

2.9. *In Vitro* Hydrolysis of Olmesartan Medoxomil in Plasma of Various Species (Report #GR 143-098). Vol. 5.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between March, 1992 and December 18, 1996. The objective of the study was to investigate the ability of plasma esterases to hydrolyze olmesartan medoxomil (OM) in various species and to characterize the OM esterase from human plasma.

Previously collected and stored plasma samples of rats, mice, rabbits, monkeys and dogs were used in the study. Human blood samples were collected from 3 healthy volunteers. Plasma samples were incubated with OM (lot #4) for 5 to 10 minutes at a concentration of 250 μM. The reaction was stopped by the addition of acetonitrile. The mixture was centrifuged and the supernatant fraction was used as the sample for measurement of olmesartan by [redacted]. In addition, plasma esterase from human samples was purified to characterize the esterase catalyzing the hydrolysis of OM.

Results

Rabbit and dog plasma showed esterase activity higher than that of human plasma. The rat plasma showed very low activity, only about 9% of human activity (Table 2.9.1).

TABLE 2.9.1
OLMESARTAN MEDOXOMIL HYDROLYZING ACTIVITY OF PLASMA

Species	Activity (nmol/min/mg)
Rabbit	41.58
Dog	26.11
Human	[redacted]
Mouse	6.30
Monkey	1.91
Rat	0.97

The purified esterase from human plasma showed a single protein band and the molecular weight was estimated to be 48,500. The N-terminal amino acid sequence (up to 20 amino acids) completely agreed with that of arylesterase. OM-hydrolyzing activity was not inhibited by diisopropylfluorophosphate (DFP), which is a potent inhibitor of various esterases other than arylesterase, or by eserine. N-ethylmaleimide (NEM) inhibited OM-hydrolyzing activity by 11.54% at 0.1 mM and by 13.69% at 0.5 mM. Antibodies raised against plasma OM esterase inhibited the activity of the purified esterase by 95.1%. The activity of the purified enzyme for hydrolysis of OM was determined at concentrations of OM ranging from [redacted] μM. The calculated Km (concentration at which the reaction develops half the maximum velocity) and Vmax (maximum velocity) values of OM for the purified esterase were 0.237 mM and 3.26 nmol/min/mg, respectively. The results of this study indicated that the esterase that catalyzes the hydrolysis of OM in human plasma was arylesterase.

In another study (Report #GR 143-036), the sponsor had demonstrated that human serum albumin (HSA) is partially responsible for the hydrolysis of OM in human plasma. Separation of OM hydrolyzing enzymes from human plasma ([redacted] revealed two peaks of activity, the first peak containing about 40% of the total activity and the second containing 60%. The first peak was identified as albumin and the second peak corresponded to proteins other than albumin. Incubation with anti-albumin antibody completely inhibited the hydrolysis of OM by purified albumin but only inhibited approximately 40% of the activity of plasma. DFP produced a 74% inhibition of hydrolysis of OM, while NEM and eserine had no effect. However, esterase(s) other than albumin are also involved in the hydrolysis of OM in plasma, with the ratio of the hydrolyzing activity of albumin to that of the other esterase(s) approximately 4:6.

2.10. Urinary, Fecal and Biliary Excretion of Radioactivity and Metabolites After Oral and I.V. Administration of ^{14}C-Olmesartan Medoxomil to Rats (Report #RAM 140-006). Vol. 6.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between October 1992 and April 1993. The study investigated the metabolites of OM in urine, feces and bile after oral and intravenous administration of 5 mg/kg ^{14}C-Olmesartan medoxomil to male rats.

The male Wistar- [redacted] rats used to determine urinary and fecal metabolites (4/route) were 8 weeks of age and weighed 238 to 244 gm and the animals used to determine biliary metabolites (3/route) were 8 to 10 weeks of age and weighed 235.6 to 305.5 gm. [^{14}C]OM (lot No. D-921006) was dissolved in dimethylacetamide by warming and then diluted with polyethylene glycol 400 and physiological saline to a concentration of 5 mg/ml. This dosing solution was administered once to the animal intravenously (duration of administration not given) *via* tail vein or orally *via* stomach tube, at a dose of 5 mg/kg.

For determination of urinary and fecal excretion, rats were housed individually in metabolic cages. Urine and feces were collected at 24-hour intervals up to 72 hours after administration; urine samples for the 48- and 72-hour time points were combined with the cage wash. Radioactivity was determined by [redacted] and metabolites in urine and fecal samples were determined by [redacted] For determination of biliary excretion, bile duct cannulated animals were housed individually and bile samples were collected at intervals of 0 to 3, 3 to 6 and 6 to 24 hour after administration. To determine the ratio of re-absorption, 1 ml of the 0 to 3 hour original bile sample collected after IV administration was re-administered orally to another four bile duct-cannulated male rats. Bile samples were then collected at the same intervals as above. Radioactivity in bile was determined by [redacted] and the metabolites in bile were determined by [redacted]

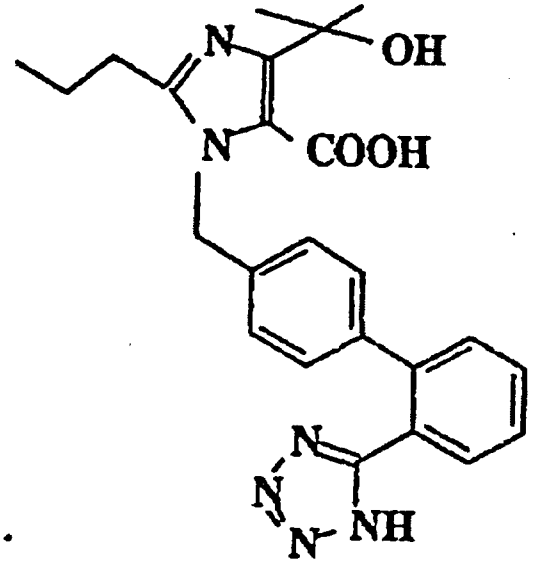
Results

The main metabolite observed in the urine, feces, and bile was the active metabolite, olmesartan. It accounted for 74 to 91% of the total radioactivity for all routes of excretion and administration studied (Table 2.10.1). Thus, OM was considered to be hydrolyzed to olmesartan immediately after administration and excreted very rapidly. After oral but not IV administration, bile contained substantial amounts of olmesartan glucuronide. Therefore, the conjugation reaction was considered to occur in the intestinal tract, leading to the biliary excretion of this metabolite only after oral administration. After IV administration, the unchanged compound was not observed in the feces, and almost all the radioactivity was detected as olmesartan. Since the absorption ratio of OM was calculated to be approximately 50%, unabsorbed OM was considered to be hydrolyzed during the passage through the GI tract, and excreted in the feces.

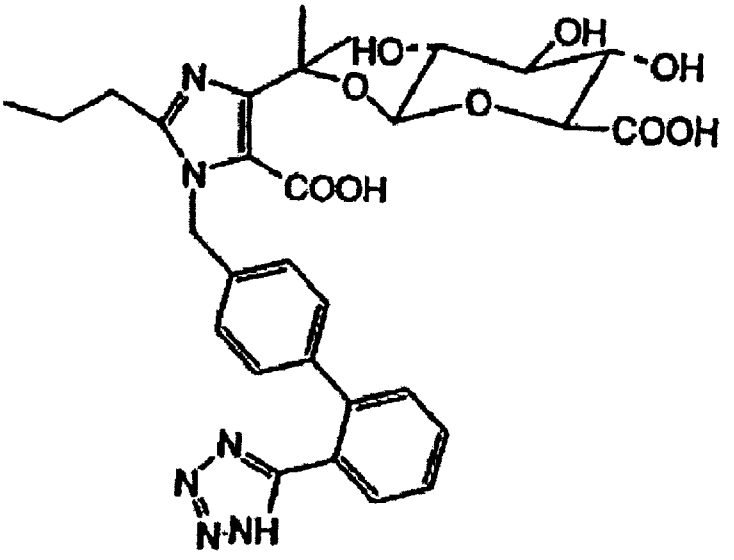
TABLE 2.10.1
METABOLITES OF OLMESARTAN MEDOXOMIL IN URINE, FECES, AND BILE OF RATS AFTER ADMINISTRATION OF 5 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL (MEAN VALUES)

	% Radioactivity[a]					
	Urine		Feces		Bile	
Compound	Oral	IV	Oral	IV	Oral	IV
OM	1.99	6.21	3.20	0.54	1.10	1.19
Olmesartan	82.90	75.73	89.23	91.36	74.11	89.66
Olmesartan glucuronide	10.70	6.17	0.38	0.45	20.54	0.29

[a] Percent of radioactivity observed in urine, feces, or bile



Olmesartan



Glucuronide conjugate of olmesartan (only in rat)

Most of the radioactivity administered was excreted during first 24 hours after administration and the main route of excretion was the fecal pathway. There were no differences in the ratios of the urinary and fecal excretion between IV and oral administration (Table 2.10.2).

Regarding the biliary excretion of radioactivity, most of it occurred during the first 3 hours after IV or oral administration. Excretion during the 0-3 hr and 0-24 hr periods was lower after oral administration than that after IV administration. In a separate experiment, the bile sample from the IV dosed rats was re-administered orally to a separate group of rats. During the 24 hrs re-administration, only 20.87% of the re-administered radioactivity was recovered in the bile (Table 2.10.3).

TABLE 2.10.2
EXCRETION OF RADIOACTIVITY IN URINE AND FECES AFTER ADMINISTRATION OF 5 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL TO MALE RATS.
MEAN VALUES FOR EXCRETION OF RADIOACTIVITY (% OF DOSE)

	Oral		IV	
Time Period	**Urine**	**Feces**	**Urine**	**Feces**
0-24 hr	1.40	93.64	1.81	93.15
24-48 hr	0.08	2.16	0.07	2.09
48-72 hr	0.04	0.19	0.07	0.20
0-72 hr	1.52	95.98	1.95	95.44
Total	97.50		97.39	

The results of this study demonstrated that the main route of excretion of radioactivity after administration of ^{14}C-olmesartan medoxomil was the fecal pathway via biliary excretion. Since most of the radioactivity was found to be excreted within 24 hours after administration, a rapid excretion was indicated. Biliary excretion after IV administration was approximately 94% and after oral administration was approximately 50%; the absorption ratio (49.89/94.07) was calculated to be 53.03%. A similar estimation of the absorption ratio based on the urinary excretion is theoretically not possible because of low values. The unabsorbed OM was considered to be hydrolyzed during the passage through the GI tract and excreted in the feces.

TABLE 2.10.3
EXCRETION OF RADIOACTIVITY IN BILE AFTER ADMINISTRATION OF 5 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL TO MALE RATS
MEAN VALUES FOR EXCRETION OF RADIOACTIVITY (% OF DOSE)

Time Period	**Oral**	**IV**	**Re-administered Bile**
0-3 hr	27.89	82.98	10.98
3-6 hr	10.39	7.22	4.57
6-24 hr	11.61	3.87	5.33
0-24 hr	49.89	94.07	20.87

After oral re-administration of the bile sample, about 21% of the administered radioactivity was re-excreted in the bile; the re-absorption ratio (20.87/94.07) was calculated to be 22.19%. Since the radioactivity recovered in the bile was almost exclusively olmesartan, the re-absorption ratio based on the biliary re-excretion data indicates that the absorption of olmesartan is lower than that (40%) of parent compound. Similar observations on the poor absorption of olmesartan compared to OM have been reported for other studies (see sections 2.1 and 2.11).

2.11. Pharmacokinetics and Urinary Excretion After Oral Administration of Olmesartan Medoxomil to Dogs (Report #PD5 139-022). Vol. 6.

This non-GLP study was conducted by Biopharmaceutical Research Laboratory, Product Development Labs, Sankyo Co., Ltd., Fukuroi, Shizuoka 437, Japan, between January 1992 and December 1992. The objective of the study was to determine the pharmacokinetics and bioavailability of olmesartan after oral dosing with olmesartan medoxomil (OM) and olmesartan in dogs.

Male beagle dogs (12 to 15 months old and weighing between 9.3 and 12.4 kg) were fasted for approximately 18 hr prior to dosing. OM (lot #5) at doses of 12.5 (n=3), 25 (n=6), or 50 mg/dog (n=3) and olmesartan (lot #5) at a dose of 20 mg/dog (n=6) were administered orally by stomach tube. In a crossover design, pentagastrin-pretreated dogs (same animals one week later; details of treatment with pentagastrin not given) received 25 or 20 mg (n=5 each) OM or olmesartan, respectively. Doses were not adjusted for body weight. Pentagastrin-pretreated dogs were studied because gastric pH may play an important role in the dissolution and absorption of OM. Pentagastrin is a potent physiological secretagogue that is released from the pyloric antrum by vagal stimuli. It stimulates the secretion of gastric acid, pepsin, and intrinsic factor. Both OM and olmesartan were solubilized in dimethylacetamide. Blood samples for analysis of plasma concentrations of olmesartan were taken at 0.083, 0.25, 0.5, 0.75, 1, 2, 4 and 6 hours after dosing. Urine was collected 0-24 hr after dosing.

Results

After oral administration of OM, plasma concentrations of olmesartan reached peak level at 30, 20 and 15 min in normal dogs receiving 12.5, 25 and 50 mg, respectively. The corresponding $AUC_{0-\infty}$ values were 0.19, 0.40, and 0.94 μg.hr/ml, demonstrating a linear relationship with dose. $T_{1/2}$ values at all doses were almost similar (0.76 to 0.88 hr) (Table 2.11.1). Thus, the data suggest that the absorption of OM after oral administration in normal dogs is almost constant within the range of these doses.

Pharmacokinetic parameters (determined from plasma concentrations of olmesartan for OM at a dose of 25 mg and olmesartan at a dose of 20 mg) in normal and pentagastrin-treated dogs were compared. In normal dogs, after administration of OM, the mean plasma concentration reached a peak level of 0.33 μg/ml at 30 min and then declined to 0.05 μg/ml at 2 hr. With olmesartan, the mean plasma concentration reached a peak level of 0.08 μg/ml at 30 min and then declined to 0.04 μg/ml at 2 hr. Half lives were similar (0.88 and 0.89). A 2-fold difference in the mean AUC value was noted between the prodrug and its metabolite. Absolute bioavailability was calculated by comparing the oral AUC values (0.40 and 0.19 with OM and olmesartan, respectively) with the AUC values determined after IV administration in another study (report # PD5 139-006). In that study, IV administration of 25 mg OM and 20 mg olmesartan in male beagle dogs gave similar AUC values (2.74 and 2.83 μg·h/ml, respectively). The bioavailability for normal dogs was 14% for OM and 6.7% for olmesartan. This demonstrates the improved oral absorption of the pro-drug over the metabolite. Similar observations were made in the rat (see section 2.1).

TABLE 2.11.1
PHARMACOKINETIC PARAMETERS OF OLMESARTAN IN NORMAL AND PENTAGASTRIN-PRETREATED DOGS GIVEN A SINGLE ORAL DOSE OF OLMESARTAN MEDOXOMIL OR OLMESARTAN BY SOLUTION (MEAN VALUES)

Parameter	Normal				Pentagastrin	
	Olmesartan Medoxomil			Olmesartan	OM	Olmesartan
	12.5mg	25 mg	50 mg	20 mg	25 mg	20 mg
Tmax (hr)	0.50	0.33	0.25	0.71	0.45	1.35
Cmax (μg/ml)	0.17	0.33	1.36	0.09	0.58	0.06
Vd/F (L/body)	72.00	70.00	59.00	247.00	42.00	387.00
T½ (hr)	0.76	0.88	0.78	0.89	0.70	0.80
Cltot/F (L/hr/body)	84.00	59.00	47.00	121.00	43.00	210.00
$MRT_{0-\infty}$ (hr)	1.11	1.32	1.14	2.37	0.98	2.11
$AUC_{0-\infty}$ (μg·hr/ml)	0.19	0.40	0.94	0.19	0.50	0.15
BA		14		6.7	17.7	5.3

Absorption of OM (25 mg/kg) and olmesartan (20 mg/kg) from solutions after administration to normal dogs was compared with absorption in pentagastrin-treated dogs. A slight increase in C_{max} and AUC with decreases in volume of distribution and total clearance from the body were observed in pentagastrin-treated dogs in comparison to normal dogs receiving OM. On the other hand, an opposite effect was observed with olmesartan (Table 2.11.1). The bioavailability for pentagastrin-pretreated dogs was 17.7% for OM and 5.3% for olmesartan. The results suggest that absorption of OM (as measured by AUC for olmesartan) was approximately 2 times higher in normal dogs and approximately 3 times higher in pentagastrin-pretreated dogs than it was for olmesartan. Thus it is concluded that the variation of gastric pH (pentagastrin lowers pH from 5-6 to 1-2) may affect the absorption of OM but not olmesartan.

The sponsor conducted a 2nd study (report #PD5 139-036) in normal and pentagastrin-treated male beagle dogs receiving OM (25 mg/dog) and olmesartan (20 mg/dog) orally by capsule (n=6/group). In this study, male dogs were 13-31 months old and weighed 10.3 to 12.0 kg and were fasted for 18 hr prior to dosing. As in the previous study, a crossover design was employed. Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 4, 6 and 8 hours after dosing. Absolute bioavailability was calculated by comparing the oral AUC values with the AUC value of 2.83 μg·hr/ml obtained after IV administration of olmesartan at a dose of 20 mg (see above). The bioavailability for normal dogs was 11.7% for OM and 5.7% for olmesartan suggesting (a) two times higher BA for OM than olmesartan and (b) better oral absorption with solution (14.1% and 6.7%, respectively) than capsule. The bioavailability in pentagastrin-pretreated dogs was 22.6% for OM and 8.1% for olmesartan (Table 2.11.2). These values are higher than those observed with solution administration (17.7% and 5.3%, respectively).

TABLE 2.11.2
PHARMACOKINETIC PARAMETERS OF OLMESARTAN MEDOXOMIL IN NORMAL AND PENTAGASTRIN-PRETREATED DOGS GIVEN A SINGLE ORAL DOSE OF OLMESARTAN MEDOXOMIL OR OLMESARTAN BY CAPSULE (MEAN VALUES)

Parameter	Normal		Pentagastrin	
	Olmesartan Medoxomil	Olmesartan	OM	Olmesartan
	25mg	**20 mg**	**25 mg**	**20 mg**
Tmax (hr)	1.50	1.58	1.67	2.20
Cmax (µg/ml)	0.14	0.06	0.24	0.07
Vd/F (L/body)	200.00	430.00	97.00	347.00
T½ (hr)	1.52	1.74	1.56	1.83
Cltot/F (L/hr/body)	66.00	127.00	32.00	95.00
$MRT_{0-\infty}$ (hr)	2.98	3.23	3.03	3.70
$AUC_{0-\infty}$ (µg·hr/ml)	0.33	0.16	0.64	0.23
BA	11.7	5.7	22.6	8.1

Additionally, urinary excretion of olmesartan was determined in both normal and pentagastrin-treated dogs receiving OM or olmesartan orally by solution and by capsule. Cumulative 24 hr urinary recoveries of olmesartan were comparable among groups (Table 2.11.3).

TABLE 2.11.3
URINARY EXCRETION (0-24 hr, % OF DOSE) OF OLMESARTAN IN NORMAL AND PENTAGASTRIN-PRETREATED DOGS GIVEN SINGLE ORAL DOSE OF OLMESARTAN MEDOXOMIL OR OLMESARTAN BY SOLUTION OR CAPSULE (MEAN VALUES)

Orally by Solution (Report #PD5 139-022)						Orally by capsule (Report #PD5 139-036)			
Normal				Pentagastrin		Normal		Pentagastrin	
Olmesartan Medoxomil			Olm	OM	Olm	OM	Olm	OM	Olm
12.5mg	**25 mg**	**50 mg**	**20 mg**	**25 mg**	**20 mg**	**25 mg**	**20 mg**	**25 mg**	**20 mg**
1.8	2.2	1.5	1.2	2.1	1.0	1.5	1.0	3.1	1.2

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2.12. Pharmacokinetics, Protein Binding, Metabolism and Excretion After Single Oral Administration of ^{14}C-Olmesartan Medoxomil to Dogs (Report #RAM 140-040). Vol. 6.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between February 1993 and June 1993.

Three male beagle dogs with a mean body weight of 11.5 kg were fasted from the evening prior to dosing and were fed 6 hr after drug administration. The dosage was 5 mg/kg ^{14}C-olmesartan medoxomil (lot No. D-930123) administered once orally by a stomach catheter. Dosing solutions were prepared in dimethylacetamide with addition of PEG400. Blood samples for analysis of plasma concentrations of olmesartan were taken at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours after dosing. Protein binding was determined from plasma samples collected at 0.25, 1 and 2 hours after dosing using [redacted] Dogs were housed individually in metabolic cages and urine and feces were collected at 24 hr intervals up to 8 days (192 hr) after dosing; after the first sampling urine samples were combined with the cage wash. Radioactivity in whole blood, plasma, urine and feces was measured by [redacted] Metabolites in urine and feces were determined by [redacted]

Results

The plasma concentration of radioactivity reached a maximum level (2.3 µg equiv/ml) at 0.5 hours after oral administration and decreased mono-exponentially, declining to 0.1 µg equiv/ml at 24 hours after administration. The half-life and AUC $_{0\text{-}24}$ were 1 hour and 3.53 µg equiv·hr/ml, respectively. The concentration of radioactivity in whole blood followed a similar time course. The ratio of the radioactivity in the blood cells to plasma was very low at the early time period (0.4% at 15 minutes) but gradually increased over time, reaching 21.5% to 54.3% during the period from 2 hr up to 24 hours after administration. The values at later time points were considered to be less reliable due to the low level of radioactivity at these time points. Olmesartan was the main component of the plasma radioactivity (95.89% at 15 minutes) and, as such, the time course of plasma concentration of olmesartan was similar to that of plasma radioactivity. Therefore, the distribution of the radioactivity to the blood cells was concluded to be low, and most of the radioactivity in the blood was considered to be present in the plasma.

TABLE 2.12.1
PLASMA RADIOACTIVITY AFTER ORAL ADMINISTRATION OF ^{14}C-OLMESARTAN MEDOXOMIL TO DOGS

Parameter	Mean ± SE
Tmax (hr)	0.50 ± 0.00
Cmax (µg/ml)	2.33 ± 0.16
T½ (hr)	1.00 ± 0.21
$AUC_{0\text{-}24}$ (µg·hr/ml)	3.53 ± 0.64

Plasma protein binding ratios were 96.6%, 96.6%, and 96.4% at 0.5, 1 and 2 hr after 5 mg/kg ^{14}C-olmesartan medoxomil administration, respectively.

The [redacted] of urine and feces showed no unchanged compound. The active metabolite, olmesartan, was detected as the sole radioactive spot, comprising 96.77 and 99.80% of the urinary and fecal metabolites, respectively. Metabolites other than olmesartan were not detected. The metabolic profile of the plasma was similar to those of the urine and feces. At 15 min after oral administration, olmesartan comprised 95.89% of the plasma radioactivity (Fig. 2.12.1), and a very low amount (1.23 to 5.84%) of the unchanged compound was detected at several time intervals (2, 4, 6, 8 and 24 hr after administration). At each data point, the metabolic profile was the same as that observed at 15 min after administration. Since olmesartan is the main component in the plasma radioactivity, the time course of the plasma concentration of olmesartan was quite similar to that of the plasma concentration of the total radioactivity. Thus, OM was considered to be hydrolyzed to olmesartan immediately after administration, and excreted very rapidly.

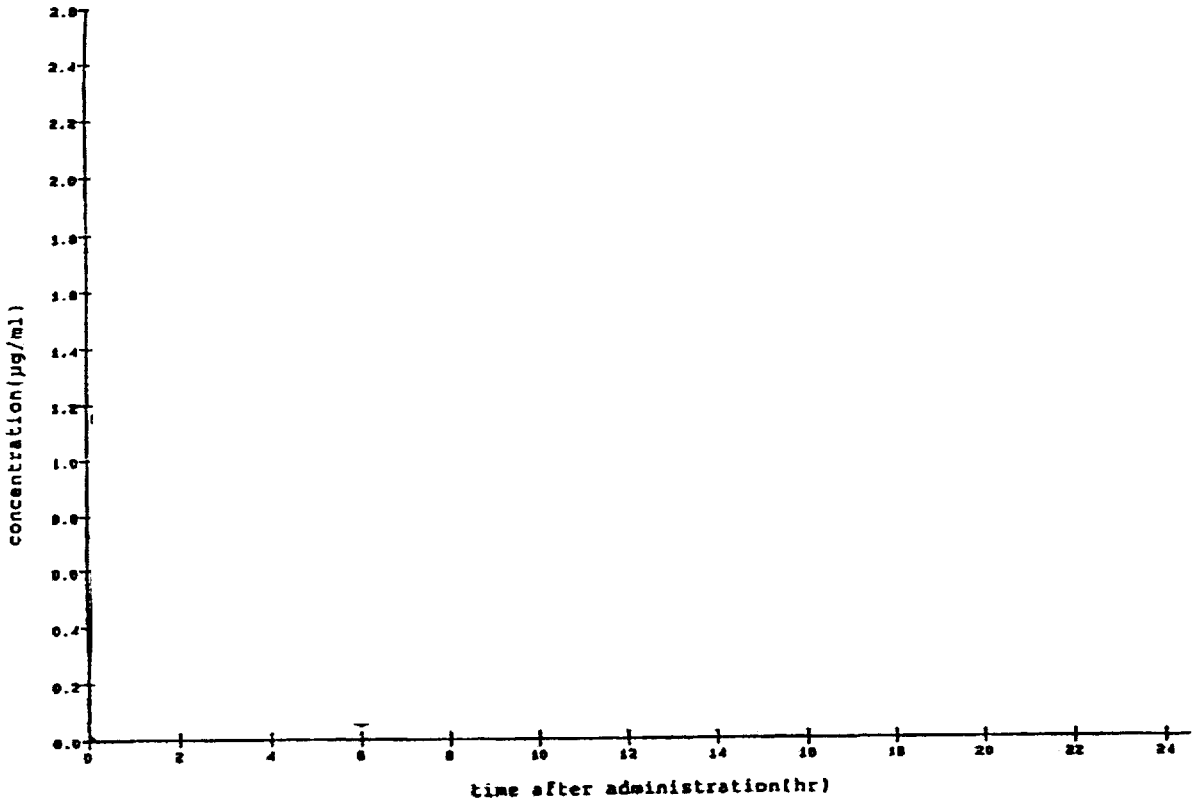

Fig. 2.12.1.: Plasma concentration of ^{14}C-olmesartan following single oral administration of 5 mg/kg ^{14}C-olmesartan medoxomil to male dogs

Radioactivity was excreted primarily in the feces, with the majority of the administered dose excreted within the first 24 hours. During the first 8 days, 8.7% and 83.4% of the radioactivity was excreted in the urine and feces, respectively (total of 92.1%, Table 2.12.2). Olmesartan medoxomil was not observed in either urine or feces, with the total amount of radioactivity

accounted for by olmesartan. The results demonstrated that the main route of excretion of OM in dogs is the fecal pathway.

TABLE 2.12.2
EXCRETION OF RADIOACTIVITY (% OF DOSE, MEAN VALUES) IN URINE AND FECES AFTER ORAL ADMINISTRATION OF 5 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL TO BEAGLE DOGS

Time Period	Urine	Feces	Total
0-24 hr	6.36	49.83	56.19
24-48 hr	1.89	29.80	31.69
48-72 hr	0.20	2.78	2.98
72-96 hr	0.14	0.75	0.89
96-144 hr	0.05	0.22	0.27
144-192 hr	0.01	0.06	0.08
Total	8.66	83.44	92.11

In a separate study, three male beagle dogs received a single IV administration of 1 mg/kg [^{14}C]olmesartan medoxomil (Report #GR 142-029). Urine and feces were collected at 24-hour intervals up to 8 days after dosing; after the first sampling, urine samples were combined with the cage wash. Radioactivity in urine and feces was determined by [redacted]. This study, like the oral study, demonstrated that radioactivity was excreted primarily in the feces, with the majority of the administered dose excreted within the first 24 hours (Table 2.12.3). Olmesartan medoxomil was not observed in either urine or feces, with the total amount of radioactivity accounted for by olmesartan.

TABLE 2.12.3
CUMULATIVE EXCRETION OF RADIOACTIVITY (% OF DOSE, MEAN VALUES) IN URINE AND FECES AFTER IV ADMINISTRATION OF 1 MG/KG ^{14}C-OLMESARTAN MEDOXOMIL TO BEAGLE DOGS

Time Period	Urine	Feces	Total
0-24 hr	19.07	69.81	88.88
0-48 hr	20.07	76.75	96.82
0-72 hr	20.14	77.85	97.99
0-120 hr	20.20	78.10	98.30

2.13. *In Vitro* Binding of Olmesartan to Mouse, Rat, Dog and Human Serum Proteins (Report #RAM 140-053). Vol. 6.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between May 1993 and August 1993.

^{14}C-olmesartan was prepared by incubating ^{14}C-olmesartan medoxomil with dog plasma for 30 minutes at 37°C. The mixture was centrifuged and the resulting supernatant was concentrated and spotted on [redacted] plates. The radioactive spot of olmesartan was confirmed, scraped from the plate, extracted, and purified using [redacted]

Individual serum samples were prepared from pooled blood samples obtained from male ddY mice, male Wistar [redacted] rats, male beagle dogs, and from healthy human volunteers (two males and one female). ^{14}C-olmesartan was incubated with serum from mice, rats, dogs, and humans at 37°C for 5 minutes at final concentrations of 1 to 100 μg/ml. In addition, the binding of ^{14}C-olmesartan to human serum albumin (HSA, 10 mg/ml), human serum α_1 acid glycoprotein (HAAG, 10 mg/ml) and human serum globulin (10 mg/ml) was determined after incubation at 37°C for 5 minutes at a final concentration of 1 μg/ml. After the incubation, ultrafiltration was performed and the radioactivity in the filtrate was measured to determine free drug concentration. The binding ratio was calculated as percent of total radioactivity.

In other experiments, the binding constant (K) and number of binding sites (n) were determined for HSA and HAAG using concentrations of ^{14}C-olmesartan ranging from 40 to 300 μM under the above conditions. The binding site on HSA was determined by using competitive inhibitors, diazepam, digitoxin or warfarin where each has a characteristic binding site on HSA. This was accomplished by incubating HSA (6.4 mg/ml) and ^{14}C-olmesartan (1 and 100 μM) in the presence of 100 μM diazepam, digitoxin or warfarin at 37°C for 5 minutes. Fluorescent probes were used to determine the specific binding sites (site I or site II) on HSA.

Results

The mean extent of binding to serum proteins in all species appeared to be independent of the concentration used. The binding percentage of the drug remained fairly high and constant for all species examined (94 to 99.3%) (Table 2.13.1). The free fraction of olmesartan in mouse and dog plasma was higher than the free fraction of olmesartan in human and rat plasma. In human serum, the protein binding ratios of olmesartan to the HSA and HAAG were found to be high (96 to 99.4), while globulin showed a binding ratio of only 13.3%.

The binding ratios of various concentrations of ^{14}C-olmesartan (40 to 300 μM) to HSA solution at the constant concentration of 100 μM was plotted by the Scatchard plot method. The plot showed biphasic binding sites with parameters for the first binding site of $K_1 = 1.6 \times 10^{-7}$M and $n_1 = 0.84$ and for the second binding site: $K_2 = 4 \times 10^{-5}$M and $n_2 = 2.0$. On the other hand, the binding to α_1 acid glycoprotein was monophasic with binding parameters of $K_1 = 1.3 \times 10^{-5}$ M and $n_1 = 0.4$.

TABLE 2.13.1
PERCENT PROTEIN BINDING OF ^{14}C-OLMESARTAN IN VARIOUS SPECIES, AND PERCENT BINDING TO PURIFIED HUMAN SERUM PROTEINS

Olmesartan Conc.	Mouse	Rat	Dog	Human	HSA	HAAG	Globulin
1 µg/ml	95.4	98.6	95.7	98.8	99.4	96.0	13.3
10 µg/ml	96.6	99.0	95.5	99.3	---[a]	---	-
100 µg/ml	94.4	98.3	94.0	99.1	---	---	---

[a] Not determined

There was no inhibition of binding of ^{14}C-olmesartan to HSA by diazepam and digitoxin. In contrast, warfarin inhibited binding of ^{14}C-olmesartan to HSA by 3.8% and 8% at olmesartan concentrations of 1 and 100 µM, respectively. Additionally, olmesartan decreased the fluorescence intensity of bound dansyl-L-arginine (the probe for the warfarin site) by approximately 20% and had no effect on the fluorescence intensity of bound dansyl-sarcosine (the probe for the diazepam site) demonstrating that olmesartan has affinity for the warfarin site on albumin.

In summary, this study demonstrated that olmesartan is highly bound to serum proteins of mice, rats, dogs and humans. Determination of the binding constants to HSA and α_1 acid glycoprotein demonstrated that olmesartan was mainly bound to albumin and fluorescence probe studies indicated that olmesartan was bound to the warfarin site on albumin.

2.14. Inhibitory Effects of Olmesartan on Drug-Metabolizing Enzyme Activities in Human Liver Microsomes (Report #GR 144-063). Vol. 6.

This non-GLP study was conducted by Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan, between June 1997 and February 1999. The objective of the study was to investigate the inhibitory effects of olmesartan on the drug-metabolizing activities of 7 different molecular isoforms of cytochrome P450.

A pooled human liver microsome fraction was prepared by combining the liver microsomes from 10 male subjects. The fraction was incubated with olmesartan (lot #4) at concentrations ranging from 1 to 500 μM, using substrates specific to the isoforms of cytochrome P450 (Table 2.14.1). Effects of typical inhibitors of each P450 isoform were also investigated as a positive control.

TABLE 2.14.1
EXPERIMENTAL DESIGN

P450 isoform	Enzyme	Substrate and Conc., μM		Protein conc., mg/ml
CYP1A1 and 2	7-ethoxyresorufin deethylase	7-ethoxyresorufin	10	0.5
CYP2A6	Coumarin 7-hydroxylase	Coumarin	50	0.2
CYP2C19	Mephenytoin hydroxylase	S-mephenytoin	400	1.0
CYP2C8 and 9	Tolbutamide hydroxylase	Tolbutamide	1000	0.5
CYP2D6	Bufuralol hydroxylase	Bufuralol	10	1.0
CYP2E1	Chlorzoxazone hydroxylase	Chlorzoxazone	400	0.5
CYP3A4	Nifedipine oxidase	Nifedipine	100	0.5

Results

Olmesartan inhibited the activity of 7-ethoxyresorufin deethylase (CYP1A1, 1A2) by 0.88% at 10 μM and by 9.83% at 500 μM. The activity of S-mephenytoin hydroxylase (CYP2C19) was inhibited by 0.91% and 30.10% at concentrations of 10 and 500 μM, respectively. The activity of tolbutamide hydroxylase (CYP2C8 and 9) was inhibited by 6.69% and 41.77% at concentrations of 10 and 500 μM, respectively. The activity of chlorzoxazone hydroxylase (CYP2E1) was inhibited by 20.10% and 8.35% at concentrations of 10 and 500 μM, respectively. It may be noted that a large variation was found in the inhibition percentage with all the above enzymes, and no correlation was observed between the inhibitory effect and the olmesartan concentration. There was no inhibitory effect of olmesartan on other enzyme activities.

The results of this study thus demonstrated that olmesartan would have only minor effects on drug-metabolizing enzymes. Further, the maximum plasma concentration of olmesartan found in the clinical trials with olmesartan medoxomil was approximately 10 μM. At this concentration, olmesartan showed no inhibition of drug-metabolizing enzymes, except for CYP2E1, the isoform for which there was no correlation between effect and concentration of olmesartan.

2.15. Plasma Levels of Olmesartan After Single Oral Dose Administration of Olmesartan Medoxomil: Interspecies Comparison

Species (strain)	Route	Dose mg/kg/d	Sex	C_{max} µg/ml	T_{max} h	$T_{1/2}$ h	$AUC_{0-\infty}$ µg.h/ml	F	Ref. Sec #
Rat (Wistar)	Oral[1] (in CMC)	5	M	1.02	2.5	2.09	4.92	27.8	2.1
		10	M	1.43	2.0	2.34	11.10	31.3	
	(in soln)	10	M				13.18		
	Oral[1,2] (in soln)	1	M	0.30[3]	0.56	1.12	0.65[4]		2.2
			F	0.28[3]	0.44	1.44	0.74[4]		
	Oral[2] (in soln)	5	M	5.18[3]	2.0	3.5	27.6[4,5,6]		2.4
Dog (Beagle)	Oral[1] (in soln)	1.2[7]	M	0.17	0.50	0.76	0.19		2.10
		2.3[7]	M	0.33	0.33	0.88	0.40	14.1	
		4.5[7]	M	1.36	0.25	0.78	0.94		
	Capsule	2.3[7]	M	0.14	1.50	1.52	0.33	11.7	
	Soln[1,2]	5	M	2.33	0.50	1.00	3.53[6]		2.11
Human[8]	Tablet	20 mg	M	0.50	2.0	18.06	3.31	25.6	866-108
	Suspen-sion	20 mg	M	0.37	2.0	18.06	2.82	21.4	
	Soln[2]	20 mg	M	0.42-0.75	0.5-2.0	9-18	1.8-3.2		SE-866/13

The parent compound, OM, is present as the methyl ester of olmesartan. There are 16 mg equivalents of olmesartan in 20 mg OM (biopharmaceutics review).
F: % of drug bioavailable, AUC_{oral} /$AUC_{i.v.}$
1: fasted from the evening prior to dosing
2: ^{14}C form used
3: unit, µg equivalent/ml
4: unit, µg equivalent.hr/ml
5: AUC_{0-24h}
6: total radioactivity, expressed as µg equivalent of OM/ml
7: Dose in mg/dog is converted to mg/kg using an average body weight of 11 kg. For details see the study report
8: overnight fast, healthy volunteers