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Toxicokinetic Analysis of Losartan During Gestation and Lactation in the Rat

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ABSTRACT Previous developmental and reproductive toxicity studies in rats with losartan, a potent AT_1-selective angiotensin II (AII) receptor antagonist, correlated maternal treatment during gestation day (GD) 15–20 with irreversible renal abnormalities in the F_1 generation (Spence et al., '95a,b). Continued treatment through lactation was also associated with increases in pup mortality and decreases in pup body weights that persisted through weaning. The studies presented here were undertaken to quantify fetal and neonatal exposure to losartan when administered to the dam by oral gavage during early gestation, late gestation, and lactation.

Following daily oral dosing of 135 mg/kg/day on GD6-15, fetal drug levels were negligible. However, losartan and its active metabolite, EXP3174 (L-158,641) were readily detectable in fetal plasma on GD 20 (estimated AUC values, 50.70 and 167.70 μg/hr/ml, respectively) and maternal milk during lactation (1.61 and 1.67 μg/ml, respectively).

These studies suggest that the relative increased sensitivity of the fetus as compared to the neonate for losartan-induced renal lesions is related to the degree of exposure which is dependent on the time of administration (early gestation vs. late gestation/lactation) and the route of exposure (transplacental or through the milk). Furthermore, the maximum exposure to losartan and EXP3174 correlates with the ontogeny of the renin angiotensin system on approximately GD 17 and the critical period for losartan-induced renal lesions (GD15-20). The data support the hypothesis that the observed adverse fetal and neonatal effects are pharmacologically mediated, presumably through the lack of AT_1 receptor stimulation.

Losartan is a potent AT_1-selective angiotensin II (AII) receptor antagonist that has been recently approved and marketed under the name Cozaar for the treatment of hypertension (Siegl, '93; Weber, '92). In previous developmental and reproductive toxicity studies in rats with losartan, maternal treatment during late gestation and lactation was associated with decreases in pup body weights, increases in pup death during the preweaning and postweaning intervals and irreversible histopathologic renal abnormalities in the F_1 generation (Spence et al., '95a,b). These abnormalities consisted mainly of dilatation of the renal pelvis, edema of the renal papilla, medial hypertrophy of intracortical arterioles, chronic renal inflammation and irregular scarring of the renal parenchyma (Spence et al., '95a). There was also drug-induced hypertrophy of myocytes surrounding the intracortical renal arterioles that contained renin granules in greater numbers than control (Spence et al., '95a). Functionally, the morphological renal abnormalities were associated with impaired urine concentrating ability, as evidenced by reduced urine osmolarity and diuresis. Other studies have also noted similar adverse effects when losartan was administered to weanling rats (Gomez et al., '93; Friberg et al., '94).

Because both the renin and angiotensin genes (Campbell and Hadener, '86; Kalinyak and Perlman, '87) and AII receptors (Mendelsohn et al., '83, '84; Wilkes et al., '85) are regulated in a tissue specific fashion, it has been suggested that the renin angiotensin system (RAS) has autocrine and paracrine functions that may be independent from its function in cardiovascular homeostasis. In addition, AII has been shown to play a significant role in cell growth and differentiation of murine proximal tubule cells in vitro (Wolf and Neilson, '90) and the lack of AII receptor stimulation may impair renal development and subsequent physiologic function. In support of this hypothesis, AII receptors (AT_1 subtype) are abundant in the newborn rat kidney (Grady et al., '91; Tufro-McReddie, '93).

The association between the period of maximum exposure, beyond gestation day (GD) 15, the onset in expression of the RAS in the rat (on approximately GD 17; see Gomez et al., '93) and the critical period for losartan-induced renal lesions (GD 15–20) suggest that the adverse effects on losartan exposed neonates are

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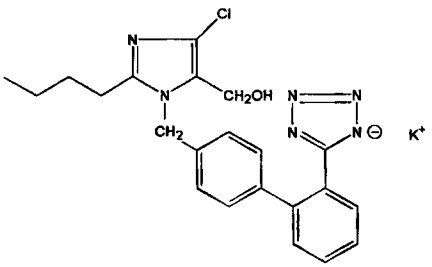
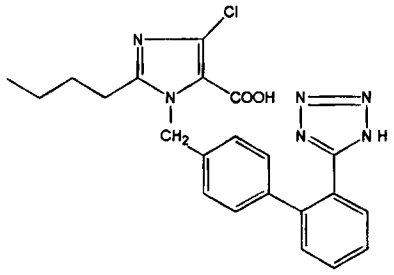
losartan:



EXP3174 (carboxylic acid metabolite):



L-158,338 (internal standard):

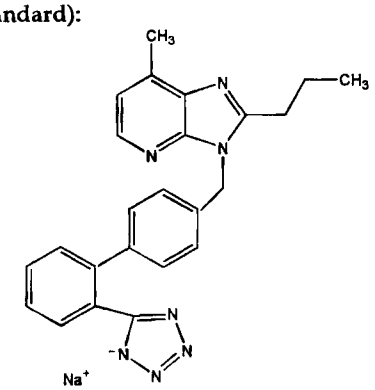


Fig. 1. Chemical structures of losartan, EXP3174 (major active acid metabolite) and L-158,338 (internal standard used for HPLC quantification).

pharmacologically mediated, presumably through the lack of AT_1 receptor stimulation. The studies presented here were undertaken to quantify fetal and neonatal exposure to losartan and its major active metabolite EXP3174 (Fig. 1) when administered to the dam by oral gavage during early gestation, late gestation, and lactation.

MATERIALS AND METHODS

Preparation of test articles

Losartan (Merck Research Laboratories, Rahway, NJ) was dissolved in deionized water (vehicle) and administered by oral gavage at a dosing volume of 5 ml/kg. Since the compound is a potassium salt, a factor of 1.09 was used to calculated doses of the base compound. The control article was deionized water and was also administered by oral gavage at a dosing volume of 5 ml/kg.

Animals, mating, and housing

All Sprague-Dawley female rats [Crl:CD®(SD) BR, Charles River Laboratories, Raleigh, NC] were approximately 11 weeks of age and weighed approximately 221–316 g at the start of each study. For mating, each female was housed with one untreated male of the same strain and examined daily for the presence of copulatory plugs in the vagina or cage pan. The day that copulatory plugs were found was considered gestation day (GD) 0, and the females were assigned to the study and housed singly. Room temperature was maintained at 21°–24°C in an atmosphere of 45% and 55% relative humidity. Room lights were set for alternating 12-h period of light and dark throughout the study. All animals had free access to food (Purina Certified Rodent Chow #5002) and water. Body weights were recorded at regular intervals throughout all studies to calculate the appropriate dose per kg (data not included). Animals were observed daily for adverse physical signs of toxicity.

Early gestation toxicokinetic study

The dams received 135 mg/kg/day of losartan by oral gavage on GD 6–15. This dose was selected based on findings from a rat developmental toxicity study and a female fertility study in which there were no effects on maternal body weight gain at doses of $\leq$100 mg/kg/day (Spence et al., 1995b). In the developmental toxicity study there was no effect on fetal body weight at doses up to and including 200 mg/kg/day. In the female fertility study there were significant ($P \leq 0.05$) treatment-related decreases in F_1 pup body weights during PND0-21 at all doses tested (25, 100, and 300/200 mg/kg/day). Therefore, a dose of 135 mg/kg/day was used with the expectations of producing some moderate maternal toxicity without creating any fetal effects.

On GD 15, dams were divided into seven time point subgroups for sample collection, with four rats per time point. Following ether-air anesthesia, approximately 1.0 ml of blood per time point was collected from the orbital sinus or inferior vena cava at 0.5, 1, 2, 3, 5, 7, and 24 hr post-dose on GD 15, using heparinized tubes. Fetal and placental tissues were collected at 1, 3, 5, 7, and 24 hr after treatment on GD 15. Fetal and placental tissues were pooled separately by litter and the number and pooled weights of the fetuses and placentas were recorded. The blood samples were centrifuged (5°C) and the plasma isolated and stored (−20°C) until analyzed for drug content. Fetal and placental tissues were quick-frozen on dry ice, and stored (−20°C) until analyzed for drug content.

Late gestation toxicokinetic study

The dams received 135 mg/kg/day of losartan by oral gavage on GD 15–20. Dose level selection was based on data from the aforementioned developmental toxicity and female fertility studies (Spence 1995b) and to make direct comparisons to the early gestation toxicokinetic study.

On GD 20, females were divided into six time point subgroups for sample collection, with three rats per time point. Following ether-air anesthesia, approximately 1.0 ml of blood per animal was collected from the inferior vena cava at 1, 2, 3, 5, 7, and 24 hr postdose, using heparinized collection tubes. Fetal blood samples were collected from the umbilical vessels with heparinized capillary tubes (same time points as above), pooled by litter, centrifuged and the plasma isolated and frozen (−20°C) until analyzed.

Secretion in milk study

The dams received 100 mg/kg/day of losartan by oral gavage on GD 15 through lactation day (LD)21. Based on results of a previous study when losartan was administered during late gestation and lactation (Spence et al., 1995b) a dose level of 100 mg/kg/day was selected to represent a moderately developmentally toxic dose. In that study, significant ($P \leq 0.05$), treatment-related, time- and dose-dependent decreases in pup weights occurred in all drug-treated groups as compared to controls during the lactation interval (Spence et al., 1995b). Based on those data (Spence et al., '95a,b), it is evident that the manifestations of the developmental toxicity of losartan are most apparent during lactation. Therefore, a slightly lower dose of losartan was administered in this study (100 mg/kg/day) as opposed to the early and late gestation studies (135 mg/kg/day).

Milk and maternal blood samples were collected from three dams each on LD 7, 14 and 21. Following ether–air anesthesia, dams were bled from the orbital sinus approximately four hours postdose on LD 7, 14, and 21, using heparinized collection tubes. Blood was centrifuged and the plasma was isolated and frozen (−20°C). Immediately following blood collection, each female was injected with oxytocin (1 unit, i.m., Parke-Davis). Approximately 5 min later, milk was collected by aspiration into individual polyethylene scintillation vials (~1–3 ml per female over a 10-min period). All milk and plasma samples were stored at −20°C until analyzed for drug content.

Bioanalytical methods

Plasma and tissue levels of losartan and its active metabolite (EXP3174) were quantified by reverse-phase high-performance liquid chromatography HPLC (Fig. 1). Briefly, the procedure involved the addition of a constant, known quantity of an internal standard (L-158,338) to the plasma matrix. Following acidification, the analytes were extracted into a methyl-t-butyl ether/acetonitrile mixture and back-extracted into dilute sodium hydroxide. An aliquot was injected into a C_{18} reverse-phase HPLC column under a gradient mobile phase consisting of acetonitrile and phosphate buffer, pH = 7.0. The analytes were monitored with UV detection at 254 nm. A standard curve was prepared by adding known quantities of losartan and EXP3174 to the appropriate control biological specimens. The concentration of each analyte was determined using the equation resulting from a linear regression analysis of the standard curve data. The detection limit of the method was approximately 0.25 μg/ml for losartan and 0.050 μg/ml for EXP3174. Placental and fetal samples were homogenized in four volumes of distilled water. Homogenates were prepared for HPLC analysis using similar methods to those described for plasma samples.

The area under the plasma concentration versus time curve was calculated using the mean plasma concentration data for a specific analyte during the 0–24-hr period post-dosing and by applying the trapezoidal rule. Half-life estimates were made using data obtained from the log-linear portion of the mean plasma durg concentration versus time curves.

RESULTS

Early gestation toxicokinetic study

Following administration of Losartan at 135 mg/kg/day the drug was rapidly absorbed and cleared from the maternal plasma (Table 1, Fig. 2). The maximum peak plasma levels (C_{max} = 36.5 μg/ml) occurred at one hour following the last dose. No parent drug was detected in the maternal plasma at 24 hr post-dose; the plasma half-life of losartan was estimated to be approximately 3 hr. Losartan and EXP3174 represented approximately 34% and 50%, respectively, of the total drug entities present in the systemic circulation in a 24-hr period. The rapid elimination of drug from the systemic circulation was due in part to metabolic conversion of losartan to EXP3174. Specifically, the area under the curve (AUC) values for losartan and EXP3174 were 88.0 and 128.9 μg/hr/ml, respectively (Table 1). The primary active metabolite (EXP3174) reached peak plasma levels at approximately 2 hr and was still present at 24 hr postdose. Two minor metabolites, an N-2 tetrazole glucuronide and an unidentified polar metabolite, represented approximately 2% and 14% of the remaining total drug entities present in the systemic circulation in a 24-hr period (data not shown).

Concentrations of losartan and EXP3174 were substantially lower in the placental and fetal tissues (Table 1). The placental C_{max} values for losartan and EXP3174, that occurred at approximately 1 hr, were 7.08 and 3.0 μg/g, respectively; estimated AUC values were 27.7 and 31.6 μg/hr/g. Only in a few instances were any of these drug-related entities detected in the fetus. These findings suggest that placental transfer of

TABLE 1. Losartan early gestation toxicokinetic study mean plasma (UG/ML) and tissue (UG/G) levels of losartan and EXP3174 following repeated oral administration of 135 MG/KG/day of losartan on GD6-15

	Maternal plasma				Placenta				Fetal			
	Losartan		EXP3174		Losartan		EXP3174		Losartan		EXP3174	
Hour	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E	Mean	S.E.
0.5	17.40	6.25	4.82	2.29	a		a		a		a	
1	36.46	10.09	20.90	3.28	7.08	1.65	3.00	0.45	0.10		0.06	
2	22.30	8.80	28.93	7.70	a		a		a		a	
3	4.59	0.82	13.63	2.18	1.79	0.28	2.97	0.55	*	*	*	*
5	2.63	0.49	9.14	2.67	1.09	0.24	1.98	0.45	*	*	*	*
7	1.85	0.44	4.18	1.18	1.02	0.05	1.45	0.38	*	*	*	*
24	*	*	0.41	0.02	0.20	0.11	0.40	0.02	*	*	*	*
AUC	88.00	nd	128.90	nd	27.70	nd	31.60	nd	*	*	*	*

a: Tissues were not collected at this time point.
*: Below the limits of detection.
AUC: (Area Under the Curve), expressed as ug.hr/ml(g), calculated for the 0–24hr period post-dose.
nd: not determined.
S.E.: Standard error.
4 rats/timepoint; fetal and placental tissues pooled separately by litter.

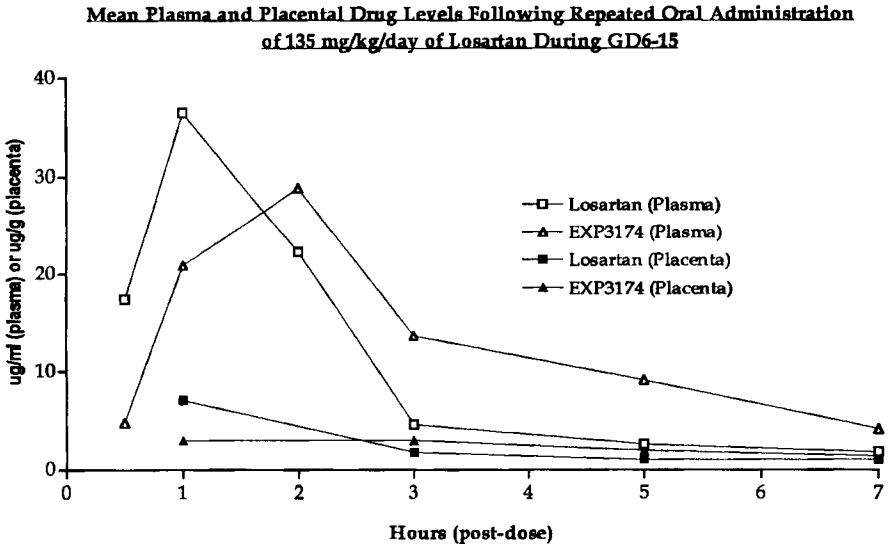


Fig. 2. Summary results of the early gestation toxicokinetic study.

losartan and its active acid metabolite (EXP3174) are minimal early in gestation.

Late gestation toxicokinetic study

Following administration of losartan at 135 mg/kg/day on GD 15–20, the mean peak maternal plasma concentration of losartan occurred at approximately 2 hr (64.68 μg/ml) and declined with a plasma half-life of approximately 5 hr; the mean drug concentration at 24 hr (0.48 μg/ml) was <1% of the mean C_{max} concentration (Table 2, Fig. 3). The systemic exposure, as measured by the AUC, was 194.8 μg/hr/ml.

The mean peak fetal losartan plasma concentrations were lower than the maternal losartan plasma concentrations. This finding is reflected in both the C_{max} and estimated AUC values. The losartan fetal estimated AUC value was 50.7 μg/hr/ml. These data indicate that losartan is readily transferred to the fetus during the latter part of pregnancy.

Pregnant rats readily metabolize losartan to EXP3174. EXP3174 exhibited a mean maternal plasma C_{max} (38.97 μg/ml) at three hours postdose on GD 20. The estimated maternal plasma EXP3174 half-life was approximately 4 hr. The maternal plasma EXP3174 estimated AUC was 282.6 μg/hr/ml and was approximately 1½ times higher than the maternal plasma estimated AUC of the parent drug, losartan. A substantial amount of EXP3174 was detected in the fetal plasma (Table 2). The mean concentrations remained relatively constant (approximately 6–7 μg/ml)

TABLE 2. Losartan late gestation toxicokinetic study mean plasma levels (UG/ML) of losartan and EXP3174 following repeated oral administration of 135 MG/KG/DAY of losartan on GD15-20

	Maternal				Fetal				Maternal/Fetal		Fetal
	Losartan		EXP3174		Losartan		EXP3174		Losartan	EXP3174	EXP3174/Losartan
Hour	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	Mean	Mean
1	17.83	4.98	9.17	2.60	0.71	0.19	4.63	1.92	27.30	2.80	6.5
2	64.68	13.60	34.95	2.47	4.07	1.16	6.40	0.62	17.00	5.50	1.6
3	25.56	7.83	38.97	6.92	3.92	0.96	7.70	1.55	6.60	5.30	2.00
5	5.20	1.08	16.30	4.14	3.20	0.76	6.98	1.01	1.70	2.30	2.20
7	6.23	1.05	15.04	0.33	2.97	0.36	7.77	1.45	2.30	2.10	2.60
24	0.48	0.20	0.50	0.09	0.60	0.09	6.37	0.94	0.90	0.10	10.60
AUC	194.80	nd	282.60	nd	50.70	nd	167.70	nd	3.84	1.69	

AUC: (Area Under the Curve), expressed as ug.hr/ml, calculated for the 0-24hr period post-dose.
nd: not determined.
S.E.: Standard error.
3 rats/timepoint; fetal samples pooled by litter.

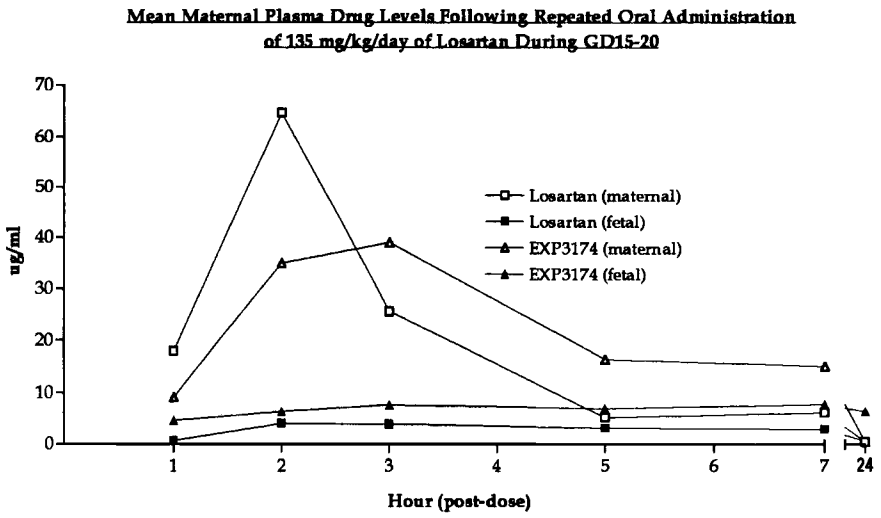


Fig. 3. Summary results of late gestation toxicokinetic study.

at most of the time points studied and remained elevated at 24 hr postdose. Fetal levels of EXP3174 were 1.6–10.6 times higher than fetal losartan levels, suggesting that the fetus acts as a deep compartment for this polar active acid metabolite (Table 2).

Secretion in milk study

Following the oral administration 100 mg/kg/day of losartan on GD 15 through LD 21 mean maternal plasma drug levels at four hours post-dose were similar regardless of the lactation day (Table 3, Fig. 4): 3.9, 3.0, and 3.2 μg/ml, on LD7, 14 and 21, respectively. These levels suggest that steady-state drug concentrations were attained during the lactation interval. The corresponding mean plasma concentrations of EXP3174 were 10.5, 12.4, and 15.3 μg/ml on LD 7, 14, and 21, respectively.

As observed for maternal rat plasma, the mean losartan concentrations in rat milk did not change considerably during the lactation period from LD 7 to 21 (Fig. 4). The mean drug levels were 1.2, 2.0, and 1.7 μg/ml milk for LD 7, 14, and 21, respectively. Although losartan was detected in rat milk, the milk to plasma ratios indicate that the losartan milk concentrations were <50% of the corresponding maternal plasma concentrations in over 75 percent of the rats. The milk to plasma ratio of EXP3174 was under 0.20 in >85% of the rats (individual animal data not presented). Unlike the maternal plasma, in which concentrations of EXP3174 were consistently higher than losartan, in milk mean EXP3174 and losartan concentrations were similar.

DISCUSSION

The maternal estimated AUC values of losartan and EXP3174 were more than two-fold greater in the late gestation study as compared to the early gestation study (Tables 1, 2). Although the reasons for these dif-

TABLE 3. Losartan secretion in milk study mean plasma and milk concentrations (UG/ML) of losartan and EXP3174 following repeated oral administration of 100 MG/KG/DAY of losartan on GD15-LD21

Lactation Day	Milk				Plasma				Milk/Plasma			
	Losartan		EXP3174		Losartan		EXP3174		Losartan		EXP3174	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
7	1.16	0.21	0.96	0.23	3.86	0.45	10.46	2.50	0.30	0.04	0.10	0.01
14	1.96	0.50	2.42	0.60	3.00	0.55	12.44	0.97	0.76	0.34	0.19	0.04
21	1.71	0.25	1.63	0.16	3.15	0.07	15.22	2.93	0.54	0.07	0.12	0.03
Overall	1.61	0.21	1.67	0.28	3.34	0.25	12.71	1.34	0.54	0.12	0.13	0.02

All samples collected 4 hr post-dose.
S.E.: Standard error.
3 rats/timepoint.

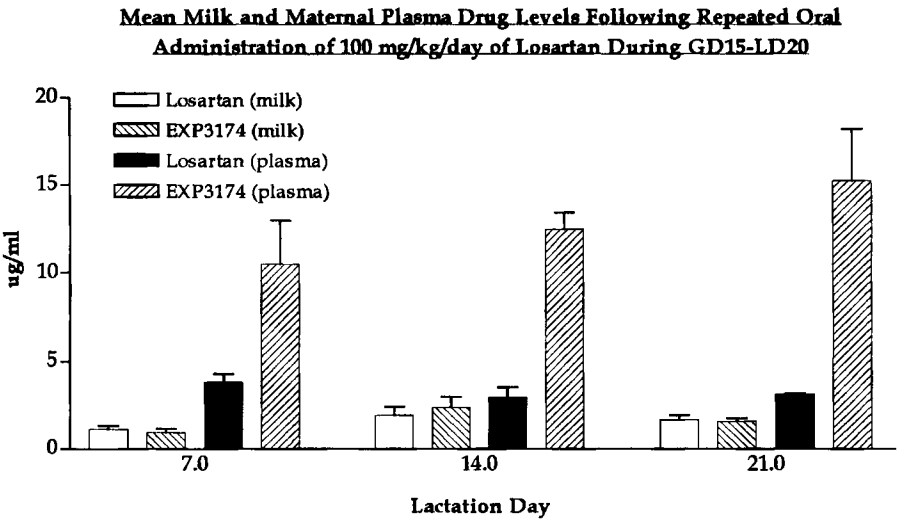


Fig. 4. Summary results of the secretion in milk study.

ferences are not evident from these studies they may be due to differences in the rates of drug absorption and/or elimination during late gestation, as compared to early gestation. During the later part of pregnancy gastrointestinal myoelectric activity has been noted to decrease (Scott et al., '83) while gastrointestinal transit time has been noted to increase (Datta et al., '74), and this may allow greater and more efficient intestinal absorption of losartan in late gestation as compared to early gestation.

The early gestation toxicokinetic study demonstrated negligible placental uptake and fetal transfer of losartan when administered to the dam during GD 6–15. By contrast, losartan and EXP3174 were readily detectable in the rat fetus with maternal administration during GD 15–20. These data correlate with a previous fostering/cross fostering study (Spence et al., 1995a), in which treatment of the dam with 100 mg/kg/day during GD 15–20 was associated with irreversible renal lesions in the F_1 generation. Conversely, when similar doses of losartan were administered during GD 6–15 there were no adverse effects on the F_1 generation (Spence et al., 1995a).

Increased fetal deposition of drugs with increasing gestational age has been noted for other drugs, most notably indomethacin (Klein et al., '81; Momma and Takeo, '87). These findings may be due, in part, to changes in permeability of the interhemal membrane of the placenta and increased placental blood flow during the later part of pregnancy (Olanoff and Anderson, '80; Sonawane and Yafee, '86). However, advancing placental age is not always associated with an increase in permeability to drugs. The degree of drug binding to plasma proteins also influences placental transfer, in that only the unbound or free drug is able to diffuse across the placenta. Decreased maternal serum protein binding during the later part of pregnancy has been noted for several drugs (Dean et al., '80). In the rat, Stock et al. ('80) demonstrated that serum protein binding of several drugs reach its lowest point on GD 20 and returns to normal two days after parturition. Increased free drug fraction associated with the later stages of

pregnancy may be associated with increased placental transfer to the fetus. Based on those studies the possibility exists that the fetus would be exposed to increasing amounts of losartan and EXP3174 as term approaches, allowing increased levels of drug in the fetal compartment.

The association between the critical period for losartan-induced renal lesions (GD 15–20), the onset in expression of the RAS in the rat (on approximately GD 17) (Gomez et al., '93), the increase in fetal exposure in late gestation, and the demonstrable exposure to losartan and EXP3174 through suckling during lactation, suggest that the observed adverse fetal and neonatal effects are pharmacologically mediated. Previous studies (Spence et al., '95a) have demonstrated increased renin expression in the myocytes of renal cortical arterioles of weanling rats exposed to losartan in utero (Spence et al., '95a), suggesting decreased negative feedback inhibition of renin synthesis caused by a lack of AII receptor stimulation. As mentioned previously, AII has been shown to play a significant role in cell growth and differentiation of murine proximal tubule cells in vitro (Wolf and Neilson, '90) and AII receptors (AT_1 subtype) are abundant in the newborn rat kidney (Grady et al., '91; Tufro-McReddie, '93).

Therefore, the lack of AII receptor stimulation during the development of the RAS may impair renal development and subsequent physiologic function. In support of this hypothesis oral administration of high doses of the angiotensin I converting enzyme (ACE) inhibitors enalapril (Robertson et al., '86), quinapril (Dostal et al., '91), and rentiapril (Cozens et al., '87) during late gestation and lactation have also been associated with treatment-related increased pup mortality decreases in pup body weight gain.

As noted previously, oral administration of losartan to the dam during GD 15–20 was associated with severe renal abnormalities in the F_1 generation (pelvic dilation, medial hypertrophy of intracortical arterioles, chronic renal inflammation and scarring of the renal parenchyma). These findings may correlate with higher exposure to the fetus during late gestation as compared to exposure to the neonate through suckling during lactation. However, irreversible histopathologic renal abnormalities have been observed in adult rats following direct subcutaneous administration of losartan (10 and 30 mg/kg/day), enalapril (10 mg/kg/day) or captopril (20 and 40 mg/kg/day) to the neonate during postnatal days 3–23 (Friberg et al., '94). Taken together, these studies suggest that the relative increased sensitivity of the fetus as compared to the neonate for losartan-induced renal lesions is related to the degree of exposure, which may be dependent on the time of administration (early vs. late gestation/lactation) and the route of exposure (transplacental or through the milk).

Following a single therapeutic dose of 50 mg of Losartan in humans, estimated losartan and EXP3174 AUC values (0.5 and 2.2 μg/hr/ml, respectively; Smith et al., submitted) were considerably lower than those observed in the early and late gestation studies presented in these studies. However, the doses tested in these studies were above the no adverse effect level for effects on F_1 offspring (increased mortality, renal lesions and decreased body weight gain; Spence et al., 1995a,b). Therefore, the relative fetal safety of therapeutic doses of losartan, when administered to pregnant women, cannot be determined from the toxicokinetic data presented. Case reports of human pregnancies have indicated that there is an association between ACE inhibitor treatment, a similar class of compounds, during the second and third trimesters of pregnancy and adverse fetal outcome associated with fetal hypotension, anuria-oligohydramnios, growth restriction, pulmonary hypoplasia, hypocalavaria, and renal tubular dysplasia (Schubiger et al., '88; Barr and Cohen, '91; Hanssens et al., '91; Pryde et al., '93). Although the mechanism underlying the fetotoxicity and associated fetal renal failures in humans is not well understood, blockade of the AT_1 receptor with losartan during the maturation of the RAS may result in similar toxicity as that observed with ACE-inhibitors.

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