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THE PHARMACOKINETICS OF SALICYLATE IN THE PREGNANT WISTAR RAT

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ABSTRACT:

Sodium salicylate, in a single dose of 50 mg/kg, was administered by iv injection to nonpregnant female and 20-day pregnant Wistar rats. Blood samples (for serum) and urine were collected and analyzed for salicylate, gentisic acid, salicyluric acid, and salicyl glucuronides by HPLC. Pregnant rats showed a significant decrease in body weight-normalized total clearance but no change in absolute total clearance of salicylate. On a body weight-adjusted basis there was a slight increase in the apparent volume of salicylate distribution in pregnancy but this increase becomes highly significant if expressed in absolute terms. The biological half-life of salicylate was significantly increased in late pregnancy. Serum protein binding of salicylate is decreased in pregnancy relative to nonpregnant females but in both groups binding shows a concentration dependence. The partial clearances of both salicyluric and gentisic acids were reduced by pregnancy in the rat whereas that of the salicyl glucuronides appeared unchanged. This latter result in intact pregnant animals contrasts with previously reported decreases in glucuronyltransferase activity in isolated liver preparations from pregnant rats.

Most studies of xenobiotic metabolism during pregnancy have concentrated on *in vitro* measurements of hepatic oxidative and reductive biotransformations, rather than those involving conjugation reactions (1–6). Where *in vitro* studies of conjugation reactions have been reported, they have generally examined the quantitatively important glucuronic acid conjugation pathway (2, 7–9). These studies have indicated that the specific activity of hepatic glucuronyltransferase is decreased in late pregnancy. The exceptions to this trend are the results of Vaisman *et al.* (10), who failed to find any change in bilirubin conjugation during pregnancy, and Pacifici and Rane (11) who reported an increase in morphine glucuronide formation with livers from 21-day pregnant rats.

Studies in the intact pregnant rat have again been primarily concerned with drugs whose elimination is limited by aromatic hydroxylation (12–15). These studies have all shown total body clearances that correlate with the decreased hepatic microsomal hydroxylase activity of late pregnancy. However, whole animal studies with compounds cleared primarily by conjugation reactions have been less extensively studied.

The widely used analgesic and anti-inflammatory salicylates are excreted as their glucuronide and glycine conjugates, together with smaller amounts of hydroxylated derivatives, and as unchanged drug (16). It has been suggested that salicylate pharmacokinetics are changed during pregnancy (17, 18); therefore, the present study investigates the influence of pregnancy both on the pharmacokinetics of salicylate and on its metabolic profile in the intact rat.

Materials and Methods

Animals. Female hooded Wistar rats at similar levels of sexual maturity, being between 18 and 23 weeks old and weighing 200–260 g, were used for this study. They were housed under conditions of controlled temperature and lighting, with free access to food and water at all times.

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Pregnant rats were obtained by placing males in cages of females for 15 hr overnight. Individual pregnancies were confirmed by palpation of the abdomen on the 18th day, at which time the rats were cannulated for iv dosing. All pregnant rats used were in their 20th day of gestation on the day of study and weighed between 256 and 320 g. Control rats (200–242 g) were taken from the same breeding colony after removal of the pregnant rats.

Pregnant animals had an indwelling silicone rubber/polyethylene cannula implanted in the right external jugular vein under ether anaesthesia (19) on the eighteenth day of pregnancy. The animals were placed in individual metabolic cages and allowed to recover for approximately 48 hr before receiving an iv injection of sodium salicylate. Nonpregnant control animals were similarly treated.

Drug Administration and Blood and Urine Sampling. Sodium salicylate (Sigma Chemical Co., St. Louis, Mo.) was dissolved in water and each rat received a single iv dose equivalent to 50 mg of salicylic acid per kg of body weight, as sodium salicylate, injected through the cannula. This was followed by repeated aspiration and reinjection of the blood to ensure complete administration of the dose. The cannula was finally cleared with saline. The pregnant and nonpregnant control groups each contained five rats.

Eight individual blood samples were taken of both control and pregnant rats at selected intervals from 1 to 24 hr. In each case, 0.4 ml of blood with withdrawn into a syringe through a three-way stopcock to rinse the cannula; a sample of 0.35 ml was withdrawn into a second syringe and the first 0.4 ml was then reinjected, followed by about 0.4 ml of saline to replace the blood removed and clear the cannula. Cannula dead-space was about 0.07 ml. Urine was collected for 24 hr after iv dosing.

Analytical Procedures. *Salicylate in Serum.* Aliquots of serum (100 μl) were added to 200 μl of methanol, vortexed, and centrifuged at 3000*g* for 10 min; a 25-μl aliquot of the supernatant was injected onto a C_{18} μ-Bondapak column and eluted with methanol/0.005 M aqueous tetramethylammonium phosphate (Pic A reagent; Waters Associates, Milford, MA), pH 7.5, (30:70, v/v). The flow rate was 1.2 ml/min, with detection at 280 nm. Because there was no evidence of salicylate metabolites in serum samples, only standards of sodium salicylate were used. These were made up of six samples prepared in drug-free serum and covering the range of 25 to 300 μg of salicylate/ml. These standards were treated in an identical manner to the serum samples from salicylate-treated rats. The retention time for salicylate was 17 min.

Salicylate Metabolites in Urine. Urine was filtered through a 0.4-μm cellulose acetate membrane (Oxoid, London) in a Swinney adapter

(Sartorius, Gohingen, W. Germany) to remove particulate matter. Samples (25 μl) were injected onto a C_{18} μ-Bondapak column and eluted as described for serum samples except that metabolites were measured at 313 nm to eliminate interference, found at 280 nm, from other components of urine.

Standards of sodium salicylate, gentisic acid, and salicyluric acid (Sigma) covering a concentration range of 25 to 500 μg/ml were added to drug-free urine and measured as above. The retention times for gentisic acid, salicyluric acid, and salicylate were 8, 14, and 17 min, respectively.

Salicyl glucuronides were estimated by measuring total salicylate, as above, after incubating 200 μl of urine with 400 μl of 0.067 M KH_2PO_4 buffer, pH 5, and β-glucuronidase (2500 units) at 37°C for 24 hr and subtracting from this the value of free salicylate obtained from identical urine samples not so treated.

Serum Protein Binding. Blood was obtained by exsanguination via the abdominal aorta, under light ether anaesthesia, and immediately centrifuged. Serum samples (1 ml) from both pregnant and nonpregnant rats were then spiked with sodium salicylate to give final concentrations of 300, 200, 100, or 50 μg of salicylic acid/ml. The protein-free filtrate was obtained by immediate centrifugation for 10 min at 37°C using an MPS-1 and Centrifree Micropartition System with YMT membranes (Amicon, Sydney, Australia). From these filtrates, the free fraction of salicylate for each individual animal at each salicylate concentration was measured.

HPLC Instrumentation. The HPLC system consisted of a model 6000A solvent delivery system, a model U6K universal injector, a model 440 absorbance detector, and a 30 cm × 3.9 mm C_{18} μ-Bondapak column with an average particle size of 10 μm (Waters). Peak heights were recorded with a 10 mV dual channel recorder (Omniscribe Waters). Chromatography was performed at ambient temperature.

Pharmacokinetics. The serum concentration of salicylate was plotted as a function of time after iv administration of the drug. The data obtained fitted a one-compartment model. The following pharmacokinetic parameters were calculated from the data from each animal. Elimination rate constants (K_{el}) were determined by linear regression analyses and from these values half-lives ($t_{½}$) were calculated from the relationship $t_{½} = 0.693/K_{el}$; total area under the serum concentration *vs.* time curve (AUC) by the trapezoidal rule to the last data point (C_t), plus the additional area from C_t to infinity by C_t/K_{el}; total body clearance (CL_T) from the relationship CL_T = iv dose/AUC; and the apparent volume of distribution (V_d) from $V_d = CL_T/K_{el}$. Partial clearances to the major metabolites and renal clearance of unchanged salicylate were determined as the product of total clearance and the fraction of the dose excreted as a metabolite or as salicylate. Statistical significance of differences between the means of control and pregnant groups were determined by Student's t test.

Results

The pharmacokinetic parameters calculated from the serum-drug concentration *vs.* time data from five 20-day pregnant and five nonpregnant rats after an iv bolus injection of sodium salicylate equivalent to 50 mg of salicylic acid/kg are summarized in table 1. These figures show that the absolute total body clearance of salicylate was unchanged whereas the absolute apparent volume of distribution increased significantly in pregnancy. However, when these parameters were normalized to accommodate the significant increase in the weight of the pregnant rats, there was a significant reduction in the total body clearance, with only a slight increase in the apparent volume of salicylate distribution. The biological half-life of salicylate was also significantly extended in the pregnant rat.

Salicylate metabolites were not detected in the serum samples taken from rats so urine was collected for 24 hr after the salicylate injection and then analyzed for major metabolites. These results showed that pregnant rats excreted a smaller fraction of the administered dose in 24 hr than did their nonpregnant controls, which is consistent with the increase in half-life. However, there were also changes in the pattern of the metabolite profiles, with significant reductions in the hydroxylation product gentisic acid and the glycine conjugate salicyluric acid, but an increase in glucuronic acid conjugates (table 2).

The time-averaged partial metabolic clearances of salicylate to gentisic and salicyluric acids were both significantly reduced in pregnancy, when expressed as body weight-normalized clearance values (table 3). Expressed on the same body weight basis, there is no difference between the partial metabolic clearances of salicylate to glucuronide conjugates in either group. However, if this data is expressed in terms of absolute partial metabolic clearances (not shown) glucuronide formation is significantly increased whereas the partial metabolic clearances of salicyluric and gentisic acids remain significantly depressed.

The serum protein binding of salicylate, covering a range of concentrations from 50 to 300 μg of salicylic acid, is recorded in fig. 1. These show that salicylate binding is decreased in the pregnant rat relative to the nonpregnant female and that binding in both groups decreases in an essentially parallel fashion with increasing salicylate concentrations.

Discussion

Lin and Levy (20), when considering the pharmacokinetics of acetaminophen, drew attention to the problems of explaining the results obtained because of the large increase in body weight associated with pregnancy in the rat. Similarly, the interpretation of pregnancy-induced changes in salicylate pharmacokinetics depends on how the results are presented (table 1).

If the total body clearance and apparent volume of distribution are expressed in absolute terms for the whole animal, then these results suggest that there is a large increase in the volume of distribution of salicylate without any significant change in its clearance from the 20-day pregnant rat. However, if these values are normalized for body weight, then the increase in the apparent volume of distribution of salicylate is minimal but the total body

TABLE 1

Influence of pregnancy on salicylate pharmacokinetics in rats

Results expressed as the mean ± SD, $N = 5$.

	Nonpregnant Females	Pregnant Females (20 days)	Statistical Significance of Difference
Body Weight	208 ± 12 g	285 ± 19 g	$p < 0.01$
Total Body Clearance	(a) 3.2 ± 0.3 ml/min	(a) 3.3 ± 0.2 ml/min	NS[a]
	(b) 15.7 ± 1.3 ml/min/kg	(b) 11.6 ± 1.1 ml/min/kg	$p < 0.01$
Volume of Distribution	(a) 39.1 ± 3.0 ml	(a) 62.7 ± 4.2 ml	$p < 0.01$
	(b) 188 ± 15 ml/kg	(b) 221 ± 19 ml/kg	NS
Half-life	8.5 ± 0.7 hr	12.8 ± 1.7 hr	$p < 0.01$

[a] NS, not significant.

TABLE 2

Influence of pregnancy on the composition of the 24-hr urinary excretion products of salicylate administration

Dose was 50 mg/kg iv. Results, expressed as percentage of the dose, are the mean values ± SD, $N = 5$.

Urinary Excretion Product	Nonpregant Females	Pregnant Females (20 days)	Statistical Significance of Difference
	% of dose		
Salicylic Acid	43.0 ± 7.8	36.8 ± 8.6	NS[a]
Gentisic Acid	9.5 ± 2.3	4.0 ± 0.9	$p < 0.01$
Salicyluric Acid	15.8 ± 2.5	5.6 ± 1.0	$p < 0.01$
Salicyl Glucuronic Acids	14.4 ± 1.2	19.1 ± 3.7	$p < 0.05$
Total	82.7 ± 12.4	65.5 ± 9.8	$p < 0.05$

[a] NS, not significant.

TABLE 3

Influence of pregnancy on the renal clearance of salicylate and the time-averaged metabolic clearance of salicylate to its major metabolites in the rat

Results, expressed as ml/min/kg, are the mean values ± SD, $N = 5$.

	Nonpregnant Females	Pregnant Females (20 days)	Statistical Significance of Difference
	ml/min/kg		
Renal Clearance to Salicylate	6.51 ± 1.6	4.42 ± 1.3	$p < 0.05$
Partial Clearance to Gentisic Acid	1.52 ± 0.34	0.48 ± 0.22	$p < 0.01$
Partial Clearance to Salicyluric	2.52 ± 0.57	0.66 ± 0.24	$p < 0.01$
Partial Clearance to Salicyl Glucuronic Acids	2.33 ± 0.62	2.24 ± 0.53	NS[a]

[a] NS, not significant.

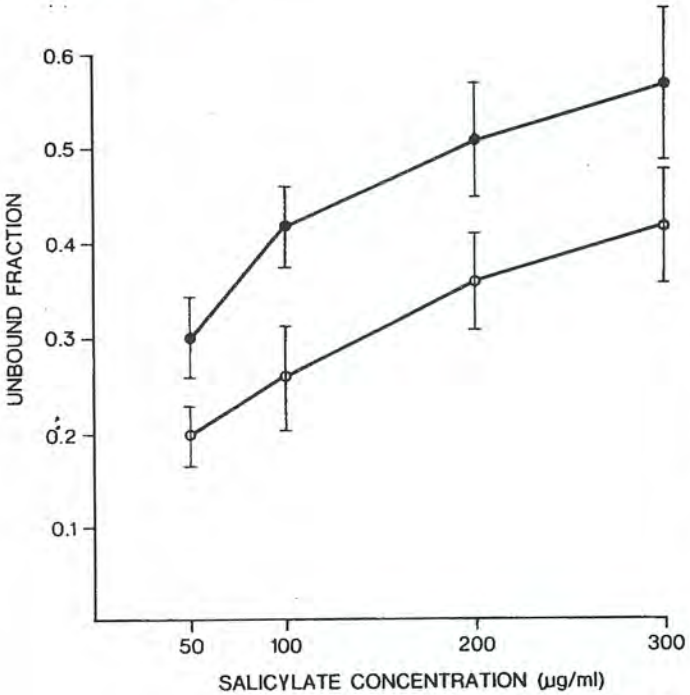

FIG. 1. *The concentration dependence of the unbound fraction of salicylic acid in the serum of nonpregnant female (○) and 20-day pregnant rats (●).*

Each *point* represents the mean of the unbound fractions of salicylic acid ± SD; $N = 4$. Significant differences exist between the pregnant and nonpregnant female groups at each concentration measured. In all cases, $p < 0.05$.

clearance is significantly reduced. The half-life of salicylate is significantly increased in the pregnant rat.

Although a case can be made for using either method of expressing the results, pregnancy is associated with a significant increase in body weight and an even greater relative increase in liver weight at day 20 of gestation (4). Therefore, for drugs whose clearance is primarily controlled by hepatic metabolism, it would appear that body weight-normalized data reflect more accurately changes due to pregnancy than do absolute values. Thus, the present results suggest that pregnancy is associated with a reduction in total body clearance of salicylate.

The values shown for partial metabolic clearances (table 3) will not necessarily reflect the absolute differences between the pregnant and nonpregnant rats inasmuch as the total amount of administered dose recovered in 24 hr differs between the two groups (table 2). However, the differences in partial metabolic clearances shown are such as to indicate that the metabolic profile of salicylate is significantly altered by pregnancy. The problem in extending the collection period to maximize the recovery of the administered dose is the observation that the period between day 20 of pregnancy and parturition is associated with major changes in hepatic monooxygenase activity (4). This could make interpretation of metabolic profiles more difficult.

Factors that influence the hepatic clearance of drugs are intrinsic enzyme activity, blood flow to the liver, and the fraction of total drug that is free or not bound to protein. Although some drugs are so rapidly metabolized by liver enzymes that their metabolism is limited by their transport to this organ, there is no evidence that this would be the case for salicylate if normal liver blood flow occurs. Certainly, there is no evidence that liver blood flow, which has been shown to be of the order of 66 ml/min/kg in the rat, (21) is reduced in pregnancy. Rather it has been speculated that the increase in liver size during pregnancy could lead to an increase in liver blood flow (20). Therefore, the changes in metabolic clearance of salicylate must be the result of changes in either one or both of the other two parameters.

Previous studies using equilibrium dialysis techniques have suggested that the serum protein binding of several drugs, including salicylate, is significantly reduced by pregnancy (22). The present studies, using ultrafiltration methods, confirm this response and also show that there is an essentially parallel concen-

tration dependence of this binding in both the pregnant and nonpregnant rat. Any increase in free fraction of salicylate in pregnant rats should facilitate the metabolic clearance of this drug. The fact that metabolic clearance is not so increased emphasizes that the reduction in intrinsic enzyme activity during pregnancy is quite significant. The decreased gentisic acid formation found in this study correlates well with the reduced aromatic hydroxylation by microsomal preparations isolated from this rat species in late pregnancy (4, 15).

The conjugation of salicylate with glycine also falls significantly but not the formation of salicyl glucuronides for which, in terms of total amount of salicyl glucuronides formed in the 24-hr period, there is actually a significant increase during pregnancy. This increased formation of salicyl glucuronides is contrary to expectation on the basis of numerous *in vitro* studies with hepatic microsomal preparations from pregnant rats. These have almost all recorded a decreased glucuronyltransferase activity during pregnancy. The present results do, however, more closely reflect the minority *in vitro* results reported by Vaisman *et al.* (10) and Pacifici and Rane (11). This apparent contradiction with the majority of *in vitro* studies could result from the differences in the *in vitro* substrate and enzyme concentrations involved, compared with those in whole animal studies. Generally *in vitro* enzyme studies use saturating substrate concentrations whereas in the present studies it is unlikely that the concentration of salicylate could saturate the available enzyme over the 24-hr measuring period. However, these findings do highlight the problem that can arise from the direct extrapolation of results from isolated tissue studies to establish the response of intact animals to more therapeutic concentrations of drug substrates.

Finally, the results of the present study are at variance with two previous studies (17, 18). These workers have reported that, although there was a similar significant increase in its volume of distribution, the total clearance and half-life of salicylate were not significantly changed by pregnancy.

Although the present results were generated from serum-salicylate concentration data which, consistent with the results of Gabrielsson *et al.* (23), showed apparent first-order linear decline, the formation of some salicylate metabolites are capacity limited. Thus, the differences recorded may be due to the different dose levels between this and the two previous studies, inasmuch as Lin and Levy (20) have shown that the relative total clearances of acetaminophen are significantly different at different dose levels in pregnant and nonpregnant rats. These differences may be particularly relevant to salicylates, which are administered over a wide range of doses.

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