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HETEROGENEITY OF CHANGES ON THE DISPOSITION OF ASPIRIN IN RATS WITH CCl_4-INDUCED CHRONIC LIVER DAMAGE

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Abstract—The profile of urinary salicylate metabolites was determined after the oral administration of acetylsalicylic acid (ASA) to CCl_4-cirrhotic rats, CCl_4-cirrhotic rats treated with colchicine for 1 month, and control groups. The following enzymatic activities were determined: liver and plasma ASA-esterase, liver UDP-glucuronyltransferase, and liver aniline hydroxylase. The time–course of plasma concentration of salicylates in similar groups was followed after the intraperitoneal administration of salicylic acid (SA) or gentisic acid (GA). The cirrhotic animals showed a lack of urinary glucuronates and an increase in urinary gentisic and salicylic acids. The activities of plasma and liver ASA-esterases were increased significantly in cirrhosis, whereas aniline hydroxylase was reduced and UDP-glucuronyltransferase remained unchanged. The plasma half-lives of salicylates were reduced in the cirrhotic animals regardless of the administered parent compound. Colchicine treatment reversed almost completely the alterations. The heterogeneity of liver metabolic dysfunctions present in chronic liver disease was demonstrated. It is emphasized that the pharmacokinetic alterations produced by liver damage are the result of a complex set of factors involving changes in the hepatic circulation, protein binding, and the existence of other routes of elimination.

The liver is the main site of drug metabolism and also influences the pharmacokinetics of some drugs through biliary excretion [1]. The hepatic clearance of drugs can be modified in liver diseases according to variations in the following determinants: (1) activity of drug-metabolizing enzymes (intrinsic hepatic clearance), (2) hepatic blood flow, (3) drug binding (availability of albumin binding sites), and (4) anatomical arrangement of hepatic circulation [2]. Each of these factors can vary in a complex manner during liver diseases [2, 3]. Additionally, the change in the overall rate of elimination of a given drug during these conditions is also dependent on the relative extent of hepatic extraction and the coexistence of alternative routes of elimination [4]. The variety of possible combinations of these factors may explain the variation of effects of liver diseases on drug metabolism and elimination which are far more unpredictable [5, 6] than the respective effects of changes in kidney function upon the elimination of drugs by this organ. Thus, conflicting results have been reported on the metabolism of drugs in patients with hepatic dysfunction and, in some cases, there is a poor correlation between the changes in the biological half-life of a drug and those found in the specific enzymatic activity of microsomal liver oxidase measured in biopsy samples [7–14].

It has been known that chronic treatment of rats with CCl_4 produces liver fibrosis and a set of biochemical and histological changes that closely resemble most aspects of human portal cirrhosis [15, 16]. Colchicine, on the other hand, is a drug currently used in the treatment of experimental and clinical cirrhosis which reverses most of the histological and biochemical signs of CCl_4-induced liver cirrhosis in rats [17–19].

Aspirin disposition is initiated by a rapid deacetylation by plasma and tissue esterases [20]. Salicylate is then subjected to various metabolic transformations in the liver and the metabolites are excreted by the kidney [21]. The hepatic metabolism of salicylates involves several enzyme systems, i.e. conjugation with glucuronic acid requires a microsomal enzyme not related to the mixed-function oxidase [22], oxidation to gentisic acid is a typical mixed-function oxidase reaction coupled to cytochrome P-450 [22], and the formation of salicyluric acid is a non-microsomal conjugation [22]. Thus, by studying salicylate disposition we can explore the manner in which experimental liver cirrhosis affects the metabolic functions of the hepatocytes at different levels and the correlation between this and the overall pharmacokinetics of salicylate.

In this study we have determined the effects of CCl_4-induced liver cirrhosis on the profile of urinary metabolites of aspirin after its oral administration to rats as well as on the time–course of the plasma concentrations of total salicylates after the intraperitoneal injection of either acetylsalicylic acid (ASA), salicylic acid (SA) or gentisic acid (GA). We also evaluated the effects of colchicine on the CCl_4-induced changes in the metabolism and elimination of salicylates.

MATERIALS AND METHODS

General treatment of animals. Male Wistar rats, weighing 50–100 g initially and fed *ad lib.* a Purina Chow diet, were used to induce cirrhosis. Liver

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cirrhosis was produced by intraperitoneal administration of 0.15 ml of CCl_4 in mineral oil three times a week for 8 weeks. The ratio of CCl_4: oil used was 1:7 in the first week, 1:6 in the second week, 1:5 in the third week, and 1:4 thereafter. The cirrhotic rats were divided into two groups. While CCl_4 administration continued for 4 more weeks, one group received no additional treatment, whereas colchicine was given to the other group (10 μg/rat per day p.o. 5 days a week). Two additional groups of rats that served as controls received i.p. injections of equivalent doses of mineral oil instead of CCl_4. Colchicine was added to one of these groups according to the schedule mentioned before.

Previous experiments published by us and other authors have proven that this scheme of CCl_4 administration consistently produces the biochemical and histological patterns that characterize liver cirrhosis [18, 19].

Metabolism of ASA. For 48 hr after the end of the aforementioned treatments, ten animals of each group were kept in metabolic cages and allowed free access to food and water. The urine of the first 24 hr was collected for blank determinations, and at the end of this time ASA (20 mg/kg) dissolved in 1 ml of water was administered by means of an intragastric tube.

A further collection of urine was made for the 24-hr period following ASA administration. The total volume of urine was measured, and aliquots were taken for the determination of the total amount of salicylates eliminated and for the separation of salicylate metabolites by thin-layer chromatography performed on silica gel HF-254 precoated plates. Solvents used for development were benzene: diethyl ether: acetic acid: methanol, 120:60:8:2, by vol. The spots visualized under u.v. light were scraped off for thorough extraction with water. Salicylate and its metabolites were measured as described by Trinder [23]. The identification of each spot was carried out by comparison with the R_f values of the pure standards developed in parallel. The determination of salicylate glucuronides required a hydrolytic step as described by Trinder [23]. The identity of glucuronates was confirmed in pilot experiments by incubation with β-glucuronidase by the method of Fishman *et al.* [24].

Determination of enzyme activities. Seventy-two hours after the end of the general treatments, separate sets of ten animals of each group were bled by heart puncture under light diethylether anesthesia, serum was separated, and the livers were removed quickly. Livers were homogenized in phosphate buffer, pH 7.4, and centrifuged at 15,000 $g \times 30$ min at 4°; the supernatants containing the microsomal fraction were used as enzyme source.

ASA-esterase acvitity was measured in both sera and microsomes containing fraction of livers by the method of Spenney [25]. The activities of aniline hydroxylase and UDP-glucuronyltransferase were also determined in liver fractions containing microsomes (supernatant of 15,000 g centrifugation) according to the methods described by Mazel [26] and Dutton and Storey [27] respectively.

Time–course of plasma concentration of salicylates. Additional sets of thirty rats of each group were taken 72 hr after the end of the general treatments. Each set was divided into three subsets of ten rats each for intraperitoneal administration of 20 mg/kg of either ASA, SA or GA. Blood samples were taken by ocular puncture at 0, 5, 10, 20, 30, 40, 60 and 90 min after drug injection. Total salicylate concentrations in plasma were determined by the method of Trinder [23], and the individual pharmacokinetic parameters were obtained by adjusting the data to a one-compartment open model assuming an instantaneous absorption of the drug and using a program for the log-linear regression in an Apple computer.

Statistics. Statistical analysis was done with *t*-tests applied to the differences between several samples, according to the procedure for the completely randomized experimental design described by Brown and Hollander [28]. A difference was considered significant when $P < 0.05$.

RESULTS

The metabolites collected in the urine over 24 hr after ASA administration accounted in all animals for virtually 100% of the administered dose. Those metabolites were salicylic acid, gentisic acid, salicyluric acid and glucuronates. In the rats treated only with mineral oil the cumulative excretions (expressed as percent of the dose, mean ± SD) were: glucuronides, 36 ± 2%; salicyluric acid, 30 ± 2%; gentisic acid, 18 ± 2%; and salicylic acid, 13 ± 1%. An almost identical pattern was observed in the group treated only with colchicine. CCl_4-Cirrhotic rats showed a virtual lack of the glucuronides in urine which compensated for the increased ($P < 0.001$) amount excreted as gentisic acid and salicylic acid. Colchicine treatment of cirrhotic rats for 1 month partially reversed these alterations (Fig. 1).

Table 1 shows the data of the enzymatic activities determined in serum and liver. ASA-Esterase activities in both liver and serum were augmented ($P < 0.001$) in the cirrhotic group and were returned to normal by colchicine co-treatment. UDP-Glucuronyltransferase activity in the liver did not change with any treatment; conversely, aniline hydroxylase activity determined in the same fraction was reduced significantly in the cirrhotic animals, and this function was also improved by colchicine.

As can be seen in Table 2, the plasma half-lives of total salicylates after the injection of ASA, SA and GA were shortened significantly ($P < 0.001$) in the cirrhotic rats, and colchicine treatment again was effective in reversing those alterations. The time–course of total salicylates in plasma after an intraperitoneal injection of either ASA, SA, GA followed first-order kinetics which fitted well to a one-compartment open model ($r^2 > 0.95$ in all cases) (Fig. 2). There were no differences between the half-lives of salicylates after injection of either ASA or SA, whereas the half-life of salicylates after GA was significantly shorter in all the groups (Table 2). The apparent volume of distribution was similar in all the groups (data not shown).

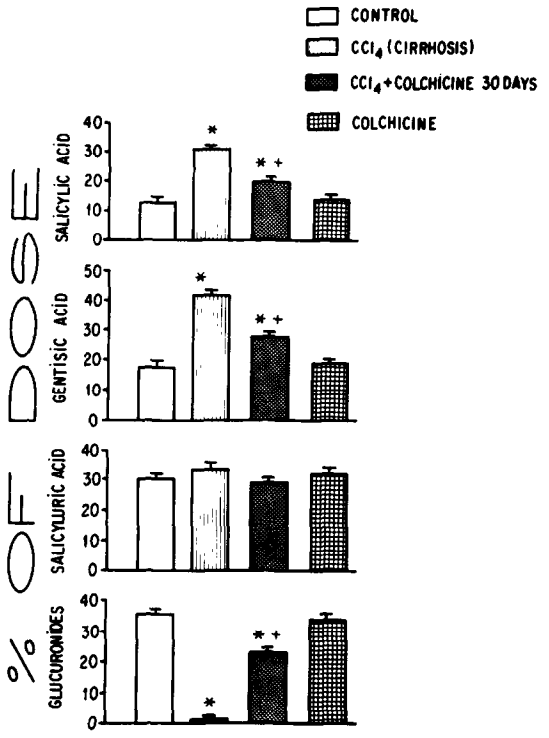

Fig. 1. Cumulative excretion of salicylic acid and its metabolites recovered in the urine over 24 hr after an oral dose of ASA (20 mg/kg). The results are expressed as percent of the administered dose and represent the mean ± SD of ten animals in each group. Key: (*) significantly different from control, $P < 0.005$; and (†) significantly different from CCl_4-cirrhotic group, $P < 0.005$.

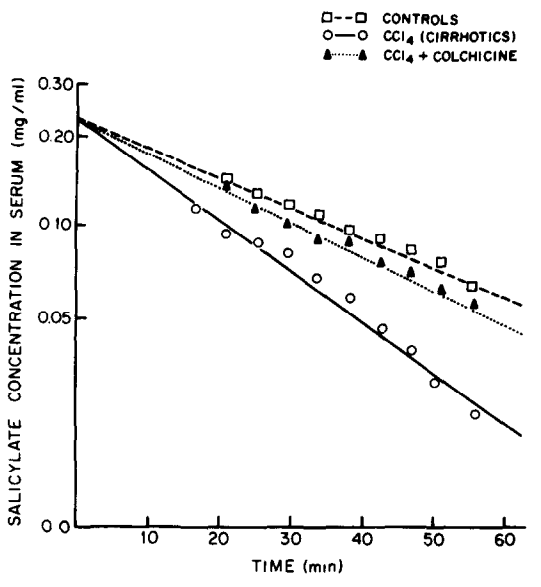

Fig. 2. Time–courses of serum salicylate levels in representative animals of different groups after the intraperitoneal injection of acetylsalicylic acid (20 mg/kg).

DISCUSSION

In the cirrhotic animals, there was a remarkable contrast between the enzymatic activities determined in the microsome-containing fraction and the profile of urinary metabolites. On one hand, the basal glucuronyltransferase activity was preserved and yet the urinary excretion of glucuronates was virtually abolished. On the other hand, the *in vitro* activity of aniline hydroxylase in the same group was reduced significantly, although gentisic acid excretion was actually augmented. This apparent paradox provides a further illustration of the heterogeneity of the changes in drug metabolism and disposition that occur in liver cirrhosis.

Desmond and co-workers [29] showed that the acute exposure of rat livers to CCl_4 leads to a reduction of the total amount of glucuronidase activity (after detergent solubilization of the microsomal fraction) although the basal activity is even enhanced [29].

In the conditions of our assay, we measured the basal glucuronidase activity. It is therefore possible that the total amount of the enzyme could have been reduced and this, rather than the basal activity, could be more relevant for salicylate glucuronidation *in vivo*.

We cannot exclude that the reduced formation of glucuronates *in vivo* may reflect an extensive decrease in liver blood flow during chronic liver injury as well as a change in the arrangement of the intrahepatic circulation, i.e. portal:systemic shunting which has been thoroughly documented in pre-

Table 1. Enzymatic activities determined in serum and liver fractions of rats

Group	ASA-Esterase		UDP-Glucuronyl-transferase	Aniline hydroxylase
	Liver ($nmol \cdot mg^{-1} \cdot min^{-1}$)	Serum ($nmol \cdot ml^{-1} \cdot min^{-1}$)	Liver ($nmol \cdot mg^{-1} \cdot min^{-1}$)	Liver ($ng \cdot mg^{-1} \cdot min^{-1}$)
Control	1.90 ± 0.30	14.40 ± 1.00	0.084 ± 0.008	1.70 ± 0.27
CCl_4 (cirrhotics)	2.90 ± 0.60* ($P < 0.001$)	27.80 ± 3.00* ($P < 0.001$)	0.075 ± 0.007	0.55 ± 0.05* ($P < 0.001$)
CCl_4 + colchicine 30 days	1.80 ± 0.25	12.80 ± 0.90	0.081 ± 0.007	1.58 ± 0.07
Colchicine	1.80 ± 0.30	13.90 ± 2.00	0.079 ± 0.008	1.68 ± 0.22

Values are the means of separate determinations in duplicate assays from ten different animals ± SD.
* Compared to control group.

Table 2. Half-lives of total salicylates after the intraperitoneal injection of either acetylsalicylic acid (ASA), salicylic acid (SA) or gentisic acid (GA)

Group	$T_{1/2}$ (min)		
	ASA	SA	GA
Control	35.0 ± 1.0	35.0 ± 0.8	12.4 ± 0.5
CCl_4 (cirrhotics)	19.0 ± 1.0* ($P < 0.001$)	18.5 ± 0.9* ($P < 0.001$)	9.0 ± 1.0* ($P < 0.001$)
CCl_4 + colchicine 30 days	32.0 ± 1.0	32.0 ± 0.9	12.5 ± 0.7
Colchicine	26.0 ± 0.8	35.0 ± 1.0	13.0 ± 1.0

Each value represents the mean ± SD from ten animals.
* Compared to control group.

vious studies [5, 30]. There has been described a differential effect of changes in hepatic blood flow on the intrinsic clearance of drugs depending on whether they are of low or high extraction [31]. Moreover, the increased concentration of unconjugated bilirubin which presumably is present in the cirrhotic liver with cholestasis may compete for glucuronidation with salicylates, thus decreasing the net rate of salicylate glucuronidation in spite of the unaffected basal activity of the enzyme found *in vitro*. Gentisic acid formation, on the other hand, may be less sensitive to changes in hepatic blood flow and, although the specific activity of the enzyme involved in its formation could be reduced, the concentration of salicylates that reached the liver could have been well below the saturation level for the enzyme and, therefore, the spared capacity of the liver to metabolize salicylates by this route was enough to compensate for the otherwise decreased formation of glucuronates, accounting in that way for the increased excretion of gentisic acid in the cirrhotic rats.

It is remarkable that, in contrast with other drugs [6], salicylates showed no changes in the volume of distribution during liver failure, that the changes in the half-lives of salicylates reflected a change in the total body clearance. This, in turn, could be increased in cirrhotic animals mainly at the expense of a higher rate of urinary excretion by glomerular filtration of unbound metabolites. Additionally, it was evident that gentisic acid has a shorter half-life than salicylic acid and perhaps the increased formation of gentisic acid in the cirrhotic animals could contribute to the observed decrease of the half-life of salicylates.

The ASA-esterase activity in liver and plasma was increased in cirrhotic animals. However, the normal rate of hydrolysis of ASA is so high compared to that of the other reactions that its contribution to the overall changes is unlikely to be important.

In addition to the well known effects of colchicine on some biochemical and histological indicators of experimental and clinical cirrhosis [17–19], it also partially reserves some of the observed pathological changes in drug metabolism and disposition. This seems more likely to be associated with the general improvement of the liver condition rather than being a specific effect on drug-metabolizing systems, since in the scheme of treatment given in this study colchicine alone had no effects on salicylate metabolism and disposition. Colchicine prevents the modifications in lipid membrane composition of the hepatocyte induced by CCl_4 [18, 19, 32, 33]. This effect might be particularly relevant to the functioning of transport and enzyme systems related to drug metabolism.

We have extended the observations made by other authors [11, 34–44] that the pharmacokinetic alterations found in chronic liver diseases cannot simply be correlated to the degree of dysfunction of the corresponding enzyme systems measured *in vitro*, but represent a complex interaction among alteration in liver hemodynamics, protein binding, and the alternative routes of elimination. For highly lipophilic compounds whose excretion is critically dependent upon their hepatic biotransformation into more polar metabolites, we can expect a greater impact of liver disease on the half-life of elimination, whereas in the case of salicylates, and perhaps some other instances, the parent compounds are polar enough to guarantee their more or less rapid elimination by the kidney. Thus, no change or even a decrease in the plasma half-lives of this family of compounds can be found.

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