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Effect of Carbon Tetrachloride on Hepatic Transport of Ouabain in Developing Rats (39634)

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Newborn rats are immature in their ability to excrete drugs by the liver. For example, Klaassen (1) observed that the biliary excretion of sulfobromophthalein (BSP) is low in newborn rats, resulting in plasma concentrations of BSP that are five times higher in newborns than in adults. Similarly, hepatic excretion of ouabain is low in young rats, and this is considered to be responsible for the high toxicity of ouabain in immature rats (2).

Although young rats have a low capacity to excrete drugs by the liver, hepatic excretory function may be enhanced in these animals by hepatic microsomal enzyme stimulators such as phenobarbital, pregnenolone-16α-carbonitrile, and spironolactone (3). These agents also enhance biliary function in adult rats, but the stimulation observed in adults is qualitatively different from the effect in young rats (3). The enhancement of biliary function in adult rats involves increases in bile flow and transport of drugs (such as ouabain) from liver to bile. Thus, stimulation with these agents results in reduced concentrations of ouabain in plasma and liver. In contrast, the principle effect of these microsomal enzyme stimulators in young rats is to increase the transport of ouabain from plasma into liver such that young animals have less ouabain in plasma but higher concentrations in liver (3). These results suggest that stimulation of the hepatic excretory mechanism is possible at two sites within the hepatocyte; enhancing uptake (sinusoidal) and secretion (canalicular).

It was of interest to determine whether the "site" of altered biliary function following acute liver damage was also age dependent. Carbon tetrachloride (CCl_4) is an agent widely used to produce experimental liver damage in laboratory animals (4) and biliary function is depressed following CCl_4 poisoning as indicated by retention in plasma of drugs administered secondarily. The effect of CCl_4 on hepatic drug transport however is not specific. Altered transport of both anions (5-8) and neutral compounds (9) by the liver has been demonstrated following CCl_4 although these compounds are transported by separate mechanisms (10).

Liver slices and isolated perfused livers from CCl_4-treated adult rats showed no differences in BSP uptake, but biliary excretion of BSP was decreased following CCl_4 (5). Retention of BSP in plasma following CCl_4 was primarily due to decreased biliary excretion associated with decreased bile flow (6). Ouabain (neutral compound) retention in plasma was accompanied by accumulation of the drug in the liver (9). These results are consistent in that CCl_4 appears to have a specific effect on the transport of drugs from liver to bile and does not appear to alter the uptake of drugs from plasma into liver.

However, CCl_4 treatment may result in a reduction in BSP uptake in isolated perfused rat livers when measured soon after poisoning (7). Furthermore, a decrease in the maximal removal rate of indocyanine green (ICG) from plasma has been observed following CCl_4 where hepatic uptake was exclusively measured (8). Thus, the results suggest that liver damage may be quantitated at two sites within the hepatocyte.

The effect of CCl_4 on hepatic transport may be age dependent and the purpose of studies described in this paper was to determine the effect of CCl_4 on ouabain transport in developing rats. The hepatic transport of ouabain was chosen as the index of biliary function because ouabain is known to be actively transported by rat liver, is not metabolized appreciably in the rat (11), and is transported to a lesser extent following CCl_4 (9).

Materials and methods. Lactating female rats with litters of eight male offspring

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(Spartan Research Animals, Inc., Haslett, Mich.) were housed in shoe box cages and were allowed food and water *ad libitum*. Offspring were separated from mothers at 4 weeks of age.

Rats of 14, 20, 24, 32, and 45 days of age received a single ip injection of corn oil (5 ml/kg) or CCl_4 (1 ml/kg) prepared in corn oil. This dose of CCl_4 is known to alter biliary function in adult rats (6, 9). The administration of CCl_4 across litters was arranged so that both control and CCl_4-treated animals were obtained from each litter. Twenty-four hours following administration of CCl_4, animals were lightly anesthetized with ether and injected with [^{3}H]-ouabain (1 mg/kg) via the tail vein. The specific activity of [^{3}H]ouabain was 250 μCi/mg and was prepared by mixing nonradioactive ouabain (Sigma Chemical Co.) with randomly labeled [^{3}H]ouabain (New England Nuclear). Thirty minutes following administration of ouabain, rats were anesthetized with ether, a blood sample was taken (with a heparinized syringe) by cardiac puncture, and the entire liver and small intestine were rapidly removed. The 30-min time period was chosen to compare the effect of CCl_4 in developing animals directly with previous reports on the effect in adults (9).

Since young animals are too small for collection of bile, the amount of radioactivity in the intestine was measured to estimate biliary excretion of ouabain (3). The intestine was homogenized in 5 or 10 ml of water and a 200-μl aliquot of whole homogenate was added to 15 ml of Dimilume counting solution (Packard Instrument Co.). Samples of liver (100 mg) and plasma (200 μl) were placed in scintillation counting vials and solubilized overnight at 40° with 1 ml of Soluene-100 (Packard Instrument Co.) before addition of 15 ml of Dimilume counting solution. Radioactivity in all samples was estimated with a Packard Model 3380 liquid scintillation spectrometer equipped with automatic external standard for quench correction.

Statistical analysis of data were made by analysis of variance (12) and the level of significance was chosen as $P < 0.05$.

Results. The effect of CCl_4 on hepatic transport of ouabain in rats of various ages is shown in Fig. 1. CCl_4 treatment resulted in significantly higher plasma concentrations of ouabain at every age tested. Coincident with the retention of ouabain in plasma, CCl_4-treated animals excreted less ouabain

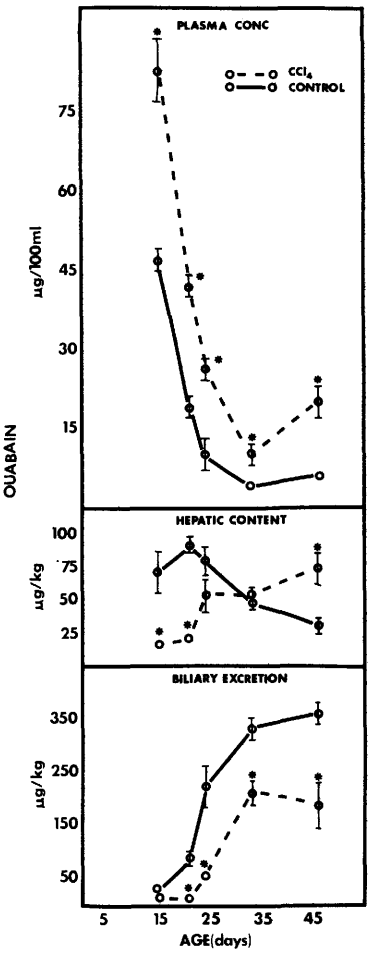

FIG. 1. Rats of ages ranging from 14 to 45 days were injected ip with corn oil or CCl_4 (1 ml/kg) prepared in corn oil. After 24 hr, rats were injected with [^{3}H]ouabain (1 mg/kg) via the tail vein. Thirty minutes following ouabain, animals were sacrificed following cardiac puncture, and plasma, liver, and intestine were analyzed for tritium. Hepatic and intestinal ouabain content are factored by kilograms body weight. Each point represents the mean (± SE) of five to eight rats obtained from two or three litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. * indicates that values are significantly different from corresponding control ($P < 0.05$).

into bile. The lower amount of intestinal (biliary) ouabain was significant except in 15-day-old animals.

Of particular interest is the effect of CCl_4 on hepatic concentration of ouabain. In young animals (less than 25 days of age), CCl_4 treatment resulted in significantly less ouabain in the liver, suggesting impaired uptake. In contrast, 46-day-old CCl_4-treated animals had significantly more ouabain in the liver. Inasmuch as the 46-day-old group retained ouabain in plasma and excreted less into the intestine, the high concentration of ouabain in liver is due to impaired transfer of ouabain from liver to bile.

Although ouabain was retained in plasma of 25- and 33-day-old treated rats and less intestinal ouabain was detected in these animals, no significant differences were observed in the hepatic concentration of ouabain (Fig. 1).

Discussion. The major effect of CCl_4 on hepatic transport of ouabain in adult rats was a decrease in the transport of ouabain from liver to bile (9). The data presented here are consistent with this conclusion in that the CCl_4-induced retention of ouabain in plasma was accompanied by accumulation of the drug in liver in 46-day-old rats (Fig. 1). This effect, however, appears to be age dependent. Indeed, treated rats younger than 25 days of age could not effectively accumulate ouabain from plasma into liver (Fig. 1).

From the data presented in this study it would be difficult to ascertain the mechanism for the age difference. One possibility may be that CCl_4 acts at a different "site" on the parenchymal cell in young rats than in adults. This would appear to be true, since the effect of CCl_4 on hepatic concentration of ouabain is exactly opposite in rats of different ages.

Since it is believed that CCl_4 toxicity is mediated through a toxic metabolite (13–15) and young animals are immature in their ability to metabolize drugs (16, 17), the toxicity of CCl_4 may be expected to be age dependent. From this explanation, however, one might expect a difference in the dose of CCl_4 required to elicit toxicity but not in the "site" of damage. It is important to note that the dose of CCl_4 used in this study (1 ml/kg) was enough to cause retention of ouabain in plasma but did not result in death to animals at any age tested. Furthermore, although newborn (1-day-old) rats are resistant to CCl_4 toxicity, Dawkins (18) observed that the 7-day-old rat appeared to be as sensitive as the adult to the hepatotoxic action of CCl_4. Since the youngest rat used in this study was 15 days old, the age difference in the qualitative effect of CCl_4 on ouabain transport was probably not due to differences in the extent of CCl_4 toxicity.

In adults, CCl_4-induced drug retention in plasma could be partly due to decreased hepatic uptake. Indeed, for organic anions (e.g., BSP and ICG), depressed uptake was observed following CCl_4 (7, 8). However, BSP and ouabain retention in plasma (in adults) is thought to be mainly due to decreased hepatic secretion into bile (6, 9). Nevertheless, CCl_4-induced hepatic damage is not specific and appears to affect both sinusoidal and canalicular transport depending upon the experimental design or the test compound used. Retention of ICG in plasma following CCl_4 was due to depressed uptake under experimental conditions designed to measure only uptake (8). Furthermore, in adult rats, the rate-limiting step in BSP transport is hepatic secretion, not uptake (19, 20) which accounts for the observation that retention of BSP in plasma following CCl_4 was primarily due to decreased biliary excretion (6). Similarly, in adult rats, hepatic extraction of ouabain is six times that for BSP (21) which would make detection of an alteration in ouabain uptake following CCl_4 unlikely.

The results of this investigation demonstrate that the effect of CCl_4 in young rats is qualitatively different from the effect observed in adults. These results are similar to observations made by Klaassen (3) on the effect of hepatic stimulators on ouabain transport in developing rats. Since the biological responses (liver damage with CCl_4 vs hepatic microsomal enzyme stimulation with phenobarbital) induced with these chemicals are markedly different, age may be an important variable in characterizing the effect of drugs on biliary function. That is, like CCl_4, phenobarbital may affect the

molecular correlate of both hepatic uptake and biliary secretion of drugs in all ages, but the developmental status of the animals is important in determining the *in vivo* characterization of the response. The present results suggest that at some time during postnatal development (approximately 33 days) there is a shift in the rate-limiting step in the hepatic transport of drugs and that experimental treatments may simply act to exaggerate age differences that already exist.

Summary. CCl_4 depressed hepatic excretory function in adult and developing rats. In this regard, plasma ouabain concentrations were significantly higher and biliary (intestinal) ouabain was significantly lower in the treated animals. Of particular interest was the observation that hepatic ouabain was significantly lower than controls in CCl_4 treated young rats (decreased uptake into liver), but significantly higher than controls in treated older rats (decreased secretion from liver into bile). These results suggest that the "site" of altered biliary function following CCl_4-induced liver damage is age dependent.

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