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Age-Related Pharmacokinetics of Ouabain in Rats[1] (41397)

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Abstract. The pharmacokinetics of ouabain was studied in 2- and 6-month-old rats in an attempt to determine the factors responsible for the marked difference in the plasma disappearance of ouabain from rats of these two ages. Biliary excretion of the two groups was also compared. Plasma concentration of ouabain in older rats was significantly higher than that of the young rats. The plasma concentration data for both groups were fitted to the triexponential equation $C_t = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$. A significant increase was observed in both B and the area under the plasma concentration verses time curve (AUC) in the older rats, while significant decreases were observed in the mean plasma clearance and volume of distribution. The slope of the terminal phase (β) was similar in both groups. No significant difference was observed in the cumulative biliary excretion or the biliary excretory rate. The age-associated difference in plasma elimination of ouabain appears to be due to a difference in volume of distribution rather than decreased biliary excretion of ouabain.

Recently the importance of aging on drug disposition has been emphasized in patients (1–3) and in experimental animals (4–8). These differences in drug disposition may result from progressive physiological changes in metabolism, excretion, tissue distribution, and blood flow. These alterations would be especially important for cardiac glycosides since they have a narrow margin of safety between therapeutic and toxic levels. Children and immature animals have been reported to have a different sensitivity to digitalis than adults (9–16). This may be due to age-related differences in both the pharmacokinetics and pharmacological effects of the drug. Glantz *et al.* (6) have demonstrated an age-related difference in the pharmacokinetics of ouabain in dogs which may be due to alteration of its volume of distribution. Kitani *et al.* (7) noted a marked difference in the plasma disappearance and biliary excretion of ouabain between young rats (3 months old) and older rats (6, 18, and 24 months old). They suggested that differences in bile production may be important for the age-related effects.

The purpose of this study was to determine the pharmacokinetics of ouabain in 2- and 6-month-old rats in an attempt to elucidate the factors responsible for this age-related difference.

Materials and Methods. Adult Sprague–Dawley (Bio-Lab., White Bear, Minn.) male rats (270–550 g) were divided into the two groups, i.e., 2 months old (the younger group) and 6 months old (the older group). The average body weights of the two groups were 290 ± 4 and 512 ± 13 g. The rats were anesthetized with ethyl carbamate (urethane, 1000 mg/kg, ip). The femoral vein and artery were cannulated with polyethylene tubing (PE-50) for drug administration and blood sampling, respectively. The common bile duct was cannulated with PE-10 tubing and bile was sampled at 5-min intervals. The rectal temperature of the rats was maintained at 37°C with a heat lamp. The injection was made over 30-sec intervals using a 250-μl syringe.

[^{3}H]Ouabain (12 Ci/mmole) and nonlabeled ouabain (analytical A grade) were purchased from New England Nuclear Corporation, Boston, Massachusetts, and Sigma Chemical Company, St. Louis, Missouri, respectively. Ouabain was dissolved in physiological saline and mixed with

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[^{3}H]ouabain before administration (0.5 mg/kg). The concentration of [^{3}H]ouabain in the plasma was determined in a Packard Tri-Carb counter (Packard Instruments Corporation, Downers Grove, Illinois) after an aliquot (50 – 100 μl) was placed in a scintillation vial containing 10 ml of 3a70B scintillation fluid (Research Products International Corp., Elk Grove, Ill.). Bile volume was measured gravimetrically using 1.0 as the density of bile. [^{3}H]Ouabain in bile (25-μl aliquot) was quantitated as described above.

Pharmacokinetic parameters were calculated by a nonlinear iterative least-squares method (SALS; (17)) with a digital computer. Area under the concentration time curve (AUC), mean plasma clearance (Cl_p), and volume of distribution (Vd_β) were determined from the constants of the triexponential equation using conventional equations (18).

Statistical analysis was performed using Student's t test with $P = 0.05$ as the minimal level of significance.

Results. The rapid disappearance of ouabain from plasma after iv administration to 2- and 6-month-old rats is shown in Fig. 1. The plasma concentration of ouabain in older rats was significantly higher than that in the younger rats, especially during the early time periods. The plasma concentration data of ouabain for both groups were fitted to the triexponential equation $C_t = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ and the derived pharmacokinetic constants are listed in Table I. In the older rats, a significant increase in both B and AUC was observed while decreases were observed in mean plasma clearance (Cl_p) and Vd_β. The slope of the terminal phase (β) was similar in the two groups.

The biliary excretion of ouabain is shown in the two panels of Fig. 2. No significant difference was observed between the two groups in either biliary excretory rate or cumulative biliary excretion. About 55% of

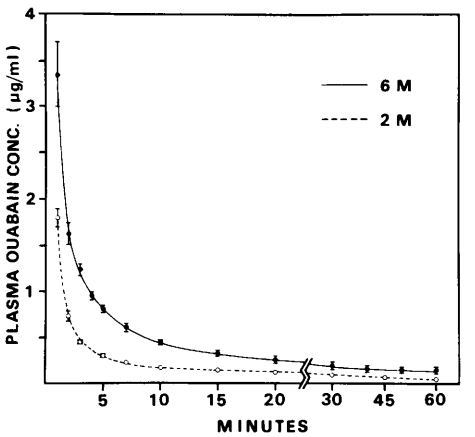

FIG. 1. Plasma disappearance curves of ouabain after intravenous injection of 0.5 mg/kg. 6M (6 months old), 2M (2 months old). Each point and vertical bar represents the mean and SE of four rats.

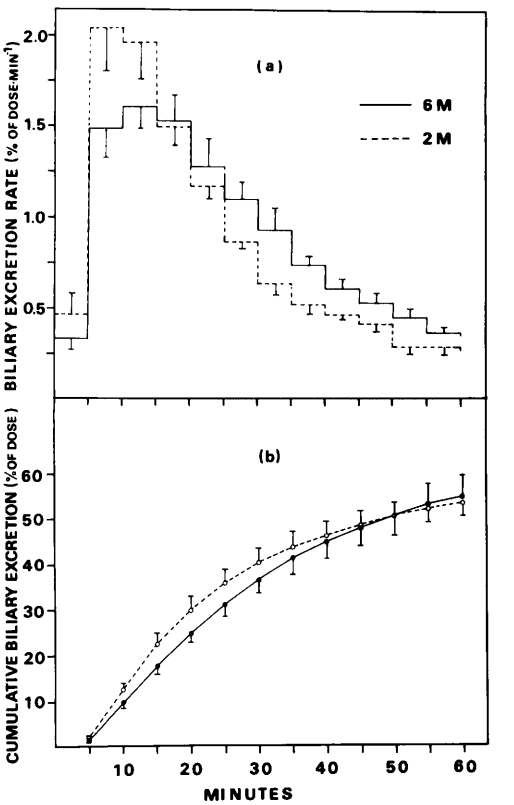

FIG. 2. Biliary excretion of ouabain after intravenous injection of 0.5 mg/kg. Panel (a) represents biliary excretion rate. Solid line represents 6M (6 months old), while broken line represents 2M (2 months old). Panel (b) represents cumulative biliary excretion curves. Each point and vertical bar represents the mean and SE of four rats. (*) Significantly different from the younger rats ($P < 0.05$).

ouabain was excreted into bile by both groups of rats during the first 60 min after administration. The average bile flow rates for younger and older rats were significantly different, 64.9 ± 2.7 (mean ± SE, n = 5) and 29.3 ± 2.0 (n = 5) μl min^{-1} kg^{-1}, respectively).

Discussion. Newborn rats are more sensitive to ouabain than older rats (13, 14). The newborns have a six times longer plasma half-life of ouabain and a decreased biliary excretory rate. This age-associated difference was recently demonstrated using isolated hepatocytes to be due to low hepatic uptake capacity of neonate rats (19). In contrast to those studies in newborn rats, Kitani *et al.* (7) have reported differences in plasma elimination and biliary excretion of ouabain between 3- and 6-month-old rats.

In the present study, we examined the pharmacokinetics of ouabain in 2- and 6-month-old rats and found a significant difference in the volume of distribution (Vd_β) with no change in the elimination rate constant (β) (Table I). These results suggest the differences between the young and older rats is not due to altered elimination but rather a difference in the volume of distribution of ouabain, as has been suggested for dogs (6).

The plasma disappearance of ouabain is very rapid and about 90% of the dose is actively excreted into bile without biotransformation (20). In this study no significant difference in biliary excretory rate or in cumulative biliary excretion was shown between the two age groups. Thus, it is unlikely that the higher plasma concentration of ouabain in the older rats is due to a decreased biliary excretion ability, even though the average bile flow rate for the younger rats was significantly greater (about two times).

Two possible explanations for the age-associated difference in the volume of distribution were suggested by previous investigators. One is the age-associated differences in the size of various body fluid spaces which would be especially important for ouabain because it binds only slightly to plasma proteins. Glantz *et al.* (6) demonstrated that the plasma volume, interstitial fluid, extracellular fluid, and total body water were all significantly greater, per kilogram of body weight, in young dogs, which most likely accounts for the age-associated volume of distribution differences. The other suggestion is that the age-associated difference may be related to the amount of Na^+-K^+ ATPase, the main ligand for the tissue distribution of cardiac glycosides (21). Age-related differences in Na^+-K^+ ATPase concentration have been reported for the rat brain and spinal cord (22), as well as for guinea pig and mice myocardial tissue (23, 24). Kroening and Weintraub (24) reported that different concentrations of Na^+-K^+ ATPase with age had an effect on tissue distribution, red blood cell distribution, and uptake by heart tissue slices of [^{3}H]digoxin.

Although rats are relatively insensitive to

TABLE I. PHARMACOKINETIC PARAMETERS FOR OUABAIN

Parameter	2 months (n = 4)	6 months (n = 4)
P (μg·ml^{-1})	8.47 ± 1.74	21.0 ± 11.4
A (μg·ml^{-1})	0.77 ± 0.20	1.97 ± 0.48
B (μg·ml^{-1})	0.19 ± 0.04	0.42 ± 0.07*
π (min^{-1})	1.94 ± 0.311	2.44 ± 0.69
α (min^{-1})	0.318 ± 0.070	0.291 ± 0.053
β (min^{-1})	0.023 ± 0.003	0.027 ± 0.005
AUC (μg·ml^{-1}·min)	14.9 ± 1.12	33.1 ± 8.56*
Mean plasma clearance (Cl_p) (ml·min^{-1}·kg^{-1})	34.1 ± 2.40	18.2 ± 4.31*
Vd (ml·kg^{-1})	1460 ± 248	559 ± 69.9*

* Significantly different from the younger rats ($P < 0.05$).

cardiac glycosides (25), the pharmacologic action of these drugs in many species is influenced by age. The age-related differences in pharmacokinetics may be important in explaining the different sensitivity of young and old animals to cardiac glycosides.

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