Biliary Excretion of Conjugated Hydroxyl Benzodiazepines after Administration of Several Benzodiazepines to Rats, Guinea Pigs, and Mice

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Abstract □ The extent of biliary excretion of diazepam, *N*-demethyldiazepam, *N*-methyloxazepam, and oxazepam was studied in rats, guinea pigs, and mice. The intravenous administration of diazepam and *N*-demethyldiazepam was not followed by the excretion of these compounds or the corresponding hydroxylated free metabolites *N*-methyloxazepam and oxazepam, respectively, in the bile of the three animal species. In rats, no conjugated derivatives were detected in the bile; in guinea pigs and mice, 3 and 4% and 13 and 33% of diazepam and *N*-demethyldiazepam, respectively, were recovered as conjugated oxazepam. The injection of *N*-methyloxazepam resulted in a lower excretion of conjugated *N*-methyloxazepam and oxazepam in rats than in guinea pigs or in mice. When oxazepam was injected intravenously, conjugated oxazepam was 6.5 and about 10 times higher in the bile of guinea pigs and mice, respectively, than of rats. The fact that hydroxylated metabolites are excreted as conjugated derivatives more by mice and guinea pigs than by rats may suggest the hypothesis of a more extensive enterohepatic circulation of these compounds in mice and guinea pigs than in rats.

Keyphrases □ Benzodiazepines, conjugated hydroxyl—biliary excretion in rats, guinea pigs, mice □ Biliary excretion—conjugated hydroxyl benzodiazepines in rats, guinea pigs, mice □ Diazepam and *N*-demethyldiazepam—biliary excretion in rats, guinea pigs, mice □ Oxazepam and *N*-methyloxazepam—biliary excretion in rats, guinea pigs, mice

Diazepam is metabolized through at least two different pathways, N_1-demethylation and C_3-hydroxylation (1–3). The two metabolites *N*-demethyldiazepam and *N*-methyloxazepam are metabolized through hydroxylation and *N*-demethylation, respectively, to form the final common metabolite oxazepam (4). The extent of these processes is different according to the animal species both *in vitro* (5, 6) and *in vivo* (7, 8).

To consider all the factors involved in the metabolism of benzodiazepines, it was of interest to measure the excretion of these drugs in the bile. The conjugated derivatives of hydroxylated benzodiazepines possess a molecular weight close to the one considered the threshold for biliary excretion. It has been suggested (9) that conjugated metabolites (glucuronic acid, sulfate, or glycine derivatives) below a molecular weight of around 400 are mostly excreted through the urine, while above that weight they may be partially eliminated through the bile. Since the metabolism and the plasma half-life of benzodiazepines differ according to the various species, the biliary excretion was studied in rats, guinea pigs, and mice.

MATERIALS AND METHODS

Male Sprague-Dawley rats (body weight 200–250 g.), male albino guinea pigs (body weight 300–350 g.), and male Swiss mice (body weight 30–32 g.) were used in all experiments. The rats and mice were anesthetized with an intraperitoneal injection of urethan, 1.25 g./kg. (solution 25% w/v).

The guinea pigs first received a light ether anesthesia for the time of the surgical operation; then the injection of the benzodiazepine was enough to maintain the animals immobile.

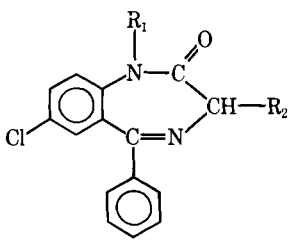
Each animal received, by intravenous injection, 5 mg./kg. of the drug under study (diazepam, *N*-demethyldiazepam, *N*-methyloxazepam, and oxazepam)[1] dissolved in a solvent containing propylene glycol, glycofurol, benzyl alcohol, and water (30:30:2:48). Control animals received an equivalent volume of this solvent. No significant changes of the biliary excretion were observed with control animals compared to benzodiazepine-treated animals. At least four animals were used for each drug.

The bile duct was surgically exposed by a midline incision and cannulated with a polyethylene tube (0.10–0.20 mm.). The surgical condition was the same in the rats, guinea pigs, and mice, except that in mice and guinea pigs the gallbladder was excluded before the cannulation of the bile duct. The muscle and the skin were sewed in two separate layers, and the bile was collected for 3 hr. Since it has been shown (10) that hypothermia develops under the conditions of these experiments, a heating lamp was used over each animal to maintain a normal body temperature.

The pH of an aliquot of the collected bile was adjusted to 7.2 and the amount of unconjugated benzodiazepine was determined. Another aliquot of the bile was incubated for 1 hr. at 37° and pH 4.5 with β-glucuronidase and arylsulfatase from *Helix pomatia*[2] (50 μl./ml. of bile), and the benzodiazepines were determined again. With these experimental conditions, a complete hydrolysis of the glucuronides of the hydroxylated metabolites was accomplished. Diazepam and its metabolites were measured by GLC as previously described (11).

RESULTS AND DISCUSSION

Table I summarizes the results obtained. The intravenous administration of diazepam and *N*-demethyldiazepam is not followed by the excretion of these compounds or the hydroxylated derivatives (*N*-methyloxazepam and oxazepam) in the bile of rats and guinea pigs. In rats, no conjugated derivatives were detected in the bile; in guinea pigs and mice, 3 and 4% and 13 and 33% of diazepam and *N*-demethyldiazepam, respectively, were recovered as conjugated oxazepam.



chemical structure of diazepam and its metabolites

diazepam: $R_1 = CH_3$, $R_2 = H$
N-demethyldiazepam: $R_1 = H$, $R_2 = H$
N-methyloxazepam: $R_1 = CH_3$, $R_2 = OH$
oxazepam: $R_1 = H$, $R_2 = OH$

[1] Drugs used in this work were kindly supplied by U. Ravizza, Muggiò, Milan, Italy.
[2] Biochemia.

Table I—Biliary Excretion of Conjugated Hydroxylated Benzodiazepines in the Rat, Guinea Pig, and Mouse[a]

Drug Administered (5 mg./kg. i.v.)	Percent of Administered Dose ± *SE*					
	N-Methyloxazepam			Oxazepam		
	Rat	Guinea Pig	Mouse	Rat	Guinea Pig	Mouse
Diazepam	—	—	—	—	3.0 ± 0.8	13.1 ± 1.9
N-Demethyldiazepam	—	—	—	—	4.0 ± 0.7	33.4 ± 3.9
N-Methyloxazepam	8.5 ± 0.2	20.8 ± 1.1	9.9 ± 1	Traces	8.1 ± 0.8	23.0 ± 0.8
Oxazepam	—	—	—	5.3 ± 0.4	34.7 ± 3.4	49.7 ± 5.3

[a] The figures are the averages of four determinations and represent the conjugated forms present in the bile collected during 3 hr. after drug administration. No diazepam or *N*-demethyldiazepam, in the free or conjugated form, was found in the bile and no free *N*-methyloxazepam or oxazepam was found after administration of the four benzodiazepines. The method permits detection of amounts higher than 0.15% of the administered benzodiazepines.

The administration of *N*-methyloxazepam resulted in a lower excretion of conjugated *N*-methyloxazepam and oxazepam in rats than in mice or in guinea pigs. When oxazepam was given by the intravenous route, the excretion of conjugated oxazepam was 6.5 and about 10 times higher in the bile of guinea pigs and mice, respectively, than of rats. The low biliary excretion of administered oxazepam in the rat may indicate a more rapid biotransformation of this compound in the rat than in the other two species, according to previous studies (4). The method utilized allows for the detection of the benzodiazepines at a level not lower than 0.15% of the administered dose.

As far as the nature of the conjugated hydroxylated benzodiazepines, other studies in progress have established that they are mostly glucuronides. The fact that the anesthesia was different in the guinea pigs was not significant, because when guinea pigs were anesthetized with urethan the biliary excretion of conjugated oxazepam after administration of oxazepam was similar to the excretion obtained with ether anesthesia. The excretion of conjugated oxazepam in guinea pigs after administration of diazepam or *N*-demethyldiazepam was interesting, because these two compounds are not hydroxylated by guinea pigs liver microsomal preparations (12) and oxazepam was detected only in traces *in vivo* in blood, brain, or adipose tissue (13). In the three species considered, the order of importance in the biliary excretion of C_3-hydroxylated benzodiazepines is: mice > guinea pigs > rats. It is remarkable that the blood and tissue half-life after oxazepam administration follows the same order as far as species is concerned, suggesting that the enterohepatic circulation of oxazepam may be an important factor in sustaining oxazepam blood levels.

In rats, unlike guinea pigs and mice, oxazepam can be methylated on the hydroxyl group and the seven-member ring can be broken (14).

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