Biliary Excretion of [^{14}C]Temazepam and its Metabolites in the Rat

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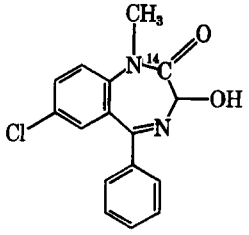
Abstract □ The excretion of temazepam and its *N*-desmethyl metabolite, oxazepam, and their respective *O*-conjugates was examined following a single intravenous dose of [^{14}C]temazepam to two groups of bile fistula rats, with and without bile replenishment to the animals *via* duodenal cannulae. During an 8-hr collection period, the two groups produced virtually identical bile volumes, and there were no significant differences between them in the amount of total radioactivity, free temazepam, or the identified metabolites in the bile, as determined by TLC and liquid scintillation counting. Elimination of the radioactive dose was rapid during 0–8 hr, with a half-life of ~1 hr. Approximately 85–90% of the administered radioactivity was recovered in the bile: <1% as free temazepam, 3% as oxazepam, and ~10% as their *O*-conjugates.

Keyphrases □ Temazepam—^{14}C-labeled, biliary excretion metabolites in the rat □ Excretion, biliary—[^{14}C]temazepam and its metabolites in the rat □ Metabolism—biliary excretion of [^{14}C]temazepam in the rat

Temazepam (7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one) is a benzodiazepine hypnotic agent recently introduced in the United States. The pharmacokinetic profile of temazepam in humans as well as in mice, rats, and dogs have been described (1). Orally administered temazepam is well absorbed in all species studied. However, there is considerable interspecies variation with respect to drug excretion and metabolite patterns in blood and urine. In humans, temazepam is completely metabolized prior to excretion, with 80% of the dose eliminated in the urine and 12% in the feces. The major metabolite is its *O*-conjugate with glucuronic acid, while *N*-desmethyl temazepam (oxazepam) and its *O*-conjugate have also been identified. In animal models, particularly the rat, biliary excretion appears to play a more important role, with as much as 78% of the dose recovered in the rat feces. Therefore, the effect of possible enterohepatic circulation must be considered in the interpretation of pharmacokinetic data obtained from these animal species.



[^{14}C]temazepam

Previous studies using radiolabeled drug in the rat (1) have shown that free temazepam and oxazepam constitute ~40% of the radioactivity in blood, while neither these compounds nor their *O*-conjugates appears in more than trace quantities in the urine. Similar information on the excretion of temazepam in bile is not available. Since the rate and extent of GI reabsorption depend in part on the nature of the compound excreted in the bile, the present study was undertaken to examine the extent of biliary excretion of temazepam and its known metabolites in rats with bile fistula, following a single intravenous dose of the parent compound. Furthermore, the possible effects of bile depletion due to bile duct cannulation on bile flow and biliary excretion of the drugs were also investigated.

EXPERIMENTAL

Animal Preparation—Six male Wistar strain rats[1], average weight ~250 g, were equally divided into two groups, A and B. The bile duct of each rat was cannulated proximal to the liver under light ether anesthesia. In addition, a silicone tube[2], 0.019-cm i.d., was inserted into the duodenum of each rat in group A opposite the sphincter of Oddi and secured by ligation with a surgical silk suture. The tubings were passed subcutaneously and the distal end exteriorized through a stab incision at the back prior to closure of the abdomen with sutures.

The rats were housed individually in special metabolism cages with covers that allowed passage of the cannulae (2). The cover also served as a restraint for the rat, while allowing some freedom of movement inside the cage.

The duodenal cannulae of the rats in group A were subsequently connected *via* 0.013-cm i.d. silicone pump tubes[3] to a peristaltic pump[4]. Previously collected control rat bile was pumped into these rats at a rate of 0.9 ml/hr, approximately the rate of bile secretion of rats of this size.

Dosing and Sample Collection—The radioactive temazepam[5] (labeled at the 2-position of the benzodiazepin structure with carbon-14, specific activity 52.25 μCi/mg) was diluted with nonradioactive drug to a final specific activity of 26.13 μCi/mg. The [^{14}C]temazepam dose, 2 mg/kg, was prepared as a 2.5 mg/ml solution in a phosphate buffer (pH 7.3) containing 40% polyethylene glycol 400 and 10% ethanol. After recovering from anesthesia, each rat received 0.2 ml of the solution as a single, rapid injection *via* the tail vein. Food and a 5% solution of glucose in Ringer's solution were provided *ad libitum*.

The bile of each rat was collected quantitatively at hourly intervals for 8 hr postdosing. All bile samples were stored frozen until analyzed.

Analysis of Radioactivity—Radioactivity was measured in a liquid scintillation counter[6]; the quench correction and efficiency of the counter were determined using ^{14}C-labeled hexadecane[7] of known specific activity as an internal standard. The bile and dose preparations were assayed directly by counting aliquots in a scintillation cocktail consisting of 2,5-bis-2-(5-*tert*-butylbenzoxazolyl) thiophene[8] in toluene (8.3 g/liter).

Thin-Layer Chromatography—Free temazepam and oxazepam were determined by TLC on silica gel[9]. A 1–2 μl bile sample was applied to the chromatographic sheet, which was subsequently developed in ethyl acetate–methanol–ammonium hydroxide (15:4:1). Standard solutions obtained by adding temazepam and oxazepam to control bile were developed concurrently, and the R_f values of these two compounds were determined by visualization under UV light (254 nm)[10].

The sample strip was cut into eight 1.5-cm sections and placed indi-

[1] Royal Hart, Middletown, N.Y.
[2] Acculab, Norwood, N.J.
[3] SMA Flow-Rated Pump Tubes, Technicon Instruments Corp., Tarrytown, N.Y.
[4] Technicon Auto-Analyzer II Peristaltic Pump, Technicon Instruments Corp., Tarrytown, N.Y.
[5] Synthetic Tracer Laboratory, Sandoz, Inc., East Hanover, N.J.
[6] Model 2450, Packard Instrument Co., Downers Grove, Ill.
[7] Packard Instrument Co., Downers Grove, Ill.
[8] BBOT, scintillation grade, Packard Instrument Co., Downers Grove, Ill.
[9] Silica Gel 13181 with fluorescent indicator, Eastman Kodak Co., Rochester, N.Y.
[10] Chromato-Vue, Ultra Violet Products, San Gabriel, Calif.

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Table I—Mean Bile Volume and Biliary Excretion of Administered Radioactivity in Rats with (group A) and without (group B) Bile Replenishment

Time Period, hr	Bile Volume, ml[a]		Percent of Dose[b]	
	Group A	Group B	Group A	Group B
0–1	0.93 ± 0.058[c]	0.87 ± 0.21	52.4 ± 8.7	43.9 ± 2.5
1–2	0.88 ± 0.076	0.85 ± 0.13	18.5 ± 1.6	23.3 ± 2.5
2–3	0.82 ± 0.029	0.83 ± 0.15	8.0 ± 1.1	10.8 ± 3.6
3–4	0.83 ± 0.12	0.85 ± 0.26	3.5 ± 0.62	5.5 ± 2.3
4–5	0.83 ± 0.058	0.82 ± 0.25	1.8 ± 0.57	2.9 ± 1.7
5–6	0.87 ± 0.058	0.80 ± 0.17	0.97 ± 0.23	1.6 ± 1.2
6–7	0.87 ± 0.12	0.70 ± 0.17	0.46 ± 0.11	0.93 ± 0.61
7–8	0.90 ± 0.10	0.73 ± 0.15	0.31 ± 0.076	0.57 ± 0.29
0–8	6.9 ± 0.34	6.5 ± 1.5	85.9 ± 7.7	89.5 ± 6.2

[a] No significant differences between groups, $p > 0.05$. [b] No significant differences between groups, $p > 0.05$, except the 1–2 hr period, $p < 0.05$. [c] Standard deviation, $n = 3$.

Table II—Mean Biliary Excretion (0–3 hr) of Temazepam and Some Metabolites in Rats with (group A) and without (group B) Bile Replenishment

Compound	Percent of Dose[a]	
	Group A	Group B
Temazepam	0.5 ± 0.3	0.5 ± 0.2
Temazepam Conjugate	4 ± 7	3 ± 4
Oxazepam	3 ± 0	3 ± 1
Oxazepam Conjugate	5 ± 4	7 ± 4
Total Identified Compounds	13 ± 10	12 ± 9

[a] No significant differences between groups, $p > 0.05$.

vidually in scintillation vials. The ^{14}C-labeled drug or metabolite was eluted by the addition of 5 ml of methanol. The scintillation cocktail (15 ml) was then added and the radioactivity determined by direct counting in a liquid scintillation counter. Thus, the radioactivity from the zones corresponding to temazepam and oxazepam, as well as total radioactivity in the bile sample, were obtained.

Similarly, total (free plus conjugated) temazepam and oxazepam were determined by the above procedure after enzymatic hydrolysis of the glucuronate or sulfate conjugates. Aliquots (100 μl) of bile were hydrolyzed by diluting with 100 μl of pH 5.2 acetate buffer, adding an excess (20 μl) of a mixture of enzymes including β-glucuronidase and sulfatase[11], and incubating at 37° for 2 hr.

Statistical Analysis—The effects of bile depletion on bile flow and biliary excretion of temazepam and its metabolites were examined by comparing the data obtained from the two groups of rats, using the two-tailed t test.

RESULTS

The mean bile flow and biliary excretion of administered radioactivity, together with statistical analysis, are given in Table I. There were no significant differences in the volume of bile produced by the two groups of rats during each hourly interval. The secretion rate appeared constant during 0–8 hr; total bile volumes produced in this period were 6.9 and 6.5 ml for rats with and without bile replenishment, respectively.

In the rats with bile replenishment *via* duodenal cannulae (group A), 52% of the radioactive dose was recovered in the bile in 1 hr, and the excretion rate gradually decreased to 0.3%/hr during 7–8 hr. Total biliary excretion in 8 hr was 86% of the dose. Similar results were obtained from the rats without bile replenishment (group B); comparison by t tests showed no signficant differences in the biliary excretion of the two groups during all but one (1–2 hr) collection interval. Total 8-hr biliary excretion in group B was 90% of the administered radioactivity.

Linear regression analysis of the logarithmic values of the excretion rates in Table I *versus* the midpoint of the time periods yielded overall elimination rate constants of 0.73 hr^{-1} ($r = -0.99$) for group A and 0.63 hr^{-1} ($r = -1.00$) for group B (3). These corresponded to half-lives of 1.0 and 1.1 hr, respectively, for the rats with and without bile replenishment.

Since nearly 80% of the dose in both groups was excreted in the bile during the first 3 hr postdosing, only these bile samples were analyzed for temazepam, oxazepam, and their conjugates. TLC analysis enabled separation of temazepam and oxazepam from other metabolites on the sheet; R_f values were 0.61 for temazepam and 0.50 for oxazepam. Thus, the relative amounts of temazepam, oxazepam, and their conjugates in each bile sample were calculated and, combined with the data in Table I, yielded the biliary excretion of each compound as a percent of the dose. The mean 0–3-hr cumulative excretion, together with statistical analysis, are shown in Table II.

Biliary excretion of free temazepam constituted <1% of the dose, while an additional 3–4% was found as temazepam conjugate. The *N*-desmethyl metabolite, oxazepam, and its conjugate in the bile accounted for 3 and 5–7% of the dose, respectively. Thus, only 12–13% of the dose was recovered in the bile as these four identified compounds. Again, there were no significant differences in the excretion of each compound by the two groups of rats.

DISCUSSION

Previous experiments in the rat have shown that the depletion of bile caused by bile duct cannulation could result in a 10- to 20-fold increase in bile salt synthesis and a decrease in the amount of bile excreted; these changes occurred within 1–2 weeks (4). The potential effects of such changes in the biliary excretion and recycling of drugs are well recognized, and sophisticated instruments have been designed for enterohepatic circulation studies in order to eliminate these artifacts (5). In the present study, however, comparison of data obtained from bile fistula rats without bile replenishment *versus* those from the rats with duodenal cannulae showed no significant differences between the two groups in the rate of bile secretion, probably due to the relatively short collection period. Thus, the results would suggest that the loss of bile has little effect on experiments of short duration (<1 day). Also, the excretion of total radioactivity, free temazepam, and the identified metabolites in the bile are unaffected by bile depletion.

Results of this study confirmed the biliary route as the major excretion pathway of temazepam in the rat. Furthermore, although temazepam, oxazepam, and their *O*-conjugates accounted for nearly 50% of the radioactivity in blood (1), they were present in much smaller proportions in the bile of rats. Less than 1% of the [^{14}C]temazepam dose was recovered unchanged, the rest extensively metabolized prior to rapid biliary excretion. Nevertheless, the clinically inactive metabolites (6) may be reverted, *e.g.*, through deconjugations, to the parent compound in the GI tract (7) and subsequently reabsorbed, thus contributing to the overall effectiveness of the temazepam dose. The demonstrated importance of the biliary excretion route warrants further studies to determine the significance of enterohepatic circulation for this drug.

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[11] Glusulase, Endo Laboratories, Garden City, N.Y.