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Enterohepatic circulation of radioactivity following an oral dose of [^{14}C]temazepam in the rat

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The enterohepatic circulation of radioactive material after administering [^{14}C]temazepam was evaluated in three sets of male Wistar strain rats connected in pairs by bile duct-duodenum cannulae. After a single oral dose (10 mg kg^{-1}) to the donor rat, the excretion of radioactivity in the urine and faeces of both rats and in the bile of the recipient rat was determined. Mean total recovery of the administered radioactivity was 92·2%. Based on the amount remaining in the donor rat (gastrointestinal tract and faeces), 81·7% of the dose was absorbed by the donor. The total amount recovered from the recipient, 69·4% of original dose (85·1% of donor's absorbed dose), represented the amount excreted in the donor's bile. Similarly, 54·1% of the original dose (77·9% of the transferred biliary excretion from donor) was reabsorbed by the recipient, and the biliary excretion from this animal (45·9% original dose) accounted for 86·% of the amount reabsorbed.

The pharmacokinetics of the benzodiazepine hypnotic agent temazepam in man and animals have been summarized (Schwarz 1979). While the animal models are useful in predicting the absorption efficiency of the drug in man, they show vast differences from man with respect to drug elimination pathways. In man, approximately 80% of a radioactive dose is excreted in the urine, 70% as temazepam conjugates (glucuronate or sulphate), whereas in animals, particularly the rat, biliary and faecal excretion are predominant.

A recent study in the rat (Tse et al 1983) showed that 85–90% of an intravenously administered dose of [^{14}C]temazepam was excreted in the bile within 8 h post-dosing. Less than 1% of the dose was recovered as temazepam, 3% as the *N*-desmethyl metabolite oxazepam, and ca 3 and 7% as the conjugates of temazepam and oxazepam, respectively, as determined by thin-layer chromatography and liquid scintillation counting. The remainder were unidentified metabolites believed to be pharmacologically inactive (Fuccella et al 1977). However, the metabolites may be changed back to the parent drug in the gastrointestinal tract (Boxenbaum et al 1979) and subsequently reabsorbed, thus contributing to the overall effectiveness of temazepam. No quantitative information on the biliary recycling of temazepam is currently available.

The present report describes the enterohepatic circulation of drug-related material after administering [^{14}C]temazepam in male Wister strain rats linked in pairs by bile duct-duodenum cannulae. The disposition characteristics after single oral doses to donor rats were studied by determining the excretion of radioactivity in the urine and faeces of both rats (donor and recipient) and in the bile of the recipient rat.

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MATERIAL AND METHODS

Animal preparation

Male Wistar strain rats (Charles River), average weight ca 250 g, were used. Bile duct cannulation was performed under ether anaesthesia in two rats. In addition, a silicone tubing (Acculab), 0·019 cm i.d., was inserted into the duodenum of the rat designated the recipient, and secured by ligation. The tubings were run subcutaneously and the distal end exteriorized through an incision at the back, before closure of the abdomen with sutures. The duodenal cannula of the recipient rat was subsequently connected via a 0·013 cm i.d. Silastic SMA flow rated pump tube to a peristaltic pump (Technicon Instruments Corp.).

The bile from the rat designated the donor was collected in a 10 ml graduated centrifuge tube. After a reservoir of 1 ml had accumulated, the bile was pumped into the duodenum of the recipient rat at 0·9 ml h^{-1} (approximately the rate of bile production by rats of this size, Tse et al 1983).

Three pairs of rats were so prepared. The animals were housed individually in special metabolism cages which allowed the rats freedom of movement while permitting complete collection of excreta from both donor and recipient (Tse et al 1983). Food and a 5% solution of glucose in Ringer solution were freely available.

Table 1. Excretion of radioactivity by the donor after receiving a 10 mg kg^{-1} oral dose of [^{14}C]temazepam.

	Time (h)	Percent of dose: Rat pair no. 1	2	3	Mean ± s.d.
Urine	0–8	0	2·9	0·1	1·0±1·6
	8–24	2·9	0·4	2·7	2·0±1·4
	24–48	0·3	**	0·1	0·1±0·2
	Total	3·2	3·3	2·9	3·1±0·2
Cage wash		0·4	0·4	0·9	0·6±0·3
Faeces	0–8	**	**	0	0
	8–24	**	0	0·2	0·1±0·1
	24–48	**	**	0·1	0 ±0·1
	Total	0	0	0·3	0·1±0·2
Stomach		24·3	19·3	6·7	16·8±9·1
Small intestine	At	0·9	1·9	0·2	1·0±0·9
Large intestine	death	0·1	0·9	0·3	0·4±0·4
G.I. tract total		25·3	22·1	7·2	18·2±9·7
Body cavity Fluid	At death	**	2·4	**	0·8±1·4
Bile reservoir wash		0	0·4	0·2	0·2±0·2

** No sample.

Dosing and sample collection

The radioactive temazepam (Sandoz, Inc., labelled at the 2-position of the benzodiazepine structure with carbon-14, specific activity 52·25 μCi mg^{-1}) was diluted with non-radioactive drug to a final specific activity of 5·23 μCi mg^{-1}. The [^{14}C]temazepam dose, 10 mg kg^{-1}, was prepared as a 2·5 mg ml^{-1} suspension in 0·5% aqueous carboxymethylcellulose. After the rats had recovered from anaesthesia, and the transfer of bile had started, 1 ml of the suspension was administered by gavage to each donor.

Bile, urine, and faeces were collected at designated intervals for 48 to 72 h post-dosing, depending on the survival time of the rats. Each cage was washed with ethanol and water after the final collection, and the cage wash was retained. At the end of the study period, the entire gastrointestinal tract was removed. All samples were stored frozen until analysed.

Analysis of radioactivity

Radioactivity was measured in a liquid scintillation spectrometer (Model 2450, Packard Instrument Co.). The bile, urine, and cage wash were assayed directly by counting aliquots in a scintillation cocktail consisting of 2,5-bis-2(5-t-butylbenzoxazolyl)thiophene in toluene (8·3 g litre^{-1}). Faecal and tissue homogenates were air-dried and combusted in a sample oxidizer (Model 306, Packard Instrument Co.). Dose preparations were assayed by both the direct and combustion methods. The quench correction and efficiencies of the oxidizer and counter were determined using ^{14}C-labelled hexadecane of known specific activity as an internal standard.

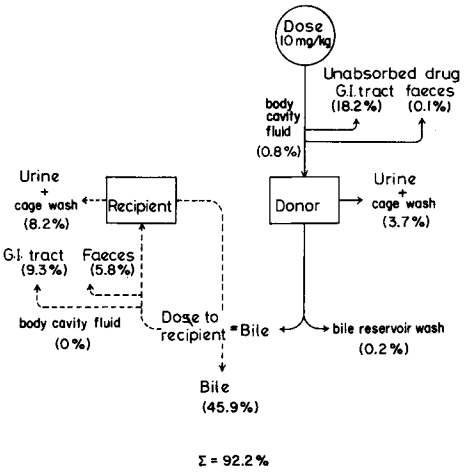

FIG. 1. Enterohepatic circulation of radioactivity in a pair of bile duct-duodenum cannula-linked rats after an oral dose of [^{14}C]temazepam to the donor.

Enterohepatic circulation

From the individual data from each rat pair, the enterohepatic circulation of temazepam was examined quantitatively as described by the equations below Table 4. It was assumed that no significant drug-related material existed in the tissues at the conclusion of the study, as supported by the virtually complete recovery of the administered radioactivity (>92%, Fig. 1).

RESULTS

The excretion by the donor rats is shown in Table 1 and the biliary excretion of radioactivity and the excretion in urine and faeces by the recipient rats in Tables 2, 3. The mean data were calculated to the time of death* which in all cases was 19 h or greater. A diagrammatic description of the mean data from the rat pairs is given in Fig. 1.

After an oral dose of [^{14}C]temazepam to the donor rat, 3·1% of the administered radioactivity was excreted in the urine. The rate of urinary excretion declined from 0·1% h^{-1} during the 0–8 h interval to 0·01% h^{-1} between 24 and 48 h. A relatively small amount of radioactivity was recovered in the faeces, 0·1% in 48 h. At the time of death, 18·2% of the original dose was present in the gastrointestinal

* Rat pair 1: D, 46 h; R, 46 h. Rat pair 2: D, 19 h; R, 72 h. Rat pair 3: D, 48 h; R, 52 h.

Table 2. Biliary excretion of radioactivity by the recipient after a 10 mg kg^{-1} oral dose of [^{14}C]temazepam to the donor.

Time (h)	Percent of dose: Rat pair no. 1	2	3	Mean ± s.d.
0–2	0	1·0	0·1	0·4 ± 0·6
2–4	0·2	0·5	1·7	0·8 ± 0·8
4–6	0·9	1·0	3·4	1·8 ± 1·4
6–8	1·1	2·3	2·7	2·0 ± 0·8
8–24	19·5	39·8	23·3	27·5 ± 10·8
24–death	22·8	4·6	12·7	13·4 ± 9·1
Total	44·5	49·2	43·9	45·9 ± 2·9

tract, most (16·8% of original dose) remaining in the stomach.

Of the total radioactivity administered to the donor, 6·5% was recovered in the urine of the recipient rat. In these rats the urinary excretion rate increased from 0·03% h^{-1} (0–8 h) to 0·2% h^{-1} (8–24 h) and subsequently decreased to 0·08% h^{-1} (24–48 h). Faecal excretion in recipient rats accounted for 5·8% of the original dose. A much larger portion of the original dose (45·9%) appeared in the bile of the recipients. At the time of death, the gastrointestinal tract of recipients contained 9·3% of the original radioactivity, the bulk of which was in the large intestine.

At post-mortem the donor of rat pair 2 and the recipient of rat pair 3 showed the presence of fluid in the body cavity. Analysis for radioactivity showed that the former contained 2·4% of the original dose, while the latter had 0·1%.

The total recovery of radioactivity in the excreta and the gastrointestinal tract at the time of death was 92·4% for rat pair 1, 95·6% for pair 2, and 88·6% for pair 3.

As shown in Table 4, 81·7% of the oral dose to the donor rat was absorbed during the sampling period, and 85·1% of that absorbed dose was excreted in the bile. In the recipient rat, 77·9% of the radioactivity in the bile transferred from the donor (54·1% of the original dose) was reabsorbed. Finally, 86·0% of the reabsorbed radioactivity was again excreted in the bile.

DISCUSSION

In the present study, three pairs of rats were linked so that the absorption and biliary excretion of temazepam could be monitored through two cycles. Initial attempts to introduce bile from the donor directly into the duodenum of the recipient rat via polyethylene tubing were unsuccessful, apparently

Table 3. Urinary and faecal excretion of radioactivity by the recipient after a 10 mg kg^{-1} oral dose of [^{14}C]temazepam to the donor.

	Time (h)	Percent of dose: Rat pair no. 1	2	3	Mean ± s.d.
Urine	0–8	0·1	0·1	0·4	0·2 ± 0·2
	8–24	0·6	2·6	6·2	3·1 ± 2·8
	24–48	0·2	0·6	5·1	2·0 ± 2·7
	48–death	**	0·2	3·3	1·2 ± 1·9
	Total	0·9	3·5	15·0	6·5 ± 7·5
Cage wash		1·2	0·7	3·3	1·7 ± 1·4
Faeces	0–8	0	**	0	0
	8–24	**	**	**	0
	24–48	4·3	6·0	**	3·4 ± 3·1
	48–death	**	7·0	**	2·3 ± 4·0
	Total	4·3	13·0	0	5·8 ± 6·6
Stomach		0·1	0	0·1	0·1 ± 0·1
Small intestine	At	2·5	0	0·5	1·0 ± 1·3
Large intestine	death	10·0	0·6	14·2	8·3 ± 7·0
G.I. tract total		12·6	0·6	14·8	9·3 ± 7·6
Body cavity fluid	At death	**	**	0·1	0 ± 0·1

due to constriction of the cannula within the duodenum resulting in obstruction of bile flow. Consequently, an abnormal fraction of the dose (>80%) was excreted in the urine, a phenomenon previously observed under similar conditions by Kinugasa et al (1981). Use of the peristaltic pump and thick-walled silicone tubing for bile delivery in the present study eliminated this problem, yielding consistently reliable data both within and between rat pairs. By properly adjusting the delivery rate of the pump to mimic that of bile production, the time course of enterohepatic circulation was relatively unaltered. Although sterile techniques were not employed, the survival time for the rats was adequate to complete the experiments. Previous investigators (Greenslade et al 1980; Parker et al 1980) have applied similar methods but with a much shorter duration (24 h) than those employed here. Antibiotics were not used during or after surgery in order to avoid disturbing the microbial flora in the gut, which could alter the metabolism and reabsorption characteristics of temazepam.

An earlier study in this laboratory (Tse et al 1983) showed that bile loss from the rat has minimal effects on bile production and biliary excretion of tempazepam, at least during the first 24 h post-dosing when the bulk of radioactivity was excreted. Therefore, while the present experimental design inevitably

Table 4. Enterohepatic circulation of radioactivity in pairs of bile duct-duodenum cannula-linked rats.

Parameter	Rat pair no. 1	2	3	Mean ± s.d.
1. Donor, % of original dose absorbed	74·7	77·9	92·5	81·7 ± 9·5
2. Donor, % of original dose excreted in bile	63·5	67·5	77·3	69·4 ± 7·1
3. Donor, % of absorbed dose excreted in bile	85·0	86·6	83·6	85·1 ± 1·5
4. Recipient, % of original dose re-absorbed	46·6	53·4	62·3	54·1 ± 7·9
5. Recipient, % reabsorption of transferred biliary excretion from donor	73·4	79·6	80·8	77·9 ± 4·0
6. Recipient, % of original dose excreted in bile	44·5	49·2	43·9	45·9 ± 2·9
7. Recipient, % of reabsorbed dose excreted in bile	95·5	92·1	70·5	86·0 ± 13·6

1. Donor, percent of dose absorbed

$$= 100 - (\text{Faeces})_D - (\text{GI Tract})_D \quad \text{(eqn 1)}$$

where the parentheses indicate percent of dose present in the excreta or tissue specified, and the subscript represents donor (D) or recipient (R) rat.

2. Donor, percent of dose excreted in bile

$$= (\text{Bile Reservoir Wash})_D + (\text{Bile})_R + (\text{Urine})_R + (\text{Cage Wash})_R + (\text{Faeces})_R + (\text{GI Tract})_R + (\text{Body Cavity Fluid})_R \quad \text{(eqn 2)}$$

3. Donor, percent of absorbed dose excreted in bile

$$= (\text{eqn 2})/(\text{eqn 1}) \times 100\% \quad \text{(eqn 3)}$$

4. Recipient, percent of dose reabsorbed

$$= (\text{Bile})_R + (\text{Urine})_R + (\text{Cage Wash})_R + (\text{Body Cavity Fluid})_R \quad \text{(eqn 4)}$$

5. Recipient, percent reabsorption of transferred biliary excreta from donor

$$= (\text{eqn 4})/[(\text{eqn 2}) - (\text{Bile Reservoir Wash})_D] \times 100\% \quad \text{(eqn 5)}$$

6. Recipient, percent of dose excreted in bile

$$= (\text{Bile})_R \quad \text{(eqn 6)}$$

7. Recipient, percent of reabsorbed dose excreted in bile

$$= (\text{eqn 6})/(\text{eqn 4}) \times 100\% \quad \text{(eqn 7)}$$

In deriving the above equations, the cage wash was assumed to be due to urinary contamination and, therefore, included as a part of the adsorbed dose.

deviated from true in-vivo conditions, it offered some insight into the quantitative aspects of enterohepatic circulation. Previous studies using tritium labelled temazepam have shown that the drug is well absorbed (>80%) after a single oral dose in the rat, and that approximately 60% of the dose is excreted in the bile (Schwarz 1979). Results of the present study supported these findings. In addition, it could be shown that 77·9% of the radioactivity in the donor bile (or 54·1% of original dose) was reabsorbed into the systemic circulation of the recipient. Thus, any radioactivity excreted in the bile, not due to unchanged temazepam (<1% of the dose (Tse et al 1983)), must be in the form of soluble, absorbable metabolites or is converted to temazepam or absorbable metabolites in the gastrointestinal tract before reabsorption.

The present data also demonstrated that 86·0% of the reabsorbed radioactivity was again excreted via the bile, virtually to the same extent as the biliary excretion during the first cycle (85·1%). Thus, the enterohepatic circulation of temazepam and/or metabolites may continue for several cycles, each time losing a small fraction to the urine and faeces. Biliary excretion and the reabsorption process appear to be the predominant factors in the overall elimination of this compound.

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