Pharmaceutical Research, Vol. 7, No. 5, 1990

Report

Disposition and Metabolic Profiling of the Penetration Enhancer Azone. I. *In Vivo* Studies: Urinary Profiles of Hamster, Rat, Monkey, and Man

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Received May 22, 1989; accepted November 6, 1989

Chain-labeled ^{14}C-Azone was intravenously administered to hamster, monkey, and rat, to compare its metabolic profile with that obtained previously in humans after dermal application. Azone-derived radioactivity was excreted predominantly in the urine for both hamster and monkey, which is similar to the disposition in humans. Metabolic profiling in urine revealed extensive systemic metabolism to occur in all species studied. The main fraction of the metabolites was most polar in man, followed by rat, monkey, and hamster. Traces of the parent compound were detectable only in hamster urine. Although some of the polar major human metabolites were also present in rat urine, the animals were unsuitable for collecting metabolites of Azone observed in humans. In rats, complete cleavage of the dodecyl side chain was ruled out by administering Azone that had been labeled at two distinct positions of the molecule. Additionally, oral administration of Azone to rats resulted in the same metabolic profile as intravenous administration, indicating that gastrointestinal metabolism does not occur or is similar to systemic metabolism.

KEY WORDS: Azone; disposition; laurocapram; metabolic profiling, man, monkey, hamster, rat; penetration enhancers.

INTRODUCTION

The penetration enhancer 1-dodecylazacycloheptan-2-one (Azone, Nelson Research, Irvine, CA) has been used to promote the percutaneous absorption of a wide variety of pharmaceutical compounds, such as antibiotics (1), 5-fluorouracil (2), triamcinolone acetonide (3), metronidazole (4), and verapamil (5), mainly in *in vitro* experiments. Its toxicity is low (6), and it is nonirritant to human skin even when applied as the pure compound (7). Its percutaneous absorption through human skin is low. While over 97% of the administered dose was recovered from the skin, less than 0.5% of the dose was detected in the excreta, predominantly in the urine (8,9). In human urine at least three polar metabolites were detected (9,10), while Azone was not metabolized in the stratum corneum during skin passage (10). Because of the low percutaneous absorption of Azone in humans, it was impossible to obtain sufficient quantities of metabolites for structure elucidation. Therefore, the systemic metabolism of Azone was investigated in the hamster, monkey, and rat after i.v. injection in order to determine whether animals can be used as a source of common metabolites for their structure elucidation.

Radioactive Azone was used to trace the parent drug and its metabolites. Additionally, the impact of the route of administration and the position of the ^{14}C-label in the Azone molecule were studied in rats.

MATERIALS AND METHODS

Chemicals

1-Dodecylazacycloheptan-2-one [*dodecyl*-1-^{14}C] and 1-dodecylazacycloheptan-2-one [*carbonyl*-^{14}C] were obtained from Atomlight, North Billerica, MA, and NEN, Boston, MA, respectively. Reference to these materials is as chain- and ring-labeled Azone, respectively. The specific activity was 8.18 and 9.2 mCi/mmol for chain- and ring-labeled Azone, respectively. The radiochemical purity of the compounds was determined by isocratic RP-HLPC using a previously described system (10). The radiochemical purity was found to be 95.1 and 95.5% for chain- and ring-labelled Azone, respectively.

All other materials were of analytical grade and obtained from Merck, Darmstadt, FRG.

Animal Studies

Intravenous Administration

Chain-labeled Azone was intravenously administered to

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hamsters, monkeys, and rats. After collection all biosamples were immediately weighed and frozen at −20°C.

Hamster Study. A male Syrian hamster (*Mesocricetus auratus;* approximately 140 g) was anesthetized with i.p. pentobarbital and polyethylene cannulae were inserted into the bile duct and the femoral vein. The dosage consisted of 1.22 μmol Azone, containing 10.0 μCi chain-labeled Azone, dissolved in 0.1 ml propylene glycol, and was given as a bolus injection into the femoral vein. For a period of 24 hr, bile was collected via the cannula and urine in a beaker placed under the animal.

Monkey Study. Conscious male (two) and female (two) cynomolgus monkeys (*Macaca fasicularis*) received a bolus injection into the cephalic vein of approximately 0.15 mg Azone/kg, dissolved in 0.8 ml ethanol. As body weights varied from 3.0 to 3.9 kg, the amounts administered to the animals varied from 0.49 to 0.65 mg of Azone, i.e., 1.7–2.4 μmol, corresponding to 14.4–19.1 μCi of the chain-labeled tracer. Urine and feces were collected separately for a period of 5 days.

Rat Study. Two male Wistar rats (approximately 270 g) were anesthetized with i.p. urethane. The bile duct and the urine bladder were cannulated, while the penis was ligated to avoid loss of urine. The dosage consisted of 0.25 μmol of Azone, containing 2 μCi of the chain-labeled tracer, dissolved in 1.0 ml of Krebs–bicarbonate solution containing 4% BSA, and was administered as a bolus injection into the jugular vein. Bile and urine were collected over a period of 3 hr, after which the liver, kidneys, brains, lungs, fat, spleen, heart, and stomach were removed, and the blood was collected.

Impact of the Position of the Isotope and the Route of Administration in Rats

Chain- and ring-labeled Azone was intravenously and orally dosed to rats. Administered amounts, composition of the dosages, and study performance were similar to those for the rat study described above. In the case of oral administration, the dosing solution was injected into the stomach via a cannula.

Human Study: Dermal Application

The application of 175 μmol of pure Azone, containing 75 μCi of the chain-labeled tracer, to the volar aspect of the forearm of volunteers for 12 hr under occlusion, has been described in detail (9). In this study, urine and feces were collected for 5.5 days following administration of the dose.

Analytical Procedures

Total Radioactivity Assessment

Weighed aliquots of the urine and bile (approximately 50 mg) were pipetted into scintillation counting vials and analyzed for ^{14}C radioactivity by liquid scintillation counting (LSC) after the addition of 3 ml RiaLuma (Lumac, Landgraaf, The Netherlands), using a Packard Minaxi Tri-Carb B4450 counter (Packard Instruments, Groningen, The Netherlands).

Feces were homogenized in 2–3 vol distilled water. Weighed aliquots of the homogenate were combusted in a Harvey Biological Material Oxidizer, Model OX-200 (Bloomsfield, NJ), and the radioactive carbon dioxide was absorbed directly in 15 ml Oxifluor CO_2 (NEN, Boston, MA). The efficiency of the combustion procedure was 94.4%. Samples were analyzed for ^{14}C radioactivity using an Isocap 300 counter (TM Analytic, Chicago).

Rat organs were rinsed and homogenized in saline. Aliquots of blood or tissue homogenate were analyzed as described by Gerding *et al.* (11).

Metabolic Profiling of the Urine Samples

Animal urines were injected directly into an HPLC system described previously (9). The sample volume depended on the concentration of radioactivity and was 100 μl (hamster), 20 μl (monkey), or 50 μl (rat). Concentrations of radioactivity in human urine were too low to be directly injected into the HPLC system. Therefore, the radioactive compounds were extracted and treated as described previously (9).

Two gradient elution patterns were used: (i) a linear gradient from 100% phosphate, 0.01 *M*, pH 6.8, to 100% methanol in 60 min, followed by a methanol flush of 15 min, and a flow rate of 1.0 ml/min, for hamster urine; and (ii) a concave gradient from 100% phosphate, 0.01 *M*, pH 6.8, to 100% methanol in 60 min, followed by a methanol flush of 15 min, and a flow rate of 1.0 ml/min, for monkey, rat, and human urine.

Column effluent fractions of 1 min were collected and, after vigourous shaking with 4 ml RiaLuma, counted by LSC for 5 min or a statistical accuracy of 0.5%.

RESULTS AND DISCUSSION

The recovery and disposition of radioactivity in the urine and feces or bile after intravenous administration to the hamster, monkey, and rat are given in Table I. In the hamster and monkey, the total recovery was approximately 80%. This value was substantially lower in the rat, which is probably due to the short duration of the rat experiment (3 hr). In order to investigate a possible incomplete elimination of radioactivity within the 3 hr of the rat study, blood and organs of the rat were analyzed for remaining radioactivity. The radioactivity was found to be present mainly in the kidneys (5.4 ± 1.6% of the administered dose), liver (3.2 ± 1.1%), and blood (1.8%). Only minor amounts were retrieved in the fat, brain, spleen, heart, and lungs (less than 0.5% for each individual organ). The low amounts in the lung (0.1%) make

Table I. Recovery and Disposition of Radioactivity in Urine and Feces or Bile After Intravenous Administration of Chain-Labeled ^{14}C-Azone to the Hamster, Monkey, and Rat

Species	*N*	Recovery (%)	Disposition (%)	
			Urine	Feces/bile
Hamster	1	71.5	91.4	8.6
Monkey	4	88.1 ± 14.1	94.6 ± 5.4	5.4 ± 5.4
Rat	2	38.5 ± 22.2	77.4 ± 7.3	22.6 ± 7.3

expiration unlikely as a possible route of elimination of Azone-derived radioactivity. The total percentage of the dose retrieved in the organs and blood was 9.9 ± 0.7%, thereby increasing the total recovery of radioactivity in the rat to 48.4%. The missing radioactivity is expected to have remained in the rest of the animal.

In all species, Azone-derived radioactivity was excreted mainly by the kidneys, and urine is therefore considered the main receptacle of the metabolites. During the 3-hr rat study, over 75% of the excreted radioactivity was retrieved in the urine. This value is close to that for hexamethylene lauramide (85%), a structural homologue of Azone (12). The urinary excretion values of Azone in the hamster and monkey (>91%) closely resemble that of chain-labeled Azone-derived radioactivity after dermal application to humans, which accounted for 97.2% of the total absorbed amount.

The urinary metabolic profiles of hamster, monkey, rat, and man are shown in Figs. 1A–D. These profiles were assessed by fractionating and analyzing the column effluent for radioactivity after separation by RP-HPLC using concave gradient elution for monkey, rat, and man urines. In order to obtain a better separation of the individual hamster metabolites, a linear elution pattern was used, resulting in a shift toward shorter retention times for the medium polar compounds, whereas the retention times of the nonpolar compounds, eluting during the methanol flush, remained virtually unaffected. For example, the nonpolar parent compound, Azone, underwent a slight shift: from 63 to 65 min in the concave gradient to 60 to 63 min in the linear gradient.

No differences were observed among the radiochromatograms of the individual monkeys, rats, or humans. When qualitatively comparing the profiles of the individual species, the bulk of the metabolites is most polar in man, followed by rat, monkey, and hamster. It is remarkable that the hamster is excreting unchanged Azone, whereas in all other species Azone undergoes complete bioconversion. On the other hand, various peaks appear to be present in the profiles of more than one species. For instance, the cluster seen at approximately 40 min in the hamster urinary profile elutes after 56 min in the concave gradient elution pattern and then coincides with the least polar metabolite(s) in the monkey profile. The major peaks in the rat and monkey urine coincide as well, whereas some peaks of the human profile can also be seen in that of the rat. Rather small amounts of the monkey and hamster cluster at 56 min in the concave gradient have been detected in the human urine (9). These similarities were corroborated with other HPLC systems.

Nevertheless, it becomes clear from Fig. 1 that the animals tested cannot be used directly in the elucidation of the major human metabolites. The latter are too polar and do not occur in the animals except, perhaps, for one metabolite eluting after 14 min in the rat. Yet the animals may be suitable to provide insight in the initial routes of metabolic conversion.

Chain- and ring-labeled Azone was intravenously and orally administered to rats. Disposition in the urine was independent of the tracer and route of administration, and overall recovery was 68.8 ± 11.3 and 53.2 ± 8.1%, respectively.

The urinary metabolic profiles are shown in Fig. 2. Independent of the position of the isotope, some radioactivity was found to elute at the Azone position in the chromatograms after oral administration. However, no unchanged

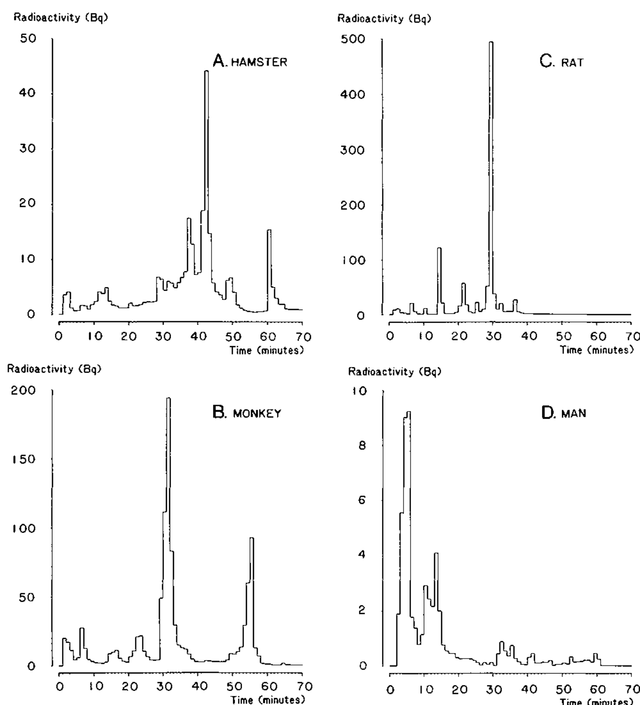

Fig. 1. Urinary metabolic profiles of chain-labeled ^{14}C-Azone-derived radioactivity following linear (A) and concave gradient elution (B–D) after i.v. administration to the hamster (0–24 hr; A), the monkey (4–12 hr; B), and the rat (60–90 min; C) and after dermal application to humans (12–24 hr; D).

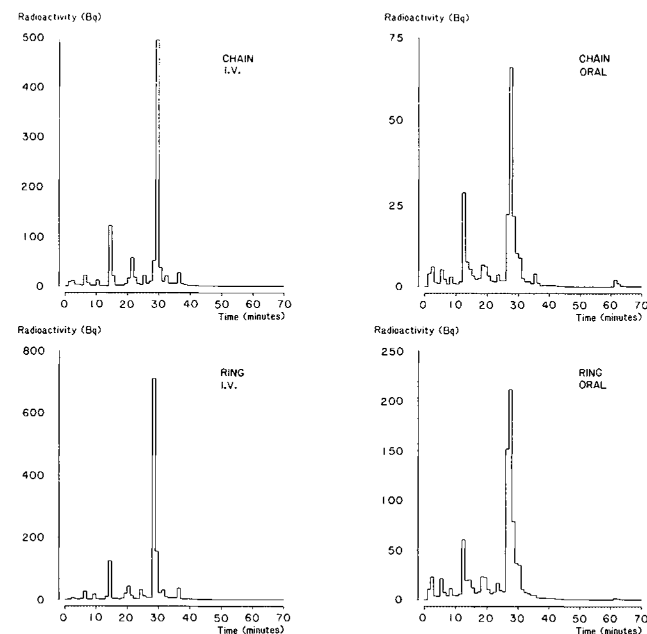

Fig. 2. Urinary metabolic profiles of ^{14}C-Azone-derived radioactivity after i.v. (left; 60–90 min) and oral administration (right; 90–120 min) of chain-labeled (top) and ring-labeled (bottom) Azone to rats.

Azone was detectable after intravenous administration. Apart from this observation and minor differences in the relative abundance of the individual peaks, the urinary metabolic profiles, after oral and intravenous administration, are essentially the same. It can therefore be concluded that in this species gastrointestinal metabolism of Azone does not occur or is similar to systemic metabolism.

Furthermore, the metabolic profile proved to be independent of the position of the label in the Azone molecule, as no differences could be seen in the profiles after using chain- or ring-labeled Azone. This result indicates that all metabolites contain the intact caprolactam ring and the α-carbon atom of the side chain, as only moieties containing the radioactive isotope are detected.

ACKNOWLEDGMENTS

The authors thank Dr. D. K. F. Meijer and Mr. R. Oosting, (Department of Pharmacology and Therapeutics) for useful discussions and assistance in performing the rat experiments, respectively. These investigators were supported in part by Nelson Research, Irvine, California.

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