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ELIMINATION OF 2′,3′,5′-TRI-*O*-ACETYL-6-AZAURIDINE IN THE RAT AND IN MAN

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Abstract—After oral administration about 45 per cent of 2′,3′,5′-tri-*O*-acetyl-6-azauridine (TA-6-azauridine) is eliminated in the urine of rats, as well as in man, in the form of its deacetylation products. In the urine of rats, TA-6-azauridine is excreted almost exclusively in the form of free 6-azauridine whereas in man a substantial amount of mono-*O*-acetyl-azauridine (MA-6-azauridine) also was found. Furthermore, about 35 per cent of the dose was found in the form of MA-6-azauridine in the faeces of rats collected during 48 hr after the administration. Neither TA-6-azauridine nor its deacetylation products are excreted in the bile of rats. 6-Azauracil was not detected as a deribosylation product of 6-azauridine neither in the urine or faeces nor in the bile of rats under study.

IN OUR previous paper[1] we studied the *in vitro* deacetylation of 2′,3′,5′-tri-*O*-acetyl-6-azauridine (TA-6-azauridine)† which is an oral formulation of the cytostatic agent 6-azauridine.‡ 6-Azauridine should not be given orally because some deribosylation by intestinal microorganisms may occur with the formation of a less active and much more toxic compound, 6-azauracil.[2] Another advantage of TA-6-azauridine which led recently to new indications for human therapy, is its more prolonged persistence in the organism as compared to intravenously administered 6-azauridine.[3]

It is known that the deacetylation of TA-6-azauridine proceeds gradually *via* di-*O*-acetyl-6-azauridine (DA-6-azauridine) and mono-*O*-acetyl-6-azauridine (MA-6-azauridine)—the position of the acetyl groups was not estimated—to free 6-azauridine as the ultimate product, which, after being phosphorylated in the organism, is held responsible for the effects of the drug.[4] Striking differences were found in the rate of deacetylation in the blood plasma of rodents and some other species including man,[1] and these led us to compare the fate of TA-6-azauridine *in vivo* in the rat and man. Because of the limited sensitivity of the chromatographic method it was not possible to determine the levels of TA-6-azauridine and its deacetylation products in the plasma with respect to the low doses (100 mg/kg) administered to psoriatic patients. For this reason our evaluation of the deacetylation intensity *in vivo* is based on the amounts of the compounds detected in the urine.

An extensive study by Williams[5] indicates that the presence of a larger polar group, coupled with a molecular weight of over 200 facilitate the excretion of drugs into

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† Proprietary name: Azaribine®.

‡ Proprietary name: Riboazauracil®.

the bile, hence we have studied in rats the elimination of TA-6-azauridine, as well as its deacetylation products, in the bile.

EXPERIMENTAL

TA-6-azauridine, 6-azauridine and 6-azauracil were obtained from Messrs Spofa, Prague. 2′,3′-Di-*O*-acetyl-6-azauridine and 5′-mono-*O*-acetyl-6-azauridine, used as standard samples of intermediate products, were synthetized in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

Six Wistar albino rats of both sexes weighing 200–250 g were administered per kilogram, orally 300 mg of TA-6-azauridine in aqueous solution by means of a stomach tube. The animals were then held in glass metabolism cages, with food and water accessible *ad lib.* The samples of urine were collected at different time intervals after the administration of the drug, and aliquots were used for chromatographic analysis. In four rats the samples of faeces were collected from the metabolic cages during 48 hr after the beginning of the experiment. After mixing the faeces with a 5-fold amount of water, the resulting suspension was centrifuged and the supernatant fraction was analyzed chromatographically.

In the scope of a controlled clinical trial evaluating the therapeutic effect of TA-6-azauridine on psoriasis,[6] portions of the urine excreted obtained at different time intervals after the ingestion of the drug from four patients (three men and one woman), each of whom was given the drug orally, 100 mg/kg in tablet form.

For estimation of the biliary excretion, the technique originally described by Bohdal and Novák[7] was used. In eight Wistar albino rats, laparotomy was performed under ether anesthesia and two polyethylene canulas (diameter 1 mm) were inserted into the *ductus choledochus*, one proximally toward the liver and the other distally toward the duodenum. After the muscle layer of the incision had been sutured both canulas were led under the skin to the neck region for exit from the body. By connecting them one to the other by means of rubber tubes and a U-formed polyethylene tube the bile was allowed to enter the duodenum whereas by disconnecting them the bile could be collected during the experiment. One week after the operation the animals were put into metabolic cages and the bile was collected at intervals of 2–6 hr, during the 24-hr period after the oral administration of 500 mg of TA-6-azauridine per kg.

Aliquots of untreated urine (0·03–0·06 ml) and of untreated bile (0·1–0·2 ml) as well as aliquots of supernatant fractions obtained from faeces (0·1–0·2 ml) were applied to chromatographic paper (Whatman No. 3). TA-6-Azauridine and its deacetylation products were separated using the solvent system *n*-butanol–acetic acid–water (10:2:5). Since the deribosylation of 6-azauridine to 6-azauracil by intestinal microflora could not be excluded the spots corresponding to MA-6-azauridine were eluted by water and rechromatographed in another system, namely isopropanol–ammonia–water (7:2:1) in order to distinguish MA-6-azauridine from 6-azauracil.

The compounds were visualized on the chromatograms in ultraviolet light utilizing standard samples of the compounds for identification. The absorbing zones were cut out, eluted by water and the amount of the compounds was calculated from the extinctions measured at 262 mμ by means of a spectrophotometer ($E_{mol} = 6120$ for 6-azauridine as well as for its acetylated derivatives and 5600 for 6-azauracil were used).

RESULTS

The excretion of TA-6-azauridine and its derivatives in the urine of rats is presented in Table 1 and that of psoriatic patients in Table 2. The total amount of the drug excreted in the urine is approximately 45 per cent of the ingested dose. It appears that only two compounds, i.e. free 6-azauridine and MA-6-azauridine were detected in the urine of both rats and men. There is, however, a marked difference in the amount of the drug excreted as MA-6-azauridine in the two species: whereas in the rat the average amount was less than 0·5 per cent of the total dose, more than a 10-fold greater percentage was found in the urine of the patients.

The bile of rats did not contain detectable amounts of either TA-6-azauridine (including its deacetylation products) or of 6-azauracil. On the other hand, the rats excreted in the faeces about 40 per cent of the total dose within 48 hr. Parallel to the findings in the urine only 6-azauridine and its monoacetylated derivative were detected in this case (Table 3). In the faeces, however, the proportion of the two metabolites were reversed: there were only traces of 6-azauridine, while the bulk of the material was MA-6-azauridine.

TABLE 1. TIME COURSE OF URINARY EXCRETION OF THE METABOLITES OF 2′,3′,5′-TRI-*O*-ACETYL-6-AZAURIDINE AFTER ITS ORAL ADMINISTRATION IN THE RAT (300 mg/kg of body weight)

Compound	Hours after administration			
	2	4	8	12
2′,3′,5′-Tri-*O*-acetyl-6-azauridine	0	0	0	0
Di-*O*-acetyl-6-azauridine	0	0	0	0
Mono-*O*-acetyl-6-azauridine	0·2 (0–0·6)*	0·3 (0–1·0)	0·4 (0–1·3)	0·4 (0–1·3)
6-Azauridine	23·0 (19·2–36·8)	38·6 (29·0–48·2)	43·3 (34·8–51·8)	43·3 (34·8–51·8)
6-Azauracil	0	0	0	0

* The values are expressed as the mean percentage of the total dose administered with limits of confidence for P = 0·95 (calculated from six rats).

TABLE 2. TIME COURSE OF URINARY EXCRETION OF THE METABOLITES OF 2′,3′,5′-TRI-*O*-ACETYL-6-AZAURIDINE AFTER ITS ORAL ADMINISTRATION IN MAN (100 mg/kg of body weight)

Compound	Hours after administration			
	2	4	8	12
2′,3′,5′-Tri-*O*-acetyl-6-azauridine	0	0	0	0
Di-*O*-acetyl-6-azauridine	0	0	0	0
Mono-*O*-acetyl-6-azauridine	6·4 (1·7–11·1)*	6·7 (1·5–11·9)	6·8 (1·7–11·9)	6·8 (1·7–11·9)
6-Azauridine	28·8 (20·2–37·4)	38·2 (27·9–48·5)	45·3 (40·1–50·5)	45·6 (36·8–54·4)
6-Azauracil	0	0	0	0

* The values are expressed as the mean percentage of the total dose administered with limits of confidence for P = 0·95 (calculated from four patients).

TABLE 3. TIME COURSE OF THE EXCRETION OF THE METABOLITES OF 2′,3′,5′-TRI-*O*-ACETYL-6-AZAURIDINE AFTER ITS ORAL ADMINISTRATION (300 mg/kg of body weight) IN THE FAECES OF RAT

Compound	Hours after administration		
	12	24	48
2′,3′,5′-Tri-*O*-acetyl-6-azauridine	0	0	0
Di-*O*-acetyl-6-azauridine	0	0	0
Mono-*O*-acetyl-6-azauridine	18·3 (8·3–28·3)*	28·8 (22·3–34·9)	38·6 (32·7–44·5)
6-Azauridine	0·7 (0·6–0·8)	1·0 (0·4–1·6)	1·4 (0·7–2·1)
6-Azauracil	0	0	0

* The values are expressed as the mean percentage of the total dose administered with limits of confidence for P = 0·95 (calculated from four rats).

DISCUSSION

The results of our experiments in rats, which show that TA-6-azauridine is excreted almost exclusively by the kidney as free 6-azauridine would speak for a much higher deacetylation rate of TA-6-azauridine occurring *in vivo* as compared to results obtained *in vitro*.[1] The higher extent of deacetylation *in vivo* is compatible with the presence of non-specific esterases in the epithelium of the intestinal mucosa[8,9] which could participate in the deacetylation of TA-6-azauridine during the absorption process.

The fact that in the absence of biliary excretion, however, not more than 45 per cent of the administered dose of TA-6-azauridine is excreted in the urine, implies that a considerable part of the drug is not absorbed. In fact, about 40 per cent of the administered dose was found in the faeces of rats within 48 hr after administration. With respect to the almost exclusive excretion of TA-6-azauridine as free 6-azauridine by the kidney in this species it seems unlikely that an active secretory process could be responsible for the transport of relatively high amounts of MA-6-azauridine from plasma into the intestinal lumen. On the other hand, if it is supposed that the absorption of TA-6-azauridine from the intestine is not complete the relatively high amounts of MA-6-azauridine in the faeces would indicate that this compound is retained in the gastrointestinal tract. According to Handschumacher *et al.*[4] TA-6-azauridine is hydrolyzed by either dilute alkali or acid, so that its deacetylation may at least partly occur in the acid environment of the stomach as well as in the essentially neutral milieu of the gut. Another possibility is the deacetylation through the activity of the intestinal microflora, which finds strong support in the recent investigations of Scheline[10–13] which demonstrate the role of intestinal microorganisms in the decomposition of a variety of drugs and organic compounds. Fully consistent with this view is the deribosylation of 6-azauridine when administered orally[2] and the capacity of *Lactobacilli* to deacytylate thiacetazone.[14] Nevertheless, 6-azauracil was not detected in the faecal matter indicating, that the acetylation of the 6-azauridine molecule prevents deribosylation, which would lead to a much less efficient and much more toxic compound.

The excretion of TA-6-azauridine in rats almost exclusively as free 6-azauridine differs markedly from our results obtained in man, according to which a considerable proportion of MA-6-azauridine was also detected in the urine. This corresponds well

to the earlier findings of Handschumacher *et al.*[4] and Grafnetterová *et al.*[15] When the more rapid deacetylation *in vivo* is taken into account, however, the differences in the excretion patterns of TA-6-azauridine between rat and man correlate well with the results of our experiments *in vitro* where after incubation with rat blood plasma TA-6-azauridine was deacetylated more rapidly than in humans.[1]

On the other hand, it is evident that acetylation of 6-azauridine cannot be regarded merely as a formulation suitable for oral administration, because it results in important alterations in the chronic tolerance at least in the rat, thus, the chronic toxicity of TA-6-azauridine in rats is higher, as compared to that of free 6-azauridine,[16,17] but no analogous shift in the toxicity was observed in man.[6]

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