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DECREASE OF SERUM PYRIDOXAL PHOSPHATE LEVELS AND HOMOCYSTINEMIA AFTER ADMINISTRATION OF 6-AZAURIDINE TRIACETATE AND THEIR PREVENTION BY ADMINISTRATION OF PYRIDOXINE

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6-Azauridine triacetate, i.e. 2',3',5'-triacetyl-6-azauridine, generically termed azaribine, is nearly completely de-acetylated metabolically, either during or after its absorption from the intestine. Much of the 6-azauridine formed undergoes intracellular phosphorylation to yield 6-azauridine 5'-monophosphate, which functions as an inhibitor of orotidine-5'-monophosphate (OMP) decarboxylase, the terminal (and a rate-limiting) enzyme in the biosynthesis de novo of uridine-5'-monophosphate (UMP) (1,2).

Another biochemical effect of this drug, reported in our previous studies (3-8), is an interference with the metabolism of various amino acids, which results in biochemical changes resembling those observed in inborn hyper-β-alaninemia, homocystinuria and hyperhistidinemia.

A unifying hypothesis to account for these changes is the inhibition of functions that involve pyridoxal phosphate, a coenzyme utilized in the metabolism of the amino acids that is altered after the oral administration of azaribine (7,8). Although several studies have indirectly supported our hypothesis (8,9), evidence for interference with the levels of pyridoxal phosphate, as a result of the oral administration of azaribine, has been lacking.

This preliminary report, broadening our previous observation (10), presents evidence that highly significant changes in levels of pyridoxal phosphate in the serum occur after the administration of azaribine to rabbits, an experimental animal model found to be predictive for the changes in the levels of amino acids caused by this drug in man (5,8). It also indicates that these changes can be prevented by simultaneous administration of pyridoxine.

New Zealand albino rabbits of both sexes, weighing 1.5 to 2.5 kg, were used in two experiments. The animals were accommodated in the Animal Resource Facility, University of New Mexico School of Medicine, 2 weeks before the start of the experiment. They were housed separately in metal cages and were kept on a standard diet of Wayne's Rabbit Ration (Allied Mills, Inc., Chicago, IL). In the first experiment, six rabbits were administered 6-azauridine triacetate and six served as control. In the second experiment, in addition

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to the control and azaribine-treated animals, a third group of six rabbits treated with azaribine and pyridoxine was included.

In both experiments, azaribine was administered for 5 days by gastric gavage in two doses daily; these doses totaled 1 g/kg body weight, of which the first half was given 6 hr before the second half. The controls were administered the same volume of drinking water by gastric gavage at the same time intervals. Pyridoxine in 10% solution, with daily doses of 50 mg/kg divided into two equal portions, was administered orally by gavage at the same time intervals with azaribine. Blood samples were taken before treatment and after the administration of each second daily portion of the drug on the third or fourth and fifth days. The animals were fasting for at least 2 hr before each blood sample was drawn. Serum samples were analyzed for pyridoxal phosphate by the enzymic assay previously described (11). Student's paired T-test was used for the statistical analysis of the changes in the concentration of pyridoxal phosphate in the serum after the oral administration of azaribine, in comparison to the pre-treatment values. Trichloroacetic acid supernatant fractions were prepared and analyzed for homocystine using the standard technique for analysis of physiological fluids on a Beckman 120 C amino acid analyzer equipped for high ($\leq$ 0.2 nMole/ml) sensitivity (12). Differences in the incidence of homocystinemia ($\leq$ 0.2 nMol/ml) during azaribine treatment were statistically evaluated using the χ^2 test.

In our first experiment, azaribine caused a rather dramatic decrease in the levels of pyridoxal phosphate in the serum, while significant changes of the coenzyme did not occur in the control group. After oral administration of azaribine, the levels of pyridoxal phosphate dropped from the pre-treatment values of 56.07 ± 17.69 ng/ml to 18.87 ± 13.77 ng/ml at 78 hr and continued to decline to the concentrations of 6.46 ± 7.99 ng/ml at 126 hr. The changes in the levels of pyridoxal phosphate at 78 and 126 hr after beginning the oral administration of 6-AzUrd-TA were statistically significant at the $P \leq 0.05$ level. In the control group of animals, the pre-treatment values of pyridoxal phosphate (53.82 ± 9.01 ng/ml) were not significantly ($P \leq 0.05$) changed, and values of 74.27 ± 19.48 and 38.17 ± 9.01 ng/ml were found 78 and 126 hr after the start of drinking-water gavage.

The results of our second experiment conducted under the same conditions are summarized in Table 1. The pre-treatment serum pyridoxal phosphate levels again decreased significantly ($P \leq 0.01$) during 102 and 126 hours of azaribine treatment, while the control group of animals again did not experience this decrease. In the group treated with azaribine and pyridoxine, a significant decrease ($P \leq 0.05$) of serum pyridoxal phosphate levels did not occur.

Table 1.

Biochemical Changes During 6-AzUrd-TA Treatment

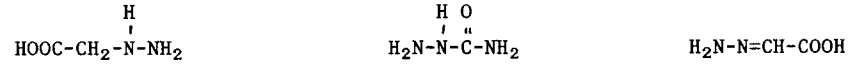
	Serum pyridoxal phosphate (ng/ml)			
	Pre-treatment	Treatment		
		Fourth day		Fifth day
Group 1 (Controls)	57.60 ±13.86	54.43 ±8.90		56.31 ±13.03
Group 2 (Azaribine)	77.72 ±17.20	29.32 ±15.45	*	7.36 ±6.24
Group 3 (Azaribine & pyridoxine)	56.80 ±7.70	72.49 ±10.08		56.00 ±12.12

*Homocystine levels ranging from 4.8 to 111.9 nMol/ml measured in half of the azaribine-treated animals.

In both experiments, homocystine levels were measured in serum samples before and during treatment. Homocystine was not found in any sample from the control group rabbits nor was it found in any of the pre-treatment samples of the azaribine or azaribine-combination groups of animals. While homocystine levels ranging from 4.8 to 111.9 nmoles/ml (47.07 ± 41.31 mean ± SEM) occurred in six of the twelve azaribine-treated animals in 4-5 days of study, no homocystine levels were found at the same time in any of the six animals treated with the azaribine and pyridoxine combination. The differences between the azaribine and control groups or between azaribine and azaribine pyridoxine groups were significant at the $P \leq 0.05$ level.

The results of our studies demonstrate that orally administered 6-azauridine triacetate causes, in rabbits, a significant decrease in the levels of pyridoxal phosphate in the serum. These findings support our unifying hypothesis for the explanation of the hyperaminoaciduria induced by 6-azauridine triacetate as attributable to an interference with the pyridoxal phosphate coenzymes (7,8). While previous studies suggested the depression of pyridoxal phosphate by azaribine only indirectly (8,9), or in an uncontrolled fashion (10), this study reveals the direct and unequivocal evidence of this effect.

The mechanism by which azaribine interferes with pyridoxal phosphate is not known. It is possible that catabolites of 6-azauridine may be responsible for this effect. About 5% of the administered drug has been reported to be catabolized by the cleavage of the triazine ring (2,13); some of the possible compounds formed from the aglycone of azauridine that could react with pyridoxal phosphate are shown in the structural diagrams below.* Since most of the drug and its metabolites are excreted in the urine within 6 hr after oral administration, the initial dramatic decrease in the levels of pyridoxal phosphate in the serum (10) could possibly be explained by its binding to a rapidly formed catabolite.



Our study also reveals for the first time that both azaribine-induced pyridoxal phosphate depletion and homocystinemia can be prevented by simultaneous administration of pyridoxine. Azaribine is a drug with an established activity in psoriasis (15, 16), mycosis fungoides (17, 18), polycythemia vera (19) and trophoblastic malignancies (20, 21). Unfortunately, azaribine has been withdrawn from the market, because of an apparently increased incidence of thromboembolic episodes reported in patients with severe psoriasis treated with the drug (22-24). Pyridoxal phosphate depletion and homocystinemia observed after azaribine administration in this study are presumptively related to the thrombogenic side-effects of this drug (8,23). On the other hand, additional information is needed to account for the reportedly low frequency (i.e. only 4%) of thromboembolic episodes in well over 500 patients with severe psoriasis (22) often treated for many weeks with large doses (60-200 mg/kg daily) of azaribine (25).

The reversal of the biochemical side-effects of azaribine by the administration of pyridoxine, as presented in this paper, appears to be a useful approach, leading to restoration of the clinical usefulness of azaribine in the treatment of both neoplastic and non-neoplastic diseases.

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