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HEMOLYSIS IN MICE TREATED WITH DEOXYCOFORMYCIN, AN INHIBITOR OF ADENOSINE DEAMINASE

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Deoxycoformycin, a potent inhibitor of adenosine deaminase (E.C. 3.5.4.4), is toxic to lymphoid tissue in mice (1), and has shown some success in the treatment of human leukemia (2); it is now being further evaluated in clinical trials. Lymphoid cell toxicity following deoxycoformycin treatment is presumed to be exerted by deoxyadenosine (AdR), or its metabolite deoxyadenosine triphosphate (dATP), or both. This conclusion is based on observations that AdR is excreted in the urine of patients with inherited adenosine deaminase deficiency (3) and that their erythrocytes contain dATP (4); the cytotoxicity of AdR to lymphatic cells is well established (e.g. 5).

Here we report that, in addition to toxic effects toward lymphoid cells, deoxycoformycin treatment of mice leading to the accumulation of AdR and dATP can also have detrimental effects on erythrocytes; such effects have not previously been reported.

Female ICR Swiss mice (25-30 g) were injected intraperitonially with 10 mg/kg of deoxycoformycin (gift of Dr. G. A. LePage and of Parke Davis and Co.) dissolved in 0.154 M sodium chloride. Erythrocyte counts and hematocrits were determined by standard techniques. Adenosine triphosphate (ATP) and dATP concentrations in neutralized perchloric acid extracts of erythrocytes were measured spectrophometrically using high performance liquid chromatography.

The dose of deoxycoformycin used in these experiments is high (10 mg/kg) relative to that required to inhibit adenosine deaminase activity in some mouse tissues (0.5-1.0 mg/kg) (e.g. 6). However, this high dose has been found necessary to inhibit adenosine deaminase activity in mouse intestine (unpublished results), and we have observed that this dose of deoxycoformycin is important for AdR metabolism *in vivo*. Thus erythrocyte dATP concentrations 24 h after deoxycoformycin treatment were 2-fold greater when 10 mg/kg was used than when the dose was 1 mg/kg, and injected AdR disappeared from plasma much more rapidly at 1 mg/kg than at 10 mg/kg.

Effects of deoxycoformycin treatment on mouse erythrocytes are reported in Table 1. In attempts to accentuate the effects of deoxycoformycin alone, some mice also received intraperitonial injections of 400 mg/kg AdR (an arbitrary dose), given 20 min after the deoxycoformycin. Erythrocyte counts were reduced 16% in mice treated for 17 h with deoxycoformycin, and 20-25% in animals treated for 24 h. A greater, but more variable effect was produced when hematocrits were measured. These were reduced 22-29% at 17 h, and 31-50% at 24 h.

These results strongly suggest that deoxycoformycin treatment of mice induces the lysis of erythrocytes. This conclusion is confirmed by observations that the number of circulating reticulocytes increases 2-3 fold when mice were treated with deoxycoformycin for three to five days, a response that would be expected to follow a decrease in erythrocyte mass; the increased reticulocyte count also indicates that deoxycoformycin did not reduce circulating erythrocyte levels through toxic effects on erythrocyte precursors in bone marrow. Qualitatively, hemoglobin (and not intact erythrocytes) often was observed in the urine of mice treated 17-24 h with deoxycoformycin; in mice that also received AdR, this was quite consistently observed. Similarly, hemoglobin was also observed in the plasma of mice treated 17-24 h with both deoxycoformycin and AdR; this was also noted in many but not all mice treated with deoxycoformycin alone.

TABLE 1

Effects of deoxycoformycin treatment on mouse erythrocytes.*

Treatment	Time (h)	Erythrocytes (x 10^9/ml)	Hematocrit (%)	dATP (nmoles/ 10^9 cells)	ATP (nmoles/ 10^9 cells)
None		7.57	42	nil	49
Deoxycoformycin	7	7.50	41	0.6	21
Deoxycoformycin	17	6.37	30	5.4	18
Deoxycoformycin	24	5.70	29	6.0	22
Deoxycoformycin + AdR	7	7.40	42	18.0	11
Deoxycoformycin + AdR	17	6.35	33	12.9	17
Deoxycoformycin + AdR	24	5.45	21	9.5	21

*Groups of three mice received intraperitoneal injections of 0.154 M sodium chloride, 10 mg/kg deoxycoformycin, or 400 mg/kg AdR, as indicated. Results are representative of those obtained in three experiments.

Table 1 also shows that deoxycoformycin treatment was associated with a progressive increase in dATP concentrations in erythrocytes (there was no dATP in erythrocytes of control mice). The additional administration of exogenous AdR elevated dATP concentrations even higher, but these concentrations decreased with time.

Erythrocytes of deoxycoformycin-treated mice also contained lowered concentrations of the normal metabolite, ATP. ATP concentrations decreased 57% within 7 h, and remained in this range for the remainder of the period studied. In mice also treated with AdR, the ATP concentration at 7 h was decreased 78%, but at 17 and 24 h it was about the same (decreased ca. 50-60%) as in mice treated with deoxycoformycin alone.

Inasmuch as lowered ATP concentrations are associated with increased hemolysis in transfused stored blood (7), and hereditary abnormalities of erythrocyte metabolism (8), the decreases in ATP concentrations observed here in deoxycoformycin-treated mice may be related to the low hematocrits, hemoglobin in plasma and urine, etc.; this relationship, however, cannot be considered proven. The metabolic basis for lowered erythrocyte ATP concentrations in deoxycoformycin-treated mice is the subject of continued investigation.

That deoxycoformycin treatment can have effects on erythrocytes as well as lymphoid cells has obvious potential consequences for the clinical use of this drug, and precise dose-response relationships for the two types of effects will have to be worked out. The present results also enlarge our view of AdR toxicity, and it seems likely that the biochemical mechanism by which hemolysis is induced is different than that by which lymphoid cells are killed.

REFERENCES

1. J.F. Smyth, R.C. Young and D.M. Young, Cancer Chemother. Pharmacol. 1, 49 (1978).

2. J.F. Smyth, M.M. Chasin, K.R. Harrap, R.H. Adamson and D.G. Johns, Proc. Am. Assoc. Cancer Res. 20, 47 (1979).

3. H.A. Simmonds, A. Sahota, C.F. Potter and J.S. Cameron, Clin. Sci. Molec. Med. 54, 579 (1978).

4. M.S. Coleman, J. Donofrio, J.J. Hutton, L. Hahn, A. Daoud, B. Lampkin and J. Dyminski, J. Biol. Chem. 253, 1619 (1978).

5. B.S. Mitchell, E. Mejias, P.E. Daddona and W.E. Kelley, Proc. Natl. Acad. Sci. U.S.A. 75, 5011 (1978).

6. G.A. LePage, L.S. Worth and A.P. Kimball, Cancer Res. 36, 1481 (1976).

7. R.I. Weed and P.L. Lacelle, in Red Cell Membrane, Structure and Function (Eds. G.A. Jamieson and J.J. Greenwalt), p. 318, J.B. Lippincott Co., Philadelphia (1969).

8. W.N. Valentine and K.R. Tanaka, in The Metabolic Basis of Inherited Disease, 4th ed. (Eds. J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson), p. 1411, McGraw-Hill Book Co., New York (1978).