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Effects of 2′-deoxycoformycin infusion on mouse phosphoribosyl pyrophosphate synthetase*

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Since the discovery of the association of the loss of adenosine deaminase (ADase) activity with some cases of severe combined immunodeficiency disease [1], the role of purine metabolism in immune response has become a rapidly developing area of investigation. A causal relationship between purine synthesis and the response of lymphocytes to stimulation with antigens or mitogens has been reported [2, 3]. Allison *et al.* [4] have suggested, based on their studies of the ADase inhibitor coformycin, that the deficiency of ADase limits lymphocyte responses to antigens and mitogens because the adenine ribonucleotides that accumulate inhibit the synthesis of phosphoribosyl pyrophosphate (PRibPP). This compound is a key intermediate in purine nucleotide biosynthesis by both the *de novo* and recycling pathways. It is, in turn, regulated by its synthesizing enzyme, PRibPP synthetase (EC 2.7.6.1). The present investigation is designed to explore the effect of a potent ADase inhibitor, 2′-deoxycoformycin (DCF), on the activity of various purine metabolizing enzymes, including PRibPP synthetase.

C57BL/6J mice, maintained on Purina rat chow and tap water supplemented with Terramycin, were infused intraperitoneally as described previously [5] at a constant rate of 0.4 $mg \cdot kg^{-1} \cdot day^{-1}$ of DCF in 0.9% sodium chloride for 5 days. Isolated mouse tissue cells used in enzyme assays were homogenized in ice-cold 5% (w/v) sucrose solution [0.125 M sucrose, 0.03 M KCl, 0.07 M Tris–HCl buffer (pH 7.4), and 1 mM dithiothreitol]. The homogenates were centrifuged at 100,000 *g* for 60 min in a Spinco L2 preparative ultracentrifuge. Supernatant fractions thus prepared were assayed for the activities of HPRTase and APRTase. Pellets were resuspended in one-third volume of original homogenate buffer containing 2% of added Triton X-100. The suspended pellets were homogenized again in the Triton solution before centrifugation at 10,000 *g* for 1 hr. The clarified preparation was used for PRibPP synthetase assays. The splenocytes and thymocytes were prepared by gently teasing the organs apart with forceps over a fine tea strainer into tissue culture medium. The cell suspensions were filtered through nylon wool packed syringe barrels before they were used to prepare homogenates for enzyme assays.

HPRTase and APRTase activities were determined by the conversion of [8-^{14}C]hypoxanthine and adenine into their respective nucleotides in the presence of PRibPP as described previously [6, 7]. The assay procedure for PRibPP synthetase has also been described previously [8, 9]. The synthetase reaction (in the presence of ATP and R-5-P) was coupled with the APRTase reaction, and the PRibPP produced by the former was measured by the conversion of [^{14}C]Ad into [^{14}C]AMP by the added APRTase. The protein content of each prepared sample was assayed by the method of Lowry *et al.* [10].

The *in vitro* effects of 1–10 mM DCF on mouse HPRTase, APRTase and PRibPP synthetase activities of either mouse hemolysates or jejunal mucosa homogenates were determined. No inhibitory effect of DCF, even at a 10 mM concentration, was observed with any of the three purine metabolizing enzymes. PRibPP synthetase isolated from various DCF infused animal tissues was compared with control values obtained from C57BL mice that had been infused with PBS for the same length of time. Table 1 shows that the effect of DCF infusion on mouse PRibPP synthetase activity is tissue specific. Thymus enzyme activity was completely abolished upon infusion, while liver, jejunum, lung and spleen were about equally affected, with

Table 1. Effect of DCF infusion of mouse tissue PRibPP synthetase activity*

Tissues	PRibPP synthetase activity [nmoles·(mg protein)$^{-1}$·min^{-1}] Control	DCF infused	% Inhibition PRibPP synthetase	ADase‡
Spleen	687.3 ± 82.2†	254.3 ± 117†	63	85
Liver	8.4	1.9	72	71
Kidney	1.9	1.8	6	74
Lung	10.2	3.3	68	67
Jejumun	19.5	5.5	72	27
Colon	72.8	75.1	0	70
Splenocytes	668.7	276.1	59	—
Thymocytes	1862.8	9.94	100	91
Erythrocytes	377.6	382.2	0	100

* Assays were performed on pools of three animal tissues.
† Mean ± S.E., obtained from three separate animal tissues, assayed individually.
‡ Data obtained previously [5].

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Table 2. Effects of DCF and tumor extract infusion on the activity of various purine metabolizing enzymes of mouse jejunal mucosa*

Enzyme source and drug treatment	PRibPP synthetase	HPRTase	APRTase
	[nmoles · (mg protein)$^{-1}$ · min^{-1}]		
Control	18.6 ± 2.8	4.0 ± 0.3	4.7 ± 0.6
Tumor extract infused	42.9 ± 15.0	4.2 ± 2.5	4.7 ± 1.4
DCF infused	2.3 ± 1.5	2.7 ± 1.6	4.8 ± 2.8
Tumor + DCF	23.9 ± 12.2	3.8 ± 1.9	4.4 ± 1.0

* Each value is the mean ± S.E. for three to six animals.

59–72 per cent decreases of enzyme activities. No significant inhibition of the PRibPP synthetase activity of erythrocyte, colon, and kidney was observed. For comparison of the DCF effect, the last column in Table 1 is the per cent inhibiton on mouse tissue ADase obtained previously under similar treatment [5].

Transplantable colon tumor 38 was used [11] in the second set of experiments. A 50% tissue homogenate in 0.9% saline solution was prepared. The homogenate was clarified by centrifugation at 13,000 *g* for 30 min. A 0.04-ml portion of the clarified supernatant fraction was infused into each mouse per day for 5 days. Continuous infusion of tumor extract into C57BL mice caused a 2-fold increase in PRibPP synthetase activity of the mouse jejunum, as compared to the 88 per cent inhibition induced by DCF infusion in this particular experiment (Table 2). Infusion of the DCF and tumor extract together seemed to have suppressed the change that occurred by the infusion of either effector alone. No significant effects on APRTase under the same treatment were observed. The DCF infusion caused a slight inhibition of HPRTase activity from the mouse jejunum mucosa.

These results show that, even though 2-deoxycoformycin is not an inhibitor for PRibPP synthetase *in vitro*, it exerts a great effect on this enzyme upon prolonged infusion of the drug. The effect is tissue specific, greatest with the lymphoid system, i.e. thymus and spleen, differentially effective against jejunum but not colon enzyme, and suppressive to the enzyme in liver and lung but not in kidney and erythrocytes.

The tissue-specific effect of DCF on PRibPP synthetase is not surprising, since we have previously reported that there are distinct differences between the PRibPP synthetase from human erythrocytes and that from other tissues and that tissue-specific PRibPP synthétase variants are expected [8]. We have further shown that, unlike PRibPP synthetase from erythrocytes, the enzyme from nucleated tissues, e.g. jejunum and colon, is mainly membrane associated [9], and a characteristic difference between the enzyme from these two sources was observed.

The facts that PRibPP synthetase activity is found associated with cell membrane, and that upon exposure of human lymphocytes to mitogen the level of PRibPP increases considerably within minutes of the stimulation are consistent with the view that purine synthesis is possibly involved in the cellular regulation of proliferation. The parallel suppressive effect of the DCF upon the cellular immune response and that of the PRibPP synthetase activity further correlates the function of this enzyme with the activity of the cellular immune system.

Tissue specific responses of adenosine deaminase and other enzymes to DCF treatment have been reported from our laboratory [5]. Unlike ADase, however, PRibPP synthetase of erythrocytes, colon, and kidney is unaffected by this inhibitor, whereas jejunal enzyme is severely inhibited. Since there is no *de novo* synthesis in erythrocytes and since jejunal mucosa cells have a significantly higher turnover rate than the other tissue cells, it seems reasonable to assume that DCF might directly or indirectly be affecting the synthesis of PRibPP synthetase.

ADase is the only enzyme that has been reported to be altered in ADase-deficient severe combined immunodeficiency disease (SCID). If the effect of DCF infusion mimics the inherited metabolic disorder and if PRibPP synthetase is involved, then erythrocytes will certainly not be the proper cells to evaluate the metabolic impairment of SCID.

The parallel suppressive effects of DCF upon the ADase and PRibPP synthetase activities appear consistent with the theory that the accumulated adenine ribonucleotides caused by the deficiency of the former enzyme inhibit the reaction of the latter [4]. However, from the results of the tumor extract infusion experiment, the relationship between these two enzymes might be more complicated.

The effects of tumor or its extract on ADase and PRibPP synthetase are opposite, it enhances the PRibPP synthetase activity but depresses the ADase activity [5, 12]. At the same time neither DCF nor tumor extract has any effect on APRTase which is readily inhibited by various purine nucleotides [13]. These observations indicate that adenosine *per se* or its nucleotides are not likely to be solely responsible for the observed changes on PRibPP synthetase. To explain different effects of DCF and tumor extract upon this enzyme one could assume that, besides adenosine nucleotides, there are other metabolites that are derived from adenosine, e.g. adenosylhomocysteine, that might be the actual effectors of PRibPP synthetase. Many biological alterations are secondary effects of a primary change on a specific target enzyme. One cannot but recognize the similarity of this case to that of Lesch–Nyhan disease and HPRTase-deficient gout [14]. Deficiency of HPRTase in affected patients causes an increase in cellular levels of PRibPP. An increase in APRTase activity is observed in these same patients and it was found to be to the stabilizing effect of the excess PRibPP on the enzyme rather than to alteration in enzyme synthesis or kinetic properties [15, 16].

It is known that excess adenosine inhibits pyrimidine synthesis [17]. This decrease in PRibPP synthetase activity might be the mechanism responsible for such inhibition.

Thus, the clearly defined gentic defect, loss of ADase activity, can exert its ultimate biologic effect at quite a distance from the primary site, and compounds inhibiting this enzyme, among others, may express their action at a distance.

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