Lack of inhibition of purine nucleoside phosphorylase and adenosine deaminase in patients treated with azathioprine

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Deficiency of the purine salvage enzymes purine nucleoside phosphorylase (PNP) and adenosine deaminase (ADA) are known causes of immunodeficiency. Evidence for inhibition of these enzymes was sought in 16 patients on azathioprine therapy by testing for deoxyguanosine (PNP deficiency) and deoxyadenosine (ADA deficiency) in urine using a novel phosphorescence method. These abnormal nucleosides were not found in urine of azathioprine treated patients or in 30 normal controls but were easily detected in urine from proven cases of PNP and ADA deficiency suggesting lack of *in vivo* inhibition of PNP and ADA by azathioprine.

Keywords azathioprine purine nucleoside phosphorylase adenosine deaminase

Introduction

The mode of action of azathioprine is unknown. In 1968 Krenitsky and his co-workers found that it inhibited the red blood cell enzyme purine nucleoside phosphorylase (E.C. 2.4.2.1) non-competitively albeit at a high substrate concentration (inhibition constant 0.22 mM). At the time the significance of this was not appreciated. We now know that deficiency of this enzyme is associated with profound impairment of T lymphocyte function and the excretion of large amounts of deoxyguanosine in urine (Giblett *et al.*, 1975; Cohen *et al.*, 1976; Stoop *et al.*, 1977). Moreover, deficiency of the enzyme adenosine deaminase (E. C. 3.5.4.4) has also been found to be associated with severe impairment of immunity (Giblett *et al.*, 1972). Both T and B lymphocyte function is reduced and abnormal amounts of deoxyadenosine are excreted in urine (Donofrio *et al.*, 1978; Mills *et al.*, 1978; Simmonds *et al.*, 1978). To determine whether azathioprine produces immunosuppression by inhibiting these enzymes we have used a very sensitive phosphorescence method for detecting deoxyguanosine and deoxyadenosine in urine of patients taking the drug.

Methods

Urine samples were obtained from eight patients with kidney transplants and eight patients with bullous skin disease treated with azathioprine (2mg/kg body weight). Urine samples from a child (S.B.) aged 3 months with known purine nucleoside phosphorylase deficiency and another (S.Y.) aged 2 months with adenosine deaminase deficiency were kindly donated by Dr H. A. Simmonds, Guy's Hospital, London. Control urine samples (30) were obtained from healthy volunteers.

Deoxyguanosine and deoxyadenosine for use as standards were purchased from Sigma Chemical Company, Dorset, England. Stock solutions were prepared in water and urine and stored at 4°C. They were replaced every 2 weeks. Cellulose thin layers (Art 5552) supplied by Merck, Darmstadt were 0.1 mm thick. They were non-activated, without fluorescent indicator and mounted on aluminium sheets. Those mounted on plastic sheets were found to be less satisfactory owing to high background phosphorescence.

Samples of urine and standard solutions of nucleosides were applied, 1 µl of each, with a

Figure 1 Photograph of an X-ray film which was placed on a cellulose thin layer chromatogram, immersed in liquid nitrogen, after exposure to u.v. light at 254 nm. 1 μ l was applied of each of the following:

1. Deoxyadenosine (1 nmol) in water.
2. Deoxyadenosine (1 nmol) in normal urine.
3. Adenosine deaminase deficiency urine (S.Y.).
4. Deoxyguanosine (1 nmol) in water.
5. Deoxyguanosine (1 nmol) in normal urine.
6. Purine nucleoside phosphorylase deficiency urine (S.B.).
7. Urine of a kidney transplant patient on azathioprine therapy.
8. Urine used as a solvent in (2) and (5).
9. Control urine.

microlitre syringe over a 1 cm line which was 2 cm from the edge of the sheet. The most effective solvent system for separation of deoxyguanosine and deoxyadenosine from other urinary constituents was sodium acetate (3%) containing lithium chloride (3 g/100 ml): methanol (30/70 v/v). Development of the chromatogram to a height of 12 cm took 100 min. Detection of phosphorescence was done in an ordinary polystyrene box that had been painted black (Maddocks & Davidson, 1975). A central well had been cut out of the floor of the box to hold liquid nitrogen and this was covered by a black painted steel plate. The chromatogram was then placed on the steel plate in the box and liquid nitrogen poured over it until it was just covered. Viewing of the chromatogram was done in a dark room after exposing it to u.v. light (254 nm) for a few seconds. The colour of the phosphorescent bands was noted and the duration of light emission measured by eye using a stop watch. A permanent record of the chromatogram was obtained by laying a medical X-ray film (Singul-X, Ceaverken Strangner AB, Sweden) on it as soon as the UV light had been switched off and exposing the film for 20 s.

Results

Deoxyguanosine and deoxyadenosine were easily detected in the urine of patients with purine nucleoside phosphorylase and adenosine deaminase deficiency respectively (Figure 1 and Table 1). None of the 30 urine samples from normal control subjects and the 16 patients taking azathioprine contained deoxyguanosine or deoxyadenosine.

Discussion

Azathioprine therapy, in this study, was not associated with abnormal urinary excretion of either deoxyguanosine or deoxyadenosine in any of the 16 patients investigated. However, these nucleosides were easily detected in the urine of known cases of purine nucleoside phosphorylase and adenosine deaminase deficiency. As the technique used for detecting deoxyguanosine and deoxyadenosine was very sensitive these results indicate that immunosuppression by azathioprine is not a result of inhibition of purine nucleoside phosphorylase or adenosine deaminase.

We wish to thank Sheffield Area Kidney Association and Chesterfield & District Kidney Association for financial support.

Table 1 Properties of substances detected in urine in adenosine deaminase deficiency (S.Y.) and purine nucleoside phosphorylase deficiency (S.B.).

	<i>R_F</i>	<i>Band colour</i>	<i>Decay time (s)</i>	<i>Detection sensitivity (pmol)</i>
Deoxyadenosine in water	0.44	Blue	16	32
Deoxyadenosine in urine	0.44	Blue	16	32
Urine (S.Y.)	0.44	Blue	14	
Deoxyguanosine in water	0.50	Blue	7	60
Deoxyguanosine in urine	0.50	Blue	7	60
Urine (S.B.)	0.49	Blue	7	

References

- Cohen, A., Doyle, D., Martin, D. W. J. & Amman, A. J. (1976). Abnormal purine metabolism and purine overproduction in a patient deficient in purine nucleoside phosphorylase. *New Engl. J. Med.*, **295**, 1449–1454.
- Donofrio, J., Coleman, M. S., Hutton, J. J. & Daoud, D. A. (1978). Overproduction of adenosine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. *J. clin. Invest.*, **62**, 884–887.
- Giblett, E. R., Amman, A. J., Sandman, R., Ward, D. W. & Diamond, R. K. (1975). Nucleoside phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet*, **ii**, 1010–1013.
- Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972). Adenosine deaminase deficiency in the patients with severely impaired cellular immunity. *Lancet*, **ii**, 1067–1069.
- Krenitsky, T. A., Elion, G. B., Henderson, A. M. & Hitchings, G. H. (1968). Inhibition of human

- purine nucleoside phosphorylase. Studies with intact erythrocytes and the purified enzyme. *J. biol. Chem.*, **243**, 2876–2881.
- Maddocks, J. L. & Davidson, G. S. (1975). Separation and detection of picomole quantities of azathioprine metabolites. *Br. J. clin. Pharmac.*, **2**, 359–360.
- Mills, G. C., Goldbnum, R. M., Newkirk, K. E. & Schmalstieg, F. C. (1978). Urinary excretion of purines, purine nucleosides and pseudouridine in adenosine deaminase deficiency. *Biochem. Med.*, **20**, 180–199.
- Simmonds, H. A., Sahota, A., Potter, C. F. & Cameron, J. S. (1978). Purine metabolism immunodeficiency: Urinary purine excretion as a diagnostic screening test in adenosine deaminase and purine nucleoside phosphorylase deficiency. *Clin. Sci. mol. Med.*, **54**, 579–584.
- Stoop, J. W., Zegers, B. J. M. & Hendrickx, G. F. M. (1977). Purine nucleoside phosphorylase deficiency and cellular immunodeficiency. *New Engl. J. Med.*, **296**, 651–655.

(Received August 6, 1984,
accepted September 23, 1984)