The entrapment of polyethylene glycol-bound adenosine deaminase (Pegademase) in human carrier erythrocytes.

BRIDGET E. BAX, LYNETTE D. FAIRBANKS, MURRAY D. BAIN, H. ANNE SIMMONDS and RONALD A. CHALMERS.

Paediatric Metabolism Unit, Department of Child Health, St George’s Hospital Medical School, London, SW17 ORE and "The Purine Research Laboratories, Guy’s Hospital, London, SE1 9RT.

Severe combined immunodeficiency disease due to an absence of ADA (EC 3.5.44) is an inherited and, if untreated, severe and often lethal disorder. ADA catalyses the deamination of adenosine and 2-deoxyadenosine to inosine and 2-deoxyxynosine respectively for either salvage and re-utilisation or for metabolism to uric acid and excretion. A deficiency in ADA results in an accumulation of its substrates and a preferential phosphorylation of 2-deoxyadenosine to dATP by a normally minor pathway. It is the cellular accumulation of dATP which is thought to impair lymphocyte differentiation and proliferation and thus prevent the effective functioning of the immune system [1,2].

ADA deficiency is responsive to enzyme replacement therapy with the licensed pharmaceutical polyethylene glycol-conjugated adenosine deaminase (Pegademase, Enzon) [3]. Therapy with Pegademase is expensive and invasive; expensive because of the high pharmaceutical production costs, and the rapid clearance from the vascular compartment; invasive because of the frequent infusions required to maintain therapeutic plasma levels. In this study, we investigated the entrapment of Pegademase (molecular weight 90 000) in human carrier erythrocytes with the future aim of extending the in vivo drug half-life and maintaining therapeutic plasma levels. In this study, we investigated the entrapment of Pegademase (molecular weight 90 000) in human carrier erythrocytes with the future aim of extending the in vivo drug half-life and maintaining therapeutic plasma levels, thus reducing the dosage, frequency of infusions and treatment costs. The effect of polyethylene glycol groups on the extent of enzyme entrapment was also investigated by loading carrier erythrocytes with native ADA (molecular weight 33 500).

Energy-replete carrier erythrocytes were prepared using a hypo-osmotic dialysis procedure [4,5]. Seven volumes of washed, packed erythrocytes were mixed with three volumes of PBS (136.89 mmol/l NaCl, 2.68 mmol/l KCl, 8.10 mmol/l Na2HPO4, 1.47 mmol/l KH2PO4, pH 7.4) containing varying concentrations of either Pegademase or Type VI ADA (Sigma) and placed into dialysis tubing with a molecular cut-off of 12 000 Daltons. Dialysis was against 150 ml hypo-osmotic phosphate buffer (5 mmol/l KH2PO4/K2HPO4, pH 7.4) at 4°C with rotation at 6-12 rpm for 90 or 180 minutes with Pegademase or for 90 minutes with ADA. The lysed erythrocytes were resealed by dialysis against 150 ml PBS supplemented with adenosine, MgCl2 and glucose (all at 5 mmol/l), pH 7.4 with continuous rotation for 60 minutes at 37°C. Endogenous ADA activity was measured in controls prepared by lysing and re-sealing in the absence of Pegademase or ADA (unloaded carrier erythrocytes). The carrier erythrocytes were washed three times in supplemented PBS with centrifugation at 10g.

Endogenous ADA activity and entrapment Pegademase and ADA activity were assayed by following the deamination of adenosine to inosine. Diluted and haemolysed carrier erythrocytes were incubated with 3.75 mmol/l adenosine in 100 mmol/l phosphate buffer for 1 hour. The reaction was stopped with 40% trichloroacetic acid and the supernatant was extracted with water-saturated diethyl ether. The reaction products were separated by HPLC using a Waters trimodular system with a Spherisorb ODS2 column and an isocratic tetrabutyl ammonium bromide and ammonium acetate buffer.

Fig.1 shows the entrapment of Pegademase and ADA as a function of enzyme units added to the dialysis tubing. Logarithmic scales are used on both axes. In each case the amount of enzyme entrapped increased with concentration. Doubling the hypo-osmotic dialysis time from 90 to 180 minutes caused a consistent three-fold increase in Pegademase entrapment when less than 20 units of enzyme were added. At higher enzyme concentrations this effect was abolished. Entrapment of native ADA after 90 minutes was considerably higher than that of Pegademase at either 90 or 180 minutes. Using a total of 200 units per dialysis, ADA entrapment was 518% and 425% of that observed with Pegademase at 90 and 180 minutes respectively.

These results demonstrate the successful entrapment of a substantial proportion of free ADA. Intriguingly, the reasons for polyethylene glycol attachment (prolonging circulation half-life, reducing immunogenicity and prevention of ADA binding to ADA-complexing proteins) may in the carrier erythrocyte system be redundant, thus suggesting a potential therapeutic use of free ADA-loaded carrier erythrocytes in treating ADA deficiency.

This work was supported by the Wellcome Trust.