THE ENCAPSULATION OF THE ANTI-SICKLING AGENT L-LYSYL-L-PHENYLALANINE (LYS-PHE) IN INTACT ERYTHROCYTES

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The biochemical lesion in sickle cell disease is due to the presence in the erythrocyte of an abnormal haemoglobin (HbS) which differs from normal haemoglobin (HbA) by the genetic substitution of a valine residue for the normal glutamate residue at position 6 of each beta-globulin chain. When HbS is deoxygenated it becomes insoluble and aggregates into long chain polymers. The cytosol of the carrier erythrocyte undergoes gelation and assumes a characteristic 'sickle shape'. One therapeutic option is to use agents which inhibit gelation and inhibit polymerization of HbS without altering the oxygen binding capability. One such agent is Lys-Phe. Although Lys-Phe has shown promise in laboratory studies the clinical experience with this drug has been disappointing possibly due to the relative permeability of the erythrocyte membrane to this drug (Franklin et al 1983). We have attempted to encapsulate this drug in erythrocytes using a modified preswell technique involving hypotonic swelling. By using returned loaded erythrocytes blood levels of the dipeptide may be sustained by slow release in vivo leading to longer contact between abnormal cells and the anti-sickling agent. In addition by introducing Lys-Phe into intact cells containing HbS we can follow the effect of interactions between HbS and the dipeptide on sickling. ^{3}H-Lys-Phe and ^{14}C-Lys-Phe was synthesized by a method kindly provided by our sponsers G D Searle & Co Ltd. The radiolabelled compounds were mixed with unlabelled dipeptide. The dipeptide was encapsulated in erythrocytes by our standard procedure (Pitt et al 1983). Optimum conditions for loading were found to be 860 μg/ml packed cells (haematocrit value 76) with an encapsulation efficancy of 16.3% of added drug. Of this 2.3% was associated with the cell membrane. Very little Lys-Phe was encapsulated until the cells were swollen to their maximum value prior to lysis. This observation agrees with that of Pitt et al 1983 that the membrane pores open immediately prior to lysis. When loaded cells were labelled with fluorescein and returned to the rats circulation the survival half-life of the cells was found to be 10 days. In in vitro dialysis experiments over 3h the rate of release of the dipeptide from erythrocytes was about 2.2% of the drug encapsulated per h.Lys-Phe was also encapsulated by treating erythrocytes with amphotericin-B (Deuticke et al 1973). A loading of 9% was obtained but the cells had poor survival characteristics when returned to the circulation (half-life 5 days). Consequently the hypotonic swelling was the preferred mode of loading. To test the efficacy of the preparation we obtained erythrocytes from a male West Indian patient from Birmingham general hospital. Portions of the erythrocytes were used to encapsulate Lys-Phe and others were left untreated. On deoxygenation and microscopic examination over 70% of untreated cells sickled under low oxygen tension but where the cells contained encapsulated Lys-Phe only 10-15% sickled. This observation is consistent with poor transport across the membrane being responsible for the drugs relatively disappointing performance in clinical trials.

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