IN VIVO SURVIVAL OF HUMAN ENERGY-REPLETE CARRIER ERYTHROCYTES

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1. INTRODUCTION

Erythrocytes have been proposed as carriers of encapsulated therapeutic agents. Encapsulation of therapeutic agents within erythrocytes with a normal mean cell life range of 89 to 131 days (normal half-life of 19 to 29 days) should limit the vascular clearance of the administered drug thus reducing the dosage and frequency of therapeutic interventions.

We have recently demonstrated that therapeutic enzyme entrapment can be increased by extending the hypo-osmotic dialysis time of human carrier erythrocytes (Bax et al., 1996a; 1996b). However to be successful as a sustained therapeutic agent delivery system it is essential to first demonstrate that carrier erythrocytes have a near physiological survival time in vivo. We have previously reported in preliminary form the in vivo survival of human carrier erythrocytes (Bax et al., 1996c). We now report the in vivo survival of unloaded carrier erythrocytes prepared using the two different hypo-osmotic dialysis times that we employed in our in vitro entrapment studies.

2. MATERIALS AND METHODS

2.1. Volunteers

Nine healthy volunteers (4 females and 5 males) aged 21 to 43 years (mean 25.6) were used in this study. Ethical approval was granted by the Local Research Ethics Committee.
2.2. Blood Preparation

Sterile materials and aseptic conditions were used throughout. Forty ml of blood were collected and placed into 2 tubes containing 4 ml anticoagulant citrate dextrose BP (n=2) or 200 units heparin BP (n=7). The blood samples were centrifuged for 10 minutes at 1100g; the supernatant plasma was removed and kept for later use and the buffy coat was discarded. The erythrocytes were washed twice in cold (4°C) phosphate buffered saline (PBS), pH 7.4 (2.68 mmol/l KCl, 1.47 mmol/l KH₂PO₄, 136.89 mmol/l NaCl, 8.10 mmol/l Na₂HPO₄) and centrifuged for 10 minutes at 1100g.

2.3. Carrier Erythrocyte Preparation

Energy-replete carrier erythrocytes were prepared using a hypo-osmotic dialysis technique (Sprandel, Hubbard and Chalmers, 1980; 1981). Washed and packed fresh erythrocytes (10.5 ml) were mixed with cold PBS to a final hematocrit of 70%. Five ml of cell suspension were placed into each of three dialysis bags (molecular weight cut-off of 12,000 daltons, Medicell International Ltd, London) which were then sealed at both ends with clips. Each dialysis bag was placed into a container and supported firmly by wedging the dialysis clips against the container side. Dialysis was against 150 ml hypo-osmotic phosphate buffer, pH 7.4 (5 mmol/l KH₂PO₄, 5 mmol/l K₂HPO₄) at 4°C in a LabHeat refrigerated incubator (Boro Labs Ltd, Berkshire) for 90 (n=6) or 180 (n=3) minutes, with rotation at 6 rpm. The lysed erythrocytes were resealed by transferring the dialysis bags to containers holding 150 ml PBS supplemented with 5 mmol/l adenosine, 5 mmol/l glucose and 5 mmol/l MgCl₂ (supplemented PBS), and continuing rotation at 6 rpm in the incubator, now at 37°C, for 60 minutes. The energy-replete carrier erythrocytes were washed three times in 3 volumes supplemented PBS with centrifugation at 100g for 15 minutes and finally pooled.

2.4. Labelling of Carrier Erythrocytes with Sodium [⁵¹Cr] Chromate

Carrier erythrocytes were labelled using a standard [⁵¹Cr] erythrocyte-labelling technique (International Committee for Standardization in Haematology, 1980); the washed and packed cells were gently mixed with 0.75 MBq Sodium [⁵¹Cr] chromate BP (Amersham International, Buckinghamshire) and allowed to stand at room temperature for 30 minutes. Unbound chromium was removed with either 100 mg ascorbic acid BP (100 mg/ml, Evans Medical, Leatherhead) which reduces the chromate ion to the non-permeable chromic followed by a single wash in supplemented PBS (n=7) or by 3 washes in supplemented PBS (n=2). Following resuspension in an equal volume of autologous plasma, the carrier erythrocytes were injected slowly over a period of 5 minutes into the volunteer’s arm vein.

2.5. Assessment of Carrier Erythrocyte Survival

*In vivo* survival was assessed by monitoring the disappearance of label from the circulation; 10 ml blood samples were taken from a vein in the opposing arm 15, 30, 60, 120 and 180 minutes after injection, then twice in the first week and weekly until activity was not noticeably above background. To check for intra vascular haemolysis, plasma was measured for [⁵¹Cr] activity. Urinary excretion of label was assessed in 7 volunteers by making 24 hour urine collections for the first 72 hours after injection. [⁵¹Cr] activity in the