Human Erythrocytes as Drug Carriers: Loading Efficiency and Side Effects of Hypotonic Dialysis, Chlorpromazine Treatment and Fusion with Liposomes

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Purpose
Human red blood cells (RBCs) are emerging as a highly biocompatible microparticulate drug delivery system. Loading of RBCs with therapeutics has focused on hypotonic dialysis, a rather disruptive method. We investigated loading of stored RBCs with enzymes of various molecular weight using hypotonic dialysis (HD), pretreatment with chlorpromazine (CPZ) and fusion with liposomes using RBCs. Along with loading efficiency, all methods were tested for the induction of side effects; next to hemolysis, we also addressed morphological changes and phosphatidylserine (PS) exposure which has been recognized as a critical parameter associated with premature RBC removal and induction of transfusion-related pathologies. We also assessed RBC deformability to predict whether loaded red blood cells can function as long-time circulating drug carriers.

Methods
Loading efficiency was determined by flow cytometry and enzyme-specific quantitative assays. Cellular localization of the loaded enzymes, as well as morphology of loaded cells, was assessed by multichannel confocal microscopy. Hemolysis induced by the different treatments was determined by spectrophotometric measurement of free hemoglobin. PS exposure was measured by flow cytometry, immediately after treatment and after 12-hour recovery. Cell deformability was observed by a bead-sorting device, that mimics the mechanical stress and deformation that RBCs experience during passage through the spleen.

Results
The efficiency of loading using hypotonic dialysis decreased with increasing molecular weight of the enzyme. For liposomes and chlorpromazine, loading efficiencies were higher and independent of enzyme molecular weights (Figure 1A). While hypotonic dialysis always induced a high degree of hemolysis, irreversible modifications in the morphology of the cells and PS exposure, the side effects that were induced by loading using CPZ and liposomes were limited. In particular, PS exposure, although high immediately after treatment, returned to physiological levels after recovery (Figure 1B). Retention and deformability studies using a spleen-mimicking device showed that RBCs treated with CPZ and liposomes behave like physiological RBCs, while HD led to very fragile and poorly deformable RBCs.

Conclusion
We conclude that CPZ and liposomes as alternative loading techniques can provide a solid and safe alternative to osmosis-based methods, as at least for stored erythrocytes they are superior in both loading efficiency and induction of side effects.