ACTIVE TRANSPORT OF ORGANIC ANIONS ACROSS THE HUMAN ERYTHROCYTE MEMBRANE

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Erythrocytes, which constitute more than 40% of the blood volume and about 3% of the body mass, are an important, although frequently neglected, detoxification organ. The main route of xenobiotic metabolism in erythrocytes goes through the glutathione conjugation process, and subsequently comprises removal of the conjugates via the membrane active transport systems. In human erythrocyte membranes, the presence of two ATP-Binding Cassette (ABC) superfamily transporters, MRP1 and MRP5, was demonstrated. MRP1 appears to be the main erythrocyte membrane transporter of glutathione S-conjugates and oxidized glutathione. MRP5 is mainly considered a cyclic nucleotide transporter, but its substrate specificity is broad, and this protein probably also transports glutathione conjugates and other organic anions. Thus, both proteins seem to constitute the “glutathione S-conjugate pump” of the human erythrocyte membrane. Interestingly, a controversy still exists concerning the nature of the “glutathione S-conjugate pump”, with some authors identifying it with the RLIP76 protein.

We compared the transport of different fluorescent anions – fluorescein, 5/6-carboxyfluorescein, 2',7'-bis-(2-carboxyethyl)-5/6-carboxyfluorescein and calcein – across the human erythrocyte membrane, using both the intact cell and isolated membrane vesicle models. Each compound was transported with different efficiency and with a different sensibility towards the classic inhibitors of organic anion transport. MRP1 was recognised as the main transporter of 2',7'-bis-(2-carboxyethyl)-5/6-carboxyfluorescein, but not of calcein. 5/6-carboxyfluorescein seems to be transported by a different transport system to MRP1 and MRP5.

The ATPase activity of the human erythrocyte membrane stimulated by glutathione conjugates was also analysed. The stimulation of this activity by oxidised glutathione was shown for the first time in a membrane. However, none of the studied fluorescent anions show the ability to stimulate ATPase activity. Also, studies on mrp1-/- and mrp5-/- mice excluded the possibility of engaging MRP1 or MRP5 in the observed measurable ATPase activity of the human erythrocyte membrane stimulated by organic anions.