2,6-Di(isobutyrylamino)hexanoic Acid as a Potential Therapeutic Agent for the Treament of Sickle Cell Disease

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2,6-Di(isobutyrylamino)hexanoic acid (DIBAH) was designed to compete with the B^6-valine residue of sickle cell hemoglobin for the hydrophobic pocket formed by B^{85}-phenylalanine and B^{88}-leucine of the hemoglobin molecule[1] and to interact directly with the erythrocyte membrane to improve cell flexibility. DIBAH was synthesized by reacting two equivalents of isobutyric anhydride and one equivalent of 2,6-diaminohexanoic acid. DIBAH was tested on both purified hemoglobin and erythrocytes obtained from sickle cell anemia patients.

The method of Poillon[2] was used to evaluate the effect of DIBAH on the solubility of deoxyhemoglobin S. DIBAH was found to increase the solubility of deoxyhemoglobin S. At concentrations of 10, 20, 30, and 40 mM, the relative concentration ratios ($C_{sat'}/C_{sat^o}$) of deoxyhemoglobin S were 1.050, 1.090, 1.152, and 1.177 respectively. The effect of DIBAH on cell deformability index was determined using an Ektacytometer (Technicon) which had been fitted with an oxygen gradient apparatus.[3,4] DIBAH lowered the partial pressure of oxygen at which cells begin to lose their deformability. Under a completely oxygenated condition, 20 mM DIBAH increased the deformability index of sickle erythrocytes from 0.45 to 0.50 after an incubation period of one hour. Under reduced oxygen tension, cells preincubated with 20 mM of DIBAH started to become rigid at a partial pressure of oxygen of about 76 mmHg. Control cells began to get rigid at a partial pressure of oxygen of about 125 mmHg. At a partial pressure of oxygen of 80 mmHg, control cells lost 50% of their original cell deformability index, but cells preincubated with 20 mM DIBAH had 100% of their original cell deformability index. At a partial pressure of about 55 mmHg cells preincubated with DIBAH lost 50% of their original cell deformability index, but control cells were fully rigid (FIGURE 1). Osmotic scan ektacytometry (FIGURE 2) showed that the DIBAH shifted the osmotic deformability spectrum of cells to the right of the control spectrum giving an indication that the compound may be hydrating the cells. However using the Coulter Counter Model 2F with a Coulter Model MHR computer attachment, no significant volume increase in mean cell volume was detected. Therefore it is reasonable to assume

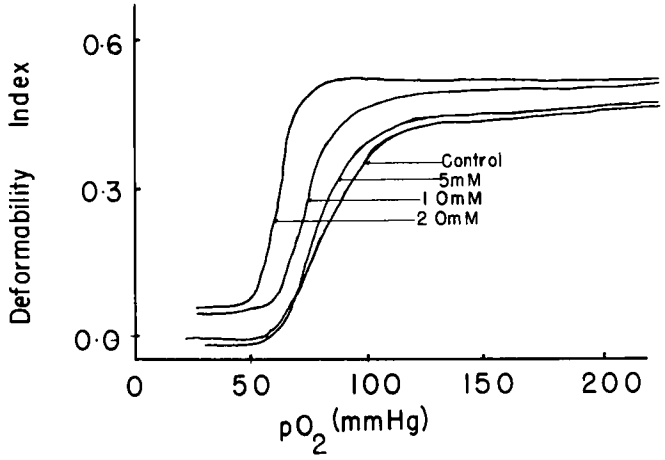

FIGURE 1. Oxygen scan ektacytometry of sickle erythrocytes treated with various concentrations of 2,6-di(isobutyrylamino)hexanoic acid.

that 2,6-di(isobutyrylamino)hexanoic acid interacts directly with the erythrocyte membrane to improve its flexibility.

It is concluded that DIBAH would improve the deformability of sickle erythrocytes and delay the onset of intracellular polymerization of sickle hemoglobin, thus allowing sickle erythrocytes to deform readily in the microvasculature. Therefore, if DIBAH is applied to cells in the model "the microcirculation and tissue damage in sickle cell

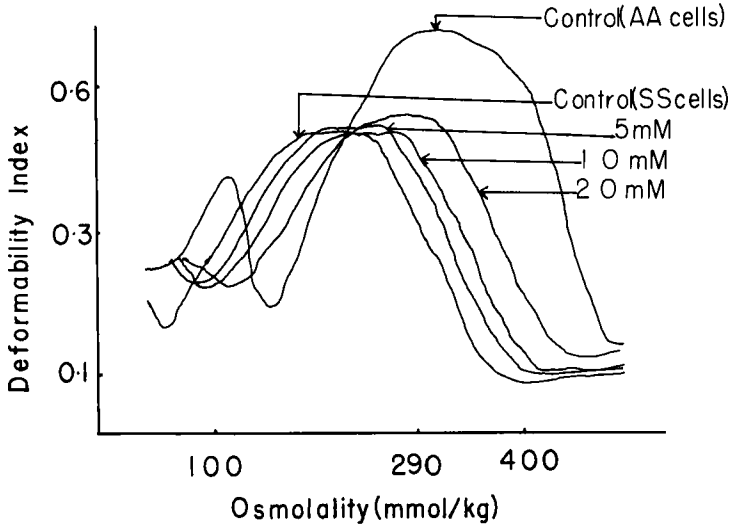

FIGURE 2. Comparison of osmotic scan ektacytometry of normal erythrocytes and sickle erythrocytes treated with various concentrations of 2,6-di(isobutyrylamino)hexanoic acid.

anemia" by Noguchi and Schechter,[5] cells will be able to escape entrapment in the microvasculature. An analogue of DIBAH that would affect the sickle erythrocyte in this manner at a lower concentration would be a potential therapeutic agent for the treatment of sickle cell disease.

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