Effect of Etidronate Disodium on Filterability of Sickle Cell Erythrocytes

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Abstract □ The effect of etidronate disodium on the deformability of human sickle cell erythrocytes was determined. A filtration process that demonstrated changes in trapping of ^{51}Cr-tagged sickle cells in the filtration apparatus was used to evaluate drug effects. Compared to the nontreated control, etidronate disodium [disodium dihydrogen (1-hydroxyethylidene)diphosphonate] reduced the trapping of sickled cells at low concentrations and increased trapping at high concentrations. These results indicate that etidronate disodium alters the deformability of sickled erythrocytes.

Keyphrases □ Etidronate disodium—effect on filterability of sickle cell erythrocytes □ Erythrocytes—sickle cell, effect of etidronate disodium on filterability □ Blood cell membrane—deformability of sickled erythrocytes, effect of etidronate disodium □ Sickle cell anemia—deformability of erythrocytes, effect of etidronate disodium

Sickle cell anemia has been studied intensively for the last decade. It is a unique disease in that its etiology is well known. The link between the genetically determined structural defect in the hemoglobin molecule and the clinical manifestations of the disease make it an excellent system for study by molecular biologists.

BACKGROUND

Despite the well-understood cause of the disease, some details about the sickling process are poorly understood. For example, it was shown only recently that the erythrocyte membrane may play an important role in the sickling process (1). Measurements of the deformability of erythrocytes as a function of time after deoxygenation showed that erythrocytes containing sickle cell hemoglobin became less deformable before any appreciable gelation of the sickle cell hemoglobin occurred. This finding is taken as evidence that the erythrocyte membrane is not only abnormal but that it also contributes significantly to the rigidity of the sickled erythrocyte.

One factor considered to be responsible for the abnormal membrane rigidity of the sickled erythrocyte is the presence of a high level of calcium in the cell (2). This calcium is expected to bind to the erythrocyte membrane, altering its deformability much as it does in normal erythrocytes (3). Another factor involved in the process is hemoglobin binding to the erythrocyte membrane. It was shown that the extent of hemoglobin binding to the membrane influences membrane rigidity and that normal hemoglobin binds to the sickled erythrocyte membrane to a greater extent than to the normal erythrocyte membrane (4).

Knowledge of the role of the erythrocyte membrane in deformability and its association with abnormally high levels of calcium has led to a new approach in the search for agents useful for therapeutic treatment of sickle cell disease. Investigators have begun looking for compounds that alter the calcium content of membranes (1). Anesthetics perform this function, and at least one, procaine hydrochloride, was shown to improve the filterability (deformability) of sickled erythrocytes (5). This approach has the potential of producing new and useful compounds for the treatment of sickle cell disease.

During early clinical trials of a diphosphonate-based bone scanning agent[1], patients with sickle cell disease had a high soft tissue uptake of the scanning agent in their extremities. Since diphosphonates adsorb strongly to calcium salts, it was suggested that this uptake might be due to the adsorption of the scanning agent to calcium in the erythrocytes trapped in the extremities. If this adsorption did occur, the deformability of the erythrocyte might perhaps be altered by etidronate disodium, which was the diphosphonate used in the scanning agent. Consequently, an investigation was initiated to determine if etidronate disodium alters sickle cell erythrocyte deformability.

EXPERIMENTAL

The filterability of erythrocyte suspensions generally is accepted as a measure of the deformability of the erythrocytes (6). Consequently, filterability was chosen as the parameter to determine the effects of etidronate disodium on deformability. The specific technique was adapted from the method of Wagner *et al.* (4).

Approximately 10 ml of blood was obtained from individual patients with sickle cell anemia whose hematology was well characterized. The samples were washed three times in an isotonic buffer solution containing 107 m*M* NaCl, 6 m*M* glucose, 400 mg of albumin/ml, and pH 7.4, 30 m*M* phosphate. The cells were tagged with sodium [^{51}Cr]chromate and treated with either etidronate disodium[2] or potassium cyanate.

The tagging was accomplished by splitting the sample in thirds. One-third of the sample (the negative control) was suspended in the isotonic buffer with 50 μCi of sodium [^{51}Cr]chromate and incubated for 1 hr at 37°. Another third (the positive control) (2) was suspended in a solution containing sodium [^{51}Cr]chromate as described and the same concentrations of glucose, albumin, and phosphate as the standard buffer but also containing potassium cyanate. The potassium cyanate concentration was varied from one experiment to the next, with the isotonicity of the solutions maintained by addition of sodium chloride to the buffer. The remaining one-third of the sample was suspended in buffer and treated in the same manner as the positive control, except that etidronate disodium was substituted for potassium cyanate.

The suspensions then were incubated for 1 hr at 37°. After incubation, the cell suspensions were washed three times with the isotonic buffer and were suspended in a ratio of 1:9 with unlabeled washed cells from a normal healthy donor to a final hematocrit of 1%. At this point, cell suspensions were stored overnight at 4°. The cells were equilibrated the next day to a partial pressure of oxygen of 26 mm Hg at 37° and filtered through 5-μm

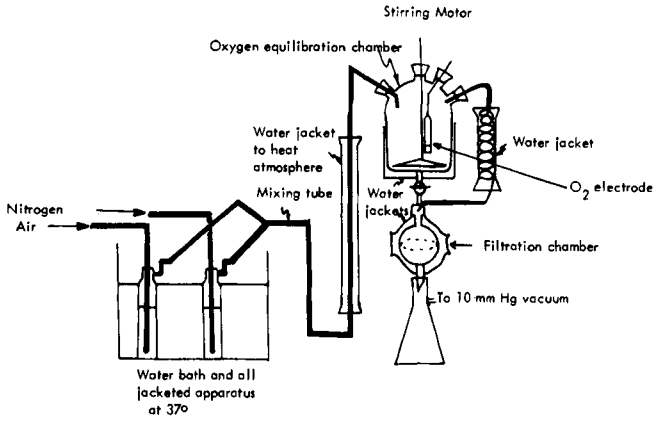

Figure 1—*Schematic diagram of the filtration apparatus. The erythrocyte suspension is introduced into the oxygen equilibration chamber where the partial pressure of oxygen and temperature are monitored. The air–nitrogen mixture is saturated with water and heated to 37° before it is introduced into the equilibration chamber. When the system is equilibrated (partial pressure of oxygen of 26 mm Hg and temperature of 37°), ~5 ml of suspension is transferred directly to the filtration chamber. Then a 10-mm Hg vacuum differential is applied to the filtration system, and the suspension passes through the filter.*

[1] Osteoscan.

[2] Procter & Gamble.

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Table I—Filtration Data for Untreated and Potassium Cyanate-Treated Controls and Etidronate Disodium-Treated Cells

Treatment	Treatment, m*M*	Percent of Cells Trapped ± *SD*	*p*[a]
Control		10.8 ± 3.9	
Potassium cyanate	107	0.88 ± 0.13	<0.01
Etidronate disodium	107	18.7 ± 4.5	<0.01
Control		44.3 ± 11.4	
Potassium cyanate	107	13.9 ± 0.8	<0.01
Etidronate disodium	10.7	36.4 ± 6.7	<0.11
Control		31.4 ± 5.8	
Potassium cyanate	1.0	20.2 ± 4.8	<0.01
Etidronate disodium	1.0	20.6 ± 6.8	<0.025
Control		58.2 ± 3.5	
Potassium cyanate	1.0	50.5 ± 11.1	<0.10
Etidronate disodium	0.10	44.4 ± 5.8	<0.001
Control		33.6 ± 4.5	
Potassium cyanate	107	5.1 ± 0.5	<0.001
Etidronate disodium	0.001	35.5 ± 11.0	NS

[a] The *p* values were determined using a single-tailed Student *t* test.

polycarbonate filter disks with a 10-mm Hg pressure differential. The filtration apparatus is described in Fig. 1. Following filtration, the filter paper and filtrate were counted in a well-type scintillation counter, and the percentage of cells that were not deformable enough to pass through the 5-μm pores of the filter disk were determined as:

$$\% \text{ trapping} = \frac{\text{number of trapped cells}}{\text{total number of cells}} \times 100 - \frac{\text{cpm from filter disk}}{\text{cpm from filter disk + cpm from filtrate}} \times 100 \quad \text{(Eq. 1)}$$

The percent trapping is taken as a measure of deformability. The larger the trapping, the lower is the deformability.

RESULTS AND DISCUSSION

The results from five filtration experiments are shown in Table I. The values reported are the means (±*SD*) of five filtrations per treatment group. The filtrations for all three treatments in each experiment were completed on the same day.

The average trapping for the controls in each experiment varied from 10.8 to 58.2%. Since the blood samples each came from different donors, this variability was not unexpected. It may have been due to the difference in the sickle cell hemoglobin content or the oxygen affinity of the erythrocytes from individual to individual.

Cyanate is effective in increasing the deformability of sickled erythrocytes and was included in these experiments as a positive control on the procedure (4). The average trapping for the cyanate-treated samples was significantly less than the control trapping in each experiment over the range of 1.0–107 m*M* cyanate. Consequently, it is believed that this apparatus and these procedures were capable of detecting changes in deformability.

In these experiments, etidronate disodium altered the deformability of the sickled erythrocytes. At high concentrations (107 m*M*), the deformability of the erythrocytes was decreased; at lower concentrations

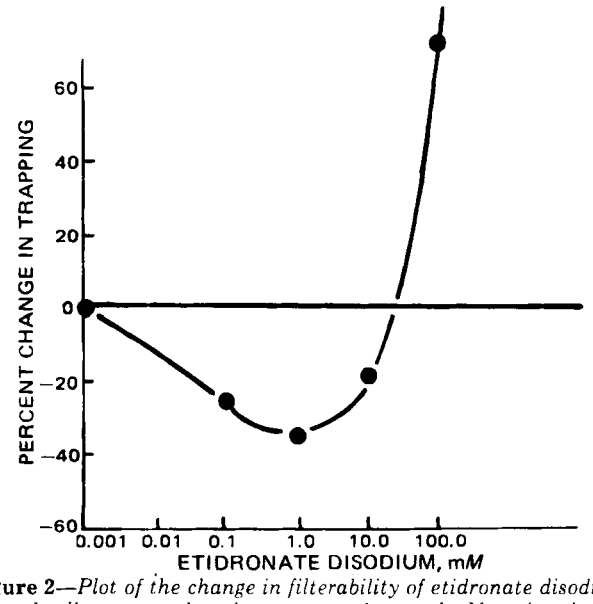

Figure 2—*Plot of the change in filterability of etidronate disodium-treated cells compared to the nontreated controls. Negative changes in trapping indicate increased cell (membrane) deformability, whereas positive changes indicate decreased deformability.*

(0.10–10 m*M*), the deformability of the cells was increased significantly. This biphasic response is quite obvious in Fig. 2, although the position of the minimum is subject to some uncertainty since different blood samples were used at each concentration. This type of biphasic effect is unusual but not unique. For example, isoproterenol and the prostaglandins exhibit a biphasic response on erythrocyte deformability. It is believed that this type of response is produced by the action of the drug on specific membrane receptors (7).

Since filtration experiments generally are considered to be a measure of membrane deformability (6), and since etidronate disodium produces an effect similar to that produced by the membrane actions of prostaglandin and isoproterenol, it is tempting to conclude that the filterability changes seen with etidronate disodium result from an interaction of etidronate disodium and the erythrocyte membrane. However, since sickle cell hemoglobin gelation is known to influence sickle cell erythrocyte filterability, such a conclusion cannot be reached from this work alone.

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