CYCLODEXTRIN-INDUCED HEMOLYSIS AND SHAPE CHANGES OF HUMAN ERYTHROCYTES IN VITRO

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Cyclodextrins (CyDs) at higher concentrations were found to cause hemolysis of human erythrocytes in the order of \( \beta^- > \alpha^- > \gamma\)-CyD in isotonic solution. Biphasic effects of CyDs were observed for the osmotic and heat-induced hemolysis; i.e. the protection at relatively low CyD concentrations and stimulation at higher CyD concentrations. From the scanning electron microscopic observations, CyDs induced shape changes of membrane internalization type on erythrocytes. CyDs caused the release of cholesterol from erythrocyte membrane in the order of \( \beta^- > \gamma^- > \alpha\)-CyD. These results clearly indicate that CyD-induced hemolysis is probably a secondary event resulting from the membrane disruption which elicited the removal of membrane components from erythrocytes.

Keywords — \( \alpha\)-, \( \beta\)-, and \( \gamma\)-cyclodextrins; human erythrocytes; hemolysis in vitro; scanning electron microscopy; membrane internalization; release of cholesterol

Cyclodextrins (CyDs), cyclic oligosaccharides, have been successfully applied pharmaceutically, to improve the physical and chemical properties of the drug molecules through inclusion complex formation.\(^1\)\(^2\) Although an improvement of bioavailability of drugs is one of the important practical applications of CyDs, most of them are studied for oral dosage forms. Recently, CyDs have been found to be negligibly toxic even in the parenteral administration.\(^2\) With the above observation in mind, we attempted to investigate the possible utility of CyDs as an injecting agent. In our previous studies, the protection of drug-induced hemolysis by CyD complexation was devised to prevent the local irritation induced with drugs.\(^3\)\(^4\) Under those experimental conditions, CyDs themselves affected little or no noticeable changes in membrane fragility and morphology of erythrocytes, because of the relatively low concentration of CyDs. However, we recently found that sufficiently higher concentrations of CyDs caused the hemolysis of erythrocytes significantly. In these continuing investigations, we report here the effects of three CyDs (\( \alpha\)-, \( \beta\)-, and \( \gamma\)-CyDs) at higher concentrations on human erythrocyte membrane in vitro.

Figure 1 shows the hemolytic effects of CyDs on human erythrocytes in isotonic solution. The hemolytic activity of CyDs was observed in the order of \( \beta^- > \alpha^- > \gamma\)-CyD, where hemolysis was initiated at 3 mM \( \beta\)-CyD, 6 mM \( \alpha\)-CyD, and 16 mM \( \gamma\)-CyD, respectively. Figure 2 shows the effects of CyDs on the osmotic and heat-induced hemolysis. CyDs gave biphasic effects against osmotic fragility of erythrocytes, with a protective effect at relatively low CyD concentrations and a stimulative effect at higher CyD concentrations (Fig. 2-A). The protective phase for \( \beta\)-CyD was not remarkable, while the maximal protection was given at 5 mM and 10 mM for \( \alpha\)- and \( \gamma\)-CyDs, respectively. In a similar concentration range, the biphasic effects of CyDs were observed for heat-induced hemolysis (Fig. 2-B). Contrary to hypotonic hemolysis, \( \beta\)-CyD showed rather large protective effect in compari-
son with those of α- and γ-CyDs. Figure 3 shows some scanning electron micrographs of human erythrocytes treated with CyDs. Upper photographs (Fig. 3-A, B, C) show the shape changes by lower CyD concentrations at which protective effects are observed for the osmotic and heat-induced hemolysis. Lower photographs (Fig. 3-D, E, F) show the shape changes by higher CyD concentrations at which hemolysis is initiated. CyDs were found to induce the membrane internalization at lower and higher concentrations. However, the extent of shape changes induced with three CyDs was distinctly different; i.e. α- and γ-CyDs caused erythrocytes to become cup-shaped, leading to spherical cells with invaginated vesicles in the interior, while β-CyD caused them to become partial swelling even at critical hemolytic concentration. From these observations, the extent of shape changes induced with three CyDs appeared to be correlated with the protective effects on the osmotic resistance. The protective effects of CyDs may be ascribed to the membrane expansion,5) as could be expected from the results of Fig. 3. CyDs may also alter the fluidization of the membrane lipids6) to protect the erythrocytes.

FIG. 2. Effects of CyDs on Hypotonic Hemolysis (A) and Heat-Induced Hemolysis (B) of Human Erythrocytes

a) Hemolysis in the absence of CyDs was taken as 1.0. Osmotic pressure (120±10 mOsm) and temperature (54±2°C) were chosen to cause about 50% hemolysis of erythrocytes in the absence of CyDs.

● : α-CyD; △ : β-CyD; □ : γ-CyD.
To gain insight into the mechanism of CyD-induced hemolysis, the effects of CyDs on membrane components were preliminarily investigated, since the hemolytic action of three CyDs seems to be somewhat different from each other. It was found that CyDs caused the release of some membrane components such as cholesterol, phospholipids, and proteins from erythrocytes, as reported in glycerin-induced hemolysis.\(^9\) Interestingly, the species and amounts of released components were depending upon the cavity size of CyDs. For example, at prelytic concentrations, the percentages of released cholesterol from erythrocytes were 0%, 19.9%, and 2.0% for 5 mM \(\alpha\)-CyD, 2 mM \(\beta\)-CyD, and 15 mM \(\gamma\)-CyD, respectively.\(^*\) These results clearly indicate that CyD-induced hemolysis is probably a secondary event resulting from the membrane disruption which elicited the removal of membrane components from erythrocytes.

From the safety point of view, it should be reminded that parenteral administration of a large dose of CyDs should be refrained. Therefore, it is necessary to select the cavity size and concentration of CyDs by considering the inclusion ability and safety, where \(\gamma\)-CyD may be particularly useful as an injecting agent among three CyDs.

REFERENCES


\(^*\) The stability constant for inclusion complex of cholesterol with \(\alpha\)-CyD was significantly small compared with those for \(\beta\)- and \(\gamma\)-CyD complexes.


