PROTECTIVE EFFECTS OF CYCLODEXTRINS ON DRUG-INDUCED HEMOLYSIS \textit{IN VITRO}

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$\beta$-Cyclodextrin ($\beta$-CyD) significantly protected the human erythrocytes from hemolysis and shape changes induced with chlorpromazine and flufenamic acid in isotonic solution. A good correlation between the stability constants of inclusion complexes ($\beta\rightarrow\gamma\rightarrow\alpha$-CyD) and the inhibitory effects on drug-induced hemolysis was found. From the observations of drug uptake into erythrocytes and changes in surface tension, the protective effects of CyDs appeared to be due to the decrease in effective concentration of drug through inclusion complexation rather than the direct interaction of CyDs with erythrocyte membrane.

\textbf{Keywords}— drug-induced hemolysis \textit{in vitro}; protective effect of cyclodextrin; stability constants of inclusion complexes; chlorpromazine; flufenamic acid; scanning electron microscopy; drug uptake into erythrocytes; surface tension

The drug-induced hemolysis provides serious problems, particularly from the viewpoint of local safety in the injection sites, and limits the development of pharmaceutical formulations. Cyclodextrins (CyDs) have been successfully applied in pharmaceutical field, by improving the physical and chemical properties of the drug molecules through inclusion complex formation.$^{1,2}$ The present paper deals with the effects of CyDs ($\alpha$-, $\beta$-, and $\gamma$-CyD) on the hemolysis induced with amphiphilic drugs \textit{in vitro}. In this study, for hemolytic agents, chlorpromazine (CPZ) was selected as cationic drug and flufenamic acid (FA) as anionic drug, since their hemolytic activities$^{3-5}$ and inclusion complexation$^{6,7}$ were well documented.

Figure 1 shows the hemolytic effects of CPZ and FA on human erythrocytes in the absence and presence of three CyDs in isotonic solution. CyDs decreased the hemolytic activities for both drugs in order of $\beta\rightarrow\gamma\rightarrow\alpha$-CyD, where $\beta$-CyD inhibited the hemolysis almost completely. This indicates that the smaller or larger CyD cavity than $\beta$-CyD is less effective to protect the drug.

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{figure1.png}
\caption{Hemolytic Effects of CPZ and FA on Human Erythrocytes (1%) in the Absence and Presence of CyDs (1$\times$10$^{-3}$M) in 10 mM Isotonic Phosphate Buffer (pH 7.4) at 37\degree C.}
\end{figure}

Percent hemolysis is expressed by the ratio of the absorbance at 543 nm of hemoglobin released from erythrocytes after incubation (5 min) with drug to the absorbance after the complete hemolysis of erythrocytes in water.

$\bigcirc$: drug alone; $\bullet$: drug + $\alpha$-CyD; $\triangle$: drug + $\beta$-CyD; $\square$: drug + $\gamma$-CyD.
induced hemolysis.

Figure 2 shows some scanning electron micrographs of human erythrocytes treated with CPZ and FA in the absence and presence of β-CyD. At relatively lower concentrations of the drugs, CPZ caused the erythrocytes to become

![Images of erythrocytes]

**FIG. 2. Scanning Electron Micrographs of Erythrocytes Treated with CPZ (6× 10⁻⁵ M) and FA (1.2× 10⁻³ M) in the Absence and Presence of β-CyD**

Magnification of the photographs is 7000×.

(A): control; (B): β-CyD (2× 10⁻³ M); (C): CPZ; (D): CPZ + β-CyD (1× 10⁻³ M); (E) FA; (F): FA + β-CyD (2× 10⁻³ M).

**TABLE I. Relationship between the Results of Inclusion Complexation and Drug-Induced Hemolysis of Human Erythrocytes**

in 10 mM Isotonic Phosphate Buffer (pH 7.4) at 37°C

<table>
<thead>
<tr>
<th>System</th>
<th>Hemolysis (%)</th>
<th>Amount of uptake ((× 10⁷ \text{ mol}))</th>
<th>Apparent surface tension (dynes/cm)</th>
<th>Stability constant (M⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPZ c)</td>
<td>FA d)</td>
<td>CPZ e)</td>
<td>FA f)</td>
</tr>
<tr>
<td>Without CyDs</td>
<td>100</td>
<td>100</td>
<td>1.3</td>
<td>2.3</td>
</tr>
<tr>
<td>With α-CyD</td>
<td>100</td>
<td>100</td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td>With β-CyD</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>With γ-CyD</td>
<td>23</td>
<td>37</td>
<td>0.5</td>
<td>1.8</td>
</tr>
</tbody>
</table>

a) Experimental conditions were the same as those in Fig. 1.

b) Determined from UV absorption change (pH 7.0, 25°C); see ref. 6, 7.

c) CPZ, 7× 10⁻⁴ M; CyDs, 1× 10⁻³ M.

d) FA, 2× 10⁻³ M; CyDs 1× 10⁻³ M.

e) CPZ, 2× 10⁻⁴ M; CyDs, 1× 10⁻³ M.

f) FA, 1× 10⁻³ M; CyDs 1× 10⁻³ M.

g) CPZ, 3× 10⁻⁴ M; CyDs, 1× 10⁻³ M.

h) FA, 1.7× 10⁻³ M; CyDs 1× 10⁻³ M.
cup-shaped (Fig. 2-C), while FA caused them to become crenated (Fig. 2-E). With higher drug concentrations, the erythrocytes became spherical and lysed. When β-CyD was added to the erythrocyte suspensions, the shape changes induced with CPZ and FA were significantly prevented, as shown in Fig. 2-D and 2-F, respectively. β-CyD itself had no effect on the discocytic form of erythrocytes under this experimental condition (Fig. 2-B). Similar results were obtained for α- and γ-CyDs. The order of protective effect was β-→ γ→ α-CyD, consistent with that of reduction in drug-induced hemolysis.

To elucidate the protective mechanism of CyDs, some factors responsible for the drug-induced hemolysis8 were preliminarily investigated. As shown in Table I, CyDs decreased the uptake of the drugs into erythrocytes along with the reduction of surface activities of both drugs, depending upon the magnitude of stability constants. By the addition of CyDs, no remarkable changes in osmotic pressure and viscosity of the drug solutions were observed under these experimental conditions. Moreover, CyDs showed a little protective or accelerative effect against the osmotic- and heat-induced hemolysis.

The above results indicate that the protective effect of CyDs was probably due to the decrease in effective drug concentrations through inclusion complex formation rather than the direct interaction of CyDs with erythrocyte membrane. These limited data suggest that β-CyD may be particularly useful in alleviating an injury to membrane upon injecting drugs, although the detailed mechanism should be investigated further.

REFERENCES