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UTILIZATION OF HUMAN SERUM ALBUMIN AS DRUG ADDITIVES I. STABILIZER OF PROSTACYCLIN (1)

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To examine the possibility of using human serum albumin (HSA) as a new drug additive, the stability of prostacyclin (PGI_2) in the presence of HSA was investigated kinetically in pH 7.35 phosphate buffer at 25°C. The half-life for the hydrolysis of PGI_2 was prolonged 10-fold in the presence of about 4-fold HSA molar concentration. By displacement examination using clofibric acid and phenylbutazone, the effective site on HSA for the PGI_2 stabilization was found to be near tyrosine 411 of the HSA amino acid sequence, not near tryptophan 214 as supposed in the literature.

KEYWORDS —— prostacyclin; stability; human serum albumin; stabilization; new drug additive; protein binding; binding site; displacement; clofibric acid; phenylbutazone

Since prostacyclin (PGI_2) has potent platelet anti-aggregatory activity, it is an interesting candidate for therapeutic use in cardiovascular, hematological and thrombotic disorders.(2,3) However, the chemical instability of PGI_2 (hydrolysis of the vinyl ether group)(4) in the aqueous solution prevents its use in therapy. Consequently, the stabilization of PGI_2 is required. Uekama et al.(5) reported the stabilization of PGI_2 by cyclodextrins (CD) in aqueous solution. Wynalda and Fitzpatrick(6) found that vertebrate albumin, of the various plasma constituents, delays the hydrolysis of PGI_2, prolonging its biological activity in vivo. Furthermore, they(7) reported that, in contrast with PGI_2, albumin accelerates the decomposition of prostaglandins with a β-hydroxy ketone (e.g., prostaglandin E_1), and they supposed that these effective sites of albumin are close to tryptophan 214 of the albumin amino acid sequence.(8)

We have attempted to use human serum albumin (HSA) as a new drug additive (e.g., stabilizer and solubilizer of various drugs). In this communication, we compare the stabilizing effects of β-CD(5) and HSA, and identify a PGI_2 binding site other than that supposed by Fitzpatrick and Wynalda.(7)

The reaction was carried out in pH 7.35 phosphate buffer (μ= 0.23) at 25°C. The hydrolysis rates of PGI_2 in the presence and absence of HSA were followed by high pressure liquid chromatography (JASCO, TRI ROTOR®), determining periodically the residual PGI_2. The chromatographic conditions were similar to those reported by Wynalda et al.(2)

Figure 1 shows the effects of HSA and β-CD(5) on the hydrolysis rates of PGI_2.

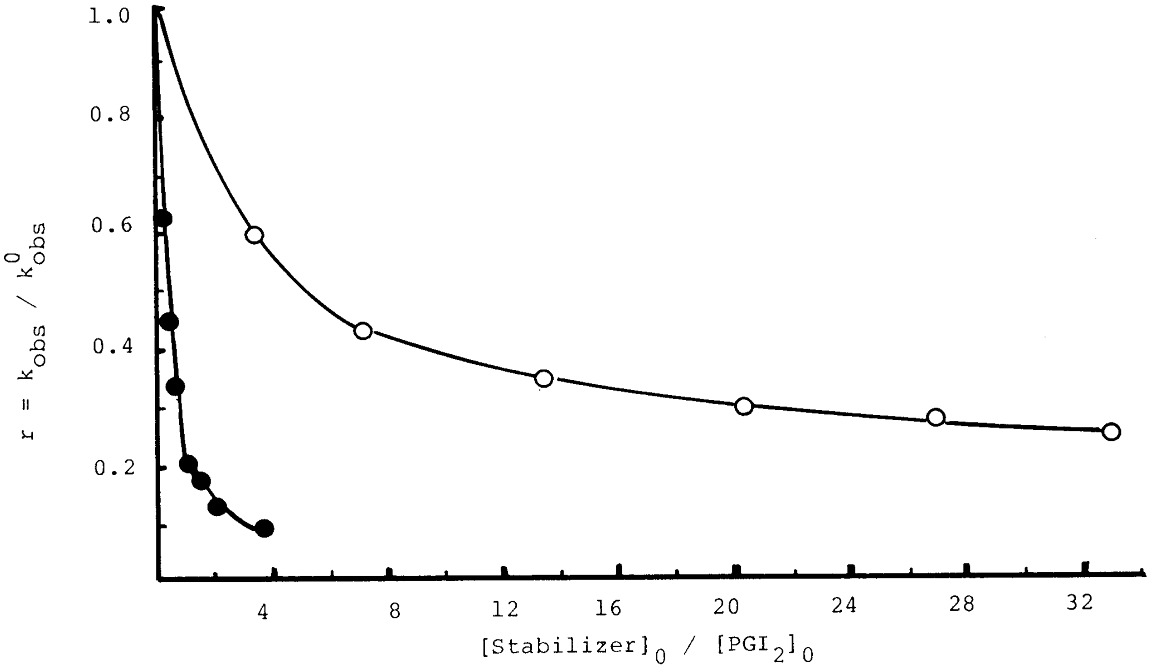

Fig. 1. Effects of HSA and β-CD on Hydrolysis Rate of PGI_2
●: HSA, pH 7.35 phosphate buffer ($\mu = 0.23$) at 25°C, $[PGI_2]_0 = 2.84 \times 10^{-4}$ (M);
○: β-CD, pH 7.0 phosphate buffer ($\mu = 0.20$) at 15°C, $[PGI_2]_0 = 3.0 \times 10^{-4}$ (M).[5]

In this figure, the ratio (r) of the pseudo-first-order rate constant (k_{obs}) for the hydrolysis in the presence of the stabilizer to that (k^0_{obs}) in its absence is plotted as a function of the molar ratio of the stabilizer to PGI_2. Evidently, the stabilizing effect of HSA is larger than that of β-CD which exhibited the largest effect among α-, β-, and γ-CD examined by Uekama et al.[5] The half-life of 2.8 min ($k^0_{obs} = 2.5 \times 10^{-1}$ min^{-1}), for example, was prolonged to 28 min ($k_{obs} = 2.5$ min^{-1}) in the presence of about 4-fold HSA over PGI_2 ($[HSA]_0 / [PGI_2]_0 \fallingdotseq 4$). This prolonged half-life of PGI_2 may be sufficient for the preparation of an intravenous injection solution, if the injection solution is made by dissolving lyophylized powdered PGI_2 and HSA in distilled water for injection.[9]

In our previous studies[10,11,12] of the distinction and identification of the drug binding sites on HSA, it was found that clofibric acid (CA) and phenylbutazone (PB) bind primarily to the R site (near tyrosine 411) and the U site (near both tryptophan 214 and lysine 199), respectively. The effects of both drugs on the stability of PGI_2 in the presence of HSA were examined to identify the PGI_2 binding site on HSA. The results are shown in Figure 2, where k'_{obs} in the ordinate is the rate constant in the presence of CA or PB. In the case of CA, k'_{obs} is larger than k_{obs} ($r' > 1$). This effect probably comes from a displacement of PGI_2 bound to the R site by CA. In contrast, PB hardly affects the k_{obs} value ($r' \fallingdotseq 1$) up to a ratio of about 2 on the abscissa. Above the ratio of 2 the r' value is larger than 1, indicating the displacement of PGI_2 by PB bound secondarily to the R site.[10,11] The effective site on HSA for the PGI_2 stabilization is , therefore, the R site, not the U site supposed by Wynalda and Fitzpatrick.[7]

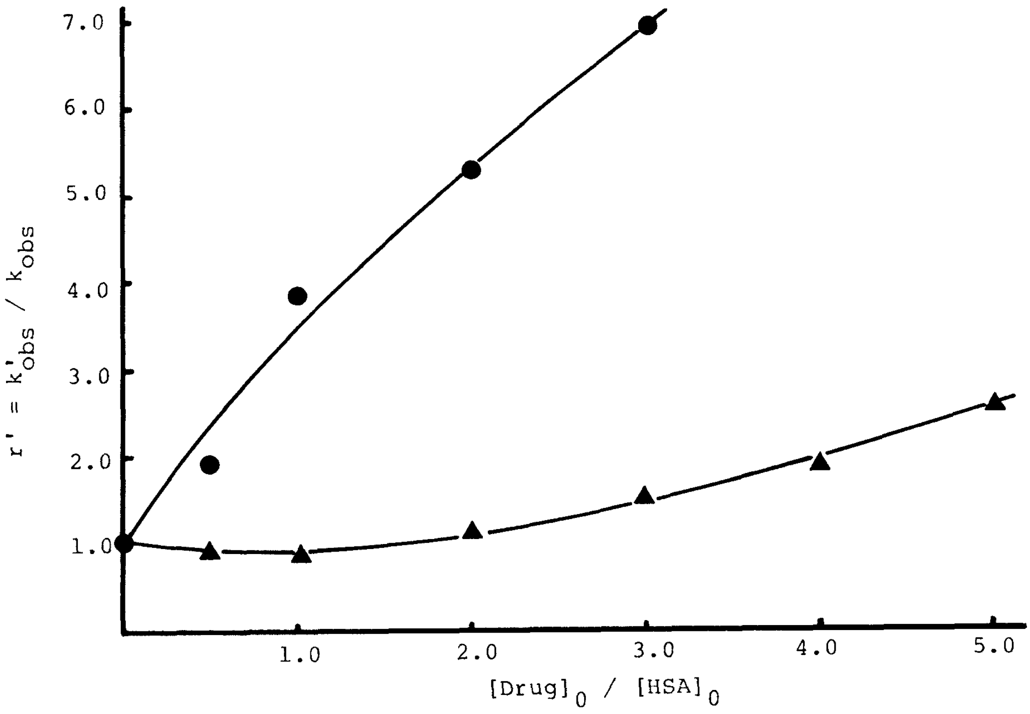

Fig. 2. Effects of CA and PB on the Stability of PGI_2 in the Presence of HSA at pH 7.35 and 25°C

● , CA ; ▲ , PB ; $[PGI_2]_0 = 2.84 \times 10^{-4}$ (M) ; $[HSA]_0 = 2.86 \times 10^{-4}$ (M).

Further studies are in progress to determine accurately the binding constant between PGI_2 and the R site by means of analog computer, and to evaluate the stability of the lyophylized powder of PGI_2 and HSA.

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