PERMEABILITY OF GLYCOSIDES THROUGH HUMAN ERYTHROCYTE MEMBRANE

Yasuhiro MATSUMOTO,* Masahiko OHSAKO and Ritsu SAKATA

Department of Pharmacy, Daiichi College of Pharmaceutical Sciences,
Tamagawa-cho 22-1, Minami-ku, Fukuoka 815, Japan

The permeability of glycosides (arbutin, salicin, glycyrritin, *p*-nitrophenyl- β -D-glucopyranoside, *p*-nitrophenyl- β -D-galactopyranoside, *p*-nitrophenyl- β -D-lactopyranoside, *p*-nitrophenyl- β -D-maltopyranoside) and their aglycons through human erythrocyte membrane was investigated. The glycosides permeated slowly, compared with their aglycons. Glycoside having disaccharide did not permeate the erythrocyte membrane. This suggested that the introduction of disaccharide to a drug significantly depresses the permeability of glycoside through erythrocyte membrane. The drug entrapped in erythrocytes was not released into the outer medium.

KEYWORDS transport; human erythrocyte; drug delivery system; glycoside; aglycon; disaccharide; targetting

As erythrocytes circulate for a long time (120 days is the average lifespan in humans), they may be accessible as a storage depot for the sustained release of drugs. The erythrocyte membranes have been used as drug delivery systems because they are biodegraded in the body.^{1,2)} As the mechanism of permeation was not elucidated completely, we need to study the matter further.

In our previous paper,³⁾ the regulation of the permeation rate and capacity through human erythrocyte membrane may be useful to control the release of drug from the erythrocyte carriers. The erythrocyte carriers-entrapped impermeable drugs can deliver selectively to the liver and spleen because most of the erythrocyte carriers are destroyed in them. The attempts to entrap the drug in the erythrocytes are carried out with protein and enzymes which are high-polymer.¹⁾ If an anticancer medicine were entrapped in the erythrocyte carriers, it might be delivered selectively to the liver or spleen. But attempts to make the low molecular weight drugs permeable have not been successful. It is important to discover substituents impermeabilized by drug bindings.

Here, we report on *in vitro* experiments on the permeation through human erythrocyte membrane of a glycoside (arbutin (ARB), salicin (SAL), glycyrritin (GLRI), *p*-nitrophenyl- β -D-glucopyranoside (NGLP), *p*-nitrophenyl- β -D-galactopyranoside (NGAP), *p*-nitrophenyl- β -D-lactopyranoside (NLP) and *p*-nitrophenyl- β -D-maltopyranoside (NMP)) and its aglycon (hydroquinone (HYD), saligenin (SALG), glycyrrhetic acid (GLRE) and *p*-nitrophenol (NP)).

Erythrocytes were prepared according to a previous report.³⁾ All reagents used were high grade. The permeability of drugs through human erythrocyte membrane was investigated by the methods described previously.³⁾ The intracellular concentration of drugs was analyzed by high performance liquid chromatography (the detector was spectrofluorometer or spectrophotometer). The erythrocyte-entrapped GLRI, NLP or NMP was prepared according to preswollen ghost methods.⁴⁾ The dissociation constant of drugs was calibrated by the usual method. The partition coefficient of drugs (*P*) was measured by the octanol-water system at 37°C. *P* showed the ratio drug concentration in octanol divided by the concentration of the molecular-form drug in water.

Table I shows *T*_{max} (the time at maximal intracellular concentration) and distribution of the drug in the suspending medium, the erythrocyte cytosol and the erythrocyte membrane. ARB and SAL, glycosides having monosaccharide and HYD, SALG and GLRE permeated human erythrocyte membrane although the drugs varied in the rate and capacity of the transport. On the other hand, the glycoside was markedly different from its aglycon in the rate of transport. In the aglycons, HYD, SALG and GLRE, the rate of transport was so rapid that the equilibrium was attained almost within one minute. In contrast to these drugs, the permeation of SAL was slower and it took 4 h before the equilibrium was attained. The

Table I. Distribution of Drugs in the Suspending Medium, the Erythrocyte Cytosol and the Erythrocyte Membrane

Drug	Medium	Fraction	Percentage (the detected amount/the total amount added) of the drug detected in each fraction			Tmax
			Suspending medium	Cytosol components	Membrane	
SAL	PBS plasma		55.9 $\pm$ 1.3	42.0 $\pm$ 2.3	0.58 $\pm$ 0.13	4 h
			57.0 $\pm$ 2.2	41.4 $\pm$ 3.4	0.87 $\pm$ 0.50	
SALG	PBS plasma		43.3 $\pm$ 1.8	58.3 $\pm$ 1.5	0.70 $\pm$ 0.20	1 min
			53.6 $\pm$ 2.4	46.4 $\pm$ 1.2	0.92 $\pm$ 0.59	
ARB	PBS plasma		68.8 $\pm$ 1.9	28.3 $\pm$ 1.2	0.86 $\pm$ 0.40	> 6 h
			68.6 $\pm$ 2.3	28.8 $\pm$ 1.0	0.67 $\pm$ 0.55	
HYD	PBS plasma		41.5 $\pm$ 1.8	60.2 $\pm$ 1.4	0.63 $\pm$ 0.41	1 min
			43.7 $\pm$ 0.4	56.2 $\pm$ 1.0	0.79 $\pm$ 0.37	
GLRI	PBS plasma		98.3 $\pm$ 1.1	5.4 $\pm$ 0.1	0.18 $\pm$ 0.13	----
			94.5 $\pm$ 1.2	4.7 $\pm$ 1.0	0.86 $\pm$ 0.40	
GLRE	PBS plasma		17.4 $\pm$ 0.4	76.9 $\pm$ 4.3	7.85 $\pm$ 1.59	1 min
			90.1 $\pm$ 0.5	3.1 $\pm$ 0.1	0.05 $\pm$ 0.01	
NP	PBS plasma		31.9 $\pm$ 1.1	66.3 $\pm$ 0.8	0.49 $\pm$ 0.29	1 min
			76.5 $\pm$ 0.9	20.2 $\pm$ 0.4	0.50 $\pm$ 0.13	
NGLP	PBS plasma		52.7 $\pm$ 1.1	46.6 $\pm$ 1.0	0.55 $\pm$ 0.12	1 h
			56.2 $\pm$ 0.9	44.1 $\pm$ 0.8	0.45 $\pm$ 0.22	
NGAP	PBS plasma		48.3 $\pm$ 0.3	52.9 $\pm$ 0.3	0.31 $\pm$ 0.10	1 h
			52.0 $\pm$ 0.8	49.7 $\pm$ 0.3	0.16 $\pm$ 0.09	
NLP	PBS plasma		95.6 $\pm$ 0.5	3.3 $\pm$ 0.4	0.35 $\pm$ 0.19	----
			95.3 $\pm$ 0.4	2.8 $\pm$ 0.5	0.35 $\pm$ 0.02	
NMP	PBS plasma		95.1 $\pm$ 1.1	3.7 $\pm$ 0.2	0.49 $\pm$ 0.18	----
			97.4 $\pm$ 0.6	3.0 $\pm$ 0.1	0.33 $\pm$ 0.08	

Erythrocytes (0.9 ml) were incubated with 1.1 ml of drug-containing PBS or plasma at pH 7.4 at 37°C until the concentration came to equilibrium (except ARB, 6 h) and then centrifuged at 2,900 x g for 15 min to give the sup. (the suspending medium containing the remaining drug) and the ppt. (erythrocytes). The erythrocytes were homogenized with one volume of distilled water and the resultant hemolysate was centrifuged at 725 x g or 16,260 x g for 15 min to give the hemolysate sup. (containing the cytosol component) and ppt. (the membrane). Data reported represent the means $\pm$ S.D. of three or four experiments.

permeation of ARB was still slower and the permeation increased fairly linearly during incubation for 6 h. The intracellular concentration was lower in permeation by ARB and SAL than HYD and SALG, respectively. GLRI, having disaccharide, barely permeated the human erythrocyte membrane, even though the ARB and SAL permeated, though the rate of transport was slow. The concentration of the permeated aglycon was higher in the permeation from PBS to erythrocytes than from the plasma to erythrocytes. In the glycoside, the rate and capacity of the transport were not influenced by the kind of suspending medium.

To reveal the origin of impermeability of GLRI having disaccharide, we examined the synthetic glycosides introduced monosaccharide or disaccharide to the NP as aglycon. The permeation of the glycosides with NP as aglycon showed tendencies similar to those above. The permeation of NP was rapid and equilibrium was attained almost within one minute. The permeation of the NGLP and NGAP with monosaccharide was slower. It took 1 h before the equilibrium was attained. The NLP and NMP with disaccharide scarcely permeated the erythrocyte membrane. The concentration of the permeating NP was appreciably higher in the permeation from PBS to erythrocytes than from plasma to erythrocytes. In the glycosides, NGLP, NGAP, NLP and NMP, the rate and capacity of the transport were not influenced by the kind of suspending medium used.

Fig. 1 Release of Glycoside and Its Aglycon from the Drug-Containing Preswollen Ghosts into the Suspending PBS. NP(■), NGLP (●), NGAP(○), NLP(□), NMP(△).

As shown in Fig 1, a behavior similar to the inward transport was also observed in the outward transport of the drugs from preswollen ghosts to the suspending medium. The outward transport of HYD, SALG, GLRE and NP was also very rapid and almost completed within one minute. GLRI, NLP and NMP entrapped in the erythrocyte interiors according to the method of preswollen ghost released very little through the human erythrocyte membrane but the ARB, SAL, NGLP and NGAP with a monosaccharide were released, though the rate of transport was slow.

Table II shows the dissociation constant and partition coefficient of the drugs. Generally, the transport of drug is fundamentally related to the partition coefficient, as the drugs permeate lipid membrane.⁵⁾ The T_{max} was related to the partition coefficient (correlation coefficient, $r = -0.98$) in glycosides, except the GLRI, NLP and NMP with disaccharide. The permeability was related to neither the dissociation constant nor the partition coefficient of drugs. It has been remarked that the permeability of a drug is related to the molecular weight (Mw). But it did not appear that the increase of Mw was related to the impermeation since GLRE (Mw, 470.69) permeates rapidly while NLP and NMP (Mw, 463.4) do not permeate at all. The impermeability may be caused by introducing disaccharide.

In this investigation, although there were various questions to consider, it was suggested that introducing disaccharide to drugs we want to deliver impermeatized the drug. An anticancer medicine applied by this mechanism may be entrapped in the erythrocyte carriers. Because most of the erythrocytes are destroyed in the liver and spleen, it may be possible that the anticancer medicine is delivered selectively in them. This investigation may be important for using erythrocyte-entrapped drugs in drug delivery systems.

REFERENCES

- 1) G.M. Ihler, "Drug Carriers in Biology and Medicine," Academic Press, London, 1979, pp. 129-153.; G.M. Ihler, R.H. Glew and F.W. Schnure, Proc. Natl. Acad. Sci., USA, **70**, 2663 (1973).; H.O. Alpar and D.A. Lewis, Biochem. Pharmacol., **34**, 257 (1985).
- 2) H.G. Eichler, W. Raffesberg, S. Gasic, A. Korn and K. Bauer, Res. Exp. Med., **185**, 341 (1985).; A. DeFlora, U. Benatti, L. Guida and E. Zocchi, Proc. Natl. Acad. Sci., USA, **83**, 7029 (1986).; H.G. Eichler, H. Rameis, K. Bauer, A. Korn, S. Bacher and S. Gasic, Eur. J. Clin. Inv., **16**, 39 (1986).
- 3) Y. Matsumoto and M. Ohsako, Yakugaku Zasshi, **110**, 120 (1990).
- 4) E. Pitt, C.M. Johnson, D.A. Jenner and R.E. Offord, Biochem. Pharmacol., **32**, 3359 (1983).; A. Tamura, N. Tominaga, T. Sato and T. Fujii, Yakuzai-gaku, **48**, 86 (1988).
- 5) J.M. Diamond and E.M. Wright, Annu. Rev. Physiol., **31**, 581 (1969).; C. Hansch and W.J. Dunn, J. Pharm. Sci., **61**, 1 (1972).

Table II. Dissociation Constant and Partition Coefficient of Drugs

Drug	pKa	log P
SAL	N ^{a)}	-1.28
SALG	10.05	0.68
ARB	9.85	-1.64
HYD	10.33	0.45
GLRI	4.93	2.80
GLRE	4.63	4.77
NP	7.07	1.90
NGLP	N	-0.46
NGAP	N	-0.66
NLP	N	-1.64
NMP	N	-1.46

a) N is nonelectrolytes.

(Received March 18, 1991)