Effect of Nonionic Surfactants on the Percutaneous Absorption of Tenoxicam

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The effect of 14 types of nonionic surfactants on the permeation of Tenoxicam (TEN) through guinea pigs from propylene glycol solution was investigated *in vitro*. The flux of TEN was significantly enhanced by poly-oxyethylene alkyl ether and polyoxyethylene monoalkyl carboxylate as hydrophilic surfactants, and by polyglyceryl trialkylate, glyceryl monoalkylate, sorbitan monoalkyl carboxylate and sorbitan trialkyl carboxylate as hydrophobic surfactants. In the presence of these surfactants, the flux was increased about 2 to 4 times, as compared to the flux without them. The mechanism of nonionic surfactant action was analyzed and classified by recent methods, the including HLB of surfactants, the solubility of the drugs in vehicle and the hemolysis of erythrocytes. The results suggested that vital factors in the enhancement of skin permeation by nonionic surfactants include the affinity of the surfactants to the stratum corneum, the solubility of the drugs in vehicle and the penetration of surfactants to the stratum corneum.

Key words　percutaneous absorption; nonionic surfactant; hemolysis; solubility; HLB; Tenoxicam

Recently, the transdermal therapeutic system (TTS) has been recognized as a drug delivery system (DDS) due to its several merits such as the control of drug concentration in the blood, and the avoidance of digestive enzymes and first pass effect by hepatic cells. The stratum corneum presents a significant barrier in percutaneous absorption. Therefore, a technical challenge lies in overcoming the barrier for the passage of the drug to a subcutaneous vessel. Technically, surfactants have been utilized to overcome the problem. This requires the efficient use of several of a surfactant's functions, including the solubility of the drugs in the vehicle, low interfacial tension ability, their penetration into the stratum corneum and so on. Sodium lauryl sulfate is the most popular skin permeation enhancer among the surfactants.[1,2] However, it is not suitable for use in a vehicle because it is a strong irritant

to the skin. In this regard, nonionic surfactants which are weak irritants can be used, and have been studied as effective enhancers of percutaneous absorption.[3-10] However, only a few surfactants have been evaluated, and are still not completely systematized. We have reported the effect of 14 types of nonionic surfactants, as external skin materials, on the permeability of the drug TEN, and have discussed the mechanism from a physicochemical viewpoint.

Experimental

Materials　Tenoxicam (TEN) was purchased from FAR-CA Co. (Milano, Italy). 14 Types of nonionic surfactants were used in the skin permeation examination *in vitro*. For this purpose, polyglyceryl (13) polyoxybutylene (13) stearyl ether (HGS-26), polyoxyethylene (20) polyoxypropylene (4) cetyl ether (PBC-34), polyoxyethylene (20) sorbitan monooleate (TO-10M), polyoxyethylene (10) oleyl ether (BO-

Table 1. Characterization of Surfactants and Their Efficiency on Percutaneous Absorption of TEN

Type of surfactants	Abbreviation	HLB	Lag time h	J $\times 10^{-5} \mu g/s \cdot cm^2$	C_v $\mu g/ml$	P $\times 10^{-8} cm/s$	Hemolysis %
No surfactant	—	—	18.4± 2.7	8.11±0.56	497	16.3±0.8	0.0±0.0
Polyglycerol (13) polyoxybutylene (13) stearyl ether	HGS-26	30.0	15.3± 1.9	5.22±0.46**	1220	4.3±0.3***	42.1±3.0
Polyoxyethylene (20) polyoxypropylene (4) cetyl ether	PBC-34	16.5	14.8±12.4	7.28±0.94	1179	6.2±0.6***	99.1±0.3
Polyoxyethylene (20) sorbitan monooleate	TO-10M	15.0	18.1± 3.0	4.46±1.36*	1073	4.2±1.2**	0.0±0.0
Sucrose monooleate	OWA-1570	15.0	15.2± 1.4	11.11±2.07	509	21.8±2.9	100.0±0.0
Polyoxyethylene (10) oleyl ether	BO-10TX	14.0	14.5± 1.1	19.21±2.97**	1539	12.5±1.4	98.2±0.3
Polyoxyethylene (7.5) nonylphenyl ether	NP-7.5	14.0	18.4± 0.8	7.00±0.56	648	10.8±0.6**	99.9±0.1
Polyoxyethylene (60) hydrogenated castor oil	HCO-60	14.0	>45.0	0.00±0.00***	1182	0.0±0.0***	0.0±0.0
Polyoxyethylene (60) sorbit teraoleate	GO-460	14.0	25.8± 0.0	3.90±1.28*	1284	3.0±1.0**	0.0±0.0
Decaglyceryl trioleate	Decaglyn 3-O	7.0	19.4± 0.5	28.48±8.55*	744	38.3±8.1	16.7±1.0
Sorbitan monooleate	SO-10	4.5	15.0± 2.6	17.03±1.42**	735	23.2±1.4*	1.7±0.4
Sorbitan trioleate	SO-30R	4.3	13.2± 4.7	12.73±1.29**	639	19.9±1.2	100.0±0.0
Glyceryl monooleate	MGO	2.6	15.1± 4.9	24.55±8.07*	1406	17.5±4.1	0.0±0.0
Dioleylphosphatidylcholine	DOPC	—	11.8± 2.7	8.96±2.08	728	8.2±2.0*	0.0±0.0
Polyoxyethylene (2) monooleate	MYO-2	4.5	13.1± 2.7	20.01±0.62***	1404	14.3±0.3	1.8±0.2
Polyoxyethylene (6) monooleate	MYO-6	8.5	14.2± 0.7	17.80±4.03*	—	—	—
Polyoxyethylene (10) monooleate	MYO-10	11.0	11.6± 4.5	17.30±1.04***	—	—	—

*p<0.05; **p<0.01; ***p<0.001.

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10TX), polyoxyethylene (7.5) nonylphenyl ether (NP-7.5), polyoxy-ethylene (60) hydrogenated castor oil (HCO-60), polyoxyethylene (60) sorbit tetraoleate (GO-460), decaglyceryl trioleate (Decaglyn 3-O), sorbitan monooleate (SO-10), sorbitan trioleate (SO-30R), glyceryl monooleate (MGO), polyoxyethylene (2, 6, 10) monooleate (MYO-2, 6, 10) were purchased from Nikko Chemicals Co. (Tokyo, Japan). Sucrose monooleate (OWA-1570) was obtained from Ryoto Co. (Tokyo, Japan). Dioleylphosphatidylcholine (DOPC) was supplied by Nippon Fine Chemical Co. (Osaka, Japan). The abbreviations and HLB values of these nonionic surfactants are shown in Table 1. Propylene glycol was purchased from Nikko Chemical Co. (Gifu, Japan). Materials of commercial grade, without further purification, were utilized.

Preparation of TEN Lotions TEN lotions were prepared as follows: 0.3 g of TEN was suspended in a mixed solution of 3.0 g of propylene glycol and 1.5 g of nonionic surfactants. 25.2 g of 33.3 mM phosphate buffer was added to the suspension and the pH was adjusted to 6.0, resulting in 1.0% TEN suspension containing 10% propylene glycol and 5.0% nonionic surfactants.

Analysis by HPLC The TEN contents in the receptor solution and the solubility of the TEN lotions were analyzed by HPLC (LC-9A, Shimazu, Kyoto, Japan). The HPLC conditions were as follows: column, Tosoh TSK gel ODS-80T$_M$ (4.6 × 150 mm); mobile phase, methanol–50 mM phosphate buffer at pH 6.0 (50 : 50); detector, UV 350 nm; column temperature, 40 °C; flow rate, 1.0 ml/min.

Procedure for Skin Permeation in Vitro Hartley strain male guinea pigs weighing 300 to 450 g were used. After cutting the hair with hair clippers and termination by exsanguination, the dorsal skin of each guinea pig was excised and mounted on a Franz type diffusion chamber. After the application of 1.0 g of test suspension to the donor skin, 1.0 ml of a receptor solution of 50 g of phosphate buffered saline containing 100 ppm of kanamycin sulfate at pH 7.4 was sampled periodically, every 3 h, until 48 h, and the permeated contents of TEN were determined by HPLC. The examination of skin permeation in vitro was performed in a closed system at 37 °C.

Calculation of the Permeability Coefficient Steady-state flux, J, is a function of both the apparent permeability coefficient, P and the solubility of TEN in 10% propylene glycol solution, C_v. It is useful only in the case of saturated suspensions. The P value can be calculated as J per C_v.

Hemolysis Blood was drawn from white male Japanese rabbits weighing 2.0 to 3.0 kg. Erythrocytes containing heparin were separated by centrifugation at 2400 × g for 5 min and resuspended after being washed in an isotonic buffer solution (0.15 M sodium chloride and 0.01 M sodium phosphate at pH 7.0). After repeating this procedure three times, a 2% (v/v) suspension was obtained. The concentration was measured at 1.14×10^8 cells per ml with a Coulter counter.

2.0 ml of erythrocyte suspension and 2.0 ml of 0.2% surfactant buffer solution were mixed in a test tube. After gentle mixing, the tubes were kept in a controlled water bath at 37 °C for 30 min and then centrifuged at 3500 × g for 15 min. The extent of hemolysis was calculated as a percentage from the absorbance of the supernatant at 540 nm. A completely hemolyzed control sample was prepared by diluting the erythrocyte suspension with a 1.0% aqueous solution of Triton X-100. A stable erythrocyte sample was prepared by the addition of isotonic buffer solution to the erythrocyte suspension.

Results and Discussion

Chemical Structure of Surfactants The effects of 14 types of nonionic surfactants on the permeation of TEN through guinea pig skin from propylene glycol solution was investigated in vitro and the flux of TEN, J, is shown in Table 1. The flux of TEN was significantly enhanced by polyoxyethylene alkyl ether and polyoxyethylene monoalkyl carboxylate as hydrophilic surfactants, and by polyglyceryl trialkylate, glyceryl monoalkylate, sorbitan monoalkyl carboxylate and sorbitan trialkyl carboxylate as hydrophobic surfactants. In the presence of these surfactants, the flux was increased about 2 to 4 times, as compared to the flux without them. Hwang[4] and Fabregas[5] had reported that a polyoxyethylene alkyl
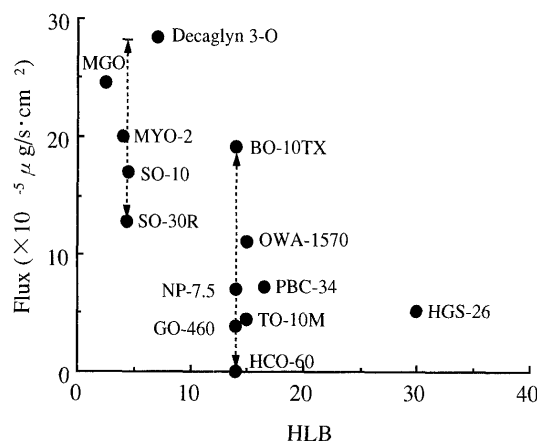

Fig. 1. Relationship between HLB of Surfactants and Flux of TEN

Each value represents the mean ± S.D. ($n = 3$).

ether such as BO-10TX, a polyoxyethylene monoalkyl carboxylate such as MYO-2, and a sorbitan trialkyl carboxylate such as SO-10 were effective enhancers of percutaneous absorption, and these results were in accordance with ours. Our results were examined in water-continuous lotions containing 10% propylene glycol, while their results were examined in oil-continuous ointments and water-continuous oil-in-water creams, respectively. Therefore, these enhancers will effectively act in any type of external vehicle for the skin.

HLB of Surfactants The HLB of the surfactants is one of the factors affecting percutaneous absorption. The relationship between the HLB of the surfactants and the skin permeability of TEN is shown in Fig. 1. The results showed high skin permeability in the presence of hydrophobic surfactants, ranging in flux from 12.78 to 28.48 ($\times 10^{-5}$ μg/s·cm^2), as compared to the hydrophilic surfactants which ranged in flux from 0 to 19.21 ($\times 10^{-5}$ μg/s·cm^2). Furthermore, the HLB of the surfactants appeared to be the dominant factor in the enhancement of skin permeation by these surfactants. Dalvi[11] and Irimoto[9] reported that hydrophobic surfactants have a strong affinity to the skin, resulting in the enhancement of skin permeability. To ensure the extent to which skin permeation is enhanced by the HLB of the surfactants, the skin permeability of polyoxyethylene alkyl carboxylate was investigated by using HLB changes produced by the polyoxyethylene chain length of its chemical structure. The result showed that skin permeation tended to decrease according to the hydrophilicity of surfactants MYO-2, MYO-6 and MYO-10 corresponding to the HLB of 4.5, 8.5 and 11.0, respectively, but the changes in permeability were small. Although the HLB of the surfactants might be one of the factors determining affinity to the skin, the extent of enhancement of skin permeation by chemical structure was superior to the HLB of the surfactants.

Solubility When skin permeation is the rate-determining step, it is known on the basis of Fick's law that the flux of the drug, J, is dependent on the multiplication of the partition coefficient between the stratum corneum and the vehicle, K, and on the solubility of TEN in the vehicle, C_v. Therefore, in the case of saturated suspensions, the flux of drugs increases with an increase in solubility caused by the addition of surfactants. On the other hand, the

partition coefficient between the stratum corneum and the vehicle will change by means of the partition of the surfactants in relation to the stratum corneum. The permeability coefficient, P, is an useful parameter in determining the dependence of changes in skin permeability on solubility. The permeability coefficient can be calculated as the division of steady-state flux by the solubility of the drugs in vehicle, and a comparison of the permeability coefficient with or without surfactants indicates the effect of the solubility of drugs on skin permeability. Steady-state flux, solubility of TEN in the vehicle and the permeability coefficient with or without surfactants are shown in Table 1. The permeability coefficients with high flux values, such as Decaglyn 3-O, SO-10, SO-30R, MGO, MYO-2 and BO-10TX, were equivalent to those without surfactants. Therefore, skin permeation depends on the increase in solubility of TEN. Moreover, other permeability coefficients with surfactants such as NP-7.5, DOPC, PBC-34, HGS-26, TO-10M, GO-460 and HCO-60, were lower, as compared to those without the surfactants. The reason for this is that the activity of the drugs was suppressed by micelle formulation, that is, by the interaction between the surfactants and the drugs, in the same manner as reported by Dalvi[11] and Cappel.[12,13] Therefore, in surfactants whose permeability coefficients are equal to that without them, the solubility was considered the main factor in their permeation mechanism, in addition to a weak interaction with the drugs.

Penetration of Surfactants to Stratum Corneum Increased membrane fluidity caused by the penetration of surfactants to the stratum corneum was reported as one of the factors in percutaneous absorption,[14] and might trigger skin irritation. At this point, sodium lauryl sulfate has been reported to be a popular surfactant causing the disorder of stratum corneum disposition.[1,2] On the other hand, Onishi[15] reported that hemolysis of the surfactants was related to the affinity between nonionic surfactants and erythrocytes or liposomes which differed from the erythrocyte lipid composition. If the penetration of the surfactants to the stratum corneum can be substituted by penetration of the surfactants to the membrane of erythrocytes, a hemolysis test will be a simple and useful method. However, hemolytic surfactants did not all permeate into the stratum corneum because the membrane of the stratum corneum was rigid compared to that of erythrocytes. Hemolysis of the surfactant solution was examined, and the penetration of surfactants to the stratum corneum was determined from the results shown in Table 1. BO-10TX and SO-30R caused high hemolysis amid high permeability. The penetration of the surfac-

tants to the stratum corneum led to an increase in the partition of TEN to the stratum corneum from the vehicle. In fact, primary skin irritation caused by the penetration of the surfactants to the stratum corneum was strong in the cases of BO-10TX and SO-30R (data was not shown). Conversely, bulky and high molecular HCO-60, high molecular TO-10M and so on which were used as pharmaceutical excipients of an injection did not cause hemolysis. These results were in accordance with Onishi's report,[15] which showed that high molecular or bulky surfactants did not cause hemolysis.

Conclusion

The effect of 14 types of nonionic surfactants on the permeation of TEN through guinea pig skin from propylene glycol solution was investigated *in vitro*. The flux of TEN was significantly enhanced by several surfactants. In the presence of these surfactants, the flux was increased about 2 to 4 times, as compared to the flux without them.

The mechanism of nonionic surfactant action was analyzed and classified using recent methods, which include the HLB of the surfactants, the solubility of the drugs in vehicle and the hemolysis of erythrocytes. The results suggested that vital factors in the enhancement of skin permeation by nonionic surfactants include the affinity of the surfactants to the stratum corneum, the solubility of the drugs in vehicle and the penetration of surfactants to the stratum corneum.

Penetration of the surfactants to the stratum corneum is a factor that causes some damage to the skin. For percutaneous absorption, ideal surfactants should have several factors such as high solubility of the drugs in vehicle, low interaction with the drugs and low interfacial tension between the stratum corneum and vehicle and, therefore, impart no damage to the skin.

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