Membrane Permeation-Controlled Transdermal Delivery System Design. Influence of Controlling Membrane and Adhesive on Skin Permeation of Isosorbide Dinitrate

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A membrane permeation-controlled transdermal delivery system (MC-TDS) of isosorbide dinitrate (ISDN), a model drug, was prepared from polyvinyl alcohol aqueous gel containing the drug, a membrane consisting of ethylene-vinyl acetate copolymer membrane and acrylic adhesive (EV-a). The permeability of ISDN through the EV-a membrane was 2.5 times higher than that through excised hairless rat skin. The ratio of plasma concentration of ISDN after application of MC-TDS on stripped (damaged) skin relative to intact skin was lower than that after application of Frandol tape-S, a marketed ISDN TDS, which suggests that the EV-a membrane might work as a control membrane for overall delivery rate of ISDN to the body. When MC-TDS stored at 30 °C for 13.5—48 h was applied to the damaged skin, however, the initial plasma concentration of ISDN was very much higher than the expected therapeutic level and was not controlled by the EV-a membrane. The initial high plasma concentration of ISDN after application of the stored MC-TDS on the damaged skin was due to migration of ISDN from the reservoir to the adhesive during storage at 30 °C. The migration of drugs into the adhesive might be an important problem in developing efficient MC-TDS.

Keywords membrane permeation-controlled transdermal delivery system; ethylene-vinyl acetate copolymer membrane; isosorbide dinitrate; acrylic adhesive; damaged skin

Recent progress in transdermal delivery system (TDS) is exemplified by the development of nitroglycerin TDS such as Transderm-Nitro, Nitro-Dur and Nitrodisc.[1] These systems were designed from the standpoint of regulated release of a systemic drug through the skin. The use of a release controlling membrane is one method to regulate the drug release. Such a membrane brings several advantages to TDS; for instance, (i) a prolonged action owing to sustained drug release,[2] (ii) almost the same *in vivo* drug delivery rate as the *in vitro* release rate[3] and (iii) prevention of extremely high plasma concentration in the event of skin damage.[4] Since the first function (i) is needed only for highly permeable drugs, such a function may be of limited applicability. Systems with the second function (ii) are desirable in principle. The plasma concentrations achieved by such systems should be independent of skin condition and application site. In such systems, however, the skin permeation rate of drugs must be high enough to allow systemic pharmacological action. High skin permeation rate of drugs may be obtained by the use of a penetration enhancer such as ethanol, medium chain esters and Azone.[5] However, too much permeation enhancement may be undesirable because of the possibility of local inflammation. Regarding the third function, if skin barrier function is remarkably decreased due to a change in skin condition, such as inflammation or scars, drug delivery rate will be still equal to the permeation rate through the controlling membrane and the plasma concentration should be maintained below the toxic concentration. Since such systems do not necessitate extremely high skin permeation rates, it may be easier to develop such systems than those with the first or second function.

In the present study, a membrane permeation-controlled TDS of isosorbide dinitrate (ISDN),[6] a model drug, with the third function (MC-TDS) was prepared, and its characteristics were examined.

Experimental

Materials Ethylene-vinyl acetate copolymer membrane (EVA, vinyl acetate content, 14%; 50—60 μm in thickness) and acrylic adhesive (7833/65, Rohm Pharma, Germany) were obtained from Mitsui Du Pont Polychemical Co. (Tokyo, Japan) and Higuchi Inc. (Tokyo), respectively. Frandol tape-S was kindly supplied by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). Polyvinyl alcohol (PVA, A-300) was from Nippon Gousei Kagaku Kogyo Co. (Osaka). ISDN was supplied by Tokyo Yakuhin Co. (Tokyo). Acetonitrile was of high performance liquid chromatography (HPLC) grade. All other reagents were of reagent grade and were used without further purification.

Animals WBN/kob male hairless rats (160—200 g, Saitama Laboratory Animals, Sugito, Saitama, Japan) were used for both the *in vitro* and *in vivo* skin permeation experiments. In experiments *in vitro*, the fresh abdominal skin was used immediately after being excised under anesthesia with sodium pentobarbital (60 mg/kg, i.p.).

Preparation The acrylic adhesive was applied on EVA to give 80—90 μm in total thickness, (EV-a).

***In Vitro* Permeation Experiments** The rat skin and/or EV-a were mounted in a 2-chamber diffusion cell.[3d] Each half-cell has a volume of 2 ml and an effective diffusional area of 0.95 cm^2. ISDN suspension (2 mg/ml) in 0.9% NaCl solution containing 0.01% Polysorbate 80 was added to the donor-side half-cell. The same solution (ISDN free) was added to the receiver-side half-cell. The cell-set was kept in a water bath at 37 °C. At predetermined times, the whole solution was removed from the receiver cell to determine the concentration of ISDN. Fresh solution was added to the receiver-side half-cell to continue the experiment.

ISDN was assayed by an HPLC system (LC-6A, Shimadzu Seisakusho, Kyoto, Japan).[7] The conditions were as follows: internal standard, ethyl *p*-hydroxybenzoate; column, 4.6 mm × 150 mm stainless steel column packed with Nucleosil $5C_{18}$ (Nagel, Germany); mobile phase, acetonitrile: water = 1 : 1; detector, ultraviolet (UV) 220 nm.

Preparation of MC-TDS ISDN (7.8 mg) and 20% PVA solution (480 mg) were sandwiched between EV-a and a backing and they were rapidly placed into a freezer at −40 °C to make MC-TDS. MC-TDS has an effective diffusion area of 4.9 cm^2 (Fig. 1). Storage time in the freezer was 16 h except in long term storage experiments (19 d).

The *in vitro* release of ISDN from MC-TDS was determined using a vertical cell.[8] Receiver solution and HPLC condition were the same as in the *in vitro* permeation experiments.

***In Vivo* Application of MC-TDS** MC-TDS taken out from the freezer was used for *in vivo* experiments immediately or after storage for 13.5—

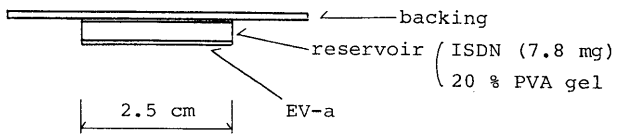

Fig. 1. Cross Section of MC-TDS

48 h at 30 °C. MC-TDS was applied on the intact skin or stratum corneum-removed skin (damaged skin)[9] of the abdominal site of hairless rats. At predetermined times, blood samples were withdrawn from the jugular vein into a heparinized syringe to measure plasma ISDN concentration.

The plasma concentration of ISDN was determined using a gas chromatograph (GC) equipped with an electron-capture detector (GC-8A, Shimadzu Seisakusho).[10] The plasma (100—250 μl) was mixed with the same volume of water, and ISDN in the mixture was extracted with *n*-hexane (2 ml) containing 74 ng/ml isomannide dinitrate (IMDN) as an internal standard. The *n*-hexane phase was removed and the remainder was shaken again with *n*-hexane (2 ml). The two *n*-hexane phases were combined and ISDN and IMDN were extracted with acetonitrile (2 ml). The acetonitrile phase was dried with N_2 gas at room temperature. The samples were reconstituted with ethyl acetate (100 μl) and injected into the GC column (1—2 μl). The conditions were as follows: column, 2 m glass column packed with OV-17 2%/Chromosorb WAW DMCS 80/100; carrier, N_2 (2 kg/cm^2); injector and detector temperatures, 180 °C; column temperature, 160 °C. The minimum detectable plasma concentration was 1 ng/ml.

Results and Discussion

Figure 2a shows the time course of cumulative amount of ISDN permeated per unit area, Q, through skin or EV-a. The steady state flux through the EV-a (49.2 μg/cm^2/h) was about 2.5 times higher than that through the skin (20.1 μg/cm^2/h). Figure 2b shows the time course of Q through the combined EV-a and skin, in which the acrylic adhesive of EV-a was attached to the stratum corneum side of the skin. The total resistance, R_t, to permeation of drugs can be expressed as follows[1b,3d]:

$$R_t = R_m + R_s \quad (1)$$

where R_m and R_s are the resistances of EV-a and skin, respectively. Since ISDN suspension was used as a drug donor in the present study, Eq. 1 can be rewritten as follows:

$$F_t = \frac{F_m \times F_s}{F_m + F_s} \quad (2)$$

where F_t, F_m and F_s are the steady state flux of ISDN from suspension through the combined EV-a and skin, EV-a, and skin, respectively. The steady state flux through the combined layer (14.8 μg/cm^2/h) is similar to that calculated using Eq. 2 (14.3 μg/cm^2/h). The calculated value is not much lower than that through skin alone (20.1 μg/cm^2/h). These result suggest that EV-a is useful to fabricate MC-TDS with the third function.

Figures 3a and b show the plasma concentration of ISDN after application of Frandol tape-S[11] (7.8 mg ISDN/9.8 cm^2/body) and MC-TDS (7.8 mg ISDN/4.9 cm^2/body), respectively. In the treatment with Frandol tape-S, a marketed matrix system, the plasma concentration after application on the intact skin rapidly reached a constant level. On the other hand, the plasma concentration after application on damaged skin rapidly increased to a peak level and then decreased slowly. It was considered that the increase and decrease in the plasma concentration were caused by a decrease of skin barrier function and a decrease of the drug content in the system, respectively. These results suggest that the delivery rate of ISDN from Frandol tape-S is limited by the stratum corneum permeability and is not controlled by the system on intact or damaged skin. In the case of MC-TDS, the plasma concentration after application on the intact skin was below the detection limit until

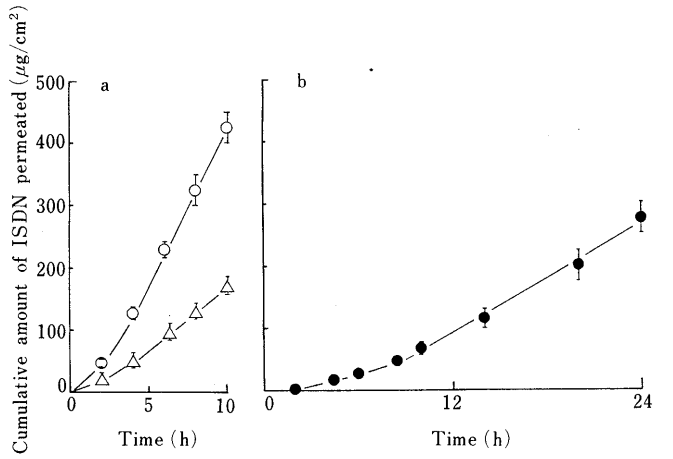

Fig. 2. Time Course of the Cumulative Amount of ISDN Permeating through Excised Hairless Rat Skin or EV-a

a, hairless rat skin (△) and EV-a (○); b, combined skin and EV-a. Data are shown as the mean ± S.D. of three experiments.

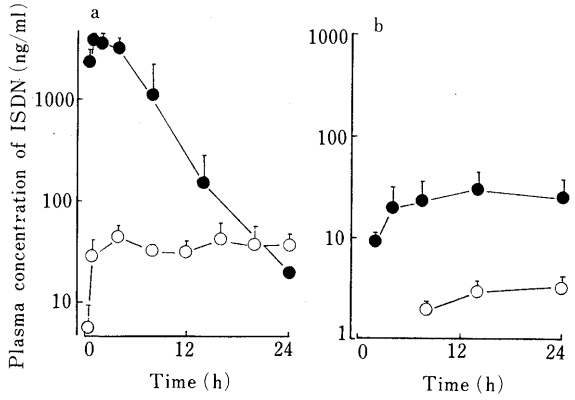

Fig. 3. Plasma Concentration of ISDN after Application of Frandol Tape-S or MC-TDS

a, Frandol tape-S ○, intact skin; ●, damaged skin. b: MC-TDS ○, intact skin; ●, damaged skin. Data are shown as the mean ± S.D. of three experiments.

4 h and it then increased slowly. On the other hand, the plasma concentration after application to damaged skin reached a constant level within 4 h and was maintained until 24 h. The plasma concentration at 24 h in the case of damaged skin was about 6 times higher than that with intact skin. Although this ratio is very low compared with the ratio of the maximum plasma concentration in the intact and damaged skin treatments with Frandol tape-S (about 85), it is 2 times higher than the expected ratio based on the *in vitro* experiment. Such difference may be attributable to a difference in the skin permeability of ISDN *in vitro* and *in vivo*. These results suggest that the delivery rate of ISDN from MC-TDS is controlled to a considerable extent by the system in the treatments on damaged skin.

A safe medication might be attained, if the plasma concentration in treatments on both intact and damaged skin is within the therapeutic range. MC-TDS could be especially suitable for transdermal delivery of drugs which have severe side effects.

The effect of pre-storage time at 30 °C on the plasma concentration after application of MC-TDS was examined. Figure 4 shows the plasma concentration of ISDN in the case of MC-TDS stored at 30 °C for 13.5—48 h. The plasma

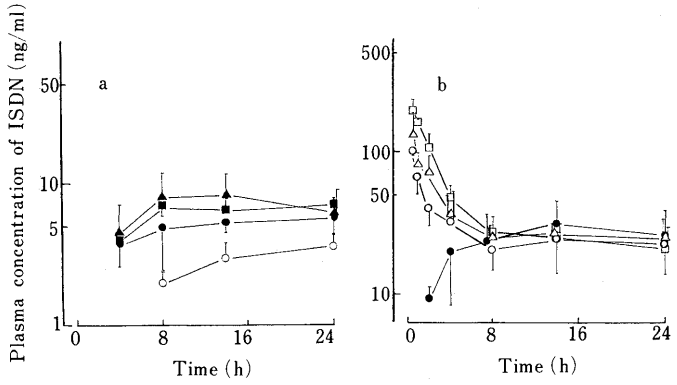

Fig. 4. Plasma Concentration of ISDN after Application of MC-TDS Stored at 30 °C

a: intact skin ○, 0 h; ●, 13.5 h; ▲, 26 h; ■, 48 h. b; damaged skin ●, 0 h; ○, 13.5 h; △, 26 h; □, 48 h. Data are shown as the mean ± S.D. of three experiments.

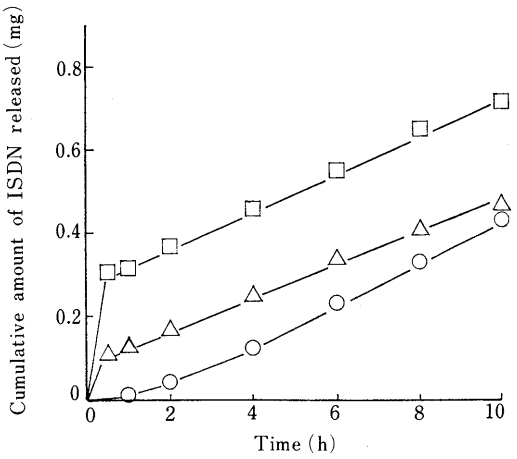

Fig. 5. Time Course of the Cumulative Amount of ISDN Released from MC-TDS Stored at 30 °C

○, 0 h; △, 13.5 h; □, 26 h; $n=1$.

concentration after application of stored MC-TDS on the intact skin was detectable within 4 h and was maintained at a fairly constant level until 24 h (Fig. 4a). Such a concentration profile is appropriate for medication. On the other hand, the plasma concentration obtained with stored MC-TDS on damaged skin increased within 30 min and then decreased to the steady state level at 8 h. This initial high plasma concentration after application of the stored MC-TDS on the damaged skin is undesirable. The change of the releasing property of ISDN from MC-TDS may be caused by migration of ISDN from the drug reservoir to the adhesive during the storage at 30 °C.

The effect of storage time at 30 °C on the release of ISDN from MC-TDS and the ISDN content in the adhesive were examined. Figure 5 shows the *in vitro* release of ISDN from MC-TDS. The steady state flux from the stored MC-TDS was similar to that from non-stored MC-TDS, but the initial burst increased with increase in the storage time. When we measured actual ISDN content in the adhesive, the ISDN content was found to increase with increase in the storage time. These results suggest that the initial high plasma concentration of ISDN in the treatments with stored MC-TDS on damaged skin is caused by the transport of ISDN from the reservoir to the adhesive during storage at 30 °C.

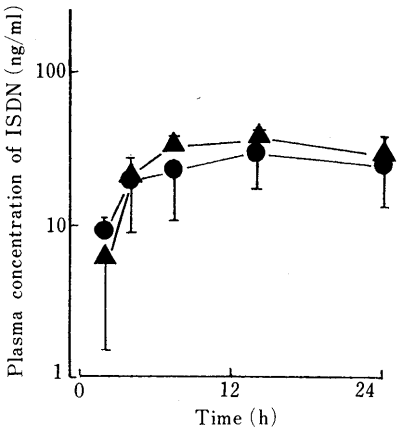

Fig. 6. Plasma Concentration of ISDN after Application on Damaged Skin of MC-TDS Frozen at −40 °C

●, stored for 16 h; ▲, stored for 19 d. Data are shown as the mean ± S.D. of three experiments.

The effect of storage on the *in vitro* release of arecoline from TDS has already been reported by Ebert *et al.*[12] They have shown the effectiveness of a user activated transdermal system (UATS). UATS is fabricated with the drug in an impermeable, pre-active state. This system is one method to prevent the undesirable initial burst of drug release.

Considering the low diffusivity of drugs in ice, the effect of freezing on the migration of ISDN to the adhesive was examined. Figure 6 shows the plasma concentration profiles of ISDN after application of MC-TDS stored for 16 h and 19 d at −40 °C on damaged skin. The two profiles were not significantly different. These results suggest that freezing can minimize the build-up of the drug level in the adhesive, and hence, prevent the initial burst.

However, these methods (UATS and freezing method) are not practically convenient. A system with an adhesive which does not solubilize much drug could be useful to obtain an easily storable preparation. High safety, sufficient adhesion and adequate drug solubility in the adhesive are needed in an efficient membrane permeation-controlled transdermal system. Studies on suitable adhesive materials will be important.

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