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Effect of penetration enhancers on isosorbide dinitrate penetration through rat skin from a transdermal therapeutic system

Halina Gabiga, Krzysztof Cal, Stanisław Janicki *

Department of Pharmaceutical Technology, Medical University of Gdańsk, Al. Gen. Hallera 107, 80-416 Gdańsk-Wrzeszcz, Poland

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Abstract

Percutaneous absorption of isosorbide dinitrate (ISDN) from a transdermal therapeutic system (TTS) with or without penetration enhancers was studied. The concentration of ISDN and its metabolites, isosorbide-5-mononitrate (IS-5-MN) and isosorbide-2-mononitrate (IS-2-MN), was determined in rat plasma during a 48 h application of TTS. The increased skin-penetration enhancing effect of oleic acid and propylene glycol in comparison to polyethylene glycol 400 and isopropyl myristate on percutaneous permeation of ISDN was shown. It was expressed by higher values of C_{max} and AUC. After the application of TTS, a lower ISDN and molar ratio of its metabolites was observed than after oral administration.

Keywords: Isosorbide dinitrate; Isosorbide mononitrate; Percutaneous absorption; Skin penetration enhancers

1. Introduction

ISDN is widely used in the treatment and prophylaxis of coronary vessel diseases (e.g. angina pectoris). Its short elimination half-life, high first-pass effect after oral administration, small molecular weight and lipophilic properties motivate transdermal drug delivery via, e.g. ointment, micro-emulsion, transdermal spray or a transdermal therapeutic system (TTS) (Menke et al., 1987; Laufen and Leitold, 1992; Kietzmann et al., 1995).

ISDN is generally metabolised in the liver, but also in the skin by glutathione transferase to isosorbide-5-mononitrate (IS-5-MN) and isosorbide-2-mononitrate (IS-2-MN). Both metabolites are also pharmacologically active and differ only in terms of pharmacokinetic parameters and vasodilatory effects (Down et al., 1974; Riviere et al., 1996).

ISDN administration in transdermal as well as in buccal forms (Danjo et al., 1994; Keller-Stanislawski et al., 1992; Nozaki et al., 1996, 1997) circumvents hepatic first-pass effect, as observed after oral administration. However, to obtain therapeutic concentrations of ISDN after transdermal administration, the application of the penetration enhancers is required.

* Corresponding author.

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In the present study, ISDN penetration into and through rat skin was examined after the application of a matrix TTS. The concentration of ISDN and its metabolites, IS-5-MN and IS-2-MN, detected in blood after the application of TTS, demonstrated the influence of penetration enhancers applied in TTS matrices.

Different molar ratios of ISDN:IS-5-MN:IS-2-MN after transdermal administration were noticed in comparison to oral administration.

2. Materials and methods

2.1. Materials

ISDN (Organika, Poland), ethylcellulose T-50, propylene glycol, isopropyl myristate (Sigma, St Louis, MO), oleic acid (Loba Feinchemie, Austria), polyethylene glycol 400 (BDH Chemicals, UK), and acrylic adhesive 'Kolakryl' were obtained from IChP Warsaw, Poland.

IS-5-MN, IS-2-MN and isoidide dinitrate (IIDN) for chromatography studies were a gift from Schwarz Pharma, Germany.

2.2. TTS

Ethylcellulose was used as a matrix polymer. Propylene glycol, polyethylene glycol 400, and oleic acid or isopropyl myristate were used as penetration enhancers. TTS matrix parameters are shown in Table 1. TTS-0 matrix was prepared without penetration enhancers and served as a control.

ISDN, penetration enhancers and ethylcellulose were dissolved in methylene chloride in the ratio 1:1.6:8 (w/w). TTS matrices were obtained by casting the solution on siliconized glass plates, followed by the evaporation of methylene chloride in an air-conditioned chamber for 24 h under following conditions: relative humidity 60–70%; temperature 20 ± 1°C.

The obtained membranes were covered with an appropriate volume of 20% acrylic adhesive solution in ethyl acetate and dried for 12 h in the air-conditioned chamber under the same conditions as above. The thickness of the adhesive layer was 30 μm. The backing sheet was a nonpermeable aluminium foil laminated with polyethylene.

2.3. In-vivo transdermal absorption of ISDN studies

Six male Wistar rats (200–210 g) were used in the in-vivo experiment. The hair of the abdominal area was carefully removed with scissors under thiopental anaesthesia (75 mg/kg, Spofa, Czech Republic). TTS matrices (2×2 cm area) were applied and immediately occluded with an adhesive tape (Leukotape, BDF, Germany) to aim full contact with the skin.

Blood samples (0.75 ml) were withdrawn from the rats tail veins into heparinized glass vessels before drug application (blank) and 1.5, 3, 5, 7, 9, 24, 48 h after dosing. The plasma was separated immediately by centrifugation at $1920 \times g$ for 10 min and stored frozen (-20°C) in glass vessels with teflon caps (Chromacol, USA) until analysis.

Penetration of ISDN into the skin was evaluated in a separate study. TTS matrix (2×2 cm area) was applied on the abdominal skin of six rats under light ether anaesthesia. The hair was removed as above. After 24 h, the matrix was

Table 1
Composition of TTS matrices

Matrices	Penetration enhancer (mg/cm^2)		Ethylcellulose	ISDN
TTS-O	–	–	26.0	3.25
TTS-PG	Propylene glycol	5.20	26.0	3.25
TTS-PEG	PEG 400	5.20	26.0	3.25
TTS-OA	Oleic acid	5.20	26.0	3.25
TTS-IPM	Isopropyl myristate	5.20	26.0	3.25

dissolved in ethyl acetate (5 ml) and treated with diphenylsulphonic acid and 25% ammonia in order to develop the yellow colour characteristic for the formation of ammonium picrate. The concentration of ISDN was assayed spectrophotometrically at 405 nm (Babko and Pilipenko, 1955). The total amount of ISDN absorbed by the skin was the difference between amounts of ISDN in the matrix before and after application.

2.4. ISDN intravenous administration

Six male Wistar rats weighing 230–260 g were used. ISDN was dissolved in physiological saline, at a concentration of 0.5 mg/ml. The drug solutions (2 ml each) were injected into the left femoral vein of the six rats restained under thiopental anaesthesia (75 mg/kg). Blood samples (0.75 ml) were obtained from the jugular vein at 5, 10, 15, 30, 60, 120, 180 min after injection and treated in the same manner as described in the in-vivo percutaneous absorption studies.

The area under the ISDN plasma concentration–time curve (AUC) after intravenous and transdermal application of ISDN was calculated by the trapezoidal method. The absolute bioavailability (F) was calculated by the following equation:

$$F(\%) = \frac{AUC_{p.c.} \cdot d_{i.v.}}{AUC_{i.v.} \cdot d_{p.c.}} \cdot 100$$

where $AUC_{p.c.}$ and $AUC_{i.v.}$ are AUC after percutaneous and intravenous administration, respectively, and $d_{p.c.}$ and $d_{i.v.}$ are the applied percutaneous and intravenous doses, respectively.

2.5. Plasma determination of ISDN and its metabolites IS-5-MN and IS-2-MN

Each plasma sample (0.3 ml) was mixed with 1 ml of saturated potassium carbonate aqueous solution and 20 μl of an aqueous solution containing 20.0 ng of IIDN as an internal standard. Then the sample was extracted twice with ethyl acetate. The ethyl acetate layers were separated and evaporated to dryness under a gentle stream of nitrogen at RT. The residue was redissolved in 20 μl of ethyl acetate. A 2 μl aliquot was injected for gas chromatography; this was carried out using a Carlo Erba HRGC 5300 Mega series apparatus equipped with an electron-capture detector (10 mCi ^{63}Ni), a 'fused silica' capillary column 30 m × 0.32 mm i.d. with a 0.25 μm stationary phase DB-5 (5% phenyl, 95% methyl siloxane, J and W Scientific, USA) with a 1 m deactivated precolumn. Chromatographic conditions were as follows: carrier-gas (hydrogen) flow rate was 2.4 ml/min; make-up gas (nitrogen) flow rate was 40 ml/min, injection by 'cold-on-column' method; oven temperature from 65°C at starting point to 190°C (increasing in 3°C/min steps); the detector temperature was 250°C. The peak areas were measured by a one-channel integrator Data Jet (Spectra Physics Analytical, USA). The recovery of ISDN, IS-5-MN and IS-2-MN was 95, 90 and 95%, respectively.

3. Results and discussion

The plasma concentration–time profiles of ISDN (A) and its metabolites IS-5-MN (B) and IS-2-MN (C) after the transdermal application of TTS matrices through the 48 h experimental period are shown in Fig. 1.

It was shown that all types of matrices assured constant plasma levels of ISDN between 5 and 24 h of administration. There was a significant difference in ISDN plasma concentration between each kind of matrix. The absorption of ISDN from TTS-0 matrix (without enhancer) was lower, in comparison to the other matrices. The plasma concentration of ISDN was increased when PEG 400 (6.3–9.6 ng/ml) or isopropyl myristate (5.3–7.8 ng/ml) was used as an enhancer, but the highest levels were found when propylene glycol or oleic acid were added to the matrix (ISDN plasma levels: 11.71–14.58 and 10.45–13.73 ng/ml, respectively).

Different plasma concentrations of ISDN, IS-5-MN and IS-2-MN between each rat were observed. The maximum calculated standard deviations (S.D.) of ISDN mean plasma levels were: TTS-0 ± 4.9, TTS-PG ± 14.5, TTS-PEG ± 4.2, TTS-OA ± 7.2, TTS-IPM ± 4.1. Higher S.D. values were observed for metabolites: TTS-0 ±

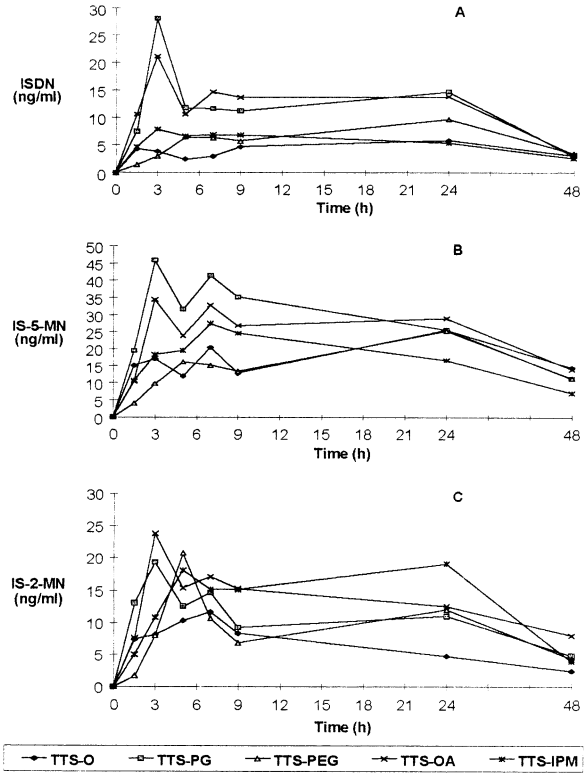

Fig. 1. Mean plasma concentrations of: (A) ISDN; (B) IS-5-MN; and (C) IS-2-MN in rats after the application of TTS matrices ($n = 6$).

16.3, TTS-PG ± 39.1, TTS-PEG ± 10.0, TTS-OA ± 25.0, TTS-IPM ± 14.5 for IS-5-MN; and TTS-0 ± 6.5, TTS-PG ± 14.3, TTS-PEG ± 20.0, TTS-OA ± 15.0, TTS-IPM ± 12.7 for IS-2-MN.

The differences were probably the consequence of the first-pass effect.

After the application of the three matrices, TTS-PG, TTS-OA, and TTS-IPM, the maximum plasma concentration of ISDN (C_{max}) was observed at $t = 3$ h, whereas for the matrices TTS-PEG and TTS-0, the C_{max} was at 24 h.

Table 2 summarises the pharmacokinetic parameters — C_{max} and $AUC_{0-48\ h}$ for ISDN and its metabolites. The highest values of C_{max} and $AUC_{0-48\ h}$ were noticed for matrices with additions of oleic acid and propylene glycol. Regarding the ISDN C_{max} value, the investigated formulations can be arranged in the following order: TTS-IPM = TTS-0 = TTS-PEG < TTS-OA < TTS-PG. The observed differences between TTS containing oleic acid or propylene glycol and the other matrices were statistically significant (Student's t-test, $\alpha < 0.05$). On the other hand, no difference was observed between TTS containing PEG or IPM and the control.

Similarly, the statistical analysis revealed that in respect of $AUC_{0-48\ h}$ values, no difference can be found for TTS modified with isopropyl myristate or PEG 400 and the promoter-free matrix (TTS-0 = TTS-IPM = TTS-PEG < TTS-PG = TTS-OA). The highest $AUC_{0-48\ h}$ values were observed for TTS containing propylene glycol and oleic acid.

Fig. 2 shows the mean concentrations of ISDN in rat plasma after intravenous administration. The high initial concentration of ISDN-750 ng/ml rapidly declined to 90 ng/ml after 30 min. This concentration drop was in line with the two-compartment model.

Table 2
Maximum plasma concentration and bioavailability parameters following percutaneous absorption of ISDN from TTS[a]

Matrices TTS	ISDN			IS-5-MN		IS-2-MN	
	C_{max} (ng/ml)	$AUC_{0-48\ h}$ (ng h/ml)	F (%)	C_{max} (ng/ml)	$AUC_{0-48\ h}$ (ng h/ml)	C_{max} (ng/ml)	$AUC_{0-48\ h}$ (ng h/ml)
TTS-O	8.4 ± 2.9	149.9 ± 128.1	4.13	30.7 ± 1.4	655.1 ± 315.2	16.1 ± 5.0	217.0 ± 77.5
TTS-PG	29.3 ± 12.7	415.0 ± 97.0	11.44	66.9 ± 28.5	963.6 ± 136.8	26.3 ± 11.5	362.6 ± 102.7
TTS-PEG	10.5 ± 3.6	230.2 ± 49.1	6.60	25.9 ± 9.0	605.9 ± 164.7	23.8 ± 18.7	324.5 ± 74.6
TTS-OA	21.4 ± 4.9	423.5 ± 165.0	11.67	45.2 ± 23.0	882.8 ± 474.3	27.7 ± 11.5	463.5 ± 230.9
TTS-IPM	9.3 ± 3.4	190.3 ± 64.4	6.29	34.7 ± 14.4	612.3 ± 201.7	22.5 ± 9.3	501.8 ± 240.9

[a] Mean ± S.D. of six experiments.

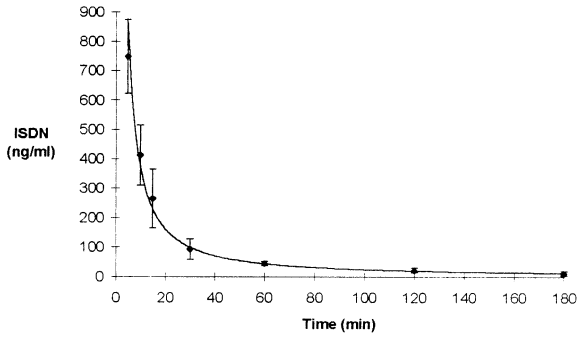

Fig. 2. Mean ISDN concentration in rat plasma after intravenous application ($n = 6$).

Table 3
Molar ratio of ISDN and its metabolites' concentrations in plasma samples (5–24 h) after application of TTS, normalised to ISDN

Matrices TTS	ISDN	IS-5-MN	IS-2-MN
TTS-0	1	5.2	2.5
TTS-PG	1	3.4	1.2
TTS-PEG	1	3.1	2.2
TTS-OA	1	2.6	1.5
TTS-IPM	1	4.3	3.3

In order to calculate the absolute bioavailability (F) of ISDN, the concentrations after transdermal and intravenous administration were compared. The results are shown in Table 2. The matrices with oleic acid and propylene glycol demonstrate similar absolute bioavailabilities (11.67 and 11.44%), which are almost double that of the matrices to which PEG 400 or isopropyl myristate were added.

This is special for all matrices tested, that the concentration levels of IS-5-MN and IS-2-MN in rat plasma calculated for the plataeu phase were 2–5 and 2–3 times greater than the concentration level of ISDN. The molar ratios of ISDN:IS-5-MN:IS-2-MN for the plataeu phase (5–24 h) for each matrix are shown in Table 3.

This result differs from the one after the oral administration of ISDN. Gomita et al. (1989) noticed significantly greater concentrations of IS-5-MN (532–599 ng/ml) in rat plasma after the oral application of ISDN (1 mg/kg) than in the present transdermal studies. The molar ratio ISDN:IS-5-MN:IS-2-MN in their study after the oral administration of ISDN was 1:25:3. After the transdermal delivery of ISDN in humans via TTS (Menke et al., 1987; Feldstein et al., 1996) and transdermal spray (Laufen and Leitold, 1992), significantly smaller molar ISDN–metabolites ratios were obtained.

Elimination of the first-pass effect after transdermal delivery results in remarkably lower blood concentrations of IS-5-MN in comparison to the oral delivery.

Fig. 3 correlates the bioavailability ($AUC_{0-24\,h}$) with the ISDN absorption into the skin from each of the matrices. The smallest quantity of ISDN (0.31 ± 0.17 mg/cm^2) was absorbed from the TTS-0 matrix (without penetration enhancer); however, the greatest quantity was absorbed from the matrix containing oleic acid (1.45 ± 0.35 mg/cm^2). It is interesting that the amounts of ISDN which penetrated into the skin in the presence of propylene glycol and isopropyl myristate were similar, and statistically insignificant, but different from each other in that the value of $AUC_{0-24\,h}$ for TTS-PG was twice that of TTS-IPM. The above results suggest that in the presence of isopropyl myristate, ISDN is well absorbed into the skin but, however, it does not penetrate across the stratum corneum barrier, as can be concluded from the small $AUC_{0-24\,h}$ observed for TTS-IPM. The retention of isopropyl myristate in the stratum corneum, documented already in the literature (Goldberg-Cettina et al., 1995), may be responsible for such effects.It was established, on

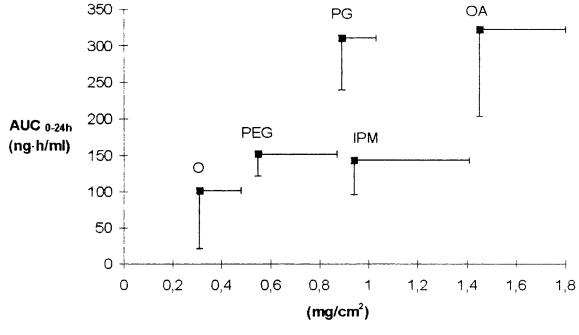

Fig. 3. Relationship between bioavailability (AUC) and concentratio of ISDN penetrating into the rat skin at 24 h application of TTS (mean ± S.D. of six experiments).

the basis of the results from the present study, that the most effective penetration enhancers for ISDN are oleic acid and propylene glycol.

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