Absorption of Isosorbide Dinitrate after Administration as Spray, Ointment and Microemulsion Patch. An In-vitro Study Using the Isolated Perfused Bovine Udder

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Abstract

The isolated perfused bovine udder is an in-vitro model, which maintains bovine udder skin with an isolated vasculature in a viable state. Using this in-vitro model, the percutaneous absorption and metabolism of isosorbide dinitrate (ISDN) was studied.

The organ was perfused with gassed Tyrode solution for up to 6 h. A region of udder skin was treated topically with 60 mg ISDN as a spray, 60 mg ISDN as an ointment and with 120 mg ISDN as a microemulsion patch of 30 cm^2. Spray and ointment were applied onto a skin region of 400 cm^2. The concentrations of ISDN and its metabolites isosorbide-2-mononitrate and isosorbide-5-mononitrate were measured in perfusate fractions by capillary column gas-liquid chromatography with electron capture detection.

Following topical administration of the different formulations, ISDN as well as its metabolites were detected in the perfusate fractions, thus demonstrating that ISDN is metabolized by the udder skin in-vitro. A maximum amount of ISDN was absorbed after administration as a spray followed by ointment and microemulsion (5, 2·5 and 1·8 μmol total organic nitrate, respectively). In contrast, the ISDN flux per cm^2 skin was significantly higher after administration of the microemulsion (64·4 pmol cm^{-2} min^{-1} for the microemulsion compared with 21·9 and 10·2 pmol cm^{-2} min^{-1} for spray and ointment).

Transdermal drug administration exhibits several advantages in therapy compared with oral or parenteral administration (Chien 1987), including the avoidance of systemic side-effects. However, the number of substances which can be used by the transdermal administration route is limited because of the poor transdermal penetration rate of many drugs. Enhanced transdermal penetration can be induced by increasing the diffusion properties of a drug (lipophilicity), by encapsulation in liposomes, or by reducing the barrier properties of the horny layer which represents the main barrier of transdermal penetration. An additional possibility is the use of microemulsions (Kemken et al 1991). Microemulsions are characterized as thermodynamically stable, clear or slightly opalescent isotropic systems. The self-emulsifying systems consist of an aqueous compound, a lipophilic compound and a surfactant or surfactant-cosurfactant mixture. The application of a saturated, water-free microemulsion with an occlusive patch causes water intake from the skin. A water-containing microemulsion develops. Because of the increased water content of the microemulsion, the solubility of the incorporated lipophilic drug is diminished resulting in an enhanced absorption rate (Kemken et al 1991). It is known that absorption rates are higher from supersaturated vehicles (Coldman et al 1972; Davis & Hadgraft 1991). An enhanced bioavailability has been

shown for β-blockers and steroids administered topically as microemulsions (Blume & Wenzel 1990; Kemken et al 1991).

Organic nitrates, which are used for the treatment of angina pectoris, are well suited for transdermal application (Coffman 1979; Murad 1990). In addition to the intravenous and the sublingual or buccal administration which ensure a high compliance, a sufficient bioavailability and a rapid onset of action, the transdermal route is a suitable application route for isosorbide dinitrate (ISDN) in patients. The drug is used frequently as a spray or ointment. ISDN is rapidly hydrolysed by a hepatic glutathione-organic nitrate reductase to mononitrates isosorbide-5-mononitrate (IS-5-MN) and isosorbide-2-mononitrate (IS-2-MN), which are less potent vasodilators than the parent compound; it is not known if ISDN is metabolized in the skin after topical administration.

The present study was performed to determine if ISDN is metabolized in the skin and to compare the rate of transdermal absorption and metabolism after topical application of different ISDN-containing formulations including a microemulsion patch. For this purpose, the isolated perfused bovine udder was used as a new in-vitro model (Arens et al 1991; Kietzmann et al 1991, 1993).

Materials and Methods

Test substances

ISDN was used as a spray (TD Spray Iso Mack Mack, Illertissen, Germany), ointment (Isoket ointment, Schwarz Pharma, Monheim, Germany) or microemulsion patch

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(ME-P-30 microemulsion patch). The patch, which is not commercially available, was 30 cm^2 and contained 120 mg ISDN.

Perfusate

As perfusion medium a gassed (95% O_2–5% CO_2) Tyrode solution (136·87 mM NaCl, 2·68 mM KCl, 1·80 mM $CaCl_2 \cdot 2H_2O$, 1·05 mM $MgCl_2 \cdot 6H_2O$, 11·90 mM $NaHCO_3$, 0·54 mM NaH_2PO_4, 6·11 mM glucose) was used. Addition of dextran to this protein-free perfusion medium has no significant effect on the transdermal drug absorption of the test compound etofenamate (Kietzmann et al 1993), and therefore no albumin or dextran was included in the Tyrode solution. The temperature of the perfusion fluid was 38·5°C. The temperature of the perfusion fluid guarantees a physiological surface temperature of the udder skin of 32–33°C. The temperature of the environment was 22°C.

In-vitro model

Details of the model have been described recently (Kietzmann et al 1993). Bovine udders were obtained immediately after the slaughter of healthy cows. The udder was dissected, and heparinized Tyrode solution (about 500 mL with 5 mg mL^{-1} heparin) was infused immediately via the arteria pudendalis externa to counteract clot formation. The udder was then transported to the laboratory and the two right udder complexes were perfused with Tyrode solution as rapidly as possible by a peristaltic pump (Masterflex 7518-10, Cole-Parmer Instruments, Chicago, USA). The perfusion pressure was 80–100 mmHg. The organ was supplied by the cannulated arteria pudendalis externa with a venous drainage via the vena epigastrica cranialis superficialis. The period of perfusion started within 30 min after slaughtering. During the perfusion period, the perfusate flux was between 60 and 100 mL min^{-1}. This perfusate flux, which is lower than the pysiological blood supply of the udder in-vivo, was chosen on the basis of earlier studies demonstrating a sufficient dermal blood supply by the infusion (Kietzmann et al 1993). At the end of the perfusion period, a trypan blue solution (1%) was infused via the arteria pudendalis externa to verify that dermal perfusion was sufficient. The present study includes only organ preparations in which the stained dermal blood vessels were visible. In addition to perfusion with trypan blue solution, microvascular perfusion was confirmed by the nearly constant skin surface temperature of 32–33°C (Kietzmann et al 1993).

Administration of ISDN and collection of perfusate and skin samples

Fifteen minutes after starting the perfusion, ISDN was administered on the udder skin. The following dosages were used in three (spray) and four udder preparations (ointment, microemulsion patch): spray, 60 mg/400 cm^2 skin (0·25 mmol/400 cm^2 skin); ointment, 60 mg/400 cm^2 skin (0·25 mmol/400 cm^2 skin); and microemulsion patch, 120 mg/30 cm^2 (0·5 mmol/30 cm^2 skin).

Perfusate fractions of 50 mL were collected before and 0·5, 1, 2, 3, 4, 5 and 6 h after ISDN administration. The collected perfusate fractions were stored at $-20°C$. After extraction with ethyl acetate, the concentration of ISDN, IS-2-MN and IS-5-MN was measured in the perfusate

Table 1. Total amount of isosorbide dinitrate (ISDN), isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) in the perfusate calculated from measured perfusate concentrations.

	Total amount of absorbed organic nitrate (μmol)		
	ISDN	IS-5-MN	IS-2-MN
Spray	3·2 ± 0·8	1·5 ± 0·2	0·5 ± 0·1
Ointment	1·5 ± 0·5	0·8 ± 0·2	0·2 ± 0·1
Microemulsion	0·8 ± 0·1	0·9 ± 0·1	0·1 ± 0·1

fractions by capillary column gas-liquid chromatography with electron capture detection (Wenzel 1993) (conditions: 5 SIL 5 CB capillary column (Chrompack, Müllheim, Germany), carrier gas flow rate 1·2 mL min^{-1}, oven temperature 145°C, detector 240°C). The recovery of ISDN, IS-2-MN and IS-5-MN was 99, 93 and 90%, respectively.

After application of ISDN-microemulsion patches, skin biopsies were taken from the centre of the treatment area and 2 and 4 cm from the margin of the patch. The concentration of the organic nitrates was measured in these skin samples.

Statistics

The total amount of ISDN absorbed within the perfusion period as well as the nitrate flux per cm^2 skin were calculated from the concentrations considering the perfusate flux. To allow a comparison of the concentration of ISDN, IS-2-MN and IS-5-MN, all results are given on a molar basis. A statistical analysis was performed by analysis of variance and by the Mann-Whitney test.

Results

Table 1 summarizes the total amounts of ISDN, IS-2-MN and IS-5-MN which were found in the perfusate within the perfusion period of 6 h. Table 2 gives the flux which was calculated from the data in Table 1. The flux was nearly constant over the period of 6 h. Fig. 1 gives cumulative time-dependent absorption rate curves for ISDN after administration as a spray, ointment or microemulsion.

After the topical administration of the ISDN spray, ointment or microemulsion, ISDN and the metabolites IS-2-MN and IS-5-MN were found in the perfusate. Calculated from the data given in Table 1, the relative amounts of ISDN, IS-5-MN and IS-2-MN (as per cent of absorbed organic nitrate) reaching the perfusate within the perfusion

Table 2. Flux of isosorbide dinitrate (ISDN), isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) via the skin.

	Flux (pmol cm^{-2} min^{-1})		
	ISDN	IS-5-MN	IS-2-MN
Spray	21·9 ± 6·5*	10·3 ± 2·3*	3·6 ± 0·8
Ointment	10·2 ± 4·7*	5·4 ± 1·7*	1·6 ± 0·5
Microemulsion	64·4 ± 17·6	65·5 ± 21·9	10·1 ± 7·4

Data are given as means ± s.e. of three (spray) and four (ointment, microemulsion patch) separate experiments. *$P < 0·05$ compared with microemulsion (Mann-Whitney test).
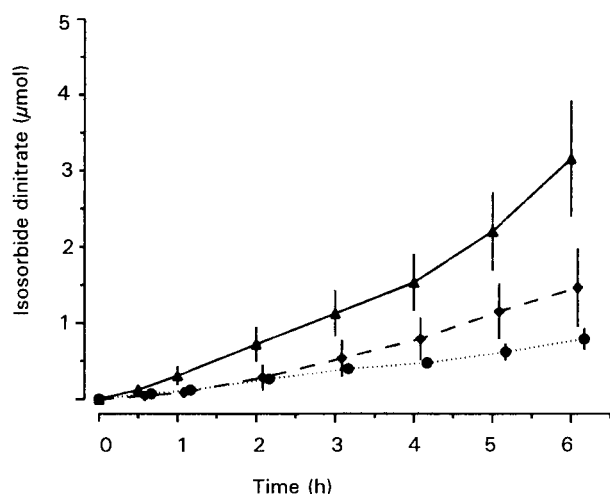
Fig. 1. Transdermal absorption rate-time curve (cumulative) of isosorbide dinitrate (ISDN) in the isolated perfused bovine udder. ▲ Spray, ◆ ointment, ● patch. Concentrations in the perfusate are given as means ± s.e. of three separate experiments.

Table 3. Distribution of isosorbide dinitrate (ISDN), isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) in isolated perfused udder skin after administration of an ISDN-containing microemulsion patch. The concentration was measured in the centre of the treatment area and 2 and 4 cm from the margin of the treatment area. Data are given as means ± s.e. of four separate experiments.

Localization	Skin (nmol cm^{-2})		
	ISDN	IS-5-MN	IS-2-MN
Centre of the treated area	170·9 ± 31·2	51·7 ± 9·2	11·2 ± 3·4
2 cm from the patch margin	60·8 ± 15·3	23·6 ± 8·6	4·1 ± 1·0
4 cm from the patch margin	15·5 ± 3·8	2·6 ± 1·1	1·5 ± 0·6

period were 61, 29 and 10%, after administration of ISDN as a spray. After treatment with the ointment and the microemulsion, the relative amounts were 60, 31 and 9% or 44, 50 and 6% of absorbed compound.

The data summarized in Table 3 show that ISDN and the mononitrates were measurable in the skin of the treatment area after administration of the ISDN-containing microemulsion patch. The concentration of the compounds decreased with the distance from the margin of the treatment area. Significant amounts of the organic nitrates were measurable at a distance of 4 cm from the patch margin.

Discussion

In-vitro models which are currently used to measure the percutaneous absorption rate of topically administered substances have both advantages and limitations compared with in-vivo models (Schaefer et al 1978; Reifenrath et al 1984; Pershing & Krueger 1987; Príborský & Mühlbachová 1990; de Lange et al 1992).

ISDN has not been previously investigated in in-vitro models so that it was not known if the drug is metabolized during skin penetration. The data presented in this study

show the rate of percutaneous penetration and the dermal absorption as well as metabolism of ISDN in bovine udder skin. The amount of total organic nitrate which reached the perfusate within the perfusion period of 6 h was 5, 2·5 and 1·8 μmol after administration of the spray, ointment or microemulsion patch. A marked variation was obvious. Within the recorded time interval of 6 h, the ISDN flux was essentially unchanged. The absorbed amount was about 2% after administration of the spray and 1 and 0·5% after treatment with the ointment and the microemulsion, respectively. From the sum of organic nitrates which reached the perfusate and which were measured in the skin (Table 3), we conclude that a significant amount of ISDN remained in the microemulsion patch. This confirms data of studies performed with healthy volunteers in-vivo (Wenzel 1993). Wenzel (1993) reported similar absorption rates of ISDN in human skin after administration of the spray, ointment or microemulsion patch. Additionally, it was apparent in healthy volunteers that maximum plasma concentrations of ISDN and its metabolites were reached within 6 h after administration of ointment or spray. In contrast, a continuous increase of the plasma concentration was found over 24 h after administration of the microemulsion patch. Using Franz cells, Wenzel (1993) measured the ISDN penetration through isolated bovine udder over 24 h and confirmed the differences amongst spray, ointment and microemulsion shown in in-vivo studies in man. It is postulated that the horny layer of udder skin functions as a rate-limiting penetration barrier similar to that of human skin. Additional studies are necessary to evaluate the penetration barrier and reservoir function of the bovine udder horny layer in detail.

For comparison of the different formulations of ISDN used in the present study, it must be considered that the dosages and the treatment areas were different. It can be concluded from the data which are given in Table 2 that the amount of organic nitrate which was absorbed per cm^2 skin was significantly higher after treatment with the microemulsion than after application of spray or ointment. This is caused by the higher ISDN concentration of the microemulsion patch compared with the spray or ointment. Additionally, an enhanced absorption rate would be caused by the increased water content of the microemulsion resulting in supersaturation (Kemken et al 1991; Davis & Hadgraft 1991). Recently, Kemken et al (1991) demonstrated an enhanced absorption rate of β-blockers topically administered as microemulsions. Horizontal diffusion of the organic nitrate, including its metabolites, was demonstrated in the skin after topical administration of the ISDN microemulsion (Table 3). Therefore, the absorption area for the topically administered drug is greater than the treated skin region of 30 cm^2. In addition to the penetration enhancing effect of the supersaturation of the microemulsion, this may be a possible explanation for the high flux of ISDN via the skin.

As demonstrated in an earlier study on the non-steroidal anti-inflammatory agent etofenamate and benzoyl peroxide (Kietzmann et al 1993), metabolism of administered drugs can be demonstrated in the isolated perfused bovine udder skin. In the present study, ISDN was metabolized to IS-2-MN and IS-5-MN. Because no metabolites were found after

incubation of ISDN in Tyrode solution over 8 h (unpublished data), it is concluded that ISDN is metabolized in bovine udder skin. The relative amounts of ISDN, IS-5-MN and IS-2-MN (as per cent of total organic nitrate) reaching the perfusate within the perfusion show that about 40 per cent of the compound was metabolized to mononitrates when the drug was administered as spray or ointment. After administration of the microemulsion patch, 56% of total organic nitrate were found in the perfusate as IS-2-MN and IS-5-MN. Therefore, the rate of hydrolysis by a dermal glutathione-organic nitrate reductase in the skin (blood vessels) seems to be saturable and relatively constant with time.

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