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Cutaneous metabolism of isosorbide dinitrate after transdermal administration in isolated perfused porcine skin

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Abstract

The purpose of this study was to determine whether isosorbide dinitrate (ISDN) was metabolized by the skin during transdermal delivery. In order to assess this, the isolated perfused porcine skin flap (IPPSF) was utilized since this in vitro model possesses a viable epidermis and intact vasculature, two attributes ideal for studying the biotransformation of a vasoactive drug. ISDN transdermal systems (20 cm^2) were applied onto IPPSFs and the venous efflux sampled repeatedly over 16 h. ISDN, isosorbide-2-mononitrate (IS-2-MN), and isosorbide-5-mononitrate (IS-5-MN) fluxes were determined using gas chromatography. Approximately 14% of parent ISDN was metabolized to IS-2-MN and 10% to IS-5-MN. These observations are important as they indicate that a fraction of ISDN is biotransformed during transdermal delivery.

Keywords: Isosorbide dinitrate; Isosorbide mononitrate; Cutaneous metabolism; IPPSF; Skin flap; Percutaneous delivery device

1. Introduction

Isosorbide dinitrate (ISDN), an organic nitrate vasodilator, has long been used clinically for the prevention of angina pectoris, vasospastic angina and congestive heart failure. An important caveat with ISDN treatment has been the development of tolerance with prolonged high blood nitrate levels, a fact which indicates a well controlled method of delivery should be necessary for therapeutic efficacy. The pharmacokinetics of ISDN have been reported (Smith et al., 1990, Straehl and Galeazzi, 1985; Platzer et al., 1982). Recent efforts have led to the study of transdermally administered ISDN (Laufen and Leitold, 1992; Hatanaka et al., 1991). Evidence suggests that ISDN is subject to considerable extrahepatic metabolism (Straehl and Galeazzi, 1985; Platzer et al., 1982), and because skin contains the glutathione-s-transferases responsible for conversion

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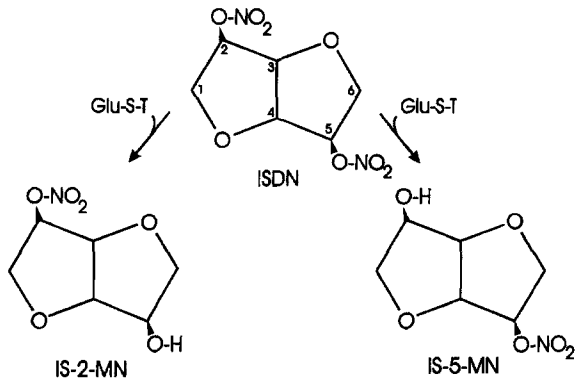

Fig. 1. Chemical structure of isosorbide dinitrate; isosorbide-2-mononitrate; and isosorbide-5-mononitrate. Glu-S-T represents glutathione-s-transferases.

of the parent compound to its two major metabolites (Kaplowitz et al., 1976, 1978), a significant part of a topical dose could be transformed first pass by cutaneous tissues. Fig. 1 is a representation of the chemical structure of ISDN and its two major metabolites, isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN). Both mononitrates are pharmacologically active in animals (Dietmann et al., 1981) and man (Stauch and Grewe, 1979). Isosorbide-5-mononitrate is the major active metabolite of ISDN, and most of the clinical activity of the dinitrate is attributed to the mononitrate. The principal pharmacological action is relaxation of vascular smooth muscle and consequent dilation of peripheral arteries and especially veins (Sifton et al., 1994).

There has been no report on direct studies of cutaneous metabolism of ISDN in the literature. In order to address the issue of cutaneous biotransformation of ISDN, we studied its absorption in the isolated perfused porcine skin flap (IPPSF). The use of porcine skin as a model for human skin has been shown to be appropriate generally (Guy et al., 1985; Reifenrath et al., 1984) and, more specifically, for nitroglycerine (Roberts and Mueller, 1990), a finding which should also apply to ISDN. Furthermore, the IPPSF has been validated as an in vitro preparation possessing normal biological cutaneous drug metabolism activity (Bikle et al., 1994; Carver et al., 1990; Chang et al., 1994) and an ability to respond to vasoactive drugs (Rogers and Riviere, 1994, Riviere et al., 1991), two attributes required to address the present problem. We report here the use of the IPPSF to measure the metabolic ability of skin on transdermally administered ISDN.

2. Materials and methods

2.1. ISDN transdermal patches

ISDN was applied to the skin flaps in transdermal patches (Pharmetrix Corporation, Lot # 9C002A). The patch contained 22 mg of ISDN in a liquid/gel reservoir-type system with an active surface area of 20 cm^2. The liquid reservoir contained ISDN, ethanol, water, a skin penetration enhancer and hydroxypropyl cellulose as a gelling agent. The reservoir was separated from the skin by a microporous polyethylene membrane and an acrylate-based pressure-sensitive skin adhesive containing the same enhancer. The backing was of occlusive polyester.

2.2. Sample and standard preparation

Isosorbide dinitrate (ISDN) was purchased as a 3% solution in ethanol from Zeneca Specialties, Wilmington, DE. IS-2-MN and IS-5-MN were purchased from Interchem Chemical Co., Paramus, NJ. All glassware was silanized with 10% (v/v) dimethyldichlorosilane in toluene to prevent drug adsorption. After being soaked for at least 1 h in the silanization reagent, the glassware was immediately rinsed with toluene and methanol before being air dried. The analytical method was based on that of Tzeng and Fung, 1991 with some modifications. Standards were prepared in ethyl acetate and standard curves were linear at least up to 16 μg/ml. The calibrating standard mixture (inserted every 10–20 injections) contained 0.4 μg/ml IS-2-MN and IS-5-MN and 1.8 μg/ml ISDN

Isomannide dinitrate (IMDN) and isomannide mononitrate (IMMN) were synthesized from isomannide (Aldrich Chemical Co., Milwaukee, WI) according to the method of Jackson and Hayward, 1960 and used as internal standards for the analogous isosorbide dinitrate and mononitrates. The perfusate samples were spiked with the internal standards (0.3 μg/ml each) and 1.0 ml aliquots of the spiked samples were loaded onto Chem Elute columns (Varian Sample Preparation Products, Harbor City, CA). After 5 min, the samples were eluted with 5 ml ethyl acetate and assayed by GC with electron capture detection.

2.3. Assay conditions

The extracts were analyzed on a Hewlett-Packard Model 5890 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a split-splitless injector containing a deactivated splitless liner. Injections (1 μl) were made with a Hewlett-Packard Model 7363A automatic sampler. The column was a fused-silica capillary (30 m × 0.32 mm i.d., 0.25 μm film) thickness (Supelco, SPB-5). The carrier gas (helium) and auxiliary gas (nitrogen) were ultra-high-purity. The carrier gas flow rate was set at 2 ml/min and the total flow (column + auxiliary) was 70–75 ml/min. The carrier and auxiliary gases were additionally purified with oxygen and moisture traps. The splitless injection mode (45 s) was used. Carrier gas flow-rate at the split vent and septum purge were 45 and 2 ml/min, respectively. The injection port temperature was set at 150°C and the detector at 225°C. The column temperature was initially set at 70°C for 0.75 min, then the oven was increased at 25°C/min to 150°C and maintained for 15 min. After each analysis, the temperature was increased at 25°C/min to 200°C and maintained for 3 min to purge residuals. Raw data capture was accomplished through a Dionex Advanced Computer Interface and peak heights were calculated with the Dionex Al 450 chromatography software.

The variability of the method was evaluated by extracting and analyzing an aqueous mixture of the three isosorbide and the two isomannide compounds exactly as the perfusate specimens were treated. The within-day coefficient of variation was 2–3% for the dinitrates and 3–9% for the mononitrates, except IS-5-MN, which was 10–15%. Even though a clean glass liner was put into the injector daily, the latter compound was problematic, because of its susceptibility to adsorption to active sites (or contamination) in the injector and column head. Using a 1 ml sample volume, 5 ml elution volume and 1 μl injection volume, the limits of quantitation were 0.02 μg/ml, 0.01 μg/ml and 0.03 μg/ml for ISDN, IS-2-MN and lS-5-MN, respectively. The recovery of IMDN and IMMN ranged from 78% to 105% and the final concentrations of ISDN, IS-2-MN and IS-5 MN in each perfusate sample was corrected accordingly.

2.4. IPPSF experiments

The IPPSF has been described in detail elsewhere (Riviere et al., 1986; Monteiro-Riviere et al., 1987; Riviere and Monteiro-Riviere, 1991). Briefly, skin flaps from female weanling Yorkshire swine ($\approx$ 20 kg each) were harvested and weighed following surgical procedures detailed elsewhere (Riviere et al., 1986), cannulated to the perfusion apparatus, placed in the perfusion chamber, and perfused predose for 1 h to assess viability. The perfusion medium consisted of Krebs-Ringer bi-

carbonate buffer, glucose and bovine serum albumin. The perfusion chamber was held at 37°C and approximately 55% relative humidity. The medium was aerated with a mixture of 95% oxygen and 5% CO_2. After the 1-h predose period, the skin flaps were fitted with the delivery device (20 cm^2 as described in the previous section), at which time the sampling schedule commenced. A nonrecirculating protocol was followed, with venous samples taken every half-hour through 5 h, then every hour through 16 h (after which the experiments were terminated). The IPPSF glucose utilization was monitored to assess viability during this 16 hour period. Additionally, temperature, arterial pressure, perfusate pH and arterial perfusate flow rate were continuously monitored. Venous efflux sample concentrations were converted to flux by multiplying by flow rate, to obtain an output flux profile for each IPPSF experiment.

3. Results

The means of the three flux profiles of the four IPPSFs are shown in Fig. 2. Table 1 lists the molar fractions of ISDN and its two metabolites found in the venous efflux. No attempt was made to predict metabolism past 16 hours. The AUC was calculated for each curve using a linear trapezoid method. Of the absorbed drug, a mean of 13.7% of the parent compound was metabolized to IS-2-MN and 10.0% to IS-5-MN in skin. The mean time of peak flux was 11 h, after which the patch was presumably being depleted of compound. Of the 22 mg of the initial dose in each patch, a range of 11.9 to 13.1 mg was recovered from each patch after termination of the four experiments. That is, a mean of 43.3 ± 2.58% of the parent drug was delivered from the patch.

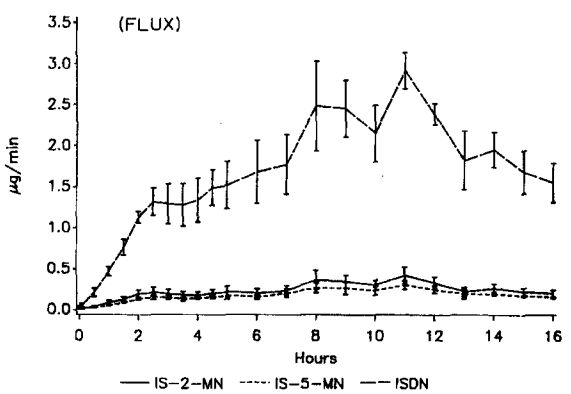

Fig. 2. Mean (± 1 S.D.) of four IPPSF venous efflux profiles of (— — —ISDN); (—IS-2-MN); and (- - -IS-5-MN).

Table 1
Molar fraction of ISDN, IS-2-MN and IS-5-MN in venous efflux of IPPSFs as a function of AUC

IPPSF #	ISDN	IS-2-MAN	IS-5-MN
1843	0.759	0.155	0.087
1850	0.715	0.153	0.132
1851	0.694	0.195	0.111
1871	0.884	0.045	0.071
Mean	0.763	0.137	0.100
S.D.	0.085	0.064	0.027

4. Discussion

As shown in Fig. 2, both IS-2-MN and IS-5-MN were found in the IPPSF venous effluent samples. The areas under these curves, converted to molarity, suggest about 13.7% (± 6.4%) of the parent ISDN was metabolized to IS-2-MN and 10.0% (± 2.7%) to IS-5-MN first pass in the IPPSF. This fraction is considerably higher than that speculated by Laufen and Leitold, 1992, who compared in vivo total biotransformations following intravenous and transdermal administration. One explanation for the discrepancy is the possibility that the dermal and systemic metabolic rates are similar, and the three compounds will always reveal the same equilibrium ratios in plasma, regardless of the site of metabolism. Indeed, this exemplifies the desirability of using isolated systems for such studies which attempt to estimate dermal metabolism using methods which require robust estimates of IV pharmacokinetic parameters.

We have previously demonstrated a significant effect of cutaneous biotransformation on in vivo disposition with parathion (Qiao et al., 1994, Qiao

and Riviere, 1995). Previous authors (Platzer et al., 1982; Straehl and Galeazzi, 1985; Laufen and Leitold, 1992) have described ISDN metabolism in vivo as rapid and nearly complete with approximately 75% being metabolized to IS-5-MN and approximately 25% to IS-2-MN. The present study suggests a different pattern in porcine skin.

In conclusion, this study demonstrates that cutaneous biotransformation of transdermally administered ISDN occurs. Finally, this work presents a potentially effective method for assessing the impact of dermal metabolism on transdermal drug delivery systems using an isolated perfused skin system.

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