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## Influence of emotional stress on pharmacokinetics of isosorbide dinitrate intraperitoneally administered to rats.

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## Abstract

The plasma level of isosorbide dinitrate intraperitoneally administered to rats stressed by foot-shock was almost the same as that in non-stressed control rats. However, levels of its metabolites, 5-isosorbide mononitrate and 2-isosorbide mononitrate, were lower in stressed rats than in non-stressed rats, suggesting that stress may influence the metabolism and/or excretion of the metabolites.

**KEYWORDS:** isosorbide dinitrate, pharmacokinetics, emotional stress, rats

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## Influence of Emotional Stress on Pharmacokinetics of Isosorbide Dinitrate Intraperitoneally Administered to Rats

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The plasma level of isosorbide dinitrate intraperitoneally administered to rats stressed by foot-shock was almost the same as that in non-stressed control rats. However, levels of its metabolites, 5-isosorbide mononitrate and 2-isosorbide mononitrate, were lower in stressed rats than in non-stressed rats, suggesting that stress may influence the metabolism and/or excretion of the metabolites.

**Key words :** isosorbide dinitrate, pharmacokinetics, emotional stress, rats

Isosorbide dinitrate (ISDN), a vasodilator, is used clinically as antianginal drug. It is well known that anginal attacks are often caused by various kinds of stress or effort (1, 2). Most patients feel fear or anxiety toward the unpredictable attack. We have previously observed that the pharmacokinetics of orally administered ISDN are influenced by emotional stress such as foot-shock. In our study, plasma levels of ISDN and its metabolites, 5-isosorbide mononitrate (5-ISMN) and 2-isosorbide mononitrate (2-ISMN), were markedly lower in rats emotionally stressed than in non-stressed control rats (3). However, it is unclear which pharmacokinetic process of ISDN is influenced by emotional stress. The present study was designed to solve this problem by determining the pharmacokinetics of intraperitoneally administered ISDN in rats emotionally stressed by electric foot-shock.

Twenty-one male Wistar rats weighing 210-230 g were used in the present experiment. They were divided into two groups: an emotion-

ally stressed (ES) group and a non-stressed control (C) group. They were housed 3-4 per cage in 26 × 36 × 25 cm plastic walled cages, and were given food and water *ad libitum*, except during the experiment. The animals were maintained on a 12h light dark cycle (lights on from 08:00 to 20:00), at a room temperature of 22-24 °C and a relative humidity of approximately 60 %.

ISDN (injection, donated by Eisai Co.) was administered intraperitoneally at a dose of 0.5 mg/kg in a volume of 1 ml/kg body weight. For the determination of ISDN and its metabolites in plasma, isomanide dinitrate (IMDN) was used as an internal standard.

A foot-shock loading box was used to apply emotional stress to the animals. The apparatus consisted of a plastic box, measuring 60 × 80 × 44 cm, with a grid floor made of stainless steel rods (5 mm in diameter) spaced at 1-cm intervals. The inside of the box was divided with white plastic walls into 12 compartments. Each compartment was 400 square cm and 44 cm in height. A direct current could be passed through the grid

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on the floor. The box was equipped with shock and tone generators (Asteck, Co.).

For determining the concentration of ISDN and its metabolites, 5-ISMN and 2-ISMN, in plasma, blood samples of approximately 5 ml were collected from the descending abdominal artery after the laparotomy under ethylether. Each sampling was performed 30 and 60 min after the drug administration. The plasma was separated by centrifugation (3,000 rpm for 10 min), and 2 ml of plasma was used for gas chromatographic determination of the concentration of ISDN and its metabolites.

Determinations of ISDN and its metabolites were performed with a gas chromatograph equipped with a  $^{63}\text{Ni}$  (10 mCi) electron capture detector (GC-ECD JGC-20KE, Nihondenshi Co.). The columns (2 mm inside diameter and 2 m length) packed with Gaschrom Q 100-120 mesh coated with 3 % OV-1 and 3 % OV-3 were used for measuring ISDN and its metabolites, respectively. The columns were previously heated for one day. The column and injection temperature were maintained at 155 °C with argon as the carrier gas. The carrier gas was followed by 10 % CH<sub>4</sub>-argon (base) under a pressure of 3.0 kg/cm<sup>2</sup>.

For the measurement of ISDN in plasma, 4 ng of internal standard (IMDN) were added to 1 ml of plasma, which was extracted twice with 4 ml of n-hexane. After evaporating to dryness, extracts were reconstituted with 100  $\mu\text{l}$  of ethylacetate. Five- $\mu\text{l}$  samples were injected into the GC-ECD. To measure 5-ISMN and 2-ISMN, after adding the internal standard to the residue obtained after the above extraction, the residue was extracted three times with 4 ml of ethylether. The extracts were evaporated and then reconstituted with 100  $\mu\text{l}$  of ethylacetate, and 5- $\mu\text{l}$  samples were injected into the GC-ECD.

The emotional stress, *i.e.*, foot-shock with pure tone (10-sec duration, 90-sec interval) was given to the animals for 30 min from immediately after the administration of ISDN. Foot-shock (1.5-2.0 mA) was given for 5 sec after the onset

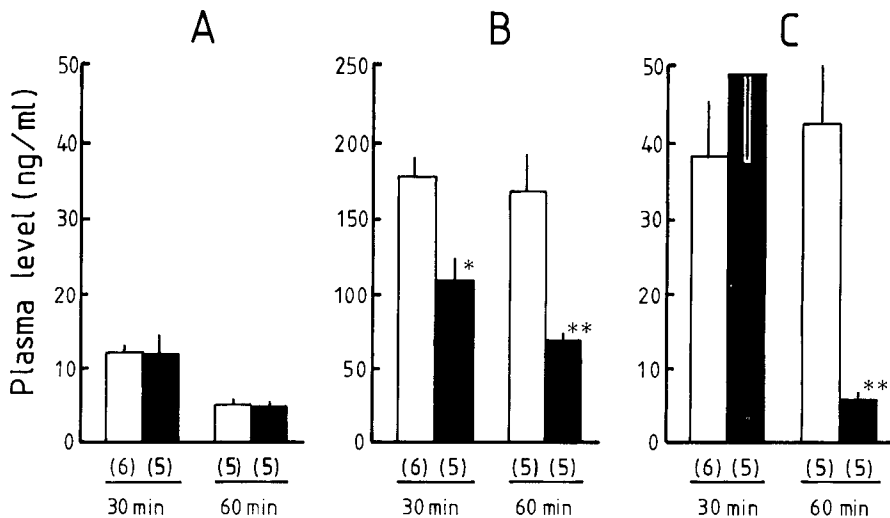
of pure tone (2,000 Hz, 5 sec), which was continued during the foot-shock. The drug was intraperitoneally administered immediately before emotional stress which was given to rats of the ES group. In the control group the drug was administered, but the emotional stress was not.

Results were evaluated statistically by means of the Student's *t*-test.

Fig. 1 shows the plasma levels of ISDN and its metabolites after the intraperitoneal injection. Mean ISDN plasma levels 30 and 60 min after the drug administration in the non-stressed control group were approximately 11.8 and 5.2 ng/ml, respectively. The ISDN plasma levels in the ES group were almost the same as those in the control group. On the other hand, the mean 5-ISMN levels 30 and 60 min after the administration were approximately 178.8 and 170.4 ng/ml, respectively. The levels of 5-ISMN 30 and 60 min after the administration were markedly lower in the ES group than in the respective control group. There were significant differences in the 5-ISMN level at 30 and 60 min ( $p < 0.05$  and  $p < 0.01$ , respectively). The mean 2-ISMN levels 30 and 60 min after the administration were approximately 38.8 and 42.9 ng/ml, respectively. The 30-min level of 2-ISMN in the ES group was not different from that in the control group, but the 60-min level was markedly lower in the ES group than in the control group. There was a significant difference in the 2-ISMN level at 60 min ( $p < 0.01$ ).

In general, it is said that various factors such as temperature, circumstances, emotionality and stress influence the action of drugs (4-7). The potentiation or decrease in a drug's effect may be due to the alternation in the sensitivity of site of drug action and/or in the pharmacokinetics.

In a previous study (3), we observed that the pharmacokinetics of ISDN orally administered was influenced by emotional stress such as foot-shock, *i.e.*, the plasma levels of ISDN and its metabolites were lower in rats emotionally stressed than in non-stressed control rats. However, it is not clear which process of the



**Fig. 1** Influence of emotional stress on plasma levels of isorbidine dinitrate (ISDN) and its metabolites in rats. ISDN was administered intraperitoneally at a dose of 0.5 mg/kg, and 30 min and 60 min later, ISDN (A) and its metabolites, 5-isorbidine mononitrate (B) and 2-isorbidine mononitrate (C), were determined. Values represent mean value  $\pm$  SEM. Asterisks indicate significant decreases from the respective control value (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ). Numbers of animals used are given in parentheses.  $\square$ , Non-stressed control group;  $\blacksquare$ , emotionally stressed group.

absorption, metabolism, distribution and/or excretion is related to that phenomenon. In the present pharmacokinetic study of ISDN administered parenterally, although the plasma level of ISDN in rats emotionally stressed was almost the same as that in non-stressed control rats, the plasma levels of the metabolites 5-ISMN and 2-ISMN were lower in emotionally stressed rats than in control rats, except for the plasma level of 2-ISMN 30 min after the administration. Considering the results of the present study on the pharmacokinetic of ISDN administered intraperitoneally and also the results of previous study concerning the pharmacokinetic of ISDN administered orally, it is suggested that not only the absorption of ISDN from the gastrointestinal tract but also its metabolism and/or excretion of its metabolites are influenced by emotional stress such as foot-shock.

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