Influence of Electric Foot Shock on Pharmacokinetics of Isosorbide Dinitrate Subcutaneously Administered to Rats

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Abstract—The influence of emotional stress on the pharmacokinetics of isosorbide dinitrate (ISDN) administered s.c. to rats was studied. The plasma level of ISDN in emotionally stressed (ES) rats was the same as that in non-stressed control rats. However, the levels of its metabolites, 5-isosorbide mononitrate (5-ISMN) and 2-isosorbide mononitrate (2-ISMN), were markedly lower in ES rats than in the control rats. On the other hand, urine ISDN and 2-ISMN excretion rates were lower in ES rats than in the control rats. These observations suggest that the pharmacokinetics of ISDN administered s.c. is influenced by emotional stress such as foot shock.

It is known that angina attacks are often caused by various kinds of stress or effort (1, 2). Almost all patients feel fear or anxiety toward an unpredictable attack. The variation in a drug's effect may be due to changes in the sensitivity of the site of drug action and/or to the pharmacokinetics. There are few reports particularly concerned with the influence of emotional stress on the pharmacokinetics.

Isosorbide dinitrate (ISDN) with vasodilator action is clinically and widely used as an antianginal drug. The major biotransformation of this drug is denitration (3). It is also said that ISDN is mainly and rapidly metabolized into mononitrates, 5-isosorbide mononitrate (5-ISMN) and 2-isosorbide mononitrate (2-ISMN), and they are pharmacologically active substances (3). We previously reported that the pharmacokinetics of ISDN administered orally was influenced by emotional stress; i.e., plasma levels of ISDN and its metabolites were markedly lower in emotionally stressed (ES) rats than those in non-stressed control rats (4). It, however, is unclear whether this phenomenon is attributed to changes of the process such as absorption, excretion and so on. If the drug is administered non-orally, at least, there is no need to consider the factor of absorption from the gastrointestinal tract. The present study was conducted to elucidate this problem by determining the pharmacokinetics, particularly the blood level and urine excretion when ISDN was administered non-orally by the s.c. route to the ES rats.

Thirty-one male rats of the Wistar strain weighing 185–240 g were used as subjects. They were divided into two experimental groups for measuring plasma level and urine level, respectively; and furthermore, each of these groups was divided into two sub-groups: the ES group and non-stressed control group. They were housed 3–4 per cage in 26 x 36 x 25-cm plastic walled cages and were given food and water ad lib except for during the experiment. The animals were maintained on a 12 hr light dark-cycle (light on from 08:00 to 20:00) and at a room temperature of 22–24°C and a relative humidity of approximately 60%.

ISDN (Nitrol injection, donated by Eisai Co.) was used in the present experiment. The drug at a dose of 0.5 mg/kg was administered s.c. at a volume of 1 ml/kg body weight in experiments for measuring the plasma and urine levels. For the determination of ISDN and its metabolites in plasma and urine, isomannide dinitrate (IMDN) was used as the internal standard.

As the apparatus for application of emo-
tional stress, a foot shock loading box was used. The details for the apparatus were described in the previous paper (4).

Approximately 5-ml blood samples were collected 30 and 60 min after drug administration for determining levels of ISDN and its metabolites, 5-ISMN and 2-ISMN, in plasma from the descending abdominal artery of rats anesthetized with ethylether. Plasma was separated by centrifugation (3,000 rpm for 10 min), and 2 ml of plasma was used for gas chromatographic (GC) determination of the concentration of ISDN and its metabolites.

For determining the level of ISDN and its metabolites in urine, each urine sample was collected 2 times from 30 to 90 min and from 91 to 150 min after s.c. administration of ISDN and p.o. administration of water (5 ml/animal) in the different subjects, using a metabolic cage (Metabolica, Sugiyamagen Iriki, Ltd.). One milliliter of urine was used for the GC determination.

The pretreatment for the GC assay was performed as follows: A) As for measuring the level of ISDN in the blood, 1 ml of plasma containing ISDN was added with 4 mg of the internal standard (IMDN) and then extracted twice with 4 ml of n-hexane. The extract was evaporated to dryness and then reconstituted with 100 μl of ethylacetate; and 5 μl was injected into the GC instrument. 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 μl of ethylacetate; 5 μl was then injected into the GC instrument. B) As for measuring the level of ISDN in urine, after adding 10 ng of IMDN as an internal standard to 1 ml of urine, the urine was extracted twice with 2 ml of n-hexane. The extracts were evaporated to dryness and then reconstituted with 100 μl of ethylacetate; 3 μl was injected into the GC instrument. To measure 5-ISMN and 2-ISMN, after the internal standard was added to 1 ml of urine, the urine was extracted with 4 ml of diethyl-ether. After evaporating to dryness the extracts were reconstituted with 1.5 ml of ethylacetate, and then 3 μl was injected into the GC instrument.

Determinations of ISDN and its metabolites in plasma and urine were performed by with a GC chromatograph equipped with a 63Ni (10 mCi) electron capture detector (GC-ECD JGC-20KE, Nihondenshi Co.; GC-ECD GC-9A, Shimadzu Co.). A column (2 mm inside diameter and 2 m length) packed with Gaschrom Q 100–120 mesh coated with 3% OV-1 and 3% OV-3 and a capillary column (Hewlett Packard OV101) were used for measuring ISDN and its metabolites in plasma and in urine, respectively. Those columns were previously heated for one day. The column and injector temperatures for measuring levels in plasma were maintained at 155°C with argon as the carrier gas. The carrier gas was followed by 10% CH-argon (base) under the pressure of 3.0 kg/cm². On the other hand, for measuring levels in the urine, the column and detector temperatures were maintained at 170 and 200°C, respectively. The carrier gas was N₂ at a rate of 50 ml/min.

The emotional stress, i.e., foot shock with pure tone, was given to the animals for 30 min (duration: 10 sec, interval: 90 sec) 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), which was continued during the foot shock. The drug was administered s.c. immediately before the exposure of emotional stress in the emotionally stressed group. In the control group, the drug was administered, but the emotional stress was not.

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

Figure 1 shows the plasma levels of ISDN and its metabolites, 5-ISMN and 2-ISMN, after the s.c. administration of ISDN. Mean ISDN plasma levels 30 and 60 min after the drug administration in the control group were approximately 33 and 12 ng/ml, respectively. The ISDN plasma levels in the ES group were almost the same as those in the control group. However, the plasma levels of the metabolites at 30 and 60 min after in the control group were approximately 208 and 199 ng/ml for 5-ISMN and approximately 32 and 21 ng/ml for 2-ISMN, respectively. The plasma levels of 5-ISMN and 2-ISMN 30 and 60 min after in the ES group were markedly lower than those in the respective control group. There
Fig. 1. Levels of ISDN and its metabolites in plasma of emotionally stressed rats. ISDN at a dose of 0.5 mg/kg was administered s.c. Each point represents a mean value (ng/ml) ± S.E.M. Asterisks in the figure indicate a significant decrease from the respective control value (**P<0.01). Numbers of animals used are given in parentheses. C: non-stressed control group, ES: emotionally stressed group.

Fig. 2. Levels of ISDN and its metabolites in urine of emotionally stressed rats. ISDN at a dose of 0.5 mg/kg was administered s.c. Each point represents a mean value (ng/hr) ± S.E.M. Asterisks in the figure indicate a significant decrease from the respective control value (*P<0.05). Numbers of animals used are given in parentheses. C: non-stressed control group, ES: emotionally stressed group, A: urine collected from 30 to 90 min after drug administration, B: urine collected from 91 to 150 min after.

were significant differences at 30 and 60 min in the 5-ISMN level (P<0.01, respectively) and in the 2-ISMN level (P<0.01, respectively) between the ES group and the control group.

Figure 2 shows the urine excretion of ISDN and 5-ISMN and 2-ISMN after the s.c. administration of ISDN. Mean excretion rates of ISDN from 30 to 90 min and from 91 to 150 min after the drug administration in the con-
trol group were approximately 70 and 3 ng/hr, respectively. Those in the ES group were approximately 38 and 6 ng/hr, respectively. There is a significant difference between the ES group and the control group in the ISDN excretion rates (P<0.05). On the other hand, the urine excretion rates of the metabolites from 30 to 90 min and from 91 to 150 min after in the control group were approximately 1,500 and 480 ng/hr for 5-ISMN and approximately 113 and 27 ng/hr for 2-ISMN, respectively. The 5-ISMN urine excretion rates from 30 to 90 min and 91 to 150 min after in the ES group were almost the same as those in the control group. The 2-ISMN urine excretion rate from 30 to 90 min in the ES group, but the 2-ISMN urine excretion from 91 to 150 min after, was lower than that in the control group. There is a significant difference between the ES group and the control group (P<0.05).

It is generally known that various factors such as temperature, circumstance, emotionality and stress influence the action of drugs (5–8). These effects may be due to the alternation in the sensitivity in a drug action site and/or the pharmacokinetics.

In the previous study (4), we have observed that the pharmacokinetics of ISDN administered p.o. was influenced by emotional stress such as electric foot shock, i.e., the ISDN and its metabolite plasma levels in ES rats were lower than those in non-stressed control rats. It, however, is unclear whether any process in absorption, metabolism, excretion and so on is mainly related to this phenomenon. In the present study, the influence of emotional stress on the pharmacokinetics of ISDN administered non-orally, i.e., by the s.c. route, was studied in the ES rats. The results showed that the plasma level of ISDN administered non-orally in the ES rats was the same as that in the non-stressed control rats, but plasma levels of the metabolites, 5-ISMN and 2-ISMN, in the ES rats were markedly lower than those in control rats. In the previous study (4), plasma levels of ISDN and its metabolites in ES rats when orally administered was lower than that in non-stressed rats. This means that the absorption of ISDN from the gastrointestinal tract when orally administered is depressed by emotional stress. In the present experiment, urine excretion rates of ISDN and 2-ISMN in ES rats were lower than those in the control rats. Considered from the urine-excretion/plasma-level ratio, the ratio for ISDN in the ES group was lower than that in the control group, and the ratios for 5-ISMN and 2-ISMN in the ES group were higher than those in the control group. These observations may indicate that the clearance of ISDN from the kidney is decreased by emotional stress and clearances of 5-ISMN and 2-ISMN are increased.

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