Contribution of Isosorbide-5-Mononitrate, a Major Metabolite of Isosorbide Dinitrate (ISDN), to the Hemodynamic Effect of ISDN Administered Orally in Conscious Dogs

Kentaro KOGI and Tetsuo SATOH*
Research Laboratories, Toa Eiyo, Ltd., lizaka, Fukushima 960-02, Japan
*Department of Pharmacology and Toxicology, Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan
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Abstract—This study was designed to determine the extent, to which isosorbide-5-mononitrate (5-ISMN) contributes to the hemodynamic effect of isosorbide dinitrate (ISDN) in conscious dogs. Test drugs (ISDN or 5-ISMN) were given orally. Either ISDN or 5-ISMN produced a decrease in blood pressure dose-dependently, the decrease in pulse pressure being specific; the pattern of blood pressure change induced by ISDN or 5-ISMN was different from that induced by nifedipine or prazosin. The effect of ISDN (2 mg/kg) was almost equivalent to that of 5-ISMN (4 mg/kg) and the effect of ISDN (4 mg/kg) to that of 5-ISMN (8 mg/kg). After administration of ISDN, both ISDN and 5-ISMN appeared in the plasma, and the effect of ISDN well-correlated with the increase in the plasma concentration of 5-ISMN. Contribution of 5-ISMN to the effect of ISDN was estimated to be about 30% from the value of the plasma concentration of 5-ISMN at 3 to 4 hr after administration, when the maximal response to ISDN occurred. Based on the data of the area under the plasma concentration curve of 5-ISMN (from 0 to 10 hr after administration), the fraction of biotransformation to 5-ISMN from ISDN was calculated to be 73.6 to 76.6% (based on moles). Because the ability of 5-ISMN to decrease pulse pressure was about 1/2 (or 41% based on moles) of that of ISDN, the contribution of 5-ISMN to the effect of ISDN was estimated to be about 30% in total, the value being similar with that estimated at 3 to 4 hr after administration.

Isosorbide dinitrate (ISDN) is an organic nitrate widely used for treatment of angina pectoris (1-3). ISDN is biotransformed extensively by the enzyme glutathione organic nitrate reductase to isosorbide-2-mononitrate (2-ISMN) and isosorbide-5-mononitrate (5-ISMN) (4-6), the latter being a major metabolite of ISDN that is pharmacologically active in animals (7, 8) and man (9). The bioavailability of ISDN, therefore, is as low as 3% (10). In contrast to ISDN, 5-ISMN can be almost completely absorbed from the gastrointestinal tract and remains almost unchanged in the body, and therefore bioavailability of 5-ISMN is near 100% (11). For this reason, 5-ISMN has been expected to be used for treatment of patients with angina pectoris (12-14).

Although the bioavailability of ISDN is low, the duration of its action is relatively long. Recently, we have suggested that the relatively long action of ISDN could be due to the relatively high and long lasting blood levels of 5-ISMN following oral administration of ISDN (15).

The purpose of the present study was to determine the extent, to which the pharmacological effect of orally administered ISDN depends on the effect of 5-ISMN that can be found in the plasma after administration of ISDN. For this purpose, we measured the blood levels of both ISDN and 5-ISMN after oral administration of ISDN or 5-ISMN and examined their pharmaco-
logical actions. In addition, the actions of ISDN and 5-ISMN were compared with those of prazosin and nifedipine.

**Materials and Methods**

**Experimental protocol:** Cross-over experiments were performed in 12 conscious mongrel dogs of either sex, weighing 7–12 kg. Dogs were divided into two groups and 2 subgroups each; and to each dog, two kinds of drugs were administered orally according to the following protocol.

I. Low dose group

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Drug Administration</th>
<th>Week 1</th>
<th>Week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I(a)</td>
<td>ISDN (2 mg/kg)</td>
<td>5-ISMN (4 mg/kg)</td>
<td>1 week</td>
</tr>
<tr>
<td>I(b)</td>
<td>5-ISMN (4 mg/kg)</td>
<td>ISDN (2 mg/kg)</td>
<td>1 week</td>
</tr>
</tbody>
</table>

In subgroup I(a), ISDN (2 mg/kg) was initially given (the first administration), and then after a week, 5-ISMN (4 mg/kg) was given (the second administration). In both cases of drug administration, plasma levels of ISDN and 5-ISMN and blood pressure were measured from the time of administration until 10 hr after administration. (When 5-ISMN was given, the plasma level of only 5-ISMN was measured, because ISDN does not appear in the plasma in this case). In subgroup I(b), 5-ISMN (4 mg/kg) was initially given (the first administration), and after a week, ISDN (2 mg/kg) was given (the second administration). In the same way, 4 mg/kg of ISDN and 8 mg/kg of 5-ISMN were given in subgroups I(a) and I(b). In each case of drug administration, both the plasma level of drugs and blood pressure were measured. Thus, the data with ISDN (2 mg/kg, 6 dogs) and those with 5-ISMN (4 mg/kg, 6 dogs) were obtained from the low dose group, and the data with ISDN (4 mg/kg, 6 dogs) and those with 5-ISMN (8 mg/kg, 6 dogs) were obtained from the high dose group.

In addition, 24 conscious mongrel dogs were used for comparative hemodynamic experiments with ISDN, 5-ISMN, prazosin and nifedipine, in which experiments were performed in a noncross-over manner. In this series of studies, only hemodynamic data were obtained.

**Drug administration:** Drugs were administered orally in both cross-over and noncross-over experiments. Each dog was fasted for 24 hr before drug administration and received test drugs with some water at the time of administration.

**Measurements of hemodynamics:** Under anesthesia with sodium pentobarbital (25 mg/kg), an arterial catheter was implanted aseptically according to our method (15, 16) described as follows. A 1.5–2.0 cm incision of the skin at the part of the right cervix was made. The right carotid artery was then exposed and carefully dissected free. An arterial catheter, which was filled with sterile physiologic saline containing sodium heparin (1000 units/ml), was introduced retrogradely through the right carotid artery. The other end of the arterial catheter was exteriorized through the skin at the neck. Experiments were performed 3–5 days after the catheter implantation. The chronically implanted catheter was connected to a pressure transducer (Nihon Kohden RP-5), and the systolic and diastolic blood pressure was measured. Heart rate was measured directly with a cardiotachometer (Nihon Kohden RT-5) triggered by the pulse of arterial blood pressure. The pulse pressure (systolic pressure–diastolic pressure) was also determined. During measurements of blood pressure, the unrestrained experimental animal was gently sitting on the floor of a sound-proof room by the side of an experimenter.

**Measurements of plasma concentrations of ISDN and 5-ISMN:** Blood samples were withdrawn before dosing and 1, 2, 3, 4, 6, 8 and 10 hr after dosing. The samples were centrifuged immediately after withdrawal, and the separated plasma was stored at −20°C until analysis was performed. Plasma concentrations of ISDN and 5-ISMN were measured by a gas chromatograph according to Kato's method (17).

**Extraction of ISDN:** Plasma was extracted with n-hexane, which was re-extracted with acetonitrile. The acetonitrile layer was transferred to another vial and then evaporated under nitrogen to near dryness at room
temperature. The residue was dissolved in ethyl acetate after addition of an internal standard (isomannide dinitrate), and a portion of the ethyl acetic solution was injected into a gas chromatograph (Hewlett-Packard, 5710A).

**Extraction of 5-ISMN:** Plasma was extracted with ethyl acetate, and the organic phase was transferred to another vial. After evaporation of the pooled ethyl acetate to near dryness, the residue was dissolved in an appropriate volume of ethyl acetate. A portion of the ethyl acetic solution after addition of an internal standard (2,4-dinitrochlorobenzene) was injected into a gas chromatograph (Shimadzu, 4BM).

**Fraction of biotransformation to 5-ISMN:** The area under the plasma concentration curve (AUC) of 5-ISMN (from 0 to 10 hr after administration) was measured when ISDN or 5-ISMN was administered. From the AUC and the amount of drugs given orally, the fraction of biotransformation to 5-ISMN from ISDN was calculated. The AUC/mg/kg of 5-ISMN when 4 mg/kg of 5-ISMN was administered was defined as 100%. In the case of ISDN administered, the fraction of biotransformation to 5-ISMN was calculated from the AUC/mg/kg to that of administered 5-ISMN (4 mg/kg).

**Reagents:** The source of the test compounds were as follows: isosorbide dinitrate (ISDN) from Toho Kasei Kogyo Co., Ltd., Japan; isosorbide-5-mononitrate (5-ISMN) from Toa Eiyo, Ltd., Japan; prazosin from Orion Corporation, Ltd., Fermion, Finland; and nifedipine from Toho Kasei Kogyo Co., Ltd., Japan.

**Statistical analysis:** Experimental results were presented as means±S.E., and the paired t-test was applied to evaluate a statistical significance of the difference between the values immediately before and after dosing. P values less than 0.05 were considered statistically significant.

**Results**

**1. Cross-over experiments**

**1-1. Hemodynamic effect of ISDN and 5-ISMN**

1-1-1. **Low dose group:** In group I(a), systolic and diastolic blood pressure, and heart rate immediately before the first administration (ISDN, 2 mg/kg) were 162±9 and 70±12 mmHg, and 111±15 beats/min, respectively; and those immediately before the second administration (5-ISMN, 4 mg/kg) were 163±10 and 73±14 mmHg, and 127±9 beats/min, respectively. In group I(b), systolic and diastolic blood pressure, and heart rate before the first administration (5-ISMN, 4 mg/kg) were 143±8 and 76±4 mmHg, and 123±16 beats/min, respectively, and those immediately before the second administration (ISDN, 2 mg/kg) were 138±2 and 69±2 mmHg, and 128±19 beats/min, respectively. Thus there was no marked difference in blood pressure and heart rate between the first and the second administrations.

The hemodynamic effect of ISDN (2 mg/kg) and that of 5-ISMN (4 mg/kg) are shown in Fig. 1 (left panel). Oral administration of either ISDN (2 mg/kg) or 5-ISMN (4 mg/kg) caused a significant decrease in systolic blood pressure, reaching its maximal reduction of 18 mmHg or 24 mmHg, respectively, 3 hr after administration and remaining decreased through 10 hr. There were not remarkable changes in diastolic blood pressure after administration of each of the agents. The mean blood pressure also decreased slightly in response to each of the agents, but the degree of changes in mean blood pressure was less than that in systolic blood pressure. Heart rate increased transiently after the administration of each of ISDN and 5-ISMN.

1-1-2. **High dose group:** In group II(a), systolic and diastolic blood pressure, and heart rate immediately before the first administration (ISDN, 4 mg/kg) were 162±6.0 and 75±5 mmHg, and 104±15 beats/min, respectively; and those immediately before the second administration (5-ISMN, 8 mg/kg) were 149±2 and 64±3 mmHg, and 104±12 beats/min, respectively. In group II(b), systolic and diastolic blood pressure, and heart rate immediately before the first administration (5-ISMN, 8 mg/kg) were 164±21 and 72±10 mmHg, and 142±10 beats/min, respectively; and those immediately before the second administration (ISDN, 4 mg/kg) were 163±18 and 73±14 mmHg, and 107±9 beats/min, respectively.
Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and heart rate (HR) after oral administration of 5-ISMN or ISDN in conscious dogs. The left panel shows the results in the low dose group, and the right panel shows the results in the high dose group. ISDN and 5-ISMN were administered in a cross-over manner in each of the groups (see Materials and Methods for description of the drug administration in a cross-over manner). Each point represents the mean±S.E. of six dogs. * Statistically significant compared with the value obtained at 0 min (P<0.05).

mmHg, and 135±7 beats/min, respectively. Thus there was no marked difference in blood pressure and heart rate between the first and the second administrations.

The hemodynamic effect of ISDN (4 mg/kg) and that of 5-ISMN (8 mg/kg) are shown in Fig. 1 (right panel). An administration of ISDN (4 mg/kg) or 5-ISMN (8 mg/kg) decreased systolic blood pressure considerably, reaching its maximal reduction of 33 mmHg or 39 mmHg, respectively, 2 hr after administration. The response of systolic blood pressure to ISDN and that to 5-ISMN generally resembled each other. Diastolic blood pressure did not change, while mean blood pressure significantly decreased after administration of each of the agents. Heart rate increased slightly after the administration of these agents.

1-2. Plasma concentrations of ISDN and 5-ISMN in relation to the decrease in pulse pressure

Administration of either ISDN or 5-ISMN produced a remarkable decrease in systolic blood pressure rather than in diastolic blood pressure; and therefore, pulse pressure was reduced by these agents. Figure 2 illustrates the comparison of the time course of the decrease in pulse pressure and the mean plasma concentrations of ISDN and 5-ISMN after oral administration of each of the agents.

1-2-1. Low dose group: The plasma concentrations of ISDN and 5-ISMN and the pulse pressure after administration of either ISDN (2 mg/kg) or 5-ISMN (4 mg/kg) are
Fig. 2. The plasma concentrations of 5-ISMN and ISDN and percent decrease in pulse pressure after oral administration of 5-ISMN or ISDN. These results were obtained from the experiments shown in Fig. 1. The left panel shows the results in the low dose group, and the right panel shows the results in the high dose group. Symbols are those given in Fig. 1.

shown in Fig. 2 (left panel). Oral administration of either ISDN (2 mg/kg) or 5-ISMN (4 mg/kg) caused a decrease in pulse pressure, reaching its maximal reduction of 29% or 30%, respectively, 3 hr after administration. There was a striking resemblance in the pattern of decrease in pulse pressure between both agents. When ISDN was administered, both ISDN and 5-ISMN appeared in the plasma. The plasma concentration of ISDN became maximal within 1 hr after the ISDN administration, then decreasing to almost zero 4 hr after administration, while the plasma concentration of 5-ISMN reached maximum 3 hr after administration and then decreased. The plasma concentration of 5-ISMN was still high and the pulse pressure was still decreased, when the plasma concentration of ISDN became almost zero. There was not a good correlation in the time course between the decrease in pulse pressure and the increase in plasma concentration of ISDN after oral administration. There was, however, a good correlation between the decrease in pulse pressure and the increase in plasma concentration of 5-ISMN. When
5-ISMN was administered, there was also a good correlation between the decrease in pulse pressure and the increase in plasma concentration of 5-ISMN.

1-2-2. High dose group: The plasma concentrations of ISDN and 5-ISMN and pulse pressure after administration of either ISDN (4 mg/kg) or 5-ISMN (8 mg/kg) are shown in Fig. 2 (right panel). Either ISDN (4 mg/kg) or 5-ISMN (8 mg/kg) decreased pulse pressure significantly, reaching its maximal reduction of 40% or 43%, respectively, 2 hr after administration. Thereafter, the decrease in pulse pressure gradually declined to 16% 10 hr after dosing of ISDN. After oral administration of ISDN (4 mg/kg), both ISDN and 5-ISMN were detected in the plasma. The plasma concentration of ISDN increased and reached maximum within 1 hr after administration and then declined rapidly, being undetectable in the plasma 6 hr after administration. However, significant decrease (26%) in pulse pressure was still observed 6 hr after the administration, when the plasma 5-ISMN was still high (620 ng/ml). Oral administration of 5-ISMN (8 mg/kg) increased the plasma levels of 5-ISMN maximally (4099 ng/ml) after 2 hr of dosing, the 5-ISMN concentration declining gradually to 578 ng/ml 8 hr after administration. There was a good correlation between the decrease in pulse pressure and the increase in plasma level of 5-ISMN in both cases of ISDN and 5-ISMN administration. Thus the results were essentially the same as those in the low dose group, although the responses were greater in the high dose group.

1-2-3. ISDN vs. 5-ISMN administration: The relationship between the plasma concentration of 5-ISMN and percent decrease in pulse pressure in dogs receiving ISDN (4 mg/kg) (Fig. 3, right panel) or 5-ISMN (4 mg/kg) (Fig. 3, left panel) is shown in Fig. 3. There was a good correlation between them after administration of ISDN (r=0.935, P<0.05) or 5-ISMN (r=0.893, P<0.05).

2. Noncross-over experiments
The hemodynamic effects of ISDN, 5-ISMN, prazosin and nifedipine were comparatively studied in this series of experiments. The systolic and diastolic blood pressure, pulse pressure and heart rate before administration of drugs were 155±3 and 77±4 mmHg, 77±3 mmHg and 116±5 beats/min, respectively. Figure 4 illustrates the maximal percent changes of blood pressure and heart rate after administration of agents. Because ISDN and 5-ISMN caused a significant decrease in systolic blood pressure without a remarkable change in diastolic blood pressure, the pulse pressure decreased markedly. On the other hand, nifedipine markedly decreased both diastolic and systolic blood pressure, the decrease in the former being greater than that in the latter. Therefore, the pulse pressure increased. Prazosin produced a significant

![Fig. 3](image-url). Linear regression analysis of the relation between plasma concentration of 5-ISMN and percent decrease of pulse pressure after oral administration of 5-ISMN (4 mg/kg) or ISDN (4 mg/kg). These results were obtained from the experiments shown in Fig. 2. Each point represents a mean±S.E.
Fig. 4. Comparison of hemodynamic responses to ISDN, 5-ISMN, nifedipine and prazosin. The hemodynamic responses include systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and heart rate (HR). * Statistically significant compared with the value obtained at 0 min (P<0.05).

Table 1. Comparison of AUC of 5-ISMN after administration of 5-ISMN or ISDN

<table>
<thead>
<tr>
<th>Drugs and doses (mg/kg, p.o.)</th>
<th>AUC_{0-10 hr} of 5-ISMN (ng·hr/ml)</th>
<th>Fraction of biotransformation to 5-ISMN (%) ( a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ISMN 4</td>
<td>10202±881</td>
<td>100</td>
</tr>
<tr>
<td>ISDN 2</td>
<td>3042±327</td>
<td>59.6 (73.6)</td>
</tr>
<tr>
<td>ISDN 4</td>
<td>6331±834</td>
<td>62.1 (76.6)</td>
</tr>
</tbody>
</table>

AUC: Area under the plasma concentration curve. \( a \): The AUC/mg/kg of 5-ISMN when 5-ISMN (4 mg/kg) was administered was defined as 100%. In the case of ISDN administered, fraction of biotransformation to 5-ISMN was calculated from AUC/mg/kg to that of administered 5-ISMN (4 mg/kg). ( ) : In terms of moles instead of mg. Each value represents the mean±S.E. of six dogs.

decrease in systolic blood pressure and a slight decrease in diastolic blood pressure, resulting in a marked decrease in pulse pressure. Heart rate increased after administration of each of the agents, and the increase in heart rate induced by nifedipine was most prominent. Thus the pattern of hemodynamic response to 5-ISMN was similar with that to ISDN, but it was considerably different from the pattern of hemodynamic response to nifedipine and also slightly different from that to prazosin.

3. Fraction of biotransformation to 5-ISMN

From the data obtained by the cross-over experiments, the fraction of biotransformation to 5-ISMN when ISDN was administered, was determined as shown in Table 1. It became clear from this table that the fraction of biotransformation to 5-ISMN when ISDN was administered was 73.6 to 76.6% (based on moles).

Discussion

There is a view that the long lasting effect of ISDN could be due to the effect of 5-ISMN, being a major metabolite of ISDN (18). The present study was designed to examine this view and determine the extent to which
the effect of ISDN depends on the effect of 5-ISMN.

First, the pharmacological effects of ISDN and 5-ISMN were studied. Figure 1 clearly shows that ISDN decreases blood pressure dose-dependently, and this effect of ISDN is similar with that of 5-ISMN. It is of interest to note that the effect of ISDN (2 mg/kg) is very similar with that of 5-ISMN (4 mg/kg), and the effect of ISDN (4 mg/kg) is also very similar with that of 5-ISMN (8 mg/kg), indicating that the effect of 5-ISMN is about 1/2 (or 41% based on moles) of the effect of ISDN.

Next, the relationship between the effect of ISDN and the plasma concentrations of ISDN and its metabolite, 5-ISMN, was studied. As an indicator of the effect of ISDN, the pulse pressure was chosen, because the pulse pressure has been shown to be a sensitive indicator of the hemodynamic response to ISDN (15, 19). In fact, the pulse pressure decreased markedly in response to either ISDN or 5-ISMN (Fig. 4). In addition, the pattern of the effect of ISDN or 5-ISMN on blood pressure was different from that of nifedipine and also different from that of prazosin (Fig. 4). Therefore, the pulse pressure can be used for evaluation of the effect of ISDN and 5-ISMN. Figure 2 illustrates the relationship between the plasma concentrations of ISDN and 5-ISMN and the pulse pressure after administration of either ISDN or 5-ISMN.

One of the most characteristic findings of the results shown in Fig. 2 is that the time course-pattern of the effect of ISDN did not parallel that of the plasma concentration of ISDN, but paralleled that of its metabolite, 5-ISMN. The administration of ISDN (2 mg/kg) produced a decrease in pulse pressure, and this effect still remained even when the plasma concentration of ISDN became zero (4 hr after administration), although the plasma concentration of 5-ISMN was as high as 467 ng/ml. As was described earlier, the administration of 5-ISMN (4 mg/kg) also produced a decrease in pulse pressure, which was similar in degree with that produced by ISDN (2 mg/kg). When the percent ratio of plasma concentration of 5-ISMN at a given time after administration of ISDN to that after administration of 5-ISMN is defined as the 5-ISMN ratio, the values of the 5-ISMN ratio at 1, 2, 3, 4 and 6 hr after administration of drugs are 21%, 19%, 24%, 33% and 44%, respectively, in the low dose group. In the high dose group, the values of the 5-ISMN ratio at 1, 2, 3, 4 and 6 hr after administration of drugs are 18%, 23%, 32%, 41% and 52%, respectively. These results suggest that 5-ISMN contributes to the effect of ISDN by 20–50% depending on the elapsed time after administration of ISDN. Other metabolites of ISDN, such as 2-ISMN, and ISDN itself should also contribute to the effect of ISDN given orally, although the extent to which they contribute to the effect of ISDN has not yet been determined. However, 2-ISMN is metabolized at a faster rate than 5-ISMN; this can be ascribed to the rapid denitration of its exposed 2-exo position rather than that of the sterically shielded 5-endo position (6–8). These facts indicate that the relatively prolonged action of ISDN is largely ascribable to 5-ISMN rather than 2-ISMN.

There is another way to calculate the extent to which 5-ISMN contributes to the effect of ISDN. According to the pharmacokinetic data, the AUC/mg/kg of 5-ISMN from 0 to 10 hr after the administration of ISDN (2 and 4 mg/kg) was 73.6 to 76.6% compared with that after the administration of 5-ISMN (4 mg/kg) (Table 1), indicating that 73.6 to 76.6% of ISDN (based on moles) is transformed to 5-ISMN in the body. This finding was in accord with data from the literature (20, 21). In addition, the effect of 2 mg/kg of ISDN was nearly equivalent to that of 4 mg/kg of 5-ISMN, suggesting that the effect of 5-ISMN is about 41% of that of ISDN based on moles. Therefore, the contribution of 5-ISMN to the effect of ISDN is 30 to 31% in total. This value accords well with the value of the 5-ISMN ratio obtained 3 to 4 hr after administration of ISDN.

From the foregoing results, it is concluded that ISDN is biotransformed to 5-ISMN, which is active in decreasing pulse pressure, and that the degree of contribution of 5-ISMN to the effect of ISDN given orally is about 30% at the time of 3 to 4 hr after administration of ISDN, when the effect of
orally given ISDN becomes nearly maximal.

References