[Chem. Pharm. Bull. 34(7)2967—2972(1986)]

Comparative Studies of the Influence of Dietary Fat on Plasma Levels of Isosorbide Dinitrate from Two Sustained-Release Products

HITOSHI TADA,* YOSHINORI SAGAE, TOSHIO SUZUKI, and KATSUO UNNO

Department of Pharmacy, Akita University Hospital, Hondo 1-1-1, Akita 010, Japan

(Received December 12, 1985)

The influence of dietary fat intake on the plasma levels and bioavailability of isosorbide dinitrate (ISDN) from two sustained-release formulations was examined in ten healthy volunteers by mean of an open crossover trial. Each subject orally received one mixed granule type product (formulation A) and one diffused-sustained-release type product (formulation B). The plasma level of ISDN rapidly increased to the maximum level within 3—5 h after administration of each product in subjects on a fatty diet. The mean enhancements were 84.5% for formulation A and 61.2% for formulation B compared with the levels in subjects on fat-free diet. The area under the curve (AUC_{0-10}) values of the former in subjects fed dietary fat were significantly greater than the preprandial figures, but no significant difference was observed with the latter product.

Keywords——isosorbide dinitrate; sustained-release product; dietary fat; pharmacokinetics; plasma level; food influence; bioavailability; human

Sustained-release isosorbide dinitrate products are clinically in common use for the prevention and treatment of angina pectoris. In Japan, two kinds of products which differ in the drug form are available on the market. According to the product information,[1,2] taking the product twice a day is recommended, and clinically it is often taken after breakfast and dinner. Several papers dealing with the *in vivo* behavior of these two products after postprandial administration have been published,[3,4] but little work has been done on the influence of food.[5] It is well known that the postprandial administration of a drug is apt to delay the absorption and affect the bioavailability, depending on the nature of the drug.[6,7] Further, among the components of food, fat is known to deley the gastric emptying time and to accelerate the excretion of bile and the absorption of drugs that are practically insoluble in water.[6]

Using sustained-release isosorbide dinitrate products, we studied the influence of fatty diet and evaluated the products on the basis of the results obtained.

Experimental

Materials——Frandol® (formulation A) and Nitrol-R® (formulation B) which each contain 20 mg of isosorbide dinitrate (ISDN) were used as sustained-release products. Formulation A (Lot. No. BY77) is a tablet consisting of soluble granules and slow-releasing granules coated with wax. On the other hand, Formulation B (Lot. No. 25041) is a capsule containing granules with a starch inner core coated with ISDN, and the outside is further coated with shellac ethyl cellulose.

Isosorbide dinitrate, isosorbide 2-mononitrate (2-ISMN), isosorbide 5-mononitrate (5-ISMN) and isomannaid dinitrate were obtained from Eisai Co., Ltd. All other chemicals used in this experiment were commercial products.

Subjects——Ten male subjects volunteered for this study, their age and weight ranges being 22—54 years and 54—76 kg, respectively. They were judged to be in good health on the basis of biochemical and hematological screenings.

Dissolution Test——A DT600 (Freund-Jasco) apparatus was used according to Method I (rotating basket method) of the Dissolution Test described in JP X. Thus, a tablet or capsule in a basket was placed in the vessel of the apparatus filled with 900 ml of the first fluid of the Disintegration Test in JP X and the basket was rotated at 100 rpm. The temperature was maintained at 37 ± 0.5 °C during the experiment. After 2 h, the fluid was replaced with JP X 2nd fluid without surfactant or with 2nd fluid containing 1% (w/v) sodium salt of cholic acid. In order to analyze ISDN, 10 ml aliquots dissolution medium was taken each hour for 8 h, and replaced with an equal volume of fresh medium.

The sample was passed through a 0.45 μm millipore filter and the filtrate (1 ml) was extracted with *n*-hexane (5 ml). The organic layer (3 ml) was transferred to a test tube and evaporated to dryness under a nitrogen stream. The residue was dissolved in the mobile phase (100 μl) containing anisole (1 μg) as an internal standard and this solution (20 μl) was injected into a high-performance liquid chromatograph (HPLC) with an ultraviolet (UV) detector (225 nm, 0.04 AUFS). The column consisted of stainless steel tubing (4.6 mm i.d. × 250 mm) packed with Hitachi Gel #3053. Methanol–water (6 : 4, v/v) was used as the mobile phase at a flow rate of 0.8 ml/min, and all operations were carried out at room temperature.

The retention times of ISDN and the internal standard were about 5 min and 6 min, respectively. The ISDN dissolved was calculated from the calibration curve (1—25 μg/ml), and expressed as percentage dissolution based on the labelled drug content as 100%.

Administration Study——Following an overnight fast, formulation A or B was orally given 30 min after breakfast according to a crossover schedule with a one-week interval between dosing. The "fat-free" breakfast was two slices of bread and drinking water (100 ml). This contained protein (8.4 g), carbohydrate (48 g) and fat (3.8 g) and the total energy was 260 kcal. Margarine was additionally taken in the fatty diet, which contained protein (8.4 g), carbohydrate (48 g) and fat (35.8 g); the total energy was 550 kcal. The subjects were fasted for a further 3 h after administration. Blood samples were drawn at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 h after dosing by venipuncture from the antecubital veins into heparinized tubes. Plasma separated by centrifugation was immediately frozen at −30 °C and stored at that temperature until the analysis of ISDN and its metabolites.

Analysis of Plasma Level of ISDN and Concentrations of Its Metabolites——A plasma sample (1 ml) in a centrifuge tube was supplemented with isomannide dinitrate as an internal standard, and the aqueous layer was extracted twice with *n*-hexane (4 ml). The organic layer was transferred to a test tube and evaporated to dryness under a nitrogen stream. The residue was dissolved in ethyl acetate (100 μl) and the solution (5 μl) was injected into a gas chromatograph with ECD (^{63}Ni 10 mCi), using a glass column (2 m × 2 mm i.d.) packed with 3% OV-1 on 100—120 mesh Gas Chrom Q. The carrier gas (N_2) flow rate was 10 ml/min and the temperatures of the injection port and column were 155 and 180 °C, respectively. The retention times of ISDN and internal standard were about 5 and 6 min, respectively. The calibration curve was linear from 0.5 to 4 ng/ml. The lower limit for analysis of ISDN was 0.5 ng/ml in plasma (1 ml), and the extraction efficiency for ISDN under the above conditions was 92.7 ± 3.7% (mean ± S.E., $n = 10$).

Isomannide dinitrate (15 ng) was added to the residual aqueous layer and it was extracted 3 times with ethyl ether (4 ml). The organic layer was evaporated to dryness and the residue was dissolved in ethyl acetate (100 μl). This solution (5 μl) was also injected into the gas chromatograph according to the method described above. In this measurement, a glass column (2 m × 2 mm i.d.) of 3% OV-3 on 100—120 mesh Gas Chrom Q was used. The retention times of 2-ISMN, 5-ISMN and isomannide dinitrate (internal standard) were about 5, 8 and 15 min, respectively. The extraction efficiencies for 2-ISMN and 5-ISMN were 96.7 ± 2.7% and 93.0 ± 3.0% (mean ± S.E., $n = 10$), respectively.

Calculation of Parameters and Statistical Analysis——The dissolution and the plasma drug concentration data were analyzed according to the model-independent moment analysis method.[8] The area under the plasma concentration *versus* time curve (*AUC*) was estimated by the trapezoidal rule.

Statistical significance was evaluated by the paired *t*-test.

Results

1. Dissolution Test

Figure 1 illustrates the time courses of ISDN release from the products. Without bile salt in the medium, there was little difference in drug release rate between formulation A and formulation B up to 2 h after initiation of the test, but thereafter formulation A gave lower values. After 8 h, the release was almost 100% in formulation B but not more than 55.6% in formulation A.

When bile salt was added to the second solution, the release rates for the two products tended to increase. Table I shows the results of moment analysis of the release value for these products.

As regards *VDT*, which is an effective parameter for comparing the time taken for a drug

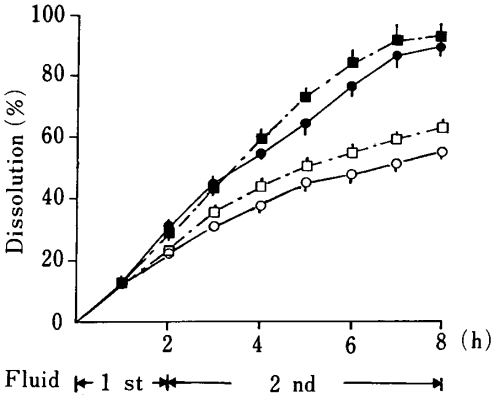

Fig. 1. Dissolution Profiles of Isosorbide Dinitrate from Two Sustained-Release Products Determined by the JP X Rotating Basket Method at 100 rpm (37 °C)

The fluid with bile salt contains 1% (w/v) sodium salt of cholic acid as the second fluid. Each point represents the mean ± S.E. of five experiments. (——), bile salt-free fluid; (- - - -), fluid with bile salt; (○□), formulation A; (●■), formulation B.

TABLE I. Mean Dissolution Time (*MDT*) and Variance of Dissolution Time (*VDT*) Estimated from Dissolution Values for Sustained-Release Products in Test Fluid[a]

Parameter	Without bile salt		With bile salt[b]	
	Formulation A	Formulation B	Formulation A	Formulation B
MDT_{0-8} (h)	3.04 ± 0.09	3.35 ± 0.05	3.10 ± 0.15	3.25 ± 0.06
VDT_{0-8} (h^2)	5.08 ± 0.25	4.68 ± 0.06	4.88 ± 0.48	3.75 ± 0.34[c]

a) First fluid of the Disintegration Test in JP X was used as a test fluid, and was replaced with the second fluid of the Test after 2 h. Each value represents the mean ± S.E. of five experiments. *b*) The second fluid contains 1% (w/v) sodium salt of cholic acid. *c*) $p < 0.05$, statistically significant difference from the same product in the fluid without bile salt.

to be released from a product, formulation A showed larger values than formulation B irrespective of the presence or absence of bile salt.

These results show formulation A to be superior to formulation B as far as sustained release *in vitro* is concerned.

2. Plasma Level of ISDN

Figure 2 illustrates changes in the plasma ISDN levels following oral administration of formulation A or B after taking fat-free diet or fatty diet.

When fat was not taken, the plasma ISDN level of the former was slightly higher than that of the latter up to 4 h after administration. Thereafter, however, the ISDN level of the former tended to decrease, and after 10 h, it was no longer detectable. In the case of formulation B, ISDN was absorbed slowly, reaching a peak level after 6 h and decreasing slowly thereafter (Fig. 2(A)).

When fat was taken, ISDN in formulation A was absorbed slowly until 3 h after administration, but a sharp rise in the plasma level was observed thereafter. In the case of formulation B, changes in the plasma level were similar to those with formulation A, but the peak level was lower than that of formulation A by 1.9 ng/ml, and the disappearance of ISDN was relatively slow (Fig. 2(B)).

3. Bioavailability in Human Subjects

Table II presents T_{max}, C_{max} and the results of the moment analysis based on the measured plasma ISDN levels.

Regarding C_{max}, there was no difference between formulation A and formulation B when fat was not taken. Intake of fat resulted in a significant increase in both cases, though C_{max} tended to be higher with formulation A. There was a significant difference in AUC_{0-10} in

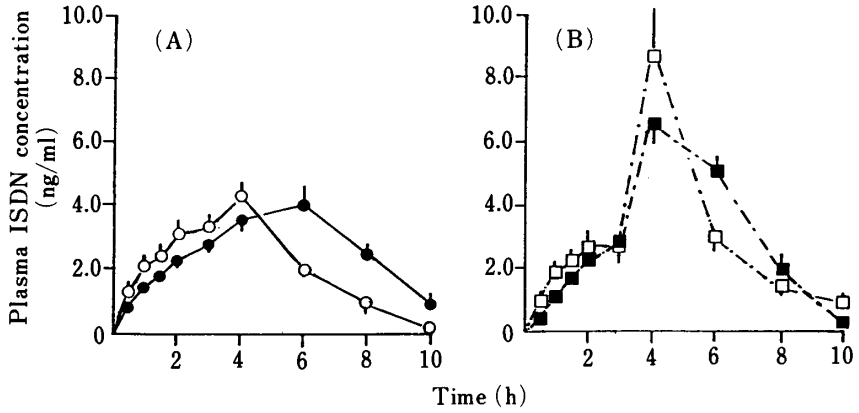

Fig. 2. Mean(±S.E.) Plasma Concentrations of Isosorbide Dinitrate in Ten Human Subjects Following Oral Administration of the Sustained-Release Products

(A), fat-free diet; (B), fatty diet.
(○□), formulation A; (●■), formulation B.

TABLE II. T_{max}, C_{max}, AUC, Mean Residence Time (MRT) and Variance of Residence Time (VRT) Estimated from Plasma Isosorbide Dinitrate Levels after Administration of Sustained-Release Products in Human Subjects[a)]

Parameter	Fat-free diet		Fatty diet	
	Formulation A	Formulation B	Formulation A	Formulation B
T_{max} (h)	3.60±0.22	5.40±0.50	3.80±0.20	4.60±0.31[d)]
C_{max} (ng/ml)	4.72±0.42	4.25±0.51	8.71±1.59[b)]	6.85±0.81[b)]
AUC_{0-10} (ng·h/ml)	20.68±1.96	26.22±2.87[d)]	30.28±3.98[b)]	30.51±2.68
MRT_{0-10} (h)	3.96±0.41	5.05±0.12[e)]	4.36±0.19[b)]	4.89±0.16
VRT_{0-10} (h^2)	3.71±0.25	5.34±0.23[e)]	4.11±0.44	3.61±0.26[c)]

a) Each value represents the mean±S.E. of ten subjects. *b*) $p<0.05$, *c*) $p<0.01$, statistically significant difference from the same product in fat-free diet. *d*) $p<0.05$, *e*) $p<0.01$, statistically significant difference from formulation A under the same conditions.

subjects given fat-free diet between the two products. Fatty diet increased AUC_{0-10} by 46.2% for formulation A and 16.4% for formulation B, on average, showing that the latter is less affected by the fatty diet. VRT_{0-10} and MRT_{0-10} were larger with formulation B in subjects given fat-free diet, suggesting that there is a difference in the rate of bioavailability between the two products, and that formulation B gives a sustained-release effect. This is in contrast to the observations in subjects given fatty diet, where little difference was observed between the two products in terms of AUC_{0-10}, MRT_{0-10} and VRT_{0-10}.

4. Plasma Levels of ISDN Metabolites

It has been reported that 2-ISMN and 5-ISMN, the main metabolites of ISDN, have weak pharmacological activity.[9,10)] We examined the plasma levels of these metabolites after administration of the products. Figure 3 illustrates the changes in the plasma levels of 2-ISMN and 5-ISMN.

In subjects given fat-free diet, the plasma levels of both metabolites reflected the changes in the plasma ISDN levels with both products. When the fatty diet was coadministered with formulation A, the 5-ISMN level tended to decrease and AUC_{0-10} decreased by about 29% as compared with fat-free diet (Table III). However, the level with formulation B was not changed by addition of fat to the diet.

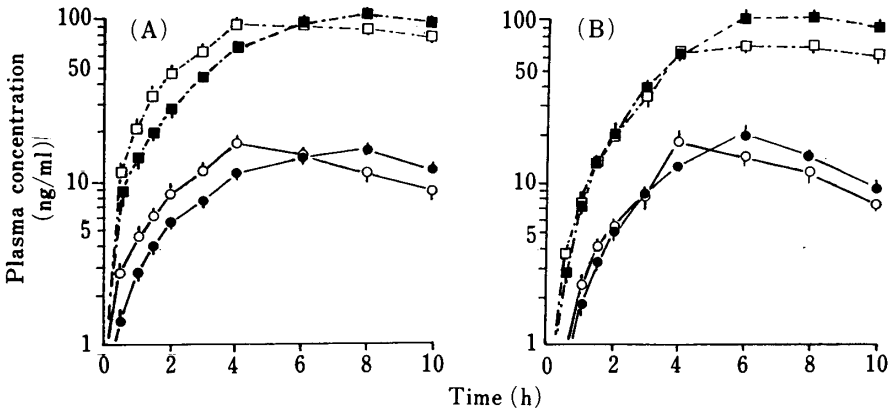

Fig. 3. Mean(±S.E.) Plasma Concentrations of Metabolites of Isosorbide Dinitrate in Ten Human Subjects Following Oral Administration of the Sustained-Release Products

(A), fat-free diet; (B), fatty diet.
(——), 2-ISMN; (- - - -), 5-ISMN; (○□), formulation A; (●■), formulation B.

TABLE III. AUC_{0-10} (ng·h/ml) of Mononitrates after Sustained-Release Products of Isosorbide Dinitrate in Human Subjects[a]

Metabolite	Fat-free diet		Fatty diet	
	Formulation A	Formulation B	Formulation A	Formulation B
2-ISMN[b]	110±11	105±12	102±13	115±10
5-ISMN[b]	708±42	705±45	504±24[c]	682±55[d]

a) Each value represents the mean±S.E. of ten subjects. *b*) 2-ISMN, isosorbide 2-mononitrate; 5-ISMN, isosorbide 5-mononitrate. *c*) $p<0.01$, statistically significant difference from the same product in fat-free diet. *d*) $p<0.05$, statistically significant difference from formulation A under the same conditions.

Discussion

The results of drug release tests *in vitro* confirmed that the rates of ISDN release from the two products were almost constant. The ISDN release rate of formulation A was lower than that of formulation B (Fig. 1). Such a sustained-release characteristic was expected to result in prolonged ISDN release *in vivo*. However, when fat-free diet was taken, the plasma ISDN level of formulation A fell off rapidly at 4 h after administration and a satisfactory duration of ISDN release was not attained. This low release rate may result in a decrease of extent of bioavailability. On the other hand, the plasma ISDN level in the case of formulation B reached a peak at 6 h after administration and ISDN tended to disappear slowly thereafter. In formulation B, therefore, the *in vitro* results seem to be reflected in the *in vivo* results to some extent.

Several studies on T_{max}, C_{max} and AUC in the postprandial state[3,4] have indicated that there was no significant difference between the two products. Our result, when a fatty diet was taken, was similar except for a marked elevation of C_{max} due to fat intake. On the other hand, a previous comparative test in the fasting state[11] found no difference in the behavior of the plasma level of ISDN or in AUC between the two products. In the case of a conventional dosage form, T_{max} and AUC were not significantly changed, but C_{max} was reduced by food ingestion.[12] However, this alteration was not reflected in the results[5] with the sustained-release product. Plasma level of ISDN appears to depend on the release rate from a sustained-release product, though food is likely to modify the rate of ISDN absorption.

Since the influence of fat was particularly great on formulation A, which is required to disintegrate, ingestion of fat appears to enhance the disintegration process of the tablets. When a large amount of fat was taken in this study, excretion of bile increased; with formulation A (in which ISDN granules are enveloped with wax), it is possible that the wax is emulsified or suspended by the surface-active action of bile acids, resulting in loss of the linkage of ISDN granules. Because such loss of linkage causes the sustained-release characteristic to be almost wholly lost in the case of Theona-P®,[13] it is surmised that the elevated C_{max} in formulation A can be attributed to the loss of linkage as an indirect result of fat ingestion. In the case of formulation B, ISDN is released by a diffused-sustained-release type of mechanism as mentioned earlier; a shellac ethyl cellulose membrane or layer controls the entry of digestive fluid and the sustained-release characteristic may be maintained to some extent even though a large amount of bile is present in the digestive fluid.

We studied the effect of bile salt in the ISDN release test and found that the release rate for both products tended to increase in the presence of bile salt, but the effect was not significant (Fig. 2). The reduction in the influence of bile salt is probably due to physiological factors, such as the movement of the digestive tract, that are not simulated in the release test.

Mizuno *et al.*[14] have reported that ISDN release from the two products is not affected by pH but is dependent on time. In our study, no marked delay in T_{max} or MRT due to the fatty diet was observed (Fig. 2 and Table II). These results also support the view that the two products are not much affected by changes of the gastric emptying time.

The peak plasma levels of 2-ISMN and 5-ISMN of these two products in subjects given fat-free diet were about 4 times and 22—26 times that of ISDN. This suggests that the duration of pharmacological action may be increased by these metabolites, considering that the biological half-life of 5-ISMN is longer than that of ISDN.[15]

The decrease in peak level of 5-ISMN from formulation A with fatty diet was small at only 27%; this may have little influence on the therapeutic effect. However, the sudden rise in ISDN level could possibly produce side-effects such as headache and/or dizziness which are often noticed after administration of plain tablets.

We conclude that formulation B is a sustained-release product that is not readily affected by fatty diet.

Acknowledgement We wish to thank Toa Eiyo Co., Ltd. and Eisai Co., Ltd. for the generous supply of ISDN products, and Mr. H. Komatsu and Miss A. Murakami for technical assistance.

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