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KINETICS OF ISOSORBIDE DINITRATE AND RELATIONSHIPS TO PHARMACOLOGICAL EFFECTS

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1 The inter-relationships among oral isosorbide dinitrate (ISDN) dose, drug pharmacokinetics and pharmacological effects were studied in 12 angina patients following single and chronic doses of 15, 30, 60 and 120 mg.

2 Significant accumulation of intact ISDN in plasma was observed after four times a day dosing at 30, 60 and 120 mg for 1 week.

3 The area under the plasma concentration *v* time curve (AUC), from 0–6 h, was shown to be proportional to dose following single doses. In contrast, AUC increased disproportionately to dose after chronic dosing.

4 Pharmacokinetic correction provided modest improvements in the dose-response relationships of ISDN.

5 Adverse hypotensive effects were observed in five patients after the single 60 mg dose. These patients showed statistically higher AUC and lower intrinsic clearance of ISDN at doses of 15, 30 and 60 mg compared to those who did not develop adverse effects. A possible relationship exists, therefore, between lower drug clearance and hypersensitivity to ISDN.

Introduction

Isosorbide dinitrate (ISDN) is widely used in patients with angina pectoris due to atherosclerotic heart disease and in patients with heart failure due to various forms of left ventricular dysfunction. Although early studies (Aronow & Chesluk, 1970; Aronow & Kaplan, 1969; Goldbarg, Moran, Butterfield, Necickas & Bermudez, 1969; Goldstein, Rosing, Redwood, Beiser & Epstein, 1971) cast doubts on the efficacy of oral ISDN *per se* and its purported longer duration of action compared to nitroglycerin, several detailed clinical studies (Danahy, Burwell, Aronow & Prakash, 1977; Kaltenbach, Tiedemann & Schellhorn, 1973; Kattus, Alvaro, Zohman & Goulson, 1979; Markis, Gorlin, Mills, Williams, Schweitzer and Ransil, 1979; Thadani, Darke, Fung & Parker, 1980a; Thadani, Fung, Darke & Parker, 1980b; Thadani, Manyari, Parker & Fung, 1980c; Winsor, Kaye & Mills, 1972) subsequently showed that ISDN, particularly in higher doses, did provide sustained hemodynamic and antianginal effects.

Very little is known about the pharmacokinetics of ISDN in patients. The relationships between ISDN dose, duration of drug administration and plasma drug concentrations have not been defined. A major goal of this study was to examine these relationships in a group of patients with angina pectoris. A sensitive and reproducible assay procedure for the determination of ISDN in plasma has been developed for this purpose. Details of this procedure, as well as data on the *in vitro* stability and protein binding of ISDN in human plasma, are also described in this report.

The effects of ISDN dose and duration of administration on the systolic blood pressure, heart rate, ST-segment depression and treadmill walking time in this group of patients have been presented elsewhere (Thadani *et al.*, 1980a, b, c). There was no general

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pattern of correlation between instantaneous plasma ISDN concentration and the pharmacological responses. Although peak response and maximum plasma drug concentration usually occurred at the same time after acute doses, the rate of decline of plasma ISDN concentrations was more rapid than those exhibited by the pharmacological effects. While increases in the acute ISDN dose usually brought about an enhancement in circulatory effects, no quantitative dose-response relationship could be demonstrated (Thadani *et al.*, 1980a, b). This may, in part, be due to individual differences in ISDN pharmacokinetics. It was thought that a better correlation might be obtained by using a parameter such as the area under the plasma concentration *v* time curve (AUC) instead of dose since the former represents the dose corrected for individual differences in systemic drug availability and clearance. We have therefore determined the relationship between AUC and pharmacological response after acute ISDN dosing. The relationship between apparent intrinsic ISDN clearance and sensitivity to adverse effects from ISDN was also explored in this study.

Methods

Twelve male adult, ambulatory patients (Table 1), with chronic, stable angina pectoris due to atherosclerotic heart disease participated in the study. Exclusion criteria included myocardial infarction within the preceding three months, unstable angina, congestive heart failure or other forms of heart disease. Medication other than sublingual nitroglycerin to relieve anginal pain was not used during the course of the study. Patients receiving digitalis and diuretic therapy were not admitted to the study nor were patients with significant renal, hepatic or small bowel disease.

Acute doses

Patients received either ISDN or placebo according to the schedule shown in Table 2. Each dose was administered in the morning. The dose of ISDN was increased stepwise; a randomized dosing order was not used so as to protect patients from abrupt exposure and possible adverse reaction to high ISDN doses. Patients were assigned randomly to one of three groups according to the day on which they would receive the initial placebo tablet. On the last study day all patients received an additional placebo dose. The ISDN dose was not increased if side effects (e.g. headache, hypotension) were too severe at one of the submaximal doses.

The heart rate and blood pressure of each patient were recorded before and at 1, 2, 4 and 6 h after administration of the ISDN dose or a placebo. Modified lead V5 was recorded at 1 min intervals for 2 min in the supine and standing positions. The average values for the heart rate during ten consecutive beats were measured from the electrocardiogram and the blood pressure was measured by sphygmomanometry. Exercise tolerance was assessed (Thadani *et al.*, 1980b) via treadmill walking time until the onset of angina (P_1) and the development of pain severe enough to necessitate stopping (P_2). Blood samples were collected in evacuated tubes containing EDTA at 0, 0.5, 1, 2, 4 and 6 h after the ISDN or placebo dose, centrifuged and the separated plasma was stored at −20°C until assayed for ISDN as described below. During each study period, the patients were allowed normal activity without exertion.

Table 1 Summary of patient characteristics

Number	*Sex*	*Age (years)*	*Total body surface area* (m^2)*	*Approximate duration of angina (years)*	*Previous myocardial infarction*	*Smoking status*
1	M	57	2.08	3	—	—
2	M	70	1.95	0.7	—	—
3	M	56	1.84	1	uncertain	+
4	M	64	1.85	11	—	—
5	M	51	1.99	20	—	+
6	M	47	1.87	0.7	—	+
7	M	58	1.85	0.8	+	—
8	M	65	1.91	15	+	—
9	M	57	1.92	0.5	+	+ (pipe)
10	M	59	1.86	3	—	—
11	M	60	1.91	4	—	—
12	M	42	2.34	6	—	—

*According to nomogram in Documenta Geigy, Scientific Tables, 7th ed.

Table 2 Summary of dosing regimens

	Day	Group 1*	2*	3*
Acute doses:	1	Placebo	15 mg	15 mg
	4	15 mg	30 mg	30 mg
	7	30 mg	Placebo	60 mg
	10	60 mg	60 mg	120 mg+
	13	120 mg+	120 mg+	Placebo
	16	Placebo	Placebo	Placebo
Chronic doses:	17–23	Placebo	Placebo	Placebo
(four times a day)	24–30	Placebo	Placebo	Placebo
	31–37	15 mg	15 mg	15 mg
	38–44	30 mg	30 mg	30 mg
	45–51	60 mg	60 mg	60 mg
	52–58	120 mg	120 mg	120 mg

*Group 1: patients numbers 2, 8 and 9; Group 2: patients numbers 3, 5, 6 and 10; Group 3: patients numbers 1, 4, 7, 11 and 12. The uneven distribution was caused by drop-out of a patient in Group 1.
+Omitted if patient is found to be sensitive to the 60 mg dose.

Chronic doses

After completion of the acute studies, the patients were treated with placebo four times daily for 2 weeks and the haemodynamic and exercise data were again recorded. The patients were then placed on ISDN, 15 mg four times daily for several days. The dose of ISDN was then doubled at weekly intervals to a maximum of 120 mg four times daily (Table 2). Blood samples, haemodynamic and exercise data were collected after the last dose at each dosage level.

Extended sampling of plasma ISDN concentrations

In a brief follow-up study, plasma ISDN concentrations were monitored for up to 24 h to examine the terminal elimination phase of ISDN. A single angina patient, male, age 59 years, nonsmoker with angina pectoris who had been receiving ISDN 60 mg four times daily as part of his therapy was enlisted. Plasma concentrations were determined on two occasions, *viz:* (i) for 24 h after a single morning dose of 60 mg which was part of the patient's continuing therapy and (ii) 72 h after the last dose, another dose of 60 mg was given and blood sampling was again carried out for 24 h.

ISDN assay

Analysis of ISDN in plasma was performed using a modification of a reported (Yap, McNiff & Fung, 1978) assay for nitroglycerin. A plasma sample (0.2 ml), to which 10 μl of 10 M silver nitrate solution was added in a microcentrifuge tube, was extracted with an equal volume of pesticide-quality hexanes. Mixing was achieved by rapidly injecting the organic solvent into the plasma with a 0.2-ml Kirk pipette affixed to a 1-ml tuberculin syringe. The upper (organic) layer was then removed with a Pasteur pipette. This extraction step was repeated a total of 20 times and the organic extracts were combined. The multiple extraction procedure, though seemingly cumbersome, was completed within 5–10 min.

A chlorinated pesticide internal standard was added to the combined extracts. Heptachlorepoxide (Supelco, Inc.) was used for determination of ISDN concentrations estimated to be above 10 ng/ml while dichlorodiphenyltrichloroethane (DDT, Supelco, Inc.) was used in samples containing less than 10 ng/ml of ISDN. Under our conditions of assay, the apparent pesticide concentrations inherent in the plasma samples were found to be negligible compared to those added. The combined extracts with added internal standard were evaporated under a stream of pre-purified grade nitrogen to about 20 μl and 1–5 μl was injected into a gas chromatograph equipped with a ^{63}Ni electron capture detector. Glass columns (2 mm i.d. × 1.85 m) were packed with 3% SP-2401 on 100/120 Supelcoport and conditioned overnight at 200°C prior to use. Inlet, column and detector temperatures were set at 200°, 150° and 200°C respectively. Either nitrogen or argon-methane (95/5) was used as a carrier gas and maintained at a flow rate between 50 and 100 ml/min.

Calibration standards of drug and internal standard in hexane were used to prepare a daily calibration curve to correct for slight variations in detector sensitivity. The amount of drug present in a sample was calculated by the peak-height ratio method. Standards were prepared and plasma recovery studies were performed using neat ISDN which was

isolated from its lactose adsorbate (courtesy Stuart Pharmaceuticals, Wilmington, DE, 19897) and standardized (United States Pharmacopeia, 1975). All glasswares were treated with dichlorodimethylsilane including a final rinse with pesticide-quality methanol prior to drying.

Protein binding

Fresh and previously frozen plasma obtained from normal, healthy subjects was spiked with ISDN in the concentration range of 1–100 ng/ml. Using a 1-ml acrylic plastic dialysis cell and regenerated cellulose membrane of 0.001 inch thickness (Union Carbide), each 0.8 ml aliquot of plasma was dialyzed to equilibrium against an equal volume of phosphate buffer (pH 7.4, isotonic) at 37°C for 6 h. Following dialysis, plasma and buffer phase concentrations were determined and the free fraction was calculated. Protein binding was also examined in four patient plasma samples covering the same ISDN concentration range.

Calculations

AUCs were computed using the spline method on a Hewlett-Packard 9825A desk top computer. The apparent intrinsic hepatic clearance (Cl_I), which is equal to the systemic clearance (Cl_s) divided by the fraction of the dose absorbed as intact drug (F), assuming complete absorption and no prehepatic metabolism, was calculated as shown in equation 1:

$$Cl_I = \frac{\text{Oral dose}}{\text{AUC}} \quad (1)$$

The peak pharmacological effect (in treadmill walking time, standing systolic blood pressure, heart rate, etc.) was expressed as % change of control value corrected for placebo effects (equation 2):

$$\text{Peak effect, \%} = \left[\frac{E_t - E_o}{E_o} - \frac{(P_t + P_t') - (P_o + P_o')}{P_o + P_o'} \right] \times 100\% \quad (2)$$

where

E_t = maximum effect observed after ISDN dosing
E_o = effect at time zero
P_t, P_t' = effects observed during the two placebo regimens at the corresponding time when E_t was observed
P_o, P_o' = effects observed at time zero during the two placebo regimens.

Results

ISDN analysis

The retention times for ISDN, heptachlorepoxide and DDT were 7.6, 14.3 and 30 min respectively. The metabolites of ISDN, *viz:* isosorbide-2-mononitrate and isosorbide-5-mononitrate are much more polar than ISDN and they partitioned very poorly into the hexane extraction solvent. When hexane solutions containing high concentrations of these metabolites were directly injected on to the column, they were found to give retention times between those of nitroglycerin (3.4 min) and ISDN, and their peaks showed considerable tailing. In plasma samples spiked with 3 ng/ml ISDN, 800 ng/ml isosorbide-2-mononitrate and 1600 ng/ml isosorbide-5-mononitrate, the peak height ratio of ISDN to internal standard was identical to that obtained in the absence of metabolites. Thus, the method is free from interference from nitroglycerin and ISDN metabolites.

Recovery data of ISDN from spiked plasma are shown in Table 3. Over a three-decade range of drug concentrations (ca. 0.1–100 ng/ml) recovery at each concentration was shown to be essentially complete (92%), with the mean coefficient of variation at 9.4%.

Table 3 Recovery of ISDN from spiked human plasma

Concentration (ng/ml plasma)	*Number of determinations*	*% Recovery (mean ± s.d.)*
0.097*	6	92±7
0.97	7	98±11
1.83	6	82±15
9.7	7	110±6
97	7	79±8

*From 0.5 ml samples of plasma, extracted with equal volume aliquots of hexane.

The assay was found to be relatively stable in day-to-day operations. Over a period of 1.5 months monitored for reproducibility, the calibration curve of peak height ratio *v* amount ratio (drug to internal standard) showed fairly minimal fluctuations in the slope (0.573 ± 0.072, mean ± s.d., $n = 30$). The intercept was small compared to the slope, and its value was not distinguishable from zero (−0.038 ± 0.054, mean ± s.d., $n = 30$). A packed column used daily (10–20 injections) could usually be maintained for 2–4 months before a replacement was needed. The injection septum (Microsep F-174, Supelco, Inc.) was changed daily at the end of the day.

ISDN stability in stored plasma samples

DiCarlo & Melgar (1969) showed that nitroglycerin degradation in rat serum is rapid, with a reported half-life of about 20 min at 37°C. Laufen, Scharpf & Bartsch (1978) suggested that ISDN might undergo similar rapid degradation in plasma. Thus, in their assay, ISDN plasma samples were either processed immediately for further analysis or deep frozen until the analysis was to take place, not later than 8 h after withdrawal of sample. We found that ISDN was considerably more stable than nitroglycerin in human plasma. At 37°C, the half-life of *in vitro* plasma ISDN degradation was about 55 h. Some ISDN samples from patients were re-assayed at intervals up to 16 months of storage at −20°C. The decrease in ISDN concentrations was not significant (mean < 2%).

ISDN plasma protein binding

Equilibrium dialysis experiments suggest that ISDN is not extensively bound to human plasma proteins. Preliminary studies indicated that binding equilibrium was achieved in 6 h. There was no apparent difference in the values of free fraction obtained using plasma from normal healthy individuals (fresh and frozen) and those from patients (frozen plasma). Nor was there an apparent dependency of protein binding on ISDN concentrations in the range of 1–100 ng/ml. In 17 plasma samples tested, the free fraction was found to be 0.72 ± 0.12 (mean ± s.d.).

Plasma concentrations in angina patients

Figure 1 shows the plasma ISDN concentrations after acute (a) and chronic dosing (b) at 15, 30, 60 and 120 mg. Drug absorption appeared to be rapid in all treatments except in the case when a single dose of 60 mg was administered (peak concentration at 2 h as opposed to 0.5–1 h observed in other treatments). This apparent delay in absorption was not evident when the same dose (and the same dosage form), was given under chronic therapy. Plasma samples collected during the placebo periods showed no apparent ISDN concentrations, confirming the specificity of the assay.

The elimination half-life of ISDN in man has been reported (Assinder, Chasseaud & Taylor, 1977) to be about 30 min after sublingual doses of 5 mg. Thus, the sampling period of up to 6 h would be expected to yield terminal slopes of log concentration *v* time plots which are linear, unless drug absorption is very much prolonged or disposition is multiphasic. Inspection of Figure 1 shows that the elimination phases were not monoexponential, with the curvilinear relationship being more evident after chronic dosing. In one patient whose plasma concentrations were monitored up to 24 h on two occasions (Figure 2), the disposition of ISDN showed a clear bi-exponential characteristic, with half-lives of approximately 1.5 h and 4 h for the α and β phases, respectively.

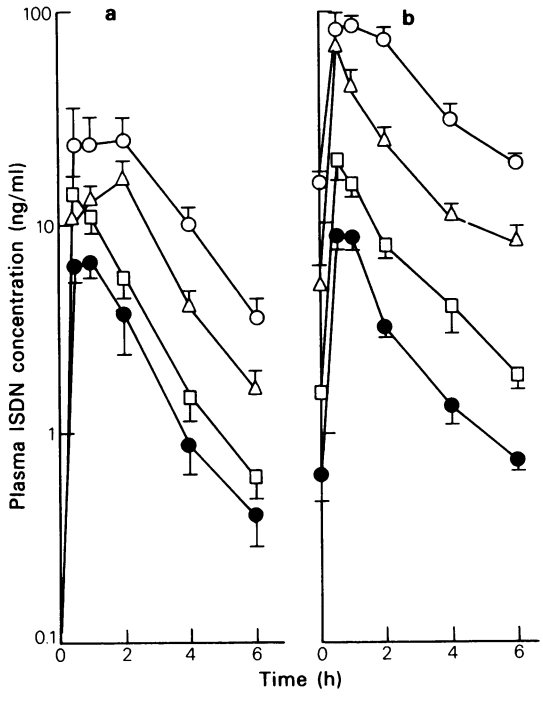

Figure 1 Plasma ISDN concentrations (mean ± s.e.mean) after acute (a) and chronic (b) doses of ISDN in twelve patients. (●) 15 mg; (□) 30 mg; (△) 60 mg; (○) 120 mg.

Plasma ISDN concentrations after chronic dosing were, in general, higher than those obtained after the comparable single doses (Figure 1). Individual values of AUC from 0–6 h, are tabulated in Table 4. Statistical differences in this parameter between acute and chronic dosing, by paired *t*-test, was observed for doses at 30, 60 and 120 mg. A more meaningful comparison would be between $AUC_{0-\infty}$ obtained after single doses and AUCτ obtained after chronic doses, where τ is the dosing interval (in this case, 6 h) during steady state administration.

Because of the unexpected finding of slower ISDN elimination in the present study, the data obtained did not permit rigorous determination of β half-life, and hence the residual AUCs in both the acute and chronic dosing studies. However, if the β half-life of 4 h (Figure 2) was used for calculation, the $AUC_{0-\infty}$ values can be estimated to be 17.9 ± 13.9, 29.8 ± 15.0, 59.8 ± 23.9 and 114 ± 30.7 ng ml^{-1} h at single doses of 15, 30, 60 and 120 mg respectively. These values are only greater than their correspond-

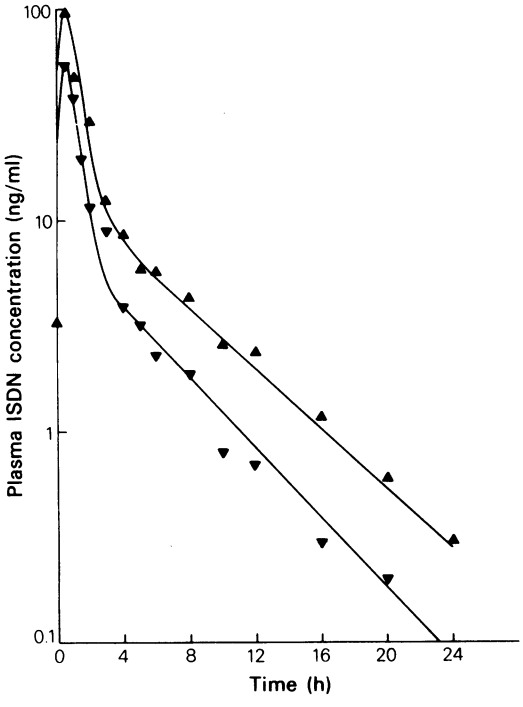

Figure 2 Extended monitoring of plasma ISDN concentrations of one patient at two different occasions. Top curve (▲) was obtained after a 60 mg dose after chronic therapy and bottom curve (▼) was obtained after another 60 mg dose 72 h later.

ing AUC_{0-6} values by about 25%. Statistical difference between the estimated $AUC_{0-\infty}$ after single dose and AUC_{0-6} after chronic dose was still obtained at 30 mg ($p < 0.01$), 60 mg ($P < 0.0005$) and 120 mg ($P < 0.0005$) of ISDN.

No apparent relationships were found between age, total body surface area and AUC. Although the study was not designed to examine the effect of smoking on ISDN kinetics, the data suggested no dramatic difference in ISDN pharmacokinetics between smokers and non-smokers in this patient group.

Pharmacological responses

The correlation coefficients (r) obtained when peak effect of each pharmacological response was linearly regressed against log dose and log AUC after single doses of ISDN are shown in Table 5. None of the pharmacological responses were significantly related to log dose. However, significant correlations were obtained between log AUC and several pharmacological responses, *viz:* walking time to P_1, standing heart rate, standing systolic and diastolic blood pressures.

During the acute dose phase of the study, five patients were found to be more sensitive to ISDN doses than others, and they exhibited severe enough hypotensive reactions at 60 mg that they were exempted from the highest single dose (120 mg). In Table 6 are listed the pharmacological and pharmacokinetic parameters after the 60 mg single ISDN

Table 4 AUC (0–6 h) as functions of ISDN dose and duration of drug administration

	AUC (ng ml^{-1} h)							
	15 mg		*30 mg*		*60 mg*		*120 mg*	
Patient number	*Single dose*	*Chronic dose*	*Single dose*	*Chronic dose*	*Single dose*	*Chronic dose*	*Single dose*	*Chronic dose*
1	5.9	17.1	14.6	29.0	39.4	77.7	53.1	232 (292)*
2	6.7	22.4	9.0	32.7	22.3	81.8	68.4	299
3	48.4	19.2	49.3	40.4	56.8	109	—	191
4	19.0	26.0	42.2	70.3	70.9	256	—	553
5	11.3	14.6	25.4	42.0	45.5	111	131	332
6	13.1	20.2	25.4	45.6	49.3	115	95.2	261
7	11.3	12.8	13.6	52.7	42.2	97.4	143	243
8	14.7	15.3	28.4	38.9	43.3	147	—	291
9	25.9	26.3	43.6	65.3	81.3	189	—	336
10	9.9	11.6	18.5	16.7	43.0	55.3	71.9	208
11	11.9	25.7	33.5	63.0	88.8	236	—	511
12	9.1	12.0	16.8	27.5	36.2	121	90.1	185
Mean	15.6	18.6	26.7	43.7	51.6	133	93.2	304
s.d.	11.7	5.5	13.1	16.5	19.5	63.0	33.2	118
s.e.mean	3.4	1.6	3.8	4.8	5.6	18.2	12.5	34.1
P	NS		< 0.005		< 0.0005		< 0.0005	

*Second measurement 1 week later.

Table 5 Comparison of correlations between log dose *v* peak effect and log AUC *v* peak effect after single doses of ISDN ($n = 43$)

*Effect**	*Log dose v peak effect* r+	P‡	*Log AUC v peak effect* r+	P‡
WT to P_1	+0.28	NS	+0.41	<0.01
WT to P_2	+0.12	NS	+0.17	NS
HR (standing)	+0.16	NS	+0.42	<0.01
HR (lying)	+0.12	NS	+0.22	NS
SBP (standing)	−0.16	NS	−0.42	<0.01
SBP (lying)	−0.16	NS	−0.26	NS
DBP (standing)	+0.03	NS	−0.30	<0.05
DBP (lying)	+0.09	NS	−0.10	NS
ST-segment depression	−0.12	NS	+0.07	NS

*WT at P_1 = treadmill walking time until the onset of angina; WT at P_2 = treadmill walking time until development of severe pain; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure.
+r = Pearson's correlation coefficient.
‡Critical ratio Z-test (N > 30), NS: $P > 0.05$.

Table 6 Pharmacological and pharmacokinetic parameters in patients exhibiting an apparent difference in ISDN sensitivity

*Parameter**	*Patients with adverse reactions at 60 mg* (n = 5)	*Patients with no apparent adverse reactions at 60 mg* (n = 7)	P+
WT to P_1	64 ± 43	91 ± 67	NS
WT to P_2	25 ± 45	31 ± 14	NS
HR (standing)	55 ± 27	26 ± 11	0.03
HR (lying)	24 ± 11	13 ± 17	NS
SBP (standing)	−45 ± 5	−33 ± 11	0.009
SBP (lying)	−31 ± 5	−24 ± 5	0.04
DBP (standing)	−45 ± 10	−22 ± 10	0.003
DBP (lying)	−23 ± 6	−16 ± 12	NS
ST-segment depression	−47 ± 34	−35 ± 37	NS
Cl_I at 15 mg	14.4 ± 5.9	28.0 ± 8.6	0.013
Cl_I at 30 mg	13.2 ± 3.0	31.8 ± 12.3	0.008
Cl_I at 60 mg	15.7 ± 4.8	26.7 ± 8.3	0.012
Cl_I at 15, 30 & 60 mg	14.4 ± 4.5	28.8 ± 9.7	5×10^{-6}

*All parameters (mean ± s.d.) calculated after the 60 mg acute dose. For explanation of parameter, see footnote of Table 5. Values are % changes at peak.
+Student's *t*-test, NS: $P > 0.05$.

dose of those who exhibited adverse reactions and those who did not. The 'sensitive' group showed statistically greater changes in standing heart rate, standing and lying systolic blood pressure and standing diastolic blood pressure. Interestingly, the apparent intrinsic clearances of ISDN at 15, 30 and 60 mg were also statistically significantly lower in this group. When the correlations between log dose or log AUC and effect were re-examined after separating the patients into a 'sensitive' and a 'non-sensitive' group, however, no improvement in correlation was observed.

The pharmacological responses observed after chronic dosing were much attenuated compared to those observed after acute dosing, a phenomenon which is consistent with the development of nitrate tolerance, as reported elsewhere (Thadani *et al.*, 1980a, c). Those patients who exhibited severe adverse reactions to a single dose of 60 mg could be, and were, titrated up to 120 mg four times a day without untoward effects.

Discussion and Conclusions

Results of this study (Figure 1) suggested that the elimination of ISDN in human plasma may be at least bi-phasic. In the one individual from whom plasma

samples were obtained over 24 h, bi-exponential decay was clearly evident (Figure 2). The data suggested that ISDN elimination may not be as rapid as previously believed.

Figure 3 shows the relationships between AUC and dose after acute and chronic dosing of ISDN. While dose appeared to be linearly related to AUC after acute dosing, an increase in dose brought about a larger than proportional increase in AUC after chronic dosing. If it is assumed that ISDN disposition in plasma follows bi-exponential decay, and that chronic dosing in this study had achieved steady state, then the AUC obtained over the 0–6 h dosing interval (τ) after chronic dosing can be mathematically described by equation 3 (Gibaldi & Perrier, 1975):

$$AUC_\tau = \frac{FD}{aV_d\beta \cdot \beta} \quad (3)$$

where F is the fraction of the dose (D) which reaches the systemic circulation intact, and $aV_d\beta$ is the apparent volume of distribution and β is the disposition rate constant. When the AUCτ is significantly larger than the $AUC_{0-\infty}$ obtained after a single dose over the same time interval, as was found in the present case, the difference might be contributed by any combination of the three derived parameters in equation 3, *viz:* β, F and $aV_d\beta$. Each of these pharmacokinetic parameters is now examined in some detail to determine the degree of contribution it makes to the observed apparent drug accumulation upon chronic dosing.

Drug accumulation occurs when the dosing interval τ is less than about five times the disposition half-life of the drug. Thus, some increases in plasma ISDN concentrations after chronic dosing at 6 h intervals are to be expected if the β half-life is 4 h. Furthermore, there may also have occurred an increase in β half-life after repeated doses. The following arguments suggest, however, that β, by itself, is unlikely to account for drug accumulation after chronic dosing.

If $AUC^s_{0-\infty}$ represents the AUC obtained from time 0 to ∞ after a single dose, then (Gibaldi & Perrier, 1975)

$$AUC_\tau = AUC^s_{0-\infty} = AUC^s_{0-6} + AUC_{6-\infty} \quad (4)$$

where AUC^s_{0-6} and $AUC^s_{6-\infty}$ are the respective areas covering from time 0 to 6 h and from 6 h to time infinity after a single dose. The values of AUC^s_{0-6} can be obtained from Table 4 while the residual area, $AUC^s_{6-\infty}$, can be approximated by C_6/β where C_6 is the plasma ISDN concentration at 6 h. It must be assumed that absorption is complete and distribution equilibrium is established well by 6 h. Results in Figure 2 show that the concentrations at 6 h are in the β phase, suggesting that this assumption is reasonable. The requisite β and β half-life which can explain the difference between AUC and AUC^s_{0-6} can then be estimated by equation 5 and equation 6, respectively:

$$\beta = C_6/(AUC_\tau - AUC^s_{0-6}) \quad (5)$$

and

$$T_{\frac{1}{2}}\beta = \frac{0.693\,(AUC_\tau - AUC^s_{0-6})}{C_6} \quad (6)$$

Using mean data and equation 6, $T_{\frac{1}{2}}\beta$ was found to be dose-dependent and would have to be 5.2, 18.7, 34.7 and 40.8 h for the doses 15, 30, 60 and 120 mg respectively if drug accumulation arises solely from a slow and hidden elimination phase. In the patient whose plasma concentrations were monitored up to 24 h, the $T_{\frac{1}{2}}\beta$ in both occasions was found to be only about 4 h after 60 mg doses. This information, albeit somewhat limited in scope, appears to indicate that ISDN accumulation in plasma after chronic administration may likely arise from other or additional factors besides a slow and undetected elimination half-life.

Apparent increases in plasma concentrations after chronic dosing may also be due to an increase in the systemic availability (F) as a function of duration of administration. F can range from 0 to 1 and is a product of two parameters, *viz:* (i) the fraction of dose that is absorbed across the gastrointestinal tract, f_a and (ii) the fraction of the absorbed dose which is not subjected to presystemic metabolism during its first passage through the gastrointestinal tract and the liver, f_m. Chasseaud, Down & Grundy (1975) administered [^{14}C]-ISDN (total dose, 5 mg) to two healthy subjects and found that 99% and 94% of the radioactivity were excreted in the urine of these individuals. In both cases, less than 1% of the dose was recovered in the faeces collected up to 120 h. These data suggested that, for ISDN in man, f_a can be approximated to unity, and the value of F is primarily dependent on f_m. The magnitude of this parameter has become the central issue of debate regarding the rationality of oral organic nitrate therapy (Krantz & Leake, 1975). Needleman, Lang & Johnson (1972) showed that when organic nitrates were given orally or infused into the portal vein in rats, negligible blood concentrations of intact drug were obtained. Since these workers found human liver biopsy samples to have a similar enzyme capacity for organic nitrate metabolism as found for rats, they concluded that orally administered organic nitrates are essentially destroyed during their passage through the liver (i.e., f_m approaches zero) and little, if any, intact drug is available in the systemic circulation to produce vasodilator action. Krantz & Leake (1975) strongly refuted Needleman's conclusion based on their collective experience. The composite clinical evidence on the efficacy of oral ISDN that was cited previously

also suggests that f_m may not be negligible in man. Because no intravenous ISDN data in humans is available, a rigorous quantitative determination of f_m is not possible. Assinder *et al.* (1979) showed that oral ISDN at a 5 mg dose was 58% bioavailable compared to the sublingual route in 6 normal subjects. Although sublingual absorption may be less than 100% when compared to a parenteral dose, this result at least allowed the assignment of a maximum value of 0.58 to f_m. Although results of this study showed that significant intact ISDN reaches the systemic circulation after oral dosing, the magnitude of F in this patient group could not be directly estimated. Chronic oral ISDN, up to 720 mg daily, resulted in prolonged high plasma ISDN concentrations, as well as high levels of the two mononitrate metabolites (Shane, Iazzetta, Chisholm, Berka & Leung, 1978). They suggested that 'chronic high oral dosage of ISDN saturates the intra-hepatic biotransformation process, and allows high concentrations of ISDN and its metabolites to enter the systemic circulation'. The non-linear dependency of AUC *v* dose after chronic dosing observed in this study (Figure 3), is, by itself, consistent with this suggestion. However, examination of the relationship between AUC and dose after acute dosing in the same patients showed it to be linear (Figure 3). Clearly, if saturation in first-pass metabolism does occur, it would have to be present to different degrees in acute *v* chronic therapy.

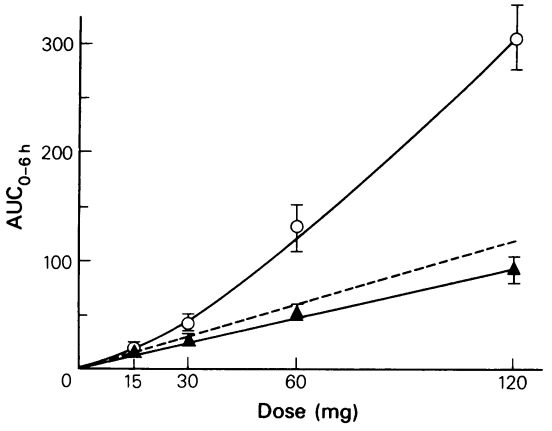

Figure 3 Relationship between AUC (0–6 h) and dose after acute (▲) and chronic (○) dosing. Points and bars represent means and standard errors, respectively. The stippled line represents AUC (0–∞) after single doses estimated by using a β half-life of 4 h.

An unknown factor is the effect of oral ISDN dose on hepatic blood flow. Since organic nitrates exert circulatory effects, hepatic perfusion could be affected after oral administration of ISDN. If a dose-dependent relationship exists between ISDN dose and hepatic perfusion, and this relationship is altered during chronic drug administration, there could be substantial changes in F as functions of dose and duration of administration.

A decrease in the volume of distribution of ISDN after chronic dosing could also bring about an increase in plasma concentration. This change, if existent, would not likely be a result of a change in protein binding because the free fraction of ISDN is similar in spiked human plasma and in plasma from patients receiving chronic ISDN. On the other hand, ISDN is known to be extensively distributed to tissues (Reed, Akester, Pranther, Tuckosh, McCurdy & Yeh, 1977), and decreases in tissue binding may result after chronic dosing. There is some preliminary evidence (Morrison, Sutton & Fung, unpublished data) that plasma clearance of ISDN may be decreased when its metabolites are co-administered by intravenous injection in rats. It is quite possible, then, that the increases in plasma ISDN concentrations after chronic dosing could come from a change in its distributive pattern in the body when its metabolites accumulate. This hypothesis is being investigated in our laboratory.

The absence of correlations between instantaneous plasma ISDN concentrations and any of the pharmacological responses suggests, at least, that these effects are not produced solely by a reversible interaction of plasma ISDN with the corresponding 'receptor' sites. This conclusion is perhaps not surprising because, for vasodilator drugs like ISDN, the magnitudes of the observed haemodynamic effects are likely to be affected by several complex and possible interactive factors. Thus, the site of hemodynamic action may likely reside in the blood vessel wall and/or other tissues, where the ISDN concentration profile may be quite different from that observed in the plasma compartment. Physiological compensatory mechanisms present in each individual may also cause significant dampening and perturbation in the overall relationship between concentration and response. In addition, the metabolites of ISDN (the 2-mononitrate and 5-mononitrate) have been shown to be pharmacologically active in animals (Wendt, 1972), and the observed responses may therefore be affected by their presence. There is also some evidence, from studies of nitrate tolerance, which suggests that the effect of nitrates in blood vessels is only slowly reversible (Needleman, Jakschik & Johnson, 1972). This phenomenon, if existent, would preclude a correlation between instantaneous concentration and effect. Plasma protein binding, however, does not appear to be a pertinent consideration here because ISDN is only slightly protein bound and the free plasma ISDN concentration is similar to the total concentration.

In spite of the multiplicity of factors which appear to govern the pharmacological responses obtained after ISDN dosing, pharmacokinetic considerations appear useful in understanding some aspects of ISDN clinical pharmacology. First, it is shown that AUC is an improved parameter compared to dose in correlating with some of the effects. However, the magnitude of the correlation coefficients remained quite small: in no case was r greater than 0.42, which suggested that variations in individual pharmacokinetics could, at best, account for $(0.42)^2 \times 100\%$ or 18% of the variance in responses.

Of more importance is the apparent difference of the intrinsic clearance in patients who exhibited adverse reactions to ISDN and in those who did not. The former group showed Cl_I values which are only about half of those of the latter group. Since $Cl_I = Cl_s/F$, a lower Cl_I reflects a lower systemic clearance or a higher F, and both occurrences are consistent with a lower metabolic capability. The data therefore suggest that adverse reactions to ISDN in certain individuals may be related to their lower ability to metabolize ISDN. Since these differences in Cl_I values are also detectable at lower doses (i.e., 15 and 30 mg), the measurement of Cl_I at a low test dose may afford some predictive possibility as to whether certain patients may be hypersensitive to ISDN at higher doses.

In summary, the interrelationships among ISDN pharmacokinetics, dose, duration of administration and pharmacological response appear complex. A better understanding of these phenomena and factors affecting them is necessary for optimizing the therapeutic usage of ISDN.

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References

ARONOW, W.S. & CHESLUK, H.M. (1970). Sublingual isosorbide dinitrate therapy versus sublingual placebo in angina pectoris. *Circulation*, **41**, 869–874.

ARONOW, W.S. & KAPLAN, M.A. (1969). Propranolol combined with isosorbide dinitrate versus placebo in angina pectoris. *New Engl. J. Med.*, **280**, 847–850.

ASSINDER, D.E., CHASSEAUD, L.F. & TAYLOR, T. (1979). Plasma isosorbide dinitrate concentrations in human subjects after administration of standard and sustained-release formulations. *J. pharm. Sci.*, **66**, 775–778.

CHASSEAUD, L.F., DOWN, W.H. & GRUNDY, R.K. (1975). Concentrations of the vasodilator isosorbide dinitrate and its metabolites in the blood of human subjects. *Eur. J. clin. Pharmac.*, **8**, 157–160.

DANAHY, D.T., BURWELL, D.T., ARONOW, W.S. & PRAKASH, R. (1977). Sustained hemodynamic and antianginal effect of high dose oral isosorbide dinitrate. *Circulation*, **55**, 381–387.

DICARLO, F.J. & MELGAR, M.D. (1969). Binding and metabolism of nitroglycerin by rat blood plasma. *Proc. Soc. exp. Biol. (N.Y.)*, **131**, 406–408.

GIBALDI, M. & PERRIER, D. (1975). *Pharmacokinetics*, ed. Swarbrick, J., vol. 1, New York: Marcel Dekker, Inc.

GOLDBARG, A.N., MORAN, J.F., BUTTERFIELD, T.K., NECICKAS, R. & BERMUDEZ, G.A. (1969). Therapy of angina pectoris with propranolol and long-acting nitrates. *Circulation*, **40**, 847–853.

GOLDSTEIN, R.E., ROSING, D.R., REDWOOD, D.R., BEISER, G.D. & EPSTEIN, S.E. (1971). Clinical and circulatory effects of isosorbide dinitrate; comparison with nitroglycerin. *Circulation*, **43**, 629–640.

KALTENBACH, M., TIEDEMANN, I. & SCHELLHORN, W. (1973). Studies on the therapeutic activity of five different long-acting nitro-derivative on angina pectoris. *Coronary Heart Disease*, ed. Kaltenbach, M., Lichtlen, P. & Friesinger, C.G., Stuttgart: Georg Theime Verlag.

KATTUS, A.A., ALVARO, A.B., ZOHMAN, L.R. & GOULSON, A.H. (1979). Comparison of placebo, nitroglycerin, and isosorbide dinitrate for effectiveness of relief of angina and duration of action. *Chest*, **75**, 17–23.

KRANTZ, J.C. & LEAKE, C.D. (1975). The gastrointestinal absorption of organic nitrates. *Am. J. Cardiol.*, **36**, 407–408.

LAUFEN, H., SCHARPF, F. & BARTSCH, G. (1978). Improved method for the rapid determination of isosorbide dinitrate in human plasma and its application in pharmacokinetic studies. *J. Chromatogr.*, **146**, 457–464.

MARKIS, J.E., GORLIN, R., MILLS, R.M., WILLIAMS, R.A., SCHWEITZER, P. & RANSIL, B.J. (1979). Sustained effect of orally administered isosorbide dinitrate on exercise performance of patients with angina pectoris. *Am. J. Cardiol.*, **43**, 265–271.

NEEDLEMAN, P., JAKSCHIK, B.A. & JOHNSON, E.M., JR. (1973). Sulphydryl requirement for relaxation of vascular smooth muscle. *J. Pharmac. exp. Ther.*, **187**, 324–331.

NEEDLEMAN, P., LANG, S. & JOHNSON, E.M. (1972). Organic nitrates: Relationship between biotransformation and rational angina pectoris therapy. *J. Pharmac. exp. Ther.*, **181**, 489–497.

REED, D.E., AKESTER, J.M., PRATHER, J.F., TUCKOSH, J.R., MCCURDY, D.H., & YEH, C. (1977). Blood and tissue levels of (^{14}C) isosorbide dinitrate after oral and intravenous administration to rat. *J. Pharmac. exp. Ther.*, **202**, 32–37.

SHANE, S.J., IAZZETTA, J.J., CHISHOLM, A.W., BERKA, J.F. & LEUNG, D. (1978). Plasma concentrations of isosorbide dinitrate and its metabolites after chronic high oral dosage in man. *Br. J. clin. Pharmac.*, **6**, 37–41.

THADANI, U., DARKE, A.C., FUNG, H.-L. & PARKER, J.O. (1980a). Dose response and duration of action of oral isosorbide dinitrate (ISDN) during sustained therapy in angina pectoris. *Am. J. Cardiol.*, **45**, 478.

THADANI, U., FUNG, H.-L., DARKE, A.C. & PARKER, J.O.:

(1980b). Oral isosorbide dinitrate in the treatment of angina pectoris. Dose response relationship and duration of action during acute therapy. *Circulation*, **62**, 491–502.

THADANI, U., MANYARI, D., PARKER, J.O. & FUNG, H.-L. (1980c). Tolerance to the circulatory effects of oral isosorbide dinitrate. Its rate of development and cross tolerance to glyceryl trinitrate. *Circulation*, **61**, 526–535.

THE UNITED STATES PHARMACOPEIA (1975), 19th rev., Easton, Pa.

WENDT, R.L. (1972). Systemic and coronary vascular effects of the 2- and 5-mononitrate esters of isosorbide. *J. Pharmac. exp. Ther.*, **180**, 732–742.

WINSOR, T., KAYE, H. & MILLS, B. (1972). Hemodynamic response of oral long-acting nitrates: Evidence of gastrointestinal absorption. *Chest*, **62**, 407–413.

YAP, P.S.K., MCNIFF, E.F. & FUNG, H.-L. (1978). Improved GLC determination of plasma nitroglycerin concentration. *J. pharm. Sci.*, **67**, 582–584.

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