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Absorption and excretion of isosorbide dinitrate and isosorbide-2-mononitrate in dogs

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In a crossover design, four male dogs were given orally or i.v. [^{14}C]isosorbide dinitrate (ISDN) or [^{14}C]isosorbide-2-mononitrate (2-ISMN) at a dose of 1 mg kg^{-1} (70–80 μCi). Virtually all of the oral dose was absorbed and all of the radioactivity was excreted in the urine. The profile of serum radioactivity was similar after all drug administrations. ISDN was rapidly denitrated, giving rise to isosorbide-5-mononitrate (5-ISMN) as a major metabolite, and 2-ISMN as a minor metabolite. The apparent elimination half-life from serum of 2-ISMN and 5-ISMN was 2–3 h. More than 50% of the serum radioactivity after [^{14}C]2-ISMN was due to unchanged drug. The apparent volume of distribution of 2-ISMN averaged 8·3 litres. The results show that, in contrast to ISDN, administration of 2-ISMN resulted in relatively high unchanged drug levels in the serum; the disposition of radioactivity after [^{14}C]ISMN was however similar to that after [^{14}C]ISDN. The findings support the concept that the concentrations of ISDN, 2-ISMN and 5-ISMN in the blood are inversely related to the rates of denitration, and that the vascular activity of the nitrates of isosorbide relates to the rates of their dinitration.

The first clinical trial with orally administered isosorbide dinitrate (ISDN) was reported in 1948 by Goldberg. In spite of some controversial reports (Krantz & Leake 1975; Jerie 1976), the clinical efficacy of orally administered ISDN as a long-acting nitrate is now generally accepted (Markis et al 1979; Thadani et al 1980). Upon administration to dogs (Sisenwine & Ruelius 1971) or man (Chasseaud et al 1975; Spörl-Radun et al 1980), ISDN appears in the blood together with isosorbide-5-mononitrate (5-ISMN) as a major metabolite and isosorbide-2-mononitrate (2-ISMN) as a minor metabolite (Sisenwine & Ruelius 1971; Chasseaud et al 1975). The relative ratios of ISDN vs 2-ISMN vs 5-ISMN found in the blood of dogs injected ISDN intravenously were 1:2:14 (Sisenwine & Ruelius 1971; Needleman 1976). In healthy subjects given ISDN sublingually, the corresponding ratios were 1:3·6:42 (Spörl-Radun et al 1980). These differences in the blood-level profiles of ISDN and its two metabolites have been ascribed to a faster rate of denitration of the sterically more exposed *exo* 2-nitrate group compared with the sterically less exposed *endo* 5-nitrate group (Sisenwine & Ruelius 1971). This, coupled with the finding that, in dogs, the coronary vasodilator potency of ISDN vs 2-ISMN vs 5-ISMN was 1:0·33:0·03, prompted Wendt (1972) to attribute the vascular activity of the nitrates of isosorbide to their denitration, the rate of which depends on the configuration of the carbon atom bearing the nitrate ester group. Convincing supporting evidence was provided by Bogaert & Rosseel (1972) by demonstrating in dogs the parallelism between vasodilator potency and configuration of the nitrate bearing carbon, i.e. the more exposed (to denitration), the higher the potency. The concentrations of ISDN, 2-ISMN and 5-ISMN in the blood are thus inversely related to their vasodilatory potency.

According to the postulate of Wendt (1972), the exposed 2-nitrate group in ISDN and 2-ISMN should be denitrated at a similar rate. We have confirmed the hypothesis by the finding of similar concentration profiles of radioactivity in the serum of dogs given [^{14}C]ISDN or [^{14}C]2-ISMN, orally and i.v. The results of these studies are presented in this report.

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MATERIALS AND METHODS

Labelled compounds

Uniformly labelled [^{14}C]glucose (New England Nuclear Corp., Boston, MA) was reduced to U-[^{14}C]sorbitol (Romaschin et al 1977), which in turn was converted to U-[^{14}C]isosorbide by refluxing in the presence of *p*-toluenesulphonic acid. The [^{14}C]isosorbide was selectively acetylated (Buck et al 1966), and the products were separated by column chromatography. The U-[^{14}C]isosorbide-5-acetate obtained was treated with acetic anhydride/nitric acid and then deacetylated to give U-[^{14}C]2-ISMN.

The fractions containing isosorbide-2,5-diacetate and isosorbide-2-acetate were combined, deacetylated, and nitrated to U-[^{14}C]ISDN. The specific activities of [^{14}C]2-ISMN and [^{14}C]ISDN were 25·1 and 22·3 μCi mg^{-1}, respectively.

The compounds were >99% radiochemically pure as determined by thin-layer chromatography (t.l.c.)-autoradiography in three solvent systems.

Animals and drug administration

Four male beagle hounds, ca 12 kg, were housed in individual metabolism cages to allow separate collection of urine and faeces. Doses with required specific activity were prepared by dissolving appropriate amounts of labelled and unlabelled compound in 0·9% NaCl (saline) containing 40% propylene glycol. In a Latin square four-way crossover design, each dog received intravenously or by gastric intubation 10 ml of the solution containing 70–80 μCi of [^{14}C]ISDN or [^{14}C]2-ISMN at a dose of 1 mg kg^{-1}. A two-week interval separated the dosings. Blood was withdrawn before each dosing and at 2, 5, 10, 15, 30 min and at 1, 2, 4, 6, 9, 12, 24, 48 and 72 h after dosing. Serum was separated by centrifugation. Urine was collected in 12 h intervals for two days, and subsequently every 24 h for up to seven days; faeces were also collected in 24 h intervals for seven consecutive days.

Analytical methods

Serum and urinary radioactivity concentrations were measured by counting 0·2 ml aliquots in 15 ml Aquasol scintillation medium (New England Nuclear, Boston, MA). Faeces were homogenized in water, aliquots of about 150 mg were digested in 2 ml of Soluene-350 (Packard Instrument Co., Downers Grove, IL), and the digests were decolourized by adding 0·5 ml isopropanol and 0·2 ml H_2O_2. Samples were counted after addition of 15 ml Omnifluor scintillation fluid (5 g litre^{-1}) (New England Nuclear).

Drug concentrations in serum were determined by a modification of the t.l.c. method described by Sisenwine & Ruelius (1971). Aliquots of 1 ml were lyophilized, and to the residue was added 1 ml of methanol. The mixture was shaken and centrifuged at 0–2 °C. At least 95% of the radioactivity from [^{14}C]ISDN and [^{14}C]2-ISMN added to dog serum was recovered in the methanol. Between 0·7 and 0·8 ml of the supernatant was concentrated to a small volume in a stream of nitrogen, and spotted on a prescored 0·25 mm Silica Gel G t.l.c. plate with 13% $CaSO_4$ binder (Anasil G, Analabs Inc., North Haven, CT). Chromatograms were developed in chloroform–methanol, 19:1 (v/v), or ethyl acetate–benzene, 1:1 (v/v). Virtually identical results were obtained with both solvent systems. The location of ISDN, 2-ISMN and 5-ISMN was determined by the use of simultaneously run authentic samples of 2-ISMN and 5-ISMN, spraying with a 1% solution of diphenylamine in ethanol, and exposure to ultraviolet light for 5 min (Dietz Jr 1967). The gel from the entire radio-chromatogram was scraped in 5 mm sections into counting vials with a Snyder-Kimble Automatic t.l.c. Zonal Scraper (Analabs). The gel was digested with 0·2 ml hydrofluoric acid and 0·2 ml water (Shaw et al 1971), and counted after addition of 15 ml Aquasol. The radioactivity associated with ISDN, 2-ISMN and 5-ISMN was expressed in ng ml^{-1} serum. The limit of detection of the method was 1 ng ml^{-1} serum.

Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer, Model 3375. Quench corrections were made by the external standard ratios method. Radioactivity content of less than 10 dpm above background was considered to be below the sensitivity of the counting method.

Data analysis

The serum concentration (C) of ISDN, 2-ISMN and 5-ISMN was calculated according to the formula

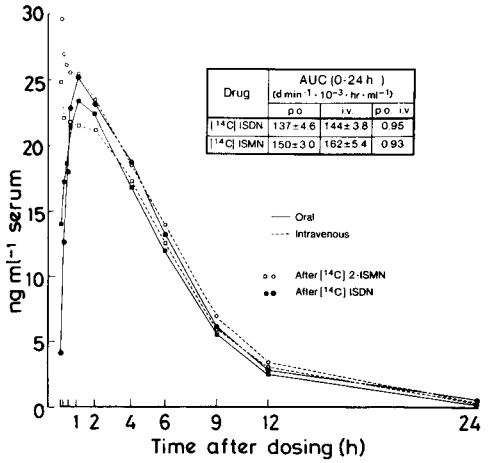
$$C = \frac{\frac{dpm_z}{dpm_p} \times T}{SA}$$

where dpm_z is the radioactivity in the t.l.c. zone corresponding to ISDN or its metabolites, dpm_p is the radioactivity on the entire t.l.c. plate, T is the total serum radioactivity (dpm ml^{-1}) before extraction, and SA is the specific activity (dpm ng^{-1}) of the dose. To calculate the concentration of 2-ISMN and 5-ISMN, SA was multiplied by 191/236, i.e. the ratio of the molecular weights of ISMN/ISDN. The total [^{14}C] (in ng ml^{-1}) in Figs 2–4 was estimated on the basis of the molecular weight of ISMN.

The pharmacokinetics of 2-ISMN and 5-ISMN were calculated by assuming a one-compartment model. The areas under the serum drug concentration/time curves (AUC) were calculated by the trapezoidal rule. The serum clearance (Cl) and apparent volume of distribution ($V_{d\ area}$) were calculated according to the formulae Cl = dose/$AUC_{0\rightarrow\infty}$ and $V_{d\ area} = Cl/K_{el}$.

RESULTS AND DISCUSSION

Similar profiles of serum radioactivity concentrations were found after intravenous and oral administration of [^{14}C]ISDN or [^{14}C]2-ISMN (Fig. 1). From the ratio of $AUC_{p.o.}/AUC_{i.v.}$ (for the 0–24 h interval), 93–95% of the radioactivity of the oral dose was absorbed.



Drug	AUC (0-24 h) (d min⁻¹ · 10⁻³ · hr · ml⁻¹)		
	p.o.	i.v.	p.o. / i.v.
[14C] ISDN	137 ± 4.6	144 ± 3.8	0.95
[14C] ISMN	150 ± 3.0	162 ± 5.4	0.93

FIG. 1. Average serum levels of radioactivity in four dogs after oral and intravenous administration of [^{14}C]ISDN and [^{14}C]2-ISMN. Data have been corrected to an exact dose of 1 mg kg^{-1} and 75 μCi/dose to allow for individual differences in the dose specifications. The AUC are expressed as mean ± s.e.m. for the four dogs.

Serum concentrations of radioactivity and of unchanged ISDN, 2-ISMN, and 5-ISMN after oral and intravenous administration of [^{14}C]ISDN (1 mg kg^{-1}) are shown in Figs 2 and 3. Peak concentrations (C_{max}) of radioactivity were found 1 h after the oral dose. Upon oral administration of [^{14}C]ISDN, C_{max} of parent drug in serum averaged 22 ng ml^{-1} (at 2 min after dosing), and <1 ng ml^{-1} was detected in the serum at 30 min after dosing. Both 2-ISMN and 5-ISMN peaked at 30–60 min; the C_{max} of 2-ISMN was about 1/12 that of 5-ISMN.

After i.v. injection of [^{14}C]ISDN, elimination of ISDN from serum was rapid, and no unchanged drug was detected at 2 h after dosing (Fig. 3). The AUC_{0-6} indicate that similar amounts of 5-ISMN were present in the serum after both routes of [^{14}C]ISDN administration (Table 1). Slightly more 2-ISMN appeared in the serum after i.v. injection than after oral administration of [^{14}C]ISDN; however, since the relative amounts of 2-ISMN were low, this difference is not considered to be pharmacologically significant. As reported earlier (Sisenwine & Ruelius

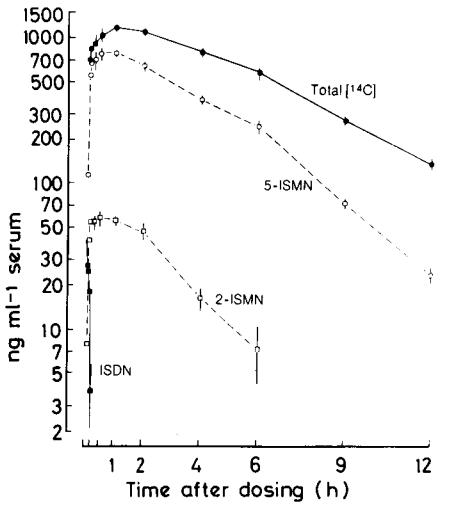


FIG. 2. Average serum levels of radioactivity, ISDN, 2-ISMN and 5-ISMN in four dogs after oral administration of [^{14}C]ISDN at a dose of 1 mg kg^{-1}. Total radioactivity ([^{14}C]) is expressed as ng equivalents ml^{-1}, based on the molecular weight of ISMN.

1971), elimination half-lives ($t_{1/2}$ ± s.e.m.) of 2-ISMN (2·2 ± 0·19 h) and 5-ISMN (2·5 ± 0·09 h) were similar in spite of the finding of about 12 times lower systemic availability (AUC_{0-6}) of 2-ISMN compared with 5-ISMN. Unlike 2-ISMN, which is metabolized mainly by denitration, the disappearance of 5-ISMN from the serum of dogs is due mainly to the transformation of the molecule to its glucuronide conjugate (Reed et al 1971).

Orally administered [^{14}C]2-ISMN was rapidly absorbed and C_{max} averaging 1100 ng ml^{-1} and

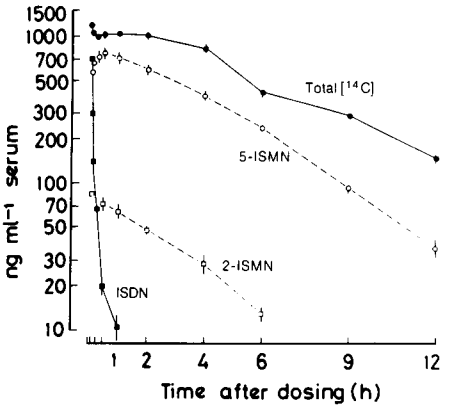


FIG. 3. Average serum levels of radioactivity, ISDN, 2-ISMN and 5-ISMN in four dogs after intravenous injection of [^{14}C]ISDN at a dose of 1 mg kg^{-1}. Total radioactivity ([^{14}C]) is expressed as ng equivalents ml^{-1}, based on the molecular weight of ISMN.

representing about 80% of total radioactivity was attained after 30–60 min (Fig. 4). The elimination pattern of radioactivity and of 2-ISMN from the serum was virtually identical after both routes of administration. Based on the AUC_{0-6} after both oral and i.v. administration of [^{14}C]2-ISMN, an average of 53% of the serum radioactivity was due to 2-ISMN (Table 1). The t½ of 2-ISMN was 1·8 ± 0·12 h, and the Cl and $V_{d\ area}$, 3280 ± 143 ml h^{-1} and 8·3 ± 0·29 litres, respectively. The $V_{d\ area}$ thus corresponded to the calculated total body water of the dog (70% of the body weight), suggesting that 2-ISMN does not accumulate in the dog tissues. It is pertinent that in tissue distribution studies in rats given [^{14}C]ISDN by i.v. injection, unchanged drug accumulated in the vascular and heart tissue, but not in the other organs examined (Reed et al 1977). In man, the pharmacokinetics of 2-ISMN was reported as follows: t½, 1·8 h; Cl, 22 litres h^{-1}; and $V_{d\ area}$, 55 litres (Chasseaud & Taylor 1981).

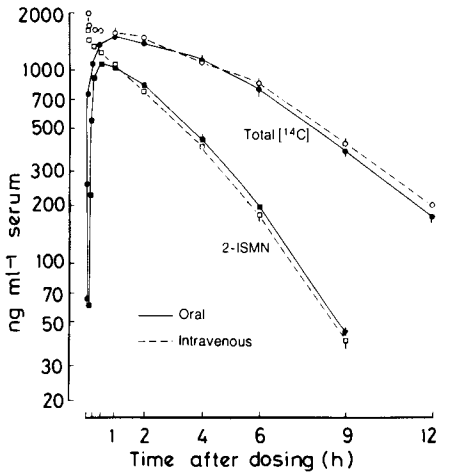


FIG. 4. Average serum levels of radioactivity and of 2-ISMN in four dogs after oral and intravenous administration of [^{14}C]2-ISMN at a dose of 1 mg kg^{-1}. Total radioactivity ([^{14}C]) is expressed as ng equivalents ml^{-1}, based on the molecular weight of ISMN.

Table 1. Areas under the serum concentration/time curves (AUC) (0–6 h)*.

Drug given	Analysed	AUC (ng h^{-1} ml^{-1}) oral	AUC (ng h^{-1} ml^{-1}) i.v.	AUC oral / AUC i.v.
[^{14}C]ISDN	Total [^{14}C]	5405 ± 124	5384 ± 62	1·00
	5-ISMN	3094 ± 161	3018 ± 61	1·03
	2-ISMN	177 ± 21	245 ± 26	0·72
[^{14}C]2-ISMN	Total [^{14}C]	5806 ± 107	6263 ± 183	0·93
	2-ISMN	3104 ± 63	3303 ± 63	0·94

* Data have been corrected to an exact dose of 1 mg kg^{-1}, and are expressed as mean ± s.e.m. for 4 dogs/group.

After both oral and i.v. administration of [^{14}C]ISDN or [^{14}C]2-ISMN, between 75–80% of the radioactivity was excreted in the urine during the first 24 h. The total excretion is shown in Table 2. After each treatment virtually all of the dose was excreted in urine.

Previous investigators have shown that denitration is the primary route of biotransformation of ISDN (Dietz Jr 1967; Reed et al 1971; Sisenwine & Ruelius 1971). The results obtained with [^{14}C]ISDN in the present study are similar to those reported by Sisenwine & Ruelius (1971), i.e. that, in dogs, orally and intravenously administered ISDN undergoes rapid denitration to give rise to 5-ISMN as a major metabolite, and 2-ISMN as a minor metabolite. Based on serum radioactivity, orally administered ISDN and 2-ISMN were rapidly and completely absorbed, and the disposition of [^{14}C]2-ISMN was similar after both intravenous and oral administration.

Table 2. Total radioactivity excretion by four dogs during 7 days after oral and intravenous administration of [^{14}C]ISDN and [^{14}C]2-ISMN in doses of 1 mg kg^{-1}.

Compound	Route	Percent of administered dose: Urine	Faeces	Total
[^{14}C]ISDN	oral	94·7 ± 1·43	0·7 ± 0·39	95·4 ± 1·31
	i.v.	91·9 ± 1·29	0·5 ± 0·20	92·4 ± 1·21
[^{14}C]2-ISMN	oral	93·9 ± 1·04	0·2 ± 0·11	94·1 ± 1·03
	i.v.	93·5 ± 0·49	0·6 ± 0·10	94·1 ± 0·48

From published data on the pharmacokinetics and pharmacodynamics of ISDN, 2-ISMN and 5-ISMN in man, it is tempting to postulate that, like the dog, the vascular activity of the nitrates of isosorbide in man also relates to the rate of their denitration. The blood-level profiles of ISDN, 2-ISMN and 5-ISMN obtained after oral (Bogaert et al 1981) or sublingual (Spörl-Radun et al 1980) administration of ISDN to man are indeed similar to those found in dogs (Sisenwine & Ruelius 1971): rapidly appearing and relatively rapidly disappearing concentrations of unchanged ISDN (t½, ~0·5 h) and 2-ISMN (t½, ~1·8 h), and rapidly appearing and slowly disappearing 5-ISMN (t½, ~7·5 h)*. According to the above postulate, in man, the pharmacodynamics of ISDN correspond to the combined rates of denitration of ISDN, 2-ISMN and 5-ISMN. The rapid onset

* Recently, a terminal half-life of 4 h was reported for ISDN in angina patients (Fung et al 1981), and of 4–5 h for 5-ISMN in health volunteers (Abshagen et al 1981; Bogaert et al 1981; Taylor et al 1981).

of action of ISDN (Michel 1981) can thus be ascribed to the rapid denitration of its exposed 2-nitrate group, followed by the action of 2-ISMN formed by the slow denitration of the 5-nitrate; the relatively long duration of action of ISDN (Fung et al 1981; Taylor et al 1981), on the other hand, can be attributed to the slow denitration of the sterically shielded 5-nitrate group of the major circulating metabolite, 5-ISMN. The expected higher potency of ISDN was borne out clinically since three times as much 5-ISMN was needed to produce the same hemodynamic effect as ISDN (Michel 1981).

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