Biotransformation of Isosorbide Dinitrate in Humans

W. H. DOWN ˣ, L. F. CHASSEAUD, and R. K. GRUNDY

Abstract □ An oral dose of ^{14}C-isosorbide dinitrate was rapidly absorbed and eliminated by human subjects. Up to 99% was excreted in the urine. The drug was completely biotransformed, nearly 50% to the principal metabolite, an isosorbide conjugate, presumably with glucuronic acid. Up to 13% was excreted as free and conjugated 5-isosorbide mononitrate, and 1% was excreted as the free 2-isosorbide mononitrate only. D-Sorbitol possibly accounted for a further 18% of the radioactivity; an unknown metabolite, less polar than isosorbide, accounted for 6%.

Keyphrases □ Isosorbide dinitrate, radiolabeled—biotransformation and excretion after oral administration, humans □ Biotransformation—isosorbide dinitrate, excretion after oral administration, humans □ Metabolism—isosorbide dinitrate, humans

Despite the widespread use of isosorbide dinitrate as a coronary dilator (1–4), there is little reported regarding its metabolism in humans. Dietz (5) detected isosorbide mononitrates, but not unchanged drug, in the urine of patients. Isosorbide dinitrate was rapidly biotransformed by isolated perfused rat liver (6) through the glutathione-dependent organic nitrate reductase system (7), which is also present in human liver (8).

Metabolism studies are greatly facilitated by the use of radioisotopes, and this paper describes the fate of ^{14}C-isosorbide dinitrate in humans.

EXPERIMENTAL

Materials—Uniformly labeled ^{14}C-isosorbide dinitrate of specific activity 2.36 mCi/mmole[1] and radiochemical purity >97% was used. Isosorbide dinitrate, isosorbide 2-mononitrate, isosorbide 5-mononitrate, and isosorbide were used as reference compounds[2].

Drug Administration—The subjects[3] did not take other drugs during the study and were under suitable medical supervision. Adverse effects attributable to the drug were not reported by the subjects.

After a 12-hr fast, each subject swallowed a gelatin capsule containing 5 mg of isosorbide dinitrate dissolved in acetone, together with 150 ml of water. The subjects fasted for a further 4 hr postadministration but took 150 ml of water hourly to maintain urine volume. All the urine and feces excreted during 5 days were collected and stored at −20°.

Extraction of Radioactivity—Samples of urine were freeze dried for 16 hr and each residue was extracted with methanol. After filtration of the residue, the methanol solution was evaporated under reduced pressure for examination by TLC, with a recovery of radioactivity of 99.6%. Feces were macerated with methanol and, after centrifugation, the radioactivity was determined in samples of the supernate and the residue.

Measurement of Radioactivity—Radioactivity was measured in a liquid scintillation analyzer[4] with automatic quench correction by external standard channels ratio (9). Urine samples and solvent extracts of urine and feces were mixed with a toluene–polyethylene glycol alkyl aryl ether[5]-based scintillator (10). Samples of the residues from solvent extractions of feces were combusted using a modified plastic bag technique (11), and the combustion products were absorbed into a β-phenylethylamine-based scintillator system (12).

TLC and Detection of Metabolites—TLC was carried out on prelayered kieselgel F_{254} plates[6] of 0.25-mm thickness. The solvent systems used were: *(a)* benzene–ethyl acetate (1:1 v/v) and *(b)* isopropanol–concentrated ammonium hydroxide (4:1 v/v).

^{14}C-Labeled metabolites were detected by autoradiography using X-ray film[7]. The radioactive areas of the silica gel were removed and measured for radioactivity in a toluene–polyethylene glycol alkyl aryl ether[5]-based scintillator gel (13). This technique gave recoveries of radioactivity exceeding 95%. Isosorbide mononitrates were located by spraying the plates with 1% (w/v) diphenylamine in ethanol and exposing to UV light for 5 min (5). They appeared as brown spots. Isosorbide was located by spraying with a metaperiodate–permanganate reagent (14), and it appeared as a yellow-brown spot on a violet background.

RESULTS

Excretion of Radioactivity by Human Subjects—Following a single oral dose of 5 mg of ^{14}C-isosorbide dinitrate, the radioactivity was readily absorbed and rapidly excreted by human subjects (Fig. 1). Up to 25% was excreted in the urine in 6 hr, 49% in 12 hr, and 78% in 24 hr. After 5 days, up to 99% of the radioactive dose had been excreted in the urine and 0.8% in the feces. The urinary excretion data show that an oral dose of ^{14}C-isosorbide dinitrate was rapidly and almost completely absorbed from the GI tract of humans.

The urinary excretion half-life for about 95% of the radioactivity was 10 hr (9.8–10.2 hr). The remainder was excreted more slowly[8].

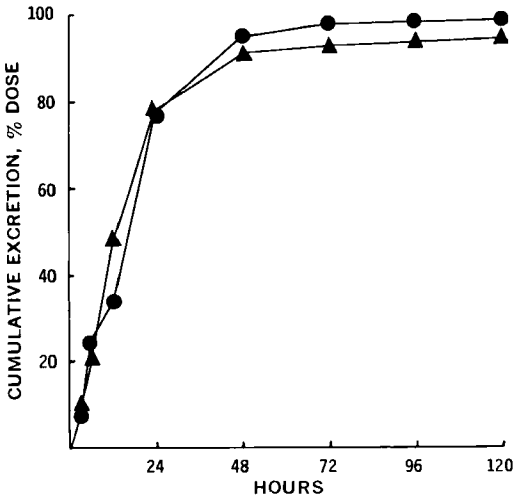


Figure 1—*Cumulative excretion of radioactivity in the urine of Subject 1 (●) and Subject 2 (▲) after an oral dose of 5 mg of ^{14}C-isosorbide dinitrate.*

[1] Synthesized by New England Nuclear Chemicals GmbH, Frankfurt, Germany.

[2] Provided by Sanol-Arzneimittel Dr. Schwarz GmbH, Monheim, Germany.

[3] With the approval of the Isotope Advisory Panel of the Medical Research Council, two male human subjects, both with normal renal and hepatic function, volunteered to take part in the studies. After the aim of the studies and the nature of the drug had been explained, the subjects gave their consent.

[4] Philips N. V., Holland.

[5] Octoxynol-9, Fisons Ltd., Loughborough, England.

[6] Merck A.-G., Darmstadt, Germany.

[7] Kodirex, Kodak Ltd., Hemel Hempstead, England.

[8] Blood also was withdrawn in this study. The results obtained will be reported elsewhere.

Table I—Urinary Metabolites of Isosorbide Dinitrate

Metabolite	R_f[a]	Administered Dose, % 0–12 hr	12–24 hr	24–48 hr	48–120 hr	Total Excreted, %
Subject 1:						
1	0–0.05	27.02	41.80	15.86	1.91	86.59
2	0.23 (5-mononitrate)	2.86	0.91	0.51	0.08	4.36
3	0.31 (2-mononitrate)	1.09	0.04	—	—	1.13
Subject 2:						
1	0–0.05	42.23	28.27	11.79	1.70	83.99
2	0.23 (5-mononitrate)	2.51	0.69	0.26	0.09	3.55
3	0.31 (2-mononitrate)	0.31	0.10	0.03	—	0.44

[a] Solvent system *a*.

Table II—Urinary Metabolites of Isosorbide Dinitrate after Deconjugation with β-Glucuronidase–Aryl Sulfatase

Metabolite	R_f[a]	Administered Dose, % 0–12 hr	12–24 hr	24–48 hr	48–120 hr	Total Excreted, %
Subject 1:						
1	0–0.05	22.29	40.71	15.93	2.24	81.17
2	0.23 (5-mononitrate)	10.03	1.30	1.45	0.06	12.84
3	0.31 (2-mononitrate)	0.78	0.06	0.08	—	0.92
4	0.57 (dinitrate)	0.38[b]	—	—	—	0.38
Subject 2:						
1	0–0.05	39.94	28.03	12.13	1.84	81.94
2	0.23 (5-mononitrate)	6.71	1.10	0.32	0.04	8.17
3	0.31 (2-mononitrate)	0.57	0.07	0.07	—	0.71
4	0.57 (dinitrate)	0.15[b]	—	—	—	0.15

[a] Solvent system *a*. [b] Excreted during 0–3 hr only.

Table III—Urinary Metabolites of Isosorbide Dinitrate after Deconjugation with β-Glucuronidase–Aryl Sulfatase

Metabolite	R_f[a]	Administered Dose, % 0–12 hr	12–24 hr	24–48 hr	48–120 hr	Total Excreted, %
Subject 1:						
1	0.10 (D-sorbitol)	4.09	6.33	3.38	0.43	14.23
2	0.55 (isosorbide)	10.86	23.18	9.86	0.63	44.53
3	0.64 (unknown)	1.90	2.78	1.11	0.26	6.05
4	0.75[b]	8.83	2.69	1.45	0.24	13.21
Subject 2:						
1	0.10 (D-sorbitol)	11.92	4.59	1.62	0.30	18.43
2	0.55 (isosorbide)	22.60	18.73	7.81	0.83	49.97
3	0.64 (unknown)	2.60	1.55	0.71	0.19	5.05
4	0.75[b]	6.20	1.67	0.83	0.21	8.91

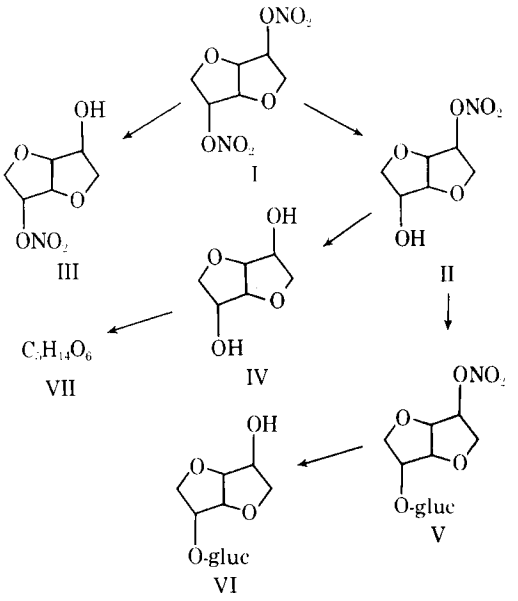
[a] Solvent system *b*. [b] R_f values: dinitrate, 0.81; 5-mononitrate, 0.76; and 2-mononitrate, 0.74.

Detection and Identification of Metabolites—The proportions of metabolites excreted in the urine of human subjects following oral administration of ^{14}C-isosorbide dinitrate are shown in Tables I–III. Isosorbide 5-mononitrate, isosorbide 2-mononitrate, and isosorbide were identified by cochromatography with authentic compounds.

More than 80% of the radioactivity was excreted in the urine as polar material and only about 4% corresponded to the 5-mononitrate (II) and 1% to the 2-mononitrate (III) (Table I). Unchanged isosorbide dinitrate (I) was only detected in trace amounts in the 0–3-hr urine (Table II). Incubation of the methanolic urinary extracts with a β-glucuronidase/aryl sulfatase preparation[9] or acid hydrolysis suggested that isosorbide 5-mononitrate was excreted partly conjugated, whereas isosorbide 2-mononitrate was excreted only as the free compound. The results (Table II) indicated that about 8% of the dose was excreted as conjugated isosorbide 5-mononitrate (V).

Most polar material was excreted as conjugated isosorbide (VI) produced by complete denitration of isosorbide dinitrate (Table

[9] Type H-2, Sigma Chemical Co. Ltd., Kingston, Surrey, England.



Scheme I—Possible biotransformation of isosorbide dinitrate in humans (gluc represents glucuronic acid)

III). Since sulfates of isosorbide (IV) or isosorbide 5-mononitrate would not be expected to be hydrolyzed by the enzyme preparation used, which contained an aryl sulfatase, it may be concluded that the conjugates are glucuronides (Tables II and III).

An unidentified metabolite, which was less polar than isosorbide (IV), accounted for about 6% of the dose and a more polar metabolite for about 16% (Table III). This latter polar metabolite was chromatographically similar to D-sorbitol (VII), which would be produced by ring opening of IV. Further characterization of the metabolites by mass spectrometry was precluded by the low dose administered resulting in only small quantities of metabolites.

DISCUSSION

Denitration of organic nitrates has been extensively studied (7, 15–21). In animals, denitration is the major biotransformation route of isosorbide dinitrate (5–8, 22–24) and this is so in humans (Tables I–III).

Identification of the radioactive components excreted in the urine showed that only traces of isosorbide dinitrate, the parent drug, were excreted (Table II). Both isomeric mononitrate metabolites were excreted, the 2-mononitrate more rapidly and in smaller amounts than the 5-mononitrate (Tables I and II). Greater production of the 5-mononitrate *(endo)* than of the 2-mononitrate *(exo)* cannot be explained on the basis of sterically assisted enzymic denitration since the chemical displacement of the 5-*endo* group is more facile (25).

Isosorbide 5-mononitrate was excreted partly conjugated and isosorbide (IV) was excreted completely conjugated, probably with glucuronic acid. In dogs, isosorbide glucuronide (VI) was apparently derived from isosorbide 5-mononitrate glucuronide (V) and not from isosorbide (IV), which was excreted unchanged when administered to the dog (22, 23). Since IV is also excreted unchanged by humans (22, 26), VI was probably derived from V in humans (Tables II and III) and not from isosorbide (IV) itself. A possible metabolic pathway for isosorbide dinitrate in humans is shown in Scheme I.

These data are qualitatively similar to those obtained from rats (24) and dogs (22, 23), except that Sisenwine and Ruelius (23) did not detect isosorbide 2-mononitrate (III) in dog urine whereas Reed *et al.* (22) did. However, larger doses were administered to the animals than the clinical dosage used in the present studies.

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