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ISOSORBIDE, ISOMANNIDE AND ISOIDIDE DINITRATE: URINARY EXCRETION IN THE RAT

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Abstract—In rats given the dinitrates of isosorbide, isomannide and isoidide orally, urinary excretion products were studied by gas chromatography and thin layer chromatography. The unchanged dinitrates are excreted in small amounts; the different mononitrates and denitrated alcohols are also present; the mononitrates are conjugated to a large extent. After administration of isosorbide dinitrate, isoidide mononitrate and isoidide are excreted in the urine, and after isomannide dinitrate administration, 5-isosorbide mononitrate and isosorbide can be found. This inversion of configuration from the endo- to the exo-position could be due to the formation of a carbonyl intermediate or to a backside displacement mechanism.

THE VALUE of isosorbide dinitrate in the treatment of angina pectoris and, more specifically, its duration of action as compared to nitroglycerin, have aroused much interest in the past few years (for discussion, see Epstein *et al.*[1]). Recently, much work has been done on the metabolic fate of substances such as nitroglycerin[2] and pentaerythritol tetranitrate;[3,4] likewise in the same year two extensive studies about the metabolic fate of isosorbide dinitrate in animals have been published.[5,6] As far as we know, no metabolic data are available about the two stereoisomers of isosorbide dinitrate: isomannide dinitrate and isoidide dinitrate. In 1939, Krantz *et al.*[7] synthetized isosorbide dinitrate and its isomer, isomannide dinitrate; in 1960, Jackson and Hayward[8] prepared another isomer, isoidide dinitrate. From the scarce data in the literature, it appears that these stereoisomers have differing pharmacologic potencies.[9–12]

Because of the absence of data about the metabolism of the dinitrates of isomannide and isoidide, we decided to analyse the substances appearing in the rat urine after oral administration of the three isomers, and to look for possible differences between the patterns seen for these substances.

METHODS AND MATERIALS

Compounds. 1,4:3,6-dianhydroglucitol dinitrate or isosorbide dinitrate (ISDN) was available as a powder (Cedona Company, The Netherlands) and was purified by recristallization from ethanol. The two mononitrates of isosorbide, exo- resp, endo-1.4:3,6-dianhydroglucitol mononitrate (2-ISMN resp. 5-ISMN) were synthetized from ISDN as described previously.[13]

1,4:3,6-dianhydromannitol dinitrate or isomannide dinitrate (IMDN) was synthetized as described by Jackson and Hayward[8] from isomannide that was obtained by the method of Hockett *et al.*[14] The IMDN was purified by column chromatography.

1,4:3,6-dianhydromannitol mononitrate, the isomannide mononitrate (IMMN), was synthetized from IMDN by acid hydrolysis.

1,4:3,6-dianhydroiditol dinitrate or isoidide dinitrate (IIDN) was synthetized as described by Jackson and Hayward;[8] the isoidide was synthetized from isoidide diacetate via isomannide ditosylate.[14,15] 1,4:3,6-dianhydroiditol mononitrate, the mononitrate of isoidide (IIMN) was obtained by acid hydrolysis from IIDN.

The synthetized products were identified and their configurational identity confirmed by proton magnetic resonance (PMR) spectroscopy, at 300 MHz, as described previously for the isosorbide mononitrates.[13]

Analytical procedure. 10 ml of urine is extracted three times with 10 ml of ethyl acetate for 30 min. The organic layers obtained after centrifugation (4000 rev/min, 10 min) are combined, filtered over water free Na_2SO_4, and evaporated under vacuum at room temperature. The residue is filtered in ethyl acetate through norite filters (No 508, ϕ 4 cm); the filtrate is evaporated under nitrogen and dissolved in 10 μl of 0·03% 1,3-dinitrobenzene (DNB) in ethyl acetate, and analysed both by thin layer chromatography and gas chromatography.

Gas chromatography. 0·4 μl of the residue is injected into a Varian Aerograph (Model 2100) gas chromatograph with flame ionization detector. The nitrogen carrier flow was 30 ml/min, hydrogen flow rate was 18 ml/min, air flow rate 370 ml/min. The column was made of glass (6 ft $\times$ 2·5 mm i.d.), containing Gas Chrom Q (60–80 mesh) (Sulpelco) packed with 3·5% QF-1 (Applied Science Laboratories). Column temperature was 130°, injection port temperature 160° and detection temperature 180°. Electrometer range was 1×10^{-11} A; attenuation 1. In these conditions, a good separation of all the products used can be obtained.[16] DNB is used as an internal standard to calculate relative retention times.

Thin layer chromatography (TLC). The rest of the residue (around 9 μl) is spotted on TLC plates (0·25 mm Silica gel F-254 on glass, Merck). The solvent used is benzene–ethyl acetate 1:1. Colouring of the nitrate groups is done by spraying with diphenylamine 1% in ethyl alcohol, and irradiation for 10 min with u.v. source. The R_f values are: ISDN: 0·84; 2-ISMN: 0·41; 5-ISMN: 0·30; IMDN: 0·80; IMMN: 0·29; IIDN: 0·88; IIMN: 0·43. Quantitation of the spots on the TLC plates is done with a Vitatron Densitometer, TLD 100.[17] For some excretion products, R_f values are too close together to allow separate quantitation on the thin layer plate. In these cases, quantitation is performed by measuring the height of the peak on the gas chromatogram, and calculating the weight by comparison with the DNB peak height using the appropriate weight per cent response factors. For the denitrated alcohols no quantitation was attempted.

Animal experiments. Male rats (Wistar strain) weighing 170–200 g were fasted overnight, and in the morning 5 ml of water was given by gavage. Four mg of either ISDN, IMDN, IIDN or IMMN, dissolved in 5 ml of water, was given via the same route 2 hr later. Urine was collected in two 4-hr periods, sometimes also during the 8–20th hr. The rats were additionally hydrated every 2 hr until the 6th hr. Each urine sample was divided into 2 parts; one was extracted immediately as already described; the other part was incubated for 20 hr at 37°, pH 5, with 10,000 U of β-glucuronidase-steroid-sulphatase (Serva, Heidelberg). Afterwards the mixture was brought to pH 7 and extraction performed. Increasing the duration of incubation to 140 hr did not appreciably change the results.

RESULTS

The extracts from the urine before administration of the nitrates to the rats did not give any peaks on the gas chromatogram, nor were there interfering spots on the thin layer chromatogram. Recovery of the different nitrates was close to 100 per cent for the 2 methods.

TABLE 1. URINARY EXCRETION PRODUCTS AFTER ISOSORBIDE, ISOMANNIDE AND ISOIDIDE DINITRATE IN THE RAT

Rat	No. 1		No. 2		No. 3		No. 4		No. 5	
	4 hr	8 hr	4 hr	8 hr	4 hr	8 hr	4 hr	8 hr	4 hr	8 hr
ISDN	7·5	4·0	14·6	0	11·9	0	14·5	0	10·7	0
2-ISMN	7·1	8·8	18·0	16·3	29·5	13·9	10·4	8·8	26·6	27·0
5-ISMN	51·6	76·9	136·2	88·9	173·8	101·6	152·7	51·0	174·3	75·6
IS	+	+	+	+	+	+	+	+	+	+
IIMN	1·8	1·1	7·6	4·6	2·6	4·0	3·4	5·6	2·7	4·1
II	0	+	0	+	0	+	+	+	0	+
Rat	No. 6		No. 7		No. 8		No. 9		No. 10	
IMDN	40·5	6·7	31·0	10·1	38·5	20·2	44·8	13·5	22·6	4·2
IMMN	49·3	31·8	45·6	29·3	82·4	66·7	80·9	44·0	76·8	24·4
IM	+	+	+	+	+	+	+	+	+	+
5-ISMN	13·3	15·9	28·6	15·0	28·0	28·0	16·7	13·2	12·2	9·6
IS	0	+	0	+	0	+	0	+	0	+
Rat	No. 11		No. 12		No. 13		No. 14		No. 15	
IIDN	6·5	0	0	0	0	0	4·7	0	0	0
IIMN	32·2	11·9	51·8	18·8	35·1	12·4	27·2	22·5	22·3	11·9
II	+	+	+	+	+	+	+	+	+	+

Urine was collected for two 4-hr periods after administration of 4 mg of isosorbide dinitrate (ISDN, upper part), isomannide dinitrate (IMDN, middle part) and isoidide dinitrate (IIDN, lower part), respectively. The dinitrates and the mononitrates (2-isosorbide mononitrate or 2-ISMN, 5-isosorbide mononitrate or 5-ISMN, isomannide mononitrate or IMMN and isoidide mononitrate or IIMN) are quantitated by densitometry and gas chromatography, and the results expressed in micrograms; the dianhydrohexitols (isosorbide or IS, isomannide or IM and isoidide or II) are not quantitated, but their presence (+) or absence (0) is ascertained by gas chromatography.

Table 1 shows the products identified by gas chromatography in non-incubated urine, after administration of the different dinitrates; quantitation is also given.

For ISDN, unchanged product is found mainly in the first 4 hr; most of it appears in the urine in the first 15 min after administration. 5-ISMN is the main metabolite while 2-ISMN is excreted in much smaller amounts. After the 8th hr, only small amounts of the mononitrates are excreted. Isosorbide is found in all rats. Small amounts of IIMN are also seen, as well as isoidide. On the thin layer plates two additional spots appear after spraying and irradiating; these spots have R_f values of respectively 0·10 and 0·27; these unidentified nitrates could not be seen on the gas chromatograms.

After administration of IMDN, appreciable amounts of the unchanged product are found, mostly in the first hours, less in the second 4 hr collection period. The mononitrate IMMN is found and continues to be excreted after the 8th hr; isomannide is also present. 5-ISMN and isosorbide are also found, as shown in Fig. 1.

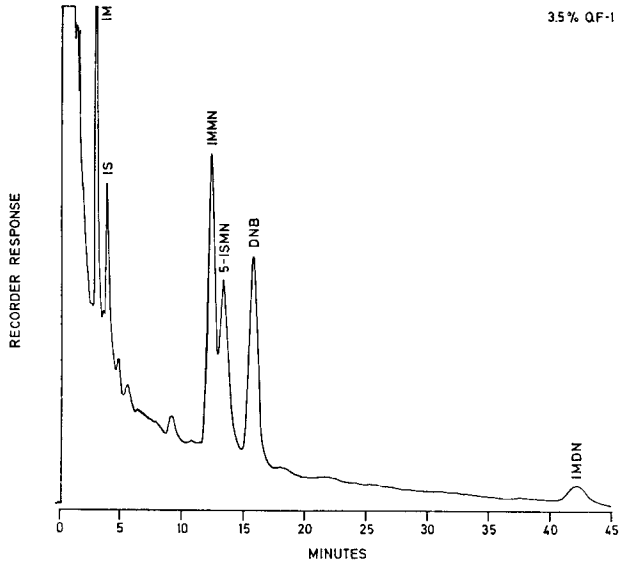

FIG. 1. Chromatogram of an extract of the urine of a rat given isomannide dinitrate, 4 mg. The urine is collected from the 4–8th hr after administration; gas chromatography with flame ionization detection, conditions as described in Materials and Methods. Abbreviations used: IM: isomannide; IS: isosorbide; IMMN: isomannide mononitrate; 5-ISMN: 5-isosorbide mononitrate; IMDN: isomannide dinitrate; DNB: 1,3-dinitrobenzene is added as internal standard, 120 ng.

After administration of IIDN, only very small amounts of the unchanged product are seen; IIMN is found in larger amounts, as is isoidide.

After incubation of the urine for 20 hr with β-glucuronidase-sulphatase, the same substances as before incubation are identified on gas chromatography; after decon-

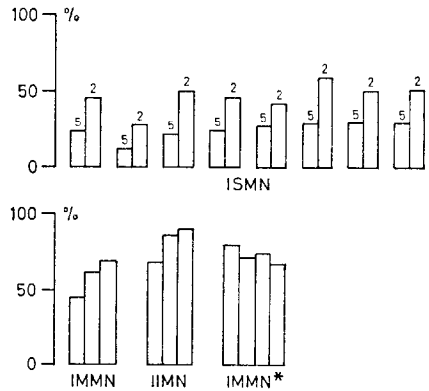

FIG. 2. Relative importance of conjugation for different mononitrates recovered from 8 hr urine of rats: 5-isosorbide mononitrate (5-ISMN) and 2-isosorbide mononitrate (2-ISMN) after administration of isosorbide dinitrate to 8 rats; isomannide mononitrate (IMMN) after administration of isomannide dinitrate to 3 rats; isoidide mononitrate (IIMN) after administration of isoidide dinitrate to 3 rats, and isomannide mononitrate (IMMN*) after administration of isomannide mononitrate itself to 4 rats. Percentage of conjugated metabolites is calculated in function of total amount. For details see text.

jugation, the amount of dinitrates does not change; Fig. 2 gives the importance of conjugation for the different mononitrates.

Finally urinary excretion was studied after administration of IMMN: unchanged product was excreted, mainly in the first 4 hr, as well as IM. Isosorbide was found in 2 out of 4 rats.

DISCUSSION

When rats are given the dinitrates of isosorbide (with one nitrate group in *exo*- and one nitrate group in *endo*-position), isomannide (with both nitrate groups in *endo*-position) or isoidide (with both nitrate groups in *exo*-position), unchanged product can be found in the urine. For ISDN this was described by Needleman and Hunter[18] in the rat; Reed *et al.*[5] and Sisenwine and Ruelius[6] did not find this in the dog. The excretion of unchanged ISDN is higher than that of unchanged IIDN but lower than that of IMDN. IIDN is only seen in very small amounts; this could be due to its higher lipid solubility[11] with higher reabsorption in the kidney tubules, or alternatively to its faster rate of denitration. Indeed, when incubated with a rat liver preparation, IIDN is metabolized at a faster rate than the other 2 dinitrates;* this is not unexpected, as the nitrate groups in exo-position are more easily available than the endo-groups, the latter being sterically shielded by the V-folded bicyclic skeleton of these sugar derivatives. IMDN is excreted in larger amounts than the 2 others, possibly due to its higher water solubility.

That 5-ISMN is excreted in much larger amounts than 2-ISMN can again be related to the fact that the 5-position is endo and lends itself less easily to enzymatic attack; it is in accordance with the findings of Sisenwine and Ruelius,[6] who found 5-ISMN to be the main metabolite in blood, and found no 2-ISMN in the urine of the dog; Reed *et al.*[5] however did not find this difference.

After administration of ISDN, IIMN and isoidide were detected. Reed *et al.*[5] found isoidide after administration of ISDN in the dog. The presence of IIMN and isoidide is not due to contamination of the ISDN given, nor to any manipulation during the extraction procedure, as ascertained by control experiments. The formation of IIMN or isoidide from ISDN, with an inversion of configuration from *endo*- to *exo*- on the 5-position, could be due, as suggested by Reed *et al.*[5] to a backside displacement (the so-called S_N2 mechanism) or to the formation of a carbonyl intermediate. Backside displacement is favoured by the presence of a leaving group such as nitrate, and of an incoming group with nucleophilic properties. UDPGA is however not easily viewed as the incoming group in this sequence, as the glucuronidation in itself requires a backside displacement from the α-configuration of UDPGA to the β-configuration of the glucuronide.[19]

We found that both isosorbide mononitrates are for a large part excreted in conjugated form. These products could be formed by conjugation of free ISMN; direct glucuronidation of ISDN, as suggested by Reed *et al.*[5] is less probable in view of the fact that glucuronidation in itself requires a backside attack, as discussed above. We found that 2-ISMN is conjugated to a large extent; Reed *et al.*[5] did not observe this in dogs, and suggested that steric hindrance would preclude a reaction such as glucuronidation in the endo-position. Buck *et al.*[20] however suggested that endo-OH groups may be reactive due to intramolecular hydrogen binding.

* N. H. Lee, unpublished results.

After administration of IMDN, IMMN is found to be conjugated to a large extent. The remarks made about conjugation on the *endo*-OH group of 2-ISMN apply here. Appreciable amounts of 5-ISMN, and also isosorbide, are present; here to, backside displacement or a carbonyl intermediate may be responsible for the change from *endo-* to *exo*-position. The view that 5-ISMN is formed from the IMDN itself is supported by the finding that after administration of IMMN, no 5-ISMN is detected; isosorbide can be found in these conditions, probably by a change from *endo-* to *exo*-position in the process of denitration of the IMMN molecule.

Our results suggest that the metabolism of the isomeric dinitrates studied proceeds by denitration, the rate of which is faster for *exo*-groups than for *endo*-groups, for which steric hindrace exists; the rat moreover is able to convert the *endo-endo* structure of isomannide to the *endo–exo* of isosorbide and the *endo–exo* structure of isosorbide to the *exo–exo* of isoidide.

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