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Micro determination of glyceryl trinitrate in biological fluids; effect of deuteration of glyceryl trinitrate on its pharmacokinetic properties

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The rate of metabolism of certain drugs may be substantially altered by substitution of the isotope deuterium for hydrogen atoms; the theoretical maximum isotope effect (K_H/K_D) is 18 and experimental values of 2–13 have been observed in biological systems (Blake et al 1975; Foster et al 1974). The kinetic effects of deuterium would be especially useful in prolonging the action of glyceryl trinitrate, which is rapidly metabolized (Armstrong et al 1979). The effect of deuteration on the disposition of glyceryl trinitrate was therefore investigated by pharmacokinetic studies using as analytical procedure our modifications of the method described by Yap et al (1978).

Materials and methods

Materials. Isosorbide dinitrate was a gift from Ayerst Laboratories Ltd., Camberley, Surrey. Lindane (γ-benzenehexachloride) (Sigma Chemicals Ltd., Poole, Dorset), diethyl ketomalonate (Aldrich Chemicals Ltd., Gillingham), g.l.c. materials (Phase Separations Ltd., Queensferry, Deeside), glass-distilled grade hexane (Rathburn Chemicals Ltd., Peebleshire, Scotland), dichlorodimethylsilane and all solvents (Analytical grade, BDH, Poole, Dorset) were used as purchased.

Synthesis of glyceryl trinitrate and deuteroglyceryl trinitrate. Glyceryl trinitrate and its fully deuterated analogue were synthesized by nitration of glycerol (Laurie 1928) or penta-deuteroglycerol respectively. The purity of glyceryl trinitrate was established on 0·25 mm silica gel plates with a solvent system of benzene–ethyl acetate–acetic acid (16:4:1 by volume), and by a g.l.c. (method described herein) to be >99%. The deuterated product had a purity >99%, as $C_3D_5N_3O_9$ by mass spectrometry.

Both the deutero-labelled and unlabelled glyceryl trinitrates were characterized by i.r. spectrometry and g.l.c.-mass spectrometry. Yields, determined colorimetrically (British Pharmacopoeia 1973), were 24% for glyceryl trinitrate, and 10% for deuteroglyceryl trinitrate; the low yields were due to losses in the purification procedure.

Pharmacokinetic determinations. Male Wistar albino rats (240–260 g) were anaesthetized with a single intraperitoneal administration of pentobarbitone sodium (Sagatal) (60 mg kg^{-1}). The right external jugular vein was cannulated and glyceryl trinitrate administered intravenously at 0·25, 0·5 and 0·75 mg kg^{-1}. The drug was also administered sublingually (0·75 mg kg^{-1}) in propylene glycol (less than 100 μl) to anaesthetized rats. Blood samples (0·5 ml) were withdrawn at regular intervals from a cannula previously inserted into the right common carotid artery and iodoacetamide (final concentration 4 mM) was added to the blood samples to terminate the denitration of glyceryl trinitrate in the blood.

Aliquots of plasma (200 μl) were extracted twelve times with 200 μl quantities of hexane. This was achieved by rapid injection of the solvent and subsequent removal of the organic layers. The extracts were pooled and isosorbide dinitrate (1·78 ng) added as a standard before concentrating the solution to 20–100 μl under a stream of N_2; 1–2 μl were injected into the chromatograph.

Gas-liquid chromatography. Chromatography was carried out using a Pye-104 gas chromatograph equipped with a heated electron capture detector (^{63}Ni). A number of columns (1·5 m × 4 mm) were initially prepared containing different types of liquid phase, OV 17, OV 1, SE 30, OV 210 and SP 2401, as used in other laboratories for the determination of glyceryl trinitrate. In each case a normal response for the standard, lindane (1 μl of a 10 pg μl^{-1} solution in hexane), was observed but no response was seen following injection of subnanogram amounts of glyceryl trinitrate. The most promising results were

Table 1. Effect of dose on the pharmacokinetics of glyceryl trinitrate administered intravenously to the rat. Results are presented as mean (with s.d.).

Dose (mg kg^{-1}) (n =)*	$t_{\frac{1}{2}}$ (min)	C_0 (ng ml^{-1})	V_D (l)	K_{el} (min^{-1})	AUC_{tot} (ng min ml^{-1})
0·25 (4)	4·7 (1·2)	17 (10)	5·4 (2·1)	0·16 (0·04)	103 (44)
0·50 (4)	4·4 (0·7)	24 (12)	7·3 (2·9)	0·16 (0·03)	152 (82)
0·75 (2)	4·5, 3·7	47, 50	10·6, 10·0	0·15, 0·19	313, 263

* No. of animals.
The half-life ($t_{\frac{1}{2}}$) was determined from semi-logarithmic plots of plasma level vs time. The initial plasma level (C_0) was estimated from the same graph following extrapolation of the curve to zero time. The other parameters were calculated from the following equations:

$$K_{el} = \frac{0.693}{t_{\frac{1}{2}}} \qquad AUC_{tot} = \frac{C_0}{K_{el}} \qquad V_D = \frac{Dose}{C_0}$$

† Correspondence.

obtained with a 3% SP 2401/Supelcoport column (Yap et al 1978); and to eliminate adsorption and to achieve a reproducible, specific, and sensitive system, the following modifications were made: (a) the elimination of all metal surfaces in the chromatography system; (b) the empty glass column was deactivated by a procedure described by De Nijs et al (1978) involving heating a pre-column containing carbowax 20 M above the maximum temperature for this liquid phase, so that degradation products flow out of the pre-column and through the empty column to deposit on active sites and so inactivate these; (c) a 5% liquid phase of SP 2401 was used in place of the original 3% SP 2401; (d) the glass wool plug was discarded to eliminate adsorption onto this material; (e) all glassware used was silanized by soaking in 5% dichlorodimethylsilane/toluene for at least 20 h,: it was then rinsed successively with toluene, water, methanol and hexane and dried before use; (f) any remaining adsorption sites were saturated by injecting a large amount of glycerol trinitrate before the analyses of plasma samples.

A 1·5 m × 4 mm int. diam. glass column packed with 5% SP 2401 on 100/120 mesh Supelcoport was finally used. The conditions employed were as follows: detector temperature, 180 °C; column temperature, 145 °C; N_2 (Air Products) flow rate, 75 ml min^{-1}; detector (pulsed mode) 50 μs; electrometer settings, 1–2 × 10^{-9} A.

Results and discussion

The pharmacokinetic properties in rats were independent of dose when the drug was administered intravenously (Table 1). The drug appears to be eliminated from plasma with a $t_{\frac{1}{2}}$ of about 4·5 min, similar to that reported by Yap et al (1978) and Maier et al (1980).

Replacement of hydrogen with deuterium in glyceryl trinitrate appears to have no significant effect on the biological half-life or any other parameter studied, when the drug was administered intravenously or sublingually (Table 2). There was only a slight increase in the volume of distribution of the drug following deuteration which may be due to the more lipophilic character of the deuterated drug. The general lack of effect observed after deuteration is not surprising in view of the type of metabolism that glyceryl trinitrate undergoes. Only a secondary isotopic effect may be expected and although this is generally small (max K_H/K_D = 1·25) it may be significant in cases where the therapeutic effect of a drug is sensitive to kinetic changes.

Glyceryl trinitrate, because of its lipophilic nature, is rapidly absorbed and extensively distributed. Its elimination obeys first-order kinetics for a single compartment model remaining constant at different dose levels (Table 1). Considerable variation was observed in the rates of elimination of the compound in different animals within a group and especially in different batches of animals (Tables 1 and 2). Similar observations have been reported by Yap et al (1978).

Di Carlo & Melgar (1970) demonstrated that glyceryl trinitrate is rapidly degraded in rat plasma at 37 °C by an enzyme system that has no cofactor requirements. They estimated that the half-life of plasma metabolism was approximately 20 min; however, these workers used plasma concentrations of the drug (22 μg ml^{-1}) far higher than those achieved after therapeutic doses. We used much lower concentrations (125 ng ml^{-1}), and the rate of degradation was much slower, the half-life being nearly 1 h. The biological half-life of glyceryl trinitrate obtained in vivo was 4–7 min, demonstrating that the plasma degradation of the drug contributes little (approx. 10%) to its overall elimination. Iodoacetamide at 4 mM was an efficient inhibitor of degradation of glyceryl trinitrate (3·75–37·5 ng in 200 μl) in plasma, giving 93–100% protection of the glyceryl trinitrate present in plasma after 5 h at room temperature. In the absence of iodoacetamide, there was a degradation of 75%. Iodoacetamide also was not removed by the extraction procedure and therefore did not interfere with the g.l.c. determination.

This degradation can be inhibited also by silver nitrate but this, when added to whole blood, gives rise to gelling (Yap et al 1978).

The g.l.c. modifications resulted in a method which

Table 2. Effect of deuteration on the pharmacokinetic properties of glyceryl trinitrate. The drug was administered intravenously (0·75 mg kg^{-1}). Results are presented as means (with s.d.).

Route of administration (n =)*	$t_{\frac{1}{2}}$ (min)	C_0 (ng ml^{-1})	V_D (l)	K_{el} (min^{-1})	AUC_{tot} (ng min ml^{-1})
Intravenous					
Deuterated (5)	6·6 (1·9)	44 (30)	6·0 (2·9)	0·11 (0·04)	359 (123)
Non-deuterated (6)	6·7 (1·0)	53 (21)	4·6 (2·0)	0·10 (0·02)	506 (164)
Sublingual					
Deuterated (4)	17·4 (1·6)	17·0 (7·2)	15·7 (6·7)	0·04 (0·004)	408 (140)
Non-deuterated (5)	14·3 (3·4)	20·0 (3·5)	11·4 (1·7)	0·05 (0·018)	426 (148)

* No. of animals.

For determination of the pharmacokinetic parameters see Table 1. Following sublingual administration the absorption of glyceryl trinitrate is rapid and therefore the same kinetic equations can be used as for intravenous injection.

was specific (metabolites are not readily extracted by the procedure used, and no endogenous compounds gave retention times close to glyceryl trinitrate), reproducible, and sufficiently sensitive to determine concentrations of the drug as low as 500 pg ml^{-1} of plasma, with only a 200 μl sample of plasma. The retention times for glyceryl trinitrate and isosorbide dinitrate were 4·5 and 9·7 min respectively. Both peaks were symmetrical and no tailing was apparent. The g.l.c. characteristics of the deutero-glyceryl trinitrate were similar to those of the non-deuterated compound. Detector response was the same for glyceryl trinitrate and deutero-glyceryl trinitrate, and was linear up to 450 pg of glyceryl trinitrate. Reproducibilities of determination from rat plasma for 4 determinations of each were means of ±10·6% at 0·375 and ±10·4% at 0·75 ng glyceryl trinitrate/200 μl of plasma, and were ±3·2% at 3·75 and ±3·9% at 7·50 ng/200 μl of plasma. Recoveries from plasma were 75–80% and were not adversely affected by the addition of iodoacetamide.

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Response of human ventricular heart muscle to histamine

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Histamine is present in large quantities in the heart and can significantly alter cardiac function (for review see Levi et al 1976; Owen 1977). Current evidence suggests that the effects of histamine on the heart are mediated by both, H_1- and H_2-receptors, the distribution and function of which seems to depend on species and region of the heart (Owen 1977). We have characterized the effects of histamine on action potential and force of contraction in human ventricular heart muscle. Histamine produced distinct changes of the action potential configuration and force of contraction which were blocked in the presence of cimetidine. The results are consistent with an increase of the calcium conductance at the myocardial cell membrane in response to H_2-receptor stimulation.

The effects of histamine on action potential and force of contraction were determined in human papillary muscles obtained from patients during cardiac surgery. Immediately after excision, the preparations were placed in cold (4 °C) oxygenated Tyrode's solution and carried to the laboratory. The time between excision and the beginning of the laboratory processing was about 90 min. In the laboratory, the muscles were transferred to a dissection chamber containing oxygenated Tyrode's solution and split into thin preparations so that the fibres ran parallel to the length of the strip. For recording electrical and mechanical activity, the preparations were mounted in a 1 ml organ bath which was continuously perfused with Tyrode's solution. One end of the preparation was connected to an inductive force displacement transducer by means of a stainless steel wire, and the other end was fixed to keep the muscle length as constant as possible. The Tyrode's solution was prepared with distilled deionized water and had the following composition (mM): NaCl, 136·9; KCl, 5·4; $MgCl_2$, 1·05; NaH_2PO_4, 0·42; $NaHCO_3$, 11·9; $CaCl_2$, 1·8; glucose, 5·6. The Tyrode's solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2; the temperature was kept at 37·0 ± 0·2 °C. All muscles were driven electrically at 0·2 Hz by rectangular pulses of 1–5 ms duration (Grass stimulator and isolation unit; intensity 10–20% above threshold). Tension was recorded under isometric conditions at the apex of the preload active tension curve. The transmembrane potential was detected intracellularly by the use of 5–15 MOhm glass microelectrodes filled with 3 M KCl.

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