PHARMACOKINETIC STUDIES OF VARIOUS PREPARATIONS OF GLYCERYL TRINITRATE

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- 1 The pharmacokinetics and pharmacodynamics of five pharmaceutical preparations of glyceryl trinitrate (GTN) have been studied in six healthy volunteers.
- 2 Peak plasma GTN levels of 1.4 ± 0.1 ng/ml were achieved at 3 min after sublingual administration (0.5 mg); 2.8 ± 0.6 ng/ml and 2.5 ± 0.3 ng/ml at 2 h and 4 h after Nitrocontin (6.4 mg) and Sustac (6.4 mg) respectively; 2.7 ± 0.1 ng/ml and 2.5 ± 0.2 ng/ml at 30 min after the cream (23 mg) and ointment (35 mg) respectively.
- 3 The mean times to reach half peak plasma GTN levels were 4.2 ± 0.7 min after sublingual GTN, 6.5 ± 0.9 h after Nitrocontin, 4.1 ± 0.7 h after Sustac, 46.1 ± 10.2 min after the ointment and 50.2 ± 39.2 min after the cream.
- 4 Plasma nitrate (NO_3) levels were undetectable after sublingual administration; peak NO_3 levels were 0.48 ± 0.03 $\mu\text{g/ml}$ at 4 h after Nitrocontin, 0.45 ± 0.02 $\mu\text{g/ml}$ at 6 h after Sustac, 0.68 ± 0.04 $\mu\text{g/ml}$ at 6 h (end of study) after the ointment and 0.63 $\mu\text{g/ml}$ at 4 h after the cream.
- 5 Plasma nitrite (NO_2) was undetectable after sublingual GTN and peak NO_2 levels were 0.48 ± 0.04 $\mu\text{g/ml}$ at 2 h after Nitrocontin, 0.48 ± 0.02 $\mu\text{g/ml}$ at 2 h after Sustac, 0.69 ± 0.02 $\mu\text{g/ml}$ at 1 h after the ointment and 0.64 ± 0.02 $\mu\text{g/ml}$ at 1 h after the cream.
- 6 Blood pressure (BP) declined throughout the 11 min of the study after sublingual GTN but did not change significantly after Nitrocontin or Sustac. After the ointment and the cream BP fell to the lowest level 30–60 min after administration and remained low throughout the 6 h of the study.
- 7 Heart rate (HR) increased to a maximum 5 min after sublingual administration with no significant increase after Nitrocontin or Sustac. After the ointment and the cream HR increased gradually to a maximum 30–60 min after administration remaining high throughout the rest of the study.

Introduction

Organic nitrates such as glyceryl trinitrate (GTN) are lipid-soluble and can therefore penetrate the cell membrane. Within the cell they are a source of the nitrite ions which mediate their smooth muscle relaxant effect (Needleman & Hunter, 1965; Needleman *et al.*, 1969). Nitrate (NO_3) and nitrite (NO_2) ions penetrate the cell membrane only poorly, but some efflux of these anions does occur and their plasma levels thus reflect their supply from GTN within the cell.

Previous pharmacokinetic studies of organic nitrate preparations in humans have not included measurement of plasma NO_3 and NO_2 concentrations. Measurement of plasma NO_3 and NO_2 levels for other reasons have generally used relatively insensitive assay techniques (Lorenzetti *et al.*, 1966; Axelrod & Engel, 1975; Williams, 1979). We have accordingly developed more sensitive gas liquid chromatographic (g.l.c.) techniques to measure free NO_3 and NO_2 levels in plasma. Using these and an established g.l.c. assay to measure GTN, we have

carried out pharmacokinetic studies of five different pharmaceutical preparations of GTN given to normal volunteers.

Methods

Protocol

Six healthy male volunteers (aged 25–32 years, weight 63–70 kg) were given single doses of each of five different GTN preparations at intervals of > 1 week. In each case the preparation was given at 10.00 h after an overnight fast. Ingestion of canned foods was avoided for > 3 days before the study.

Sublingual GTN (Evans Medical Ltd,) containing 0.5 mg GTN was administered by placing a tablet under the tongue for 5 min without active sucking or chewing. Blood was sampled before treatment and at 1, 3, 5, 7, 9 and 11 min after administration. Nitrocontin (Napp Laboratories Ltd) and Sustac (Pharmax

Ltd) tablets each containing 6.4 mg GTN were administered orally. Blood samples were taken before treatment and at 0.5, 1, 2, 4, 6 and 10 h after administration. Doses of GTN cream (Napp Laboratories Ltd) and GTN ointment (Marion Laboratories Ltd) containing 23 mg and 35 mg GTN respectively, were administered by applying 2.5 in from a standard dispensing tube to the anterior chest wall, spreading the preparation with a gloved hand over an area of 36 sq. in., and covering the area with a polythene sheet for 6 h. Blood samples were taken before treatment and at 0.25, 0.5, 1, 2, 3, 4 and 6 h after application.

Supine blood pressure (BP) and heart rate (HR) were measured by the same observer at the times indicated in Figure 3 using a standard mercury sphygmomanometer (diastolic phase 5).

Assay techniques

(a) *GTN assay* Venous blood samples were collected in iced heparinised glass tubes containing 50 μ l 0.1 N silver nitrate to prevent *in vitro* metabolism of GTN (Yap *et al.*, 1978). Samples were immediately centrifuged at 800 g for 15 min at 4°C. Supernatant plasma (3 μ l) was transferred to a 10 ml capacity glass tube and 10 μ l of 25 ng/ml γ -lindane in hexane were added to each sample as internal standard. Hexane was used instead of ethylacetate as previously described (Rosseel & Bogaert, 1973) because it is more easily purified and was found to extract fewer unwanted plasma constituents. γ -lindane was used instead of isosorbide dinitrate as previously described (Rosseel & Bogaert, 1973; Yap *et al.*, 1978; Armstrong *et al.*, 1979) because it is more soluble in hexane and has a shorter retention time. Double-distilled hexane (3 μ l) was added to each sample and the samples were agitated on a flat bed shaker for 8 min. The samples were then centrifuged at 800 g for 10 min at 4°C and the hexane layer was transferred to a glass conical tube cooled to 0°C on ice. The extraction was repeated three times. The three hexane layers were combined and evaporated under an air stream to near dryness. The final volume after evaporation was not critical since all calculations were based on the peak height of the internal standard. The concentrated hexane solution was agitated using a rotor mixer for 5–10 s and the samples stored at –20°C. Assays were performed within 4 weeks.

A g.l.c. apparatus (Pye-Unicam, UK Model 104) supplied with an electron capture detector (ECD) containing 10 mCiNi⁶³ as the radioactive source was used for the GTN measurements. The glass columns (150 cm long, 2 mm internal diameter) were packed with 3% SP-2401 on 100–120 mesh Supelcoport conditioned at 140°C. The columns were purged for 30 h before use. The column oven temperature was 140°C and the detector oven temperature was 200°C.

Oxygen-free nitrogen was used as the carrier gas at a flow rate of 80 ml/min. The concentrated hexane solution (5 μ l) was injected into the column using a 10 μ l g.l.c. glass syringe. A new standard curve was constructed on each experimental day by plotting the ratios of the peak heights of the γ -lindane and GTN chromatograms against the GTN concentrations. Unknown concentrations of GTN were estimated from these graphs.

The retention times for the GTN chromatogram were: air 13 s, hexane 15 s, GTN 510 s, γ -lindane 627 s. The relative retention time of GTN to γ -lindane was thus 0.81. The lower limit of GTN detection was 0.2 ng/ml. The coefficients of linear regression analysis of the calibration curves were found to be in the range of 0.93–0.99 on four different days. The intra-assay coefficient of variation for GTN concentrations of 0.5–5 ng/ml was 10.7% ($n = 6$). The mean percentage recoveries for concentrations between 0.5–5 ng/ml was $82.2 \pm 1.6\%$ (s.e. mean) ($n = 6$).

(c) *Nitrate assay* Blood (5 μ l) was centrifuged in heparinised polyethylene tubes at 800 g for 15 min at 4°C. Plasma (1 ml) was added to a clean glass tube containing 50 mg of twice washed silver sulphate. The tube was shaken for 15 min on a table shaker and centrifuged for 3 min as above. The sample was then deproteinised with 100 μ l of nitrogen-free sulphuric acid and the tube centrifuged as above for a further 5 min. The supernatant (0.2 ml) was transferred to a clean glass vial with a polyethylene stopper and 10 μ l of a 25 μ g/ml solution of nitro-ortho-xylene (NOX) were added as the internal standard followed by the addition of 1 ml of double distilled benzene and 1 ml of nitrogen-free sulphuric acid. The reaction vial was tightly stoppered and shaken for 8 min using a table shaker. The benzene layer was removed immediately using a Pasteur pipette. The nitrobenzene generated from the reaction between NO₃ and benzene was analysed by g.l.c.-ECD as follows.

The g.l.c. and ECD used were as described for the GTN assay. The columns (150 cm long, 2 mm internal diameter) were packed with 1.5% SE-30 coated on 80–100 mesh, acid-washed Chromosorb G. The column oven temperature was 125°C and detector oven temperature was 180°C. The carrier gas used was argon:methane (90:10) at a flow rate of 60 ml/min.

All the operating conditions were maintained constant throughout the study. 0.6–2 μ l of the benzene layer was injected into the g.l.c. column using a 10 μ l g.l.c. glass syringe. A standard curve was constructed on each experimental day. The chromatograms were quantified by the method described for the GTN assay, using peak height ratios. The retention times of the chromatograms were: air 56 s, benzene 62 s, nitrobenzene 245 s, and NOX 770 s. The relative retention time of nitrobenzene to NOX was thus

0.264. The lower limit of NO_3 detection was 10 ng/ml. The coefficient of linear regression analysis was in the range of 0.94–0.97 on four different days. The intra-assay coefficient of variation over the range of 0.25–2 $\mu\text{g/ml}$ was 3.6% ($n = 6$). The mean percentage recovery was $85.1 \pm 1\%$ (s.e. mean) ($n = 6$) in the range of 0.25–2 $\mu\text{g/ml}$ NO_3 .

(c) *Nitrite assay* G.l.c. measurement of NO_2 was determined by oxidation of NO_2 to NO_3 . The difference in the measurement of NO_3 before and after oxidation gave the NO_2 content. Two 0.2 ml plasma samples, prepared as described previously, were used for each measurement. One sample was treated for NO_3 measurement as described above. 20 μl of 1 N hydrogen peroxide was added to the other sample and the NO_3 content measured. NO_2 was measured from the difference between the peak heights of NO_3 before and after oxidation. The ratio of this difference to the peak height of the internal standard (NOX) was calculated. A standard calibration curve was constructed on each experimental day from plasma to which had been added known concentrations of potassium nitrite. The lower limit of sensitivity of the assay was 10 ng/ml. The standard calibration curve was linear over the range of 0.25–2 $\mu\text{g/ml}$ with a coefficient of linear regression in the range of 0.95–0.98 on four experimental days. The intra-assay coefficient of variation over the range 0.252 $\mu\text{g/ml}$ was 8.8% ($n < 6$). The mean percentage recover was $86.6 \pm 1.9\%$ (s.e. mean) ($n = 6$).

The results are expressed as mean $\pm$ s.e. mean. Student's *t*-test for paired data was used to compare data where appropriate.

Results

(a) Plasma GTN

Peak plasma GTN concentrations were 1.4 ± 0.1 ng/ml at 3 min after sublingual GTN, 2.8 ± 0.6 ng/ml at 2 h after Nitrocontin, 2.5 ± 0.3 ng/ml at 4 h (range 2–4 h) after Sustac, 2.7 ± 0.1 ng/ml at 30 min after the cream and 2.5 ± 0.2 ng/ml at 30 min after the ointment (Figure 1). Although the peak GTN concentrations reached were similar after cream or ointment, at 15 min significantly higher levels were present after the cream than after the ointment.

Calculation of plasma half-life is inappropriate because plasma levels had not achieved steady state. The mean times to reach the half-peak plasma levels from the time of the peak have therefore been used. These values were 4.2 ± 0.7 min after sublingual GTN, 6.5 ± 0.9 h after Nitrocontin, 4.1 ± 0.7 h after Sustac, 46.1 ± 10.2 min after the ointment and 50.2 ± 39.2 min after the cream.

The areas under the curves (AUC) were calculated by the trapezoidal rule. The AUCs up to 10 h after Nitrocontin and Sustac were 17.98 ± 1.10 ng ml⁻¹ h and 16.48 ± 2.08 ng ml⁻¹ h respectively and up to 6 h after the ointment and cream were 5.22 ± 0.01 ng ml⁻¹ h and 7.10 ± 1.63 ng ml⁻¹ h respectively. There was no significant difference in AUC between Nitrocontin and Sustac but the AUC of the cream was significantly greater than the ointment.

(b) Plasma NO_3

Plasma NO_3 concentrations were undetectable after

Figure 1 Mean ($\pm$ s.e. mean) plasma GTN levels following administration of sublingual GTN (●); Nitrocontin (○); Sustac (□); GTN cream (Δ); and GTN ointment (▽).

sublingual GTN. Peak plasma values were $0.48 \pm 0.03 \mu\text{g/ml}$ at 4 h (range 4–10 h) after Nitrocontin, $0.45 \pm 0.02 \mu\text{g/ml}$ at 6 h (range 4–10 h) after Sustac, $0.68 \pm 0.04 \mu\text{g/ml}$ at 6 h (range 4–6 h) (the end of the study) after the ointment and $0.63 \pm 0.036 \mu\text{g/ml}$ at 4 h (range 2–6 h) after the cream (Figure 2a).

(c) Plasma NO_2

Plasma NO_2 concentrations were likewise undetectable after sublingual GTN. Peak values were $0.48 \pm 0.04 \mu\text{g/ml}$ at 2 h (range 1–2 h) after Nitrocontin, $0.48 \pm 0.02 \mu\text{g/ml}$ at 2 h (range 2–4 h) after Sustac, $0.69 \pm 0.02 \mu\text{g/ml}$ at 1 h after the ointment and $0.64 \pm 0.02 \mu\text{g/ml}$ at 1 h (range 0.5–1 h) after the cream (Figure 2b).

(d) Blood pressure

After sublingual GTN BP declined throughout the 11 min of the study. After Nitrocontin or Sustac BP changes were small and not significant. After both the ointment or the cream systolic and diastolic supine BP fell to their lowest levels at 30–60 min and remained low throughout the 6 h of the study (Figure 3).

(e) Heart rate

After sublingual GTN HR increased to a maximum at 5 min. After Nitrocontin or Sustac, HR increased only slightly. After the ointment or the cream HR increased gradually to a maximum at 30–60 min and remained high throughout the 6 h of the study (Figure 3).

Discussion

The pharmacokinetics of sublingual GTN were similar to those previously described (Armstrong *et al.*, 1979; Wei & Reid, 1979). Data following slow release oral preparations of GTN or cutaneously absorbed preparations are scanty. A previous investigation by Blumenthal and co-workers (Blumenthal *et al.*, 1977) compared plasma GTN levels in one subject following sublingual (0.3 mg) oral (6.5 mg) and topical (16 mg) GTN administration. These workers found markedly lower plasma GTN concentrations after each of the preparations than were found in the present study. The reasons for these discrepancies in plasma GTN concentrations are difficult to explain since in the previous study no details were provided concerning the methods of administration of either the sublingual or topical preparations and no information was given on the pharmaceutical preparation of the orally administered capsule. Details of assay reproducibility were also omitted from that study. A recent report (Armstrong *et al.*, 1980) describes peak levels at 60 min following GTN ointment given to patients in heart failure and maintained plasma GTN levels for 4 h compared with our finding of peak levels at 30 min followed by a more rapid decline; these differences may be related to smaller skin area (9 sq. in.) used in that study than in ours (36 sq. in.) and to reduced hepatic metabolism in patients in heart failure. The present study shows that GTN absorption was slightly more rapid with the cream than the ointment, due possibly to its lower solubility in a water based cream. Apart from the 4 h sample subsequent levels were similar following the cream or ointment but the AUC of the cream was significantly greater than the ointment indicating a greater bio-availability of the cream than the ointment. Plasma

Figure 2 Mean ($\pm$ s.e. mean) plasma levels of (a) NO_3^- and (b) NO_2 following administration of Nitrocontin (O), Sustac ($\square$), GTN cream (Δ) and ointment (∇).

Figure 3 Mean HR (broken lines), systolic BP (open symbols, solid lines) and diastolic BP (closed symbols, solid lines) after sublingual GTN (X), Nitrocontin (O), Sustac (□), GTN cream (Δ) and ointment (▽).

levels of GTN following oral Nitrocontin or Sustac rose more slowly than after the topical preparations, reaching peak levels only after 2–4 h. Plasma levels rose slightly earlier with Nitrocontin than with Sustac, but subsequent levels were similar with these two oral preparations.

The peak GTN levels achieved following Nitrocontin, Sustac, the ointment or the cream were similar at 2.5–2.8 ng/ml and about double the peak level of 1.4 ng/ml reached after sublingual GTN. Plasma GTN levels declined to half their peak levels in 4–6 h after Nitrocontin or Sustac, and in < 1 h after the ointment or the cream.

Plasma levels of NO_3 and NO_2 were undetectable after sublingual GTN but rose to peak levels of 0.5–0.7 $\mu\text{g/ml}$ after the other four preparations. Peak NO_2 levels were always reached earlier (1–2 h) than peak NO_3 levels (4–6 h) as was found in an earlier experimental study in rats (Lorenzetti *et al.*, 1966). Interestingly although plasma GTN levels reach similar peaks after the oral or the topical preparations and declined faster after the topical preparations, the levels of NO_2 and NO_3 were higher after the topical than after the oral preparations. This unexpected observation implies that more NO_3 and NO_2 enters the blood as a net result of GTN metabolism when topically applied than when given orally.

Supine BP and HR provided a simple measure of haemodynamic effect of these GTN preparations in these normal subjects. The haemodynamic changes

(systolic and diastolic BP and HR measured at the time of the respective peak GTN plasma levels) were all significantly greater after sublingual GTN than after either of the oral preparations despite the much lower plasma GTN level after sublingual GTN. Furthermore, although the differences did not reach statistical significance, the haemodynamic changes after the topical preparations were greater than after the oral preparations despite similar peak plasma GTN levels. These haemodynamic measurements were made at 3 min after sublingual GTN 30 min after the cream or the ointment and 2–4 h after the oral preparations. It has previously been observed that GTN given i.v. induced a fall in BP in experimental rabbits whereas GTN given orally did not, despite comparable plasma GTN levels (Bogaert *et al.*, 1970). It seems likely that this apparent temporal relationship between the haemodynamic effects and the plasma levels of GTN reflects a rapid development of tolerance. We have previously demonstrated that once daily administration of sublingual GTN to normal subjects is not associated with any evidence of tolerance in respect of standing HR (Ross *et al.*, 1981). Tolerance to chronically administered isosorbide dinitrate is well documented (Thadani *et al.*, 1980). The present findings suggest however that marked tolerance to more frequent or continued exposure to GTN cannot be ignored and may be clinically important.

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(Received May 16, 1982,
accepted August 10, 1982)