SUMMARY  Pharmacokinetic analysis of nitroglycerin (GTN) has been hampered by the lack of a sensitive and specific method for measuring GTN in blood. Therefore, we examined the appearance of GTN in blood after administering 0.6 mg sublingually in 10 studies of normal volunteers. We used a gas-liquid chromatographic method with electron-capture detection and isosorbide dinitrate as the internal standard. GTN appeared in blood at 0.5 minutes, reached a peak of 2.3 ± 0.36 ng/ml at 2 minutes, fell to 50% of peak value at 7.5 minutes and was barely detectable at 20 minutes. These blood levels paralleled the changes in heart rate and systolic blood pressure. These data show rapid appearance and disappearance of GTN from blood after sublingual administration, a large volume of distribution, and a rapid rate of total body clearance that precludes the liver from being the sole elimination site. This method for analysis of GTN and isosorbide dinitrate should be helpful in defining the role of chronic nitrate therapy.

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Blood Levels After Sublingual Nitroglycerin

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NITROGLYCERIN (GTN) HAS BEEN USED for over a century, but uncertainty exists about many aspects of its use. The lack of a sensitive and specific method for measurement of GTN in blood has precluded pharmacokinetic analysis, adding to this uncertainty. Traditionally, GTN has been used in sublingual form for the therapy of angina pectoris. There is continuing interest in the development of long-acting nitrate preparations as prophylactic therapy for angina. This interest has been stimulated by recent research on the role of nitrate therapy in acute myocardial infarction and chronic congestive cardiac failure. The overall role of long-acting nitrate therapy however, has not been established.

In 1973, Rosseel and Bogaert reported a gas-liquid chromatographic method for identifying and quantitating GTN and isosorbide dinitrate (ISDN) in plasma; they also demonstrated that GTN could be recovered from blood after sublingual administration. GTN has also been recovered from blood after sublingual administration using a modification of this method. However, in both of these reports the data were obtained from a single patient.

In this investigation we attempted to establish whether a relationship exists between the blood levels of GTN and its physiologic effects after sublingual administration.

Methods

Ten studies were conducted in seven normal male volunteers (ages 24–48 years). The studies were done with the subject in the supine posture. During a 10–15-minute control period, heart rate and indirect sphygmomanometric blood pressure measurements were made to ensure stability. Eighty milliliters of blood were withdrawn through an indwelling venous
cannula in the left antecubital fossa for the preparation of a standard curve. After the control period, 0.6 mg GTN (Nitrostat, Parke Davis Co, Brockville, Ontario) was administered sublingually. Zero time was defined as the time when dissolution of the tablet was perceived, and varied from 15-90 seconds after administration. At 0.5, 2, 5, 7.5, 10, 20 and 30 minutes, 10-ml aliquots of blood were withdrawn and heart rate and blood pressure measurements recorded. Observations at 0.5 minutes were made in the last four studies only. Statistical analysis of changes from control measurements was performed using a paired t test.

**Standard Curve**

The 80-ml pool of control blood was divided into eight 10-ml samples and held at 37°C. Two hundred microliters of a solution of GTN in saline (0.025-0.15 μg/ml) was added to each sample to give duplicate standards containing 0.5, 1.5 and 3.0 ng GTN/ml blood. An equal volume of saline was added to two control samples. The samples were incubated at 37°C for 1 minute, centrifuged at 0°C and 2,000 rpm for 15 minutes and the plasma was removed. Because of variation in column sensitivity, a standard curve was run as described on each experimental day.

**Extraction Procedure**

Blood samples were immediately centrifuged at 0°C at 2,000 rpm for 15 minutes and the plasma was removed. ISDN (100 ng) was added to each 4-ml aliquot of plasma to serve as an internal standard. Fifteen milliliters ethyl ether, freshly distilled over potassium hydroxide pellets, and 0.1 ml of 0.2N NaOH were also added. After shaking for 10 minutes and centrifuging at 1,500 rpm for 3 minutes, the ether layers were decanted to clean centrifuge tubes. After the addition of a further 15 ml of ether, the procedure was repeated, the ether layers were pooled and the plasma discarded. Five ml 0.04 N H₂SO₄ was added to the pooled ether layers, and after 10 minutes of shaking and centrifugation to break the emulsion, the acid layer was discarded. Anhydrous MgSO₄ was added and the samples were shaken for 20 minutes and centrifuged for 3 minutes at 1,500 rpm. Twenty-five-milliliter aliquots were transferred to clean centrifuge tubes and evaporated to dryness on ice under a stream of nitrogen gas. The remaining residues were dissolved in 300 μl benzene and frozen at −20°C until chromatography.

**Chromatography**

At the time of chromatography, samples were thawed and 2 μl were injected into a Hewlett Packard 5700A gas-liquid chromatograph with a 44Ni radioactive source in the electron-capture detector. The 6-foot coated glass column, 2 mm I.D., 0.25" O.D. with a 4" precolumn, was packed with 10% OV-101 on chromosorb W-HP (100-120 mesh). Operating conditions were as follows: injection port, 150; oven, 130; detector, 200°C. The argon:methane (95:5) carrier gas flow rate was 55 ml/min. Under these conditions

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Representative chromatograph. The left panel shows control blood samples plus the internal standard isosorbide dinitrate. The right panel shows a sample taken 2 minutes after 0.6 mg sublingual nitroglycerin (GTN).
the retention times were 4.5 and 16 minutes for GTN and ISDN, respectively.

Before each injection, the column was loaded with a solution of GTN in benzene (10 ng/10 μl) to saturate all binding sites. Thereafter, a standard test solution containing 0.2 ng of GTN and 0.6 ng of ISDN in 2 μl of benzene was injected on the column to assess its sensitivity. The first injection on any given day was arbitrarily defined as 100%, and corrections for loss of column sensitivity were made by comparing the change in chromatographic response to the test solutions compared with the initial injection. If column sensitivity fell below 50% of its initial value, sensitivity was enhanced by replacing the precolumn. The coefficient of variation of the method at a level of 7 ng/ml of GTN in blood was 13.6% using these techniques. Studies also showed that this technique was capable of recovering 78.2 ± 7.3% of GTN added to plasma (n = 5).

Results

Figure 1 shows a chromatogram from one experiment. In the left panel is a control sample of blood plus an internal standard and in the right, a sample recorded 2 minutes after the administration of 0.6 mg sublingual GTN.

Figure 2 demonstrates a plot of heart rate, blood pressure and blood GTN during the control period and after 0.6 mg GTN. All values represent mean ± SEM for n = 10, except at 0.5 minutes, where n = 4. At 0.5 min, a small amount of GTN appeared in the blood. This was associated with a rise in heart rate and a modest fall in systolic blood pressure. The heart rate remained significantly increased over control values at 2 (p < 0.01) and 5 minutes (p < 0.02) and began to return toward control levels at 7.5 minutes. At 30 minutes, heart rate was lower than the control values. Systolic blood pressure fell maximally at 5 minutes (p < 0.01) and rose gradually toward its control value. There was no significant change in diastolic blood pressure. The blood GTN level peaked at 2 minutes (p < 0.001) and fell off rapidly, reaching 50% of its peak value at 7.5 minutes. It was barely detectable by 20 minutes.

To permit calculation of blood concentration of GTN at zero time (t0) and its half-life, (t½), we have assumed that the 0.6 mg sublingual dose was instantaneously distributed in a manner similar to an intravenous bolus. Log blood GTN concentration was plotted against time and from this relationship: t0 = 3.3 ng/ml and t½ = 4.4 min. The volume of GTN distribution,

\[ V_d \text{ (blood GTN at } t_0) \]

= 179.6 l and its total body clearance

\[ \frac{V_d \times 0.693}{t_{is}} \]

= 28 l/min.

Discussion

This study demonstrates that after sublingual administration, GTN appeared in the blood promptly and its level peaked within 2 minutes after dissolution of the tablet and then fell rapidly to levels that were barely detectable at 20 minutes. Furthermore, there was a good association between the blood levels of GTN and the time course of the physiologic effects after sublingual administration.

In our study using a blood concentration at t0 of 3.3 ng/ml, the Vd of GTN is 179.6 l and the total body GTN clearance is 28.0 l/min. Although the liver is important for GTN metabolism, this high rate of GTN clearance is much greater than hepatic blood flow, precluding it as the sole elimination site.4 The disappearance of GTN may have been hastened by denitrification in other organ systems or perhaps by blood itself.4 The short half-life of GTN in our study is in agreement with pharmacokinetic studies in rats.
quantitative determination of trinitroglycerin in human plasma

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Summary: We developed a simplified method for quantitative measurement of trinitroglycerin in human plasma using hexane extraction and analysis by gas-liquid chromatography with electron-capture detection. This assay was linear from 0.5 to 60 ng/ml. Sensitivity and reproducibility were ± 0.5 ng/ml.

We used this assay to evaluate the pharmacodynamics of trinitroglycerin in 14 patients. Maximum plasma levels were similar with trinitroglycerin given by constant intravenous infusion (1.6 ± 0.4 ng/ml (SEM)), transcutaneously (2.3 ± 0.6 ng/ml), or sublingually (1.6 ± 0.6 ng/ml). Despite similar levels and hemodynamic responses after intravenous trinitroglycerin, the dose range was wide (37.5 – 175 μg/min, n = 5), emphasizing the need to individualize therapy. In normal volunteers on no other drugs, the plasma level time course followed changes in heart rate better than blood pressure changes. Use of the trinitroglycerin assay may enhance optimization of trinitroglycerin therapy when administered by different methods.

Trinitroglycerin, or Nitroglycerin (TNG), has been used to alleviate symptoms of angina pectoris for over 100 years.1 Recently, it has also become an important therapeutic agent for chronic congestive heart failure2 and for heart failure associated with acute myocardial infarction.3 In addition, it has been found to reduce acute myocardial ischemia,4 enhance electrical stability of ischemic myocardium,5,6 and improve localized ventricular asynergy.7 Despite its widespread use, little is known about the bioavailability of TNG administered by different methods. Due partly to the lack of convenient analytical methods, there has been little data to examine the relationship between plasma concentrations and hemodynamic or clinical effectiveness.8-11 This report describes a simplified method for quantitative measurement of TNG in human plasma using gas-liquid chromatography with electron-capture detection.

We have applied this technique to evaluate the plasma TNG levels and hemodynamic responses obtained in normal volunteers and patients when TNG was administered by sublingual, transcutaneous, and intravenous methods.

Methods

Blood samples were obtained in iced heparinized glass tubes. The plasma was immediately separated and frozen (−20°C) until assayed. Five milliliters of chromatography-grade hexane were added to 5 ml of plasma containing metadinitrobenzene (DNB) as the internal standard in a screw-top glass tube. The tube
Blood levels after sublingual nitroglycerin.
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