Br J clin Pharmac 1994; **37**: 441–444

Lack of tolerance in forearm blood vessels in man to glyceryl trinitrate

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1 Nitrate tolerance is a clinical problem. The cause is not known but it has been suggested that tolerance to organic nitrates occurs within the blood vessels to reduce sensitivity to the drug. We have determined the sensitivity of human forearm resistance vessels and veins to glyceryl trinitrate (GTN) at the beginning and end of a 7 day period of GTN administration in healthy volunteers using a clinically relevant dose of transdermal drug.

2 Eight healthy volunteers completed the study which entailed measurement of change in forearm venous compliance and change in forearm blood flow following intraarterial infusions of two doses of glyceryl trinitrate (0.5 and 2.0 $\mu g\ min^{-1}$) before, after 2 h and after 7 days of transdermal GTN administration using one 10 mg patch each 24 h.

3 Changes in venous compliance and blood flow were measured by venous occlusion plethysmography using a basal infusion of noradrenaline (1 $\mu g\ min^{-1}$) to increase venous tone.

4 Noradrenaline produced the expected decrease in forearm blood flow and venous compliance. The effect of locally infused GTN on venous compliance and forearm blood flow was similar on the three study days. In particular there was no significant difference in the response to GTN following 7 days transdermal administration compared with that after 2 h.

5 We conclude from this study that local vascular tolerance to GTN is unlikely to explain the clinical problem of nitrate tolerance, and that other mechanisms such as neurohumoral activation may be important.

Keywords glyceryl trinitrate nitrate tolerance arterioles veins forearm man plethysmography

Introduction

Tolerance to organic nitrates is a clinical problem. Even within 24 h a sustained therapeutic plasma concentration of nitrates can lead to loss of drug efficacy [1]. Although there have been many studies seeking to explain this phenomenon the mechanisms responsible are still unclear (reviewed by Elkayam [2]).

Katz and coworkers [3] used forearm venous occlusion plethysmography to develop a model of nitrate tolerance. To induce tolerance volunteers wore nitrate patches which deliver a constant dose of glyceryl trinitrate (GTN) [4]. Responsiveness to nitrates was tested by giving sub-lingual GTN and measuring change in forearm venous compliance.

Such a model has the advantages of being non-subjective, easy to use and non-invasive. However, it has two major disadvantages. Firstly, sublingual GTN activates systemic reflexes and so the model does not distinguish between tolerance occurring at a local vascular level and systemic tolerance. Secondly, the degree of venodilation possible after administration of GTN is limited by lack of basal tone in the forearm veins. This reduces the sensitivity of the technique. The aim of this study was to determine whether tolerance to local administration of nitrates could be demonstrated in the forearm vessels following prolonged systemic administration. The use of forearm venous plethysmog-

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raphy with brachial artery infusion of GTN allows the measurement of responsiveness of both arterioles and veins while avoiding the activation of systemic reflexes. It can be assumed that measurements reflect the direct action of the drug assessing local vascular tolerance [5]. Cannulation of the brachial artery allows coadministration of noradrenaline with the GTN. This increases basal tone thereby increasing the sensitivity to show venodilation. In addition, the effect of GTN on both resistance arterioles and veins can be measured in the same study.

Methods

This study was approved by the Joint Ethical Committee of Grampian Health Board and the University of Aberdeen. All subjects gave written informed consent.

Subjects

Eleven healthy male volunteers (age 19–36 years) were studied. They were not taking any medication. They were asked not to consume alcohol for 24 h before each study.

Measurement of forearm blood flow and forearm venous compliance

Subjects were studied recumbent in a quiet environment. Room temperature was maintained constant (± 1° C) during each study but varied on different occasions between 22–27° C.

A 27-gauge steel cannula was inserted into the left brachial artery using 1% lignocaine anaesthesia. Drugs were infused using a constant-rate infusion pump at a rate of 1 ml min^{-1}.

Measurement of forearm blood flow was previously described [6]. Wrist and upper arm pneumatic cuffs were applied to both arms. Strain gauges were positioned around the forearms 7 cm from the olecranon. Their signal was balanced by a plethysmograph and shown on the screen of an Apple Macintosh computer via a MacLab™ interface. The subject's arms were inclined at 30° to ensure free venous drainage. During blood flow measurement, the wrist cuffs were inflated to 200 mm Hg and the collecting cuffs to 40 mm Hg. At least six measurements of forearm blood flow were performed for each infusion. Measurement of forearm venous compliance was similar to that described by Mason & Braunwald [7]. The wrist cuffs were kept inflated. Cuff zero was determined by increasing the pressure until a deflection in forearm volume was seen. Collecting cuff pressure was then rapidly increased to 20 mm Hg above cuff zero and forearm volume allowed to plateau. A second measurement was made at 40 mm Hg above cuff zero.

Protocol

The subjects were studied on three occasions: before nitrate administration, 2 h following the application of a 10 mg GTN patch (Deponit, Schwartz) and again after 7 days of continual administration of GTN patches (10 mg 24 h^{-1}). This is almost identical to the protocol used by Katz *et al.* (1991) except the duration of patch application was longer in this study.

On each study day, forearm blood flow and venous compliance were measured in both arms following sequential brachial artery infusions of saline, noradrenaline 1 μg min^{-1}, GTN 0.5 μg min^{-1} with noradrenaline 1 μg min^{-1}, GTN 2.0 μg min^{-1} with noradrenaline 1 μg min^{-1}. Each infusion was for 5 min with measurement of blood flow and venous compliance during the following 5 min.

Data analysis

The slopes of the last five blood flow measurements in each intervention were measured and averaged using a MacLab™ interface and standard software packages which then calculated forearm blood flow in ml 100 ml^{-1} forearm min^{-1}. Similarly, for venous compliance the increase in left venous volume with each cuff pressure was expressed as per cent change from that seen with noradrenaline alone.

Differences in responsiveness to GTN between the baseline, acute and chronic studies were expressed as confidence intervals and measured with paired *t*-tests. Results were only considered significant if $P < 0.05$. Results are presented as mean ± s.e. mean.

After application of a transdermal nitrate patch the background plasma concentration might be expected to make forearm vessels less responsive to brachial artery infusion of GTN even without the presence of tolerance. The most important comparison is therefore of the response to infused GTN when the patch has been applied for only 2 h (study day 2) and for 1 week (study day 3).

Results

Study subjects

Two subjects withdrew during the continuous patch treatment due to headache, fainting and nausea. Another withdrew because he misunderstood instructions and removed his final nitrate patch before the third study had been performed. Results are based on the data from the eight subjects who completed the whole study.

There was no association between room temperature and resting forearm blood flow or venodilator responses.

Forearm blood flow

Noradrenaline infusion alone (1 μg min^{-1}) resulted in a reduction in absolute blood flow of 53.4, 41.9 and 50.8% of baseline respectively on the 3 study days ($P < 0.001$). The increase in forearm blood flow with infusion of GTN is shown in Figure 1. There was no significant difference in the change in forearm blood flow ratio with GTN between any of the infusion days. Table 1 gives the 95% confidence intervals for the comparison of change in forearm blood flow response to GTN between study days 2 and 3.

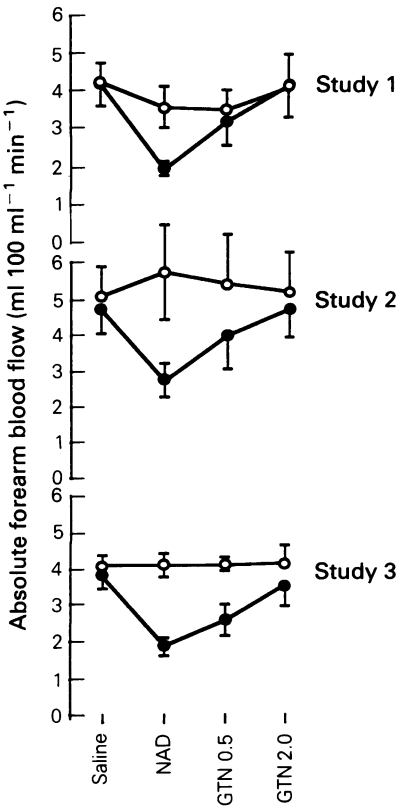

Figure 1 Absolute forearm blood flow increased with infusion of glyceryl trinitrate (GTN) on study day 1 (before GTN patch), study day 2 (2 h following patch) and after 1 week of patch aplication (study day 3). Noradrenaline (NAD) 1 μg min^{-1} was used to increase basal tone and was infused continually with GTN. Control arm flows are shown in open circles and infused arm flow in filled circles.

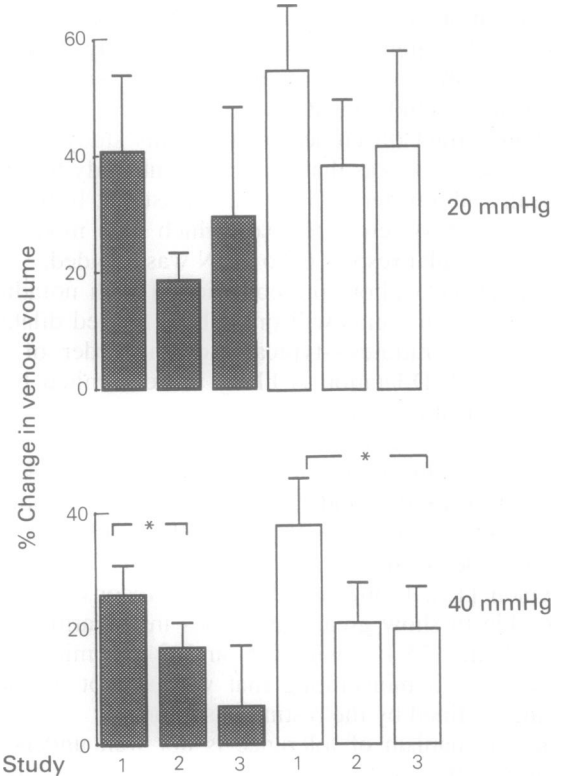

Figure 2 Venous volume increases (given as per cent increases above response with noradrenaline infusion alone) following GTN infusion on each study day (see legend Figure 1). Responses to low dose (0.5 μg min^{-1}) are shown in shaded bars and high dose (2 μg min^{-1}) in open bars. Measurements were made with cuff pressure at 20 and 40 mm Hg above cuff zero. *Indicates a significant difference ($P < 0.05$).

Table 1 Percentage change in forearm blood flow response, compared with and change in venous volume following 2 h and 7 days of transdermal GTN

	Mean change Study day 2 vs day 3 (%)	*95% confidence interval (%)*
GTN 0.5 μg min^{-1}		
Blood flow	−16.8	−76.9 to 43.3
Venous volume		
cuff pressure 20 mm Hg	10.7	−24.1 to 45.5
cuff pressure 40 mm Hg	−10.2	−30.8 to 10.4
GTN 2.0 μg min^{-1}		
Blood flow	−6.1	−73.5 to 61.2
Venous volume		
cuff pressure 20 mm Hg	10.1	−27.6 to 40.6
cuff pressure 40 mm Hg	−1.1	−20.4 to 18.2

Venous volume

The per cent changes in venous volume at 20 and 40 mm Hg are presented in Figure 2. There was no significant difference between any of the infusion days at cuff pressure 20 mm Hg. With a cuff pressure of 40 mm Hg, two of the comparisons were significantly different. It seems that after application of a patch the resulting GTN in the plasma makes the forearm vessels less responsive to brachial artery infusion of the drug, even without the presence of tolerance. The important comparison is between the 2nd and 3rd study days because on both these occasions there was background exposure to GTN from the patches. The difference in venous responsiveness to GTN between the 2nd and 3rd study days was never significant and did not alter by more than 50% (Table 1).

Discussion

Following systemic exposure to a standard clinical dose of GTN this study showed no convincing alteration in the responsiveness of forearm arterioles or veins to locally infused GTN.

The power of the study was sufficient to confidently show that the change in sensitivity to GTN did not change by more than 50% following chronic GTN exposure, although the power to detect changes in arteriolar sensitivity was less (see Table 1), perhaps due to the decreased responsiveness of arteriolar compared with vascular smooth muscle to organic nitrates [8].

These results contrast with those of Katz and colleagues [3]. Using sublingual GTN to measure venous sensitivity they demonstrated the development of tolerance between acute (2 h) and chronic (74 h) transdermal

administration of GTN using one 10 mg patch 24 h^{-1}. However the attenuation observed was only 40%. The power of the present study was not sufficient to confidently exclude this degree of tolerance.

An important difference between this study and that of Katz *et al.* [3] was that in the present study the effect of locally administered GTN was measured. In this way any effect of systemic reflexes which may modify the forearm vascular responses to GTN was avoided.

In addition, without pre-constriction with noradrenaline the forearm veins will only show limited dilatation with organic nitrates—typically of the order of 30% [3, 7, 9, 10]. Dilatation is likely to be limited by the degree of resting tone in the veins; when this is overcome, no further venodilatation is possible. In their study of the physiology of forearm veins Wood & Eckstein [11] state that under normal laboratory conditions these vesels have no resting tone. Lack of basal tone was also demonstrated in the studies of Greenfield & Patterson [12]. Using noradrenaline preconstriction we were able to show greater increases in forearm volume of 40.7 and 55.1% with 0.5 and 2 μg min^{-1} GTN respectively, demonstrating that we had not reached a 'ceiling' defined by the resting basal tone.

The mechanism of tolerance is not well understood. Theories fall into two broad categories—those suggesting that tolerance arises at a cellular level, and those suggesting that it is a systemic phenomenon.

The most favoured cellular mechanism is that proposed by Needleman [13, 14] when he showed that *in vitro* tolerance is accompanied by sulphydryl depletion. Some more recent studies have used sulphydryl donors such as acetylcysteine to prevent and partially reverse tolerance [2]. Demonstration of sulphydryl depletion requires, however, far higher concentrations of organic nitrates than are effective therapeutically.

Alternatively tolerance may be due to a systemic mechanism. It has been suggested that there is resetting of neurohumoral homeostatic mechanisms (including the sympathetic nervous system and the renin angiotensin system) leading to fluid retention and increased vasoconstrictor tone [2, 15]. This mechanism would be active at therapeutic concentrations and may more easily explain the well documented rebound vasoconstriction seen in persons exposed to very high concentrations of organic nitrates [2].

Although the power of this study did not allow an effect less than 40% to be confidently shown, it has been suggested that the marked tolerance with long term nitrate therapy is due to almost complete abolition of the effect of nitrates on blood vessels. Our study has shown that this certainly is not so, and if there is an effect it is relatively modest.

In conclusion, the results from this study suggest that at the doses used, continual exposure to GTN does not have a large effect on local responsiveness to organic nitrates which would be expected if a cellular mechanism leading to local vascular tolerance was operating. The variance seen in responsiveness to local GTN infusion meant that small changes could not be excluded with confidence. Changes in the activity of neurohumoral mechanisms which maintain venous and arterial tone would not be expected to alter local responsiveness to GTN, and therefore could not be shown by the methods used in this study.

We would like to thank Schwarz Pharma AG, Monheim, FRG for some financial support for this study.

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(Received 26 May 1993, accepted 27 January 1994)