

Effect of the nitric oxide donor, glyceryl trinitrate, on human gall bladder motility

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Abstract

Background—Nitric oxide is a major neurotransmitter in non-adrenergic, non-cholinergic (NANC) pathways. NANC inhibitory innervation has been shown in human gall bladder muscle *in vitro*; the role of nitric oxide in human gall bladder emptying however is undefined.

Aims—To study the effect of glyceryl trinitrate, a nitric oxide donor, on gall bladder emptying in healthy subjects using a randomised, double blind, crossover, placebo controlled design.

Methods—Ultrasonographic gall bladder volume was measured in the fasting state in eight healthy volunteers after randomised administration of either glyceryl trinitrate 1200 µg buccal spray or placebo spray. On two further occasions, after randomised administration of either glyceryl trinitrate 1200 µg buccal spray or placebo spray, gall bladder volumes were also measured after a liquid test meal.

Results—Glyceryl trinitrate significantly increased fasting gall bladder volume to a mean of 114% (SEM 5%) of pretreatment volume ($p=0.039$). Glyceryl trinitrate also significantly impaired gall bladder emptying between five and 40 minutes postprandially. Gall bladder ejection fraction was also reduced after glyceryl trinitrate compared with placebo (43 (6.9)% versus 68.4 (6.5)%, $p=0.016$).

Conclusions—This study shows that glyceryl trinitrate produces gall bladder dilatation in the fasting state and reduces postprandial gall bladder emptying, suggesting that nitric oxide mechanisms may be operative in the human gall bladder *in vivo*.

(Gut 1998;42:410-413)

Keywords: gall bladder motility; nitric oxide; glyceryl trinitrate

Nitric oxide (NO) was initially identified as an endothelium derived relaxing factor in 1987,¹ and since then it has been shown to have a wide variety of biological functions. In the cardiovascular system NO plays an important physiological role in controlling vascular tone. Inhibition of NO synthase, the enzyme responsible for NO production from L-arginine, causes marked vasoconstriction in the human vasculature, suggesting that there is a physiological, NO dependent vasodilator tone that is important in regulating blood flow.² Nitrovasodilators such as glyceryl trinitrate (GTN), which

has been used in the treatment of angina for over a century, act after metabolism to NO in the vasculature, with subsequent vascular smooth muscle relaxation.³

In addition to its role in the cardiovascular system, NO is a neurotransmitter both in the central and enteric nervous system.⁴⁻⁷ In the human gall bladder NADPH diaphorase staining neurones (NO producing neurones) have been characterised,⁸ and *in vitro* electrical activation of non-adrenergic, non-cholinergic (NANC) neurones produces gall bladder relaxation.⁹ Inhibition of NO synthase in guinea pig gall bladder *in vivo* increases basal gall bladder pressure, indicating that there is a continuous "tick-over" production of NO in the gall bladder which contributes to the maintenance of gall bladder volume in the fasting state.¹⁰ However, the effects of NO on human gall bladder motility have not been reported.

The aim of the present study was to examine the effect of the NO donor GTN on human gall bladder volume and emptying in healthy volunteers.

Methods

SUBJECTS

Eight healthy subjects (five females) volunteered to participate in the study. The subjects were free from any gastrointestinal symptoms and gave no history of previous gastric surgery or diabetes mellitus. They were on no medication throughout the study period. All subjects had normal gall bladders on ultrasonography. The median values for age, weight, and height were: 33 years (range 28-35), 67 kg (range 54-74), and 1.73 m (range 1.61-1.83). The median body mass index of the subjects was 21.8 kg/m² (range 20.2-24.1). Each subject gave written informed consent. Ethical approval was obtained from the East London and City Health Authority Research Ethics Committee.

STUDY DESIGN

There were two arms to the study. In the first, the response of fasting gall bladder volume to GTN and placebo was compared. In the second, the effects of GTN and placebo on postprandial gall bladder emptying after a standard liquid meal were compared.

In the fasting volume study, subjects were randomly assigned to receive GTN buccal spray (Nitrolingual, Merck-Lipha Pharmaceuticals, Middlesex, UK) 1200 µg and, on a separate day, an identical tasting placebo spray (Merck-Lipha Pharmaceuticals). Fasting gall bladder volume was measured after an eight hour fast, following which GTN or placebo

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Accepted for publication
1 October 1997

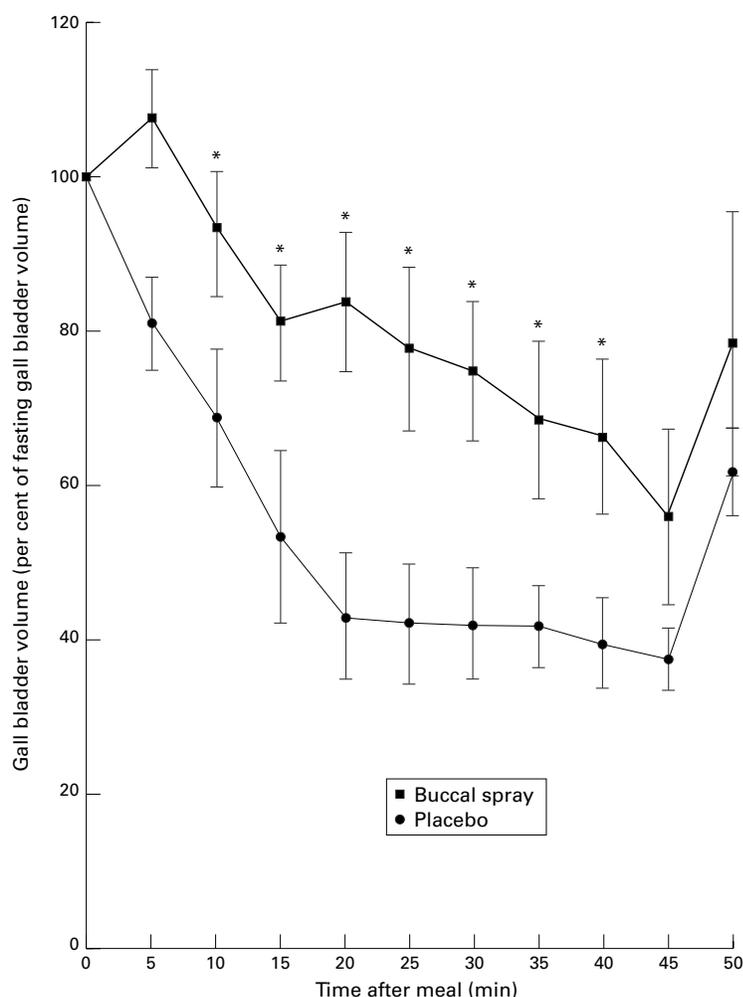


Figure 1 Effect of GTN 1200 µg buccal spray or placebo on the time course of gall bladder emptying following a liquid test meal in healthy subjects, expressed as percentage of control fasting volume. Results are expressed as mean (SEM). * $p < 0.05$.

spray was given, and gall bladder volume was measured every five minutes for 15 minutes.

For the postprandial experiment, the effect of GTN and placebo on gall bladder emptying was measured after consumption of a 250 ml liquid test meal (1.050 MJ; 14% protein, 37% corn oil, 54% carbohydrate; "Ensure", Abbott Laboratories, Kent, UK). Fasting gall bladder volume was measured after an eight hour fast and the liquid test meal was immediately ingested within two minutes under supervision. The subjects then randomly received either GTN spray 1200 µg or placebo; the alternate spray was given on a separate day. Gall bladder volume was measured every five minutes for 50 minutes, or until the gall bladder began to refill.

Table 1 Effect of glyceryl trinitrate (GTN) on fasting gall bladder volume

Time (min)	Volume (ml)	
	Placebo	GTN
0	100 (0)	100 (0)
5	96.4 (5.1)	105.9 (4.7)
10	98.0 (5.0)	114.4 (5.0)*
15	98.9 (2.5)	111.7 (5.2)

Results are expressed as mean (SEM).

* $p = 0.039$ (Wilcoxon signed rank test) *v* volume at time 0 min.

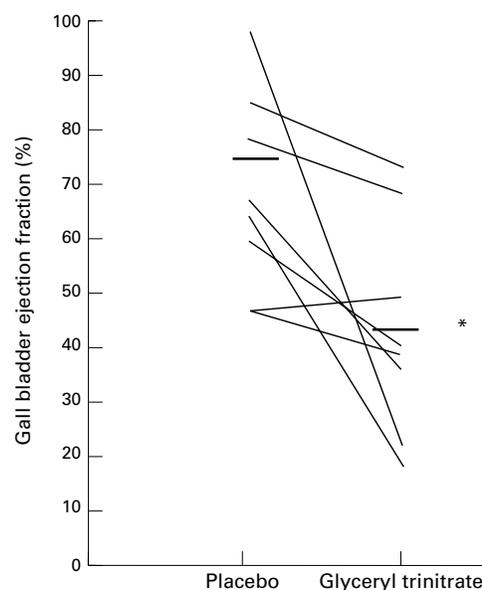


Figure 2 Gall bladder ejection fraction following a liquid test meal with either GTN 1200 µg or placebo. The solid horizontal bars represent the mean values. * $p < 0.05$.

GALL BLADDER EMPTYING

Gall bladder emptying was assessed by serial measurement of gall bladder volume using ultrasound. Studies were conducted by a radiologist using a 3.5 MHz transducer (Accuson 128XP). The radiologist was unaware of which treatment the volunteer had received. After an eight hour fast the gall bladder was scanned in the right anterior oblique position. At each time point, two separate gall bladder measurements of length, width, and height were obtained in the longitudinal and transverse planes and the mean of these measurements was used for calculation of the gall bladder volume. Gall bladder volume was determined using the ellipsoid method¹¹ as we have previously used¹²:

$$V = (L \times W \times H) \times \pi/6$$

where V is the gall bladder volume, L is the gall bladder length (sagittally), W is the width, and H is the height of the gall bladder (axially).

The gall bladder ejection fraction was calculated according to the formula:

$$EF = \frac{V_p - V_{\min}}{V_p} \times 100\%$$

where EF is ejection fraction, V_p is fasting gall bladder volume, and V_{\min} is the minimal gall bladder volume.

STATISTICAL METHODS

Non-parametric paired data were analysed using the Wilcoxon signed rank test. Values of $p < 0.05$ were considered significant. Results shown are mean (SEM) values.

Results

EFFECT OF GTN ON FASTING GALL BLADDER VOLUME

Mean fasting gall bladder volumes prior to GTN or placebo administration were similar: (20.1 (1.5) ml versus 25.1 (3.2) ml, NS). The placebo spray had no significant effect on gall

bladder volume over 15 minutes (table 1). GTN spray, however, produced dilatation of the gall bladder which was maximal at 10 minutes in 6/8 subjects. The mean increase was to 114 (5)% of fasting volume at 10 minutes ($p=0.039$).

EFFECT OF GTN ON POSTPRANDIAL GALL BLADDER EMPTYING

There was no significant difference between fasting gall bladder volume prior to GTN or placebo spray (21.3 (2.4) ml versus 19.9 (1.8) ml). Gall bladder emptying was significantly impaired after GTN administration compared with placebo at five minutes, and this effect persisted until 40 minutes (fig 1). Ejection fraction was also reduced with GTN compared with placebo (43.2 (7)% versus 68.4 (6.5)%, $p=0.016$) (fig 2).

Discussion

This study shows that in healthy humans a standard dose of buccal GTN spray produces dilatation of the gall bladder in the fasting state and impairs postprandial gall bladder emptying. In the cardiovascular system, GTN exerts its biological effect through the donation of NO, with consequent arterial smooth muscle relaxation.³ It is also likely that NO donation by GTN with consequent gall bladder smooth muscle relaxation is the mechanism underlying the effect of this drug on gall bladder volume. Nitric oxide containing neurones have been visualised using immunohistochemical methods in both the guinea pig and human gall bladder.⁸ In guinea pig studies in vivo, inhibition of NO synthase increases both resting tone and cholecystokinin (CCK) induced contraction. Moreover, NO donation in the form of sodium nitroprusside reduced the response of guinea pig gall bladder in vitro to CCK by about 80%.¹⁰ Our data provide evidence that NO pathways may be operative in the human gall bladder in vivo. However, the coordinated delivery of bile into the duodenum also requires a complex interplay between gall bladder contraction, cystic duct resistance, and sphincter of Oddi relaxation.^{13,14} In guinea pigs and rabbits, the sphincter of Oddi produces NO synthase, which acts locally to reduce sphincter tone.¹⁵ Ambulatory sphincter of Oddi manometric studies were not attempted in this study, as they are invasive and technically demanding.

Apart from a direct effect on gall bladder smooth muscle and sphincter of Oddi, NO may also indirectly reduce postprandial gall bladder emptying by reducing gastric emptying, and hence the rate of nutrient delivery to the duodenum,⁴ with consequent reduction of nutrient release of CCK and gall bladder contraction. However, as GTN affects fasting gall bladder volume in vivo, it is likely that this effect on the gall bladder is independent, at least in part, of gastric emptying.

The impairment of gall bladder emptying by buccal GTN may have implications for gallstone pathogenesis in subjects on other nitrate formulations. Gallstones arise because of a triple defect in the hepatobiliary system:

cholesterol supersaturation of gall bladder bile,¹⁶ reduced gall bladder emptying,¹⁷ and an increased rate of cholesterol crystal nucleation, owing to the presence of biliary nucleating factors.¹⁸ In animal studies, reduced gall bladder emptying precedes gallstone synthesis.¹⁹ In humans, impaired gall bladder emptying is associated with gallstone formation^{20,21}; the rate of gallstone formation may therefore be accelerated in patients on long term nitrates.

The relaxant effect of GTN on the gall bladder might have therapeutic possibilities. Hassel reported three patients with biliary colic whose pain responded rapidly to a variety of nitrate formulations (buccal GTN 400 µg, sublingual GTN 500 µg, isosorbide dinitrate 20 mg). He suggested that GTN might be a useful adjunct to standard analgesia in acute biliary colic.²²

This study used a pharmacological dose of GTN as an NO donor, and it therefore does not show definitively that NO plays a physiological role in human gall bladder motility. To determine whether NO has a physiological role would require parenteral NO synthase inhibition, but this was considered hazardous and unethical, particularly with regard to the risk of mesenteric small vessel ischaemia.²³

In conclusion, this study is the first to show a significant impairment of gall bladder emptying with buccal GTN compared with placebo in healthy human subjects. This implies that NO mechanisms may be operative in the human gall bladder, and may have implications for gallstone formation in subjects on long term nitrates.

Grant support was provided by the Frances and Augustus Newman Foundation and the Health Research Board (Ireland). This work was presented in part at the British Society of Gastroenterology (*Gut* 1996;38(suppl 1):A63) and at Digestive Diseases Week (*Gastroenterology* 1996;110:A670).

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