Chronic administration of *N*-acetylcysteine fails to prevent nitrate tolerance in patients with stable angina pectoris

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1 Reduced availability of sulphydryl groups in vascular smooth muscle cells may contribute to the development of tolerance to the action of the organic nitrovasodilators.

2 Eight patients with stable angina were treated with 10 mg of transdermal glyceryl trinitrate per 24 h, together with 400 mg *N*-acetylcysteine, a sulphydryl group donor, or matching placebo three times daily in a double-blind randomised crossover manner for two periods of 4 days with intervening washout period of 3 days off these drugs. Other therapy remained unaltered during the study.

3 Blood pressure, heart rate and symptom-limited treadmill walking time were measured in the pre-treatment control state and 4 h after starting treatment on day 1 and day 4 of each glyceryl trinitrate treatment period.

4 The changes seen on day 1 were attenuated by day 4 to an equal extent in *N*-acetylcysteine and placebo treatment periods.

5 These results suggest that chronic oral administration of *N*-acetylcysteine fails to prevent the development of tolerance to the anti-anginal or haemodynamic effects of glyceryl trinitrate.

Keywords angina glyceryl trinitrate tolerance *N*-acetylcysteine

Introduction

Daily administration of transdermal glyceryl trinitrate results in attenuation of its haemodynamic and anti-anginal actions (Parker & Fung, 1984; Reichek *et al.*, 1984). The mechanism by which this tolerance to nitrates develops is unclear. Current hypotheses include activation of neurohumoral reflexes (Fahmy & Gavran, 1985), haemodilution (Lis *et al.*, 1984) and, at a cellular level, depletion of cytosolic sulphydryl groups in vascular smooth muscle (Ignarro *et al.*, 1981; Needleman *et al.*, 1983).

Glyceryl trinitrate exerts its vasodilator actions by activating soluble guanylate cyclase to elevate cyclic guanosine monophosphate (cGMP). This process is dependent upon the presence of sulphydryl groups to form S-nitrosothiols as

active intermediates (Ignarro *et al.*, 1981). Continuous or frequent administration of glyceryl trinitrate could therefore result in intracellular sulphydryl group-depletion and tolerance to its vasodilator action. On this basis, prevention of reversal of nitrate tolerance might be achieved by repletion of sulphydryl groups from an exogenous source such as *N*-acetylcysteine. This has been demonstrated *in vitro* (Needleman *et al.*, 1973) but *in vivo* studies have given conflicting results (Hogan *et al.*, 1989; Packer *et al.*, 1987; Parker *et al.*, 1987).

We have already demonstrated failure of *N*-acetylcysteine to prevent tolerance developing to the haemodynamic (systemic blood pressure and heart rate) actions of GTN (Hogan *et al.*,

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1989). The anti-anginal action of GTN is however mediated predominantly by its venodilator activity thus reducing venous return. This in turn reduces ventricular end-diastolic pressures, myocardial wall tension and thereafter myocardial oxygen consumption. This venodilator action of nitrates occurs at a much lower dose than its arterial vasodilator action and therefore the anti-anginal effects of GTN can be seen in the absence of any haemodynamic effect on systemic blood pressure or heart rate. Given this differential action of GTN on different vascular beds it is therefore entirely possible that tolerance to the anti-anginal effects of nitrates may respond differently to *N*-acetylcysteine than that tolerance seen to the effects on systemic blood pressure and heart rate.

This study therefore investigates the effect of chronic oral dosing with *N*-acetylcysteine on the development of nitrate tolerance in patients with stable angina pectoris treated with daily transdermal glyceryl trinitrate.

Methods

Eight patients (age range 52–67 years) with chronic stable angina pectoris were studied. All

patients experienced typical angina on exertion, had positive exercise tests (chest pain together with planar or downsloping ST segment depression of 1 mm on exercise after the J point > 0.08 s) and had angiographically documented significant ($> 70\%$) luminal stenosis of one or more major coronary arteries. Patient characteristics and concurrent treatment are given in Table 1.

Ethical approval for the trial was given by the South Glamorgan Health Authority Ethics Committee and informed consent was obtained from each patient.

All eight patients were treated with 10 mg of transdermal glyceryl trinitrate (Transiderm-Nitro, Ciba-Geigy) per 24 h, together with 400 mg of *N*-acetylcysteine or matching placebo three times daily in a prospective, double-blind, randomised crossover manner for two periods of 4 days with a washout interval of 3 days off these drugs. Other nitrovasodilators were discontinued 48 h before starting the trial. All other medication including β -adrenoceptor antagonists in all cases remained unchanged.

Haemodynamic measurements and treadmill exercise testing (Bruce protocol) were performed in the pre-treatment control state and 4 h after starting treatment on day 1 and day 4 of both treatment schedules. Resting standing systolic

Table 1 Characteristics of patients included in study

Patient	Age (years)	Sex	Symptoms	Concurrent treatment*	ECG	Angiography
1	61	M	Angina uphill	Atenolol Nifedipine	Normal	2 vessel CAD Good LV
2	55	M	Angina 600 yards	Nifedipine Atenolol	T inverted Lead AvL	1 vessel CAD Good LV
3	67	M	Angina uphill	Nifedipine Aspirin	ST flattening V3–V6	3 vessel CAD Good LV
4	52	M	Angina 100 yards	Atenolol Nifedipine	Normal	1 vessel CAD Good LV
5	52	M	Angina 200 yards	Propranolol	T inverted Leads III, AvF	3 vessel CAD Good LV
6	56	M	Angina 400 yards	Atenolol Aspirin	Previous anterolateral MI	3 vessel CAD Anterior hypokinesia
7	64	M	Angina and SOB uphill CABG	Nifedipine Atenolol Frusemide Amiloride Aspirin	Previous anterior MI	2 vessel CAD Anterior akinesia
8	56	M	Angina 800 yards	Atenolol Aspirin	Normal	1 vessel CAD

Abbreviations: CABG, coronary artery bypass graft; CAD, coronary artery disease; LV, left ventricle; MI, myocardial infarction; SOB, short of breath. *All patients had access to sublingual GTN.

Figure 1 Mean ($\pm$ s.e. mean) resting upright systolic (SBP) and diastolic blood pressure (DBP, mm Hg), heart rate (HR, beats min^{-1}) and treadmill walking time (TWT, min) in the control state (C) and on days 1 and 4 during subsequent treatment with glyceryl trinitrate alone ($\square$) or in combination with *N*-acetylcysteine ($\blacksquare$). * $P < 0.025$ compared with control $n = 8$.

(SBP) and diastolic blood pressure (DBP), heart rate (HR) and symptom-limited treadmill walking time (TWT) were recorded on each occasion. Exercise was discontinued at the onset of angina of a severity similar to that which would usually cause the patient to stop and take sublingual glyceryl trinitrate.

Analysis of results was performed using two-way analysis of variance to check for period and treatment effects, followed by Student's *t*-test for paired data where indicated. Results were considered significantly different where $P < 0.05$.

Results

No significant period effect was detected ($P < 0.05$). Treatment effects are shown in Figure 1 and actual values (mean $\pm$ s.e. mean) given in Tables 2, 3, 4 and 5. In both treatment periods the acute effects of glyceryl trinitrate are demonstrated on day 1 and the development of tolerance is indicated by the reversion of these changes towards control values on day 4. It is clear that *N*-acetylcysteine did not prevent significantly the development of tolerance.

Discussion

The results illustrate the development of tolerance to the anti-anginal and haemodynamic effects of transdermal glyceryl trinitrate after 4 days treatment and show that *N*-acetylcysteine has no demonstrable effect on this.

Table 2 Mean $\pm$ s.e. mean upright systolic blood pressure (mm Hg) in the control state and on days 1 and 4 of subsequent treatment with GTN and placebo or GTN and *N*-acetylcysteine. *P* value compared with control for that treatment schedule

	GTN + placebo	<i>P</i> value	GTN + <i>N</i> -acetylcysteine	<i>P</i> value
Control	124.4 $\pm$ 2.7	—	124.2 $\pm$ 3.5	—
3 h post dose day 1	114.4 $\pm$ 5.0	< 0.025	113.3 $\pm$ 6.3	NS
3 h post dose day 4	122.5 $\pm$ 4.9	NS	125.0 $\pm$ 5.0	NS

Table 3 Mean $\pm$ s.e. mean upright diastolic blood pressure (mm Hg) in the control state and on days 1 and 4 of subsequent treatment with GTN and placebo or GTN and *N*-acetylcysteine. *P* value compared with control for that treatment schedule

	GTN + placebo	<i>P</i> value	GTN + <i>N</i> -acetylcysteine	<i>P</i> value
Control	83.3 $\pm$ 2.6	—	80.0 $\pm$ 2.9	—
3 h post dose day 1	78.1 $\pm$ 3.5	< 0.02	75.0 $\pm$ 4.8	NS
3 h post dose day 4	78.3 $\pm$ 4.8	NS	78.3 $\pm$ 4.3	NS

Table 4 Mean $\pm$ s.e. mean heart rate (beats min^{-1}) in the control state and on days 1 and 4 of subsequent treatment with GTN and placebo or GTN and *N*-acetylcysteine. *P* value compared with control for that treatment schedule

	GTN + placebo	<i>P</i> value	GTN + <i>N</i> -acetylcysteine	<i>P</i> value
Control	56 $\pm$ 2.3	—	55.3 $\pm$ 2.8	—
3 h post dose day 1	58.1 $\pm$ 1.9	NS	57.5 $\pm$ 3.8	NS
3 h post dose day 4	54.8 $\pm$ 2.7	NS	54.0 $\pm$ 1.9	NS

Table 5 Mean $\pm$ s.e. mean symptom-limited treadmill walking time (min) in the control state and on days 1 and 4 of subsequent treatment with GTN and placebo or GTN and *N*-acetylcysteine. *P* value compared with control for that treatment schedule

	GTN + placebo	<i>P</i> value	GTN + <i>N</i> -acetylcysteine	<i>P</i> value
Control	5.84 $\pm$ 0.55	—	5.18 $\pm$ 0.63	—
3 h post dose day 1	7.01 $\pm$ 0.58	< 0.01	6.85 $\pm$ 0.60	< 0.005
3 h post dose day 4	6.38 $\pm$ 0.55	NS	6.05 $\pm$ 0.65	NS

This study also provides no evidence that oral dosing with *N*-acetylcysteine potentiates the vasodilator effects of glyceryl trinitrate, as reported previously (Horowitz *et al.*, 1983).

However, only eight patients were investigated and consequently it is unlikely that a partial reversal of tolerance by *N*-acetylcysteine would be detected. The number of patients required to demonstrate such an effect would necessarily be large and the clinical significance of a marginal effect of this dose of *N*-acetylcysteine would be limited.

Previous clinical studies of the effect of sulphhydryl group replenishing agents on the development of tolerance to nitrovasodilators have given apparently conflicting results (Packer *et al.*, 1987; Parker *et al.*, 1987). Parker and colleagues (1987) showed no effect of *N*-acetylcysteine infusion once nitrate tolerance had developed in patients with angina. Packer and colleagues (1987) however, demonstrated restoration of the haemodynamic effects of intravenous glyceryl trinitrate following the administration of oral *N*-acetylcysteine in patients with chronic congestive heart failure who were previously tolerant to the effects of nitrates. The present study differed from the previous two studies in that it addressed the question whether oral administration of *N*-acetylcysteine might prevent the development of tolerance during transdermal glyceryl trinitrate treatment of patients with chronic stable angina. In this respect it is similar in design and findings to a previous report by us investigating the effects of *N*-acetylcysteine on nitrate tolerance in healthy volunteers (Hogan *et al.*, 1989).

It remains possible that the relatively high

oral dose of *N*-acetylcysteine used in the present study was still insufficient to replenish the intracellular sulphhydryl group stores (Maddock, 1980; Rodenstein *et al.*, 1978). However *N*-acetylcysteine was chosen for this study as it is available for clinical use, is relatively non-toxic in common clinical dosage and has been documented to produce availability of the active compound at high concentration, at least in lung tissue, with oral doses much less than that used in this study (Maddock, 1980; Rodenstein *et al.*, 1978). Furthermore side effects occur with higher oral doses (Packer *et al.*, 1987). Oral *N*-acetylcysteine may be a less effective donor of sulphhydryl groups than intravenous *N*-acetylcysteine for it is metabolised on its passage through the liver to cysteine which may be a less effective donor of sulphhydryl groups. Nitrate tolerance may be due to mechanisms other than sulphhydryl group-depletion. Activation of the renin-angiotensin system (Fahmy & Gavras, 1985) and plasma volume expansion (Lis *et al.*, 1984) may limit the systemic vasodilatory effects of glyceryl trinitrate responsible for some aspects of its action.

In conclusion this study demonstrates that daily dosing with *N*-acetylcysteine 400 mg three times daily failed to prevent significantly or ameliorate the development of nitrate tolerance in a group of patients with chronic stable angina during treatment with transdermal glyceryl trinitrate.

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