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N-acetylcysteine fails to attenuate haemodynamic tolerance to glyceryl trinitrate in healthy volunteers

J. C. HOGAN, M. J. LEWIS[1] & A. H. HENDERSON
Department of [1]Pharmacology and Therapeutics and Cardiology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN

1 The effects of chronic dosing with *N*-acetylcysteine (NAC), on nitrate-induced haemodynamic changes during the acute and chronic treatment of healthy volunteers with glyceryl trinitrate (GTN) patches (Transiderm nitro) has been investigated.
2 Seven volunteers were treated in a double-blind randomised crossover manner for two periods of 4 days with 20 mg of transdermal GTN/24 h together with NAC (200 mg three times daily) or matching placebo. There was a washout period of >3 days between treatment periods.
3 Haemodynamic measurements (blood pressure (BP); heart rate (HR)) at rest and following maximal treadmill exercise were performed before treatment and 4 h after starting treatment on days 1 and 4.
4 Significant haemodynamic changes as evidenced by a fall in BP and rise in HR, were seen on day 1 in both the NAC and placebo phases. By day 4 the haemodynamic changes had returned towards the pre-treatment values during both the NAC and placebo phases suggesting the development of tolerance in both treatment groups.
5 These findings suggest that concurrent administration of NAC fails to prevent the development of tolerance to GTN.

Keywords *N*-acetylcysteine glyceryl trinitrate tolerance

Introduction

Tolerance to the actions of organic nitrates is a well recognised phenomenon (Laws, 1898; Stewart, 1905; Needleman, 1970). Its development is most frequently associated with those formulations or dosage regimes which attempt to provide a constant plasma nitrate level (Thadani *et al.*, 1982; Parker & Fung 1984; Parker *et al.*, 1987a, b). In this respect, transdermal nitroglycerine patches, which deliver GTN across the skin at a constant rate over a 24 h period, have been particularly implicated (James *et al.*, 1985; Jordan *et al.*, 1985; Thadani *et al.*, 1985).

The mechanism of nitrate tolerance is uncertain. Current hypotheses include activation of the renin-angiotensin system (Fahmy & Gavras, 1985), plasma volume expansion (Lis *et al.*, 1984) and at a cellular level, diminished availability of reduced sulphydryl (SH) groups in vascular smooth muscle cells (Needleman & Johnson, 1973). These SH groups are thought to react with the nitric oxide generated from organic nitrates to form the active intermediates, s-nitrosothiols, which in turn activate the enzyme soluble guanylate cyclase, elevating levels of cyclic guanosine monophosphate (cGMP) leading to smooth muscle relaxation (Ignarro *et al.*, 1981).

It is postulated that continued exposure to nitrate reduces the availability of sulphydryl

Correspondence: Dr M. J. Lewis, Department of Pharmacology and Therapeutics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN

groups thereby leading to tolerance and that by replenishing these groups tolerance may be reversed. While this has been shown *in vitro* (Needleman & Johnson, 1973) *in vivo* human studies have been contradictory (Packer *et al.*, 1987; Parker *et al.*, 1987b).

This study was designed to investigate the effects of chronic dosing with *N*-acetylcysteine (NAC), a sulphydryl group donor in use clinically, on nitrate induced haemodynamic changes during the acute and chronic treatment of healthy volunteers with transdermal nitroglycerine patches.

Methods

Eight healthy male volunteers (age range 20–27 years) entered the study after giving their written informed consent and undergoing preliminary medical examination, 12 lead electrocardiography and routine biochemical and haematological testing. The protocol was approved by the Ethics Committee of the South Glamorgan Area Healthy Authority.

In a double-blind randomised crossover manner volunteers were treated for two periods of 4 days with 20 mg of transdermal nitroglycerine/24 h (Transiderm nitro) together with NAC (200 mg three times a day) or matching placebo. There was a washout interval of >3 days between treatment periods. Blood pressure (BP) and heart rate (HR) were measured upright (after 3 min standing) and supine (after 5 min lying) prior to commencing treatment, 4 h after starting treatment on the first day and 4 h after the start of treatment on the fourth day. These measurements were performed at rest and then following maximal treadmill exercise according to the standard Bruce protocol at the same time on each of the days (13.00 h). Each volunteer underwent two practise treadmill exercises on the day immediately preceding the start of either treatment schedule to minimise any training effect.

Finally, blood samples for GTN levels were drawn at the same time (12.00 h) on days 1 and 4 of both treatment schedules. GTN assays were performed using standard capillary gas chromatography techniques (Sioufi & Pommeier, 1985). Results were analysed using paired *t*-tests with the Dunn-Bonferroni modification and considered significantly different when $P < 0.05$.

Results

Of the eight volunteers entering the study, seven completed the whole treatment protocol. Volunteer 6 was withdrawn after developing severe headaches, nausea and vomiting following initiation of treatment. Subsequent analysis revealed him to be receiving NAC at this time.

Plasma GTN concentration

Plasma GTN concentration measured 4 h post dose on days 1 and 4 of both treatment schedules were not significantly different with respect either to day of treatment or treatment schedule (Table 1).

Acute haemodynamic effects of GTN (4 h post dose on day 1)

Significant haemodynamic changes occurred in both treatment and placebo groups after the first dose of GTN (Figures 1, 2, 3 and 4). There were no significant differences in these changes between the two groups.

Chronic haemodynamic effects of GTN (4 h post dose on day 4)

After 4 days dosing with GTN there was a marked attenuation of the haemodynamic changes seen on day 1 in both groups suggesting

Table 1 Plasma GTN concentration (nmol l^{-1}) (mean + s.e. mean)

	Active	*Placebo*
Day 1	2.53 ± 0.73	1.96 ± 0.53
Day 4	1.90 ± 0.40	2.67 ± 0.39

Table 2 Significant differences in BP (mm Hg) between active and placebo groups after 4 days of treatment with GTN

	Active	*Placebo*
Supine MBP pre-exercise	96.3 ± 2.2*	85.5 ± 4.3
Supine SBP post-exercise	134.3 ± 5.8*	122.9 ± 3.9
Supine DBP post-exercise	74.3 ± 2.8*	69.7 ± 4.5
Supine MBP post-exercise	94.3 ± 3.4***	86.2 ± 4.0

* $P < 0.05$ *** $P < 0.01$ compared with placebo values

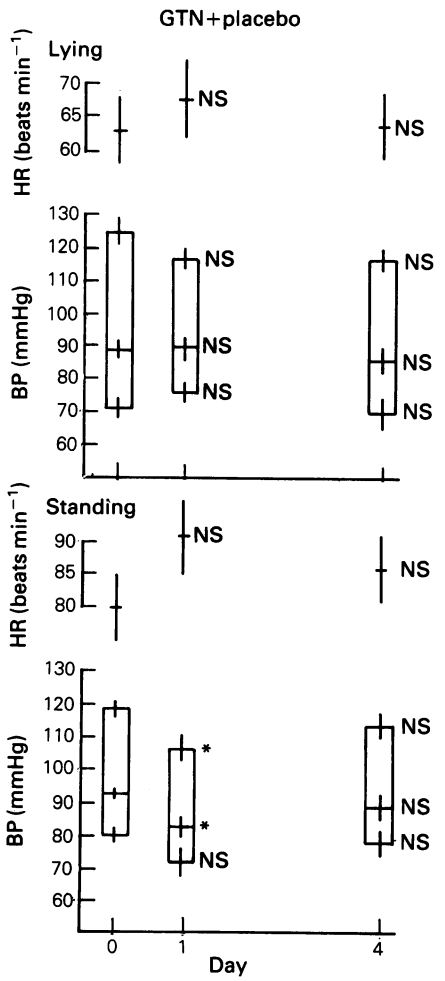

Figure 1 Mean (± s.e. mean) lying and standing heart rate (HR, beats min^{-1}); systolic, diastolic and mean blood pressure (BP, mm Hg), in the absence of treatment on day 0 and 4 h after GTN on days 1 and 4 in the presence of placebo before exercise. $*P < 0.05$ compared with control values on day 0.

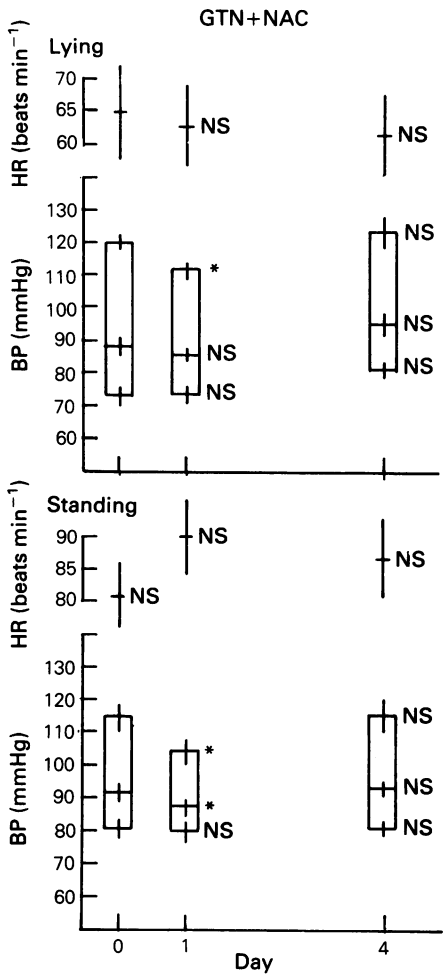

Figure 2 Mean (± s.e. mean) lying and standing heart rate (HR, beats min^{-1}); systolic, diastolic and mean blood pressure (BP, mm Hg), in the absence of treatment on day 0 and 4 h after GTN on days 1 and 4 in the presence of NAC before exercise. $*P < 0.05$ compared with control values on day 0.

development of tolerance to the organic nitrate preparation (Figures 1, 2, 3 and 4).

Comparison between the two groups at day 4 surprisingly revealed significantly greater supine blood pressure (Table 2) in the NAC than the placebo treated group but otherwise no significant differences.

Comparison of the extent of change occurring over the 4 day period between the two groups once more only showed significantly greater increases in supine blood pressures in the NAC group (Table 3) there being no other significant differences between the two groups.

Thus tolerance was seen to occur in both groups though significantly greater supine blood pressures were found by day 4 of treatment in the NAC group.

Discussion

The results of this study confirm that tolerance to the haemodynamic effects of GTN patches occurs with chronic dosing and that concomitant dosing with 200 mg three times daily of NAC fails to alter this phenomenon. That is, by day 4 of treatment heart rate and blood pressure have returned to control values irrespective of whether

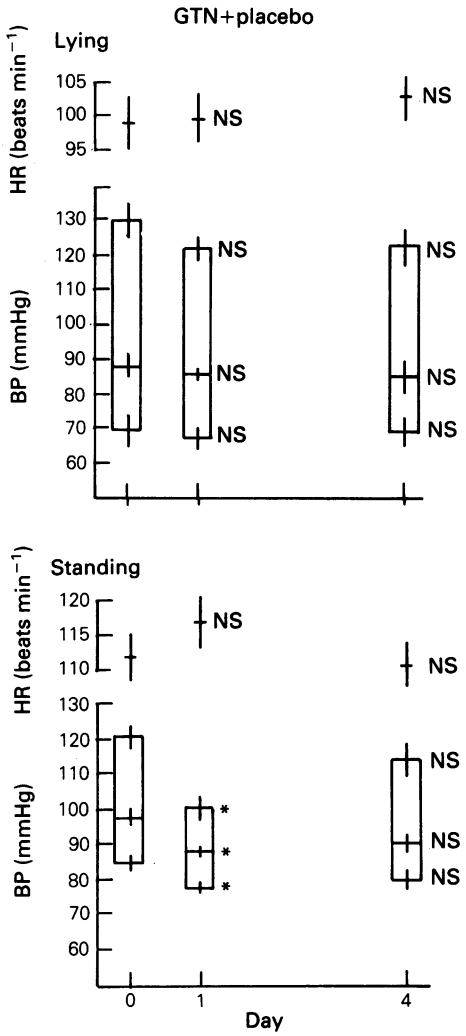

Figure 3 Mean (± s.e. mean) lying and standing heart rate (HR, beats min^{-1}); systolic, diastolic and mean blood pressure (BP, mm Hg), in the absence of treatment on day 0 and 4 h after GTN on days 1 and 4 in the presence of placebo after exercise. $*P < 0.05$ compared with control values on day 0.

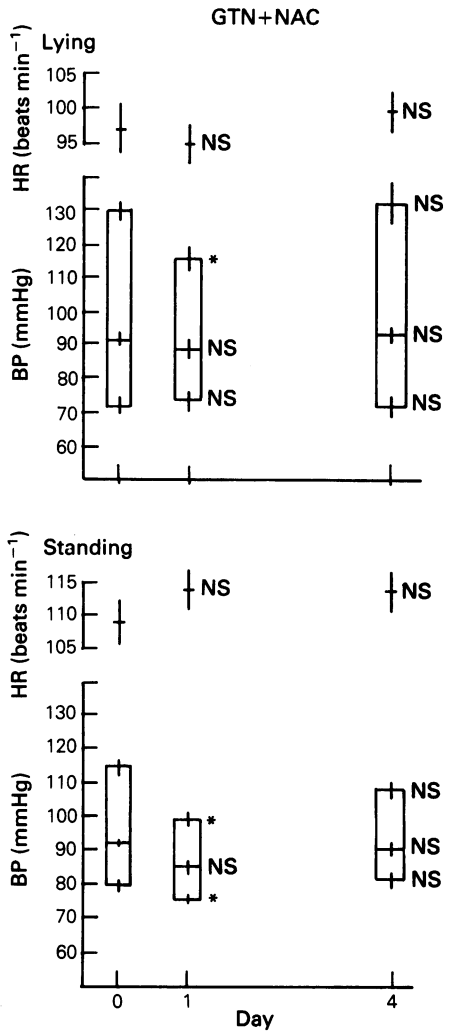

Figure 4 Mean (± s.e. mean) lying and standing heart rate (HR, beats min^{-1}); systolic, diastolic and mean blood pressure (BP, mm Hg), in the absence of treatment on day 0 and 4 h after GTN on days 1 and 4 in the presence of NAC after exercise. $*P < 0.05$ compared with control values on day 0.

Table 3 Significant differences in extent of change in BP (mm Hg) between active and placebo groups after 4 days of treatment with GTN

	Active	*Placebo*
Supine DBP pre-exercise	+8.86 ± 3.4**	−5.57 ± 3.9
Supine MBP pre-exercise	+9.89 ± 2.0***	−4.29 ± 3.8
Supine SBP post-exercise	+16.14 ± 7.1*	−0.57 ± 4.0

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ compared with placebo values

NAC was being taken. The only exceptions to this were supine MBP pre-exercise, supine SBP post-exercise, supine DBP post-exercise and supine MBP post-exercise, all of which were surprisingly higher at day 4 in the NAC treated group than in placebo. The reasons for this are obscure, as is the clinical significance, but the design of the trial doses not allow a direct effect of chronic dosing with NAC to be excluded as a cause.

The requirement for SH groups in the metabolism of organic nitrates to facilitate their smooth muscle relaxation is well documented (Needleman & Johnson, 1973; Ignarro *et al.*, 1981). It is presently thought that reduced intracellular SH groups react with the nitric oxide generated from organic nitrates to form nitrosothiols which in turn activate the enzyme soluble guanylate cyclase elevating intracellular cGMP which results in vasodilation. Chronic administration of nitrates may well deplete intracellular stores of reduced SH groups and cause tolerance. Reversal of nitrate tolerance by the addition of SH group replenishing agents has been demonstrated *in vitro* (Needleman & Johnson, 1973) but *in vivo* studies have been contradictory (Packer *et al.*, 1987; Parker *et al.*, 1987b). These latter studies both involved patients with either congestive heart failure (Packer *et al.*, 1987) or chronic angina (Parker *et al.*, 1987) and both administered NAC as a single large dose after tolerance had been established. The present study addressed the question of early and chronic administration of NAC on the development of nitrate tolerance during treatment with transdermal GTN in healthy subjects taking no other medication. The failure of NAC to impair the development of tolerance in this study may be attributable to many causes. The dose of NAC used was small and despite chronic administration may have been insufficient to generate adequate tissue levels of reduced SH groups. Furthermore NAC may not be an optimal donor of SH groups and recent *in vitro* studies have shown cysteine to be superior in this respect (Feelisch & Noack, 1987). In addition, mechanisms other than the depletion of SH groups may be important in the development of tolerance. In particular the activation of neurohumoral reflexes such as the renin-angiotensin system resulting in volume expansion and vasoconstriction in response to nitrate induced vasodilation have been implicated (Lis *et al.*, 1984; Packer *et al.*, 1985, 1987). Finally nitrates exert their haemodynamic actions to differing extents in different vascular beds. It is possible therefore that the relative development of tolerance and its reversal may occur to varying extents in different vascular beds making the overall effect negligible.

In conclusion the results of this study show that tolerance to the haemodynamic effects of GTN occurs when administered transdermally over several days. They further demonstrate that concurrent administration of NAC has little effect on this phenomenon.

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