Efficacy and Rebound Phenomenon Related To Intermittent Nitroglycerin Therapy for the Prevention of Nitrate Tolerance

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Intermittent transdermal therapy of nitroglycerin (NTG) has been recommended for the prevention of nitrate tolerance, but a rebound phenomenon has been reported to occur following removal of the NTG tape. The present study investigated the effects of intermittent NTG therapy on vasodilatory response and the intracellular production of cyclic GMP (cGMP). The study group comprised 12 healthy adults and measurements were taken of the platelet cGMP level, the venous volume (VV) (by forearm plethysmography) and the plasma levels of neurohormonal factors before and 5 min after administration of 0.3 mg of sublingual nitroglycerin (NTG) during the following 4 phases: (i) the control phase (8.00 h); (ii) the continuous phase (8.00 h; 7 days after continuous application of a 10 mg/24 h NTG tape); (iii) the intermittent application phase (8.00 h; 7 days after intermittent application of NTG tape, applied at 21.00 h and removed at 9.00 h); and (iv) the intermittent removal phase (13.00 h; 4 h after removal of the NTG tape in the intermittent phase). The percentage increase in cGMP (%cGMP) and venous volume (%VV) were significantly lower in the continuous phase than the control phase, but there was no difference between the control and the intermittent application phases. However, in the intermittent removal phase, the cGMP level before sublingual NTG, the %cGMP and the %VV were unchanged, but the VV before sublingual NTG was significantly lower than in the control phase. Plasma renin activity and the plasma level of angiotensin II were significantly increased in the control phase, the intermittent application phase, and the intermittent removal phase. In conclusion, intermittent transdermal NTG therapy prevented nitrate tolerance in the production of cGMP and vasodilation, but induced a rebound phenomenon after removal of the NTG tape. The rebound phenomenon following the tape removal may be related to some other mechanism, such as activation of neurohormonal factors. (Jpn Circ J 1998; 62: 571–575)

Key Words: Angiotensin II; Cyclic GMP; Nitrate tolerance; Nitrates; Plasma renin activity

Nitroglycerin (NTG) is widely used for the treatment of ischemic heart disease and congestive heart disease. The mechanism of nitrate action is believed to be related to the production of cyclic GMP (cGMP). Nitrate tolerance, which is the rapid attenuation of the effect of organic nitrate, is the major factor that limits the use of nitrates.1–3 The Food and Drug Administration (FDA) Cardiorenal Advisory Committee met on June 3, 1993 to discuss the implications of the nitrate tolerance phenomenon and proposed some recommendations for avoiding nitrate tolerance.4 From these recommendations, interruption of nitrate exposure for 8–12 h appears to be the best means of preventing tolerance. However, in some trials of intermittent nitrate therapy, patients with angina pectoris have experienced a rebound phenomenon during the interruption phase.5–7 The relationship between the production of cGMP and vasodilation during intermittent nitroglycerin (NTG) therapy has not been clarified. Therefore, we investigated the effects of intermittent NTG therapy on the vasodilation and the intracellular production of cGMP, and the development of a rebound phenomenon during intermittent NTG therapy.

Methods

Study Population

The study population consisted of 12 normal volunteers (mean age, 36±3 years; range 32–45 years; 8 men, 4 women) who had no history of renal or cardiac disease, and who had normal physical findings, normal serum levels of electrolyte, cholesterol and creatinine, and normal results of urine analysis. No subjects were taking medications at the time of the study.

Study Protocol (Fig 1)

The study design consisted of a series of measurements of plethysmographic vasodilator response and the platelet cGMP level before and after sublingual administration of NTG in the following 3 phases: (i) control phase; (ii) 7 days after continuous application of a NTG tape; and (iii) 7 days after intermittent application of a NTG tape. During the continuous phase, a 5 mg NTG tape was applied twice a day at 9.00 h and at 21.00 h for 7 days. The intermittent
Phase was initiated after a 7 day washout period. During the intermittent phase, a 5 mg NTG tape was applied at 21.00h, and removed at 9.00h for 7 days. The venous volume was measured and blood samples were obtained at 8.00h on day 7 of all phases. To evaluate the rebound phenomenon, measurements and blood sampling were performed 4 h after removal of the NTG tape on day 7 of the intermittent phase. An 18 gauge heparin lock was placed in the contralateral forearm to obtain venous blood samples for measurements of platelet cGMP.

The study protocol was approved by the ethics committee of KINU Medical Association Hospital, Mitsukaido, Japan, and the University of Tsukuba, Tsukuba, Japan, and written informed consent for participation in the study was obtained from all subjects.

Assessment of the Vasodilator Response to Nitroglycerin

To evaluate the vasodilator response to NTG, we measured the venous volume using a mercury-in-silastic strain gauge plethysmograph and the venous occlusion technique. The strain gauge was placed 5 cm below the antecubital crease and connected to a calibrated plethysmograph. The hand was isolated from the circulation by inflating a wrist cuff to suprasystolic pressure. A forearm occlusion cuff was inflated to 30 mmHg above cuff zero. Cuff zero is that pressure below which forearm volume remains unchanged and above which forearm volume increased. After inflation of the forearm cuff, the venous volume increased for 3–4 min until a plateau was reached. This value was considered to be the baseline (before sublingual NTG administration) venous volume (VV, ml/100 ml arm). Immediately on attaining equilibration, 0.3 mg sublingual NTG was applied with continued forearm volume recording until a new plateau was achieved or for a maximum of 5 min. The change in forearm volume from the first to the second plateau was considered to be the venodilatory effect of NTG. We used the average of three measurements obtained at 15 sec intervals for analysis.

Preparation of Platelet cGMP

Blood samples were drawn into syringes containing 5 mmol/L EDTA and a cGMP phosphodiesterase inhibitor (10^-3 mol/L 2-O-propoxyphenyl-8-azapurin-6-one dissolved in 1% triethanolamine). Samples were immediately centrifuged at 200 g for 20 min to obtain platelet-rich and platelet-poor plasma samples. Platelet-rich plasma was further centrifuged at 2500 g for 10 min, and the supernatant was discarded. The pellet was suspended in modified Tyrode’s solution (containing 0.35% bovine serum albumin and 5 mmol/L HEPES, pH 7.35) to obtain a final platelet count of 2–3×10^9 platelets/μl. Samples were stored frozen at −70°C until analysis.

Platelet cGMP Assay

For the cGMP assay, 0.5 ml of trichloroacetic acid (final concentration, 6%) was added to 1 ml of the platelet samples. After the preparation was centrifuged at 2500 g for 20 min, trichloroacetic acid was extracted four times from the supernatant with water-saturated ether. The cGMP level of the aqueous phase was measured using a commercially available radioimmunoassay kit. (Yamasa Shoyu, Choshi, Japan). The results were expressed in picomoles per 10^9 platelets. The coefficients of variation were 3.4% for the intraassay error and 11.9% for the interassay error.

Plasma Renin Activity and Angiotensin II Levels

Plasma levels of renin and angiotensin II were measured by radioimmunoassays in platelet-poor plasma. The results were expressed in ng/ml per h for plasma renin activity and pg/ml for angiotensin II. The intra- and interassay variations for plasma renin activity were 6% and 12%, respectively, and for angiotensin II were 8% and 15%, respectively.

Statistical Analysis

Results were expressed as the mean ± SD for the venous volume, plasma renin activity and angiotensin II, and as the mean ± SEM for the platelet cGMP level. Differences among all phases were analyzed by repeated-measures of analysis of variance (ANOVA) with Bonferroni’s test and the difference between before and after sublingual NTG was assessed by the Student’s t-test. A p value <0.05 was accepted as statistical significance.

Results

Heart Rate and Blood Pressure (Table 1)

The heart rate did not change in any phase. The mean blood pressure was decreased after sublingual administration of NTG in all phases. The decrease in blood pressure tended to be smaller in the continuous phase compared with that in the control phase (not significantly), but the decrease in the intermittent application phase or the control phase was similar to that in the control phase. In the intermittent removal phase, the mean blood pressure before sublingual NTG tended to be high compared with that in the control phase (not significantly).

Body Weight and Hematocrit (Table 2)

Body weight did not change in any phase. The hematocrit was significantly decreased in the continuous phase compared with the other phases.

Venous Volume (Table 3, Fig 2)

The venous volume was significantly increased after sublingual administration of NTG in all phases. There was no difference in the venous volume between the control and the continuous phases before sublingual administration of NTG. After sublingual administration of NTG, the
venous volume was significantly lower in the continuous phase than in the control phase. The venous volume in the intermittent application phase before and after sublingual administration of NTG was significantly higher than that in the control phase. However, in the intermittent removal phase, the venous volume before sublingual administration of NTG was significantly lower than in the control phase.

The percent increase in the venous volume was significantly lower in the continuous phase than in the control phase (32±8 vs 15±4%, p<0.01), but was similar in the intermittent application phase (28±7%) and the control phases. The percent increase in the intermittent removal phase (31±6%) was also similar to that in the control phase.

Platelet cGMP (Table 4, Fig 3)

The administration of sublingual NTG significantly increased the platelet cGMP level in all phases. There was no difference in the platelet cGMP level before sublingual administration of NTG among the control, continuous and intermittent removal phases. In the intermittent application phase, the platelet cGMP level before and after sublingual administration of NTG was higher compared with the other three phases. The platelet cGMP level after sublingual administration of NTG was lower in the continuous phase than in the control phase. The level in the intermittent removal phase was similar to the control phase.

The percent increase in the platelet cGMP level was significantly lower in the control phase (38±5% vs 8±2%, p<0.01). The percent increase in the intermittent application phase (33±6%) was similar to the control phase, but was significantly increased compared with the continuous phase. In the intermittent removal phase, the percent increase (40±6%) was similar to the control phase.
Platelet cGMP level was decreased during continuous application and the intermittent removal phases. The plasma level of angiotensin II was also significantly increased in the continuous, the intermittent application, and the intermittent removal phases. There was no difference in the plasma renin activity and the plasma level of angiotensin II between the intermittent application and the intermittent removal phases.

**Neurohormonal Factors (Table 5)**

Plasma renin activity was significantly increased in the continuous, the intermittent application, and the intermittent removal phases. The plasma level of angiotensin II was also significantly increased in the continuous, the intermittent application, and the intermittent removal phases. There was no difference in the plasma renin activity and the plasma level of angiotensin II between the intermittent application and the intermittent removal phases.

**Discussion**

Intermittent therapy with transdermal NTG maintained the intracellular production of cGMP and the venous volume, indicating that it prevented nitrate tolerance. However, the removal of the NTG tape resulted in a decrease in the venous volume, indicating that a rebound phenomenon had developed.

**Nitrate Tolerance**

Tolerance to organic nitrates has been demonstrated in vitro and in vivo. The mechanisms of nitrate tolerance are poorly defined, and are likely to be multifactorial; several mechanisms have been proposed. Nitroglycerin requires intracellular conversion before activating vascular smooth muscle relaxation. Ignarro et al have shown that nitrovasodilators induce the relaxation of vascular smooth muscle cells through activation of intracellular soluble guanylate cyclase, leading to increased levels of cGMP. Kukovez and Holzmann have reported the biochemical aspects of vascular nitrate tolerance. In their work, they showed a very good correlation between the percent relaxation and the accumulation of cGMP in bovine coronary arteries after treatment with NTG, nitroprusside, and 3-morpholino-sydnonimine (SIN-1). We previously reported that there was a good correlation between the percent increase in platelet cGMP level and the percent dilatation of coronary arteries, and that the intracellular production of platelet cGMP level was decreased during continuous nitrate therapy. These observations suggest that nitrate tolerance is related to blunted vasodilation due to the attenuation of intracellular production of cGMP in response to NTG. Parker et al suggested that nitrate therapy is associated with both neurohormonal activation and plasma volume expansion and that these responses may play a role in the loss of nitrate efficacy. In the present study, the plasma renin activity and the plasma level of angiotensin II increased not only in the continuous phase but also in the intermittent application and removal phases. Intermittent NTG therapy did not prevent the activation of neurohormonal factors in the present study.

**Table 5 Plasma Renin Activities (PRA) and Angiotensin II Levels (AT II)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Continuous</th>
<th>Intermittent application</th>
<th>Intermittent removal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRA (ng/ml per h)</strong></td>
<td><strong>Before</strong></td>
<td>4.8±1.6</td>
<td>9.2±2.4*</td>
<td>7.8±2.0*</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>5.1±1.8</td>
<td>9.6±2.5*</td>
<td>8.0±1.8*</td>
</tr>
<tr>
<td><strong>AT II (pg/ml)</strong></td>
<td><strong>Before</strong></td>
<td>16.1±8.3</td>
<td>28.5±9.4*</td>
<td>27.4±7.3*</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>16.2±7.1</td>
<td>29.2±8.1*</td>
<td>29.4±9.7*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; *p<0.05 vs control phase.

**Intermittent Nitroglycerin Therapy and the Rebound Phenomenon**

A number of investigations have demonstrated that intermittent therapy with NTG is an effective treatment for angina2,5,6,21,22. A recent FDA advisory meeting concluded that interruption of nitrate exposure for as little as 8–12 h is the best means of preventing or reversing tolerance. However, to our knowledge, there have been no previous investigations of the intracellular production of cGMP, the vasodilatory response and activation of neurohormonal factors during intermittent NTG therapy. In the present study, the venous volume and platelet cGMP levels before sublingual NTG were higher in the intermittent application phase than in the continuous phase. The increases in venous volume and cGMP level after sublingual NTG were also higher in the intermittent application phase than in the continuous phase. Thus intermittent application of NTG tape maintained the efficacy of the intracellular production of cGMP. The present study is the first report to confirm that intermittent NTG therapy prevents nitrate tolerance on the intracellular production of cGMP.

Our results also indicated that the rebound phenomenon after removal of the NTG tape was not demonstrated in the production of cGMP. In the intermittent removal phase, the platelet cGMP level before sublingual NTG was similar to that in the control phase. However, venous volume before sublingual NTG was lower in the intermittent removal phase than that in the control phase. A rebound phenomenon after nitrate withdrawal was first described in muntions workers who were exposed during the week to industrial concentrations of NTG and who then experienced angina, myocardial infarction and even death in the weekend period after removal from exposure. Although that situation clearly differs from the removal of a nitrate patch, two early studies of NTG patches suggested the possibility of rebound angina. These studies showed that angina attacks and the frequency of sublingual NTG use increased on the first day after removal of the NTG patch. The hemodynamic and vasodilatory responses to removal of a NTG patch have not been previously investigated. In the present study, the venous volume was decreased after removal of the NTG tape, although the platelet cGMP level was maintained. Moreover, the plasma renin activity and the plasma level of angiotensin II were increased during the application of the NTG tape. It has been known that the plasma level of NTG decreases rapidly after tape removal, and that the counterregulatory phenomenon persists for 4–6 h. Therefore, the present results suggest that there is a balance between the nitrate effects (cGMP production) and the counterregulatory forces (the activation of neurohormonal factors) before tape removal during intermittent NTG therapy, and that the rapid decline in the plasma level of NTG after tape removal is accompanied by expression of these counterregulatory influences on vasodilation.

**Conclusions**

Intermittent transdermal NTG therapy was effective for the prevention of nitrate tolerance in the production of cGMP. The rebound phenomenon following tape removal may be related to a mechanism other than decreased cGMP production, such as activation of neurohormonal factors.
References