Tolerance to Organic Nitrates: Evidence, Mechanisms, Clinical Relevance, and Strategies for Prevention

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Objective: To review the available information about nitrate tolerance, its potential mechanisms, clinical implications, and strategies for prevention.

Data Identification: A survey of the National Library of Medicine MEDLINE database and bibliographies of the reviewed articles.

Study Selection and Data Extraction: Studies were selected from the English language literature with an emphasis on recent studies and, when available, randomized placebo-controlled studies. Old studies were selected on the basis of their historical value and originality. A total of 134 retrieved articles were considered relevant and were reviewed in depth.

Results: The available information about the experimental as well as the clinical evidence for tolerance to organic nitrates has been summarized. In addition, information related to potential mechanisms, clinical implications, and possible methods for prevention have been reviewed.

Conclusions: Evidence indicates that prolonged in-vitro exposure to organic nitrates, continuous intravenous or topical administration of nitrates, and frequent in-vivo oral dosing result in the rapid development of tolerance to the peripheral as well as to the coronary vasodilatory effects of the drugs. This phenomenon leads to the rapid attenuation of the hemodynamic and anti-ischemic effects of nitrates in patients with ischemic heart disease or congestive heart failure, or both. Tolerance development seems to be dose- and time-dependent, and its main mechanism seems to be a depletion of sulfhydryl groups at the vascular cell. Although the repletion of sulfhydryl groups with the use of sulfhydryl-containing drugs may help to prevent tolerance, the efficacy and safety of this approach requires further evaluation. Intermittent therapy allowing a sufficiently long, daily nitrate-washout interval seems to be the most effective and the most safe strategy currently available for the prevention of nitrate tolerance.

Although the development of tolerance to the effects of organic nitrates was described as early as 1898 and has been shown repeatedly since then in both animals and humans, the clinical relevance of this phenomenon has been controversial. Renewed interest and intensive scientific effort in the last decade have helped to illuminate this issue and have clearly shown that tolerance, if ignored, may severely limit the benefit of nitrate therapy. This article is intended to review comprehensively the state of knowledge about nitrate tolerance, its mechanisms, its clinical relevance, and strategies for its prevention. Data were obtained using a MEDLINE search of the English language literature, bibliographies of the reviewed articles, and the author's files. The reference section of this article includes citations from all these sources. Recent studies and, when available, randomized placebo-controlled studies were emphasized. Old studies were selected on the basis of their historical value and originality.

Evidence for Nitrate Tolerance

Headache and Blood Pressure

Tolerance to the effects of organic nitrates in humans was first described by Laws (1) in 1898 and later by others (2-4) who reported immunity to throbbing headache over time in persons who were engaged in the manufacture of dynamite and isosorbide dinitrate. This immunity was reported to be lost with the discontinuation of exposure, such as during weekends or vacations. The application of a small amount of dynamite within the sweatbands of the hats worn by powder plant workers who had had contact with dynamite was reported to decrease the incidence of headache (described as "nitroglycerin head") (3, 5). Stewart (6) first described tolerance to the antihypertensive effect of nitroglycerin when he reported the loss of effect with continuous therapy despite a 160-fold dose increase. In the first prospective evaluation by Crandall and colleagues (7) in 1931, the development of tolerance to the effects of various organic nitrates on headache, heart rate, and blood pressure occurring after 3 to 60 hours of therapy was documented.

The temporary nature of the effect of continuous or repeated administration of nitrates on heart rate and blood pressure was confirmed in animal experiments (8-10). In addition, in-vitro experiments have shown a marked reduction in the relaxant response to nitroglycerin in isolated aortic preparations obtained from rats that were pretreated with nitroglycerin. The applicability of these experimental data to humans was later reconfirmed by Thadani and colleagues (II) who found tolerance to the effect of oral isosorbide dinitrate
given every 6 hours on systolic blood pressure as early as after the fifth consecutive dose, despite higher plasma isosorbide dinitrate concentration.

Angina Pectoris

To further investigate the clinical relevance of nitrate tolerance in humans, Danah Y'and Aronow (12) studied the acute and chronic hemodynamic and antianginal effects of sublingual nitroglycerin and oral isosorbide dinitrate given four times daily to patients with chronic angina pectoris. These investigators reported partial tolerance to the effects of both drugs on heart rate and blood pressure after a mean period of 5.6 months, but they failed to find an impairment of the antianginal efficacy. Because at least a 14-hour drug-free interval was allowed before exercise, however, the antianginal effect may have been restored and may have masked the previous development of tolerance. This study and a study by Lee and coworkers (13), reported shortly thereafter, showing preservation of the antianginal action of sublingual nitroglycerin after 1 month of chronic oral isosorbide dinitrate, resulted in a widely accepted conclusion that tolerance to the antianginal effect of long-acting organic nitrates and cross-tolerance to sublingual nitroglycerin were of little clinical significance (14). Continued research by Thadani and colleagues (15), however, provided contrasting results and provoked a new wave of intensive investigation about nitrate tolerance in humans. These investigators showed a dose-related increase in the length of exercise before the onset of angina, an increase lasting for approximately 8 hours after acute therapy with 15 to 120 mg of oral isosorbide dinitrate. They showed a marked attenuation of effect, in both magnitude and duration, after 2 weeks of therapy when the same dose was given four times daily. Further, the relation between dose and effect was abolished during sustained therapy, and the change in the length of exercise before the onset of angina was similar with all doses. An evaluation of isosorbide dinitrate plasma concentration showed higher levels during sustained therapy compared with acute therapy, indicating tolerance rather than accelerated metabolism of the drug. The loss of the anti-ischemic effect of oral isosorbide dinitrate with frequent dosing was confirmed by other well-designed, placebo-controlled studies (16-18).

The recent introduction of transdermal systems for the administration of nitroglycerin and investigations of their antianginal effect provided additional proof of the early development of tolerance to organic nitrates. These systems continuously release nitroglycerin by matrix diffusion-controlled or membrane permeation-controlled drug delivery systems and result in an elevated nitroglycerin blood level that persists for at least 24 hours (19, 20). The results of reported placebo-controlled studies are contradictory; some show a complete failure of transdermal nitroglycerin to enhance exercise capacity in patients with angina pectoris (21, 22), whereas other studies show a beneficial effect (23-34). In addition, the results of studies about the persistence of the antianginal effect of transdermal nitroglycerin have conflicted. Several studies showed sustained effect over 16 to 24 hours (23-28), whereas other studies showed an important initial effect lasting for 2 to 8 hours after application, with marked attenuation of efficacy thereafter (29-34). The reasons for the marked differences among the results of these studies are not entirely clear, but they probably are related to previously described difficulties associated with research of antianginal therapy, including differences in patient population, disease severity, study protocols, drug dosage, concomitant therapy, and therapeutic end points (35, 36). Other potential reasons are small study samples, spontaneous fluctuations in the frequency and the severity of angina (37), and various percentages of responders and nonresponders (26) in different studies. A recently completed large-scale, multicenter study designed to overcome the difficulties inherent in analyzing several small-scale studies confirmed the development of tolerance to the antianginal effect of transdermal nitroglycerin within the first 24 hours of therapy (38). This study, the Federal Drug Administration Transdermal Nitroglycerin Cooperative Study (unpublished data), evaluated the efficacy and the safety of the continuous administration of transdermal nitroglycerin at doses ranging from 15 to 105 mg/d in 562 patients with stable angina. The results of the study showed an important increase in total walking time before the onset of angina for all doses at 4 hours but not at 24 hours after the initiation of therapy. Additionally, despite the documented increase in walking time before the onset of angina with the use of active drug after 8 weeks of continuous therapy, this increase was similar to the level seen with use of placebo. A clinically important increase in the incidence of headache was noted initially in the nitroglycerin group. This incidence was decreased to that seen with placebo use by week 4 of chronic therapy. The strong placebo effect seen in this study may cause the apparent paradox of continued symptomatic improvement in patients treated with organic nitrates despite the development of nitrate tolerance.

The early tolerance that develops with transdermal administration of nitrates is most likely due to a loss of vascular response associated with continuous exposure to the drug. Such attenuation of the vasorelaxing effects of nitrates was reported by Needleman (9), who showed an inverse relation between nitroglycerin-mediated relaxation of isolated aortic strips and the duration of pre-exposure to the drug. The limitations of drug delivery systems that produce sustained therapeutic blood levels of organic nitrates in patients with angina pectoris have been further shown by Parker and coworkers (39), who demonstrated the development of tolerance to the anti-ischemic effect of transdermal isosorbide dinitrate within several hours of the initiation of therapy. Zimrin and coworkers (40) showed an early attenuation of the anti-ischemic effect of intravenous nitroglycerin in patients with angina pectoris. Similarly, Thadani and colleagues (41) showed the complete abolition of the effects on blood pressure, heart rate, and ischemia in nine men with angina pectoris after therapy with slow-release isosorbides-mononitrate, 50 mg and 100 mg once daily for 1 week, despite therapeutic plasma concentration.
Table 1. Evidence for Nitrate Tolerance in Patients with Angina Pectoris*

<table>
<thead>
<tr>
<th>Investigators, Year (Reference)</th>
<th>Nitrate Preparation</th>
<th>Dosage and Regimen</th>
<th>Duration of Therapy, d</th>
<th>Attenuated Variables</th>
<th>Number of Patients</th>
<th>Study Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thadani et al, 1980 (II)</td>
<td>ISDN</td>
<td>15-120 mg four times daily</td>
<td>7</td>
<td>HR, BP</td>
<td>II</td>
<td>RDPC</td>
</tr>
<tr>
<td>Thadani et al, 1982 (15)</td>
<td>ISDN</td>
<td>15-120 mg four times daily</td>
<td>14</td>
<td>HR, BP, ET</td>
<td>13</td>
<td>DBPC</td>
</tr>
<tr>
<td>Tauchert et al, 1983 (68)</td>
<td>IS,-MN</td>
<td>50 mg three times daily</td>
<td>28</td>
<td>HR, BP, PAP, CO</td>
<td>10</td>
<td>Open</td>
</tr>
<tr>
<td>Dalal and Parker, 1983 (82)</td>
<td>ISDN</td>
<td>15-50 mg four times daily</td>
<td>10</td>
<td>BP, ET</td>
<td>10</td>
<td>OBPC</td>
</tr>
<tr>
<td>Reichek et al, 1984 (29)</td>
<td>TDNTG</td>
<td>9.4-25 mg/dl</td>
<td>1</td>
<td>HR, BP, ET, STII</td>
<td>13</td>
<td>RD</td>
</tr>
<tr>
<td>Parker and Fung, 1984 (30)</td>
<td>TDNTG</td>
<td>5-45 mg/dl</td>
<td>1</td>
<td>HR, BP, ET, STII</td>
<td>17</td>
<td>DBPC</td>
</tr>
<tr>
<td>Parker et al, 1985 (16)</td>
<td>ISDN</td>
<td>30 mg four times daily</td>
<td>7</td>
<td>HR, BP, ET</td>
<td>16</td>
<td>DBPC</td>
</tr>
<tr>
<td>James et al, 1985 (31)</td>
<td>TDNTG</td>
<td>5 mg/dl</td>
<td>3</td>
<td>ET, STII</td>
<td>12</td>
<td>DBPC</td>
</tr>
<tr>
<td>Thadani et al, 1986 (32)</td>
<td>TDNTG</td>
<td>5-20 mg/dl</td>
<td>2</td>
<td>HR, BP, ET, STII</td>
<td>14</td>
<td>DBPC</td>
</tr>
<tr>
<td>Kohli et al, 1986 (129)</td>
<td>IS,-MN</td>
<td>40 mg twice daily</td>
<td>14</td>
<td>HR, ET</td>
<td>18</td>
<td>OBPC</td>
</tr>
<tr>
<td>Silber et al, 1987 (17)</td>
<td>SRISDN</td>
<td>80 mg twice daily (concentric)</td>
<td>17</td>
<td>HR, ET, EF, STII</td>
<td>12</td>
<td>RD</td>
</tr>
<tr>
<td>Thadani et al, 1987 (18)</td>
<td>ISDN</td>
<td>30 mg four times daily</td>
<td>12</td>
<td>HR, BP, ET</td>
<td>12</td>
<td>RD</td>
</tr>
<tr>
<td>Thadani et al, 1987 (41)</td>
<td>SR IS,-MN</td>
<td>50 &amp; 100 mg once daily</td>
<td>7</td>
<td>HR, BP, ET, STII</td>
<td>9</td>
<td>DBPC</td>
</tr>
<tr>
<td>Parker et al, 1987 (122)</td>
<td>ISDN</td>
<td>30 mg four times daily</td>
<td>7-10</td>
<td>HR, BP, ET</td>
<td>12</td>
<td>DBPC</td>
</tr>
<tr>
<td>Cowan et al, 1987 (123)</td>
<td>TDNTG</td>
<td>10 mg/dl</td>
<td>7</td>
<td>HR, BP, ET, STII</td>
<td>14</td>
<td>DBPC</td>
</tr>
<tr>
<td>Luke et al, 1987 (124)</td>
<td>TDNTG</td>
<td>10 mg/dl</td>
<td>7</td>
<td>HR, BP, ET</td>
<td>12</td>
<td>DBPC</td>
</tr>
<tr>
<td>FDA Cooperative Study, 1988 (38)</td>
<td>TDNTG</td>
<td>15-105 mg/dl</td>
<td>56</td>
<td>ET</td>
<td>562</td>
<td>DBPC</td>
</tr>
<tr>
<td>Zimrin et al, 1988 (40)</td>
<td>IV NTG</td>
<td>10-120 mg/dl</td>
<td>1 (25 h)</td>
<td>ET</td>
<td>10</td>
<td>DBPC</td>
</tr>
<tr>
<td>Frishman et al, 1989 (34)</td>
<td>TDNTG</td>
<td>20-100 mg/dl</td>
<td>14</td>
<td>HR, BP, ET</td>
<td>20</td>
<td>DBPC</td>
</tr>
<tr>
<td>Thadani et al, 1989 (130)</td>
<td>IS,-MN</td>
<td>50 and 100 mg/dl</td>
<td>7</td>
<td>HR, BP, ET</td>
<td>19</td>
<td>DBPC</td>
</tr>
<tr>
<td>Webster et al, 1989 (131)</td>
<td>TDNTG</td>
<td>10 mg/dl</td>
<td>10</td>
<td>ET</td>
<td>12</td>
<td>BPPC</td>
</tr>
</tbody>
</table>

* A VD02 = arteriosomous oxygen difference; BP = blood pressure; CO = cardiac output; concentric = drug given at 0800 and at 1400 h; DB = double blind; ET = exercise time; FDA = Food and Drug Administration; HR = heart rate; ISDN = oral isosorbide dinitrate; IS,-MN = isosorbide,-mononitrate; IV = intravenous; NTG = nitroglycerin; OB = open, blinded; PAP = pulmonary artery pressure; PC = placebo controlled; RD = randomized; SR = sustained release; ST/1 = ST-segment change; SV = stroke volume; TD = transdermal.

Table 1 summarizes the evidence about nitrate tolerance in patients with angina pectoris.

Congestive Heart Failure

The introduction of transdermal nitroglycerin systems resulted in intense investigations of the efficacy of this form of therapy inpatients with heart failure and was helpful in showing the early development of nitrate tolerance in this patient population. Olivari and colleagues (42) were first to report the initial effectiveness of transdermal nitroglycerin in patients with chronic heart failure and the early attenuation of most of its hemodynamic effects within the first 24 hours of therapy. Although Rajfer and coworkers (43) reported a sustained hemodynamic response to transdermal nitroglycerin, 10 to 60 mg/d, many studies using similar doses confirmed the findings of Olivari and coworkers (42) and showed the rapid development of tolerance leading to a marked attenuation of hemodynamic efficacy (44-48). Elkayam and colleagues (47, 48) showed nonpersistent hemodynamic effects even with 90 mg of transdermal nitroglycerin and a significant attenuation of initial hemodynamic changes within several hours of the initiation of therapy in responders to a very large dose (120 mg) of the drug.

The rapid development of tolerance to continuous nitroglycerin administration has also been documented in patients with chronic heart failure during intravenous infusion of the drug. Elkayam and colleagues (49) have shown an important attenuation of the hemodynamic effect occurring within the first 24 hours of therapy in approximately half of responders to the drug. The development of tolerance could not be predicted by base line hemodynamic and hormonal values or by changes in these values during nitroglycerin therapy. The results of this study were confirmed by other investigators who noted the development of tolerance during 24 to 72 hours of continuous nitroglycerin infusion in patients with congestive heart failure (50-52).

Preliminary data have indicated that frequent dosing (every 4 or 6 hours) of oral isosorbide dinitrate in patients with heart failure has also resulted in the early development of tolerance to the effect on left ventricular filling pressure (53, 54). This phenomenon is seen in many patients as early as 6 to 12 hours after the initiation of therapy. These results contrast somewhat with Franciosa and Coh's finding (55) of sustained hemodynamic effects without the development of tolerance in patients with heart failure who were treated with isosorbide dinitrate, 40 mg every 6 hours for 3 months. The withholding of therapy before the hemodynamic evaluation of the isosorbide dinitrate effect after chronic treatment in that study, however, could have allowed a sufficiently long washout interval for restoration of the nitrate effect. Table 2 summarizes the evidence about nitrate tolerance in patients with congestive heart failure.

Acute Myocardial Infarction and Unstable Angina

Nitroglycerin is often continuously administered to treat patients with acute myocardial infarction (56-58) and unstable angina (59, 60). Prolonged nitroglycerin therapy has been shown to relieve ischemia, to reduce myocardial infarction size, to enhance left ventricular ejection fraction, and to prevent left ventricular expansion and dilatation after acute myocardial infarction (56, 61).
A meta-analysis of reported data suggested an improvement in the survival of patients receiving intravenous nitroglycerin during the acute stage of myocardial infarction (58). Do these results suggest a lack of the development of nitrate tolerance in this patient population or an improvement despite the development of nitrate tolerance? The answer to these questions is not readily available and require further research. The documented early attenuation of the nitrate effect in most patients with angina and heart failure, however, may suggest that the beneficial effect of nitroglycerin occurs during the first 24 hours of therapy. Such a hypothesis is supported by the improvement in left ventricular function that is seen in patients in whom nitroglycerin treatment was begun less than 10 hours after the onset of symptoms and that is not seen in patients in whom treatment was begun more than 10 hours after the initiation of symptoms (56). In addition, a recent study showing a persistent hemodynamic improvement, despite the discontinuation of intravenous nitrate administration after several hours of therapy, in patients with acute myocardial infarction and heart failure further indicates that early relief of ischemia may cause acute and, possibly, late functional and clinical improvement (61). The high incidence of acute myocardial infarction, of recurrence of symptoms, and of need for surgical reperfusion in patients with unstable angina who receive continuously infused intravenous nitroglycerin and the significant reduction in the incidence of myocardial infarction in such patients with the use of N-acetylcysteine may be related to the development of tolerance in association with the continuous administration of nitrates (62, 63). Additional research is obviously needed to further evaluate the benefit and the limitation of continuous intravenous nitroglycerin therapy in treating patients with the acute ischemic syndrome.

Coronary Blood Flow

May and coworkers (64) measured coronary sinus blood flow before and during intracoronary administration of nitroglycerin at increasing doses and showed a dose-dependent rise in flow. This beneficial change in coronary sinus blood flow with nitroglycerin was reproducible a day later in patients who received a 24-hour intravenous infusion of saline. In contrast, an approximately 50% reduction in the effect of intracoronary nitroglycerin was shown in patients who received a continuous, intravenous nitroglycerin infusion for 24 hours. These results indicate the partial development of tolerance to the coronary effect of nitroglycerin within 24 hours of therapy. The results of this study are supported by the results of animal experiments, in which tolerance to the coronary vasodilatory effect of continuous intravenous nitroglycerin infusion developed in chronically instrumented conscious dogs by the second to third day of treatment (65).

The Incidence of Tolerance Development

The question of whether tolerance is likely to occur universally in every patient treated with organic nitrates is of great clinical significance. At present, however, only limited information is available about individual response to nitrate therapy. Elkayam and colleagues (49) reported the development of tolerance within 24 hours of the initiation of therapy in approximately half of patients with heart failure receiving an infusion of intravenous nitroglycerin. A similar incidence of the development of nitrate tolerance in the same period was reported by Dakak and coworkers (52). Roth and colleagues (48) reported partial or complete development of tolerance to the hemodynamic effect of large-dose transdermal nitroglycerin within 24 hours of the initiation of therapy in 8 of 11 patients with heart failure. Other investigators reported a loss of the initial hemodynamic effect of nitroglycerin in the majority of patients with congestive heart failure with large-dose infusion lasting up to 48 hours (50). Although Dupuis and colleagues (51) noted a sustained beneficial hemodynamic effect during a 72-hour intravenous nitroglycerin infusion in some patients with heart failure, the persistence of hemodynamic improvement even after the discontinuation of nitroglycerin infusion in their patients may suggest the continuation of initial improvement rather than a lack of tolerance. The available data therefore suggest that the incidence of tolerance in patients with chronic heart failure is related to the duration of therapy, with most patients developing tolerance within

Table 2. Evidence for Nitrate Tolerance in Patients with Chronic Congestive Heart Failure

<table>
<thead>
<tr>
<th>Investigators, Year</th>
<th>Nitrate Preparation</th>
<th>Dosage and Regimen</th>
<th>Duration of Therapy</th>
<th>Number of Patients</th>
<th>Study Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olivari et al, 1983 (42)</td>
<td>TDNTG</td>
<td>15-20 mg/d</td>
<td>24 hr</td>
<td>9</td>
<td>Open</td>
</tr>
<tr>
<td>Jordan et al, 1985, 1986 (44, 45)</td>
<td>TDNTG</td>
<td>60 mg/d</td>
<td>24 hr</td>
<td>15</td>
<td>DBP</td>
</tr>
<tr>
<td>Elkayam et al, 1985 (47)</td>
<td>TDNTG</td>
<td>90 mg/d</td>
<td>24 hr</td>
<td>11</td>
<td>Open</td>
</tr>
<tr>
<td>Packery et al, 1986 (46)</td>
<td>TDNTG</td>
<td>10-60 mg/d</td>
<td>24 hr</td>
<td>22</td>
<td>Open</td>
</tr>
<tr>
<td>Roth et al, 1987 (48)</td>
<td>TDNTG</td>
<td>120 mg/d</td>
<td>30 hr</td>
<td>11</td>
<td>Open</td>
</tr>
<tr>
<td>Elkayam et al, 1987 (49)</td>
<td>IV NTG</td>
<td>50-560 min</td>
<td>24 hr</td>
<td>40</td>
<td>DBP</td>
</tr>
<tr>
<td>Packer et al, 1987 (50)</td>
<td>IV NTG</td>
<td>6.4 g body weight per minute</td>
<td>48 hr</td>
<td>35</td>
<td>Open</td>
</tr>
<tr>
<td>Elkayam et al, 1987 (53)</td>
<td>ISDN</td>
<td>40-120 mg every 4 hours</td>
<td>30 hr</td>
<td>11</td>
<td>Open</td>
</tr>
<tr>
<td>Sharpe et al, 1987 (127)</td>
<td>TDNTG</td>
<td>10 mg/d</td>
<td>31 mo</td>
<td>10</td>
<td>Open</td>
</tr>
<tr>
<td>Elkayam et al, 1989 (54)</td>
<td>ISDN</td>
<td>40-120 g four or three times daily</td>
<td>30 hr</td>
<td>22</td>
<td>Open</td>
</tr>
<tr>
<td>Neuberg et al, 1989 (103)</td>
<td>IV NTG</td>
<td>6.4 g per minute</td>
<td>48 hr</td>
<td>10</td>
<td>Open</td>
</tr>
<tr>
<td>Dupuis et al, 1990 (51)</td>
<td>IV NTG</td>
<td>1.5 g per minute</td>
<td>72 hr</td>
<td>19</td>
<td>Open</td>
</tr>
<tr>
<td>Dakak et al, 1990 (52)</td>
<td>IV NTG</td>
<td>60-1600 ILS/min</td>
<td>24 hr</td>
<td>21</td>
<td>Open</td>
</tr>
</tbody>
</table>

* DB = double blind; ISDN = oral isosorbide dinitrate; IV = intravenous; NTG = nitroglycerin; PC = placebo controlled; TD = transdermal.
24 to 48 hours of continuous exposure to the drug. Little information is available about the incidence of the development of nitrate tolerance in patients treated for ischemic heart disease. Amidi and Shaver (66) described tolerance to the hemodynamic effect of sublingual nitroglycerin in all 25 patients with coronary artery disease receiving chronic oral therapy with isosorbide dinitrate. More data is required, however, about individual response to oral, topical, "intravenous nitrate therapy before the incidence of the development of tolerance can be established.

Relation between Dose of Nitrates and Tolerance Development

A relation between the concentration of nitroglycerin and the development of tolerance to the vasorelaxing effect of the drug was shown in in-vitro experimentations by Needleman (9) and Kukovetz and coworkers (67). These investigators found an increased magnitude of tolerance to organic nitrates with increased concentration of the drug during the pre-incubation period. Little information is available about the relation between nitrate dose and the incidence of the development of tolerance in vivo, and the data conflict. Tauchert and colleagues (68) described the dose dependency of tolerance during the treatment of patients with angina with isosorbide dinitrate. These investigators noted the development of tolerance to the hemodynamic effect as well as an effect on exercise tolerance after 4 weeks of high-dose (150 mg/d) therapy but not after low-dose (60 mg/d) therapy. These findings may be supported by reports of the sustained hemodynamic effect of low doses of transdermal and intravenous nitroglycerin in patients with heart failure (69-71), in contrast to the substantial early attenuation reported with the use of high doses (48-50). In contrast, Schneider and coworkers (72) reported a lack of relation between dose and the development of tolerance. They investigated the dose-response relation of isosorbide dinitrate and found only moderate, statistically insignificant attenuation of anti-ischemic efficacy with high doses of oral isosorbide dinitrate (480 mg/d) after 4 weeks of continuous therapy. In addition, a similar degree of attenuation of the anti-ischemic effect was reported by some investigators with both small and large doses (15 to 105 mg) of transdermal nitroglycerin (FDA Cooperative Study, unpublished data) and oral isosorbide dinitrate (15 to 120 mg) in patients with angina pectoris (11, IS).

In summary, information about the relation between nitrate dose and the incidence of the development of tolerance to the hemodynamic and anti-ischemic effects of these drugs is limited and somewhat conflicting. Further controlled studies using different doses are needed to establish the existence of such a relation.

Differential Tolerance

Stewart and colleagues (73) reported preferential venous tolerance in a dog model after 4 days of nitroglycerin administration. These investigators described true tolerance to nitroglycerin venodilation but continued arteriolar responsiveness offset by sympathetic activation induced by nitroglycerin and unmasked by autonomic blockade with hexamethonium. Similarly, Zelis and Mason (74), in a plethysmographic study, showed the development of tolerance to the venous effect of large-dose isosorbide dinitrate in normal subjects without a change in the responsiveness of the resistant blood vessels.

The relative susceptibility of the venous system to the development of tolerance shown by these studies could not be supported by other clinical investigations conducted in patients with heart disease. The rapid development of tolerance to the effect on systemic vascular resistance and arterial blood pressure has been shown in many studies of patients treated for angina pectoris or congestive heart failure (11, 15,50). Further, Leier and coworkers (75) reported the development of preferential tolerance to the effect of nitrates on the arterial circulation with preserved venous effect during chronic nitrate therapy in patients with chronic congestive heart failure. Similarly, Aronow and Danahy (12) reported the development of tolerance to the nitrate effect on systemic blood pressure despite maintenance of the antianginal effect, suggesting the preservation of the venous effect, a predominant cause for the nitrate anti-ischemic effect.

In summary, despite experimental data obtained from animals and healthy humans suggesting preferential venous tolerance to the nitrate effect, most clinical studies conducted in patients with ischemic heart disease and heart failure suggest concomitant venous and arterial tolerance.

Nitrate Cross-tolerance

Does nitrate cross-tolerance affect the efficacy of sublingual nitroglycerin? The evidence about such interaction conflicts. Bernstein and Ivy (76) reported the observation made by three patients who noticed the reduced antianginal effect of sublingual nitroglycerin while receiving long-acting nitrates. This report was followed by several investigations similarly showing cross-tolerance between long-acting nitrates and sublingual nitroglycerin. Schelling and Lasagna (77) showed an important decrease in sublingual nitroglycerin-mediated change in blood pressure and heart rate in normal volunteers while they were receiving penterylthol tetranitrate. Zelis and Mason (74) reported the loss of effects of sublingual nitroglycerin on calf blood flow and venous volume, as measured by plethysmography after 6 to 8 weeks of therapy with sustained-release isosorbide dinitrate. Similar results were reported by Manyari and colleagues (78) who showed a lack of effect of sublingual nitroglycerin on blood pressure, heart rate, and arm blood volume, as measured by radionuclide technique after 1 month of isosorbide dinitrate therapy. Thadani and coworkers (11) showed marked attenuation in nitroglycerin-induced systolic blood pressure reduction and heart rate acceleration after therapy with isosorbide dinitrate, 60 mg every 6 hours for 5 days. In a recent preliminary report, Amidi and Shaver (66) showed the effect of sublingual nitroglycerin on heart rate, systemic and pulmonary blood pressure, and left ventricular filling pressure in patients not receiving chronic nitrate therapy but in none of 25 patients receiving long-term treatment with isosorbide dinitrate.
In contrast, Aronow and Chesluk (79) showed that sublingual nitroglycerin relieved exercise-induced angina similarly in patients receiving isosorbide dinitrate and patients receiving placebo. The use of small-dose (5 mg), short-acting isosorbide dinitrate, given sublingually four times daily, however, may have prevented the development of tolerance in that study. Danahy and Aronow (12) did not show cross-tolerance to the hemodynamic and antianginal effects of nitroglycerin in patients receiving chronic oral therapy with isosorbide dinitrate. A nitrate-free interval of at least 14 hours, however, was allowed before the administration of nitroglycerin in this study.

Lee and colleagues (13) showed that the antianginal and anti-ischemic effects of sublingual nitroglycerin persisted after 1 month of isosorbide dinitrate therapy. In this study, the effect of nitroglycerin was studied 6 hours after the administration of isosorbide dinitrate. In an earlier study, Reichek and colleagues (50) found that sublingual nitroglycerin effects persisted in five patients receiving chronic therapy with nitroglycerin ointment; however, no data about the timing of drug administration were provided.

Dalal and coworkers (81) reported that the antianginal and hemodynamic effects of sublingual nitroglycerin persisted 6.5 hours after the oral administration of isosorbide dinitrate, 15 mg four times daily for 1 week, despite evidence of the development of tolerance to the hemodynamic and antianginal effects of isosorbide dinitrate. This study, however, showed a reduction in the magnitude of the nitroglycerin effect on systolic blood pressure and on the length of exercise time before the onset of angina, indicating at least a partial cross-tolerance between the two drugs. In a later study, the same investigators showed similar circulatory changes and an improvement in walking time after the administration of either sublingual isosorbide dinitrate or nitroglycerin 6.5 hours after the oral administration of isosorbide dinitrate in patients chronically treated with isosorbide dinitrate (82). A response to sublingual isosorbide dinitrate in the presence of tolerance to the oral form of the drug may indicate that the effect of sublingual nitroglycerin resulted not from a lack of cross-tolerance to isosorbide dinitrate, but possibly from a rapidly increasing nitroglycerin concentration after sublingual dosing. Packer and colleagues (50) reported a loss of hemodynamic response to oral isosorbide dinitrate in patients with congestive heart failure who developed tolerance to continuous intravenous nitroglycerin.

The effect of sublingual nitroglycerin during the continuous administration of intravenous or transdermal nitroglycerin has been evaluated by several investigators. Zimrin and associates (40) reported a significant attenuation of the anti-ischemic effect of sublingual nitroglycerin after 24 hours of continuous intravenous infusion. Similarly, a loss of sublingual nitroglycerin-mediated hemodynamic effect was noted in a group of patients with congestive heart failure 1 hour after the completion of 72 hours of intravenous nitroglycerin infusion (51). In contrast, preservation of sublingual nitroglycerin effect has been shown during therapy with buccal nitroglycerin and transdermal isosorbide dinitrate and nitroglycerin (16, 40, 83). These findings may indicate an ability to overcome nitrate tolerance with a rapid, abrupt increase in nitroglycerin concentration.

In summary, the evidence indicates, although not uniformly, nitrate cross-tolerance with attenuation of the circulatory hemodynamic and antianginal effects of sublingual nitroglycerin in patients chronically treated with long-acting nitrates. A rapid increase in plasma nitroglycerin concentration may partially overcome the tolerance and result in circulatory and antianginal effects.

Possible Mechanisms for the Development of Nitrate Tolerance

Strong evidence indicates that organic nitrates cause vasodilation via production of the cellular transmitter cyclic guanosine 3′:5′-monophosphate (cGMP) which presumably reduces intracellular Ca++ concentration by decreasing Ca++ release from the sarcoplasmic reticulum and reducing permeability to extracellular Ca++ (10, 67, 84-88). Recent investigations have shown that nitrovasodilators undergo a metabolic process resulting in the formation of vasoactive compounds (87, 88). Ignarro and colleagues (89) have suggested that after penetration of the smooth muscle cell membrane, the lipophilic organic nitrates are reduced intracellularly to inorganic nitrates via an interaction with sulfhydryl groups, with further conversion into nitric oxide. This compound then interacts with a second population of sulfhydryl groups to form a s-nitrosothiol compound. Either nitric oxide or 5-nitrosoglutathione is believed to be the final stimulant of guanylate cyclase, an enzyme that generates cGMP in the vascular wall (90, 91).

The development of nitrate tolerance has been shown to be associated with a severe reduction in tissue cGMP. This reduction results from decreased production secondary to reduced guanylate cyclase activity as well as from an increased rate of breakdown from enhanced activity of cGMP-phosphodiesterase (9, 92, 93).

Needleman and Johnson (94) hypothesized an association between nitrate tolerance and the depletion of sulfhydryl groups in vascular smooth muscle cells. This hypothesis was supported by the finding that the tissue content of sulfhydryl groups was reduced with prolonged exposure of the vascular strip to nitroglycerin (94, 95). Additional evidence that the development of nitrate tolerance depends on thiol is that only negligible tolerance is seen with thiol-independent nitrovasodilators, such as molsidomine and nitroprusside (67, 73, 91, 92, 96-98), and there is a lack of effect of nitrate tolerance on nitric oxide-induced relaxation of rabbit aorta (99). In addition, several studies have recently shown the prevention or the reversal of nitrate tolerance with the administration of sulfhydryl donors, both in vitro and in vivo (50, 68, 94, 95, 100-103). The only partial reversal of tolerance in several of these studies may suggest, however, a partial role for other mechanisms, such as the accelerated breakdown of cGMP reported with continuous exposure to organic nitrates and the possible inactivation of enzymes participating in organic nitrate metabolism (10, 50, 98, 100, 101, 103).

The activation of reflex vasoconstrictive forces offsetting the initial vasodilatory effect has been suggested as another mechanism for the development of nitrate tol
erance (104-106). Such a mechanism is supported by the reported findings that abolition of reflexes with guanethedine in anesthetized dogs prevented the development of acute tolerance to nitroglycerin (107) and that the effect of i)nitroglycerin on systemic blood pressure masked by compensatory reflexes ("pseudo tolerance") persists (73).

In addition, the reported increases in heart rate, plasma renin activity, and body weight accompanied by the development of hemodynamic tolerance (50) and the rebound increases in mean arterial pressure and systemic vascular resistance reported by some investigators after the abrupt discontinuation of continuous nitroglycerin administration (43-46) may further support the existence of neurohormonal stimulation during nitrate therapy. In contrast to these findings, other data suggest a limited role for the activation of endogenous neurohormonal systems in the development of nitrate tolerance. These data include the lack of relation between the development of tolerance and changes in values of plasma renin activity and circulating catecholamines during nitroglycerin therapy (42, 47-49); the failure to document hemodynamic rebound with abrupt withdrawal of nitrate therapy despite documentation of the development of tolerance. (47, 49); and the development of nitrate tolerance in isolated blood vessels in vitro where activation of endogenous neurohormonal systems can be clearly excluded (9, 98, 99).

Strategies for Preventing the Development of Nitrate Tolerance

Administration of Sulfhydryl Donors

Because the depletion of tissue stores of sulfhydryl groups appears to play an important role in the development of nitrate tolerance, investigators have tried to use sulfhydryl-containing compounds to regain the nitrate effect. N-Acetyl cysteine was the first agent used for this purpose. This drug is hydrolyzed in vivo to cysteine, a sulfide-containing amino acid, and was found to be more active than other sulfhydryl-containing compounds in reacting with organic nitrates (108) and in stimulating the activation of guanylate cyclase (109). N-Acetyl cysteine has been used safely as a mucolytic agent and as a cysteine source in the treatment of acetaminophen toxicity (110, 111).

Data have also shown the ability of this agent to potentiate the peripheral and coronary effect of nitroglycerin in patients with coronary artery disease and heart failure (112-114). Torresi and colleagues (101) showed the partial prevention and the reversal of tolerance to the nitrate effect on bovine coronary artery rings that were preincubated with N-acetyl cysteine.

Packer and coworkers (50) found partial restoration of the hemodynamic effect in eight patients with chronic heart failure in whom tolerance developed during continuous intravenous therapy with nitroglycerin. May and associates (64) found complete reversal of partial tolerance to the augmenting effect of nitroglycerin on coronary sinus blood flow in seven patients with coronary artery disease. Studies have also shown potentiation of the hemodynamic effects of nitroglycerin in patients with coronary artery disease and partial reversal of tolerance to nitrates in patients with heart failure when they were treated with methionine, an essential amino acid that is metabolized to cysteine (102, 103, 115).

Methionine has also been widely used for the treatment of acetaminophen toxicity (115-117) and may be more suitable for chronic therapy than N-acetylcysteine, a drug that is associated with a high incidence of gastrointestinal untoward effects (118). Preliminary data have shown an improvement in exercise performance with the use of captopril, a sulfhydryl-containing angiotensin-converting enzyme inhibitor, during chronic nitrate therapy in patients with angina pectoris (119). In addition, tolerance to the effect of nitroglycerin on venous blood volume has been shown to be prevented by captopril, but not by enalapril, a non sulfhydryl-containing angiotensin-converting enzyme inhibitor (120).

In contrast, however, several investigators could not show potentiation of the relaxant effect of nitroglycerin or reversal of nitroglycerin tolerance with cysteine in vitro (98, 121). In addition, Parker and colleagues (122) reported the lack of reversal of hemodynamic and antiischemic tolerance to oral isosorbide dinitrate with N-acetylcysteine in patients with chronic stable angina, and Dakak and coworkers (53) reported the failure of captopril to prevent the development of nitrate tolerance in patients with congestive heart failure. Fung and associates (121) recently suggested an interaction between N-acetylcysteine and nitroglycerin, but not between N-acetylcysteine and isosorbide dinitrate. These contrasting data suggest the need for well-designed, controlled studies to conclusively establish the role of sulfhydryl donors as "antinitrate-tolerance" therapy.

Intermittent Dosing

Packer and colleagues (50), who showed the development of tolerance to the hemodynamic effect of intravenous nitroglycerin when administered continuously for 48 hours and cross-tolerance to oral isosorbide dinitrate in patients with heart failure, prevented tolerance by using intermittent therapy (12 hours of therapy followed by 12 hours without therapy). A comparison of continuous and intermittent therapy with transdermal nitroglycerin, allowing 8 to 12 hours of nitroglycerin patch-free intervals, had similar results, substantiating the early development of tolerance with continuous therapy and the preservation of the antianginal and hemodynamic effects of the drug with intermittent therapy, in both patients with angina pectoris and patients with congestive heart failure (123-127).

Although most investigators have not evaluated the therapeutic effect of transdermal nitroglycerin throughout the patch application period, the investigators in the recently reported Transderm Nitro Trial Study found greater treadmill walking time, both acutely and chronically, lasting for at least 8 hours in patients treated intermittently with transdermal nitroglycerin, IS to 20 mg/d, when compared with patients treated with placebo (126).

Although oral administration of nitrates is associated with fluctuations in plasma concentrations, frequent dosing results in elevated trough plasma levels, which also may lead to the development of tolerance (128). In
fact, the relation between the dosing interval of oral nitrates and the development of tolerance has been clearly established. Silber and coworkers (17) showed the development of tolerance to the anti-ischemic effect of sustained-release isosorbide dinitrate, 80 mg every 12 hours for 2 weeks. They did not find the development of tolerance with the same dose given either once daily or eccentrically (0800 and 1400 hours). Parker and colleagues (16) compared the hemodynamic and antiischemic effects of oral isosorbide dinitrate, given four times daily with 8 hours between doses at night; buccal nitroglycerin, given three times daily with 12 hours between doses; and placebo. The results of this investigation indicated the development of tolerance during oral therapy with isosorbide dinitrate, but not during buccal therapy with nitroglycerin. The lack of tolerance to the buccal preparation was probably related to both the shorter effect of this drug's activity and the dosing frequency. Similar results were reported by the same investigators, showing tolerance to the anti-ischemic effects of oral isosorbide dinitrate, 30 mg four times daily with 8 hours between doses at night, but not when the drug was given two or three times daily, allowing a "nitrate washout" interval of 19 and 14 hours, respectively (18).

Similar findings have been recently reported with the use of the longer-acting isosorbide dinitrate metabolite, isosorbides-mononitrate. Kohli and colleagues (129) and Thadani and coworkers (130) showed tolerance to the initial effect of isosorbides-mononitrate after 2 weeks of twice-daily, concentric (0800 and 2000 hours) therapy, but not with twice-daily, eccentric (0800 and 1500 hours) therapy (130). The same investigators showed that the use of the longer-acting, slow-release isosorbides-mononitrate leads to the development of tolerance even with once-daily administration (41). Preliminary data have similarly shown a relation between the dosing interval and the development of tolerance in patients with heart failure. Elkayam and coworkers (53, 54) have shown the early development of tolerance to an isosorbide dinitrate-mediated effect on mean pulmonary artery wedge pressure with drug administration every 4 and 6 hours and the complete restoration of the effect after a washout period of 12 hours in patients with chronic heart failure.

Although the importance of a prolonged daily nitrate washout interval for tolerance prevention has been well documented, the duration of this interval varies among studies, and the time required for the restoration of nitrate efficacy is probably related to the half-lives of the various nitrate preparations. The prevention of tolerance to nitroglycerin, when given transdermally, has been shown with drug-free intervals of 8 to 12 hid. In contrast, a 4-hour transdermal nitroglycerin-free interval has been shown to be insufficient for the prevention of tolerance to the effect on exercise time in patients with chronic stable angina pectoris (131). Parker and colleagues (18) have shown the prevention of nitrate tolerance with oral isosorbide dinitrate therapy using a washout interval of 14 hours or longer, but not using a washout interval of 8 hours. A longer washout interval (17 hours) was successfully used by Thadani and coworkers (130) to prevent the development of tolerance to the antianginal effect of isosorbides-mononitrate that is seen with an interval of only 12 hours. Similarly, allowance of 17 hours between doses was needed to maintain the effectiveness of sustained-release isosorbide dinitrate in patients with angina pectoris; 12-hour interdose intervals were insufficient (17).

Although intermittent therapy with nitrates seems to be effective in preventing the development of nitrate tolerance, the majority of reported studies have only evaluated the effect of the morning dose of nitrates after the washout period. The effects of the second and third doses have not been investigated and are therefore unknown (132). In addition, nitrate dependence may lead to hemodynamic and symptomatic rebound with the discontinuation of therapy (133). Sudden withdrawal from exposure to nitrate environments was reported to cause myocardial infarction and sudden death in workers in the munitions industry who were exposed to high concentrations of nitrates (134). DeMots and colleagues (126) described a substantial increase in rest angina during nitrate-free intervals in 7% of patients with angina pectoris who received intermittent therapy with transdermal nitroglycerin. Further, during long-term therapy, patients treated with placebo exercised longer before the onset of angina before patch application ("zero hour") than did patients receiving active drug. A withdrawal phenomenon, possibly due to the activation of vasoconstrictive, neurohormonal systems, was considered by the investigators as a potential mechanism for the "zero hour effect." The clinical importance of these findings and of their mechanisms deserves further investigation.

Summary and Recommendations

Nitrate tolerance was first described over a century ago and has been shown repeatedly in both animals and humans. The clinical importance of this phenomenon, however, has been controversial. Research associated with the development of new, longer-acting nitrate formulations has shown the potentially limiting effect of tolerance on the therapeutic benefits of organic nitrates in both ischemic heart disease and heart failure. Continuous nitrate therapy has been clearly associated with rapid diminution of peripheral and coronary vasodilatory action, with marked reduction in hemodynamic and antiischemic effects. Several mechanisms seem to be involved in the development of nitrate tolerance including the depletion of sulfhydryl groups in the vascular smooth muscle cells. The use of sulfhydryl-containing compounds, such as N-acetylcysteine, methionine, and captopril, as sulfhydryl donors may be associated with the prevention and the partial reversal of tolerance. Further evaluation is needed, however, before the validity, efficacy, and safety of this approach can be established. Substantial amounts of data have shown the efficacy of intermittent nitrate therapy, which allows sufficiently long, daily, nitrate-free intervals, in the prevention of nitrate tolerance. On the basis of these data, avoiding long, continuous administration of intravenous or topical nitrates and frequent dosing of oral formulations seems prudent.

The FDA's new recommendations for the use of
transdermal nitroglycerin call for intermittent therapy, allowing a daily 10- to 12-hour patch-off period in patients with angina pectoris. Similar, daily, nitrate-free intervals seem to be adequate in patients with heart failure. Because tolerance develops in many patients within 12 to 48 hours after beginning continuous intravenous therapy, using intermittent therapy, including a nitrate-free interval of at least 8 hours, after 12 to 48 hours of continuous therapy see IJS to be rational. Frequent, uninterrupted dosing (every 4 to 6 hours) of oral isosorbide dinitrate should be avoided. Twice- or thrice-daily dosing seems to be adequate. Because of the longer half-lives of oral isosorbide dinitrate and its metabolites, a longer washout interval than that used for transdermal or intravenous nitroglycerin seems necessary to allow blood levels to fall adequately. At least a 12-hour washout period for regular oral isosorbide dinitrate and a longer period when larger-than-average doses are used therefore are recommended. On-going isosorbide, -mononitrate and the slow-release formulations of oral isosorbide dinitrate and nitroglycerin should be given either in a single-daily-dose regimen or in an eccentric regimen (for example, at 0800 and 1500 hours), allowing a sufficiently long washout period.

Stewart (6), Who in 1905 failed to overcome tolerance to the antihypertensive effect of nitroglycerin despite using a markedly increased dose, concluded that the drug was of little value because, despite "intelligent employment," its effect was rapidly lost. A great body of information obtained since that time has supported Stewart's impression that tolerance may abolish the therapeutic effect of organic nitrates. With "intelligent employment," however, this phenomenon can be prevented and the nitrate effects can be preserved.

Acknowledgments: The author thanks Alice Madrid and Lorine Villanueva for secretarial assistance and Janet Vasquez-Johnson, RN, for assistance in the manuscript preparation.

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References

40. Zhon D, Relchek N, Iogin KT, et al. Antianginal effects of