

Nitroglycerin Induces Late Preconditioning Against Myocardial Infarction in Conscious Rabbits Despite Development of Nitrate Tolerance

Michael Hill, PhD; Hitoshi Takano, MD; Xian-Liang Tang, MD; Eitaro Kodani, MD; Gregg Shirk, BA; Roberto Bolli, MD

Background—Recent studies suggest that the late phase of ischemic preconditioning (PC) can be mimicked by pretreatment with NO donors. The ability of clinically relevant NO donors to induce PC against infarction, however, has not been evaluated. Furthermore, it is unknown whether tolerance to the hemodynamic actions of nitrates also extends to their PC effects.

Methods and Results—Conscious rabbits underwent a 30-minute coronary occlusion and 3 days of reperfusion. A 60-minute intravenous (IV) infusion of nitroglycerin (NTG) ending 1 hour before occlusion reduced infarct size, indicating an early PC effect. When the time interval between NTG infusion and occlusion was extended to 24 or 72 hours, the infarct-sparing action of NTG became even more pronounced, indicating a robust late PC effect. Transdermal NTG patches elicited a late PC effect that was (1) equivalent to that induced by IV NTG, demonstrating the efficacy of transdermal NTG as an alternative form of NTG delivery for inducing late PC, and (2) similar in nitrate-tolerant and -nontolerant rabbits, demonstrating that tolerance does not extend to the PC effects of NTG.

Conclusions—In conscious rabbits, administration of NTG via either the IV or the transdermal route elicits a robust protective effect against infarction that lasts for 72 hours. The magnitude of NTG-induced cardioprotection is equivalent to that observed during the late phase of ischemic PC and is not affected by the development of tolerance. These findings reveal a new action of nitrates and support novel applications of these drugs for protecting the ischemic myocardium in patients. (*Circulation*. 2001;104:694-699.)

Key Words: nitroglycerin ■ ischemia ■ reperfusion ■ myocardial infarction ■ nitrates

The late phase of ischemic preconditioning (PC) protects against both myocardial stunning¹⁻⁶ and myocardial infarction.⁵⁻⁹ Because of this, and because of its sustained nature (3 to 4 days),^{2,7,10} PC may have considerable therapeutic applications if a clinically relevant treatment could be identified that is capable of mimicking the delayed protection conferred by exposure to an ischemic stress.

Recent studies have shown that NO plays a major role in initiating the late phase of ischemic PC^{3,4,11} and that a similar cardioprotective effect can be reproduced by pretreatment, 24 hours earlier, with NO donors in the absence of ischemia.^{5,6} The ability of exogenous NO to trigger a late PC-like phenomenon has important therapeutic implications. Several fundamental issues need to be addressed, however, before this concept can be exploited to treat patients with coronary artery disease. First, the NO donors that were tested in previous studies^{5,6} [diethylenetriamine/NO and *S*-nitroso-*N*-acetylpenicillamine (SNAP)] are not clinically available. Although nitroglycerin (NTG) induces late PC against myocardial stunning,^{12,13} it is unknown whether this salutary action also involves protection against cell death. To date, no clinically relevant NO donor has been shown to be

capable of inducing late PC against myocardial infarction. Second, the time course of NO donor-induced late PC is incompletely understood. In contrast to ischemia-induced late PC, which is known to last for 72 hours,^{2,10} NO donor-induced late PC has been studied only at 24 hours after treatment^{5,6}; there is no evidence of delayed cardioprotection by NO donors at a distance of >24 hours. Consequently, it is not known whether these agents can faithfully reproduce the time course of protection afforded by ischemic PC. Third, continuous administration of NO-releasing agents, such as organic nitrates, results in the rapid development of tolerance to their vasodilator and anti-ischemic effects.¹⁴⁻¹⁸ Because management of coronary artery disease frequently requires long-term administration of nitrates, it is important to determine whether tolerance extends to the PC effects of these agents. Finally, although SNAP has recently been shown to induce early PC,¹⁹ there is no information as to whether clinically available NO donors can also elicit an early PC effect, in addition to a late PC effect.

The present investigation was designed to address these issues. We elected to study NTG because this is the most commonly used NO-releasing agent in the treatment of

Received January 29, 2001; revision received April 11, 2001; accepted April 11, 2001.

From the Experimental Research Laboratory, Division of Cardiology, University of Louisville and Jewish Hospital Heart and Lung Institute, Louisville, Ky. Correspondence to Roberto Bolli, MD, Division of Cardiology, University of Louisville, Louisville, KY 40292. E-mail rbolli@louisville.edu

© 2001 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

coronary artery disease and because it can be administered both by the intravenous (IV) and the transdermal (TD) routes. To fully characterize the PC-mimetic potential of NTG, we examined the following specific questions: (1) Does a brief IV infusion of NTG induce delayed protection against infarction? (2) If so, how long does this protective effect persist? (3) Is NTG-induced late PC abrogated by the development of nitrate tolerance? (4) Can a late PC effect be induced by TD, rather than IV, administration of NTG? (5) Can NTG induce an early phase of PC as well? In a well-established rabbit model of ischemic PC,^{3,5,6,8} a comprehensive investigation was performed that included 3 consecutive phases (1, 2, and 3) and 12 experimental groups. All studies were conducted in conscious animals to eliminate the potentially confounding influence of factors associated with open-chest preparations, such as anesthesia, surgical trauma, abnormal hemodynamics, elevated catecholamine levels, fluctuations in body temperature, exaggerated formation of reactive oxygen species, and release of cytokines, which could, in themselves, interfere with the development of myocardial infarction and with the phenomenon of PC.^{20,21} The results demonstrate, for the first time, that NTG can faithfully recapitulate the infarct-sparing effects of the late phase of ischemic PC despite the development of nitrate tolerance, suggesting novel potential applications of nitrates in patients.

Methods

The conscious rabbit model of myocardial ischemia has been described in detail previously.^{3-6,8,22}

Phase 1: Effect of IV NTG

Group 1 (control group) underwent the 30-minute occlusion without any treatment or PC protocol (Figure 1). Groups 2, 3, and 4 were preconditioned with a sequence of six 4-minute coronary occlusion/4-minute reperfusion cycles ending 10 minutes (early PC-10 minutes group), 1 hour (early PC-1 hour group), or 24 hours (late PC group) before the 30-minute occlusion (Figure 1). Groups 5 to 8 were studied to examine the ability of NTG to induce PC. In group 5 (NTG-1 hour group), rabbits were pretreated with a 60-minute IV infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ending 1 hour before the 30-minute occlusion. In groups 6 (NTG-24 hours group), 7 (NTG-72 hours group), and 8 (NTG-96 hours group), the animals were pretreated with the same dose of NTG as in group 5, but the time interval between NTG infusion and onset of the 30-minute occlusion was extended to 24, 72, and 96 hours, respectively (Figure 1). The dose of NTG used in this investigation was chosen because pilot studies demonstrated that infusion rates $>2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ caused significant hypotension and tachycardia.

Phase 2: Effect of TD NTG

TD NTG patches that released 0.3 mg/h NTG continuously for a period of 24 hours were applied on the rabbits' skin. After removal of the patch, a 3-day interval was allowed to elapse before rabbits were subjected to a 30-minute occlusion followed by 72 hours of reperfusion (Figure 2). The reason for selecting a 3-day interval was to determine whether a single TD NTG treatment is sufficient to provide long-lasting protection against subsequent infarction. The dose of NTG chosen in phase 2 ($132 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) is similar to that previously used in several studies of NTG in rabbits.^{23,24}

Phase 3: Nitrate Tolerance

To induce nitrate tolerance, rabbits were given 1 TD NTG patch/d (0.3 mg/h) continuously over a period of 28 days (Figure 2). Seventy-two hours after removal of the last patch (day 31), nitrate-tolerant rabbits were subjected to a 30-minute coronary occlusion

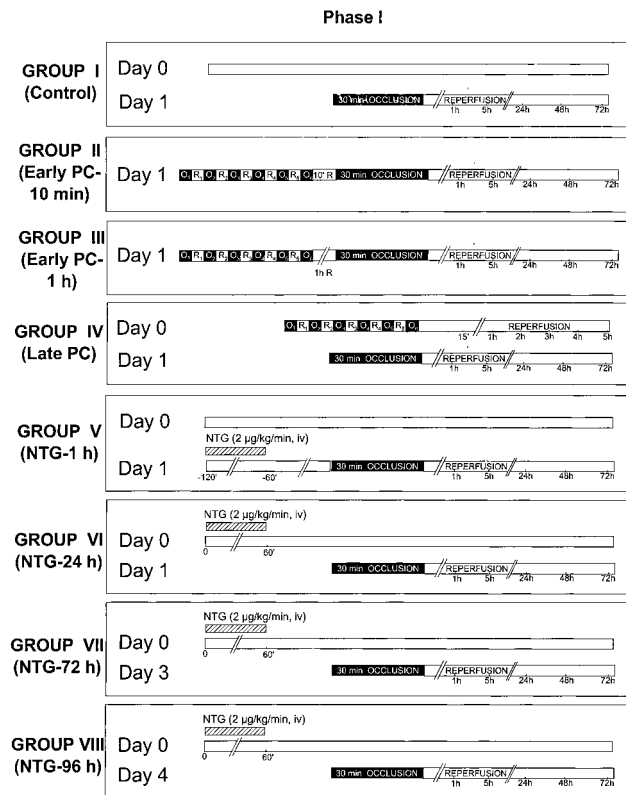


Figure 1. Experimental protocol for studies of effect of IV NTG pretreatment on subsequent myocardial infarction (phase 1).

followed by 72 hours of reperfusion. Previous studies have shown that use of TD NTG patches at this dose ($120 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) is associated with the development of tolerance to the vasodilator effects of NTG in rabbits.^{23,24}

Measurement of Regional Myocardial Function and Infarct Size

The total deficit of systolic wall thickening over the 3-day reperfusion period (an integrative assessment of the overall severity of contractile dysfunction during this time interval) was calculated as described.^{5,6,8,22} The total deficit of wall thickening provides a tetrazolium-independent index of protection.²² The size of the occluded-reperfused coronary vascular bed (region at risk) and of the infarction was determined by a previously described postmortem perfusion technique followed by staining with triphenyltetrazolium chloride and by computerized planimetry.^{5,6,8,22}

Statistical Analysis

Data are reported as mean \pm SEM. Heart rate and thickening fraction were analyzed by a 2-way repeated-measures ANOVA (time and group). Infarct sizes and risk region sizes were analyzed by a 1-way ANOVA followed by Student's *t* tests for unpaired data with the Bonferroni correction.

Results

Of the 99 conscious rabbits instrumented for the study, 13 (13%) were excluded for the reasons specified in Table 1. As shown in Table 2, there were no significant differences among the various groups with respect to the size of the region at risk. In all 3 phases of this study, the effects of NTG-induced PC were compared with those of ischemia-induced PC.

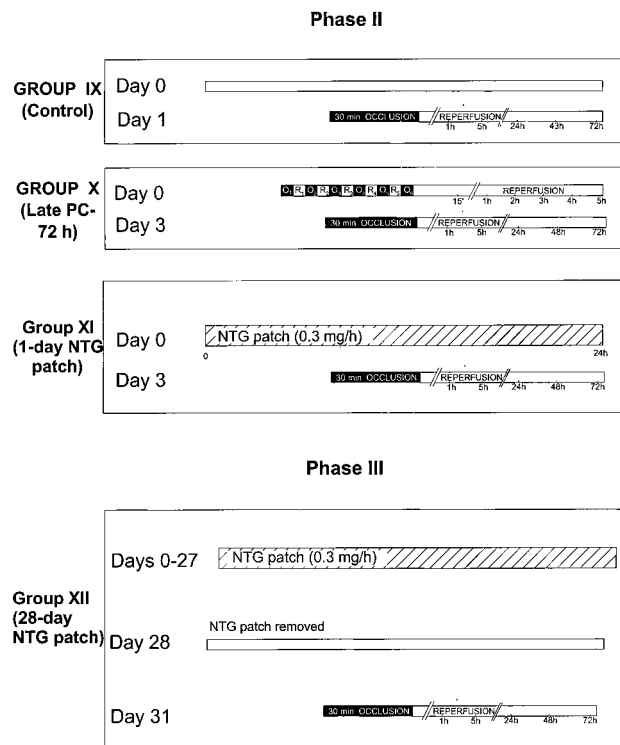


Figure 2. Experimental protocol for studies of effect of TD NTG pretreatment on subsequent myocardial infarction (phase 2) and for studies of nitrate tolerance (phase 3).

Phase 1: Effect of IV NTG

No significant changes in heart rate or arterial pressure were observed during or after the 60-minute infusion of $2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of NTG, which is consistent with previous studies in rabbits.^{12,13} As expected, when rabbits underwent a 30-minute coronary occlusion either 10 minutes or 1 hour after the 6 occlusion-reperfusion cycles, infarct size was markedly reduced, indicating an early PC effect of ischemia (Figure 3). In rabbits undergoing the 30-minute occlusion 24 hours after ischemic PC, infarct size was also reduced (late PC) (Figure 3). When rabbits were pretreated with a 60-minute IV infusion of NTG ending 1 hour before the 30-minute occlu-

sion, infarct size was significantly decreased compared with controls (Figure 3), indicating that NTG induced an early PC effect. This is in agreement with previous observations with SNAP in isolated rabbit hearts.¹⁹ When the time interval between the NTG infusion and the 30-minute occlusion was extended to 24 or 72 hours, the reduction in infarct size became even more pronounced, demonstrating that NTG elicited a robust late PC effect. When the interval between NTG infusion and the 30-minute occlusion was further extended to 96 hours, however, infarct size was indistinguishable from that measured in the control group, indicating that the NTG-induced late PC effect against infarction had disappeared.

Phase 2: Effect of TD NTG

Application of the TD NTG patch resulted in a transient decrease in mean arterial pressure at 3, 6, 9, and 12 hours; however, arterial pressure returned to baseline values by 24 hours (Table 3).

In rabbits that were subjected to the 30-minute coronary occlusion 72 hours after six 4-minute occlusion/reperfusion cycles, infarct size was significantly reduced (Figure 4), indicating that ischemic PC produces a sustained infarct-sparing effect that lasts ≥ 72 hours in this conscious rabbit model. An even greater infarct-sparing effect was observed in the rabbits that received a single NTG patch ending 72 hours before the 30-minute occlusion; the reduction in infarct size in this group (-58% versus controls) compared favorably with that observed at 72 hours after ischemic PC (-41%), indicating that pretreatment with TD NTG resulted in a delayed protective action at least equivalent to, if not greater than, that induced by ischemia.

Phase 3: Nitrate Tolerance

No significant changes in heart rate or arterial pressure were observed on days 14 and 28 of the 28-day continuous TD NTG treatment (Table 3). Thus, the early hypotensive effects of TD NTG seen in phase 2 did not persist during daily application of TD NTG patches, indicating tolerance. The development of nitrate tolerance was confirmed in a separate

TABLE 1. Reasons for Exclusion

	Group												Total
	Phase 1					Phases 2 and 3							
	1	2	3	4	5	6	7	8	9	10	11	12	
Malfunction of the balloon occluder	1	1
Ventricular fibrillation during occlusion	2	...	1	...	2	2	...	1	...	8
Small region at risk (<10% of left ventricle)	1	...	1
Technical problems during postmortem perfusion	2	2
Paraplegia	1	1
Total number of rabbits excluded	2	...	1	...	2	...	1	1	2	...	2	2	13 (13%)
Total number of rabbits assigned to each group	10	6	6	8	10	8	7	8	9	9	9	9	99
Rabbits that completed the protocol	8	6	5	8	8	8	6	7	7	9	7	7	86 (87%)

TABLE 2. Size of LV, Region at Risk, and Infarct

	n	LV, g	RR, g	Infarct, g	RR, % of LV	Infarct, % of RR	Infarct, % of LV
Phase 1							
Control	8	4.60±0.18	0.80±0.11	0.50±0.10	17.90±2.00	58.40±3.70	10.23±1.25
Early ischemic PC (10 min)	6	4.10±0.30	0.70±0.10	0.11±0.03*	16.40±2.40	15.40±2.60*	2.53±0.54*
Early ischemic PC (1 h)	5	4.20±0.30	0.80±0.10	0.12±0.01*	18.70±1.90	15.80±2.50*	2.85±0.26*
Late ischemic PC (24 h)	8	4.50±0.10	0.80±0.10	0.26±0.04*	18.10±1.50	31.40±3.00*	5.83±0.88*
NTG PC (1 h)	8	4.20±0.20	0.60±0.10	0.26±0.04*	16.10±2.20	37.30±1.50*†§	6.14±0.97*
NTG PC (24 h)	8	4.10±0.20	0.70±0.10	0.16±0.04*	16.10±1.60	18.80±3.40*‡	3.67±0.86*
NTG PC (72 h)	6	4.60±0.20	0.80±0.10	0.20±0.05*	16.60±1.70	21.80±4.50*	4.28±1.03*
NTG PC (96 h)	7	3.80±0.18	0.80±0.10	0.41±0.07	19.90±2.40	48.50±4.70	10.04±1.49
Phases 2+3							
Control	7	4.70±0.20	0.88±0.10	0.51±0.08	18.00±2.20	60.10±4.50	10.61±1.37
Late ischemic PC (24 h)	8	4.50±0.10	0.80±0.10	0.26±0.04*	18.10±1.50	31.40±3.00*	5.83±0.88*
Late ischemic PC (72 h)	9	4.10±0.20	0.80±0.30	0.27±0.10*	18.46±1.94	35.40±2.10*	6.61±0.74*
1-Day NTG patch (72 h)	7	3.90±0.12	0.90±0.10	0.20±0.02*	21.70±3.40	25.00±2.80*	5.14±0.70*
NTG tolerant (72 h)	7	4.20±0.10	0.90±0.10	0.25±0.03*	21.40±1.90	27.30±1.70*	5.79±0.49*

LV indicates left ventricle; RR, region at risk. Data are mean±SEM. The late ischemic PC (24 h) group is the same in phases 1 and 2+3.
 *P<0.05 vs control; †P<0.05 vs NTG PC (24 h); ‡P<0.05 vs late ischemic PC (24 h); §P<0.05 vs early ischemic PC (10 min) and early ischemic PC (1 h).

group of 5 rabbits by the finding that on days 14 and 28 of continuous TD NTG treatment, the hypotensive response to IV NTG was markedly attenuated compared with that observed immediately before the application of the first TD NTG patch (Figure 5). In the rabbits that underwent the 30-minute coronary occlusion 72 hours after the end of a continuous 28-day TD NTG treatment (NTG-tolerant group), infarct size was significantly smaller than in controls and was indistinguishable from that measured in the late ischemic PC groups (Figure 4), indicating that tolerance to the hemodynamic actions of NTG does not extend to the PC effects of this drug.

In all 3 phases of this study, the results of the measurements of infarct size were corroborated by the analysis of the relationship between infarct size and risk region size (ANCOVA) and by the measurements of the total deficit of wall thickening over the 3-day reperfusion period (data not shown).

Discussion

The ultimate goal of studying PC is to exploit this phenomenon for the protection of the ischemic myocardium in patients with coronary artery disease. Unfortunately, despite impressive advances in our understanding of the pathophys-

iology and mechanism of PC in experimental models, no widely applicable PC mimetic has yet been developed for clinical use. Most agents that elicit a late PC-like protection have significant side effects that limit their clinical applicability.^{25,26} In contrast, NO donors, such as nitrates, are widely used clinically and relatively well tolerated.

The present study provides significant new information that is relevant to the development of PC-mimetic therapies. The salient results can be summarized as follows: (1) In conscious rabbits, a brief IV infusion of a clinically relevant NO-releasing agent (NTG) elicits a powerful infarct-sparing effect 24 hours later; (2) this infarct-sparing effect is sustained for 72 hours and is at least equivalent to that of ischemia-induced late PC; (3) the same degree of cardioprotection as afforded by IV NTG can be elicited by TD administration of NTG (NTG patch); (4) NTG-induced late PC is not affected by the development of nitrate tolerance; and (5) in addition to a late PC effect, NTG also induces an early PC effect against infarction. Taken together, these results support a potential role of nitrates as PC mimetics in humans and provide a framework for testing these agents in the clinical arena. To the best of our knowledge, this is the first

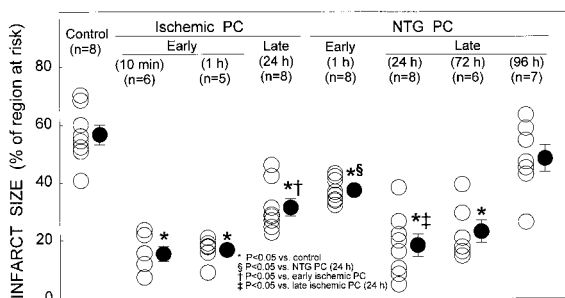


Figure 3. Myocardial infarct size. ●, Mean±SEM.

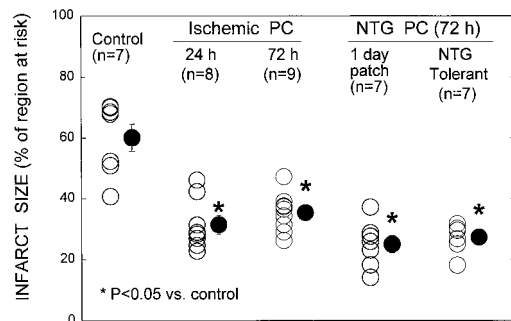


Figure 4. Myocardial infarct size (late ischemic PC-24 hours group is same as that included in Figure 3). ●, Mean±SEM.

TABLE 3. Heart Rate and Blood Pressure Before and During Administration of TD NTG

	Baseline	1 h	3 h	6 h	9 h	12 h	24 h	Day 14	Day 28
Heart rate, bpm									
12-h NTG patch group (n=5)*	246.4±8.3	276.0±5.9†	271.2±5.1†	252.0±7.7	249.6±7.8	243.2±11.4
1-day NTG patch group (n=7)	255.0±5.3	279.0±9.3
28-day NTG patch (n=7) (NTG tolerant)	240.0±11.3	232.0±9.3	246.7±4.0
Mean arterial pressure, mm Hg									
12-h NTG patch group (n=5)*	100.9±3.0	94.3±2.2	85.5±2.1†	78.7±1.5†	83.3±1.5†	85.1±3.4†
1-day NTG patch group (n=7)	82.7±2.8	84.6±1.8
28-day NTG patch (n=7) (NTG tolerant)	88.5±3.8	87.7±2.1	85.6±3.0

In the 1-day NTG patch group, measurements were taken before application of the NTG patch (baseline) and 24 h after application of the patch. In the NTG-tolerant group, measurements were taken before application of the first NTG patch (baseline) and on days 14 and 28 of the 28-day continuous TD NTG treatment. Data are mean±SEM.

*In a separate group of 5 rabbits, measurements were taken before application of the first NTG patch (baseline) and at 1, 3, 6, 9, and 12 h after application of the patch.

† $P<0.05$.

study to determine that a form of therapy widely used in patients elicits both an early and a late PC effect against infarction. In addition, this is the first study to indicate that NO donors induce cardioprotection at a distance of >24 hours and that this salutary effect is preserved despite nitrate tolerance.

Having found in phase 1 that IV infusion of NTG induces late PC, in phase 2 we tested the efficacy of TD NTG patch treatment and compared the degree of cardioprotection afforded by this route of administration with that achieved by the IV route. TD NTG patches provide a vehicle for NTG delivery that is simple, is noninvasive, does not require blood pressure monitoring, and thus is potentially widely applicable among patients with coronary artery disease. The results of phase 2 demonstrate that a single NTG patch is sufficient to induce a cardioprotective effect 72 hours later that is similar in magnitude to that achieved with IV NTG infusion. If these results are found to be applicable to the clinical arena, the PC-mimetic actions of NTG could be exploited in ambulatory patients without the need for IV treatment or hospital admission.

The development of nitrate tolerance is an important factor limiting the therapeutic efficacy of NTG in the treatment of patients with ischemic heart disease.^{15,16,27} Accordingly, in phase 3 of this study, we investigated whether tolerance to the hemodynamic actions of NTG extends to its PC-mimetic actions. To this end, rabbits were subjected to daily application of TD NTG patches for 4 consecutive weeks, and infarction was produced 72 hours after removal of the last patch. We reasoned that if nitrate tolerance prevents NTG from inducing late PC, under these conditions the reduction in infarct size should be either diminished or completely abolished. The results clearly demonstrate that the ability of NTG to elicit late PC against infarction is not hampered by the development of tolerance, even after 28 days of continuous NTG administration resulting in

marked attenuation of the vasodilator response to this drug. The dissociation between the PC-mimetic and hemodynamic actions of NTG after long-term treatment is important not only for its therapeutic implications but also from a conceptual standpoint, because it implies that different cellular mechanisms account for the 2 effects.

In conclusion, this study reveals a new, heretofore unappreciated action of nitrates that has considerable potential usefulness for the treatment of coronary artery disease. Data obtained in experimental animals must obviously be extrapolated to the clinical situation with caution. Nevertheless, the notion that in addition to its immediate anti-ischemic effects, NTG can also trigger a sustained phenotypic change that renders the heart resistant to infarction at a distance of 24 to 72 hours suggests novel applications of nitrates for protecting

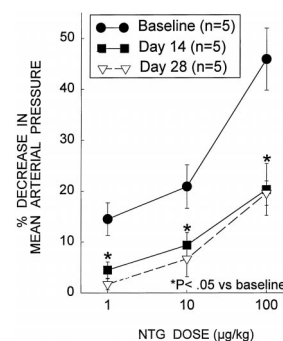


Figure 5. Change in mean arterial pressure in response to a bolus IV injection of 1, 10, or 100 µg/kg NTG before (baseline) and during (days 14 and 28) continuous daily treatment with TD NTG patches in a separate group of rabbits that underwent 28 days of continuous TD NTG patch treatment without subsequent coronary occlusion. Data are mean±SEM.

the ischemic myocardium in patients. Thus far, these drugs have been used mainly for their antianginal and preload-reducing properties. The present findings support the novel idea that nitrates might be equally useful, or perhaps even more useful, for the prophylaxis of ischemic myocellular death. Such an effect could be as important as, or even more important than, their short-term effects.

If nitrates exert PC-mimetic actions in humans, the IV route of NTG administration examined in phase 1 would be particularly feasible in patients who are hospitalized for acute coronary syndromes or for mechanical revascularization (CABG, PTCA). However, the results of phases 2 and 3 demonstrating that the same degree of infarct size reduction can be achieved with TD NTG delivery suggest that at least in principle, this therapy might be clinically feasible in a much larger spectrum of patients. The prolonged duration of NTG-induced cardioprotection observed in this study supports the hypothesis (which remains to be tested) that a protracted or even chronic PC state could be implemented by administering appropriate doses of IV or TD NTG on a regular basis every 2 to 3 days. Thus, the present results provide a rationale for studies aimed at investigating the usefulness of NTG and other nitrates as prophylactic treatments against infarction in patients with coronary artery disease. In all clinical studies that have examined the effect of nitrates in acute coronary syndromes, treatment was started either during or immediately after the index ischemic insult.^{28,29} We suggest that it might be fruitful to reexplore the role of nitrate therapy given before the onset of ischemia.

Acknowledgments

This study was supported in part by NIH grants R01-HL-43151 and HL-55757 (Dr Bolli); by American Heart Association (AHA) Ohio Valley Affiliate grant 9951533V (Dr Tang); by AHA Ohio Valley Affiliate Fellowship Award 9804558 (Dr Takano); by a Medical Research Council of Canada Fellowship Award (Dr Hill); by the Medical Research Grant Program of the Jewish Hospital Foundation, Louisville, Ky; and by the Commonwealth of Kentucky Research Challenge Trust Fund.

References

- Sun JZ, Tang X-L, Knowlton AA, et al. Late preconditioning against myocardial stunning. *J Clin Invest*. 1995;95:388–403.
- Tang X-L, Qiu Y, Park SW, et al. Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ Res*. 1996;79:424–434.
- Bolli R, Bhatti ZA, Tang X-L, et al. Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res*. 1997;81:42–52.
- Bolli R, Manchikalapudi S, Tang X-L, et al. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. *Circ Res*. 1997;81:1094–1107.
- Ping P, Takano H, Zhang J, et al. Isoform-selective activation of protein kinase C by nitric oxide in the heart of conscious rabbits. *Circ Res*. 1999;84:587–604.
- Takano H, Tang X-L, Qiu Y, et al. Nitric oxide donors induce late preconditioning against myocardial stunning and infarction in conscious rabbits via an antioxidant-sensitive mechanism. *Circ Res*. 1998;83:73–84.
- Marber MS, Latchman DS, Walker JM, et al. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation*. 1993;88:1264–1272.
- Qiu Y, Rizvi A, Tang X-L, et al. Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits. *Am J Physiol*. 1997;273(Heart Circ Physiol 42):H2931–H2936.
- Yang XM, Baxter GF, Heads RJ, et al. Infarct limitation of the second window of protection in a conscious rabbit model. *Cardiovasc Res*. 1996;31:777–783.
- Baxter GF, Goma FM, Yellon DM. Characterisation of the infarct-limiting effect of delayed preconditioning: time course and dose-dependency studies in rabbit myocardium. *Basic Res Cardiol*. 1997;92:159–167.
- Bolli R, Dawn B, Tang X-L, et al. The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol*. 1998;93:325–338.
- Banerjee S, Tang X-L, Qiu Y, et al. Nitroglycerin induces late preconditioning against myocardial stunning via a PKC-dependent pathway. *Am J Physiol*. 1999;277(Heart Circ Physiol 46):H2488–H2494.
- Shimura K, Tang X-L, Takano H, et al. Nitric oxide donors attenuate myocardial stunning in conscious rabbits. *Am J Physiol*. 1999;277(Heart Circ Physiol 46):H2495–H2503.
- Abrams J. Tolerance to organic nitrates. *Circulation*. 1986;74:1181–1185.
- Packer M, Lee W, Kessler PD, et al. Prevention and reversal of nitrate tolerance in patients with congestive heart failure. *N Engl J Med*. 1987;317:799–804.
- Zimrin D, Reichel N, Bogin KT, et al. Antianginal effects of intravenous nitroglycerin over 24 hours. *Circulation*. 1988;77:1376–1384.
- Watanabe H, Kakihana M, Ohtsuka S, et al. Randomized, double-blind, placebo-controlled study of supplemental vitamin E on attenuation of the development of nitrate tolerance. *Circulation*. 1997;96:2545–2550.
- Parker JD. Potential problems with intermittent nitrate therapy. *Can J Cardiol*. 1996;12(suppl C):22C–24C.
- Nakano A, Liu GS, Heusch G, et al. Exogenous nitric oxide can trigger a preconditioned state through a free radical mechanism, but endogenous nitric oxide is not a trigger of classical ischemic preconditioning. *J Mol Cell Cardiol*. 2000;32:1159–1167.
- Triana JF, Li XY, Jamaluddin U, et al. Postischemic myocardial “stunning”: identification of major differences between the open-chest and the conscious dog and evaluation of the oxygen radical hypothesis in the conscious dog. *Circ Res*. 1991;69:731–747.
- Schwartz LM, Verbinski SG, Vander Heide RS, et al. Epicardial temperature is a major predictor of myocardial infarct size in dogs. *J Mol Cell Cardiol*. 1997;29:1577–1583.
- Takano H, Manchikalapudi S, Tang X-L, et al. Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation*. 1998;98:441–449.
- Munzel T, Sayegh H, Freeman BA, et al. Evidence for enhanced vascular superoxide anion production in nitrate tolerance: a novel mechanism underlying tolerance and cross-tolerance. *J Clin Invest*. 1995;95:187–194.
- Munzel T, Giaid A, Kurz S, et al. Evidence for a role of endothelin 1 and protein kinase C in nitroglycerin tolerance. *Proc Natl Acad Sci U S A*. 1995;92:5244–5248.
- Brown JM, Grosso MA, Terada LS, et al. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. *Proc Natl Acad Sci U S A*. 1989;86:2516–2520.
- Meng X, Cleveland JC Jr, Rowland RT, et al. Norepinephrine-induced sustained myocardial adaptation to ischemia is dependent on alpha 1-adrenoceptors and protein synthesis. *J Mol Cell Cardiol*. 1996;28:2017–2025.
- Elkayam U, Roth A, Kumar A, et al. Hemodynamic and volumetric effects of venodilation with nitroglycerin in chronic mitral regurgitation. *Am J Cardiol*. 1987;60:1106–1111.
- ISIS-4. A randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58,050 patients with suspected acute myocardial infarction. *Lancet*. 1995;345:669–685.
- GISSI-3. Effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. *Lancet*. 1994;343:1115–1122.