Comparison of Vasoactive Response of Left and Right Internal Thoracic Arteries to Isosorbide-Dinitrate and Nitroglycerin: An In Vitro Study

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ABSTRACT  Objective: The internal thoracic artery (ITA) is the most important graft in coronary artery bypass grafting. Its distal region is, however, prone to vasospasm. We studied the effects of nitroglycerin (NTG) and isosorbide-dinitrate (DSDN) on distal segments of left versus right ITA. Methods: Rings of distal segments (6 to 9 mm proximal to bifurcation) of the human left and right ITA were studied. After baseline contraction of the rings, achieved using 60 mmol/L of KCl, they were exposed to increasing doses of ISDN and NTG (10 to 100 µg/ml), and dose-response curves were recorded. Results: The contractile response of left ITA rings to KCl were significantly lower than those of right ITA rings (1.87 ± 0.25 g versus 3.5 ± 0.61 g, p < 0.005). Both nitrates inhibited the contractile response in a concentration-dependent manner, with relaxing effects of ISDN higher than those of NTG (p < 0.01) in both left and right ITA rings. Conclusions: The distal segment of the left ITA is less prone to vasospasm than that of the right. ISDN has a considerably higher relaxant effect on this segment than NTG. We therefore recommend favoring high doses of ISDN over NTG as an antispastic measure. (J Card Surg 2003;18:279-285)
A surgical technique was recently developed, in which the ITA is dissected as a skeletonized vessel.\(^8\) The skeletonized artery is particularly long, and its spontaneous blood flow is greater than in a pedicled ITA\(^9\) allowing the use of both ITAs as grafts to practically all coronary vessels requiring revascularization. The extra length obtained with skeletonizing dissection enables more frequent use of in situ right ITA to LAD, and more frequent use of the composite technique (free right ITA on in situ left ITA) for complete arterial revascularization.\(^10\)

Postoperative coronary angiography has proved that, like other arterial conduits, ITA is prone to arterial spasm.\(^11\) Several research groups have demonstrated that nitrates given perioperatively can prevent spasm of ITAs.\(^10,12\) While nitrates have a relaxing effect on all vessel types, the extent of this effect varies considerably in vessels from different anatomical regions.\(^13,14\)

In a recently published study, we showed that the segment of the left ITA located 6 to 9 mm proximal to its bifurcation is less susceptible to spasm than the more distal segments of the ITA, and recommended avoiding the use of the very distal parts of this artery.\(^15\) In view of the recent popularity and more common use of the right ITA,\(^4,6\) we decided to compare the vasospastic response of the distal left ITA to that of the right ITA, and to evaluate the relaxation effect of nitroglycerin (NTG) and isosorbide-dinitrate (ISDN) on these arteries.

**MATERIALS AND METHODS**

**Preparation of vessels**

The left and right ITA of patients undergoing CABG were harvested in a skeletonized fashion. The skeletonized arteries were isolated gently with scissors and silver clips, without cauterization, then rings from distal segments (6 to 9 mm proximal to bifurcation) of the left and right ITA were prepared.

We took great care to ensure that there was no influence of the handling and storing of the arteries on the results. Specifically, in order to avoid any influence of the order of harvesting on outcome, harvesting of the mammaries was performed in the following fashion: In 5/10 patients in each group, the right ITA was harvested before the left ITA, and in the remaining 5/10, the left ITA was harvested before the right ITA. The second ITA was always harvested before administration of heparin and, therefore, during harvesting of the second ITA in each group, the ITA that had first been separated from the chest wall was left connected in both its proximal and distal ends. Separation of the distal edges of the two ITAs was performed only after completion of harvesting of both arteries and administration of heparin. Immediately after separation of the distal edges, the centimeter proximal to the area of the bifurcation was excised from each of the two arteries and it was placed in a cup containing oxygenated Krebs–Hensleit solution for immediate transfer to the laboratory. The segments were taken directly after harvesting of the ITA and treated according to the above protocol—they were never set aside. The samples were removed from the operating room before the patient was connected to the heart-lung machine, not at the time of performing the anastomoses. The retrieved segments of the ITA were not exposed to hypothermia, to vasoactive materials, or to circulating vasoactive substances that were administered after weaning off bypass. They were not treated either with topical or with intraluminal vasodilating agents.

The use of human ITA tissue was approved by the relevant hospital authorities.

**Organ bath technique**

Arterial rings, 3 mm in length, were suspended on wire hooks in a 100 ml jacket glass bath, with a temperature maintained at 37\(^{\circ}\)C. The modified Krebs–Henseleit (KH) buffer solution contained the following composition (mmol/L): NaCl 118; KCl 4.7; CaCl\(_2\) 2.0; MgSO\(_4\).7H\(_2\)O 1.2; KH\(_2\)PO\(_4\) 1.2; glucose 11.1; NaHCO\(_3\) 25. The perfusate was bubbled continuously with 95% O\(_2\) and 5% CO\(_2\). The upper hook was connected to a force transducer (Instrument & Control Ltd., Israel), and the lower hook was fixed on a Stalin leg. The amount of force was recorded on a Beckman Dynograph Recorder R611 (USA). Two organ bath arrangements were run simultaneously.

**Protocol**

After the rings were equilibrated without tension on the wire hooks for one hour, a normalizing procedure was performed. The resting tension applied to each ring was equivalent to that required to stretch the ring to 90% of its internal
circumference when distended with a transmural pressure of 100 mmHg. After the normalizing procedure, the rings were left to rest for 30 minutes. A steady level of active contraction was then established by adding potassium chloride (KCl 60 mmol/L). This concentration gave 60 to 80% of the maximal contraction of this agent on the left and right ITA. Cumulative concentration-response curves for ISDN and NTG were obtained by adding the drugs every 3 minutes in concentrations from 10 to 100 µg/ml to the organ chamber. Residual contraction—the contraction 30 minutes after exposure to ISDN or NTG—were measured. Curves were obtained from ten different rings for a designated part of the left and right ITA.

A concentration-response curve was obtained only once for each ring.

Tolerance studies

After performing cumulative concentration-response curves for ISDN and NTG as described in the above protocol, arterial rings were washed twice with KH solution and left in a bath with KH solution for an additional 40 minutes. The rings were then re-exposed to solutions with increasing concentrations of ISDN or NTG (from 10 to 100 µg/ml). The second concentration-response curve thus obtained was then compared to the first, in order to evaluate the tolerance of the nitrates.

In this study, we paid particular attention to carefully mount the left and right ITA rings on the wire hooks in the organ bath, to minimize damage to the endothelium. In all experiments, the presence of functional endothelium was confirmed by determining the relaxation response to 10^{-5} mol/L acetylcholine.

Drugs and chemicals

NTG was purchased from Taro Pharmaceutical Industries Ltd. (Israel); ISDN from Schwartz Farm AG (Monheim, Germany); and other chemicals were purchased from Sigma (St. Louis, USA).

Statistics

The results are presented as mean ± standard error. Statistical analysis was performed using Student’s unpaired t test. One-way analysis of variance (ANOVA) with repeated measures was used to calculate dose-responses and for comparison of groups. Significance was established at a p level of < 0.05.

RESULTS

Contraction

Incubation of the rings with KCl resulted in different contractions of the rings from the left and right ITA. In the left ITA rings, KCl induced a significantly lower contractile force than in the right ITA rings (1.87 ± 0.25 g versus 3.5 ± 0.61 g, respectively, p < 0.005).

Relaxation

ISDN induced a more pronounced relaxation of the precontracted left ITA rings than did NTG (p < 0.001, ANOVA, Fig. 1A). Specifically, maximal relaxation induced by ISDN was 46.08 ± 2.06%,
and 20.45 ± 1.82% by NTG (p < 0.02). ISDN was also more effective than NTG on the vasorelaxation of right ITA rings (Fig. 1B, p < 0.05, ANOVA). There were no differences in percentage of relaxation to increasing concentrations of ISDN and NTG between left and right ITA rings (p < 0.97 and p < 0.916, ANOVA, respectively). However, residual contraction—the contraction 30 minutes after exposure to ISDN or NTG—in the right ITA rings was significantly higher than in left ITA rings (1.74 ± 0.34 g versus 1.01 ± 0.20 g and 2.35 ± 0.52 g versus 1.49 ± 0.02 g, respectively).

**ISDN and NTG tolerance**

All rings of left and right ITA were re-exposed to increasing concentrations of ISDN or NTG after the first exposure, and cumulative concentration-response curves were obtained. ISDN induced a less pronounced relaxation of the precontracted left and right ITA rings after the second exposure (p < 0.01 and p < 0.05, ANOVA, respectively). Specifically, maximal relaxation induced by ISDN in the left ITA after the first exposure was 46.08 ± 2.06%, whereas it was only 18.27 ± 2.05% after the second exposure (p < 0.01); in the right ITA maximal relaxation was 50.35 ± 7.53 versus 27.45 ± 5.84, p < 0.05, respectively (Figs. 2A and B). NTG did not have any relaxation effect on precontracted left ITA rings after the second exposure (Fig. 2C), but it did have some relaxing effect on the precontracted right ITA rings after second exposure. However, this effect was less pronounced than the effect of the first exposure (p < 0.03, ANOVA, Fig. 2D).

In conclusion, the left ITA rings show more pronounced tolerance to NTG than to ISDN (p < 0.001, ANOVA), but right ITA rings showed the same tolerance to both nitrates (p < 1.0).

**Figure 2.** Tolerance of the left internal thoracic artery (ITA) and right ITA to isosorbide-dinitrate (ISDN) and nitroglycerin (NTG). ISDN induced a less pronounced relaxation of precontracted left ITA rings (A) and right ITA rings (B) during second exposure (p < 0.01 and p < 0.05, respectively). NTG did not have any relaxation effect on precontracted left ITA rings after the second exposure (C), but did have some relaxation effect on the precontracted right ITA rings after second exposure (p < 0.03) (D).
DISCUSSION

The ITA, like other arterial conduits, is prone to arterial spasm, particularly in its distal portion, near the bifurcation. Spasm of arterial conduits can be prevented or alleviated by perioperative administration of various nitrates. Nitrates are converted enzymatically to nitric oxide (NO) in the vascular smooth muscle cells. NO stimulates soluble guanylate cyclase, causing an increase of cyclic GMP content that results in arterial vasodilatation.

The major finding in this study is that contractility at the distal section of the right ITA is greater than that of the distal section of the left ITA. This is indicated by: (1) higher tension developed in right ITA arterial rings in response to standard concentration of potassium chloride; (2) more pronounced relaxation effect of nitrates on the left ITA rings.

We now demonstrated that the same concentration of KCl caused a higher contractile response in the right ITA rings than in the left ITA rings. The selection of 60 mmol/L concentration of KCl in the present study was based on the relatively high concentrations of KCl in the cardioplegic solutions used in our department during CABG. Previous studies have also demonstrated such differences between different arteries, i.e., a pronounced increase in contractile response of the radial artery to KCl when compared to either the ITA or the gastroepiploic arteries, and differences of reactions to KCl in different parts of the left ITA. This phenomenon might be explained by the differences in the density of voltage-dependent channels of the smooth muscle cell membrane and in the sensitivity of these channels to depolarization effect of KCl, or by the different sensitivity of the contractile apparatus to calcium.

Our observations on difference in spastic responses of the right and left ITAs might have important clinical applications, particularly for patients undergoing revascularization procedures with bilateral ITAs. Whenever possible, especially when the distal portion of the artery has a very small diameter, it is advisable not to use the more distal segments of the right ITA. In cases where the extra length obtained with the distal ITA is critical (right ITA to LAD, or right ITA in a composite graft to the PDA), we highly recommend perioperative administration of nitrates in high doses.

Our study showed better relaxing response of both ITAs to ISDN when compared to NTG. The differences in the effects of ISDN and NTG observed in the current study might, therefore, be explained by different participation of several intracellular mechanisms in the action of nitrates. It is generally accepted that the intracellular second messenger, cGMP, mediates the vascular smooth muscle relaxation elicited by nitrates.

The intracellular free calcium concentration plays a crucial role in the regulation of smooth muscle tension. Relaxation of vascular smooth muscles induced by cGMP is due to the reduction in cytosolic ionic calcium concentration. In the rabbit aorta, ISDN induced maximal values of cGMP more rapidly than NTG.

To the best of our knowledge, there is no clear-cut explanation for the difference observed between the two ITAs in response to KCl-induced vasoconstrictive stimuli, nor for the difference in vasorelaxation after treatment by nitrates. Nitrates have a heterogeneous vasodilatation effect on different types of vessels and on different parts of the same vessel. The difference in reactivity of the right versus the left ITA may be explained by different levels of sensitivity of these vessels to NO donors which may be related to different degrees of participation of the various vessel segments in each step of the intracellular signaling of nitrates action (cGMP, intracellular calcium, activation of cGMP-dependent protein kinase, the latter leading to phosphorylation of proteins that are involved in the removal of calcium from the cell and probably in some other mechanisms). Another interesting question but one that was not evaluated in the current study concerns the reactivity of ITAs in their mid-portion and proximal segments and whether these areas will demonstrate similar differences in vasoreactivity. This opens up a much wider field for subsequent experiments.

Some reports have indicated that in the vascular smooth muscle cells, ISDN and NTG modulate...
the gating of two major potassium channels, ATP-sensitive and calcium-activated, which might play an important role in controlling vascular tone by changing the membrane potential. In our experiments, potassium ions served as a membrane depolarizing agent to open voltage-operated calcium channels and, in turn, to increase calcium influx. In this study it was clearly shown that ISDN is more effective than NTG in inhibiting the contraction associated with voltage-operated channels.

Our study also confirmed previously published data indicating that nitrate tolerance induced by ISDN is lower than that induced by NTG. In addition, less pronounced hypertensive effect of ISDN compared to NTG enables patients to tolerate higher doses of the drug. The problem of tolerance to nitrates action is well known, but the molecular basis for this phenomenon is still not understood. Experiments on isolated strips of bovine coronary arteries showed that, after NTG pretreatment, the response of the strips to NTG was less sensitive with respect to relaxation and to an increasing amount of cGMP. While the addition of cysteine (the precursor of NO) during incubation was found to lessen the degree of tolerance, it did not prevent it completely. Whether the addition of cGMP or calcium can restore the vasorelaxation effect of nitrates awaits future studies.

CONCLUSIONS

The distal segment of the left ITA is less prone to vasospasm than that of the right. ISDN has a considerably higher relaxant effect on this segment than NTG. We therefore think that ISDN is a more suitable antispastic agent than NTG, and recommend its routine use in patients undergoing revascularization procedures with bilateral ITA.

REFERENCES


