

THE DIAZENIUMDIOLATE IPA/NO  
MODERATES NEOINTIMAL HYPERPLASIA  
FOLLOWING VASCULAR INTERVENTIONS VIA  
A MECHANISM DISTINCT FROM NITRIC OXIDE

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## INTRODUCTION

Nitric oxide (NO) is known to be involved in maintenance of vascular tone in healthy tissue, and to act beneficially to prevent pathologic derangements such as neointimal hyperplasia. Diazeniumdiolates have been synthesized as NO donors for over 25 years (1). Originally synthesized as a NO donor, isopropylamine NONOate (IPA/NO) was mostly disregarded because it was relatively unstable and its conversion to NO occurred in relatively low yield (2). It was realized, however, that IPA/NO released nitroxyl (HNO) at predictable rates. Several recent studies have shown that HNO is a positive inotrope (3) and a vasodilator (4). Since the positive inotropic action of HNO is unaffected by beta-blockers, and, since the vasodilatory effects of HNO are not subject to development of tolerance (5), nitroxyl donors are being investigated as agents for treatment of congestive heart failure.

While much is known about the beneficial effects of NO in the vasculature, and some is known about HNO as a vasoactive molecule, the effect of HNO on neointimal hyperplasia remains unknown. With the growing amount of evidence suggesting a therapeutic potential for HNO, we sought to investigate the organic HNO donor IPA/NO in the vasculature with respect to its ability to inhibit neointimal hyperplasia following arterial injury. Therefore, the aim of this study was two-fold: 1) to determine the effect of IPA/NO on endothelial and vascular smooth muscle cell (VSMC) proliferation and death *in vitro*; and, 2) to determine if IPA/NO inhibits neointimal hyperplasia *in vivo*. Our hypothesis is that IPA/NO will inhibit neointimal hyperplasia following vascular injury.

## EXPERIMENTAL

VSMC and human umbilical vein endothelial cells (HUVEC) were exposed to IPA/NO and proliferation was assessed by <sup>3</sup>H-thymidine incorporation. Cell

death was assayed by Guava PCA. VSMC exposed to IPA/NO were stained with propidium iodide and analyzed by flow cytometry. Morphometric analysis was performed on hematoxylin and eosin stained sections of balloon-injured rat carotid arteries (+/- IPA/NO) harvested 14 days after injury. Intravenous administration of Evans blue dye 7 days after balloon injury was used to assess the extent of re-endothelialization of injured vessels (+/- IPA/NO).

## RESULTS

IPA/NO induced a concentration-dependent inhibition of VSMC (50%,  $P < 0.001$ ) and HUVEC proliferation (55%,  $P < 0.001$ ), both of which were independent of cell death. Interestingly, IPA/NO induced a 49% increase in the S-phase population ( $P = 0.039$ ) and a 20% decrease in the G<sub>0</sub>/G<sub>1</sub> population ( $P = 0.027$ ), as compared to controls. This is distinct from the G<sub>0</sub>/G<sub>1</sub> arrest caused by NO. Morphometric analysis showed that application of IPA/NO powder (10 mg) to the external surface of the balloon-injured carotid artery caused statistically significant reductions in both the neointima (27%,  $P = 0.006$ ) and the media (33%,  $P < 0.001$ ) compared to injury alone. IPA/NO also prevented re-endothelialization, as shown by Evans blue staining.

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