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Short communication

Role of endogenous nitric oxide in the nitric oxide donor-induced plasma extravasation of mouse skin

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Abstract

The role of endogenous nitric oxide (NO) and prostanoids in the increase in microvascular permeability induced by NO donors was investigated in the mouse skin by a dye leakage method. Subcutaneous (s.c.) injection of 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene (NOC 5), 1-hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazene (NOC 18) and sodium nitroprusside dose-dependently increased local dye leakage. While indomethacin inhibited the dye leakage elicited by these NO donors, N^G-nitro-L-arginine methyl ester (L-NAME) inhibited the effect of NOC 5 and NOC 18 but not of sodium nitroprusside. These results suggest that endogenous NO, in addition to the prostanoid biosynthesis, is involved in the dermal microvascular permeability increase induced by the NOC series NO donors.

Keywords: Nitric oxide (NO) donor; Vascular permeability; Nitric oxide; Prostaglandin

1. Introduction

Vascular permeability has been studied by microvascular preparations of various organs such as skin, small intestine, mesentery, skeletal muscle and cheek pouch (Hughes et al., 1990; Kubes and Granger, 1992; Kurose et al., 1993; Noel et al., 1995; Fujii et al., 1996, 1997; Mitchell and Tyml, 1996). The prostaglandins and peptidoleukotrienes have been shown to promote vascular permeability by increasing blood flow (Williams and Morley, 1973; Campbell and Halushka, 1996). While it is clear that nitric oxide (NO) plays an important role in inflammatory states, the pro- or anti-inflammatory properties of NO may vary according to the site of the pathological process and other factors (Clancy and Abramson, 1995). The inhibitor of NO synthesis, N^G-nitro-L-arginine methyl ester (L-NAME), increased microvascular permeability in cat mesentery (Kubes and Granger, 1992), whereas L-NAME inhibited the increase in vascular permeability induced by 5-hydroxytryptamine, lipopolysaccharide, histamine in the mouse skin and hamster cheek pouch (Fujii et al., 1994, 1996; Mayhan, 1994). In the latter case, NO may increase vascular permeability by inducing endothelial contraction and possible opening of interendothelial junctions. Because of the unstable nature of NO in solution with oxygen, NO donors which generate NO in a controlled manner are frequently used in vivo (Feelisch, 1998). Sodium nitroprusside is a rapid-acting nitrovasodilator which releases NO and cyanide with a half-life ($T_{1/2}$) of 1.0–1.8 min (Ikeda et al., 1994). NOCs are a zwitterionic NO releaser which does not produce toxic substances such as cyanide (Hrabie et al., 1993). NOCs may be safer and more specific as a NO donor than sodium nitroprusside, although their biological effect on a vascular permeability has not been clarified. Among NOCs, 1-hydroxy-2-oxo-3(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC 7) releases NO most rapidly with $T_{1/2}$ of 1.7 min, followed by 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene (NOC 5) ($T_{1/2} = 7.0$ min) and 1-hydroxy-2-oxo-3,3′-bis(3-aminoethyl)-1-triazene (NOC 18) ($T_{1/2} = 78$ min) (Hrabie et al., 1993). NOC 7 showed a rapid vasodi-

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lating effect similar to sodium nitroprusside in dogs (Zhang et al., 1996). In addition, a complex relationship is emerging with regard to cross-talk between NO and cyclooxygenase pathways (Clancy and Abramson, 1995). The effect of these NO donors on microvascular permeability and intermediary roles of cyclooxygenase and endogenous NO synthesis system have not been clarified. In the present study, we evaluated the effect of different NO donors on microvascular permeability of the mouse skin and asked whether the mechanism of the vascular effect of these NO donors was the same.

2. Materials and methods

2.1. Animals and experimental procedure

The protocol of this study was approved by the animal experiment committee of the Tokyo Women's Medical University. Male ddY strain mice (Sankyo Laboratory Service, Tokyo, Japan) weighing about 35 g were used. Vascular permeability was assessed by an extravasation of Pontamine sky blue dye (Fujii et al., 1994). Five minutes after an intravenous (i.v.) injection of Pontamine sky blue (50 mg/kg), NO donors or saline (0.1 ml/site) were administered subcutaneously (s.c.) into the back of mice. L-NAME and N^G-nitro-D-arginine methyl ester (D-NAME) were given to the tail vein of mice immediately before the dye administration, and indomethacin intraperitoneally (i.p.) 30 min before. The dose of the inhibitors was chosen from our previous study (Fujii et al., 1997). Sixty minutes after injection of NO donors, the mice were killed by cervical dislocation and the entire stained area of the skin was excised. The dye was extracted from the minced skin by dispersing the tissue in a 6-ml 0.5% Na_2SO_4 in H_2O followed by an addition of 14 ml acetone. Dye concentration was colorimetrically determined at 590 nm. Although our previous studies have shown that this method to evaluate dye leakage is quite reproducible, the experiments were repeated at least twice.

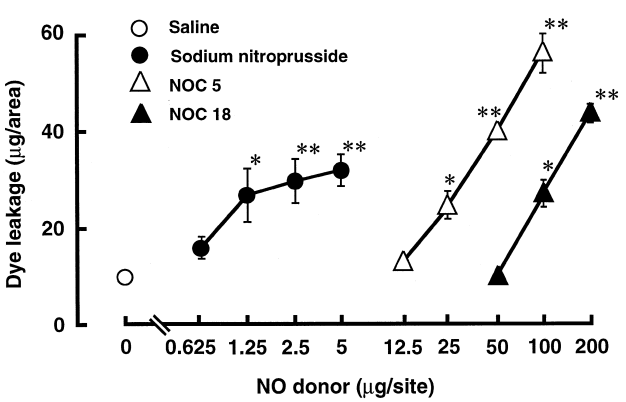

Fig. 1. Effect of dose of NO donors on the dye leakage in mouse skin. Increasing doses of sodium nitroprusside, NOC 5, NOC 18 or saline (0.1 ml/site) were given s.c. to mice 5 min after i.v. Pontamine sky blue (50 mg/kg). The dye leakage in the skin over 60 min was determined. Points and bars represent mean ± S.E.M. of five mice. $^*P < 0.05$, $^{**}P < 0.01$ vs. saline.

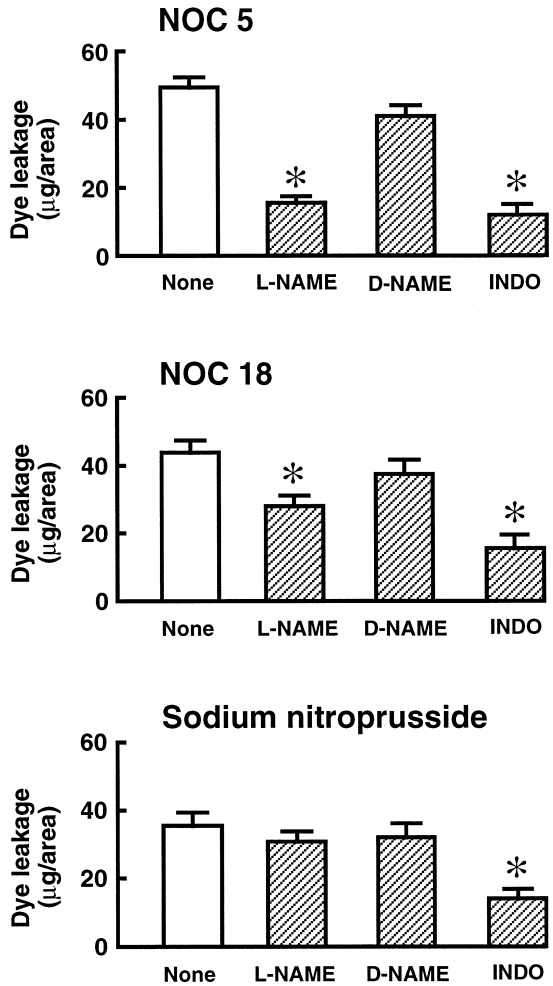

Fig. 2. Effect of inhibitors of cyclooxygenase and NO synthase on the dye leakage induced by NO donors. Mice were pretreated with or without L-NAME (10 mg/kg, i.v.) or D-NAME (10 mg/kg, i.v.) immediately before, or indomethacin (INDO) (10 mg/kg, i.p.) 30 min before the dye injection. Five minutes after the dye, NOC 5 (100 mg/site), NOC 18 (200 mg/site) or sodium nitroprusside (2.5 mg/site) was injected s.c. at the dorsal skin. The dye leakage in the skin over 60 min was determined. Columns and bars represent mean ± S.E.M. of five mice. $^*P < 0.01$ vs. none (no pretreatment).

2.2. Drugs

NOC 5 and NOC 18 were purchased from Dojindo Laboratories, Kumamoto, Japan; L-NAME HCl and indomethacin from Sigma, MO, USA; D-NAME HCl from Nova Biochem, Läufelfingen, Switzerland; and sodium nitroprusside from Wako, Osaka, Japan. Indomethacin was dissolved in a small volume of ethanol, then 50% propylene glycol was added to make 1 mg/ml stock solution.

Just prior to use, the indomethacin stock solution was diluted with 0.9% saline. Other drugs were dissolved in sterile physiological saline (0.9% NaCl) immediately before use.

2.3. Statistical analysis

Results were expressed as means ± S.E.M. of five mice and were analysed by unpaired Student's *t*-test.

3. Results

A pilot study showed that s.c. injection of NOC 5 (100 mg/site), NOC 18 (200 mg/site) and sodium nitroprusside (2.5 mg/site) into the mouse skin caused a significant local dye leakage not at 5 min but at 60 min after injection. Fig. 1 shows that the dye leakage induced by three NO donors over 0–60 min periods increased dose-dependently. The dose required for the significant dye leakage was the lowest for sodium nitroprusside followed by NOC 5 and NOC 18, while the maximum dye leakage induced by sodium nitroprusside was less than that of NOCs. To examine the role of endogenous NO and prostaglandins in the dye leakage elicited by NO donors, the effect of inhibitors of NO synthase and cyclooxygenase was investigated (Fig. 2). Although not shown, the basal dye leakage induced by saline was not significantly affected by a pretreatment with L-NAME, D-NAME or indomethacin (data not shown), which confirmed previous results (Fujii et al., 1997). The dye leakage induced by NOC 5 and NOC 18 was inhibited by an inhibitor of NO synthase, L-NAME, but not by its inactive enantiomer, D-NAME, whereas the effect of sodium nitroprusside was not altered by either L-NAME or D-NAME. In contrast, indomethacin, a cyclooxygenase inhibitor, suppressed the dye leakage induced by all three NO donors.

4. Discussion

Altered microvascular permeability is an important pathophysiology of inflammation. NO has been shown to play a role in vascular permeability change as an inflammatory mediator. We found that s.c. administration of NO donors increased the microvascular permeability in the mouse skin, confirming the previous studies with rat skin (Holzer et al., 1995; Sautebin et al., 1995). The onset of dye leakage induced by NO donors was slower than the case of 5-hydroxytryptamine and platelet-activating factor (Fujii et al., 1994, 1995), which may suggest the indirect effect of NO on the dermal vasculature. In the present study, all three NO donors tested caused an increase in microvascular permeability, sodium nitroprusside being the most potent on the weight basis followed by NOC 5 and NOC 18. Our results indicate that NO may act as a proinflammatory mediator at least in the mouse skin. Contrastingly, exposure of intestinal or mesenteric microvasculature to NO synthase inhibitors stimulated extravasation of proteins indicating antiinflammatory role of NO (Kubes and Granger, 1992; Baldwin et al., 1998). The cause of the differential microvascular response to NO among organs is not clear at present.

There are discrepancies among reports on the role of prostaglandin in the vascular effect of NO donors. Rat paw edema induced by a NO donor, SIN-1, was not inhibited by inhibitors of cyclooxygenase (Sautebin et al., 1995). The neurogenic hyperemia evoked by sodium nitroprusside and SIN-1 involves the formation of prostaglandin (Holzer et al., 1995). Our study with indomethacin revealed that the NO donor-induced permeability increase was mediated by enzymatic production of prostanoids, confirming the idea that NO stimulates cyclooxygenase pathways (Cirino, 1998).

The NO synthase inhibitor exerted a differential effect on sodium nitroprusside and NOC 5/NOC 18. Thus, an increase in dye leakage induced by NOCs was attenuated by L-NAME, whereas that by sodium nitroprusside was not inhibited. This may indicate that vascular permeability increase by NOCs is mediated by endogenous NO production at least partly. Degradation products of NOCs other than NO may activate any NO synthase, thereby making the effect of NOCs sensitive to NO synthase inhibition. Further, the maximal dye leakage induced by NOCs was larger than that of sodium nitroprusside, suggesting the release of extra NO.

5. Conclusion

We showed that the local microvascular permeability increase elicited by NO donors involves cyclooxygenase products and that endogenous NO may also play a role in the dye leakage elicited by NOC NO donors in the mouse skin.

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