Effect of S-Ethylisothiourea, a Putative Inhibitor of Inducible Nitric Oxide Synthase, on Mouse Skin Vascular Permeability

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ABSTRACT—By dye leakage in mouse skin, we evaluated the inhibition of proinflammatory stimuli-induced plasma extravasation by a putative inhibitor of inducible nitric oxide synthase, S-ethylisothiourea. A low dose of S-ethylisothiourea (5 μg/kg) mimicked aminoguanidine in inhibiting the plasma extravasation elicited by lipopolysaccharide but not by 5-hydroxytryptamine or platelet-activating factor. A higher dose of S-ethylisothiourea (10 μg/kg) inhibited the plasma extravasation induced by 5-hydroxytryptamine slightly; however, it increased the basal dye leakage. Thus, S-ethylisothiourea may be used as a relatively specific inhibitor for inducible nitric oxide synthase in vivo.

Keywords: Vascular permeability, Inducible nitric oxide synthase, Lipopolysaccharide

Endothelium-derived relaxing factor was identified as nitric oxide (NO) or a closely related molecule synthesized from arginine by nitric oxide synthase (NOS) (1). NO is a short-lived gas that is implicated in the regulation of mammalian blood vessels and other systems (2). NOS exists in 3 isozymic forms including 2 constitutive Ca2+-dependent isoforms (cNOS) from endothelial cells and neurons and a Ca2+-independent isoform (iNOS) that is induced by endotoxin or cytokines in several cells such as macrophages and endothelial cells (3). While NO produced by cNOS may have physiological roles, the overproduction of NO by iNOS may have a causal relationship with inflammatory vasodilation or septic shock (4). Therefore, specific inhibitors of iNOS may have some therapeutic value in these pathological conditions (5). While arginine analog inhibitors of NOS such as N'G-nitro-L-arginine methyl ester (L-NAME) inhibit both cNOS and iNOS, aminoguanidine is proposed as a preferential inhibitor of iNOS (6).

By using NOS inhibitors (7–10), we showed that NO is involved in the plasma leakage induced by the lipopolysaccharide (LPS) from Salmonella typhimurium and 5-hydroxytryptamine (5-HT) but not by platelet-activating factor (PAF) in the mouse skin. In addition, we found that aminoguanidine inhibited the LPS-induced plasma extravasation but not 5-HT-induced dye leakage. This finding suggests that NO produced by iNOS plays a role in the LPS-induced increase in vascular permeability as a toxin. Recently, S-ethylisothiourea (EIT) has been shown to be a potent selective inhibitor of iNOS in vitro (11, 12). Another isothiourea analog, S-methylisothiourea, has been shown to exert beneficial effects in rodent models of septic shock (13). Therefore, the purpose of this study is to examine whether EIT mimics aminoguanidine in the differential inhibitory effect on the dye leakage induced by LPS, 5-HT as well as PAF.

Changes in the dermal vascular permeability induced by LPS, 5-HT and PAF were investigated by determining Pontamine sky blue leakage in the mouse skin as described previously (7). Male ddY strain mice weighing about 35 g (Sankyo Laboratory Service, Tokyo) were injected i.v. with Pontamine sky blue (50 mg/kg). Five minutes later, LPS (400 pg/site), 5-HT (0.1 μg/site) or PAF (0.1 μg/site) dissolved in 0.1 ml saline was injected s.c. in the back of the mouse, and 0.1 ml saline was given s.c. instead of proinflammatory stimuli as a control. NOS inhibitors were injected i.v. immediately before dye injection. After 1 hr (for 5-HT and PAF) or 2 hr (for LPS), the mice were killed by cervical dislocation, and then the stained injection site of the dorsal skin was excised. The dye was extracted with a mixture of acetone and 0.5% sodium sulfate (14:6, v/v), determined colorimetrically at 590 nm.

Aminoguanidine hemisulfate, 5-HT creatinine sulfate, indomethacin, LPS from Salmonella typhimurium and L-NAME·HCl were purchased from Sigma (St. Louis, MO, USA); 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine (PAF) from Nova Biochem (Läufelfingen, Switzerland).
Switzerland); diphenhydramine HCl from Nacalai Tesque (Kyoto). EIT was synthesized as described previously (12). The other drugs were available from commercial sources. All the doses refer to the salt form of the drugs.

Results were evaluated nonparametrically by the Kruskal-Wallis method followed by the Wilcoxon rank sum test and are each expressed as a mean ± S.E.

EIT (5 and 10 μg/kg) decreased LPS-induced plasma leakage dose-dependently (Fig. 1). Whereas the lower dose of EIT did not inhibit the 5-HT-induced dye leakage, the higher dose caused a slight inhibition. Aminoguanidine, a putative iNOS inhibitor, inhibited plasma extravasation induced by LPS but not that by 5-HT, whereas L-NAME, a nonspecific NOS inhibitor, inhibited the plasma extravasation elicited by 5-HT more efficiently than that induced by LPS (Table 1). Dye leakage induced by PAF was not inhibited by any of the 3 NOS inhibitors. These findings confirm our previous reports that NO is involved in the dye leakage induced by LPS and 5-HT but not that by PAF (7–10). Presumably, iNOS is involved in the NO production in LPS-induced plasma extravasation, while cNOS plays a role in production of NO by 5-HT. The differential inhibition by the 5 μg/kg dose of EIT of the plasma leakage elicited by LPS and 5-HT was similar to the mode of inhibition by aminoguanidine rather than that by L-NAME in that EIT inhibited the effect of LPS more efficiently than that of 5-HT (Table 1). The difference of the doses employed indicates that EIT was about 1000 times more potent than aminoguanidine in inhibiting the LPS-induced dye leakage. This reflects the difference in the inhibitory potency on iNOS in vitro between the 2 drugs (11). We propose that comparison of the inhibition of LPS-induced dye leakage by EIT and aminoguanidine should provide insight into the mechanism of NO production in plasma extravasation.

![Fig. 1. Effect of S-ethylisothiourea (EIT) on the dye leakage elicited by the 3 proinflammatory stimuli. Mice were given lipopolysaccharide (LPS), 5-hydroxytryptamine (5-HT) or platelet-activating factor (PAF), s.c. in the back, and the amount of dye leaked at the injection site was determined 1 hr (5-HT, PAF) or 2 hr (LPS) later. As a control, some mice were injected with physiological saline, s.c. (0.1 ml/site). EIT was injected i.v. immediately before the proinflammatory stimuli. Columns and bars each represent the mean ± S.E. of 5–15 experiments. *P < 0.01 vs proinflammatory stimuli alone.](image-url)
leakage by a drug against its inhibition of 5-HT-induced dye extravasation may be a good parameter for screening potential iNOS inhibitors in vivo. As EIT (10 μg/kg) suppressed the effect of 5-HT slightly, the iNOS selectivity of EIT may decrease along with an increasing dose. Actually, Southan et al. (14) reported that EIT is a potent inhibitor of both eNOS and iNOS, although some investigators proposed that EIT is an iNOS-specific inhibitor (11, 12).

At 2 hr but not 1 hr after i.v. injection of a 10 μg/kg dose of EIT, EIT by itself induced a faint but generalized dye leakage in the skin (Fig. 1) that was not confined to the site of s.c. injection of proinflammatory stimuli. Prostaglandins or histamine would not play roles as mediators, because indomethacin or diphenhydramine did not inhibit the effect of EIT alone (Table 2). The dose of indomethacin is sufficient for inhibition of cyclooxygenase (15), and we confirmed that this dose of diphenhydramine inhibited the histamine (1 μg/site)-induced dye leakage completely (data not shown). At present, the mechanism of the EIT-induced delayed increase in vascular permeability is not known; however, a high dose of EIT may directly injure endothelial cells or increase the intravascular volume of the skin. The dye leakage induced by EIT by itself may hamper the proper evaluation of the potency of EIT in inhibiting the LPS-induced vascular permeability and decrease the usefulness of EIT as a possible drug for endotoxin shock.

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