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Effects of S-ethylisothiourea, a potent inhibitor of nitric oxide synthase, alone or in combination with a nitric oxide donor in splanchnic artery occlusion shock

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1 The aim of this study was to compare the effects of an intravenous infusion of a potent and non selective nitric oxide synthase inhibitor S-ethylisothiourea (Ethyl-TU) with that of a nitric oxide (NO) donor on the pathological sequelae associated with splanchnic artery occlusion (SAO) shock. In addition the effects of the combination of these two treatments were also investigated.

2 SAO shock was induced in anaesthetized rats by clamping splanchnic arteries for 45 min. Sham operated animals were used as controls. Survival time, white blood cell (WBC) count, mean arterial blood pressure, myeloperoxidase activity (MPO; studied as a quantitative means to evaluate neutrophil accumulation) and the responsiveness of aortic rings to acetylcholine (ACh, 10 nM - 10 μM) and to phenylephrine (PE, 1 nM - 10 μM) were studied.

3 SAO shocked rats had a decreased survival rate (0% survival 2 h after the release of occlusion) and survival time (76 ± 10 min), increased MPO activity in the ileum (3.39 ± 0.8 u $\times 10^{-3}$ g^{-1} tissue), a marked leukopenia and a profound hypotension. In addition aortic rings from shocked rats showed a marked hyporeactivity to PE and reduced responsiveness to ACh. Endothelium denuded aortic rings had also a marked hyporeactivity to PE.

4 *In vivo* administration of Ethyl-TU (0.1 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) significantly increased survival time and rate, improved mean arterial blood pressure, restored the responsiveness to PE, but did not change MPO activity, leukopenia or the impairment in the responsiveness of aortic rings to ACh. Addition of Ethyl-TU (2 μM) to endothelium denuded aortic rings *in vitro*, restored the marked hyporeactivity to PE. Administration of the NO donor C87-3754 (0.75 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) slightly increased survival time and reduced MPO activity and leukopenia, but did not change survival rate and mean arterial blood pressure. In addition C87-3754 restored the responsiveness of aortic rings to ACh to control values, but did not modify the hyporeactivity to PE. The combination of these two interventions produced a higher degree of protection than either Ethyl-TU or C87-3754 alone. In fact, co-administration of Ethyl-TU plus C87-3754 completely prevented mortality, reduced MPO activity, attenuated leukopenia and the profound hypotension and restored the impaired responsiveness of aortic rings to PE and ACh.

5 Our study suggests that treatment with a nitric oxide synthase inhibitor combined with an NO donor may be a new therapeutic approach to the treatment of splanchnic artery occlusion shock.

Keywords: SAO shock; nitric oxide synthase inhibition; nitric oxide donor

Introduction

Nitric oxide (NO) serves as an important intercellular mediator in the vasculature, kidney, endocrine system, and central nervous system (Palmer *et al.*, 1987; Moncada *et al.*, 1991). NO biosynthesis is the result of oxidation of a terminal nitrogen on the amino acid arginine by a class of enzymes generally referred to as the nitric oxide synthases (NOSs) (Palmer *et al.*, 1988; Marletta, 1993). NO produced by NOS (bNOS) in the central and peripheral nervous system acts as a potential neurotransmitter and may cause neuroinjury (Dawson *et al.*, 1991).

NO generated by the constitutive endothelial NOS (eNOS) contributes to the regulation of local and systemic vascular resistance, distribution of blood flow and oxygen delivery, sodium balance and arterial blood pressure (Vane *et al.*, 1990; Vane, 1994). Impaired NO synthesis may lead to pathological vasoconstriction, to tissue ischaemia with organ dysfunction, and to the genesis or perpetuation of hypertension (Dinerman *et al.*, 1993).

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Increased NO production following the induction of a distinct isoform of NOS (inducible NOS) by tumour necrosis factor-α (TNF-α) endotoxin, interleukin-1β and γ-interferon in several cell types, including macrophages and smooth muscle cells, has been shown to play an important role in the pathogenesis of inflammation and circulatory shock (Nathan, 1992).

Splanchnic artery occlusion (SAO) shock is an experimental type of shock which is the consequence of a marked ischaemia of the splanchnic region (Squadrito *et al.*, 1992). Indeed SAO shock causes an irreversible circulatory failure that results in the death of animals within 75-90 min after the release of occlusion (Squadrito *et al.*, 1991).

It has been previously shown that the L-arginine-nitric oxide pathway plays an important role in the circulatory derangements of SAO shock. In fact the marked dysfunction of this type of experimental shock is characterized by both a (i) reduction in nitric oxide generated via the endothelial and constitutive nitric oxide synthase and (ii) an overproduction of nitric oxide derived by the inducible nitric oxide synthase (iNOS) (Squadrito *et al.*, 1994).

These data would justify the use of an inhibitor of inducible nitric oxide synthase in combination with a NO donor in SAO

shock. Therefore, the aim of this study was to investigate the effects of an intravenous infusion of a potent and non-selective NOS inhibitor, S-ethylisothiourea (Ethyl-TU) (Southan *et al.*, 1995), with that of a nitric oxide (NO) donor. In addition the effects of the combination of these two treatments were also investigated.

Methods

Surgical procedures

Male Sprague-Dawley rats weighing 250–300 g were allowed access to food and water *ad libitum*. The rats were anaesthetized with urethane (1.3 g kg^{-1}, i.p.). After anaesthesia catheters were placed in the carotid artery and jugular vein. Blood pressure was monitored continuously by a Statham pressure transducer. After midline laparotomy, the celiac and superior mesenteric arteries were isolated near their aortic origins. During this procedure, the intestinal tract was maintained at 37°C by placing it between gauze pads soaked with warmed 0.9% NaCl solution.

Rats were given heparin (1,000 u kg^{-1}, i.v.) and were observed for a 30 min stabilization period before either splanchnic ischaemia or sham ischaemia. SAO shock was induced by clamping both the superior mesenteric artery and the celiac trunk resulting in total occlusion of these arteries for 45 min. After this period of occlusion the clamps were removed. Following reperfusion the rats were observed for 240 min. Sham shocked rats were subjected to all the same surgical procedures as SAO shocked rats except that the arteries were not occluded.

Evaluation of survival

S-ethylisothiourea (Ethyl-TU; 0.1 mg kg^{-1} h^{-1}), C87-3754 (0.75 mg kg^{-1} h^{-1}), or a combination of these two treatments were given by an infusion pump (Harvard Apparatus CO., Dover, MA) 1 min following the onset of reperfusion. The dose of Ethyl-TU chosen to be used in this study was the same as that of the NOS-inhibitor aminoethyl-TU that has been shown to attenuate the circulatory failure and the liver injury and dysfunction caused in the rat by endotoxin (Thiemermann *et al.*, 1995). Moreover, Ethyl-TU and aminoethyl-TU have a similar potency as inhibitors of murine iNOS (Southan *et al.*, 1995) and 0.1 mg kg^{-1} h^{-1} of aminoethyl-TU causes a substantial inhibition of iNOS activity without causing a rise in blood pressure in anaesthetized rats (Thiemermann *et al.*, 1995). The dose of C87-3754, a drug that belongs to the class of organic NO donors termed sydnonimines, was chosen in agreement with previously obtained pharmacokinetic and pharmacodynamic data in an experimental model of ischaemia-reperfusion injury. (Carey *et al.*, 1992). Survival rate and survival time were evaluated for 4 h. Control rats were injected with a same amount of 0.9% NaCl saline solution.

Arterial blood pressure

Animals were anaesthetized with urethane (1.3 g kg^{-1}, i.p.) and a cannula (PE 50) was inserted into the left common carotid artery as described previously (Caputi *et al.*, 1980). The arterial catheter was connected to a pressure transducer. The pressure pulse triggered a cardiotachometer, and arterial blood pressure was displayed on a polygraph. Arterial blood pressure is presented as mean arterial pressure (MAP) in mmHg. Rats were subjected to the same experimental protocol as described above.

Myeloperoxidase activity

Leukocyte accumulation was estimated by measuring the activity of myeloperoxidase (MPO) in intestinal mucosa, as previously described (Squadrito *et al.*, 1993). The samples were obtained at 0 and 45 min before reperfusion (release of the arterial clamp) and at 80 min after the onset of reperfusion. The samples were first homogenized in a solution containing 20 nM potassium phosphate buffer (pH 7.4), 0.01 M EDTA, 50 u ml^{-1} of a protease inhibitor in proportions of 1:10 (w:v) and then centrifuged for 30 min at 20,000 *g* at 4°C. The supernatant of each sample was then discarded and the pellet was immediately frozen on dry ice; freezing was continued for 14 h before sonication. After being thawed, the resulting pellet was added to a buffer solution consisting of 0.5% hexacyltrimethylammonium bromide (Sigma Chemical Co., St Louis, MO, U.S.A.) dissolved in 50 nM potassium phosphate buffered solution (pH 6) containing 30 u ml^{-1} of aprotinin, a protease inhibitor. Each sample was then sonicated for 1 min at intensity 2 and at a temperature of 4°C. After sonication, the samples were allowed to chill on ice for approximately 30 min, and then centrifuged for 30 min at 40,000 *g* at 4°C. An aliquot of the supernatant was then allowed to react with 0.167 mg ml^{-1} o-dianisidine dihydrochloride (Sigma Chemical Co) and 0.001% H_2O_2, and the rate of change in absorbance was measured at 405 nm in a microtitre plate reader (SLT-Labinstruments Salzburg, Austria). MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide min^{-1} at 25°C and was expressed in milliunits per gram weight ($U \times 10^{-3}$ g^{-1}) of tissue.

Leukocyte counts

Tail vein blood samples for the leukocyte count (Squadrito *et al.*, 1992) were taken at different time intervals (0 and 45 min before occlusion, and 80 min after reperfusion). The number of leukocytes ($WBC \times 10^3/mm^3$) is shown as mean ± s.e.mean.

Isolated aortic rings

Animals were killed 80 min after the onset of reperfusion. Thoracic aortae were removed and placed in cold Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.7. Then aortae were cleaned of adherent connective and fat tissue and cut into rings of approximately 2 mm in length. In some rings, the vascular endothelium was removed mechanically by gently rubbing the luminal surface with a thin wooden stick. Rings were then placed under 1 g of tension in an organ bath containing 10 ml of Krebs solution at 37°C and bubbled with 95% O_2 and 5% CO_2 (pH 7.4). All experiments were carried out in the presence of indomethacin (10 μM) in order to exclude the involvement of prostaglandins and their metabolites. Developed tension was measured with an isometric force transducer and recorded on a polygraph (Ugo Basile, Varese, Italy). After an equilibration period of 60 min during which time the rings were washed with fresh Krebs solution at 15–20 min intervals and basal tension was readjusted to 1 g, the tissue was exposed to phenylephrine (PE, 100 nM). When the contraction was stable, the functional integrity of endothelium was assessed by evaluating the vasodilator response to acetylcholine (ACh, 100 nM). The tissues were then washed regularly for 30 min.

Endothelium-dependent relaxation was evaluated with cumulative concentrations of ACh (10 nM – 1 μM) in aortic rings precontracted with PE (100 nM). Relaxation of the rings was calculated as % decrease of contractile force. Concentration-response curves were obtained by adding cumulative concentrations of PE (1 nM – 10 μM) to intact or endothelium-denuded aortic rings.

In some experiments, Ethyl-TU (2 μM) was added to the organ bath 1 h before performing the experiments.

Drugs

S-ethylisothiourea was a kind gift from Dr Christoph Thiemermann, The William Harvey Research Institute, London, U.K. C87-3754, a new sydnonimine, was a kind gift from Dr

Marco Prosdocimi, Abano Terme Italy. Acetylcholine chloride, phenylephrine hydrochloride and indomethacin were obtained from Sigma Chemical Co., St Louis, MO.

Statistical analysis

The difference between the means of groups was evaluated with an ANOVA followed by Bonferroni's test and was considered significant when $P<0.05$. Statistical analysis of survival data was done with the Fisher's exact probability test.

Results

Survival

Table 1 summarizes survival rate, percentage survival and survival time for the groups of rats subjected to SAO or sham shock. All sham rats survived the entire 4 h observation period. In contrast, all of the SAO shocked rats treated with vehicle, died within 2 h of the onset of reperfusion (survival time 76 ± 10 min) indicating that this is a severe model of shock. Administration of C87-3754 (0.75 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) increased survival time to 126 ± 7 min but did not change survival rate. Ethyl-TU (0.1 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) significantly enhanced survival time (225 ± 7 min) and survival rate (70% survival rate at 4 h of reperfusion). The combination of these two treatments produced a higher degree of protection (survival time >240 min; survival rate was 100% at 4 h of reperfusion).

Arterial blood pressure

Occlusion of the splanchnic arteries for 45 min caused a marked increase in mean arterial blood pressure (Figure 1). With the onset of reperfusion, mean arterial blood pressure progressively decreased in vehicle-treated rats (Figure 1). Ethyl-TU did not change arterial blood pressure in sham rats (Figure 1). The NO donor C87-3754 slightly decreased mean arterial blood pressure in sham operated animals (85 ± 3 mmHg and 80 ± 2 mmHg, at 15 and 60 min after the beginning of infusion, respectively). The basal value of mean arterial blood pressure was 89 ± 2 mmHg. Administration of C87-3754 did not modify the marked hypotension. In contrast, Ethyl-TU alone or in combination with the NO donor C87-3754 produced a sustained and long-lasting increase in blood pressure (Figure 1).

Myeloperoxidase activity

The kinetics of ileal leukocyte infiltration in SAO shocked rats was determined by measurement of the myeloperoxidase

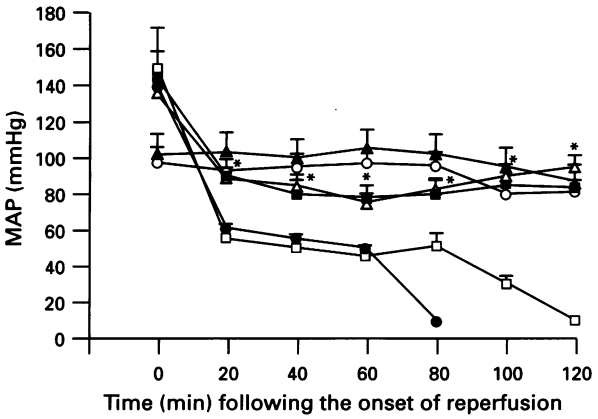

Figure 1 Effects of vehicle (1 ml kg^{-1} h^{-1}, i.v. of a 0.9% NaCl solution), C87-3754 (0.75 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion), S-ethylisothiourea (Ethyl-TU; 0.1 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) or a combination of these two treatments on mean arterial blood pressure of sham-operated rats and splanchnic artery occlusion (SAO) rats. Each point represents the mean ± s.e.mean (vertical lines) of seven experiments. (○) Sham + vehicle; (●) SAO + vehicle; (▲) Sham + Ethyl-TU; (□) SAO + C87-3754; (■) SAO + Ethyl-TU; (△) SAO + C87-3754 plus Ethyl-TU. *$P<0.01$ vs SAO + vehicle.

(MPO) activity in rats at different times: 0 and 45 min after occlusion and 80 min post reperfusion. MPO levels were significantly increased in the ileum (3.39 ± 0.8 U × 10^{-3} g^{-1} tissue; Table 2) at 80 min after reperfusion in shocked rats treated with the vehicle.

Administration of Ethyl-TU did not modify the increase in MPO activity in the ileum. In contrast, C87-3754 alone or in combination with Ethyl-TU significantly lowered the increase in ileal MPO activity (Table 2).

Leukocyte count

The administration of vehicle did not modify the white blood cell (WBC) count in sham-operated rats (Table 3). In contrast, SAO shock produced a marked leukopenia. Our data show that leukocyte count was markedly decreased at the end (80 min) of reperfusion (Table 3). The administration of Ethyl-TU did not change the degree of leukopenia while C87-3754 alone or in combination with Ethyl-TU significantly attenuated leukopenia (Table 3).

Vasodilator response to acetylcholine

Addition of PE (100 nM) to the organ bath contracted intact aortic rings (80–90% of the maximum response). These rings

Table 1 Effect of vehicle, C87-3754, S-ethylisothiourea or a combination of these two treatments on survival rate, percentage survival and survival time in sham shocked rats or splanchnic artery occlusion (SAO) shocked rats

	Time after reperfusion (h)				
	2		*4*		*Survival*
Treatment	*Surviving animals*	*(%)*	*Surviving animals*	*(%)*	*time (min)*
Sham + vehicle	10/10	100	10/10	100	>240
Sham + C87-3754	10/10	100	10/10	100	>240
Sham + Ethyl-TU	10/10	100	10/10	100	>240
Sham + C87-3754/Ethyl-TU	10/10	100	10/10	100	>240
SAO + vehicle	0/10	0	0/10	0	76 ± 10
SAO + C87-3754	3/10	30	0/10	0	126 ± 7*
SAO + Ethyl-TU	10/10	100	7/10	70*	225 ± 7**
SAO + C87-3754/Ethyl-TU	10/10	100	10/10	100	>240***

Animals received vehicle (1 ml kg^{-1} h^{-1}, i.v. of a 0.9% NaCl solution), C87-3754 (0.75 mg kg^{-1} h^{-1}), S-ethylisothiourea (Ethyl-TU, 0.1 mg kg^{-1} h^{-1}) or a combination of these two latter treatments 1 min after the onset of reperfusion. *$P<0.05$ vs SAO + vehicle; **$P<0.01$ vs SAO + vehicle; ***$P<0.001$ vs SAO + vehicle.

were relaxed in a concentration-dependent manner by ACh (10 nM – 10 μM). The relaxant effect of ACh was significantly smaller in aortic rings obtained from SAO shocked rats than from Sham-operated rats (Figure 2). Administration of C87-3754 alone or in combination with Ethyl-TU significantly improved the responsiveness of aortic rings obtained from SAO shocked rats to ACh (Figure 2). In contrast, Ethyl-TU alone did not affect the impairment of the dilator responses to ACh in rings from SAO shocked rats.

Contractile response to phenylephrine

In intact aortic rings prepared from SAO shocked rats the contractile response to PE (1 nM – 10 μM) was significantly reduced. The maximum force of contraction (g tension mg^{-1} tissue) induced by 10 μM PE in aortic rings from sham rats was 1.7 ± 0.5, whereas it was 1.1 ± 0.4 in rings from SAO shocked rats (Figure 3).

Removal of the endothelium had no significant effect on the constrictor responses elicited by PE in rat aortic rings obtained from sham operated rats (Figure 4). In contrast, when PE was tested in denuded aortae from shocked rats, it caused identical effects in aortic rings with (Figure 3) or without endothelium (Figure 4).

Administration of Ethyl-TU alone or in combination with C87-3754 significantly improved the impaired contractile response to PE in SAO shocked rats, while C87-3754 alone failed to affect the impaired contraction to PE (Figure 3).

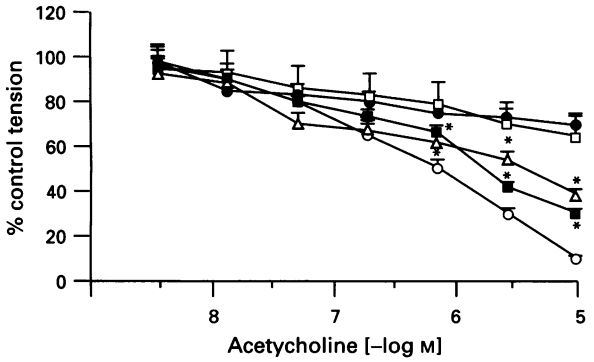

Figure 2 Relaxant effect of acetylcholine in aortic rings (contracted with phenylephrine, 100 nM) of sham-operated rats and splanchnic artery occlusion (SAO) shocked rats treated with vehicle (1 ml kg^{-1} h^{-1}, i.v. of a 0.9% NaCl solution), C87-3754 (0.75 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion), S-ethylisothiourea (Ethyl-TU; 0.1 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) or a combination of these two treatments. (○) Sham + vehicle; (●) SAO + vehicle; (□) SAO + C87-3754; (■) SAO + Ethyl-TU; (△) SAO + C87-3754 plus Ethyl-TU. Each point represents the mean ± s.e.mean (vertical lines) from six experiments. *$P < 0.05$ vs SAO + vehicle.

In endothelium-denuded aortic rings from SAO shocked rats, Ethyl-TU (2 μM), restored PE sensitivity to control values (Figure 4).

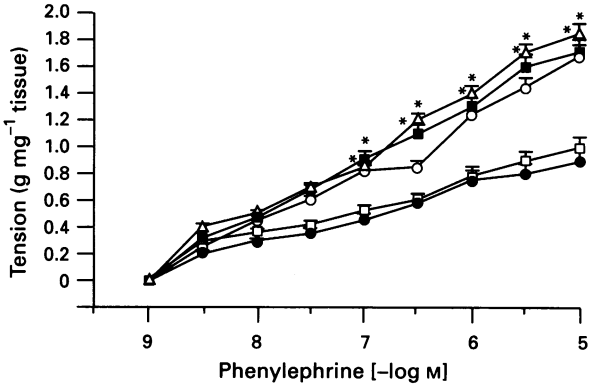

Figure 3 Contractile response to cumulative doses of phenylephrine in aortic rings from sham-operated rats and splanchnic artery occlusion (SAO) shocked rats treated with vehicle (1 ml kg^{-1} h^{-1}, i.v. of a 0.9% NaCl solution), C87-3754 (0.75 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion), S-ethylisothiourea (Ethyl-TU; 0.1 mg kg^{-1} h^{-1}, beginning 1 min after the onset of reperfusion) or a combination of these two treatments. (○) Sham + vehicle; (●) SAO + vehicle; (□) SAO + C87-3754; (■) SAO + Ethyl-TU; (△) SAO + C87-3754 plus Ethyl-TU. Each point represents the mean ± s.e mean (vertical lines) of six experiments. *$P < 0.02$ vs SAO + vehicle.

Table 3 Effect of vehicle, C87-3754, S-ethylisothiourea or a combination of these two treatments on leukocyte count (WBC) in sham shocked rats or splanchnic artery occlusion (SAO) shocked rats

	WBC ($\times 10^3/mm^3$)		
Treatment	*Basal*	*Occlusion*	*Reperfusion*
Sham + vehicle	12 ± 1.1	13 ± 1.3	11 ± 1.4
Sham + C87-3754	10 ± 1.6	12 ± 0.9	14 ± 1.5
Sham + Ethyl-TU	11 ± 1.2	11 ± 1.3	13 ± 0.9
Sham + C87-3754/Ethyl-TU	13 ± 1.3	14 ± 1.2	10 ± 0.8
SAO + vehicle	12 ± 1.4	12 ± 1.5	5 ± 1.2*
SAO + C87-3754	11 ± 1.2	13 ± 1.4	9 ± 1.1**
SAO + Ethyl-TU	13 ± 1.4	11 ± 1.3	6 ± 0.9*
SAO + C87-3754/Ethyl-TU	11 ± 1.1	13 ± 1.5	10 ± 1.7**

Animals received vehicle (1 ml kg^{-1} h^{-1}, i.v of a 0.9% NaCl solution), C87-3754 (0.75 mg kg^{-1} h^{-1}), S-ethylisothiourea (Ethyl-TU; 0.1 mg kg^{-1} h^{-1}) or a combination of these two latter treatments 1 min after the onset of reperfusion. *$P < 0.05$ vs Sham + vehicle; **$P < 0.01$ vs SAO + vehicle.

Table 2 Effect of vehicle, C87-3754, S-ethylisothiourea or a combination of these two treatments on myeloperoxidase (MPO) activity in sham shocked rats or splanchnic artery occlusion (SAO) shocked rats

	MPO activity ($u \times 10^{-3}$ g^{-1} tissue)		
Treatment	*Basal*	*Occlusion*	*Reperfusion*
Sham + vehicle	0.015 ± 0.04	0.016 ± 0.02	0.017 ± 0.04
Sham + C87-3754	0.019 ± 0.03	0.018 ± 0.04	0.020 ± 0.05
Sham + Ethyl-TU	0.021 ± 0.05	0.023 ± 0.06	0.017 ± 0.03
Sham + C87-3754/Ethyl-TU	0.016 ± 0.03	0.024 ± 0.04	0.019 ± 0.03
SAO + vehicle	0.023 ± 0.03	0.22 ± 0.04	3.39 ± 0.8*
SAO + C87-3754	0.023 ± 0.04	0.023 ± 0.05	1.19 ± 0.8**
SAO + Ethyl-TU	0.021 ± 0.04	0.018 ± 0.03	3.43 ± 0.7
SAO + C87-3754/Ethyl-TU	0.025 ± 0.08	0.021 ± 0.08	1.43 ± 1.3**

Animals received vehicle (1 ml kg^{-1} h^{-1}, i.v. of a 0.9% NaCl solution), C87-3754 (0.75 mg kg^{-1} h^{-1}), S-ethylisothiourea (Ethyl-TU; 0.1 mg kg^{-1} h^{-1}) or a combination of these two latter treatments 1 min after the onset of reperfusion. *$P < 0.05$ vs Sham + vehicle; **$P < 0.005$ vs SAO + vehicle.

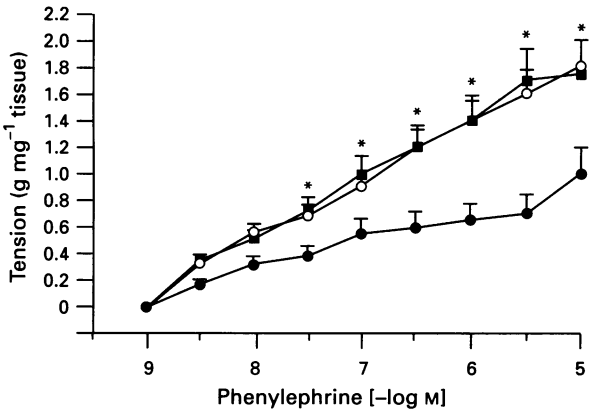

Figure 4 Effects of S-ethylisothiourea (Ethyl-TU; 2 μM) on contractile response to cumulative concentrations of phenylephrine in aortic rings without endothelium from sham operated rats or splanchnic artery occlusion (SAO) shocked rats. Ethyl-TU was added to the bath 1 h before phenylephrine. (○) Sham + vehicle; (●) SAO + vehicle; (■) SAO + Ethyl-Tu. Each point represents the mean ± s.e.mean (vertical lines) of six experiments. *$P<0.01$ vs SAO + vehicle.

Discussion

Splanchnic artery occlusion shock causes a vascular dysfunction characterized by a reduced endothelial production of NO and an increased smooth muscle production of NO derived from the iNOS isoform (Squadrito *et al.*, 1994). This latter result confirms that smooth muscle cells may generate NO via the inducible isoform of NO synthases (Busse & Mulsch, 1990). Previous findings from our laboratory have shown that endothelium-leukocyte interaction plays an important role in the pathogenesis of splanchnic artery occlusion shock. Indeed the adhesion of leukocytes to the vascular endothelium of capillaries and venules causes their extravasation in several vital organs including the ileum and the lung (Canale *et al.*, 1994).

The reduced production of NO by the endothelium may have a role in favouring the accumulation of neutrophils by the endothelium that may in turn, by releasing the deleterious oxygen species which inactivate NO, amplify and increase the endothelial dysfunction. In agreement with this hypothesis it has been suggested that NO donors are able to improve the endothelial dysfunction and to inhibit the adherence of neutrophils to the vascular endothelium (Lefer & Lefer, 1994). This suggests that in low-flow states, including circulatory shock, the use of NO donors may be of benefit. In SAO shock NO donors cause a transient increase in survival time but do not increase long-term survival rate, even though these agents reduce the endothelial dysfunction and neutrophil accumulation (Aoki *et al.*, 1990; Carey *et al.*, 1992).

In the present study we used the NO donor C87-3754. This compound belongs to a class of organic NO donors termed 'sydnonimines'. SIN-1 is the prototype compound of this class: however, SIN-1 has also been shown to release superoxide radicals so that it may not be an ideal NO donor. This problem has been rectified in the synthesis of some recent NO donors, including pirsidomine and, more specifically C87-3754 (Lefer & Lefer, 1994). The newer sydnonimines spontaneously release NO without the necessity of cellular metabolism to release the NO moiety. Moreover, they are much more effective at releasing NO that many other NO donors, so they cause a longer lasting release of NO and their EC_{50} values are low compared to those of nitroglycerin and sodium nitroprusside, respectively (Lefer & Lefer, 1994).

Rats treated with the NO donor C87-3754 exhibited only a slight increase in survival time, but they did not differ from the untreated SAO shocked rats as regards long term survival rate, mean arterial blood pressure and the marked vascular hyporeactivity to PE. However, treatment with the NO donor attenuated neutrophil accumulation in the ileum, leukopenia and the impairment of the dilator response to acetylcholine. Thus, in SAO shock impaired NO release from the endothelium may contribute to the accumulation and sequestration of neutrophils in the ileum. However, the correction of this dysfunction by administration of an NO donor does not result in a marked increase in the resistance of rats to SAO shock. This clearly indicates that other pathogenetic aspects are likely to have a key role in SAO shock.

SAO shock is also characterized by an impaired response to phenylephrine, a marked hypotension and a failure of the vasculature to respond to vasoconstrictor stimuli (Squadrito *et al.*, 1994).

This impaired vascular reactivity, as suggested for experimental haemorrhagic (Thiemermann *et al.*, 1993) and endotoxic shock (Julou-Schaeffer *et al.*, 1991), is a consequence of an overproduction of NO by the iNOS (Squadrito *et al.*, 1994). In agreement with these findings Ethyl-TU was able to revert both *in vivo* and *ex vivo* in endothelium denuded aortae the impaired vascular reactivity observed in SAO shock. Furthermore, the administration of Ethyl-TU significantly increased mean arterial blood pressure and markedly improved survival time and survival rate, thus indicating that this potent inhibitor of iNOS is effective in protecting against this type of experimental shock.

These data are in agreement with previous findings obtained in rats subjected to endotoxin shock (Szabo *et al.*, 1994). However Ethyl-TU is also a potent inhibitor of eNOS (Southan *et al.*, 1995), and, because of the endothelial dysfunction, a reduction in endothelial NO production would be detrimental in this type of circulatory shock.

The increase in blood pressure afforded by NOS inhibitors is due to inhibition of eNOS. Indeed the pressor effects of such compounds are relevant measures of their potencies as inhibitors of eNOS *in vivo* (Gross *et al.*, 1990; Misko *et al.*, 1993; Hasan *et al.*, 1993). The dose of ethyl-TU used in our experiments did not modify mean arterial blood pressure in sham rats, thus suggesting that, at least with an intravenous infusion of 0.1 mg kg^{-1} h^{-1} and under our experimental conditions (anaesthetized rats), Ethyl-TU does not affect eNOS activity.

It has been suggested that NOS inhibition combined with NO inhalation exerts protective effects in a porcine model of endotoxin shock (Klemm *et al.*, 1995).

In agreement with these previous data our results show that the administration of Ethyl-TU in combination with an NO donor causes a higher degree of protection compared to both single treatments. The combination treatment was in fact able to revert the two components of the vascular dysfunction that occurs in SAO shock: the endothelial dysfunction and the hyporeactivity to phenylephrine. Indeed, the vascular hyporeactivity to phenylephrine, due to an overproduction of smooth muscle NO derived by the inducible synthase, seems to be the most important component of the composite vascular dysfunction of SAO shock and the most prominent for the overall survival in this type of experimental shock: in fact, Ethyl-TU alone was able to protect significantly against the pathological sequelae of SAO shock.

In conclusion, we propose that treatment with a NOS inhibitor combined with an NO donor might represent a new therapeutic strategy in the treatment of circulatory shock.

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