EFFECT OF PROLONGED EXPOSURE TO TUMOUR NECROSIS FACTOR-ALPHA AND NICOTINE ON RAT MESENTERIC AND PULMONARY ARTERIES

[1]Preetpal S. Chadha, [2]James D. Moffatt & [1]Rebecca Lever, [1]Department of Pharmacology, The School of Pharmacy, University of London, WC1N 1AX. [2] The Sackler Institute of Pulmonary Pharmacology, King's College London SE1 1UL.

The pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) is associated with various vascular disorders including atherosclerosis and peripheral arterial disease. It has previously been reported that acute exposure to TNF-α inhibits endothelium-dependent relaxation in rat mesenteric arteries (Wimalasundera *et al*., 2003). Nicotine, as a constituent of cigarette smoke, has been identified as a major contributor to vascular disease and has been shown to impair endothelial-dependent relaxation in porcine carotid and coronary arteries (Conklin *et al*., 2001). In the present study, we have examined the effects of nicotine exposure on endothelial-dependent relaxation in pulmonary and mesenteric arteries, in the presence of TNF-α, a mediator produced by a variety of cells, including endothelial cells, under conditions of inflammation.

Male Wistar rats (240-260g) were sacrificed by CO_2 asphyxiation. Mesenteric and pulmonary arteries were dissected and treated with 6-hydroxydopamine (2 mM) and capsaicin (0.1 mM) for 30 minutes in order to remove neuronal influences. Following this, some vessels were incubated in DMEM containing nicotine (10^{-7} M) and TNF-α (10 ng ml^{-1}), alone and in combination, or vehicle, for a period of 24 hours in respect of pulmonary arteries and 48 hours for mesenteric arteries. 2 mm segments of artery were mounted on a wire myograph under normalised tension in oxygenated (95% O_2/5% CO_2) Krebs' buffer maintained at 37°C. Maximum contraction to KCl (120 mM) was initially determined and sub maximal tone subsequently induced using phenylephrine (0.1-10 μM) or U46619 (0.01-1 μM) in the presence of nifedipine (0.3 μM). Endothelial-dependent responses to acetylcholine (ACh) were used as a measure of endothelial function. Maximal responses to ACh (E_{max}) are expressed as percent relaxation of active tone (mean ± SEM) and differences in pEC_{50} and E_{max} determined by ANOVA followed by Bonferroni's post test.

Table 1 Pulmonary arteries (48 hour incubation)

Treatment	Diameter (μm)	pEC50	Emax	n
Vehicle	493.6 ± 30.1	6.919 ± 0.17	82.743 ± 5.92	7
Nicotine	506.3 ± 48.6	6.954 ± 0.39	69.929 ± 11.00	7
TNF-α	366.8 ± 18.2	6.713 ± 0.54	33.460 ± 2.65**	6
TNF-α + Nicotine	334.8 ± 62.6	6.718 ± 12.94	18.600 ± 3.76*** ##	4

Table 2 Mesenteric arteries (24 hour incubation)

Treatment	Diameter (μm)	pEC50	Emax	n
Vehicle	281.8 ± 16.7	7.039 ± 0.18	76.817 ± 6.32	6
Nicotine	310.9 ± 14.7	7.152 ± 0.08	87.243 ± 3.10	7
TNF-α	261.3 ± 12.8	6.773 ± 0.33	55.525 ± 8.36	4
TNF-α + Nicotine	273.3 ± 9.0	6.393 ± 0.36	40.200 ** ###	4

** P<0.01, *** P<0.001, denotes difference between vehicle control and treated vessels.
P<0.01, ### P<0.001, denotes difference between nicotine control and treated vessels.

Pulmonary arteries exposed to TNF-α for a period of 24 hours showed significantly reduced maximal endothelial-dependent relaxation in response to ACh (Table 1). Interestingly, mesenteric arteries treated with TNF-α alone for 48 hours did not differ significantly from vehicle treated vessels, however, TNF-α exposure in the presence of nicotine did significantly reduce subsequent endothelial-dependent relaxation (Table 2).

Conklin *et a*l. (2001). *J. Surg. Research*. **95** 23-31.
Wimalasundera *et al*. (2003). *Br. J. Pharmacol*. **138** 1285-1294.