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Modulation of Nitric Oxide-dependent Vascular and Platelet Function In-vitro by the Novel Phosphodiesterase Type-V Inhibitor, ONO-1505

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Abstract

We have characterized the in-vitro modulation of both nitric oxide (NO)-dependent vasodilator activity and anti-platelet function by the novel type-V phosphodiesterase inhibitor, ONO-1505 (4-[2-(2-hydroxyethoxy)ethylamino]-2-(1*H*-imidazol-1-yl)-6-methoxyquinazoline methanesulphonate).

ONO-1505 elicited vasorelaxation in the rat isolated aorta. If the concentration of ONO-1505 was $\leq 10\,\mu$M the vasorelaxation was abolished by N^G-nitro-L-arginine methyl ester (L-NAME), by methylene blue, and by endothelial denudation. Furthermore, pretreatment of the rat isolated aorta for 10 min with ONO-1505 in the presence of L-NAME potentiated vasorelaxation to the NO-donor, sodium nitroprusside. Similarly, ONO-1505, although having no effect on adenosine diphosphate (ADP)-induced rat platelet aggregation in-vitro, augmented established anti-aggregatory effects of sodium nitroprusside.

The data therefore show that the novel phosphodiesterase V inhibitor ONO-1505 augments endogenous and exogenous nitrovasodilator activity in-vitro; they also imply modulation of the NO pathway in the haemodynamic actions of this compound, previously reported in-vivo.

Phosphodiesterase activity is pivotal in the regulation of intracellular concentrations of cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP) and therefore has an important modulatory effect in signalling by these cyclic nucleotide second messengers. Phosphodiesterase V, which is cyclic GMP-specific, has been found in vascular smooth muscle and platelets and might also be present in airway smooth muscle and mast cells (Lugnier & Komas 1993; Beavo 1995). Hence, inhibitors of phosphodiesterase V relax vascular smooth muscle and oppose platelet aggregation by enhancing intracellular levels of cyclic GMP (Rapoport & Murad 1983; Murad et al 1985; Komas et al 1991). Because the major stimulant of soluble guanylyl cyclase activity is the endogenous nitrovasodilator, endothelium-derived relaxing factor (Furchgott & Zawadzki 1980) now identified as NO (Palmer et al 1987), the activity of phosphodiesterase V can be expected, ultimately, to modulate reactivity to this signalling gas in vascular smooth muscle, platelets and perhaps other sites.

We have previously described the novel combined phosphodiesterase V inhibitor and thromboxane synthase inhibitor ONO-1505 (Laight et al 1996), which has subsequently been found to reduce vascular smooth muscle cell proliferation in-vitro (Carrier et al 1997) and elicit vasodepression in-vivo (Laight et al 1997). In addition, ONO-1505 improves vascular endothelial function and blood flow in-vivo in animal models of endothelial dysfunction such as diabetes, hypercholesterolaemia and ischaemia (Kitakaze et al 1996; Konneh et al 1996; Omawari et al 1996). The aim of this study was to investigate the effects of ONO-1505 on NO-mediated vasodilation and platelet function in-vitro, to obtain further support for the rationale for its haemodynamic actions in-vivo.

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Materials and Methods

Drugs

All drugs were obtained from Sigma (Poole, Dorset, UK) except ONO-1505 (4-[2-(2-hydroxyethoxy) ethylamino]-2- (1*H*-imidazol-1-yl)-6-methoxy-quinazoline methanesulphonate) which was a generous gift from ONO Pharmaceutical (Osaka, Japan).

Vasorelaxation in rat isolated aorta

Male Wistar rats, 250–300 g, were anaesthetized by intraperitoneal (i.p.) administration of pentobarbitone sodium (60 mg kg^{-1}) and the thoracic aorta were carefully excised after a thorectomy. Aortic rings approximately 2 mm in length were mounted under a resting tension of 2 g in organ baths in physiological salt solution (PSS) gassed with carbogen and warmed to 37°C. The PSS had the composition (mM): NaCl, 133; KCl, 4·7; NaH_2PO_4, 1·35; $NaHCO_3$, 16·3; $MgSO_4$, 0·61; D-glucose, 7·8; $CaCl_2$, 2·52. Some rings had been denuded of endothelium by intimal rubbing.

After stabilization for 1 h, during which time the PSS was changed every 15 min, rings were precontracted with a concentration of noradrenaline (100 nM) which elicited approximately 90% of the maximum response and endothelial function was assessed by examining maximum vasorelaxation to acetylcholine (1 μM). After washout for 30 min, some rings were treated with enzyme inhibitors 10 min before a second precontraction with noradrenaline (100 nM), and vasorelaxation to ONO-1505 (0·3–300 μM) was then assessed. In studies examining the effect of ONO-1505 on vasorelaxation to sodium nitroprusside (1–300 nM), rings were treated with L-NAME (0·3 mM) and ONO-1505 (0·1–10 μM) for 20 and 10 min, respectively, before precontraction with noradrenaline (100 nM).

Platelet aggregation

Male Wistar rats, 300–350 g, were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and the carotid artery was cannulated. Arterial blood was collected under arterial pressure into sodium citrate (3·4%) at a final blood/citrate ratio of 9 : 1 (v/v). Citrated blood was centrifuged at low speed for 2 min to provide platelet-rich plasma (PRP) which was then incubated and stirred at 37°C for 30 s before addition of either ONO-1505 (10 μM final concentration) or its vehicle (distilled water; 4 μL). Sodium nitroprusside (1–100 μM final) or its vehicle (distilled water; 4 μL) was added 1 min later and subsequently a concentration of adenosine diphosphate (ADP) which elicited approximately 60% of maximum aggregation (1 μM final concentration) was added at the end of 2 min incubation. The aggregation response was followed turbidometrically for 3 min in a Born aggregometer. Determinations of the effect of ONO-1505 on sodium nitroprusside-evoked responses were made in matched samples of PRP.

Assessment of data

The significance of differences between two means was assessed by use of Student's *t*-test, which was paired for matched data. Multiple comparison of means was conducted by use either of repeated measures analysis of variance or one-way analysis of variance then Dunnett's test. Platelet aggregation was assessed by the maximum fall in light transmission and expressed as a percentage of matched control responses. The potency of vasodilators was assessed as the pD_2 value (the negative $\log_{10}$ of the concentration of agonist eliciting 50% of the maximum response (E_{max})). Data are expressed as means ± s.e.m. $P < 0.05$ was considered to be indicative of significance.

Results

Vasorelaxation to ONO-1505 in rat isolated aorta

Vasorelaxation to acetylcholine (1 μM) was 61·3 ± 2·9% (n = 11) during the initial contraction to noradrenaline (100 nM) and was abolished by endothelial denudation. Noradrenaline (100 nM) subsequently elicited a contraction of 1·4 ± 0·1 g in the control group and in the presence of indomethacin (10 μM) or the selective thromboxane synthase inhibitor furegrelate (10 M) (Johnson et al 1986) (all n = 11). However, noradrenaline (100 nM)-mediated contraction was greater in the presence of L-NAME (0·3 mM; 1·7 ± 0·1 g, n = 11), methylene blue (10 μM; 1·7 ± 0·2 g, n = 4) and endothelial denudation (1·7 ± 0·1 g, n = 4), with statistical significance being attained in the L-NAME (0·3 mM) group ($P < 0.01$, n = 11). ONO-1505 (0·1–300 μM) elicited vasorelaxation in noradrenaline (100 nM)-precontracted rat aorta which was unaffected by indomethacin (10 μM, n = 11) and furegrelate (10 μM) (Figure 1 and Table 1). In contrast, the initial phase of vasorelaxation to ONO-1505 (≤10 μM; $pD_2 = 5.76 \pm 0.10$; $E_{max} = 47.1 \pm 6.9\%$; n = 11) was abolished by methylene blue (10 μM, n = 4), L-NAME (0·3 mM, n = 11) and endothelial denudation (n = 4), although 100% vasorelaxation ($pD_2 < 4$) could still be achieved by ONO-1505

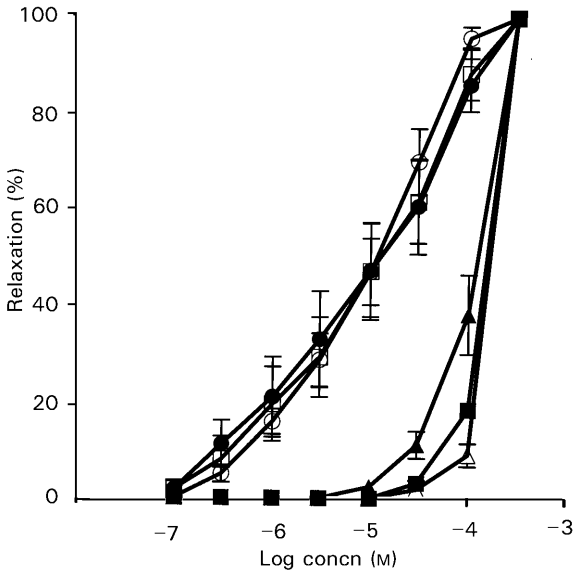

Figure 1. Vasorelaxation to ONO-1505 in precontracted rat isolated aorta: effects of control (○), indomethacin (●, 10 μM), furegrelate (□, 10 μM), L-NAME (■, 0·3 mM), methylene blue (△, 10 μM) and endothelial denudation (▲). Values are means ± s.e.m. (n = 4–11).

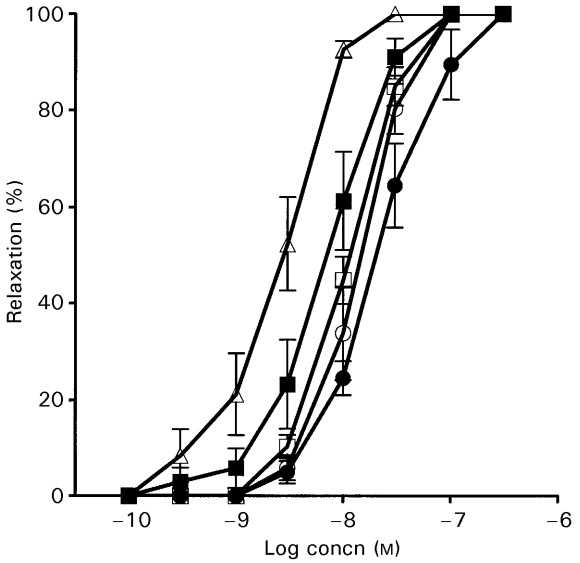

Figure 2. Effects of ONO-1505 (○ control, ● 0·3 μM, □ 1 μM, ■ 3 μM, △ 10 μM 0·3–10 μM) on vasorelaxation to sodium nitroprusside in precontracted rat isolated aorta in the presence of L-NAME (0·3 mM). Values are means ± s.e.m.

Table 1. Potency (pD_2) of vasorelaxation to ONO-1505 (0·1–300 μM) in the precontracted rat isolated aorta: effects of enzyme inhibitors and endothelial denudation.

Intervention	pD_2[a]	n
Control	5·01 ± 0·05	11
Indomethacin (10 μM)	4·97 ± 0·10	11
Furegrelate (10 μM)	4·96 ± 0·08	11
L-NAME (0·3 mM)	3·82 ± 0·19*	11
Methylene blue (10 μM)	3·76 ± 0·22*	4
Endothelial denudation	3·97 ± 0·14*	4

[a]The negative logarithm$_{10}$ of the concentration of agonist eliciting 50% of the maximum response. Values are means ± s.e.m. * $P < 0·01$ compared with control.

Table 2. Effect of ONO-1505 on the potency (pD_2) of vasorelaxation to sodium nitroprusside in the precontracted rat isolated aorta in the presence of L-NAME (0·3 mM).

Concn ONO-1505 (μM)	Sodium nitroprusside pD_2	n
Control	7·84 ± 0·09	4
0·3	7·60 ± 0·16	4
1	7·95 ± 0·06	4
3	8·16 ± 0·13*	4
10	8·63 ± 0·13*	4

Values are means ± s.e.m. * $P < 0·01$ compared with control.

(300 μM) (Figure 1 and Table 1). In subsequent experiments ONO-1505 was therefore used at concentrations $\leq$10 μM.

Effect of ONO-1505 on vasorelaxation to sodium nitroprusside in rat isolated aorta

Sodium nitroprusside elicited vasorelaxation in the isolated rat aorta with a pD_2 of 7·84 ± 0·09 (n = 4) in the presence of L-NAME (0·3 mM) (Table 2). ONO-1505 (0·3 and 1 μM) had no statistically significant effect on the pD_2 for sodium nitroprusside, although 0·3 μM ONO-1505 tended to depress vasorelaxation to sodium nitroprusside (Table 2 and Figure 2). In contrast, ONO-1505 (3 and 10 μM) evoked clear leftward displacement of the concentration–response curve for sodium nitroprusside with a maximum observed shift in pD_2 of 0·79 ± 0·07 ($P < 0·01$, n = 4) (Table 2 and Figure 2).

Effects of ONO-1505 on rat platelet aggregation

ADP elicited aggregation of rat platelets in-vitro with a pD_2 of 6·11 ± 0·04 (n = 10). ADP (1 μM) elicited readily reversible aggregation and approximately 60% of the maximum aggregatory response. Sodium nitroprusside (10 and 100 μM) depressed aggregation to ADP (1 μM) in a graded manner which was augmented by ONO-1505 (10 μM) such that aggregation in the presence of 100 μM sodium nitroprusside was effectively abolished (Table 3). Control aggregation to ADP (1 μM)

Table 3. Effect of ONO-1505 (10 μM) on inhibition by sodium nitroprusside of adenosine diphosphate (ADP) (1 μM)-induced aggregation of rat platelets in-vitro.

Concn sodium nitroprusside (μM)	Aggregation (% control)		n
	Vehicle	ONO-1505	
1	109·5 ± 8·4	104·0 ± 4·0	6
10	73·3 ± 3·7†	44·1 ± 3·9*†	6
100	25·8 ± 4·6†	1·1 ± 1·1*†	6

ADP (1 μM) elicited approximately 60% of the maximum aggregatory response. ADP (1 μM)-induced aggregation was 98·3 ± 3·9% of the control response in the presence of ONO-1505 (10 μM) alone ($P > 0{\cdot}05$, n = 6). Values are means ± s.e.m. *$P < 0{\cdot}01$ compared with the absence of ONO-1505 (vehicle control); †$P < 0{\cdot}01$ compared with corresponding aggregation in the presence of 1 μM sodium nitroprusside.

in the presence of ONO-1505 (10 μM) alone was 98·3 ± 3·9% of the response in the presence of ONO-1505 vehicle ($P > 0{\cdot}05$, n = 6).

Discussion

ONO-1505 elicited vasorelaxation of precontracted rat vascular smooth muscle which was sensitive, to comparable extents, to several chemical and physical lesions in the NO–cyclic GMP pathway. Indeed, soluble guanylyl cyclase activity, NO synthase activity or an intact endothelium was obligatory in the early vasodilator response to ONO-1505 ($\leq$10 μM). Although reasons why endothelial denudation was modestly less inhibitory than either L-NAME or methylene blue are not readily apparent, it is conceivable that the extent of endothelial denudation achieved, although adequate to abrogate the response to acetylcholine, might have been insufficient to prevent a slight residual basal NO vasodilator function.

The early, L-NAME-inhibited, vasorelaxation component, for which ONO-1505 had a potency of approximately 1·7 μM, probably reflects the augmentation of endogenous NO vasodilator function because of the inhibition of phosphodiesterase V in vascular smooth muscle (Komas et al 1991; Trapani et al 1991; Dundore et al 1991, 1993), whereas the later vasodilator response ($>$10 μM) is increasingly mediated by a component independent of the NO–cyclic GMP pathway. Because indomethacin and furegrelate were without effect, it is highly unlikely that this NO-independent component of vaso-relaxation involved action on prostanoid synthesis and is more likely to result from a relatively weak, direct relaxing effect of ONO-1505 on vascular smooth muscle.

The ONO-1505-mediated potentiation of vasorelaxation to sodium nitroprusside in the rat isolated aorta, in which prior NOS inhibition was achieved to prevent a vasorelaxant effect of ONO-1505 per se, is also consistent with enhanced intracellular accumulation of cyclic GMP in vascular smooth muscle, in this instance in response to exogenous NO (Harris et al 1989; Merkel et al 1992). This observation is in good agreement with our previous finding that ONO-1505 elicits vasodepression in the rat in-vivo during sodium nitroprusside infusion (Laight et al 1997). Whereas in this study reasons for the paradoxical depression in response to sodium nitroprusside observed at lower concentrations of ONO-1505 (0·1 μM) are unclear, it is interesting to speculate that an opposing stimulation of phosphodiesterase V activity, mediated by elevations in intracellular levels of cyclic GMP acting via protein kinase activity (Burns et al 1992; Beavo 1995; Corbin et al 1996) might lead, at least initially, to the reduced efficacy of an NO donor. This effect would be surmountable by more complete phosphodiesterase V inhibition, achieved at higher concentrations of ONO-1505, enabling the potentiation of sodium nitroprusside-evoked vasorelaxation (Harris et al 1989).

ONO-1505 was also shown to augment established anti-aggregatory effects of sodium nitroprusside in rat platelets, converting a submaximum effect of the NO donor to effective abolition of ADP-induced aggregation. It is noteworthy that ONO-1505 was unable to confer anti-aggregatory activity on a subthreshold concentration of NO donor, suggesting that a functionally relevant elevation of platelet cyclic GMP was obligatory (Sly et al 1997). Because ONO-1505 did not affect ADP-induced aggregation per se, but only modulated the anti-aggregatory activity of exogenous NO, it is unlikely that inhibition of de-novo thromboxane synthesis in platelets played any functional role in these effects.

In summary, the novel phosphodiesterase V inhibitor ONO-1505 was found to augment basal endothelial function in isolated aortic smooth muscle and promote exogenous NO-dependent vasodilation. In addition, the anti-aggregatory action of exogenous NO on platelets was potentiated. Our data therefore provide good support for the involvement of the NO pathway in the haemodynamic actions of ONO-1505 previously described in-vivo (Kitakaze et al 1996; Konneh et al 1996; Omawari et al 1996; Laight et al 1997). Furthermore, high concentrations ($>$10 μM) of ONO-1505 can be shown to recruit a vasodilator mechanism independent of the NO–cyclic GMP

pathway, at least in rat aortic smooth muscle in-vitro, which is not related to inhibition of thromboxane synthesis.

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