NITRIC OXIDE DONORS: ANTI-AGGREGATING EFFECTS AND MECHANISMS OF INHIBITION OF PLATELET AGGREGATION

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Nitric oxide (NO) donors are pharmacologically active substances that release NO *in vivo* or *in vitro*. NO is an important modulator of platelet function, inhibiting platelet adhesion and aggregation. Until recently, NO-mediated inhibition of platelet aggregation was believed to be exclusively mediated by activation of soluble guanylate cyclase (cGMP-dependent mechanism). However, there is evidence that NO can exert its biological effects by cGMP-independent mechanisms that have not yet been characterized. In this case, NO seems to be implicated in different pathways, such as inhibition of protein phosphorylation which is essential in calcium entry into platelets and/or inhibition of cell-surface receptors (such as those of adenosine 5-diphosphate (ADP), collagen and fibrinogen) involved in platelet activation by nitrosation of cysteine or nitration of tyrosine residues (1, 2). The literature on NO-donor mechanisms is not univocal: for example, S-nitrosoglutathione (GSNO) seemed to inhibit platelet aggregation by a cGMP-dependent mechanism (3) whereas Sogo et al. (1) showed a cGMP-independent mechanism.

In this study, we compared the anti-aggregating effect exerted by three NO-donors (sodium nitroprusside (SNP), S-nitrosoglutathione (GSNO) and 3-morpholino-sydnonimine (SIN-1)) in *in-vitro* tests of platelet aggregation using human and rat platelet-rich plasma (PRP). To clarify the contribution of cGMP-dependent and cGMP-independent pathways, the anti-aggregating effect of NO-donors was also evaluated using PRP pre-incubated with 1H-[1,2,4]oxadiazolo[4,3a]quinoxalin-1-one (ODQ), a selective inhibitor of soluble guanylate cyclase (sGC). Platelet aggregation was performed according to Born's method (4) and induced by ADP. NO-donors (0.5-1000 µM) were incubated with PRP for 3 minutes before addition of agonist. ODQ (100 µM) was incubated with PRP for 30 minutes before addition of NO-donors (100 µM in human PRP; 1000 µM in rat PRP).

SNP, GSNO and SIN-1 inhibited platelet aggregation in human PRP with different power, GSNO having the most powerful effect (GSNO IC_{50} = 0.55 ± 0.1 µM; SNP IC_{50} = 2.4 ± 0.6 µM; SIN-1 IC_{50} = 3.0 ± 1.0 µM). The anti-aggregating effect of the NO-donors was less in rat PRP, however GSNO was still the most powerful drug. Incubation of ODQ with rat and human PRP suggested that the anti-aggregating effect of GSNO was cGMP-independent and that SNP inhibited platelet aggregation by cGMP-dependent and independent mechanisms. SIN-1 exerted both cGMP-dependent and independent mechanisms in human PRP but a cGMP-independent mechanism in rat PRP.

Our results show that NO-donors have different anti-aggregating power and exert their effects on platelet aggregation by different mechanisms. In some cases (SIN-1 and SNP) cGMP-dependent and cGMP-independent pathways may operate simultaneously. Studies are underway to further investigate the mechanism of action of NO-donors.

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