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Systemic effects of S-nitroso-glutathione in the human following intravenous infusion

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Nitric oxide (NO) is a potent vasodilator and inhibitor of platelet aggregation. At present the clinical use of NO donors as inhibitors of platelet activation is limited by their concomitant hypotensive effect. The new NO donor S-nitroso-glutathione (GSNO) has a significant antiplatelet effect at doses that cause only a small decrease in blood pressure in rats. We have examined the antiplatelet and vasodilator properties of this nitrosothiol following systemic intravenous infusion in the human. GSNO was administered intravenously to 10 normal females of reproductive age noting changes in blood pressure, pulse and reported side effects. *Ex vivo* platelet aggregation to ADP was then performed in a platelet-ionized calcium lumiaggregometer on blood samples taken both before and after the infusions. Side effects such as headache or palpitations occurred only in two subjects at the highest infusion rate of 250 $\mu g\ min^{-1}$. Blood pressure and pulse did not vary significantly during the study. *Ex vivo* platelet aggregation in response to ADP was significantly reduced by the infusion. These results suggest that GSNO is a more potent inhibitor of platelet activation than it is a vasodilator and therefore potentially represents a more clinically useful NO donor than has so far been available where an anti-thrombotic effect is required.

Keywords nitric oxide S-nitroso-glutathione platelet aggregation

Introduction

The generation of NO from L-arginine by the vascular endothelium is crucial for the regulation of blood flow and pressure in animals and humans [1]. Nitric oxide from the vascular endothelial cell inhibits platelet aggregation and adhesion whilst the intraplatelet L-arginine-NO pathway acts as a negative feedback system to regulate platelet aggregation [2]. Platelet activation is known to play a role in the pathogenesis of a variety of human vascular thrombotic diseases and it has already been shown that NO-mediated effects are diminished in hypertension [3], diabetes [4] and atherosclerosis [5]. This suggests that supplementation of NO to these patients may be of benefit.

Unfortunately the currently available NO donors (organic nitrates including glyceryl trinitrate, isosorbide mono- and dinitrate, and direct NO donors including sodium nitroprusside and SIN-1) are of limited clinical use as anti-thrombotic agents due to dose-limiting hypotension [6]. Recent studies in the conscious rat have demonstrated that GSNO is a potent inhibitor of platelet activation, at doses at which it has a relatively minor vascular effect [7]. The present study investigated the vascular and antiplatelet properties of GSNO following systemic intravenous infusion in humans.

Methods

The study was approved by the local ethics committe, and was undertaken in healthy female volunteers (n = 10, aged 17–19 years) in the setting of a gynaecology department as part of an ongoing research programme. None of the subjects had taken drugs known to affect platelet function for the previous 2 weeks.

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Table 1 Results before, and after infusion of GSNO shown as mean (s.d.) and compared using a paired Student's *t*-test ($n = 10$)

	Before infusion	*After infusion*	*Difference mean (95% CI)*	P
Mean arterial pressure (mm Hg)	103.6 (5.8)	102.9 (3.3)	0.71 (−3.86, +5.28)	NS
Pulse (beats min^{-1})	67 (5.6)	66 (5.0)	1.00 (−4.13, +6.13)	NS
% Aggregation with 5 μM ADP	19.80 (12.35)	4.40 (5.08)	15.40 (6.52, 24.28)	0.002
% Aggregation with 10 μM ADP	40.70 (9.76)	28.80 (15.05)	13.90 (1.80, 25.99)	0.027

An 18 gauge intravenous cannula was inserted into the brachial vein and a 10 ml sample of blood was withdrawn into a syringe prefilled with 1 ml 3.15% trisodium citrate for measurement of *ex vivo* platelet aggregation [7]. Aliquots were immediately (within 20 s of collection) centrifuged at 320 *g* for 2 min to obtain platelet rich plasma (PRP). Platelet poor plasma (PPP) was prepared by centrifuging PRP at 2000 *g* for 2 min. Platelet aggregation was measured in a platelet-ionized calcium lumi-aggregometer (Chronolog). PRP (0.4 ml) was preincubated for 2 min and aggregation responses to ADP (1–10 mM) measured. The extent of aggregation was calculated as an increase in the light transmission 3 min following the addition of ADP.

Infusions were prepared with 2.5 mg GSNO in 50 ml normal saline. Following insertion of the cannula the blood pressure and pulse were allowed to stabilise over a period of 20 min with the subject supine. Infusions were commenced at an initial rate of 50 μg min^{-1} and increased by 50 μg min^{-1} every 5 min to a maximum of 250 μg min^{-1} after 20 min. This rate was maintained for a further 10 min giving a total infusion time of 30 min. After the infusion was discontinued a further 10 ml blood sample was taken from the contralateral ante-cubital fossa for repeat platelet aggregation studies. The pulse was taken every 5 min throughout the duration of the infusion and blood pressure recorded at the same time using a mercury sphygmomanometer.

Results

The results of platelet aggregation studies and cardiovascular parameters are shown in Table 1. Of the 10 subjects, 8 reported no side effects and received a total dose of 2.5 mg GSNO over 30 min. One patient reported headache and flushing and another palpitations, although clinically her pulse remained regular throughout. Both women only reported these side effects between 20 and 25 min after starting the infusion at the highest rate of 250 μg min^{-1} having received 1.8 mg of GSNO during that time.

Discussion

These results show that GSNO has a significant antiplatelet effect without alteration of systemic blood pressure or pulse. Minor side-effects were reported by two subjects. Whilst our data suggests that these side effects may not occur with infusion rates less than 200 μg min^{-1} such a restriction may also reduce the antithrombotic effect observed. Further studies are needed to test this. Nevertheless, on current evidence it seems likely that where an anti-thrombotic effect is required GSNO may represent a more clinically useful NO donor than those currently available.

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