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Short communication

# Serotonin blockade protects against early microvascular constriction following atherosclerotic plaque rupture

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**Abstract**

Early microvascular constriction following atherosclerotic plaque rupture may be mediated via serotonin and/or endothelin-1. Atherosclerotic lesions in the rabbit hindlimb underwent plaque rupture, resulting in a rapid reduction of distal flow (7.1 ± 0.7 ml/min pre-rupture versus 3.6 ± 0.6 ml/min post-rupture, $P < 0.001$) and a rise in distal microvascular resistance (10.5 ± 0.9 mm Hg min/ml pre-rupture versus 23.5 ± 3.5 mm Hg min/ml post-rupture, $P = 0.01$). Distal microvascular resistance remained elevated following endothelin-1 receptor antagonism and control vehicle, but normalised after serotonin receptor antagonism with ritanserin (10.5 ± 0.9 mm Hg min/ml pre-rupture versus 22.2 ± 6.0 mm Hg min/ml post-endothelin-1 receptor antagonism [$P < 0.05$] versus 21.6 ± 6.2 mm Hg min/ml post-control vehicle [$P < 0.05$] versus 11.6 ± 2.0 mm Hg min/ml post-ritanserin [$P$ = NS]). Early antagonism of serotonin receptors protects against distal microvascular constriction following atherosclerotic plaque rupture.


*Keywords:* Microcirculation; Reperfusion; Atherosclerosis

## 1. Introduction

Critical in the treatment of acute myocardial infarction is the successful restoration of perfusion to the infarcted myocardium. In many instances, flow in the infarct related artery remains poor even despite the successful restoration of vessel patency at the site of lesion rupture and is associated with an adverse clinical outcome (Ito et al., 1996).

Prior reports on the efficacy of vasodilators in improving microvascular flow (Marzilli et al., 2000) implicate microvascular constriction as an important contributor to impaired reflow in acute myocardial infarction. A number of mechanisms could account for such a constrictive response, including serotonin (5-HT) release from activated platelets (Willerson et al., 1989) and/or endothelin-1, released during plaque rupture (Petronio et al., 1999), or from reperfused ischaemic myocardium (Velasco et al., 1994).

We investigated whether antagonism of serotonin and endothelin-1 could attenuate microvascular constriction induced by mechanical rupture of a fibrofatty atherosclerotic plaque in the iliac artery of hypercholesterolemic rabbits. We demonstrate that ritanserin, a 5-$HT_2$ receptor antagonist, but not SB209670, a non-selective endothelin-1 ($ET_A$/$ET_B$) receptor antagonist, attenuated the vasoconstriction, suggesting a role for serotonin release in early microvascular constriction following atherosclerotic plaque rupture.

## 2. Methods

### 2.1. Animals and study design

All experiments were approved by the Alfred Hospital/Baker Institute Animal Experimentation Committee and adherent to the European Community guidelines for the use of experimental animals. Twenty Baker rabbits underwent a combination of balloon injury to the left iliac artery followed by 4 weeks of 1% cholesterol feeding to induce haemodynamically significant atherosclerotic lesions (Taylor et al., 2002). A unique feature of this model is that balloon injury is anterograde and not retrograde, hence the distal vasculature is left intact allowing study of the microvasculature.

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Four weeks after balloon injury, mechanical abrasion-induced plaque rupture. Distal flow, pressure and microvascular resistance was recorded. After plaque disruption, the effects of the 5-$HT_2$ receptor antagonist ritanserin, the endothelin-1 ($ET_A$/$ET_B$) receptor antagonist SB209670 and control vehicle on microvascular constriction were assessed.

### 2.2. Induction of atherosclerotic lesions

A combination of balloon injury to the left iliac artery to denude its endothelium and cholesterol feeding-induced atherosclerotic lesion development. Cholesterol feeding was commenced 3 days prior to the balloon injury and continued for the duration of the study. Prior to balloon injury procedure, all rabbits were administered carprofen (1.5 mg/kg s.c., Pfizer USA) and heparin (200 IU/kg i.v.). General anaesthesia was induced with Diprivan (1 mg/kg i.v., AstraZeneca USA) and maintained by 5% halothane inhalation. We have previously shown that this results in the development of localised fibrofatty atherosclerotic lesions with many of the characteristics of American Heart Association (AHA) Type IV atherosclerotic plaques susceptible to mechanical rupture (Taylor et al., 2002).

### 2.3. Plaque rupture and distal microvascular resistance

Four weeks after the initial surgery, the rabbits were again anaesthetised (as above but with heparin omitted), the left iliac artery was carefully mobilised and an ultrasonographic flow probe placed around a segment of artery distal to the atherosclerotic lesion. A fine gauge catheter was also inserted into the distal iliac artery and advanced retrogradely to the level of the probe. Distal microvascular resistance could then be calculated by dividing distal pressure by flow. In addition, a pressure transducer was attached to the carotid artery catheter allowing continuous recording of aortic pressure. A deflated angioplasty balloon was then passed back and forth across the atherosclerotic lesion to induce plaque rupture.

### 2.4. Administration of drugs

All drugs were administered via the iliac pressure catheter as a slow intra-arterial bolus over 1 min allowing direct action on the vasculature distal to the atherosclerotic lesion both pre- and post-rupture. A bolus of intra-arterial glyceryl trinitrate (25 μg) was administered at baseline and then following plaque rupture, to eliminate any local spasm at the lesion site. Ten minutes following plaque rupture, 20 rabbits were then administered either ritanserin (1 mg, Research Biochemicals, USA), SB209670 (2.5 mg, SmithKline Beecham, USA) or 1 ml of vehicle pH matched to that of the ritanserin solution. The effects of drug administration on distal microvascular resistance were then assessed 5 min following drug administration, to allow the development of a new steady state.

### 2.5. Statistics

All data are expressed as mean ± 1 S.E. Comparisons between groups are made using paired or unpaired Student's *t*-test as appropriate, or with one-way analysis of variance (ANOVA) and post-hoc subgroup analysis as required. All data analysis utilised a computerised statistics package (SPSS© for Windows, version 10.0.7).

## 3. Results

### 3.1. Haemodynamic effects of plaque rupture

Prior to plaque disruption, mean iliac pressure distal to the atherosclerotic lesion was significantly lower than mean aortic pressure (63.9 ± 4.2 mm Hg versus 72.9 ± 3.8 mm Hg, $P < 0.001$), consistent with the development of a stenotic atherosclerotic lesion. At baseline, there were no significant differences between the three treatment groups with respect to mean aortic pressure, mean distal iliac pressure or distal microvascular resistance ($P$ = NS for all comparisons, data not shown).

Following atherosclerotic plaque rupture, mean blood flow reduced rapidly (from 7.1 ± 3.3 to 3.6 ± 0.6 ml/min, $P < 0.001$, Fig. 1). Iliac pressure distal to the atherosclerotic lesion was reduced following plaque disruption (from 63.9 ± 4.2 mm Hg pre-rupture to 46.6 ± 4.5 mm Hg post-rupture, $P < 0.01$), consistent with increased local obstruction at the site of plaque rupture. In addition, there was a marked rise in distal microvascular resistance following plaque rupture (from 10.5 ± 0.9 mm Hg min/ml pre-rupture to 23.5 ± 3.5 mm Hg min/ml post-rupture, $P = 0.01$), consistent with microvascular obstruction distal to the ruptured atherosclerotic lesion. Mean aortic pressure following plaque disruption was unchanged following plaque disruption (72.9 ± 3.8 mm Hg pre-rupture versus 78.3 ± 4.2 mm Hg post-rupture, $P$ = NS).

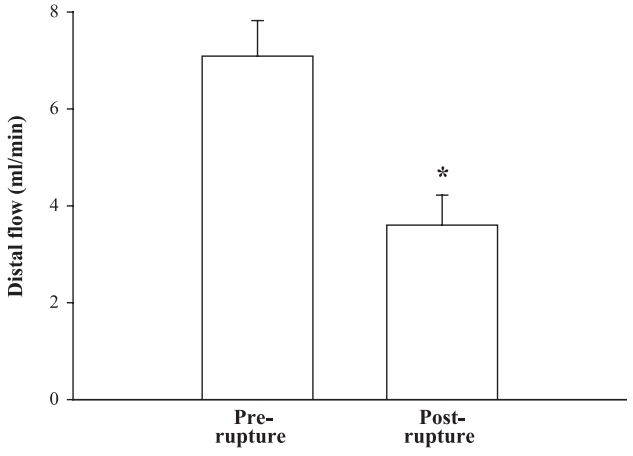


Fig. 1. Following plaque rupture, distal blood flow is significantly reduced. *$P < 0.001$.

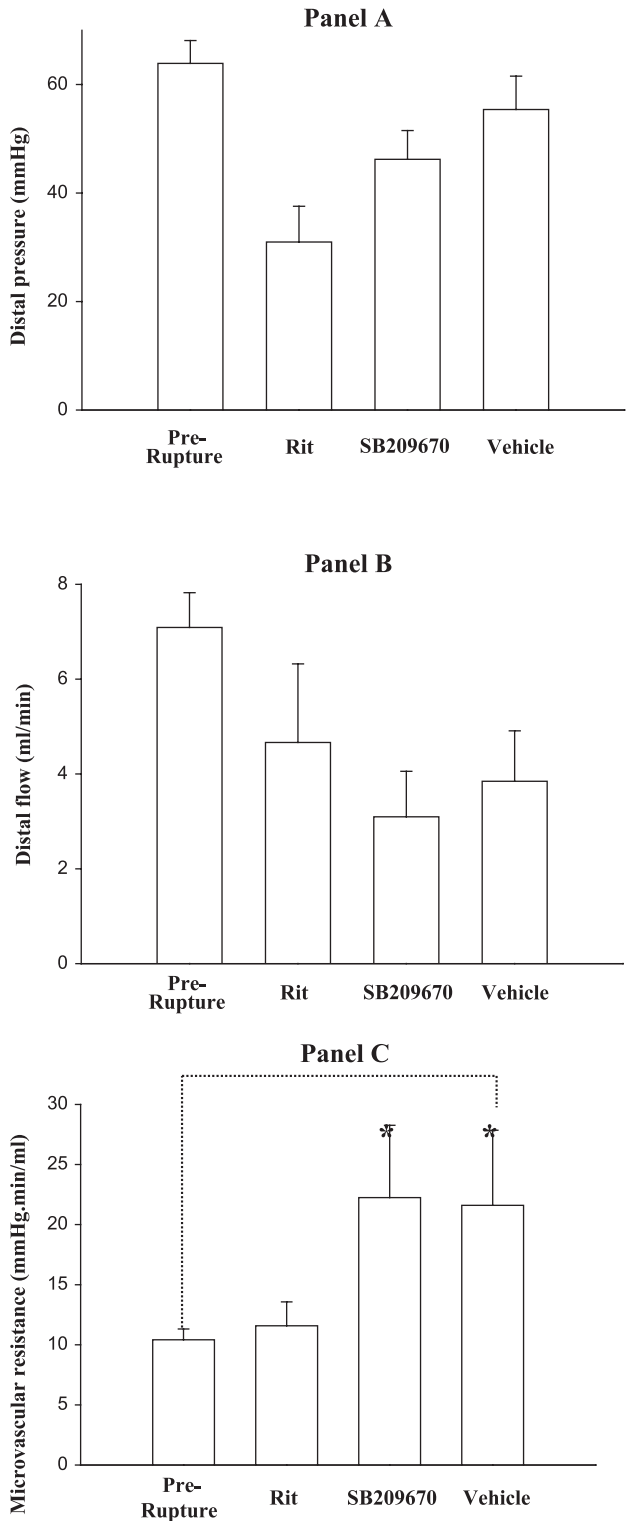


Fig. 2. There was a trend towards lower distal pressure (Panel A) and increased distal flow (Panel B) in the ritanserin-treated animals (Rit) compared with SB209670 and control vehicle following plaque rupture. Distal microvascular resistance after plaque rupture remained significantly elevated following SB209670 (22.2 ± 6.0 mm Hg min/ml) or control vehicle (21.6 ± 6.2 mm Hg min/ml) compared with pre-rupture (10.5 ± 0.9 mm Hg min/ml), whereas ritanserin treatment reduced resistance to pre-rupture levels (11.6 ± 2.0 mm Hg min/ml). *$P<0.05$ (ANOVA).

### *3.2. Systemic effects of drugs following plaque rupture*

Systemic blood pressure following plaque rupture was reduced following non-selective endothelin-1 receptor antagonism as well as the administration of ristanserin (60.4 ± 6.3 mm Hg post-SB209670 and 56.6 ± 2.0 mm Hg post-ritanserin versus 78.3 ± 4.2 mm Hg following plaque rupture, $P<0.05$ for both comparisons). In contrast, the administration of control vehicle had no effect (70.5 ± 4.0 mm Hg post-vehicle versus 78.3 ± 4.2 mm Hg following plaque rupture, $P$ = NS).

### *3.3. Effects of 5-HT$_2$ receptor antagonism, mixed endothelin receptor antagonism and placebo vehicle following plaque rupture*

Following plaque rupture, there was a trend towards lower distal pressure (Fig. 2, Panel A) and higher distal flow (Fig. 2, Panel B) in the ritanserin-treated group compared with SB209670 and placebo vehicle. Distal microvascular resistance post-rupture was significantly elevated in both the SB209670 and placebo vehicle groups compared with pre-rupture levels, whereas the administration of ristanserin was protective (Fig. 2, Panel C). In contrast, the administration of intra-arterial glyceryl trinitrate had no effect on the elevated distal microvascular resistance post-rupture (23.5 ± 3.5 mm Hg min/ml post-rupture versus 21.0 ± 4.7 mm Hg min/ml post-glyceryl trinitrate).

## 4. Discussion

Using a newly developed animal model for atherosclerotic plaque rupture, we demonstrate that following plaque rupture distal microvascular resistance is rapidly elevated via mechanisms involving 5-HT$_2$ receptors. The administration of the 5-HT$_2$ receptor antagonist ritanserin significantly reduced the elevated distal microvascular resistance, ameliorating microvascular obstruction. In contrast, inhibition of endothelin-1 $ET_A/ET_B$ receptors with SB209670 had no effect.

During atherosclerotic plaque rupture, platelets become activated (Gawaz et al., 1996), aggregate and degranulate. As a consequence, a number of vasoactive substances are released into the circulation including 5-HT (Loots and De Clerck, 1993). Activated platelets are known to initiate vascular constriction (Awano et al., 1989), and microvascular constriction in response to 5-HT is enhanced in the presence of atherosclerosis (Chilian et al., 1990). Blockade of 5-HT$_2$ receptors markedly reduces 5-HT-induced vasoconstriction in human coronary arteries ex vivo (Connor et al., 1989), and also during coronary angioplasty (Golino et al., 1994). In addition, ritanserin has been shown to attenuate renal vasoconstriction induced by serotonin (Moran et al., 1997) as well as increase sub-

cortical blood flow following photothrombotic middle cerebral artery occlusion in rats (Back et al., 1998). Local thrombus formation is also a characteristic feature of ruptured atherosclerotic lesions in the rabbit iliac artery (Taylor et al., 2002). Our findings together with those of Back et al. (1998) suggest that 5-$HT_2$ receptor antagonism may be a useful therapeutic strategy to prevent thrombus-mediated vasoconstriction due to rupture of atherosclerotic lesions.

Despite reports of release of endothelin-1 during angioplasty (Petronio et al., 1999), non-selective endothelin-1 $ET_A/ET_B$ receptor antagonism in doses that reduced blood pressure did not attenuate the elevation in microvascular resistance induced by rupture of atheromatous plaques. It is possible that endothelin-1 release from ruptured atherosclerotic lesions is more delayed and slower than serotonin, with the vasomotor effects of endothelin-1 appearing only after more prolonged periods of ischaemia (Galiuto et al., 2000). In addition, the use of a mixed endothelin-1 $ET_A/ET_B$ receptor antagonist in our study may have negated any compensatory vasodilatory effects mediated by the $ET_B$ receptor via the production of nitric oxide (Haynes and Webb, 1998). However, endothelium-dependent vasodilation is impaired in the presence of atherosclerosis, with coronary vasoconstriction in response to endothelin-1 accentuated in experimental hypercholesterolaemia, the mechanism of which is through $ET_B$ receptor-mediated vasoconstriction (Hasdai et al., 1997). As atherosclerotic lesion development in our model is enhanced by cholesterol feeding, a mixed endothelin $ET_A/AT_B$ receptor antagonist would be the most appropriate choice to block the vasoconstrictor effects of endothelin-1.

Although elevated distal microvascular resistance impedes blood flow, this was not the only contributory factor in our model, as plaque rupture also increased the trans-lesional pressure gradient. Distal microvascular resistance is however an important impediment to coronary flow, a point recently highlighted by Meuwissen et al. (2001). In addition, when extrapolating these findings to the coronary circulation differences between hindlimb and cardiac circulation need to be borne in mind. However, the atherosclerotic lesions in our animals have many similarities to AHA Type IV unstable coronary plaques (Taylor et al., 2002), inducing local thrombosis and distal flow reduction following their rupture, which overcomes many of the shortcomings of prior animal models (Badimon, 2001).

Complete reperfusion of ischaemic cardiac tissue depends not only on restoration of macrovascular patency but also microvascular flow. Our findings demonstrate that in addition to causing local obstruction due to thrombosis, plaque rupture results in microvascular obstruction via the release of serotonin. Targeting serotonin receptors responsible for vasoconstriction, in addition to conventional treatments restoring infarct vessel patency, may improve reperfusion in acute myocardial infarction.

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