Effect of aspirin on vasodilation to bradykinin and substance P in patients with heart failure treated with ACE inhibitor

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Aims We wanted to examine some of the mechanisms by which aspirin might be responsible for counteraction of the effects of ACE inhibitors. Aspirin has been reported to counteract the effects of ACE inhibitors in patients with heart failure. Despite this, there is little evidence on what the mediator of such an effect might be, although there is some evidence to implicate bradykinin and, perhaps, substance P.

Methods Twelve patients with congestive heart failure treated with an ACE inhibitor were studied on two occasions, after abstaining from aspirin for 14 days, and after 14 days of aspirin 150 mg once daily. Forearm blood flow was measured by venous occlusion plethysmography during intrabrachial infusions of bradykinin and substance P, before and after intrabrachial aspirin.

Results Bradykinin caused profound vasodilation (peak 390%, 95% confidence intervals (CI) 300, 480%; $P < 0.01$), substance P slightly less (peak 222%, 95% CI 162, 283%; $P < 0.01$). Intra-brachial aspirin had no effect on its own. The response to bradykinin was unchanged by intrabrachial aspirin (peak 404%, 95% CI 304, 504%) or oral aspirin (peak 320%, 95% CI 209, 431%; $P = 0.2$). The response to substance P was unchanged by intrabrachial aspirin (peak 226%, 95% CI 171, 281%) or oral aspirin (peak 220%, 95% CI 142, 297%; $P = 0.86$).

Conclusions Aspirin has no effect on the vasodilator response to bradykinin and substance P in patients with heart failure treated with an ACE inhibitor. Neither bradykinin nor substance P is likely to contribute to the reported interaction between aspirin and ACE inhibitors.

Keywords: ACE inhibitors, aspirin, bradykinin, heart failure, substance P

Introduction

There is continuing controversy surrounding the concomitant use of aspirin and angiotensin converting enzyme (ACE) inhibitors in patients with congestive heart failure due to left ventricular systolic dysfunction [1]. The results of clinical haemodynamic studies [2] and subgroup analysis of clinical trials [3] have generated the hypothesis that aspirin may counteract some of the beneficial effects of ACE inhibitors. Whilst this hypothesis continues to await a definitive answer, some progress might be made by studying some of the potential mechanisms for such an

interaction. Any interaction between aspirin and ACE inhibitors must almost certainly involve prostaglandins [4]. The most usual explanation for the interaction between aspirin and ACE inhibitors therefore involves bradykinin, as ACE inhibitors are reported to potentiate the effects of bradykinin by inhibition of its breakdown [5], and bradykinin is reported to exert at least some of its effects via prostaglandins [6]. It is therefore an inhibition by aspirin of prostaglandin-mediated bradykinin-induced effects of ACE inhibitors which is said to be responsible for their interaction. We sought to determine whether or not aspirin actually has any effect on the response to bradykinin, in patients with heart failure treated with an ACE inhibitor. We also chose to look at the effect of aspirin on the response to substance P, as this has also been reported to be potentiated in some way by ACE inhibitors [7], and may too exert its effects via prostaglandins [8].

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Methods

Patients

Twelve patients with chronic heart failure secondary to left ventricular systolic dysfunction confirmed by echocardiography were studied (12 patients gave more than 80% power to exclude a 33% reduction in response assuming a response with a standard deviation of 40%). All patients were clinically stable on constant doses of vasodilator and diuretic drugs for at least 3 months, with no peripheral oedema or pulmonary congestion, and none had uncontrolled hypertension, untreated hypercholesterolaemia or diabetes mellitus requiring insulin treatment. All patients were receiving treatment with an ACE inhibitor (enalapril 10 mg twice daily in the majority). All medications apart from aspirin were kept constant throughout the period of study. Patient characteristics are summarized in Table 1. The study was conducted with the approval of the West Ethics Committee (Glasgow). All patients gave full written informed consent.

Protocol

Each patient was studied on two occasions, after 14 days of no aspirin at all, and after 14 days of 150 mg aspirin daily, having abstained from all other aspirin, aspirin-containing medications or aspirin-like medications during that time. This dose is in the middle of the range previously demonstrated by us to inhibit vasodilation to arachidonic acid in

the forearm of patients with heart failure [9]. Patients took their usual medications including aspirin therapy as directed 6 h before attendance on the day of study. Patients abstained from alcohol, tobacco and caffeine for 24 h before each study and attended fasted for at least 6 h. Bradykinin was infused at 10, 30 and 100 pmol min⁻¹ for 3 min at each dose and substance P was infused at 1, 2 and 4 pmol min⁻¹ for 3 min at each dose. Aspirin was then infused at 1 mg min⁻¹ for 15 min. Bradykinin and substance P were then reinfused in the same order as before (the order of infusion having been randomized). For the other study, patients attended after taking oral aspirin for 14 days, and only the first part of the study (bradykinin and substance P infusion) was repeated. Each patient therefore had measurement of responses to bradykinin and substance P off all aspirin therapy, after intra-arterial aspirin and after oral aspirin.

Measurements

Studies were performed with patients lying supine in a quiet clinical laboratory maintained at a constant temperature of 23–25°C. After local anaesthesia with 1% lignocaine (Astra Pharmaceuticals) a 27-gauge steel needle (Terumo Medical Corp.) was inserted into the non-dominant brachial artery and connected to a constant rate infusion pump (IVAC P1000, Alaris Medical Systems) via a 16-gauge epidural catheter (Portex Ltd). Physiological saline solution (0.9%, Baxter Healthcare Ltd) was infused at 1 ml min⁻¹ for at least 20 min before drug infusion. Blood flow was measured simultaneously in the infused and noninfused arms by venous occlusion plethysmography using indium/gallium-in-Silastic strain gauges applied to the widest aspect of each forearm. To obtain blood flow measurements hand circulation was excluded by inflation of wrist cuffs to 220 mmHg and upper-arm cuffs were inflated to 40 mmHg to obstruct venous outflow for 12 out of every 16 s. Voltage output from a Hokanson plethysmograph (Hokanson Corp.) was transferred via a MacLab 4e analogue-to-digital converter (AD Instruments) to a Macintosh personal computer (PowerMac, Apple Computer Inc.) for analysis using Chart software (version 3.2.8; AD Instruments). Plethysmographic recordings were made for a period of 2.5 min at 10 min intervals during saline infusion and at 3–5 min intervals during drug infusion. The last five measurements from each 2.5 min recording period were transferred from Chart to Excel software (version 7.0, Microsoft Corp.), averaged, and the mean percentage change from baseline in the ratio of flow between the infused and noninfused arms was calculated. Provided blood pressure remains constant, increases in blood flow represent vasodilation and decreases in blood flow represent vasoconstriction. This method is well-validated, using the noninfused arm as a contemporaneous

Table 1 Patient characteristics.

Patient characteristic	n
Sex	10 M/2 F
Age (years) (mean ± s.d.)	63 ± 6
Primary diagnosis	
IHD	10
IDC	2
NYHA functional class	
II	7
III	4
IV	1
LVEF (%) (mean ± s.d.)	20 ± 7
Current smoker	4
On:	
Aspirin	9
ACE inhibitor	12
Enalapril 10 mg twice daily	9
Diuretic	12
Digoxin	3
Nitrate	1
β-adrenoceptor blocker	3
Statin	6

control and a means of distinguishing the effects of drug infusion from any other external or environmental factors [10, 11]. Blood pressure and pulse rate were manually recorded in the noninfused arm at 5–10 min intervals throughout.

Drugs

Bradykinin and substance P was obtained from Clinalfa AG and both were dissolved in normal saline. Aspirin was obtained from Synthelabo France and dissolved in normal saline. All drugs were always used within 2 h of final preparation and destroyed thereafter.

Statistical analysis

All results are expressed as mean values with 95% confidence intervals in the text and mean values with standard errors in the figures. Between groups differences were compared using two-factor analysis of variance with replication (Microsoft Excel 97). Differences were considered statistically significant at a value of $P < 0.05$.

Results

General effects

Local infusion of bradykinin, substance P and aspirin caused no adverse or systemic effects, and patients did not report any discomfort. Pulse rate, blood pressure, and forearm blood flow in the noninfused forearm did not change significantly during the study days, and there were no significant differences in baseline pulse rate, blood pressure or baseline forearm blood flow between the two different study days.

Effect of bradykinin

Bradykinin caused vasodilation. There was a clear dose-response relationship, with peak vasodilation at the highest dose of $100 \text{ pmol min}^{-1}$ (390%, 95% CI 300, 480%; $P < 0.01$; Figure 1).

Effect of substance P

Substance P caused vasodilation. There was a clear dose-response relationship, with peak vasodilation at the highest dose of 4 pmol min^{-1} (222%, 95% CI 162, 283%; $P < 0.01$; Figure 2).

Effect of aspirin

Intra-arterial aspirin had no discernible effect on forearm blood flow on its own. The response to bradykinin was

Figure 1 Effect of bradykinin on forearm blood flow before and after intra-arterial and oral aspirin (□ BK = no aspirin; □ BK + A(i.a.) = after intra-arterial aspirin; ■ BK + A(O) = after 14 days oral aspirin: $P < 0.01$ for dose-effect; $P = 0.2$ for treatment effect; $P = 0.92$ for interaction between the two).

Figure 2 Effect of substance P on forearm blood flow before and after intra-arterial and oral aspirin (□ SP = no aspirin; □ SP + A(i.a.) = after intra-arterial aspirin; ■ SP + A(O) = after 14 days oral aspirin: $P < 0.01$ for dose-effect; $P = 0.86$ for treatment effect; $P = 0.996$ for interaction between the two).

unaffected by intra-arterial aspirin (peak 404%, 95% CI 304, 504%; Figure 1) or oral aspirin (peak 320%, 95% CI 209, 431%; $P = 0.2$; Figure 1). The response to substance P was unaffected by intra-arterial aspirin (peak 226%, 95% CI 171, 281%; Figure 2) or oral aspirin (peak 220%, 95% CI 142, 297%; $P = 0.86$; Figure 2). There was no evidence

of any interaction between aspirin pretreatment and the dose-response of bradykinin ($P=0.92$) or substance P ($P=0.996$).

Discussion

In this study in the forearm of patients with heart failure, we have shown that exogenous bradykinin and substance P cause vasodilation, that intra-arterial aspirin has no discernible effect on its own, that intra-arterial aspirin has no effect on the response to bradykinin or substance P, and that 14 days oral aspirin 150 mg once daily has no effect on the response to bradykinin or substance P.

Effect of bradykinin in heart failure

This is one of the first studies to examine the effects of exogenous bradykinin in patients with heart failure. Bradykinin has been well studied in healthy volunteers [12] and in patients with endothelial dysfunction [13], but not, except by us, in patients with heart failure [14, 15], despite the importance of ACE inhibitors and bradykinin potentiation in heart failure. Our findings confirm that bradykinin is a potent vasodilator in patients with heart failure, as it is in subjects without heart failure. Furthermore, they generate the hypothesis that this may be one endothelium-dependent response which is not impaired in heart failure. Although it is possible that an otherwise impaired response to bradykinin might be 'corrected' by ACE inhibitor treatment (given that ACE inhibitors do potentiate the effects of bradykinin by inhibition of its breakdown), our findings were actually remarkably similar to those in healthy volunteers also treated with an ACE inhibitor, albeit acutely rather than chronically [16].

Effect of substance P in heart failure

Unlike bradykinin, substance P has been studied in heart failure, as well as other models of endothelial dysfunction, although the results of this have been somewhat inconsistent. The only other study to look at the effects of substance P in the forearm of patients with heart failure found that vasodilation was unimpaired, allowing for the lower basal blood flow in patients compared with controls [17]. Another study examined endothelium-dependent responses in the lower limb, and found that the response to substance P was reduced in patients compared with controls, albeit to a lesser extent than the response to acetylcholine [18]. Other studies have been *in vitro* [19] or in animal models of heart failure [20]. We found obvious vasodilation to substance P in our patients with heart failure treated with an ACE inhibitor. Although comparison with the effects of bradykinin suggests this vasodilation is quite modest, the molar doses of substance P were

more than an order of magnitude smaller. The role of substance P as a potent vasodilator, even in heart failure, at least when treated with an ACE inhibitor, is confirmed.

Effect of intra-arterial aspirin

Only one previous study has looked directly at the effects of intra-arterial aspirin and found a reduction in forearm blood flow [21]. For the dose of 1 mg min^{-1} which we used, however, the reduction was only 8%, and was not significant. Higher doses of 3 mg min^{-1} and 10 mg min^{-1} were used, with more significant effects, but these would be equivalent to the oral anti-inflammatory dose-range of 325 mg–1.5 g rather than the cardio-protective dose-range of 75 mg–300 mg. Because of this, also, their effects could by no means be guaranteed to be confined to the forearm. Of course the difference between our findings and previous findings could relate to a difference between health and disease, but it is difficult to see how aspirin could have a deleterious effect in healthy volunteers which it does not have in patients with heart failure (unless the vascular effects of aspirin could in some way be equated with the vascular effects of heart failure).

Interaction of bradykinin and aspirin

Our findings show no significant effect of aspirin, whether intra-arterial or oral, on the vasodilator response to bradykinin in patients with heart failure treated with an ACE inhibitor. The effects of bradykinin are frequently said to be at least partially aspirin-sensitive and this sensitivity is frequently invoked as a potential mechanism for the reported negative interaction between aspirin and ACE inhibitors. Despite this, the evidence that aspirin actually affects the vascular effects of bradykinin is patchy. There is no doubt that aspirin blocks the algescic effects of bradykinin [22], indeed this is probably central to the analgesic activity of aspirin and aspirin-like drugs [23]. There are also well-validated instances of inhibition of smooth muscle motor responses to bradykinin by aspirin [24, 25], and yet the effect of aspirin on vasomotor responses to bradykinin is much more variable. There seems no doubt that aspirin blocks the release of vasoactive prostanoids by bradykinin [6, 26–28], but there are at least four reports [5, 6, 26, 29] that aspirin has no effect on the vasodilator response to bradykinin. Most of the reports that aspirin does block the vasomotor effects of bradykinin have been *in vitro* [30, 31] or in animals [31–33]. Our findings reiterate the fact that aspirin does not block the vasodilator effect of bradykinin, and extend that finding to patients with heart failure treated with an ACE inhibitor.

Interaction of substance P and aspirin

Our findings show no significant effect of aspirin, whether intra-arterial or oral, on the vasodilator response to substance P in patients with heart failure treated with an ACE inhibitor. Potentiation of substance P by inhibition of its breakdown is frequently invoked as a possible cause of side-effects such as cough in patients treated with an ACE inhibitor [34, 35], even though the most direct examination of the interaction between substance P and an ACE inhibitor has failed to find any evidence of any such effect [36]. Any evidence that the effects of substance P were mediated by prostaglandins and blocked by aspirin might still be highly relevant to the reported negative interaction between aspirin and ACE inhibitors. As with bradykinin, there is little doubt that aspirin blocks the analgesic effects of substance P [37], and again, this is somewhat central to the analgesic effect of aspirin. As with bradykinin, the evidence that aspirin blocks the vasodilator effects of substance P is patchy, venodilation apparently being blocked [38], and arterial vasodilation (in atherosclerosis) apparently not [39]. Our findings reiterate the fact that aspirin does not block the vasodilator effect of substance P, at least in patients with heart failure treated with an ACE inhibitor. This could be quite important if, as has been reported, substance P levels are elevated in heart failure [40] and further elevated by treatment with ACE inhibitors [7].

Limitations

To examine the interaction of ACE inhibition and aspirin more directly, a two-by-two factorial design would have been appropriate, however, it would have been very difficult to justify giving these heart failure patients placebo in place of their ACE inhibitor. We do not have any appropriate controls, however, we were not interested in a difference between health and disease, but in examining the pharmacology of these very commonly used drugs in patients in whom they are often used together. We do not have any information about conventional measures of endothelial function in these patients, however, there is a limit to the length of these studies and the number of infusions they can be expected to tolerate. These short-term local responses may not necessarily concur with previously described systemic or long-term responses, but it is inescapable that local and systemic, and short and long-term studies are required for full definition of any response.

Conclusions

We have shown that exogenous bradykinin and substance P cause vasodilation in patients with heart failure, that there is no effect of intra-arterial aspirin on its own, and that

there is no effect of aspirin, intra-arterial or oral, on the responses to bradykinin and substance P. Demonstration of these responses is novel in patients with heart failure, and is only partly consistent with previous work *in vitro*, in animals and in healthy volunteers. Whilst these findings leave open the issue of whether bradykinin or substance P contributes to the nonangiotensinergic side-effect of ACE inhibitors, they do suggest that vasodilation to bradykinin or substance P, in the forearm at least, is unlikely to contribute to the reported interaction between aspirin and ACE inhibitors. Proponents of this hypothesis may need to look elsewhere for other molecules to incriminate, perhaps, for example, angiotensin-(1-7) [17, 41].

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