Cyclosporin treatment does not impair the release of nitric oxide in human coronary arteries

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Abstract

Objective—It has been hypothesised that compromised endothelial function can contribute to the toxic manifestations associated with cyclosporin therapy. In vitro animal studies have implicated inhibition of release of the endothelium derived relaxing factor, nitric oxide; however, this has not been investigated in human tissue. The present study investigated the effect of cyclosporin A on nitric oxide release in human coronary arteries.

Design—Study of in vitro organ bath preparations and in vivo angiographic measurements in the coronary circulation.

Patients—For the in vitro experiments coronary arteries were harvested from the excised hearts of 10 patients requiring transplantation for reasons other than ischaemic heart disease. Three of these patients were being re-transplanted for obliterative bronchiolitis and had been receiving cyclosporin for a mean of 22 months. The in vivo study was performed on a group of 12 cardiac transplant recipients who were clinically well 1–5 years postoperatively and were not undergoing allograft rejection at the time of assessment.

Results—Isolated vessel segments in vitro relaxed in a dose dependent manner in response to substance P (10^{-11} – 10^{-7} mol/l). The maximum response was 76.6 (7.4)% of the response to 1 µg/ml glyceryl trinitrate. Incubation with 1000 and 2000 ng/ml cyclosporin reduced the response to 63.0 (11.5)% and 62.2 (11.1)% respectively; this was not statistically significant. In segments taken from the explanted hearts of three patients requiring re-transplantation, the mean maximum response was 78.0 (11.0)% and there was no correlation between maximum response in segments from each patient and the duration of cyclosporin therapy. The effect of intracoronary substance P in 12 cardiac transplant recipients was also examined (mean cyclosporin blood concentration 228.9 (42.8) ng/ml). The mean maximum dilatations measured as the percentage diameter change induced by substance P and isosorbide dinitrate were 22.1 (3.2)%

and 26.0 (2.5)% respectively. There was no correlation between the degree of endothelium mediated vasodilatation in response to substance P and cyclosporin concentration.

Conclusions—The nitric oxide response was preserved in the coronary arteries of patients exposed to cyclosporin. The mechanisms that initiate cyclosporin associated toxicity remain to be elucidated.

The outcome in patients after heart transplantation has been considerably improved by cyclosporin therapy.¹ None the less, several cyclosporin associated toxic manifestations have been encountered, particularly hypertension^{2,4} and renal damage.^{5–9} The underlying mechanisms involved in the pathogenesis of these manifestations remain largely undefined. Animal studies have implicated the disruption of endothelium dependent and independent dilatation^{10–13} as well as alteration in vascular response;^{14,15} in human tissue, cyclosporin induced hypertension was associated with sympathetic neural activation.¹⁶

The effect of cyclosporin on the human coronary arterial system could have important clinical implications. Though the use of cyclosporin therapy has coincided with a decrease in accelerated atherosclerosis in grafted coronary arteries in transplant recipients,¹⁷ there is some evidence that cyclosporin can have an adverse effect on the ability of the endothelium to secrete prostacyclin¹⁸ and to enhance the production of endothelin.¹⁹ Endothelial dysfunction could have potentially harmful effects on the vessel wall and myocardium.

In a previous study we showed that acute exposure to cyclosporin at concentrations of 100–500 ng/ml did not affect the ability of the endothelium of human coronary arteries to release the endothelium derived relaxing factor, nitric oxide, in response to substance P.²⁰ After short term exposure in vitro it seems that this vasodilatory mechanism is preserved. To elucidate further the influence of cyclosporin on the endothelium we examined the effect of short term and long term exposure to therapeutic and toxic doses of cyclosporin on endothelium dependent relaxation in the human coronary circulation in vitro and in vivo.

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Patients and methods

IN VITRO STUDY

Epicardial coronary arteries were harvested from seven patients undergoing heart or heart-lung transplantation for reasons other than ischaemic heart disease; they were judged to be free of atheromatous plaque both macroscopically and microscopically. After dissection, they were placed in cold, modified Tyrode's solution (composition (mmol/l): NaCl 136.9, NaHCO₃ 11.9, KCl 2.7, NaH₂PO₄ 0.4, MgCl₂ 2.5, CaCl₂ 2.5, glucose 11.1, disodium EDTA 0.04) and gassed with 95% oxygen/5% carbon dioxide for no more than eight hours.

Forty four vessel segments, 3–5 mm long, were suspended in 5 ml organ chambers between two L-shaped metal hooks, one of which was fixed and the other attached to a Grass FTO-3C strain gauge transducer connected to a Grass 79D polygraph set up to monitor and record changes in segment tension. The temperature of the bath was maintained at 37°C and the Tyrode's solution was continually gassed.

A preload of 50 mN was applied to each segment and after a stabilisation period of 40–60 minutes during which "relaxing-out" occurred and a steady baseline was reached. Potassium chloride (90 mmol/l) was applied to assess tissue viability. After washout of the potassium chloride and when a steady baseline was reached, the segments were exposed for three hours to cyclosporin (powder form donated by Sandoz). The cyclosporin was initially dissolved in methanol to give a stock solution of 0.5 mg/ml methanol; subsequent dilutions in Tyrode's solution gave final bath concentrations of 1000 and 2000 ng/ml. This dilution procedure gives a uniform solution of cyclosporin in Tyrode's solution as assessed by high performance liquid chromatography (unpublished observation). During the three hour incubation period the bath solutions were renewed once. Control incubations were performed in Tyrode's solution or methanol (at a bath concentration of methanol that was identical to that used when it was used as the diluent). A level of precontraction, optimally 60–70% of the potassium response, was then induced with cumulative doses of the thromboxane-mimetic U46619. The relaxation profiles of the preparations were examined by comparing the responses after application of substance P (10^{-11} to 10^{-7} mol/l) with those generated by 5.3×10^{-6} mol/l of glyceryl trinitrate. The ability of the vessels to respond to glyceryl trinitrate was measured against the constriction produced by U46619. In a separate study, 15 segments removed from three patients undergoing re-transplantation of the heart and lung for obliterative bronchiolitis were assessed for endothelium function as described above. These patients had been shown to have angiographically normal coronary arteries and had been treated with immunosuppressive doses of cyclosporin for a mean of 22 months; in the six week period before re-transplantation blood concentrations of the cyclosporin parent compound had

ranged from 43 to 258 ng/ml, and on the day before the operation concentrations were between 40 and 95 ng/ml.

IN VIVO STUDY

The effect of intracoronary substance P (1.4–24 pmol/min) and isosorbide dinitrate (2 mg for 2 minutes) infusion was also examined in 12 cardiac transplant recipients who were clinically well 1–5 years postoperatively. These substances possess only vasodilatory properties, and their use in this study was approved by the district ethics committee. The percentage changes in diameter were measured by a computerised analysis system (CAAS-Pie Data Medical). The blood concentrations of cyclosporin were simultaneously measured by a conventional radioimmunoassay targeted against the cyclosporin parent compound (INCSTAR Corporation). This assay measures total cyclosporin concentration in whole blood.

DATA ANALYSIS

For each patient in the in vitro studies some segments were used as controls while others were treated with only one concentration of cyclosporin. A mean value was obtained for each treatment within a patient, and these means were then averaged to give an overall mean (SEM) for each treatment for the group of patients as a whole. Treatments were compared by an unpaired *t* test, and significance was assigned at *p* < 0.05.

For the in vivo study the substance P and isosorbide dinitrate measurements were taken in duplicate from each patient and the means calculated. An overall mean (SEM) was then calculated for each treatment and compared by an unpaired *t* test. Linear regression analysis was applied to examine the relation between blood concentration of cyclosporin and the response to substance P and isosorbide dinitrate.

Results

In vitro study—We studied vessels removed from hearts at time of transplantation (3 hour cyclosporin incubation—acute effect) or re-transplantation (chronic effect). Substance P

Figure 1 Effect of cyclosporin on the relaxation induced by substance P (10^{-11} – 10^{-7} mol/l) in human epicardial coronary arteries (○ = control, 16 segments from 7 patients; △ = 1000 ng/ml cyclosporin, 14 segments from 7 patients; ● = 2000 ng/ml cyclosporin, 14 segments from 7 patients). Responses are mean (SEM) of segments from seven patients, measured as percentage of relaxation in response to 5.3×10^{-6} mol/l glyceryl trinitrate. **p* < 0.05.

Figure 2 Effect of cyclosporin (Cy) (100–2000 ng/ml) on the relaxation induced by 5.3×10^{-6} mol/l glyceryl trinitrate (GTN) in human epicardial coronary arteries. Open columns: control = 12 segments from four patients; 100 ng/ml cyclosporin = 12 segments from four patients; and 500 ng/ml cyclosporin = 12 segments from four patients. Responses are means (SEM) of segments from four patients. Hatched columns: control = 16 segments from seven patients; 1000 ng/ml cyclosporin = 14 segments from seven patients; and 2000 ng/ml cyclosporin = 14 segments from seven patients. Responses are means (SEM) of segments from seven patients measured as percentage of constriction in response to U46619.

caused dose dependent relaxation in all vessel segments. In the control tissue maximum relaxation was 76.6 (7.4)% in response to 10^{-7} mol/l substance P. This was unaffected by incubation with the methanol vehicle. After incubation with 1000 ng/ml and 2000 ng/ml cyclosporin the maximum relaxations were 63.0 (11.5)% and 62.2 (11.1)% respectively. This reduction in the vasodilatory response was not statistically significant. However, at low concentrations of substance P (10^{-10} and 10^{-7} mol/l) the dilatatory response was significantly reduced ($p < 0.05$) (fig 1). Cyclosporin caused a mild but non-significant reduction ($p > 0.05$) of the relaxations produced by 5.3×10^{-6} mol/l glyceryl trinitrate: 1000 ng/ml, 82.3 (4.3)%, 2000 ng/ml, 82.0 (6.4)%, control, 92.8 (2.4)% (fig 2). This trend was not seen when lower concentrations of cyclosporin were used.²⁰

Segments of coronary artery from the re-transplanted patients had a mean maximum response to 10^{-7} mol/l substance P of 78.0 (11.0)%, which was similar to that of the control tissue. The dose response curves were parallel for each patient although the maximum response varied (fig 3). There was no correlation

Figure 3 Relaxations to substance P (10^{-11} – 10^{-7} mol/l) in 15 segments of epicardial coronary artery from three patients undergoing re-transplantation for obliterative bronchiolitis (○, mean of three patients). Patient 1 (■) 2 days cyclosporin therapy; patient 2 (●) 30 months cyclosporin therapy; patient 3 (□) 37 months cyclosporin therapy.

Figure 4 Correlation curves for blood cyclosporin concentrations and the in vivo responses to (A) substance P (SP) and (B) isosorbide dinitrate (ISDN) measured as percentage vessel diameter change after intracoronary SP (1.4–24 pmol/min) and ISDN (2 mg for 2 min) infusion in 12 cardiac transplant recipients assessed 1–5 years postoperatively.

between maximum response and duration of cyclosporin treatment.

In vivo study—We examined angiograms of the coronary arteries of 12 transplant recipients 1–5 years postoperatively. The mean maximum dilatations in response to substance P and isosorbide dinitrate were 22.1 (3.2)% and 26.0 (2.5)% respectively. These were significantly different from the control value ($p < 0.05$) but not significantly different from each other. There was no correlation between the blood concentration of cyclosporin and the response to either substance P or isosorbide dinitrate (fig 4A and B).

Discussion

We examined the short and long term consequences of therapeutic and toxic exposure to cyclosporin in vitro and in vivo. We showed that cyclosporin does not affect the ability of human coronary arteries to release nitric oxide in response to substance P.

Nitric oxide, released from vascular endothelium, is thought to be important in guarding against vasospasm and platelet aggregation.^{21–23} The consequences of compromised nitric oxide release are now considered to be far-reaching in the human vascular system.^{24–26} Loss of the protective anti-spastic and anti-aggregatory properties of nitric oxide seems to predispose toward constrictor and thrombotic events. Hypertension, renal damage, and atherosclerosis could be caused or aggravated by malfunction of the endothelium.

Nitric oxide release was reduced in animal preparations treated with cyclosporin. Specific endothelial damage was shown to be associated with the duration of cyclosporin administra-

tion. Thus after three hours¹⁰ and five days¹¹ the ability of the endothelium of rat aortic rings to produce nitric oxide was affected but the complete response to endothelium independent vasodilatation with nitrates was retained. However after three weeks¹³ and eight weeks¹¹ the smooth muscle response was reduced; so the assessment of a specific effect on the release of endothelium derived nitric oxide is difficult. We also found that in the isolated rat heart the endothelium dependent and independent responses were reduced by cyclosporin. In human tissues there seems to be a loss in the sensitivity of the relaxatory response at low concentrations of substance P; however, the nitric oxide and nitrate maximum responses are not significantly compromised. This holds true for a range of cyclosporin concentrations in both acute and chronic experiments. At high doses in vitro there was a blunting of the nitrate response which did not reach statistical significance. It is therefore possible that prolonged exposure to toxic concentrations of cyclosporin may produce smooth muscle damage.

The results obtained in coronary arteries from re-transplantation can be regarded as preliminary data from a small group of patients. They serve to confirm that the preservation of the response as measured in the in vivo study is reproducible in the isolated organ bath and that the duration of cyclosporin treatment does not correlate with inhibition of nitric oxide release.

Despite the results from the in vitro experiments, it is still possible that patients who were treated with cyclosporin for months and years have altered endothelial function. This question cannot be adequately addressed by in vitro techniques because we could only obtain a small number of arteries from patients who had been receiving cyclosporin. Therefore, the in vivo study was designed to examine the consequences of chronic exposure of the endothelium to therapeutic concentrations of cyclosporin. The mean vasodilatory response to substance P was not significantly different from the responses obtained in normal non-transplanted patients with no coronary artery disease.²⁷ Chronic exposure to cyclosporin did not seem to alter endothelial function as assessed by the ability of substance P to release nitric oxide in the coronary arteries of cardiac transplant patients. We used substance P to assess endothelial function because it has a vasodilatory action only. Previous studies of acetylcholine infusion in transplant recipients suggested endothelial dysfunction because it caused vasoconstriction.²⁸ However, acetylcholine produced vasoconstriction in any vessel when a high enough dose was used,²⁹ which makes it an unreliable agent for the examination of endothelium mediated dilatation.

Cyclosporin affected some endothelial functions; factor VIII concentration increased³⁰ while prostacyclin production was impaired¹⁸; concentrations of endothelin, the potent endothelium derived constrictor, were raised.¹⁹ However, our study has shown that the capacity to release nitric oxide is preserved in human coronary endothelium. Cyclosporin

does inhibit endothelial prostacyclin production,¹⁸ although there has been conflicting experimental evidence on prostaglandin excretion in cyclosporin nephrotoxicity.³¹ It seems that interaction of cyclosporin with endothelium derived vasodilators has no direct role in the genesis of hypertension, renal damage, and accelerated graft atherosclerosis in the transplant recipient. It is more likely that the vasospasm and vascular damage associated with cyclosporin toxicity is caused by a combination of effects on constrictor influences (such as endothelin¹⁹ and thromboxane³²⁻³⁴) as well as a possible direct effect of cyclosporin on smooth muscle.¹⁴⁻³⁵⁻³⁷ These may be superimposed on the observed increase in sympathetic nerve activity.¹⁶

We conclude that the nitric oxide response remains unaffected in coronary arteries of patients exposed to cyclosporin. The mechanisms of vascular damage in patients receiving cyclosporin need further elucidation if we are to evolve rational methods of preventing this important complication.

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