Effect of ischaemic preconditioning on regional release of inflammatory markers

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ABSTRACT

Systemic markers of inflammation may be increased in patients after percutaneous coronary intervention. In the present study, we evaluated whether IP (ischaemic preconditioning) attenuated inflammation by activating K_{ATP} (ATP-sensitive potassium) channels in patients undergoing coronary angioplasty. Patients ($n = 36$) undergoing angioplasty of a major left coronary artery were allocated randomly to one of four groups: a control group, a group receiving nicorandil (an agonist of K_{ATP} channels), an IP group or an IP group pretreated with glibenclamide (an antagonist of K_{ATP} channels). To measure the release of sCD40L, P-selectin and myeloperoxidase from the ischaemic region, blood samples were drawn simultaneously from the ascending aorta and the great cardiac vein before and 15 min after coronary angioplasty. At 15 min after angioplasty, a significant increase in sCD40L and P-selectin levels in the great cardiac vein in the control group was observed. IP- and nicorandil-treated patients did not show a significant change in sCD40L and P-selectin levels in response to angioplasty. However, the IP-induced attenuation of sCD40L and P-selectin release was abolished by administering glibenclamide. The change in myeloperoxidase levels mirrored those of sCD40L and P-selectin. The levels of inflammatory markers in the aorta remained stable throughout the study. Patients undergoing angioplasty had increased sCD40L and P-selectin levels in the ischaemic region. In conclusion, IP abolished angioplasty-induced myeloperoxidase release by preventing activated platelet-induced P-selectin release via a K_{ATP}-channel-initiated pathway. Therefore, in addition to its primary effect on cardioprotection, IP may also provide beneficial anti-inflammatory effects on the interaction between platelets and neutrophils.

INTRODUCTION

PCI (percutaneous coronary intervention) has been widely performed in the management of coronary atherosclerosis, and has been shown to elicit an inflammatory response by activation of platelets and leucocytes [1]. Numerous inflammatory factors representing regulatory pathways might be involved, such as CD40L (CD40 ligand; also known as CD154), P-selectin and MPO (myeloperoxidase). Furthermore, activated platelets expressing both CD40L and P-selectin can trigger the up-regulation of MPO in leucocytes in a P-selectin-dependent manner [2].

CD40 and CD40L, members of the TNF (tumour necrosis factor) family, are expressed on endothelial cells, vascular smooth muscle cells, mononuclear cells and platelets [3]. CD40L is stored in the cytoplasm of resting platelets and is rapidly presented on the surface after activation

Key words: angioplasty, coronary disease, inflammation, ion channels, ischaemic preconditioning, platelets, reperfusion.
Abbreviations: CAD, coronary artery disease; CD40L, CD40 ligand; i.c., intracoronary; IL, interleukin; IP, ischaemic preconditioning; K_{ATP} channel, ATP-sensitive potassium channel; MLR, myocardial lactate extraction ratio; MPO, myeloperoxidase; PCI, percutaneous coronary intervention; sCD40L, soluble CD40L; sIL2R, soluble IL-2 receptor; TNF, tumour necrosis factor.
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[3]. The surface-expressed CD40L is subsequently cleaved, generating a soluble, but functional, fragment, sCD40L (soluble CD40L) [4]. Platelet-derived CD40L up-regulates pro-inflammatory mediators, such as the expression of adhesion molecules and pro-inflammatory cytokines, and induces chemokines [3]. Elevated sCD40L levels predict an increased cardiovascular risk in healthy subjects and in patients with CAD (coronary artery disease) [5,6]. Blockade of the CD40/CD40L system may inhibit the interaction between platelets and endothelium, and hence limit inflammation in response to coronary intervention. Therefore sCD40L has been suggested as a potential therapeutic target to modulate vascular inflammation and possibly influence cardiovascular risk.

P-selectin, mainly located in α-granules of platelets, is a major determinant of platelet and neutrophil interaction [7]. The proximity of the endothelium to circulating leucocytes makes it an important early target for neutrophil adherence and subsequent neutrophil-mediated damage [7]. Intravital microscopic analysis of leucocyte rolling in postischaemic microvessels has revealed a major role for P-selectin [8]. Indeed, a monoclonal antibody to P-selectin [9], as well as the absence of P-selectin expression in knockout mice [10], significantly abrogate neutrophil sequestration. Neutrophils have been implicated as mediators of lethal injury after reperfusion to coronary vascular endothelium and cardiomyocytes [11]. One of the principal mediators secreted upon neutrophil activation is MPO, an indirect measurement of neutrophil infiltration.

Myocardial IP (ischaemic preconditioning) is a potent endogenous mechanism of cardioprotection in which short periods of myocardial ischaemia result in resistance of the myocardium to subsequent ischaemia [12]. The earliest demonstration of the profound biological effect of IP was the ability to decrease infarct size [12]; however, it cannot be obtained in clinical settings. Thus surrogate end points have been used, including ST-segment shifts in the surface or i.c. (intracoronary) ECG, metabolic markers such as lactate and ATP, and the release of creatine kinase and troponin [13]. We have demonstrated previously [14] that IP is mediated, at least in part, by the mitochondrial K_{ATP} (ATP-sensitive potassium) channel. The opening of these channels may be important in IP, because inhibition of K_{ATP} channels with glibenclamide abolishes the cardioprotective effects of IP in both experimental and clinical studies [15,16]. The use of agents (nicorandil) to open this channel may mimic a physiological response which acts to attenuate ischaemic injury [16]. Previous studies have shown that, in additional to its anti-ischaemic effect, IP attenuates the expression of inflammatory markers such as IL (interleukin)-1β, IL-6 and TNF-α [17]. However, nothing is known about the effect of IP on plasma levels of sCD40L and P-selectin. To assess whether IP influences the inflammatory process in patients undergoing coronary intervention in the present study we measured the effect on circulating and coronary levels of sCD40L, P-selectin and MPO during periprocedural coronary intervention. We have also investigated whether the observed anti-inflammatory effects of IP are caused by the activation of K_{ATP} channels through use of glibenclamide, a K_{ATP} channel blocker, and nicorandil, a K_{ATP} channel agonist.

METHODS

Study population

The study was conducted prospectively. All patients fulfilled the entry criteria: (i) a history of chronic stable angina pectoris $\geqslant 3$ months and a positive stress thallium or exercise test for myocardial ischaemia; (ii) no history of previous myocardial infarction or pathological Q waves, or bundle branch block on an ECG that could have interfered with the interpretation of ST-segment changes; (iii) a single proximal or mid-epicardial left coronary artery lesion; and (iv) successful balloon angioplasty resulting in residual stenosis $< 30\,\%$ in diameter. We have demonstrated previously [18] that the PCI procedure may fail to elicit IP in elderly patients $\geqslant 65$ years of age. To avoid the confounding factor, only patients < 65 years of age were included. Because P-selectin may be affected by different shear force within the stenotic segments [19], patients were highly selected with similar diameter stenosis between 70 and 90 %. To ensure that the collateral circulation of the study patients was homogenous, patients with angiographically visible collateral blood at baseline were excluded. All patients had no history of diabetes mellitus. Patients were instructed to avoid non-steroid anti-inflammatory agents, because such drugs shed selectin [20], with the exception of aspirin (100 mg/day). We excluded patients taking clopidogrel, which inhibits CD40L expression of platelets [21]. Medication, including calcium channel and β-adrenergic blockers was withheld, except aspirin for 24 h before the procedure. Any patients who had taken nitroglycerine within 4 h of catheterization were excluded from the present study.

To determine the potential role of K_{ATP} channel in IP-related inflammatory markers, glibenclamide (10 mg) was administered orally 60 min before catheterization with a continuous infusion of 10 % dextrose at the same time. Although glibenclamide is an antagonist of K_{ATP} channels, there are many potential non-specific targets of glibenclamide, including the inhibition of Na^+ channels and the opening of Ca^{2+} channels [22]. These alternative effects could confound the interpretation of the findings of the present study. To confirm further whether activation of K_{ATP} channels was mandatory for IP, patients treated with nicorandil were assessed. Nicorandil was administered intravenously at a dose of 80 μg/kg of body weight, which has been shown to specifically activate

mitochondrial K_{ATP} channels without the interference of a nitrate effect [23]. To ensure that neither the technical manoeuvres (e.g. blood sampling and catheter manipulation) nor the contrast medium used resulted in changes in sCD40L, P-selectin and MPO, ten patients undergoing coronary angiography without angioplasty were selected and matched according to age and sex.

The study was approved by the Institutional Review Board, and all subjects provided written informed consent before participation.

Study protocol

Catheterization procedures

Because of circadian fluctuation in selectin levels [24], cardiac catheterization was performed between 13.00 and 15.00 hours. Only one study patient was enrolled on any particular day. Diagnostic left heart catheterization and angiography were performed as described previously [25]. After completion of the diagnostic catheterization, intravenous heparin was supplemented to maintain activated clotting time at 300–350 s, and a 6F Judkins guiding catheter was advanced to the ostium of the left coronary artery. To assess collateral flow during coronary occlusion, a 0.36 mm Doppler wire (FloWire; Cardiometrics Inc.) was first introduced through a standard angioplasty-type Y-connector attached to the angiographic catheter. The wire tip was positioned such that a characteristic and stable flow velocity waveform was obtained. Collateral blood flow was defined as retrograde or persistent antegrade flow during balloon occlusion as described previously [25]. The distal segment of the guide wire was placed 2–3 cm beyond the balloon catheter tip. The external end of the guide wire was connected to the chest lead by a sterile alligator clamp to record an i.c. ECG. Multiple pairs of perpendicular views (90°) of the left and right coronary arteries were obtained. The precise angle, skew rotation and table height of each projection were recorded so that the projection could be duplicated. Quantitative measurements of coronary artery dimensions were made using a computer-based edge enhancement technique (DCI System; Philips), as described previously [25]. For each lesion, the view showing the most severe degree of stenosis was used for analysis. Ionic contrast medium was used in all patients to prevent platelet degranulation induced by non-ionic contrast medium [26]. Blood pressure and heart rate were monitored continuously during the procedure. Patients were not medicated with sedatives.

Angioplasty procedure

After angiographic collateral assessment and i.c. ECG monitoring, the lesion was crossed with a balloon. The optimal balloon size was determined by quantitative evaluation of the coronary artery diameters adjacent to the stenosis. After the balloon was positioned across the lesion, the patients underwent two 2 min balloon inflations separated by 1 or 2 min intervals of reperfusion (Figure 1) with the Doppler guide wire remaining across the lesion at the same site for each successive recording. A recovery time of 1 min was adequate to re-establish baseline non-ischaemic conditions assessed by i.c. ECG and cardiac pain. Previous studies have shown that an interval of more than 2 min between balloon inflations is required to achieve IP during angioplasty [27]. During the reperfusion intervals, the angioplasty balloons were withdrawn from the stenotic site and the guide wire was left at the same position. Because balloon pressure is a determinant of cardiac pain during coronary angioplasty [28], balloon pressure was kept identical during the first and second inflations in each patient, with inflation pressures ranging from 6.0–10.0 atm (where 1 atm ≡ 101.325 kPa).

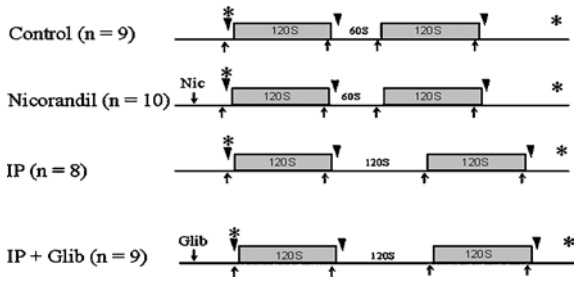

Figure 1 Summary of study protocol

Grey boxes indicate the period of balloon inflation. Arrows indicate when intracoronary ECGs and chest angina score were determined. Arrowheads indicate when the lactate measurements were taken simultaneously from the great cardiac vein and the aorta. Asterisks indicate when sCD40L, P-selectin and MPO measurements were made simultaneously from the great cardiac vein and the aorta. Glib, glibenclamide; Nic, nicorandil.

Assessment of myocardial ischaemia

The i.c. ECG was recorded on-line at a paper speed of 25 mm/s during the two balloon inflations and at selected times after deflation. Calibration was performed at the beginning of the procedure (1 mV = 5 mm). ST-segment elevation was evaluated by two observers, who viewed the ECGs in a random and blinded manner. Differences in interpretation were resolved by consensus. Changes in ST-segment levels at baseline were used as the control, and the differences in ST-segment levels between the control and at the ends of the first inflation and the second inflation were compared to evaluate the severity of myocardial ischaemia.

To confirm myocardial ischaemia during balloon inflation, selective catheterization of the great cardiac vein was attempted successfully. Simultaneous samples of the aortic root and the great cardiac vein at the same speed were obtained for measurements of lactate content. MLR

(myocardial lactate extraction ratio) was calculated using the following formula: $[(L_{AO} - L_{GCV})/L_{AO}] \times 100$ where L_{AO} and L_{GCV} represent plasma lactate concentrations in the aortic root and in the great cardiac vein respectively.

Prior to coronary angioplasty, patients were informed that they might develop chest pain during the balloon inflation. Immediately before termination of balloon inflation, patients were asked to quantify the intensity of cardiac pain by using a visual-analogue scale of 0–10 [no pain (0) to the most severe pain (10)], as described previously [29].

Laboratory measurements

Because of a local release of sCD40L, P-selectin and MPO levels at the dilated sites, blood samples from the aortic root and the great cardiac vein were obtained simultaneously for measurements of local inflammatory marker production at baseline and at the end of the study. Because P-selectin may be released into the plasma within 10–20 min of reperfusion [30], sampling was performed 15 min after the last balloon inflation. In the patients undergoing coronary angiography, blood samples were obtained from the aorta and the great cardiac vein immediately before and 15 min after the coronary angiography procedure.

Plasma samples were thawed once and analysed in duplicate for sCD40L, P-selectin, MPO and sIL2R (soluble IL-2 receptor), a reliable marker for T-cell activation [31], using a commercially available ELISA kit with an assay reproducibility of > 95 % (R&D Systems).

Previous studies have shown that both increased glucose and insulin levels enhance release of P-selectin [32]. To determine the confounding roles of glucose and insulin in P-selectin levels, sinus blood samples, reflecting local concentrations for glucose and insulin concentrations, were assayed.

Blood (10 ml) was collected in 3.8 % citrate (9 parts blood to 1 part sodium citrate) and was used for the biochemical analysis and sIL2R, sCD40L, P-selectin and MPO levels. Myocardial release ratio was calculated using the formula: $[(C_{GCV} - C_{AO})/C_{AO}] \times 100$ where C_{GCV} and C_{AO} represent plasma concentrations in the great cardiac vein and in the aortic root respectively.

Statistical analysis

Continuous variables are expressed as means ± S.D. Values were analysed using ANOVA among the groups. A two-way repeated-measures ANOVA was used to search for possible effects of IP and glibenclamide on the measurements of i.c. ECG, lactate, sCD40L, P-selectin, MPO and sIL2R and, if an F value was found to be significant, a two-tailed Student t test for paired observations with Bonferroni's correction was used to test for significant differences. Visual analogue scales were analysed using the Wilcoxon signed rank test. χ^2 analysis was used for categorical variables, and Fisher's exact test was used for patient numbers less than five. Linear regression models were used to compute the association between the severity of myocardial ischaemia and the levels of inflammatory markers in IP groups alone or in combination with glibenclamide. Probability values are two-tailed, and a value of $P < 0.05$ was considered to be statistically significant.

Table 1 Clinical characteristics of study subjects

Values are means ± S.D., or number (%). ARB, angiotensin receptor blocker; Glib, glibenclamide; LAD, left anterior descending artery; LCX, left circumflex artery.

	Treatment group			
Parameters	Control	Nicorandil	IP	IP + Glib
n	9	10	8	9
Age (years)	51 ± 4	48 ± 6	50 ± 4	49 ± 5
Gender (male/female)	7/2	7/3	6/2	7/2
CAD risk factor				
Hypertension (*n*)	5 (56)	6 (60)	4 (50)	5 (56)
Smoking (*n*)	3 (33)	3 (30)	3 (38)	3 (33)
Total cholesterol (mg/dl)	245 ± 31	241 ± 36	246 ± 21	239 ± 35
Triacylglycerols (mg/dl)	213 ± 47	245 ± 82	236 ± 91	241 ± 53
Medication (*n*)				
ARB	2 (22)	3 (30)	2 (25)	3 (33)
β-Blocking agents	4 (44)	3 (30)	4 (50)	4 (44)
Calcium blocker	2 (22)	3 (30)	3 (38)	3 (33)
Statins	4 (44)	6 (60)	5 (63)	5 (55)
Vessel disease (*n*)				
LAD	6 (66)	7 (70)	6 (75)	7 (78)
LCX	3 (33)	3 (30)	2 (25)	2 (22)

RESULTS

A total of 43 consecutive patients were enrolled, but seven of these patients (16 %) had one or more of the exclusion criteria, leaving a total of 36 participants who were randomized into four groups (Figure 1). The clinical features of the patients are shown in Table 1. There were no significant differences among the study groups for patient age, gender or the frequency of cardiovascular risk factors. Coronary stenosis was reduced similarly among the groups. Glucose levels remained stable throughout the study (Table 2), whereas insulin concentrations were significantly increased in patients given glibenclamide. The balloon pressure used was similar in each group, and there was no myocardial injury in any patients after angioplasty, as determined by ECG.

Haemodynamics

No significant changes in mean blood pressure and heart rate among the four groups at baseline and after

Table 2 Blood glucose, insulin, haemodynamic characteristics and Doppler collateral circulation in the study subjects

Values are mean ± SD. Glib, glibenclamide; SBP, systolic blood pressure.

	Treatment group			
Parameters	Control	Nicorandil	IP	IP + Glib
n	9	10	8	9
Blood glucose (mg/dl)	90 ± 5	87 ± 7	92 ± 5	86 ± 6
Insulin (μ-units/ml)	7.4 ± 2.1	8.2 ± 1.8	9.3 ± 2.5	104 ± 19
SBP (mmHg)				
Baseline	134 ± 10	131 ± 15	128 ± 10	125 ± 17
Inflation 1	128 ± 11	129 ± 16	132 ± 15	131 ± 12
Inflation 2	130 ± 12	125 ± 12	124 ± 13	119 ± 12
Heart rate (beats/min)				
Baseline	72 ± 6	76 ± 9	75 ± 8	77 ± 14
Inflation 1	78 ± 5	74 ± 12	73 ± 14	73 ± 9
Inflation 2	76 ± 6	81 ± 7	77 ± 12	83 ± 12
Collaterals (cm)				
Inflation 1	3.2 ± 0.6	3.0 ± 0.4	3.0 ± 0.5	3.2 ± 0.5
Inflation 2	3.1 ± 0.5	3.0 ± 0.4	3.3 ± 0.7	3.0 ± 0.8
Degree of stenosis (%)				
Before angioplasty	81 ± 6	85 ± 5	84 ± 5	84 ± 3
After angioplasty	8 ± 8	8 ± 3	9 ± 5	9 ± 6

the first and second angioplasty procedures were detected (Table 2). Rate pressure product, an index of oxygen consumption, was comparable among the four groups. The quantitative variables for assessment of the collateral circulation obtained during the first and second balloon inflations indicated the presence of low-grade collaterals, which did not differ among the study groups.

Myocardial ischaemia

Cardiac pain

All patients had no symptoms before each balloon inflation. In the control group, the severity of chest pain was similar between the first and second inflations (Table 3). Chest pain in the IP group during the second inflation was significantly less than during the first inflation, indicating effective IP. In contrast, cardiac pain was significantly increased in IP patients pretreated with glibenclamide.

i.c. ECG

Before each inflation, there was no ST-segment shift in the i.c. ECG. In the control group, the mean ST-segment shift during the second balloon inflation was similar to that observed during the first inflation (Table 3). In the IP patients, the ST-segment shift was significantly less ($P < 0.0001$) during the second inflation than during the first inflation, consistent with IP (Table 3). The reduction in the ST-segment shift afforded by nicorandil in the first inflation (−59% compared with the first inflation in the IP group) was similar to that afforded by IP (−50% during the second compared with the first inflation). Patients given glibenclamide developed a higher ST-segment shift during the second inflation than that in the IP group alone (Table 3).

Lactate measurements

The respective baseline values were positive and similar, indicating the absence of significant lactate production in the pre-angioplasty state. MLR was more negative in the control group than in the IP group during the second inflation, indicating less lactate production from

Table 3 Myocardial ischaemia assessed by subjective chest pain, values of ST-segment shift and MLR throughout the study

Values are means ± S.D. ST-segment shift was defined as the differences in ST-segment levels between baseline and end of the first and second inflations. $^*P < 0.05$ compared with inflation 1 within the same group; †$P < 0.05$ compared with the controls sampled at the same time. Glib, glibenclamide.

	Treatment group			
Parameters	Control	Nicorandil	IP	IP + Glib
n	9	10	8	9
Chest pain (arbitrary units)				
Inflation 1	5.7 ± 1.0	3.4 ± 0.8†	5.8 ± 1.4	5.4 ± 0.9
Inflation 2	5.4 ± 1.1	3.6 ± 1.1†	3.9 ± 0.8*†	6.0 ± 0.9
ST-segment shift (mV)				
Inflation 1	1.03 ± 0.14	0.41 ± 0.06†	0.98 ± 0.13	0.93 ± 0.13
Inflation 2	1.01 ± 0.15	0.41 ± 0.02†	0.49 ± 0.13*†	1.06 ± 0.16
MLR (%)				
Baseline	19 ± 11	9 ± 27	15 ± 27	10 ± 23
Inflation 1	−120 ± 57	−42 ± 35†	−142 ± 72	−129 ± 33
Inflation 2	−127 ± 30	−45 ± 19†	−35 ± 26*†	−156 ± 49

Table 4 sCD40L, P-selectin and MPO activity sampled from the aorta in the four treatment groups, along with a group of patients undergoing angiography alone

Values are means ± S.D. For the treatment groups, Before procedure and After procedure relate to before and after angioplasty respectively, whereas, in the angiography alone group of subjects, this refers to before and after angiography respectively. $^*P < 0.05$ compared with the angioplastied patients in the four treatment groups sampled at the same time. Glib, glibenclamide.

	Treatment group				
	Control	Nicorandil	IP	IP + Glib	Angiography
n	9	10	8	9	10
sCD40L (ng/ml)					
Before procedure	1.66 ± 1.47	1.28 ± 1.15	1.24 ± 1.11	1.24 ± 1.10	0.83 ± 0.78*
After procedure	1.64 ± 0.94	1.44 ± 1.45	1.31 ± 1.21	1.44 ± 1.34	0.88 ± 0.82*
P-selectin (ng/ml)					
Before procedure	94 ± 24	81 ± 22	96 ± 24	84 ± 29	70 ± 19*
After procedure	91 ± 24	89 ± 16	85 ± 17	86 ± 11	72 ± 21*
MPO (ng/ml)					
Before procedure	24 ± 10	23 ± 7	25 ± 11	24 ± 7	19 ± 10*
After procedure	24 ± 8	25 ± 6	23 ± 5	26 ± 5	16 ± 9*

ischaemic myocardium. The benefits of metabolic features were abolished after administering glibenclamide prior to IP.

Inflammatory markers

No significant changes in the levels of sIL-2R before and after coronary angioplasty either in the aorta or great cardiac vein among the groups was observed (results not shown).

sCD40L level in the aorta was significantly higher in patients undergoing angioplasty compared with the patients undergoing coronary angiography (Table 4). In the ten subjects who underwent coronary angiography, no change in sCD40L levels was observed before and after angiography in samples from the aorta and the great cardiac vein (results not shown). In contrast, the myocardial release ratio of sCD40L was increased significantly 15 min after angioplasty compared with those before angioplasty in the control group (Figure 2). The mean levels of sCD40L remained stable in the aorta during the procedure in all of the groups. The increase in sCD40L level in the great cardiac vein between baseline and after angioplasty was 67% less in IP patients compared with controls. The attenuated release of sCD40L in the IP group was abolished when glibenclamide was administered.

Changes in P-selectin and MPO in the aorta and great cardiac vein before and after angioplasty mirrored the changes in sCD40L (Figure 2).

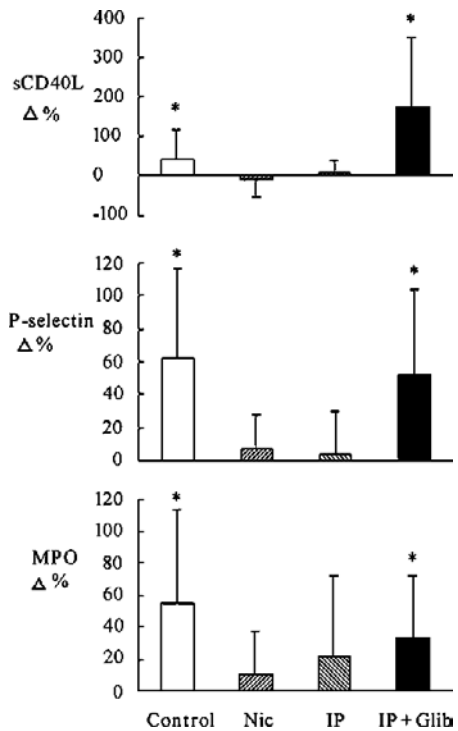

Figure 2 Myocardial release ratio of sCD40L, P-selectin and MPO between the great cardiac vein and aorta in response to angioplasty in the four groups

Myocardial release ratio was calculated as described in the Methods section. Data are expressed as means ± S.D. Glib, glibenclamide; Nic, Nicorandil; $^*P < 0.05$ compared with the nicorandil- and IP-treated groups after angioplasty.

Correlation of IP with inflammatory markers

To assess whether the observed effect of IP of attenuating the increase of inflammatory markers after angioplasty was due to the prevention of ischaemic events, Pearson's correlation was performed between ST-elevation, as a surrogate of myocardial ischaemia, and sCD40L, P-selectin and MPO sampled from the great cardiac vein 15 min after the end of the second balloon inflation. The

correlation was not significant (adjusted R^2=0.10, 0.18, 0.21 for sCD40L, P-selectin and MPO respectively; all the P values were not significant), suggesting that IP-induced myocardial energy preservation did not play a role in attenuating inflammatory markers.

DISCUSSION

The present study confirms that brief periods of myocardial ischaemia during PCI result in the up-regulation of inflammatory markers and provides direct evidence for the first time in humans that increased inflammatory markers can be attenuated by IP, suggesting that IP may limit the activation of platelets and neutrophils assessed by cytokine release. This attenuated inflammatory response in the IP group can be attributed to the activation of K_{ATP} channels, because the channel blocker, glibenclamide, inhibited the IP-induced effect. Our results are consistent with previous studies showing the persistence of *in vivo* platelet activation despite the conventional use of aspirin [33]. Inhibition of platelet activation and a reduction in the up-regulation of sCD40L and P-selectin led to a decrease in the release of MPO from neutrophils. Thus the attenuated release of inflammatory markers induced by IP can be recognized as an integral part of the benefits of IP in addition to its primary cardioprotective infarct-limiting and anti-arrhythmic effects.

Our conclusions are supported by three lines of evidence. First, coronary angioplasty resulted in the up-regulation of inflammatory markers, consistent with the notion that inflammatory markers are activated immediately in ischaemia/reperfused heart [34]. The increased regional inflammatory markers after angioplasty may provide a clue to the mechanisms of myocardial injury of the culprit lesion. Besides the atherosclerotic arterial wall, inflammatory markers may also originate from myocardial inflammation in response to plaque rupture and microembolization [35]. Microembolization results from a fissuring/rupturing of the atherosclerotic plaque in an epicardial coronary artery. Thus the site of inflammation may be the atherosclerotic arterial wall and also the microembolization-related myocardium at risk. No such changes in leucocyte and platelet function were found in subjects who underwent routine coronary angiography. Secondly, the angioplasty-induced increase in inflammatory markers can be attenuated by IP. IP may target cellular processes involved in leucocyte recruitment as end effectors of this phenomenon. IP abrogated the increase in P-selectin levels thus preventing neutrophil infiltration, as P-selectin-dependent leucocyte rolling is a requisite step in this process. Since the attenuation of the angioplasty-induced up-regulation of inflammatory markers by IP was unchanged in the aorta, it would appear that this effect is not non-specific and alterations in inflammatory marker levels are confined to regions of the ischaemic myocardium. Thirdly, activation of K_{ATP} channels serves as an effector of IP in the myocardium. As leucocyte activation can be prevented by administration of exogenous K_{ATP} channel agonists, it is tempting to postulate that K_{ATP} channel activation underlies the anti-adhesive actions of IP. This finding was corroborated further by the inhibition of this effect in the group receiving glibenclamide, a K_{ATP} channel blocker, prior to IP.

IP and inflammation

The mechanisms by which IP affects the inflammatory markers remain undefined. However, several factors can be excluded. (i) Haemodynamics and collateral circulation. Clearly, IP did not exert any haemodynamic effects nor was it associated with an increase in myocardial collateral blood flow. Previous studies have shown that attenuated P-selectin levels may result from less myocardial ischaemia [36]. Ischaemia alone activates endothelial cells to express adhesion molecules on their membrane surface [36]. On the basis of the correlation between inflammatory markers and ST-elevation, myocardial ischaemia only explains 10–21 % of the variation in inflammatory markers. Therefore the attenuated release of inflammatory markers by IP cannot be attributed solely to myocardial ischaemia. This finding reinforces further the notion that MPO is a prerequisite, rather than a consequence, of myocardial injury. (ii) Technical factors. The data obtained in the patients undergoing coronary angiography, who had no changes in inflammatory marker levels, indicate that the increase in these levels after angioplasty was not dependent on some technical factors related to the procedures, such as femoral artery puncture, cardiac catheterization or contrast medium injection. (iii) Differences in the severity of coronary stenosis. Although stenosis-induced shear forces have been shown to increase release of P-selectin [21], it is not a major determinant of the observed changes, because the patients were randomly allocated with similar severities of coronary stenosis among the groups. (iv) Differences in insulin concentrations. Insulin has been shown to modulate platelet and leucocyte activity [37]. Although glibenclamide stimulates insulin secretion, as shown in the present study, the increased insulin levels cannot be a confounding factor in interpreting P-selectin levels, because P-selectin levels from the glibenclamide-treated group were similar to those in the control group.

Our results confirm several studies that have shown that coronary angioplasty is associated with platelet activation [1]. The mechanism responsible for enhanced inflammation by PCI is still unclear. sCD40L and P-selectin derived from platelets share common features, but show different biological activity. Platelets express and secrete CD40L within seconds of activation, and sCD40L levels gradually increase through the cleavage and release of the membrane-bound form. Similarly,

P-selectin is expressed in both endothelial cells and platelets where, upon activation, it may be secreted rapidly into the plasma. The rapid increase in P-selectin levels after coronary angioplasty is likely to represent mobilization of a preformed pool of this adhesion molecule, because *de novo* protein synthesis generally requires 3–6 h [38].

Previous data have suggested a link between the anti-neutrophil effect of IP and activation of K_{ATP} channels [39], and that neutrophil inhibition is a mechanism by which K_{ATP} channel openers may reduce reperfusion injury. Our present results are not consistent with a recent study [40]. Hu et al. [40] found that the effect of IP induced by volatile anaesthetics to attenuate neutrophil adherence was not related to the use of glibenclamide in isolated rat hearts. An explanation for the apparent discrepancy between these findings may be related to methodological differences, including those related to species, and protocols. In fact, our results are consistent with the findings by Mizumura et al. [39], showing that bimakalim reduced transmural MPO activity by activating K_{ATP} channels in the ischaemic myocardium. These data support an important role for K_{ATP} channels in neutrophil activation during reperfusion.

Clinical implications

The clinical implications of the present findings are of interest, as increased levels of inflammatory markers have been shown to be associated with immediate vasoconstriction and late restenosis after angioplasty [41]. The association between IP and an attenuated inflammatory response is consistent with the view that IP is associated with a beneficial short- and long-term outcome [41]. Cipollone et al. [5] have shown that the post-procedural level of inflammatory markers such as sCD40L is predictive of late restenosis. Activated platelets release growth factors, and platelet adhesion to the subendothelial matrix might contribute to proliferation of vascular smooth muscle cells. Our present data show attenuated levels of inflammatory markers in patients undergoing IP, thus potentially reflecting improved results during long-term follow up. The lower level of inflammation may explain, at least in part, the view that IP reduces in-hospital adverse ischaemic events, as well as the risks of death or non-fatal myocardial infarction at 1 year [41]. Furthermore, the attenuated release of IP-related MPO, a key player in ventricular remodelling, is consistent with a recent study [42] showing that IP protects against subsequent ventricular remodelling.

Study limitations

There were several limitations of the present study. First, previous studies have shown that both platelets and T-lymphocytes can express CD40L [43]; however, the relative capacity of each cell type to release sCD40L per unit of blood remains unclear. The lack of an effect on sIL2R makes the direct effects of IP on T-lymphocytes an unlikely explanation for our present findings. These results may point to platelets as the major source of plasma sCD40L in patients undergoing angioplasty; however, definite determination of the source of elevated sCD40L will require further investigation.

We did not measure changes in surface expression of P-selectin on circulating platelets by flow cytometry. Two variant cDNAs for P-selectin have been identified by alternative splicing of mRNA, one predicting a soluble form of the molecule lacking the transmembrane domain and the other predicting a membrane-bound molecule. Soluble P-selectin may be considered as an overflow of P-selectin and thus a barometer of P-selectin regulation [43]. Lorant et al. [43] have shown that soluble P-selectin had similar behaviour to the membrane-bound molecule.

From our present study, it is uncertain whether the changes in inflammatory markers could persist for >15 min in patients undergoing coronary angioplasty. However, other studies have provided evidence for the presence of persistent inflammation after coronary angioplasty, consistent with the view that inflammation is not merely a manifestation of acute myocardial ischaemia [44]. In fact, in an experimental study, local inflammatory activation of endothelial cells and leucocytes lasts up to 30 days after balloon injury in the rabbit aorta [45].

Finally, the present study was limited by the lack of long-term observations on the incidence of restenosis and coronary events in the different treatment groups. Patients with the highest levels of P-selectin release after angioplasty might develop vascular occlusion and/or myocardial infarction [1]. It remains to be seen whether inflammatory markers assessed in the present study have any predictive value with regard to thrombotic complications after angioplasty. Additional observations are needed to evaluate the clinical significance and the therapeutic implication of this finding.

Conclusions

The results of the present study suggest that the mechanisms responsible for the clinical benefits of IP may not be limited to metabolic inhibition, but may also involve the inhibition of inflammation by attenuating the local release of sCD40L and P-selectin, and neutrophil-mediated MPO via the activation of K_{ATP} channels. IP resulted in the reduction of inflammation after coronary angioplasty, which is in agreement with its clinical effect in reducing procedural complications and the progression of restenosis.

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