Interleukin-1 receptor antagonist alters the response to vessel wall injury in a porcine coronary artery model

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Abstract

Objective: To determine the influence of IL-1 on the arterial response to experimental injury in porcine models of percutaneous coronary intervention (PCI).

Methods: An intravenous (i.v.) bolus of 0.5 mg/kg followed by a subcutaneous (s.c.) infusion of 2 mg/kg/24 h of human IL-1 receptor antagonist (IL-1ra) inhibited neutrophil recruitment in response to intradermal IL-1. Using this dose regimen, five groups of pigs were studied: Group 1, oversized balloon angioplasty of 2 coronary vessels (14-day infusion, 28th day sacrifice and analysis); Groups 2, 3, 4, and 5, oversized stenting of 2 coronary vessels (Group 2: 14-day infusion, 28th day analysis; Group 3: 14-day infusion, 14th day analysis; Group 4: 28-day infusion, 28th day analysis; Group 5: 28-day infusion, 90th day analysis). Neointimal area was quantified by standard means.

Results: In Group 1, IL-1ra resulted in a 23% decrease in neointimal area (p = 0.04); in Group 2, a 34% increase (p = 0.001); in Group 3, a 38% decrease (p < 0.0001); in Group 4, a 34% decrease (p = 0.0004); and in Group 5, a 41% decrease (p = 0.00001).

Conclusions: IL-1ra was associated with a sustained, significant reduction in neointima after vessel wall injury as long as it is given for the duration of the stimulation of the IL-1 system, in this case at least 28 days. This suggests that therapies based on the antagonism of IL-1 may modulate the coronary artery response to injury.

1. Introduction

The treatment of coronary artery disease has been revolutionized by the introduction of percutaneous methods of revascularisation. Initially balloon angioplasty (BA) was used to dilate discrete proximal stenoses of epicardial coronary arteries. In the early 1990s, endoluminal coronary stenting was introduced as a procedure that increased the safety and applicability of this technique. Currently there is continued growth in the rate of percutaneous coronary intervention (PCI) throughout the world and there are no signs that rates of coronary revascularisation have reached saturation point.

A major limitation of both BA and stenting has been the issue of restenosis — the return of symptoms of coronary insufficiency after an apparently successful procedure. In the case of BA this was based on recoil of the vessel and neointima formation [1]. Stenting with bare-metal stents led to a small reduction in angiographic and clinical restenosis by the elimination of recoil, but the issue of neointima formation remained the basis of significant rates of restenosis [2]. The recent introduction of stents eluting cytostatic agents has had beneficial effects on restenosis rates [3,4] although there remains concerns over the use of this strategy in the setting of acute coronary syndromes, as

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well as the possible adverse effects consequent upon delayed or incomplete vessel healing.

The cell biology of restenosis has been extensively studied in the last few years. Restenosis arises from a stereotypic response to injury by the arterial wall, characterized by neointima formation. The neointima is formed from recruited vascular smooth muscle cells that migrate, proliferate and produce an extracellular matrix that forms the tissue, which re-narrows the vessel lumen [5]. Consequently, there have been considerable efforts in the investigation of anti-proliferative therapies for the amelioration of this response for therapeutic benefit [6].

The stereotypic response to vascular injury follows a classical inflammatory paradigm. Injury causes an up-regulation of adhesion molecules in the vessel wall which in conjunction with adherent thrombus direct leucocyte accumulation. In the case of balloon injury, leucocytes are predominately neutrophils whereas in the more chronic situation of atherosclerosis, monocytes predominate [7].

Inseparable from white cell recruitment is the synthesis and release of inflammatory signalling molecules that transduce the inflammatory signal (cytokines) and direct white cell migration (chemokines). Amongst the inflammatory cytokines, Interleukin-1 (IL-1) is central and important in the initiation of many inflammatory signals from bacterial products, tissue injury and activation of complement. The agonistic proteins of the family, IL-1α and β, signalling over the type 1 IL-1 receptor (IL-1R1), provoke a wide range of inflammatory signals that incite the synthesis of down-stream, signalling cytokines such as IL-6 and IL-8. This system is very tightly regulated by natural antagonists; IL-1 receptor antagonist (IL-1ra), and a non-signalling receptor IL-1R2 which can either be antagonistic at the cell surface or shed and act as a soluble receptor, scavenging for circulating IL-1 [8].

IL-1ra acting as a pure antagonist of signalling over the type 1 receptor has been proposed as a potential therapeutic agent. In man, trials are on going for the use of IL-1ra in chronic inflammatory diseases that include rheumatoid arthritis. Used at high doses and administered chronically by subcutaneous (s.c.) infusion it has been shown that IL-1 agonist signalling can be completely interrupted [9]. IL-1ra gene polymorphism (carriage of *2 allele) has been associated with a lower incidence of restenosis after coronary stenting [10,11]. As this polymorphism has functional significance, these findings provide further evidence that alteration in an individual’s inflammatory genotype may modulate the blood vessel response to injury, via an IL-1 dependent mechanism.

Laboratory studies have suggested that IL-1 may be involved in the vessel wall response to injury. IL-1 is a smooth muscle mitogen, dependent upon autocrine platelet derived growth factor production by vascular smooth muscle cells. Administration of IL-1 to the porcine coronary artery causes neointima formation [12]. BA of porcine coronary arteries induces vessel wall IL-1 production [13]. The absence of IL-1ra in mice promotes a 250% increase in neointima formation after injury [14].

In this study we report the results of a study where human recombinant IL-1ra has been administered to pigs immediately prior to and then for periods between 14 and 28 days following coronary BA and coronary stenting. The results indicate a critical role for IL-1 in the coronary artery wall response to injury.

2. Methods

2.1. Animals

In vivo work was performed on normo-lipemic, domestic, crossbred Yorkshire White pigs (Sus scrofa). They were of either sex, obtained from a UK Home Office designated supplier weighing 18–20 kg and were fed unmodified pelleted chow and had free access to water. All procedures were performed under appropriate UK Home Office licences, had been subject to local ethical review and conformed to NIH standards. After an overnight fast, animals were sedated with intramuscular azaperone (12 mg/kg, Janssen Animal Health). Anaesthesia was induced by intravenous (i.v.) propofol, (4 mg/kg, Zeneca Pharmaceuticals) and maintained by inhaled enflurane (4–6%) in oxygen (1 l/min) via endotracheal tube.

2.2. In vivo pharmacokinetics of IL-1ra release

In all animal studies when IL-1ra was compared to vehicle, animals were randomised in a double-blind manner to either one treatment. ‘2ml2’ Alzet miniosmotic pumps (Charles River Ltd, Kent, UK) were primed and filled according to manufacturer’s instructions. ‘2ml2’ pumps elute at a flow rate of 5 µl/h for 14 days. To achieve the desired concentration of 2 mg/kg/24 h, three pumps were inserted per animal. Pumps were inserted into the groin crease. Plasma samples were taken from animals (n = 3) immediately pre-implantation to 28 days post-implantation. To ensure high circulating levels of IL-1ra were present at the time of experimental injury and therefore inhibition of IL-1 possible as soon as the vessel wall was injured, two more animals were studied that had received an intravenous bolus of IL-1ra (0.5 mg/kg) as well as subcutaneous pump insertion. Plasma levels were taken from 0 h to 24 h post bolus. IL-1ra levels were quantified by ELISA. These data showed that in all of the animals undergoing pump implantation without removal at 14 days, IL-1ra levels remained detectable until 18 days. In all cases, when “14-day” pumps were left in situ, analysis at 21 days revealed absent or negligible levels of systemic IL-1ra. In this manuscript, this discontinuation phase is referred to as a 14-day infusion. For IL-1ra to be circulating at the time of injury, which for stoichiometric reasons is important, it was necessary to give all animals an i.v. bolus in conjunction with a s.c. infusion.
2.3. In vivo assessment of IL-1 antagonism by IL-1ra

Neutrophil recruitment to intradermal (i.d.) injections of porcine IL-1α and IL-1β was used as an assay of in vivo IL-1 antagonism by the selected dose of human IL-1ra. Animals were anaesthetised, the pumps filled with IL-1ra (2 mg/kg/24 h), inserted and the i.v. bolus (0.5 mg/kg) of IL-1ra given immediately prior to the first i.d. injection on the ventral surface of the abdomen. Following pump insertion, animals were given a 0.1 ml i.d. injection of either IL-1α or IL-1β, at 300 U, 1000 U, 3000 U or 10,000 U at 24 h, 6 h, 4 h, 2 h, 1 h or 0 h pre-death. Normal saline was used as a control. 3–5 separate skin sites were injected per time-point. Sites of injection were marked with a marker pen. At time point 0, the pig was sacrificed by lethal injection and the skin on the ventral surface harvested. Each injection site was removed and skin was either placed into formalin for 24 h followed by PBS (for histology) or snap-frozen into liquid nitrogen (for myeloperoxidase assay). To ensure continued inhibition once plasma levels were maintained by pump alone, the i.v. bolus and pump implantation 3 days prior to the i.d. injections.

2.4. Measurement of neutrophil recruitment in skin samples

Myeloperoxidase was used as a surrogate marker for neutrophils recruited to skin injection sites. A myeloperoxidase assay was used as previously described [15]. Briefly, injection sites were removed from liquid nitrogen and thawed. Each sample was weighed and cut into small pieces. 2 ml of 0.5% HTAB (hexa-decyl-trimethyl-ammonium bromide) using 10 mM MOPS (3-(N-morpholino) propane-sulfonic acid) at pH 7 as the diluent was added to each sample. Each sample was homogenised using a small rotor, the resulting solution centrifuged at 4000 g for 20 min at 4 ºC. Porcine peripheral blood neutrophils were used as a standard. These were prepared by Percoll density gradient. 3,3′,5,5′-tetramethylbenzidine (TMB) was used as the coloured substrate for the assay and MPO levels were determined using ultraviolet spectrophotometry and Labsystems Genesis V3.05 software. Spleen was used as positive control for each assay.

2.5. Immunohistochemistry of porcine skin

Tissue sections of skin were rehydrated (submerged in xylene for 10 min, then sequentially into 100% alcohol, 100% alcohol, 90% alcohol and 70% alcohol for 1 min). Slides were incubated in 3% v/v hydrogen peroxide for 10 min. Tissue was blocked with 10% normal goat serum and 1% dried milk powder for 30 min. Following a 1-h incubation with the primary myeloperoxidase antibody, sections were incubated with biotinylated goat anti-rabbit secondary antibody (Vector Laboratories Inc., UK). Horseradish peroxidase-conjugated (HRP-conjugated) ABC amplification system (Vector Laboratories Inc.) was used with all antibodies and reactivity was visualized with 3,3-diaminobenzidine (DAB) substrate. Tissue was counterstained with Carazzi’s for 1 min and dehydrated through an ethanol series. The slides were mounted in DPX mounting medium and viewed using light microscopy.

2.6. Experimental coronary artery injury: balloon angioplasty (BA) or stent placement

Animals were anaesthetised and underwent either BA or intracoronary stent deployment as previously described [16]. For all experiments, quantitated digital angiography was used to determine the site of balloon inflation such that the balloon/artery ratio was 1.25:1 (a 3.5 mm balloon in a 2.8 mm section of the artery). Animals received i.v. heparin (2500 IU) during the procedure and those undergoing intracoronary stent placement also received 150 mg of aspirin orally, 24 h before surgery and daily thereafter for 5 days. Circulating levels of IL-1ra were confirmed in a representative number of animals from each Group by ELISA as before (data not shown). Five groups of 12 animals were studied (6 receiving IL-1ra and 6 vehicle, apart from Group 5 where there were 4 animals in each group). Group 1 (14-day infusion, 28-day analysis) underwent oversized BA using a 3.5 mm × 20 mm balloon to both the left anterior descending (LAD) and right coronary artery (RCA). The balloon was inflated 3 times at 8 atmospheres for 10 s. Group 2 (14-day infusion, 28-day analysis), Group 3 (14-day infusion, 14-day analysis), Group 4 (28-day infusion, 28-day analysis) and Group 5 (28-day infusion, 90-day analysis) all underwent intracoronary stent placement with a BiodivYsio™ (Abbott Vascular Devices, Redwood City, Ca) 3.5 mm stent to the LAD and RCA. Stents were deployed with a single balloon inflation at 8 atmospheres for 30 s. Animals were allowed to recover and were sacrificed at the 14th, 28th or 90th day with an overdose of intravenous pentobarbitone (Scheme 1). No deaths occurred in any of the treatment groups and no stent thrombosis was observed.

2.7. Pathological evaluation

Immediately after sacrifice, the hearts were harvested. For the BA-treated arteries, hearts were perfusion-fixed with 10% buffered formalin at 100 mmHg for 20 min via the aortic stump. Arteries were cut into 2-mm blocks from proximal to distal vessel, placed into 10% buffered formalin...
for a further 24 h, paraffin wax embedded and serially sectioned. Following stent implantation, vessels were flushed with 0.9% normal saline, the stented arterial segments explanted, placed into 10% buffered formalin for 24 h and then into PBS. The stented arterial segments were embedded into Technovik 8100 resin as previously described [17]. For the analysis of vessel wall inflammation at 18 h, the stented segments were explanted immediately postmortem, the vessel dissected open and the stent removed. The tissue was then fixed in 10% buffered formalin, paraffin-embedded and stained with haematoxylin and eosin for histological analysis. The inflammatory response following vessel wall injury is maximal at 18 h [13] and therefore later time-points were not studied.

2.8. Histomorphometric analysis

For the BA-treated vessels, only sections that showed a breach in the internal elastic lamina of the vessel wall were studied. Rupture of the internal elastic lamina in this model can only occur by balloon injury, by identifying these sections and eliminating others, only sections that have undergone injury are used for analysis. These were stained with Miller’s elastin and van Gieson’s stain and viewed under light microscopy. The stented vessels were all embedded in methylmethacrylate resin. Each artery was serially sectioned using a diamond edged saw. The section was ground and polished resulting in a section of artery, 20–30 μm thick. This method leaves the stent in situ and the tissue undamaged, enabling accurate assessment of tissue morphology [17]. Sections of artery were examined using a quantitative method of histomorphometric analysis (Lucia Image Analysis Software, Nikon, UK). The cross-sectional areas of the lumen, neointima, media, adventitia, stent and whole vessel were recorded and an injury score for each section determined, as previously described [16,18]. For the BA group, sections that were incomplete had branch distortion or tissue loss were rejected. For the stented vessels, sections were rejected if there was an incomplete ring of struts, presence of a large side-branch or distortion. On average 4–6 sections were analysed per BA vessel and 6–10 per stented vessel. A corrected neointimal area was calculated for each section to eliminate differences in neointima due to vessel size and injury. To do this, the raw neointima area was divided by both the vessel area and the corrected injury score for each section. A mean of all sections was then calculated. Analysis was operator blinded and two independent observers have measured all sections. There was no significant difference between the values obtained (p > 0.85 for all measurements with <10% magnitude of difference between the two observers).

2.9. Statistical analysis

All data are expressed as mean (S.E.M.). A Kolmogorov–Smirnov test (SPSS version 11.5) was used to determine whether the data were normally distributed. Once normality was confirmed, histological measurements were analysed by unpaired Student’s t-test.

3. Results

3.1. Pharmacokinetics of IL-1ra release from pumps

Following an initial peak, IL-1ra release from pumps was constant with a plasma concentration of 150–250 ng/ml. Peak elution occurs immediately after the i.v. bolus has been given and by 1 h, the serum concentration has reached steady state with serum levels of 150–250 ng/ml (Fig. 1A). This level is maintained for 18 days after which the level decreases (Fig. 1B). When a subcutaneous infusion is given alone, it takes 8 h for peak elution to occur, following which steady state levels of 150–250 ng/ml are achieved.

![Fig. 1. Plasma concentration of IL-1ra following an intravenous bolus of 0.5 mg/kg and implantation of subcutaneous pumps that release IL-1ra at 2 mg/kg/24 h. (A) First 10 h of elution: there is a rapid peak elution immediately post bolus of 2700 ng/ml, which then rapidly falls to a steady state level of 150–250 ng/ml. (B) The levels remain constant up to 18 days, following which, levels fall below 100 ng/ml and by 23 days, no IL-1ra is eluted.](https://academic.oup.com/cardiovascres/article-abstract/68/3/493/310996)
3.2. IL-1ra inhibition of cutaneous inflammation

IL-1ra inhibited the effect of i.d. injection of IL-1α and IL-1β. In all the doses studied, IL-1β resulted in greater neutrophil accumulation than IL-1α. The maximal inflammatory response for IL-1β occurred with doses of 1000 U and greater, 4–6 h pre-death (data not shown). For all subsequent experiments, IL-1β only was used at a dose of 1000 U and injections were performed from 6 h pre-death. i.v. bolus (0.5 mg/kg) of IL-1ra, with optimum pump dosing, led to near complete inhibition of IL-1β in terms of neutrophil recruitment (Fig. 2A). Immunohistochemistry showed near complete abolition of MPO positive cells in response to IL-1ra treatment (representative sections are shown in Fig. 2B and C).

3.3. IL-1ra inhibition of early arterial inflammation after stenting

IL-1ra markedly inhibited the inflammatory infiltrate 18 h after stenting (Fig. 3A and B).

3.4. IL-1ra effects on BA response (Group 1)

50 sections had injury and were suitable for analysis in the IL-1ra-treated group and 51 sections in the vehicle-treated group. Histological examination of all sections revealed similar injury scores for both groups (19.14 (1.47) for IL-1ra and 21.94 (1.00) for vehicle, \( p = \text{NS} \)). No differences between LAD and RCA with regard to injury

![Fig. 2. (A) Neutrophil accumulation (as assessed by MPO assay) in response to intradermal IL-1β (1000 U). Values for pigs that received no IL-1ra (●, \( n = 1 \)). Neutrophil accumulation in animals that received both an intravenous bolus and subcutaneous infusion of IL-1ra at day 0 (■, \( n = 2 \)) and day 3 (▲, \( n = 1 \)). Immunohistochemistry of pigskin following intradermal injection of 1000 U of IL-1β. (B) Representative section of pigskin, stained for MPO following intradermal injection of IL-1β, 6 h pre-death in an animal that has not received IL-1ra. (C) Corresponding time-point in an animal that had an intravenous bolus of IL-1ra and insertion of IL-1ra eluting subcutaneous osmotic pumps. MPO positive cells (see arrow) can be seen within the skin tissue (B) but no positive cells are seen in animals that have received IL-1ra (C).

![Fig. 3. IL-1ra inhibition of acute inflammation (18 h) after stenting. (A) Vehicle-treated animal; (B) IL-1ra-treated animal. IL-1ra markedly inhibited the inflammatory infiltrate seen after stenting.

![Table 1 Summary of histomorphological measurements from BA-treated porcine coronary arteries (Group 1)](https://academic.oup.com/cardiovascres/article-abstract/68/3/493/310996/497)

<table>
<thead>
<tr>
<th></th>
<th>IL-1ra</th>
<th>Vehicle</th>
<th>( p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel area (mm²)</td>
<td>3.77 (0.14)</td>
<td>3.69 (0.14)</td>
<td>NS</td>
</tr>
<tr>
<td>Neointima area (mm²)</td>
<td>0.44 (0.04)</td>
<td>0.57 (0.04)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lumen area (mm²)</td>
<td>1.44 (0.08)</td>
<td>1.29 (0.17)</td>
<td>NS</td>
</tr>
<tr>
<td>% Internal elastic lamina breach</td>
<td>19.14 (1.47)</td>
<td>21.94 (1.00)</td>
<td>NS</td>
</tr>
<tr>
<td>Intima: Media ratio</td>
<td>0.84 (0.09)</td>
<td>1.27 (0.12)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Results are expressed as mean (S.E.M.) and comparisons are made between IL-1ra- and vehicle-treated animals within the same group.

![Fig. 4. Tissue response to BA. (A) Effect of IL-1ra on neointima area following BA. IL-1ra significantly inhibits the neointimal response to BA. IL-1ra infusion was for 14 days and analysis was at 28 days. (B) Section of coronary artery from an animal that received vehicle. Both sections have been stained with Miller’s elastin and van Gieson’s stain. Both show a 23% breach in the internal elastic lamina.](https://academic.oup.com/cardiovascres/article-abstract/68/3/493/310996/497)
response were detected. All results were collected for final group analysis and the complete quantitative histomorphometry data are summarised in Table 1. IL-1ra resulted in a 23% decrease in neointimal area (\(p = 0.01\)) and a 34% decrease in intima: media ratio (\(p = 0.0001\)) (Fig. 4A). There was a trend for a bigger lumen in the IL-1ra-treated group although this did not reach significance. Representative sections of IL-1ra- and vehicle-treated coronary arteries are shown in Fig. 4B and C.

### 3.5. IL-1ra and porcine coronary stenting [Group 2 (14-day infusion, 28-day analysis)]

112 sections were suitable for analysis in the IL-1ra group and 98 sections in the vehicle group. Histological examination of all sections showed similar amounts of injury for both groups (1.07 (0.07) for IL-1ra and 1.26 (0.06) for vehicle, \(p = \text{NS}\)). No differences between LAD and RCA with regard to injury response were detected. All results were collected for final group analysis and the complete quantitative histomorphometry data are summarised in Table 2. There was a 34% increase in corrected neointimal area (\(p = 0.001\)) and a 13% smaller lumen (\(p = 0.02\)) with IL-1ra treatment than in controls. Fig. 5A and B show representative histological sections.

### 3.6. IL-1ra and porcine coronary stenting [Group 3 (14-day infusion, 14-day analysis)]

83 sections were analysed in the IL-1ra group and 82 in the vehicle group. The injury score was similar in each group (0.73 (0.06) in IL-1ra and 0.79 (0.07) in vehicle, \(p = \text{NS}\)). No differences between LAD and RCA with regard to injury response were detected. All results were collected

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### Table 2

Summary of histomorphological measurements following intracoronary stent placement

<table>
<thead>
<tr>
<th>Group 2</th>
<th>IL-1ra</th>
<th>Vehicle</th>
<th>Neointima area (mm²)</th>
<th>Lumen area (mm²)</th>
<th>Modified Schwartz injury score</th>
<th>Corrected neointima area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel area (mm²)</td>
<td>8.29 (0.07)</td>
<td>8.49 (0.08)</td>
<td>2.42 (0.12)</td>
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<td>3.41 (0.12)</td>
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<td>p-value</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
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<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Group 3</td>
<td>IL-1ra</td>
<td>Vehicle</td>
<td>Neointima area (mm²)</td>
<td>Lumen area (mm²)</td>
<td>Modified Schwartz injury score</td>
<td>Corrected neointima area</td>
</tr>
<tr>
<td>Vessel area (mm²)</td>
<td>9.61 (0.09)</td>
<td>9.34 (0.15)</td>
<td>0.97 (0.07)</td>
<td>1.64 (0.12)</td>
<td>5.46 (0.13)</td>
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<td>p-value</td>
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<td>NS</td>
<td>NS</td>
</tr>
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<td>Group 4</td>
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<td>Neointima area (mm²)</td>
<td>Lumen area (mm²)</td>
<td>Modified Schwartz injury score</td>
<td>Corrected neointima area</td>
</tr>
<tr>
<td>Vessel area (mm²)</td>
<td>9.42 (0.1)</td>
<td>9.45 (0.12)</td>
<td>2.62 (0.14)</td>
<td>2.90 (0.2)</td>
<td>3.46 (0.14)</td>
<td>3.19 (0.17)</td>
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<tr>
<td>p-value</td>
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<td>NS</td>
</tr>
<tr>
<td>Group 5</td>
<td>IL-1ra</td>
<td>Vehicle</td>
<td>Neointima area (mm²)</td>
<td>Lumen area (mm²)</td>
<td>Modified Schwartz injury score</td>
<td>Corrected neointima area</td>
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<tr>
<td>Vessel area (mm²)</td>
<td>9.24 (0.1)</td>
<td>9.51 (0.1)</td>
<td>1.93 (0.17)</td>
<td>3.27 (0.25)</td>
<td>3.76 (0.19)</td>
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<tr>
<td>p-value</td>
<td>NS</td>
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<td>0.00001</td>
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</table>

In all cases, results are expressed as mean (S.E.M.) and comparisons are made between IL-1ra- and vehicle-treated animals within the same group.

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Fig. 5. Tissue response to intracoronary stent placement. These photomicrographs were taken at 14, 28 or 90 days after oversized stent placement into porcine coronary arteries. (A, C, E and G) IL-1ra-treated animals; (B, D, F and H) vehicle-treated animals. (A and B) Group 2 animals i.e. 14-day infusion and 28-day time-point. For a similar degree of injury, there is more neointima in the IL-1ra-treated group although this did not reach significance. Representative sections of IL-1ra- and vehicle-treated coronary arteries are shown in Fig. 4B and C.
for final group analysis and the complete quantitative histomorphometry data are summarised in Table 2. In contrast with Group 2, IL-1ra treatment resulted in a 38% decrease in corrected neointimal area ($p = 0.001$) and a 33% increase in lumen area ($p = 0.0001$) compared with vehicle. Representative sections are shown in Fig. 5C and D.

3.7. IL-1ra and porcine coronary stenting [Group 4 (28-day infusion and 28-day analysis)]

70 sections were analysed in the IL-1ra group and 65 in the vehicle group. No differences between LAD and RCA with regard to injury response were detected. All results were collected for final group analysis and the complete quantitative histomorphometry data are summarised in Table 2. There was significantly more injury in the IL-1ra-treated animals compared with vehicle (1.49 (0.006), 1.19 (0.07), $p = 0.001$) and due to this, no difference was seen in the raw neointima area. However, when the correction for injury was made, as for Group 3, there was 33% decrease neointima formation ($p = 0.0004$) and a 9% bigger lumen ($p = \text{NS}$) in the IL-1ra-treated group compared with vehicle. Fig. 5E and F are representative sections from animals studied.

3.8. IL-1ra and porcine coronary stenting [Group 5 (28-day infusion, 90-day analysis)]

54 sections were suitable for analysis in the IL-1ra group and 44 sections in the vehicle group. Histological examination of all sections showed similar amounts of injury for both groups (1.52 (0.07) for IL-1ra and 1.56 (0.09) for vehicle, $p = \text{NS}$). No differences between LAD and RCA with regard to injury response were detected. All results were collected for final group analysis and the complete quantitative histomorphometry data are summarised in Table 2. There was a 41% increase in corrected neointimal area ($p = 0.000001$) and a 21% bigger lumen ($p = 0.005$) with IL-1ra treatment than in controls. Fig. 5G and H show representative histological sections.

4. Discussion

Antagonism of IL-1 by IL-1ra inhibits neointima formation after coronary artery injury and stenting in a porcine model. In the case of BA, 14-day therapy of IL-1ra resulted in a 23% decrease in neointima area. In the case of stenting, IL-1ra inhibited neointima formation for the duration of the infusion, but after cessation of the infusion around 14 days, there was a ‘catch-up’ phenomenon, with a 34% larger neointima in IL-1ra-treated arteries than vehicle. Animals that receive a 28-day infusion with an analysis at 90 days have 41% less neointima than equivalent vehicle-treated animals. It can therefore be concluded that the activation of the IL-1 system lasts for about 14 days after balloon angioplasty whereas after stenting, the critical time for the infusion appears to be 28 days.

Previous work has highlighted the role of IL-1 in this process, in IL-1ra knockout mice, injury to the femoral artery by an externally applied vascular cuff results in increased neointima formation [14]. In comparison with that work, this study is the first to show that IL-1 is directly involved in the vessel wall response to injury and in neointima formation after intracoronary stenting and is also the first to administer a purely anti-inflammatory agent to inhibit neointima formation. The agents in current clinical use for suppression of neointima such as sirolimus and paclitaxel are in the main, cytostatic [3,4]. An anti-inflammatory strategy using IL-1ra in the coronary setting is novel and, if these results translate to man may prove an effective alternative or additional treatment in arterial disease. It may be that certain patterns of coronary heart disease (e.g. acute coronary syndromes), which are associated with ‘inflammatory’ lesions, may be particularly suitable for an anti-inflammatory approach in the context of therapeutic PCI.

IL-1ra is a large protein with inhibitory properties upon the Type 1 IL-1 receptor. Agonistic IL-1 cytokines signal inflammatory events via this receptor, which is blocked by IL-1ra. In general terms, IL-1ra blocks new IL-1 signalling events over the Type 1 receptor, rather than acting by competitive displacement binding. Blockade of the Type 1 IL-1 receptor is the only known anti-inflammatory property of IL-1ra [8]. Those data presented here, which show a powerful modulatory effect upon the early inflammatory infiltrate and the neointimal response to injury, are conclusive evidence that IL-1 has a critical role in this process.

IL-1, together with TNF and complement, in a variety of complex inflammatory models, processes and diseases are viewed as having an apical position in the signalling cascade that produce the multiplicity of effects seen in these situations. This apical position indicates not only the relative importance of these agents (i.e. lack of redundancy) but is also proven when they are antagonised. A notable example would be the inhibition of TNFa and IL-1 in rheumatoid arthritis [19]. The results reported here suggest that IL-1 may have such an apical position in the arterial response to injury.

There are multiple potential sources of IL-1 at the time of arterial injury. Adherent platelets may produce IL-1, as well as the recruited inflammatory cells. In addition, endothelial cells, which re-grow and cover the site of injury, may also produce IL-1. IL-1 has many inflammatory effects upon endothelial cells and vascular smooth muscle cells, plausibly associated with neointima formation and notable amongst these is the autocrine induction of PDGF by vascular smooth muscle cells [20]. A differential but concomitant expression of IL-1β, IL-1ra, IL-1R1 and IL-1R2 mRNA expression in neointima formation has been shown following balloon angioplasty in rat carotid arteries.
again highlighting the importance of the IL-1 in this response [21].

The increase in neointima formation seen in Group 2 animals, when IL-1ra is discontinued between 14 and 18 days and analysis conducted at 28 days and inhibition of this by a 28-day IL-1ra infusion, suggests that this is an IL-1-mediated event since there are no other reported functions of IL-1ra other than inhibition of IL-1 type 1 receptor signalling. The reduction of neointima at 28 days when the IL-1ra was continued for the complete duration of the study indicates not only the importance of IL-1 in this process but also the duration of the IL-1 mediated response following stenting. The fact that at 90 days there is sustained suppression of neointima, despite cessation of therapy at 28 days again highlights the critical role of IL-1. The Group 2 stenting response also contrasts with the Group 1 response (balloon angioplasty alone with 14-day IL-1ra and analysis at 28 days), when a reduction in neointima was induced. Since BA is a ‘one-off’ injury, it appears that a 14-day infusion of IL-1ra is sufficient to have inhibitory effects upon distant (28-day) responses. The Group 2 response, however, suggests that the chronic injury resulting from stent placement causes a more chronic inflammatory stimulus. This has to be presumed to arise from the vessel wall cells as inflammatory white cells and platelets are not a prominent feature at 14 days and beyond.

The therapeutic implications of these results are at least twofold. First, there can be little doubt that IL-1ra is a potential therapeutic agent for the treatment of proliferative vascular responses. The potential application of such an anti-inflammatory treatment is clearly of some excitement in the context of coronary artery disease, which is now viewed as an inflammatory process in terms of both atherogenesis and mechanisms of disease presentation. Secondly, these results suggest that the duration of such therapy will be crucial for its success. The sustained neointimal inhibition seen at 90 days following cessation of IL-1ra therapy at 28 days implies that 28 days of therapy is enough to prevent ‘catch-up’ to occur. In order to observe any benefit from IL-1ra therapy, antagonism must be maintained for the total duration of the stimulation of the IL-1 system. The duration of the healing response and neointima following stent implantation is still imprecisely understood but clinical studies as well as experience suggest that the process may continue up to 9 months [22]. We can only speculate that in a human 28 days of therapy will be enough for clinical utility and by analogy with cytostatic stent-based delivery many weeks are needed. In reality, this will require the development of a stent-based delivery system for IL-1ra. IL-1ra is commercially available and in clinical use for the treatment of rheumatoid arthritis. To date, no clinically significant side effects following IL-1ra treatment have been documented [23].

All such animal model based results have their limitations. In the case of the porcine coronary artery model, the most obvious of these is the absence of disease, the short duration of the experiments relation to clinical effects and the young age of the animals. Not withstanding this, the model has correctly predicted clinical responses, both the beneficial effects of sirolimus and paclitaxel and the deleterious effect of gold stent coatings [24–26]. In the case of sirolimus, a significant attenuation of neointimal growth in the pig coronary artery was seen 4 weeks after oversize balloon injury when sirolimus was infused systemically. This was accompanied by inhibition of phosphorylation of key cell-cycle promoters [27]. Two years later, Suzuki et al implanted polymer-coated, sirolimus-eluting stents in the coronary arteries of 16 pigs and disclosed a 51% reduction in in-stent neointimal area compared with bare metal stents [28]. This value (51%) turned out to be less than the corresponding clinical benefit seen in the First-In-Man and the RAVEL studies, which revealed remarkable results in patients [3,29]. However, more recently, Carter examined the long-term effect of sirolimus-eluting stents in the pig coronary artery. In contrast to their moderately good mid-term (28 days) results in that earlier paper, they showed complete attenuation of the benefit at 90 and 180 days [30]. The magnitude of inhibition of neointima response found in our studies is therefore in keeping with these studies and does indicate an important and potentially useful clinical effect.

In conclusion, IL-1ra has beneficial effects upon the coronary artery response to injury. The results indicate that the duration of such therapy needs to be for at least 28 days.

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References


