Inhibition by leukocyte depletion of neointima formation after balloon angioplasty in a rabbit model of restenosis

Ashley M. Miller\textsuperscript{a}, Allan R. McPhaden\textsuperscript{b}, Roger M. Wadsworth\textsuperscript{a}, Cherry L. Wainwright\textsuperscript{a,\ast}

\textsuperscript{a}Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, Scotland, UK
\textsuperscript{b}Department of Pathology, Glasgow Royal Infirmary, Glasgow, Scotland, UK

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Abstract

Objective: The aim of the current study was to examine neointima formation in balloon injured left subclavian artery of rabbits subjected to two different methods of leukocyte depletion at the time of injury. Methods: Angioplasty of the left subclavian artery was performed in leukopenic male New Zealand White rabbits. Depletion of circulating leukocytes was induced by either mustine hydrochloride or an antibody against leukocyte common antigen (anti-LCA) before angioplasty. Left and right subclavian arteries were removed 28 days after injury for morphological analysis and measurement of neointimal size. At the same time, leukocytes were isolated from autologous rabbit blood for \textsuperscript{51}Cr-labelling for assessment of leukocyte adhesion to injured and non-injured artery segments. Results: Leukopenia decreased neointima formation in injured arteries (neointimal area was 0.09$\pm$0.03 mm$^2$ in mustine-treated arteries, \(n=8\), vs. 0.56$\pm$0.07 mm$^2$ in control arteries, \(n=7\); \(P<0.001\) and 0.07$\pm$0.01 mm$^2$ in anti-LCA treated arteries, \(n=9\), vs. 0.22$\pm$0.04 mm$^2$ in non immune serum-treated arteries, \(n=9\); \(P<0.001\)). Adventitial fibrosis was also significantly (\(P<0.05\)) decreased by both leukopenic interventions. Neither medial nor adventitial area was modified in any of the groups. No differences in leukocyte adhesion were observed between injured and non-injured arteries in any of the experimental groups at the 28 day time point. Conclusion: These results suggest that leukocytes play a major role in the development of two of the major characteristics of the response to balloon injury, namely formation of neointima and adventitial fibrosis, that currently limit the success of clinical angioplasty. Elucidation of the fine mechanisms involved in leukocyte-mediated injury may lead to the discovery of novel therapeutic targets for the prevention of restenosis. \textcopyright 2001 Elsevier Science B.V. All rights reserved.

Keywords: Leukocytes; Angioplasty; Restenosis; Histo(patho)logy; Coronary disease

1. Introduction

Restenosis following successful percutaneous coronary revascularisation continues to represent a major problem limiting the clinical efficiency of this procedure. The underlying mechanisms are comprised of a combination of effects from vessel recoil, negative vascular remodelling, thrombus formation and neointimal hyperplasia [1]. The use of intracoronary stent placement to address the problem of arterial remodelling has resulted in a reduced occurrence of restenosis in nearly all patient and lesion subsets [2]. However, the incidence of in-stent restenosis is still unacceptably high due to an increased proliferative response associated with the use of stents, leading to an increase in neointimal formation [3]. The mechanisms underlying neointimal formation remain unclear, although platelet activation, release of growth factors and mitogens, smooth muscle cell proliferation and extracellular matrix deposition are all believed to play important roles (reviewed recently in [4]). There is increasing evidence to suggest that leukocytes are activated after angioplasty and one of the first studies to investigate this demonstrated that granulocytes were activated following balloon angioplasty in humans [5]. Subsequent studies revealed an increased expression of adhesion molecules on the surface of leukocytes [6,7] and an increase in soluble adhesion

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\textsuperscript{\ast}Corresponding author. Tel.: +44-141-548-2405; fax: +44-141-552-2562.
E-mail address: c.l.wainwright@strath.ac.uk (C.L. Wainwright).

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molecules in the plasma [8,9] of patients following angioplasty.

While the activation of inflammatory mechanisms following injury may be indicative of a beneficial action of leukocytes to aid in the late (i.e., several days after injury) repair processes, there is good experimental evidence that these processes are activated very soon after injury. For example, there is early intense expression of ICAM-1 in balloon-injured rat carotid artery [10]. Furthermore, we have shown recently in a rabbit model of balloon injury to the subclavian artery that there is a marked increase in adhesion of leukocytes to injured vessels 24–48 h after injury [11] which correlates with an increase in expression of E-selectin, P-selectin and VCAM-1 [10]. Taken together, these findings suggest an early detrimental role for leukocytes in neointimal formation. Indeed, the ability of a monoclonal antibody to ICAM-1 to suppress intimal hyperplasia in a rat carotid artery injury model [10] supports this hypothesis. Thus an early response to injury may involve increased leukocyte adhesion and subsequent migration into the artery wall, with subsequent release of cytotoxic mediators (including superoxide anion and proteolytic enzymes [12]), cytokines (such as interleukin-1β and tumour necrosis factor-α [13]), platelet activating factor and leukotrienes [14], and growth factors [15]. The release of these mediators could further damage the artery wall, amplify the effects of platelets, cause vasoconstriction, stimulate further leukocyte adhesion and smooth muscle cell proliferation and migration.

All of the above evidence for leukocyte involvement in restenosis is, however, circumstantial. The aim of the current study was to provide a more direct demonstration of the importance of leukocytes in neointima formation by determining the extent of neointimal formation 4 weeks after balloon angioplasty in leukopenic rabbits. To address this aim we employed two different interventions to induce leukopenia — the cytotoxic drug mustine and an antibody to leukocyte common antigen (anti-LCA). Both interventions were administered in a regimen aimed at inducing a depletion of leukocytes over the first few days following angioplasty, with subsequent recovery, which allowed us to pinpoint the time during which leukocytes may play a detrimental role in the restenosis process. To evaluate the effects of these interventions on the recovery of vascular and leukocyte function, we also assessed the extent of leukocyte adhesion to injured and non-injured vessels at the end of the study.

2. Methods

2.1. Animals

A total of 38 male New Zealand White rabbits (2–2.5 kg) underwent balloon angioplasty and were allocated to one of four experimental groups (control, mustine, preimmune serum and anti-LCA) prior to the angioplasty procedure. Five rabbits (two controls, two mustine-treated and one anti-LCA rabbit) did not survive the entire 4-week experimental period. Thus the final group numbers were: (i) control (n=7); (ii) mustine (n=8); (iii) preimmune serum (n=9); (iv) anti-LCA (n=9). All surgical procedures were performed under a Project Licence issued under the UK Home Office Animals (Scientific Procedures) Act 1986 (PPL 60/1988).

2.2. Depletion of circulating leukocytes

Leukocyte depletion was induced by either pretreatment with mustine hydrochloride [16] or administration of an antibody against leukocyte common antigen [17]. Mustine hydrochloride (dissolved in saline) was administered intravenously via the marginal ear vein (1.7 mg/kg) and animals used 4 days later for balloon angioplasty. The antibody against leukocyte common antigen (anti-LCA) was also administered intravenously via the marginal ear vein (1 mg/kg) and animals used 2 days later for balloon angioplasty. The doses of both interventions were chosen on the basis of published literature [16,17] and the timing of the angioplasty procedure was determined by a series of preliminary studies in four rabbits to identify the time after intervention at which leukocyte counts were maximally depleted. Controls for the mustine-treated rabbits received saline (vehicle for mustine) during the operative procedure. Controls for the antibody treated animals received the same volume of the IgG fraction of a preimmune mouse serum. This was prepared by the use of caprylic acid fractionation [18], followed by precipitation with sodium acetate buffer and extensive dialysis against saline. Total leukocyte counts were measured using an automated cell counter (Medonic Cell Analyser CA460, Sweden) in blood samples withdrawn from the marginal ear vein before drug administration, just prior to the angioplasty procedure (to ensure leukocyte depletion had occurred) and thereafter at 1-week intervals for 4 weeks to measure leukocyte recovery.

2.3. In vivo balloon angioplasty

An in vivo rabbit model of restenosis previously developed in this laboratory was used in this study [19]. Rabbits were sedated by an intramuscular injection of 0.3 ml/kg i.m. Hypnorm® (fluanisone–fentanyl citrate mixture) and anaesthesia was induced and maintained with a mixture of 2% nitrous oxide and 1.5–2% halothane in oxygen. All animals were given preoperative antibiotic cover (100 mg ampicillin i.m.) and heparin (500 units i.v.). The left femoral artery was exposed by blunt dissection and a 3.0 mm balloon angioplasty catheter (Advanced Cardiovascular Systems Temecula, CA, USA) containing a 0.014-inch steerable guide wire was introduced into the artery. The catheter was advanced into the left subclavian
artery under fluoroscopic control (Siemens Memoskop and Siremobil 2), ensuring the balloon was positioned with the centre point about 1 cm from the first rib. The contrast-filled balloon was then inflated twice to 10 atmospheres for 30 s. A third inflation to 8 atmospheres was performed and the balloon was withdrawn by half its own length to ensure endothelial damage. The balloon was deflated, withdrawn and the wound to the femoral artery was sutured. All animals were given 0.15 mg i.m. buprenorphine (Vetergesic®) immediately after surgery as analgesic cover.

2.4. Leukocyte isolation and radioactive labelling

Animals were euthanised 28 days after angioplasty by an injection of a Sagatal® (sodium pentobarbitone, 60 mg/ml) and heparin (1000 U/ml) mixture into the marginal ear vein. The chest was then opened, the pericardium removed and blood extracted from the pulmonary artery via a large bore 19G needle into sterile 20-ml syringes containing 2 ml of 3.8% sodium citrate. Blood was immediately transferred into 10-ml centrifuge tubes containing 2 ml of 6% dextran solution and left to sediment at room temperature for 2 h. The leukocyte rich upper layer was then removed and centrifuged at 300 g for 5 min. The resultant cell pellet was hypotonically lysed with distilled water to remove any contaminating erythrocytes and layered onto 1 ml of Histopaque® (1.077 g/ml density). A second centrifugation for 20 min at 300 g yielded a leukocyte pellet which was resuspended in Hank’s balanced salt solution (HBSS). Isolated cells were counted using an automated cell counter (Medonic Cell Analyser CA460, Sweden). Leukocyte yield was adjusted to 1×10⁶ cells/ml by adding an appropriate amount of HBSS.

Leukocytes were radioactively-labelled by adding 185 kBq of ⁵¹chromium (specific activity 37 MBq/ml, Na₂CrO₄ in 0.9% saline, half-life 27.7 days) to the leukocyte suspension containing approximately 1×10⁶ cells/ml in HBSS. This was allowed to proceed for 1 h at 37°C with agitation every 10 min to increase chromium uptake. Leukocytes were then washed twice and resuspended in HBSS.

2.5. Subclavian artery dissection

Following blood removal from the pulmonary artery both left (injured) and right (non-injured) subclavian arteries were dissected out and immediately placed in Krebs–Henseleit solution of the following composition (mM): NaCl, 118.3; NaHCO₃, 25.0; glucose, 11.1; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2 and MgSO₄, 2.5. Arteries were cut into 2–3 mm rings and each ring was assigned a number to ensure that the same tissue ring was used from each animal in subsequent experiments. The first ring immediately adjacent to the aortic arch was discarded on both arteries and the next four rings from both left and right subclavian arteries were used for further studies. The second and fourth rings were immersed in neutral buffered formal saline (100 ml of 40% formaldehyde mixed with 900 ml of phosphate buffer (4 g of NaH₂PO₄·2 H₂O and 6.5 g of NaHPO₄ per litre)) for morphological analysis. Perfusion fixation was not possible in these studies since the third and fifth rings were required for leukocyte adhesion measurements. These rings were stored in Krebs–Henseleit solution bubbled with 95% O₂–5% CO₂ and maintained at 37°C until the assay was performed following preparation of labelled leukocytes.

2.6. Leukocyte adhesion assay

The adhesion of leukocytes to injured and non-injured arteries was determined using a method described previously [11]. Briefly, artery rings were opened longitudinally and pinned out luminal side upwards onto Sylgard blocks and placed inside a humidified chamber maintained at 37°C. A 5-µl volume of the ⁵¹chromium-labelled leukocyte suspension was then added to the luminal side of the artery, taken from the same animal and left to adhere for 30 min. The tissues were then gently washed with HBSS to remove any non-adherent cells and adhesion quantified by taking duplicate counts in a gamma counter (Packard Bell Multiprias). Leukocyte adhesion was calculated as a percentage of the cells added of that which remained adherent to the artery after washing.

2.7. Morphological staining

All samples were given a code before morphological processing, which was only broken when all analyses were complete. The artery rings were fixed for 24 h, embedded in paraffin wax and two 3-µm cross-sections were cut from each artery ring. Sections were stained with haematoxylin and eosin, mounted and viewed in the microscope.

2.8. Morphological analysis

Semi-quantitative analysis of vessel morphology and the response to vessel injury was performed using a scoring system (on a scale of 0–3, where 0=normal; 1=mild; 2=moderate; 3=severe) was performed by an individual blinded to the status of the vessel in all cases. The following parameters were employed to identify signs of injury: (i) disruption of endothelial cover; (ii) disruption of the internal elastic lamina (extent of breakage as an indicator of the severity of injury); (iii) extent of neointimal growth into the artery lumen; (iv) extent of disruption of the media and external elastic lamina (including breakage of the external elastic lamina, medial damage and smooth muscle cell disruption, evidence of calcification and giant cell reactions); (v) extent of adventitial fibrosis (increased collagen deposition associated with increased numbers of fibroblast nuclei). Quantitative analysis of the area of each of the vessel layers (adventitia, media and neointima) was achieved using computerised planimetry.
2.9. Statistical analysis

All data are expressed as the mean±standard error of the mean of n animals. Student’s unpaired t-test was employed to determine the significance of changes in leukocyte counts. In leukocyte adhesion studies data is expressed as percentage leukocyte adhesion. A Student’s paired t-test was employed to determine the significance of changes in leukocyte adhesion between right non-injured and left injured subclavian arteries in control and drug-treated groups. The areas of the different vessel layers and degree of neointima in injured arteries between control and drug-treated animals are expressed in mm² and were compared using one-way ANOVA followed by a Tukey test. For statistical comparison between neointimal area in mustine injures arteries and anti-LCA injured arteries a Student’s unpaired t-test was used. When P<0.05 the results were taken to be statistically significant.

2.10. Materials

Mustine hydrochloride was purchased from Knoll (Nottingham, UK) and was prepared by adding 10 ml sterile 0.9% NaCl solution to 10 mg mustine hydrochloride on the day of each experiment. Anti-human LCA antibody and preimmune mouse serum were a gift from SAPU (Law Hospital, Carluke, Scotland). Caprylic acid was purchased from Sigma (Poole, Dorset, UK). Hypnorm® was purchased from Janssen (Beerse, Belgium), Amphipen (100 mg/ml) from Mycofarms (Cambridge, UK) and heparin from Leo Laboratories (Buckinghamshire, UK). Vetergesic® was obtained from Reckitt & Colman Pharmaceuticals (UK) and Sagatal® was purchased from Rhône Mérieux (Harlow, Essex, UK). Sodium citrate, sodium chloride and sodium acetate were all purchased from BDH (Poole, Dorset, UK). Harris haematoxylin and eosin, dextran (Mw ~500 000), HBSS and Histopaque 1077 were all purchased from Sigma. Sodium chromate radioisotope was obtained from Amersham (Little Chalfont, Buckinghamshire, UK). Neutral buffered formal saline was purchased from Rimon (UK).

3. Results

3.1. Leukocyte counts

Four days after mustine administration leukocyte count had decreased from a mean of 4.9±0.8×10⁶ cells/ml to 0.4±0.1×10⁶ cells/ml, a decrease of ~92% (Fig. 1a).

Fig. 1. Reduction in circulating leukocyte count by (a) mustine hydrochloride (1.7 mg/kg) or (b) anti-LCA (1 mg/kg) given intravenously to rabbits 4 days (mustine) or 2 days (anti-LCA) prior to balloon angioplasty of the left subclavian artery. Both interventions induced approximately 90% depletion of leukocyte count by the time of angioplasty. Gradual recovery of leukocyte count commenced approximately 10 days after induction of leukopenia, with almost full recovery by the end of the study, 28 days after angioplasty.
Two days after anti-LCA treatment, the leukocyte count had decreased from a mean of $4.3 \pm 0.2 \times 10^6$ cells/ml to $0.4 \pm 0.1 \times 10^6$ cells/ml, resulting in a leukocyte population 10% of normal at the time of angioplasty (Fig. 1b). Follow up counts at weekly intervals showed leukocyte counts had returned to normal levels by week 4 in both groups.

3.2. Morphological changes following angioplasty

Previous studies from our laboratory have shown that in this model the early response (i.e. within the first 8 days) following injury involves eccentric endothelial denudation (often associated with overlying thrombus), infiltration of inflammatory cells and the presence of proliferating smooth muscle cells in the media, with some evidence of neointimal formation [11,19]. By 4 weeks after injury the vessels exhibit marked neointimal formation with extracellular matrix deposition and an intact, functional endothelial layer [11,20]. In the present study, the non-injured right subclavian arteries in all treatment groups were identical, with no evidence of artery damage (Figs. 2 and 3), indicating no direct effect of drug treatment on vascular morphology. In contrast, the injured left subclavian arteries from controls, mustine and antibody-treated rabbits (Figs. 2 and 3) all had evidence of neointimal proliferation 28 days after balloon angioplasty and all had intact endothelial layers. Qualitative scoring demonstrated a similar extent of medial necrosis and calcification and internal elastic lamina rupture in all groups of rabbits. However, the extent of adventitial fibrosis was significantly less in both the mustine treated (score $1.25 \pm 0.25$) and anti-LCA treated (score $1.11 \pm 0.26$) rabbits in comparison to injured arteries from the corresponding control rabbits (scores $2.29 \pm 0.15$ and $2.22 \pm 0.15$ for mustine control and preimmune serum control rabbits respectively; $P<0.05$ for both drug groups). In addition, three mustine control arteries and two preimmune serum treated arteries had evidence of foreign body giant cells (macrophages which fuse together yield giant cells with two or more nuclei), indicating an inflammatory response. No leukocytes were detected morphologically in injured artery segments taken from either of the leukopenic groups.

3.3. Effect of leukopenia on neointimal size

No neointima was present in the uninjured right subclavian arteries of either the control groups or the mustine-treated or anti-LCA-treated rabbits. Quantification of the vessel layer areas revealed that mustine treatment markedly decreased neointimal area in injured arteries, from $0.56 \pm 0.07$ mm$^2$ in the control group to $0.09 \pm 0.03$ mm$^2$ in mustine-treated arteries ($P<0.001$, Fig. 4a). No difference was seen in the areas of media (Fig. 4b) or adventitia (Fig. 4c) between control and mustine-treated arteries. Anti-LCA treatment also inhibited neointima formation in injured arteries, from $0.22 \pm 0.04$ mm$^2$ in serum-treated arteries to $0.07 \pm 0.01$ mm$^2$ in anti-LCA treated arteries ($P<0.001$, Fig. 5a). Similar to the findings with mustine, no difference was seen in the areas of media (Fig. 5b) or adventitia (Fig. 5c) between serum and anti-LCA treated arteries.

3.4. Leukocyte adhesion assay

No significant differences in leukocyte adhesion were observed between injured and non-injured arteries in either the control vs. mustine-treatment or preimmune serum vs. anti-LCA treatment groups (Table 1).

4. Discussion

Increasing experimental evidence supports the concept that leukocytes interact with the vessel wall following angioplasty and can modulate and enhance the pathophysiological response associated with arterial injury. A positive correlation between leukocyte count and severity of coronary artery disease, as evaluated by coronary angiography, has been described [21]. Furthermore, it has been shown that despite standard aspirin and heparin therapy, monocyte and neutrophil activation and leukocyte-platelet complexes after coronary angioplasty are higher in patients experiencing late clinical events including restenosis, myocardial infarction and unstable angina [5].

This present study demonstrates for the first time that depletion of circulating leukocytes at the time of angioplasty markedly decreases the development of neointimal thickening in a rabbit subclavian artery model 28 days after injury. While the effects of mustine pretreatment are most likely due to leukocyte depletion, there are several alternative explanations for its ability to prevent neointimal formation. As a cytotoxic drug, mustine may reduce neointimal formation by a direct action on the vascular tissue. However, histological examination of the right uninjured arteries of mustine-treated rabbits indicated no evidence of any direct cytotoxic effect of the mustine. Furthermore, several previous studies have shown cytotoxic drugs to be ineffectual against restenosis. For example, both colchicine [22] and methotrexate [23] failed to prevent restenosis in patients or neointimal formation in an experimental pig model, respectively. In contrast, combination therapy with vincristine and actinomycin D resulted in less smooth muscle cell hyperplasia 3 days after endothelial denudation in rabbit aorta [24]. However, leukocyte counts were not measured in any of these studies so it is not known whether these were affected and could account for the variation in results.

An alternative explanation for the effect of mustine on neointimal formation may be related to an effect on platelets, which are fragments of a larger megakaryocyte cell produced in the bone marrow. One previous study has
Fig. 2. Representative morphological cross-sections of subclavian arteries (Haematoxylin & Eosin staining) (a) control right non-injured, (b) mustine-treated right non-injured, (c) control left injured and (d) mustine-treated left injured arteries. Control injured arteries display a thick layer of neointima (NI) which extends into the artery lumen (L) and evidence of damage to the media (M). Mustine-treated injured arteries display a mild degree of neointimal formation. No neointima was found in non-injured arteries. Magnification ×400.
shown that induction of thrombocytopenia with an antibody against platelets can reduce intimal lesion formation in a rat model of restenosis [25]. Since platelet counts were not performed in this present study, and hence it is not known whether or not the mustine-treated rabbits were also thrombocytopenic, this cannot be ruled out as a contribut-
Fig. 3. Representative morphological cross-sections of subclavian arteries (haematoxylin and eosin staining) (a) serum-treated right non-injured, (b) anti-LCA-treated right non-injured, (c) serum-treated left injured and (d) anti-LCA-treated left injured arteries. Serum-treated injured arteries display a thick layer of neointima (NI) which extends into the artery lumen (L) and evidence of damage to the media (M). Anti-LCA injured arteries display a mild degree of neointimal formation. No neointima was found in non-injured arteries. Magnification ×400.
Fig. 3. (continued)
effect of anti-LCA on platelets is unlikely to account for the inhibition of neointimal formation since LCA is not present on megakaryocytes [26]. What the present studies do not tell us, however, is which subtypes of leukocytes are responsible for playing a role in restenosis, and what degree of leukocyte depletion is required for an effect, since we did not perform differential cell counts and both interventions induced a very profound leukopenia. This opens up a range of possibilities for future studies to look at specific antibodies targeting different subtypes of leukocytes.

One interesting aspect of the beneficial effect of the two
interventions on the response to injury is the reduced of adventitial fibrosis (assessed qualitatively) compared to untreated control rabbits, albeit without any change in adventitial areas in any of the groups. The probable reason for this finding is that the adventitia is normally a loose connective tissue layer, hence rather than an increase in area being observed after injury, an increase in density is seen. Adventitial fibrosis results from the proliferation of adventitial fibroblasts, which form a fibrotic scar around the injury site and subsequently migrate into the neointima. This event is considered to contribute to negative vascular remodelling associated with clinical restenosis [27] and a recent study has suggested that it is the predominant mechanism for luminal narrowing following a single balloon injury in a pig model of coronary angioplasty [28]. The importance of controlling adventitial fibrosis to improve the long-term clinical outcome of balloon angioplasty has been highlighted recently in an experimental study in dogs given intra-arterial irradiation therapy at the time of angioplasty. While the ‘short-term’ success rate of the procedure was good, adventitial fibrosis was found to increase with increasing irradiation dose and became evident after 2 and 5 years following irradiation [29]. However, it is conceivable that adventitial fibrosis, which is a very late response in the restenosis process, is not complete by 28 days after injury and that the effects seen here in the present studies show simply a delay in this process. Clearly further studies employing a later time point for assessment of adventitial fibrosis are required to address this question. Nevertheless, a drug intervention that reduces adventitial fibrosis, as well as neointimal formation, would serve the patient better in the long-term.

It is likely that leukocytes exert their detrimental effects on neointimal formation at a relatively early stage after injury, since in the present studies adhesion of leukocytes to injured control arteries 28 days after angioplasty did not differ from non-injured arteries, reflecting a return to normal leukocyte–endothelial cell interactions. Our previous study, where we demonstrated that increased leukocyte adhesion to injured arteries peaks at 24–48 h after injury [11], supports this hypothesis. It should also be noted that neither mustine nor anti-LCA treatment had any effect on leukocyte adhesion to either injured or non-injured artery segments 28 days after injury (i.e. at the time we know neointimal formation to be complete in this model [19]), and that leukocyte counts had returned to normal by this time. This is important as it shows that the inhibition of neointimal formation had not simply been delayed until there were sufficient numbers of circulating leukocytes present in order to infiltrate the injured area. It also indicates that leukocyte function at this time was normal and therefore these cells are available to participate in late repair processes.

Although this study is the first to demonstrate that leukocyte depletion prevents neointimal formation, several other studies have employed different approaches to interfering with leukocyte function postangioplasty. A monoclonal antibody to ICAM-1 significantly suppresses intimal hyperplasia in the rat model of restenosis [10], whereas a monoclonal antibody to CD11/CD18 did not decrease the restenotic process in an atherosclerotic rabbit arterial injury model [30]. Blockade of the selectins sialyl-Lewis α and P-selectin can also reduce both neointimal hyperplasia [31,32] and the acute interaction of leukocytes and platelets at the site of arterial injury [33,34].

While it is now becoming apparent that leukocytes play a role in restenosis development, the mechanism(s) underlying this remain to be fully determined. The initial stimulus causing local leukocyte activation is likely to be the short bout of ischaemia followed by restoration of blood flow caused by balloon inflation [14]. In addition, endothelial damage resulting in the exposure of membrane receptors for immunoglobulin and complement, would promote subsequent leukocyte activation and the increased expression of leukocyte adhesion molecules. Indeed, a number of studies have demonstrated increased levels of a range of adhesion molecules in coronary sinus blood collected from patients immediately after elective coronary angioplasty [8,9,13] and on the vessel wall following experimental balloon angioplasty [10,11,35]. This increase in adhesion molecules would be expected to facilitate the influx of leukocytes into the injured artery wall that has been observed in both the intimal and medial layers in experimental models of neointimal formation [36–38]. Once within the artery wall the leukocytes could induce tissue injury and contribute to neointimal formation and restenosis by several mechanisms: (i) the release of cytotoxic mediators (including oxygen-derived free radicals, lysosomal hydrolases and leukotrienes) that would injure nearby cells and possibly also directly activate excessive cell growth [39]; (ii) synergism with platelets to amplify injury [40] and to stimulate smooth muscle cell proliferation [41]; (iii) the release of various cytokine and growth factor products [42] with consequent stimulation of smooth muscle cell proliferation and migration. The latter may be the main mechanism by which leukocytes influence neointimal growth. The release of cytokines also stimulates the upregulation of adhesion molecules, which

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**Table 1**

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<tr>
<th>Treatment</th>
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<th>Leukocyte adhesion (%)</th>
<th>Right non-injured</th>
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*No significant differences were found between left and right arteries in either group.*
would facilitate further leukocyte adhesion [43] and thus perpetuate the cycle of leukocyte adherence and damage to the artery wall.

5. Conclusions

The results from the present study strongly suggest that leukocytes may play an important role in the development of neointima, and indeed adventitial fibrosis, following vascular balloon injury. Induction of leukopenia has recently been employed for patients undergoing cardiopulmonary by-pass surgery to attenuate leukocyte-mediated inflammation and organ reperfusion injury [44]. If we can identify the major leukocyte subtype contributing to neointimal formation and the optimum extent of leukocyte depletion required to prevent restenosis, selective leukocyte depletion would be a useful therapeutic approach for restenosis prevention.

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References


