Interleukin 18 binding protein (IL18-BP) inhibits neointimal hyperplasia after balloon injury in an atherosclerotic rabbit model

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Objectives: Interleukin 18 (IL18) is an interferon (IFN)-gamma-inducing factor and a proinflammatory and proatherogenic cytokine. IL18 binding protein (IL18-BP) functions as an IL18 inhibitor. This study was designed to investigate whether systemic administration of IL18-BP could inhibit neointimal hyperplasia and arterial lipid deposition.

Methods: New Zealand white, male rabbits were fed with a 21% fat, 0.15% cholesterol diet. The left superficial femoral artery (SFA) was de-endothelialized with a 2F arterial embolectomy catheter. IL18-BP (5 μg, 10 μg, or 25 μg), or 0.9% saline (control) was administered by i.v. bolus during surgery. Rabbits were followed-up at 2 and 4 weeks. Intima-media (I/M) and lumen-whole artery (L/A) area ratios, and luminal areas were measured. Serum lipid levels, liver enzymes, and kidney function were evaluated. Inflammatory cells were quantified and further verified with immunohistofluorescence staining. The extent of lipid deposition in the artery wall was quantified with Oil Red O (ORO) staining employing Zeiss AxioVision 4.6.3. Image analysis software. Lipid laden cells including macrophages were evaluated by transmission electron microscopy (TEM).

Results: Intravenous IL18-BP 5 μg, 10 μg, and 25 μg significantly reduced I/M ratios compared with the control group at both 2 and 4 weeks. There was no significant difference between the 5 μg and 10 μg dose groups. However, at 10 μg, IL18-BP significantly increased L/A ratio more than either the 5 μg IL18-BP or control groups. The high fat diet caused significant elevation of serum lipids at 4 and 6 weeks. IL18-BP had no effect on blood lipid levels. Lipid deposit in the thoracic aorta of the control group at 6 weeks was more than at 4 weeks (P = .025). Administration of IL18-BP inhibited the lipid deposition at 4 weeks (not significant) and 6 weeks (P = .012 to .008) compared with its control group. Lipid laden macrophages (foam cells), as well as endothelial cells and smooth muscle cells were seen in the descending thoracic aorta after 6 weeks of a high fat diet by ORO, immunohistofluorescence staining, and TEM. The lipid laden cells were not seen in either of IL18-BP groups. IL18-BP 10 μg significantly inhibited mono/macro adherence and infiltration in the SFA after balloon-injury at 2 weeks after surgery.

Conclusion: A single intravenous dose of IL18-BP significantly decreased arterial neointimal hyperplasia, improved lumen to artery ratio after balloon-injury and also prevented arteriosclerosis progression. (J Vasc Surg 2008;47:1048-57.)

Clinical Relevance: A single intravenous dose of IL18BP decreased neointimal hyperplasia and improved arterial L/A ratios in an atherosclerotic balloon-injury animal model. These preliminary results suggest that IL18BP may be a promising molecular approach to inhibit neointimal hyperplasia and arteriosclerosis progression following coronary and peripheral angioplasty.

Over the past decade, atherosclerosis has been recognized as a complex inflammatory and immune response disease mediated by cytokines and chemokines at both the systemic and local levels.1 Both in vitro and in vivo studies have demonstrated that the cytokine IFN-γ is important in atherogenesis.2-4 Interleukin 18 (IL18), a member of the IL-1 cytokine family, is a potent IFN-γ-inducing factor.5,6 It was first found in Kupffer cells and MO,5 and later in osteoblasts,7 chondrocytes,8 and keratinocytes.9 Expression of interleukin-18 was also detected in human atherosclerotic plaques and was related to plaque instability.10 In a rat carotid artery angioplasty model using high density oligonucleotide micro array (Affymetrix) and real time quantitative RT-PCR, we have shown that IL18 gene expression was upregulated starting at day 1, peaking at day 4, and lasting to day 14 following angioplasty.11

Several investigators have shown that IL18 is a pro-inflammatory and pro-atherogenic cytokine.12-17 Recently, IL18 has also been demonstrated to induce rat aortic smooth muscle cell (SMC) proliferation18 and human coronary artery SMC migration.19 Therefore, IL18 is also a promitogenic cytokine. IL18 binding protein (IL18-BP) is a constitutively expressed and secreted protein and functions as an IL18 inhibitor.20,21 It was shown that in vivo electrotransfer of
an expression plasmid DNA encoding for murine IL18-BP prevented fatty streak development in the thoracic aorta of apoE knockout mice and slowed progression of atherosclerotic plaque development. Maffia et al reported that neutralizing IL18 with anti-rat IL18 IgG significantly reduced neointimal formation in a nonatherosclerotic rat carotid artery balloon injury model. The present study was designed to determine if the systemic administration of IL18-BP could inhibit neointimal hyperplasia and arterial fatty streak development in an atherosclerotic rabbit femoral artery balloon angioplasty model.

METHODS AND MATERIALS

This study was approved by The Animal Care and Use Committee of the University of Massachusetts Medical School. All animal procedures were in accordance with the Guide for the Care and Use of Laboratory Animals. Atherosclerotic animal model: male New Zealand white (NZW) rabbits (body weight 3.50 ± 25 kg; Harlan, Indianapolis, Ind) were fed with a high fat diet containing 21% fat, 0.15% cholesterol (5TKW, TestDiet Co, Richmond, Ind), which was continued throughout the study. After 2 weeks the rabbits were subjected to surgery. Serum lipid levels, liver and kidney functions were evaluated prior to starting the high fat diet and then on the day of surgery, 2 weeks later, and at termination.

Surgical procedure

New Zealand rabbits were intubated and anesthetized using intramuscular ketamine (50 mg/kg,) and xylazine (5 mg/kg), intubated and maintained with inhaled 1% to 2% Isoflurane. Crystiben (Pencillin G Benzaprine and Penicillin G Procaine) 150,000 units IM was given preoperatively. Heparin 100 units/kg was given i.v. 5 minutes before artery clamping. The left superficial femoral femoral artery (SFA) was denuded by fully expanding a 2F arterial embolectomy balloon catheter three times within the SFA. Analgesic buprenorphine (0.03 mg/kg) was given subcutaneously (SC) prior to surgery and a transdermal fentanyl patch (1/2 to one of 25 μg/hr patch) was placed on the back skin for 2 days postoperatively for pain relief. The animals were randomly selected to receive an i.v. bolus of 0.9% saline control or IL18-BP recombinant mouse IL18-BPd/Fe chimera (R&D Systems, Inc., Minneapolis, Minn) at 5 μg, 10 μg, or 25 μg, prior to reconstituting SFA blood flow. The anti-rabbit IL18-BP is not commercially available. The amino acid sequence of mouse IL18-BP is 90.6% identical to rat. For uniformity, matched control and IL18-BP group animals underwent surgery on the same day under the same conditions. The rabbits were followed-up at 2 weeks and 4 weeks postoperatively.

Specimen collection

On the day of termination, the animals were anesthetized and intubated as described above. Heparin 100 units/kg was administrated intravenously. Both femoral arteries were harvested following antegrade cannulation and perfusion with 0.9% saline at 110 to 120 mm Hg pressure, followed by perfusion fixation with 10% buffered formalin. Segments of the experimental arteries including the common femoral, superficial femoral, and popliteal arteries were removed en bloc and fixed in 10% buffered formalin. Bilateral SFA samples (about 2.5 cm in length) were cross-sectioned into three segments: proximal, middle, and distal thirds. These segments were processed for paraffin embedding, sectioning 5 μm thick, and hematoxylin-eosin (HE) staining. The descending thoracic aortas were removed and fixed in 10% buffered formalin. The ascending aortas were snap frozen with optimum cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek, Torrance, Calif) and kept at −80°C for cryo-section. Specimens were identified by the rabbit ID number only and all subsequent morphometric area measurements and analyses were performed in a blinded fashion.

Histology

Two representative HE stained cross-sections of the proximal, middle, and distal superficial femoral artery were analyzed using a digital imaging computerized planimetry equipped a cooled CCD camera (Roper Scientific, Princeton Instruments Inc., Acton, Mass) attached to an inverted microscope (Olympus IX-70, Olympus Corp., Tokyo, Japan). The images were analyzed using imagingJ software. The lumen, intima, media, and whole artery areas were measured. The area ratios of intima to media (I/M), and lumen to whole artery (L/A) were calculated.

Blood laboratory parameters. The serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, very-low density lipoprotein (VLDL), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), and creatinine levels were measured prior to starting the high fat diet, 2 weeks later on the day of surgery and at termination.

Oil Red O (ORO) staining. The extent of fatty streak was evaluated in the descending thoracic aorta, which had been fixed in 10% buffered formalin. A segment of the descending aorta was longitudinally opened, washed with Milli-Q water (Millipore Corp., Billerica, Mass). After removal of the adventitia, the artery was pined on a cork board. The exposed lumen was processed for ORO (Cat No K043, Poly Scientific R&D Corp, Bay Shore, NY) staining. The images were taken with a Leica light stereomicroscope at ×0.42 and ×3.25. Lipid deposit on the inner surface was quantified using Zeiss AXIOVision software. The cross-sections (5 μm) of the descending thoracic aorta were also cryo-sectioned and subjected to ORO staining.

Immunohistofluorescence staining. The cryo-sections of the descending thoracic aorta were subjected to immunofluorescence staining for identification of lipid laden cells including macrophages or foam cells. The SFA paraffin blocks were sectioned for immunohistofluorescence staining to identify inflammatory cells and to confirm the findings on HE stain slides. Neutrophils
and T cells were identified with a primary monoclonal mouse anti-rabbit antibody MCA805 (AbD Serotec, Raleigh, NC) and visualized with second antibody Rhodamine Red-X-conjugated AffiniPure goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, West Grove, Penn). Macrophages were identified with monoclonal mouse anti-rabbit macrophage RAM11 primary antibody (DakoCytomation, Inc., Carpinteria, Calif), and visualized with fluorescein (FITC)-conjugated AffiniPure F(ab’)-fragment goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories). The nucleus was stained with DAPI, which was contained in VECTASHIELD mounting medium (Vector Laboratories, Burlingame, Calif). Images were taken with Zeiss imager.M1 microscope.

**Evaluation of inflammatory cells.** The HE stained cross-section of SFA samples were quantified for polymorphonuclear neutrophils (PMN), lymphocytes, monocytes/macrophage (mono/macro) adhesion, and infiltration with microscopy, Zeiss imager.M1, under ×20. The inflammatory cells were counted from the whole field of the sample section. There were three SFA samples for each time period (2 weeks and 4 weeks) and for each treatment group (control, IL18-BP 5 μg, 10 μg, and 25 μg). Each artery contains three segments (proximal, middle, distal) each of which was duplicated. Therefore, each data point in the Fig 1, B represents the mean of 18 sections. The inflammatory cells were further verified with immunohistofluorescence staining.

**Transmission electron microscopy (TEM).** The segments of the descending thoracic aorta after 6 weeks high fat diet with or without IL18-BP 10 μg were evaluated with TEM for identification of lipid laden cells or fatty streak.25

**Statistical analysis**

The effects of treatment, follow-up time, artery segment, and so on were evaluated by analysis of variance (ANOVA) for mixed models (ie, factorial repeated measures design) by restricted maximum likelihood (REML). In the presence of significant main or interaction effects, pairwise comparisons were performed using the Tukey-Kramer multiple comparisons procedure using the actual fitted covariance structure. The distributional characteristics of the outcome variables were assessed using the Kolmogorov-Smirnov goodness-of-fit test for normality on
model residuals. All computations were performed using SAS 9.1.3 Proc Mixed and SPSS 14 statistical softwares.

The results of lumen area, neointima area, media area, whole artery area, neointima to media (I/M) ratio, and lumen to artery (L/A) ratio, are expressed as the mean ± SEM of three to five experimental animals. The effect of ratios or lumen area vs artery segment, treatment, and length of follow-up were evaluated. Differences were considered significant at the 95% confidence level (P < .05).

The effects of dosage and time on PMN, lymphocytes, and mono/macro were evaluated by generalized linear mixed modeling (GLMM) assuming a negative binomial distribution, which allowed for the effects of both categorical and continuous covariates such as time and dosage modeling them either as groups or as linear relationships with separate slopes for groups. Models were fit by restricted maximum likelihood estimation using the SAS Proc GLIMMIX procedure. Models were constructed by first fitting saturated models and then eliminating higher order interaction terms that were not significant until a parsimonious model resulted that contained significant factors and covariates as determined by the type III fixed effects tests for those effects. In the presence of significant differences among means, pairwise comparisons were made using Tukey’s Honestly Significant Difference (HSD) test utilizing the estimated covariance matrix to account for correlated observations.

RESULTS

A total of 50 NZW male rabbits were used in this study with average body weight 3.53 ± 0.34 kg. Sixteen rabbits were fed an atherogenic chow but did not undergo angioplasty. The treatment group was composed of 29 animals that were also fed an atherogenic diet and underwent a left SFA angioplasty and received 0.9% saline control or IL18-BP at three different dose levels. Five rabbits, either from control group or IL18-BP groups, were excluded that died of anesthetic complications.

Histology

The morphologic appearance of the cross-section of left femoral artery following balloon-injury is shown in Fig 1. The circumferential neointima was obvious in the saline control group by postoperative week 2 and 4. Visual inspection shows that the neointimal thickness decreases with systemic administration of IL18-BP. Leukocytes ad-

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**Table.** Histomorphometric measurement areas of lumen, neointima, media, and whole artery (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Lumen (mm²)</th>
<th>Neointima (mm²)</th>
<th>Media (mm²)</th>
<th>Whole artery (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 wk postop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.75 ± 0.30</td>
<td>0.43 ± 0.06</td>
<td>0.46 ± 0.06</td>
<td>2.75 ± 0.34</td>
</tr>
<tr>
<td>0.9% saline</td>
<td>1.11 ± 0.22</td>
<td>0.23 ± 0.04</td>
<td>0.37 ± 0.04</td>
<td>1.81 ± 0.40</td>
</tr>
<tr>
<td>5 µg</td>
<td>2.07 ± 0.30</td>
<td>0.26 ± 0.03</td>
<td>0.47 ± 0.03</td>
<td>3.06 ± 0.30</td>
</tr>
<tr>
<td>25 µg</td>
<td>1.74 ± 0.32</td>
<td>0.13 ± 0.02</td>
<td>0.39 ± 0.05</td>
<td>1.96 ± 0.25</td>
</tr>
<tr>
<td>IL18-BP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 wk postop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.90 ± 0.19</td>
<td>0.51 ± 0.04</td>
<td>0.44 ± 0.03</td>
<td>2.88 ± 0.23</td>
</tr>
<tr>
<td>0.9% saline</td>
<td>1.55 ± 0.24</td>
<td>0.34 ± 0.07</td>
<td>0.38 ± 0.03</td>
<td>2.43 ± 0.20</td>
</tr>
<tr>
<td>5 µg</td>
<td>2.02 ± 0.18</td>
<td>0.41 ± 0.03</td>
<td>0.55 ± 0.02</td>
<td>3.01 ± 0.16</td>
</tr>
<tr>
<td>10 µg</td>
<td>2.54 ± 0.20</td>
<td>0.32 ± 0.04</td>
<td>0.41 ± 0.07</td>
<td>3.27 ± 0.20</td>
</tr>
</tbody>
</table>

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**Fig 2.** Effect of IL18-BP on neointima to media area ratio (I/M ratio), lumen to whole artery area ratio (L/A ratio), and luminal area of the femoral artery 2 weeks and 4 weeks after balloon-injury. Mean ± SEM, N = 3-5. Each bar represents 18 to 30 sections of the left balloon-injured carotid artery from three to five animals.
hetered to the luminal surface and infiltrated into the media can be seen in Fig 18 at ×10.

**Morphometric analysis.** The results of neointima formation and luminal preservation are shown in Fig 2. The table lists the data for lumen, neointima, media and whole artery areas. Intima to media (I/M) ratio following balloon-injury the I/M ratio decreased significantly both at 2 weeks and 4 weeks after surgery in the IL18-BP groups.

**Fig 3.** Blood lipid panel before and after 2 weeks, 4 weeks, and 6 weeks of high fat diet.

**Fig 4.** A, Lipid deposition on the inner surface of rabbit descending thoracic aorta with and without IL18-BP after 4 weeks and 6 weeks high fat diet (A, ORO stain); Calcium deposition in the ascending thoracic aorta without IL18-BP after 4 weeks high fat diet (B, HE stain). C, Percentage area of lipid deposition on the inner surface of a segment of the descending thoracic aorta. Data represent the percentage of total lipid deposition area on 4.38 cm² surface. Two animals of each group were analyzed.
compared with the control group ($P < .002$ and .0001, respectively). Among the IL18-BP groups, there was no statistical difference between 5 μg vs 10 μg, but the higher dose of 25 μg group showed a significant decrease in the I/M ratio compared with the lower dose groups. No significant difference was found between the three segment locations of the artery. The effects of different dosages of IL18-BP (5 μg, 10 μg, and 25 μg) on the percentage
decrease of I/M ratio compared with control groups were 40.2%, 45.5%, and 66.2% at 2 weeks postop and 17.1%, 30.6%, and 56.3% at 4 weeks, respectively (Fig 2).

Lumen to whole artery (L/A) ratio. IL18 10 μg significantly increased the L/A ratio compared with the control group and to the IL18-BP 5 μg group at both 2 weeks and 4 weeks. There was no statistical difference between 5 μg and control. IL18-BP 25 μg did not further increase the L/A ratio compared with IL18-BP 10 μg group.

Lumen area measurements did not show any clear pattern of significance at different time points or among different experimental groups. Because of variations in the diameter of the superficial femoral artery, even between rabbits of the same weight, we found L/A ratio to be more representative than simple measurements of luminal area.

Atherosclerosis evaluation

Blood lipid profile. Blood cholesterol, LDL, HDL, VLDL, and cholesterol to HDL ratio were not significantly increased after consuming 2 weeks of a high fat diet. However, they were significantly increased after 4 weeks, and increased continuously at 12 weeks (P < .01). Triglyceride levels were decreased at 2 weeks, 4 weeks, and 6 weeks compared with before high fat diet (P < .02, .005, and .066, respectively). There were no statistical differences of blood lipids between 0.9% saline control and IL18-BP treatment groups (Fig 3).

Lipid deposition. With ORO staining lipid deposition in the inner surface of the descending thoracic aorta was seen as small red dots in the control group after 4 weeks of a high fat diet. The lipid deposition was further increased with higher density and bigger dot size at 6 weeks (Fig 4, A). IL18-BP 5 μg, 10 μg, or 25 μg decreased fat deposition at 4 weeks (Fig 4, A, upper row) and at 6 weeks (Fig 4, A, bottom row).

Calcium deposits were seen in the ascending thoracic aorta at 4 weeks. (Fig 4, B, HE stain).

The results of quantification of the extent of lipid deposition on the inner surface of a segment of the descending thoracic aorta are shown in Fig 4C, which demonstrates the percentage of total lipid deposition area on 4.38 cm² surface. Two animals of each group were analyzed. Lipid deposition for the control group at 6 weeks was significantly greater than control at 4 weeks as well as each of IL18-BP treatment groups at 4 and 6 weeks. IL18-BP reduced lipid deposition by 32.3% to 78.5% compared with its control. There were no significant differences between different dosages of IL18-BP treatment.

Fig 5A demonstrates the ORO staining of the descending thoracic aorta after 4 and 6 weeks of a high fat diet. At 6 weeks, the lipid is clearly seen in the mono/macrophage, endothelial cells, and smooth muscle cells, but it is not seen in the IL18-BP treatment groups.

Images of ORO and immunohistofluorescence stained cross-sections of the descending thoracic aorta after 6 weeks of a high fat diet are shown in Fig 5B. There were lipid laden macrophages (foam cells), endothelial cells, and SMC. Transmission electron microscopy of the same specimen showed one macrophage containing lipid in a plasma cell (foam cell) undergoing diapedesis between endothelial cells, and another beneath the endothelium. Fig 5C (×8000). There were no lipid laden cells seen in the descending thoracic aorta treated with IL18-BP 10 μg (image not shown).

Liver and kidney functions. The high fat diet had no effect on liver function after 4 weeks as measured by SGOT and SGPT. However, SGOT levels were significantly elevated with high dose of IL18-BP 25 μg at 6 weeks compared with pre-high fat diet (P < .0321). IL18-BP 5 μg and 10 μg had no significant liver impairing effect. SGPT was mildly elevated (NS) (Fig 6). The gross appearance of the liver appeared normal at the time of termination. There was no impairment of kidney function as measured by BUN and creatinine.
Inflammatory cell reaction. Immunofluorescence staining for PMN, lymphocytes and mono/macro of the cross-sections of the SFA after balloon angioplasty are shown in Fig 7A. The quantitative cell counts are shown in Fig 7B. The administration of IL18-BP did not significantly affect the presence of PMN and lymphocytes at any time point compared with the control group. At 2 weeks, IL18BP 10 μg, however, significantly decreased monocytes compared with the control and the other two treatment groups (P < .0002, < .0002 and =.0036, respectively). At 4 weeks, mono/macro was less in IL18-BP 5 μg and 10 μg groups (NS).

DISCUSSION

IL18, a potent IFN-γ-inducing cytokine is important in the development of atherosclerosis. It also induces SMC proliferation and migration. IL18 gene expression was shown to elevate after balloon angioplasty and detected in human atherosclerotic plaques and in stabilized plaque. Therefore, inhibition of IL18 would be an efficient approach in the treatment of clinical atherosclerotic cardiovascular patients. A molecule that has biologic functions of both inhibiting neointima formation and atherosclerotic progression would be of great clinical utility.

The pro-atherosclerotic signaling pathways for cytokine IL18 involves a key role for mature IL18 in the regulation of IFN-γ synthesis. Additionally, IL18 also augments the production of various other mediators implicated in atherogenesis such as, cytokines IL-1ß and the intracellular adhesion molecule (ICAM)-1. Furthermore, IL18 activates Th1 responses, which dominate during human atherogenesis and the Th1 lymphocyte subpopulation express the receptor for IL18. IL18 is therefore a proinflammatory and proatherogenic cytokine. The mechanism of action of IL18 involves binding of the heterodimeric IL18 receptor complex, comprised of the IL-1 receptor-related protein (IL-1Rrp), termed IL18Ra, and the IL-1 receptor accessory protein-like (IL-1RacPL), termed IL18Rβ. Overexpression of both IL18 and IL18Ra/β were found within human atheroma in situ compared with normal arterial tissue, and the ligation of the receptor on cultured endothelial cells (ECs), smooth muscle cells (SMC), and macrophages, and in human artery SMC. It is therefore reasonable to expect that systemic blockage of IL18 using IL18-BP may reduce the proatherogenic response.

Several signaling pathways for the IL18 pro-mitotic mechanism have been identified. It was reported that IL18 induced CXCL16 expression in rat aortic smooth
mRNA expression at day 7, and NF-κB activation at day 14.

The present study showed that following 6 weeks of a high fat diet, containing 21% fat and 0.15% cholesterol, distinct lipid laden macrophages (foam cells), endothelial cells, and smooth muscle cells developed in the thoracic aorta of control animals but not in either of the IL18-BP treated groups. At the same time, the administration of IL18-BP had no effect on serum lipid levels. Mallat et al.23 also found that serum cholesterol and HDL levels were no different between IL18-BP and control group in apoE knockout mice. Our results and those of Mallat suggest that the atherogenic mechanism of action of IL18-BP is from a direct inhibition of IL18 binding to IL18 receptors, rather than an indirect response to an increase in serum lipid and cholesterol levels.

This study also found that a single dose of IL18-BP produced a significant decrease in neointimal hyperplasia 2 and 4 weeks after balloon-injury of the superficial femoral artery, measured by intima to media ratios. Furthermore, the reduction was related to the dose of IL18-BP. These findings confirm the findings of others23 that IL18-BP blocks the inflammatory and mitogenic properties of IL18 that are the key to the development of neointimal hyperplasia.

The accelerated development of hyperplasia, which we observed in this animal model, is in contrast to the usual time course for the development of neointimal hyperplasia in humans, which ranges from months to a year or more. If IL18-BP proves to be an effective inhibitor of hyperplasia in humans, would this short-time course limit its applicability or is there a means to extend the up-regulation of mRNA to maintain suppression of hyperplasia? Would repeated doses of IL18-BP be necessary to prolong the inhibition of the hyperplasia? What are the rabbit blood IL18 and IL18BP levels? The answers to these questions will come from a more thorough understanding of the pharmacodynamics of IL18-BP and its potential for long-term inhibition of hyperplasia and atherosclerosis.

CONCLUSIONS

This study demonstrated that a single intravenous dose of IL18-BP significantly decreased arterial neointimal hyperplasia and improved lumen to artery ratio after balloon-injury and also prevented arteriosclerosis progression. If the safety and efficacy of IL18-BP can be confirmed in other animal models IL18-BP could be of potential benefit to inhibit neointimal hyperplasia following coronary and peripheral angioplasty.

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AUTHOR CONTRIBUTIONS

Conception and design: JML
Analysis and interpretation: JML, BC, IJ
Data collection: JML, PD, GH
Writing the article: JML, BC
Critical revision of the article: JML, BC, ME, MR
Final approval of the article: BC, JML
Statistical analysis: SB, JML
Obtained funding: BC, JML, ME
Overall responsibility: JML, BC

REFERENCES


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