Deficiency of Long Pentraxin PTX3 Promoted Neointimal Hyperplasia after Vascular Injury

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Aim: Pentraxin 3 (PTX3) is a novel marker for the primary local activation of innate immunity and inflammatory responses. Although clinical and experimental evidence suggests that PTX3 is associated with atherosclerosis, the relationship between PTX3 and vascular remodeling after wall injury remains to be determined. We investigated the effects of PTX3 on neointimal hyperplasia following wire vascular injury.

Methods: PTX3 systemic knockout (PTX3-KO) mice and wild-type littermate (WT) mice were subjected to wire-mediated endovascular injury. At four weeks after wire-mediated injury, the areas of neointimal and medial hyperplasia were evaluated.

Results: The PTX3-KO mice exhibited higher hyperplasia/media ratios than the WT mice after wire injury, and the degree of Mac-3-positive macrophage accumulation was significantly higher in the PTX3-KO mice than in the WT mice. Furthermore, the PTX3-KO mice showed a much greater increase in the number of PCNA-stained cells in the vascular wall than that observed in the WT mice.

Conclusions: A deficiency of PTX3 results in deteriorated neointimal hyperplasia after vascular injury via the effects of macrophage accumulation and vascular smooth muscle cell proliferation and migration.


Key words: Vascular remodeling, Pentraxin 3, Inflammation, Proliferation

Introduction

Despite recent advances in treatment strategies, ischemic heart disease remains the major cause of death in both developing and industrialized countries1). Although drug-eluting stents can be used to strongly inhibit coronary restenosis after percutaneous coronary intervention (PCI), a meta-analysis of randomized clinical trials showed that coronary restenosis continues to be a significant problem2, 3). Therefore, identifying novel molecules involved in the onset of restenosis after PCI will help to expand new treatment options for the prevention of restenosis. Neointimal hyperplasia is an essential stage in the development of restenosis as well as atherosclerosis4). Experimental and clinical studies have demonstrated that proinflammatory proteins play a fundamental role in the initiation and progression of neointimal hyperplasia and atherosclerosis5). Pentraxin 3 (PTX3), a 42-kDa secreted glycoprotein, is an acute-phase inflammatory protein of the pentraxin superfamily. While PTX3 shares similarities with classic short pentraxins, such as C-reactive pro-
tein (CRP) and serum amyloid P component, it contains an unrelated long N-terminal domain coupled to a C-terminal pentraxin domain and differs in its gene organization, cellular source and ligands. PTX3 is released from several cell types, particularly dendritic cells, mononuclear phagocytes, macrophages, smooth muscle cells, fibroblasts and endothelial cells, in response to primary inflammatory signals. PTX3 was initially described as an early marker for the primary local activation of innate immunity and inflammatory responses.

There is clinical and experimental evidence linking PTX3 to atherosclerosis and coronary restenosis. However, the relationship between PTX3 and vascular remodeling remains unclear. Therefore, we evaluated the effects of PTX3 loss on vascular remodeling after vascular injury.

**Methods**

**Animal Treatment**

Male PTX3 systemic knockout (KO) mice and littermate wild-type (WT) mice 10-12 weeks of age with a C57BL/6 background were used in the present study. The PTX3-KO mice were kindly supplied by Dr. Mantovani (Istituto Clinico Humanitas IRCCS, Rozzano, Italy). All animals were handled according to the animal welfare regulations of Yamagata University, and the study protocol was approved by the Animal Subjects Committee of Yamagata University. The investigation conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

**Mouse Model of Vascular Injury**

The mice were anesthetized with pentobarbital sodium (50 mg/kg) via intraperitoneal injection. Transluminal arterial injury was induced by inserting a straight spring wire (0.38 mm in diameter) into the left femoral artery, as previously described. The wire was left in place for one minute to denude and dilate the artery. In the sham-operated animals, the same procedure was performed, except for wire insertion. The arteries were harvested at days 7 and 28 after the operation. Blood pressure and heart rate were recorded according to the tail cuff volume-oscillometric method (UR-5000; UEDA Ltd, Japan) in an awake state.

**Histological and Immunohistochemical Studies**

Four weeks after vascular injury, the femoral arteries were removed and fixed in 10% neutral-buffered formalin overnight. The femoral arteries were cut into 5-μm-thick cross-sections (200-μm intervals), which were stained with Elastica van Gieson. The average of three sections for each animal tissue was used to determine the lesion size. For immunohistochemical staining, paraffin-embedded sections (5 μm thick) were deparaffinized and blocked with 5% skim milk. Antibody distribution was visualized according to the streptavidin-biotin-peroxidase complex technique. The sections were incubated with anti-PTX3 antibodies (Santa Cruz), anti-Mac-3 antibodies (BD Biosciences), anti-proliferating cell nuclear antigen (anti-PCNA) antibodies (Santa Cruz) or nonspecific IgG and then counterstained with hematoxylin.

For PCNA staining, the number of labeled proliferating cells and total cells in the neointima and media was counted in all sections for each specimen, and labeling indices were calculated as the labeled cell-to-total cell.

**Statistical Analysis**

The data are presented as the mean ± the standard error of the mean (SEM). Student’s t-test or a two-way ANOVA followed by the Bonferroni test was used for comparisons between groups. A P-value less than 0.05 was considered to be statistically significant. The statistical analyses were performed using a standard statistical program package (JMP version 8; SAS Institute Inc., Cary, North Carolina).

**Results**

**PTX3 Expression in the Femoral Arteries after Vascular Injury**

We investigated the time-dependent expression of PTX3 in the femoral arteries after the sham and wire-mediated vascular injury in the C57BL/6 wild-type mice. Neointimal hyperplasia was found in the femoral arteries on day 28 after wire-mediated vascular injury in the WT mice. Although PTX3 was not detected on immunohistochemistry in the arteries without surgery, the PTX3 expression was increased in the wire-injured arteries on day 28. The PTX3 mRNA expression on days 7 and 28 after vascular injury was significantly higher than that observed in the sham-operated arteries (6.2 ± 1.6- and 2.4 ± 0.6-fold increase, respectively; P<0.05).

**Infiltration of Inflammatory Cells after Vascular Injury**

We suspected that the infiltration of macrophages into the injured arteries may be increased in the mice. Accordingly, there was a greater increase in the number of Mac-3-positive macrophages in both
KO mice after wire injury than in the sham-operated mice. Importantly, the wire injury (Fig. 3A) PTX3-KO mice showed a much greater increase in the number of PCNA-stained cells in the vascular wall than that observed in the WT mice (Fig. 3B).

**Neointimal Hyperplasia after Vascular Injury**

At baseline and four weeks after vascular injury, no significant differences were observed in body weight, heart rate or systolic blood pressure between the WT and PTX3-KO mice (data not shown). Fig. 4A and 4B show representative sections of femoral arteries in the WT and PTX3-KO mice at four weeks after the sham operation and wire injury, respectively. Fig. 4C shows representative sections of femoral arteries in the WT and PTX3-KO mice at four weeks after wire-mediated injury. The PTX3-KO mice after wire injury than in the sham-operated mice. Importantly, the wire injury (Fig. 3A) PTX3-KO mice showed a much greater increase in the number of PCNA-stained cells in the vascular wall than that observed in the WT mice (Fig. 3B).
Fig. 2. Immunostaining for macrophages (Mac3) in the vascular wall with injury in the wild-type (WT) and pentraxin-3-knockout (PTX3-KO) mice

Mac3 staining of the vascular wall with wire-mediated injury in the WT and PTX3-KO mice (A). The arrowhead indicates the internal elastic lamina. (B) Quantitative analysis of Mac3-positive cells in the neointima in the WT and PTX3-KO mice. The values are expressed as the mean ± standard error of the mean. *P<0.05. n=8 in each group.

Fig. 3. Immunostaining for proliferating cells with proliferating cell nuclear antigen (PCNA) in the vascular wall, with and without vascular injury, in the wild-type (WT) and pentraxin-3-knockout (PTX3-KO) mice

(A) PCNA staining of the vascular wall with and without wire injury in the WT and PTX3-KO mice. The arrowhead indicates the internal elastic lamina. (B) Quantitative analysis of PCNA-positive cells in the neointima and media in the WT and PTX3-KO mice. The values are expressed as the mean ± standard error of the mean. *P<0.05; **P<0.01. n=8 in each group.
vascular injury by increasing cell proliferation and macrophage infiltration. PTX3 is produced and released by several cell types, particularly dendritic cells, mononuclear phagocytes, macrophages, smooth muscle cells, fibroblasts and endothelial cells, in response to primary inflammatory signals. PTX3 binds to and regulates complement component C1q, apoptotic cells, some mice also exhibited an increased neointimal area and higher neointima/media ratio after wire injury than the WT mice (Fig. 4C).

**Discussion**

In the present study, we found that a deficiency of PTX3 promotes neointimal hyperplasia after endo-

Fig. 4. Histopathology of the femoral arteries in wild-type (WT) and pentraxin-3-knockout (PTX3-KO) mice
Histological features of the sham-operated mice (A) and wire injury (B) femoral arteries. Cross-sections of all arteries were obtained four weeks after vascular injury and stained with Elastica van Gieson. The arrow and arrowhead indicate the external and internal elastic lamina, respectively. (C) Quantitative comparison of the arterial neointima, media and neointima/media ratio in the WT (white bars) and PTX3-KO mice after wire injury (gray bars). The values are expressed as the mean ± standard error of the mean. *P<0.05. n=8 in each group.
In clinical studies, patients undergoing elective coronary stenting with restenosis have been found to have higher plasma PTX3 levels than those without restenosis. Therefore, PTX3 is considered to be associated with the mechanism of restenosis after PCI. Camozzi et al. reported that one endovascular injection of the recombinant adeno-associated virus PTX3 gene is sufficient to inhibit neointima formation after balloon injury in rat carotid arteries. In that study, PTX3 was considered to be a potent inhibitor of the FGF2-dependent activation of smooth muscle cells. Moreover, adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and platelet/endothelial cell adhesion molecule-1 (PECAM-1), are significantly increased in PTX3 and apolipoprotein E double-knockout mice. In our loss-of-function study, PTX3 deficiency showed a similar worsening effect on neointimal hyperplasia after vascular injury. Interestingly, in PTX3-KO mice, vascular injury significantly increases the number of proliferating cells in neointimal lesions as well as the levels of infiltrating macrophages and inflammatory cytokines. These findings suggest that, in addition to inhibiting FGF2, PTX3 regulates inflammation in the artery after injury. Infiltrating macrophages may contribute to the increase in these adhesion molecules. We obtained similar results for tube cuff-mediated perivascular vascular injury (data not shown), where the PTX3 expression was primarily detected in the adventitia. Considering these data, PTX3 may play an important role in an “outside-in” hypothesis, in which perivascular inflammation is initiated in the adventitia and progresses inward toward the intima.

Conclusion

A deficiency of PTX3 promotes neointimal hyperplasia after vascular injury via macrophage accumulation and VSMC proliferation and migration. These results suggest that PTX3 is a promising molecular target for reducing neointimal hyperplasia after PCI.

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Disclosures

None.

References


