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Proresolving lipid mediators resolvin D1 and protectin D1 isomer attenuate neointimal hyperplasia in the rat carotid artery balloon injury model

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ABSTRACT

Background: Specialized proresolving mediators from ω-3 polyunsaturated fatty acid may control resolution of inflammation. We evaluated the influence of two specialized proresolving mediators, resolvin D1 (RvD1) and protectin D1 isomer (PD1 iso) on neointimal hyperplasia after balloon injury.

Materials and methods: Sprague Dawley male rats at 12-14 wk of age were injured as a model of balloon angioplasty. Then, 1 μg/rat of RvD1 or PD1 iso was administered intravenously via the tail vein immediately and 2 d after angioplasty. The proliferation of injured artery and the infiltration of leukocytes, monocytes, and macrophages at 3 d after injury were evaluated by immunostaining. The activity of the inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) in the injured artery at 3 d after injury was evaluated using an enzyme-linked immuno sorbent assay kit. The proliferation of the neointima was evaluated by calculating the ratio of the neointimal and medial areas using specimens at 14 d after injury.

Results: RvD1 and PD1 iso attenuated proliferation of medial cells ($P < 0.05$) and infiltration of leukocytes ($P < 0.05$) and monocytes/macrophages ($P < 0.01$). Although both RvD1 and PD1 iso mitigated NFκB activity ($P < 0.01$), RvD1 attenuated this activity more strongly ($P < 0.01$). RvD1 decreased neointimal hyperplasia by 37.3% ($P < 0.01$), whereas PD1 iso decreased neointimal hyperplasia by 31.8% ($P < 0.05$) (RvD1 *versus* PD1 iso: $P = 0.51$).

Conclusions: RvD1 and PD1 iso reduced the activity of inflammatory transcription factor NFκB within the injured artery and attenuated inflammatory cell

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infiltration, leading to a reduction in early inflammation and subsequent neointimal hyperplasia.

Introduction

Today, the number of patients with peripheral arterial disease and cardiovascular disease is increasing globally.[1,2] Although endovascular (angioplasty, stenting) or surgical (bypass graft, endarterectomy) interventions have resolved cardiovascular and peripheral arterial diseases, restenosis of the artery remains untreated.[3]

In atherogenesis, endothelial dysfunction leads to polymorphonuclear neutrophil, monocyte/macrophage, and T cell infiltration. As a result, cytokines and transcription factors, including nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), are activated, thereby stimulating vascular smooth muscle cell proliferation and infiltration into the lumen. This ultimately generates a neointima (NI).[4,5] Today, atherosclerosis is recognized as chronic inflammation of the arterial wall[6] and at the restenotic artery, migration of vascular smooth muscle cells caused by arterial injury is recognized as a NI.[7]

Recently, the resolution of inflammation was thought to be an active, rather than a passive, process, as previously thought.[8-10] Four families (lipoxins, resolvins, protectins, and maresins) of specialized proresolving mediators (SPMs) were detected.[11] Lipoxins were derived from arachidonic acid, and the others were derived from ω-3 and ω-6 polyunsaturated fatty acid. Resolvins are divided into the two groups according to their origin. One is resolvin D series (RvD), which is derived from docosahexaenoic acid, and the other is resolvin E series derived from eicosapentanoic acid. Similarly, protectins derived from docosahexaenoic acid are called protectin D (PD).

In a previous *in vivo* study, resolvin D1 (RvD1) and RvD2 have been reported to attenuate vascular smooth muscle cell activation and neointimal hyperplasia in a rabbit balloon injury model.[12,13] Meanwhile, lipoxinA4[14] and maresin 1[15] have also been reported to attenuate neointimal hyperplasia in a mouse carotid artery ligation model. Yet, to date, no studies have assessed the efficacy of protectins in an injured artery. Protectin D1 isomer (PD1 iso) is a newly detected lipid mediator.[16] In previous *in vitro* studies, PD1 iso was found to decrease the level of reactive oxygen species as well as inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase release from neutrophils.[16] In a mouse model of sepsis, PD1 iso was found to improve the survival rate and reduce the incidence of multiple organ injury. Furthermore, phagocytosis of peritoneal macrophages and the level of M2 macrophages in the septic peritoneum both increased.[17] The aim of this study was to evaluate the influence of resolvin D1 and PD1 iso on neointimal hyperplasia in a manner that was simpler than in previous studies. Previously, SPMs were administered by local intraarterial,[12] intraperitoneal,[15] or perivascular wraps or gels.[13] We administered treatment intravascularly twice via the tail vein. Here, we compared NFκB activity, inflammatory cell infiltration, and neointimal hyperplasia across three groups (control, RvD1, and PD1 iso).

Materials and methods

Animal experiments

All animal experiments were performed under a protocol approved by the University of Tokyo Animal Care Committee. Sprague Dawley rats (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) of 12-14 wk of age (370-470 g) were fed a normal diet and subsequently used in all experiments. Only male rats were used to avoid the effect of the sex cycle in all experiments. Animals were anesthetized for surgical procedures with an intraperitoneal injection of a mixture of ketamine (Daiichi Sankyo Company limited, Tokyo, Japan) and xylazine (Bayer Yakuhin Ltd, Osaka, Japan) for appropriate anesthetic depth. The process of generating a carotid artery balloon injury model was described previously.[18,19]

A midline incision of the neck was made, and the left common carotid artery and bifurcation was exposed. A two Fr Fogarty balloon (Edwards Lifesciences, Irvine, CA) was inserted from the external carotid artery to the aortic arch. When the tip of the balloon catheter reached the aortic arch, we inflated the catheter by 0.02 mL and injured the entire common carotid artery three times with rotation. After the injury, the external carotid artery was ligated just distal to the bifurcation by a 4-0 silk thread. The common carotid artery was harvested 3 d and 14 d after the injury for each experiment.

Chemicals

RvD1 (7S,8R,17S-trihydroxy-4Z,9 E,11E,13Z,15E,19Z-docosahexaenoic acid) and PD1 iso (10(S),17(S)-dihydroxy-4Z,7Z,11E,13Z,15E,19Z-docosahexaenoic acid) were purchased from the Cayman Chemical Company (Ann Arbor, MI).

Systemic administration of SPMs

RvD1 or PD1 iso (1 μg/rat for each dose) or 1% ethanol (ethanol with phosphate-buffered saline [PBS]) as vehicle was administered via the tail vein. These SPMs or vehicle solutions were administered just after the balloon injury (d 0) and on d 2 after the injury. In previous studies on SPMs in a neointimal hyperplasia model, the frequency of administration ranged from one to five times, with both regimens resulting in a reduced incidence of neointimal hyperplasia.[12,15] We administered treatment at d 0, in accordance with previous studies, and at 2 d after injury, at which time cell proliferation of the arterial wall peaked.[19]

To evaluate acute effects, rats ($n = 6$/group) were euthanized at 3 d after injury. Injured common carotid arteries were harvested and immediately frozen in OCT (Sakura Finetek Japan Co. Ltd, Tokyo, Japan). Cryosections were used for