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ORIGINAL ARTICLE

Dietary Supplementation with Omega-3 Polyunsaturated Fatty Acids Modulate Matrix Metalloproteinase Immunoreactivity in a Mouse Model of Pre-abdominal Aortic Aneurysm

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Background Two-day infusion of angiotensin II to apolipoprotein E-deficient ($ApoE^{-/-}$) mice provides a model of pre-abdominal aortic aneurysm. Long chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) have anti-inflammatory effects. This study examined the effect of an eight-week low or high n-3 PUFA diet in $ApoE^{-/-}$ mice on matrix metalloproteinase (MMP) expression and elastin degradation.

Methods $ApoE^{-/-}$ mice were fed a low or high n-3 PUFA diet for eight weeks prior to two-day infusion with angiotensin II. The omega-3 index, MMP-2, MMP-9, TIMP-1, and TGF-β1 immunoreactivity, and elastin fragmentation were measured.

Results The omega-3 index with the low and high n-3 PUFA diet was 3.78% and 13.03%, respectively. MMP-9 immunoreactive stain intensity was lower in mice fed the high, compared to the low n-3 PUFA diet in endothelial cells (suprarenal aorta), and inflammatory cells (suprarenal and infrarenal aorta). Inflammatory cells had higher TIMP-1 and TGF-β1 stain intensity in mice fed the high, compared to the low n-3 PUFA diet (suprarenal aorta). MMP-2 immunoreactivity was unaffected by diet. A non-significant trend for reduced elastin fragmentation was observed in mice fed the high n-3 PUFA diet.

Conclusion Dietary supplementation with n-3 PUFAs may have protective anti-inflammatory effects mediated through modulation of MMPs and TIMPs.

Keywords Abdominal aortic aneurysm • Matrix metalloproteinases • Long chain omega-3 polyunsaturated fatty acids • Transforming growth factor-β1 • Cytokines • Elastin

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Introduction

Abdominal aortic aneurysm (AAA) is a cardiovascular disease (CVD) of increasing prevalence and incidence [1]. AAA is characterised by progressive dilation of the aorta as a result of inflammatory, apoptotic and proteolytic processes damaging the aortic wall [1,2]. AAA is defined as a permanent dilation of the infrarenal aorta to at least 30 mm, or 1.5 times the expected infrarenal aortic diameter [1,2]. When peak wall stress exceeds the local strength of the aortic tissue, rupture ensues [3]. Ruptured AAA is associated with a mortality rate up to 90% [2], and is responsible for an estimated 7,000 deaths each year in the United States alone [1]. Open surgery and endovascular repair are currently the only validated therapeutic options recommended for AAA [1]. However, surgical repair costs in excess of US$20,000 per patient and carries an operative mortality rate up to 5% [4–6], highlighting the need to identify non-surgical treatments capable of slowing AAA expansion and minimising AAA associated complications.

Collagen and elastin provide tensile strength and elasticity to the aortic wall. Matrix metalloproteinases (MMPs) have a central role in tissue remodelling through the degradation of these matrix proteins [7]. A dynamic equilibrium between MMPs and their tissue inhibitors (TIMPs) is crucial for tissue destruction and repair homeostasis, with TIMPs binding to the catalytic site of the MMPs to reduce their activity [7]. A 1:1 stoichiometric balance normally exists between MMPs and TIMPs, with an imbalance in this ratio resulting in a loss of tissue destruction and repair homeostasis [7]. In particular, excess MMP-2 and MMP-9, and absence of TIMP-1 potentiate aneurysm formation and rupture [8–10]. Cytokines, including monocyte chemoattractant protein-1 (MCP-1), interleukin-1β (IL-1β), and transforming growth factor-β1 (TGF-β1) are implicated in the pathogenesis, or in some instances in the protection against the development of abdominal aortic aneurysm [11–13]. MCP-1 is highly expressed in AAA, and exposure of human aortic smooth muscle cells to MCP-1 increased MMP-9 expression and activity [11]. Neutralisation of TGF-β1 lead to an increase in MMP-9 gelatinolytic activity in mouse abdominal aortic segments, indicative of a possible protective effect of TGF-β1 against extracellular matrix destruction [12].

The long chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory activities following their incorporation into cell membrane phospholipids [14]. Recently, it has been shown that supplementation of BALB/cA mice with a diet fortified in EPA (10% wt/wt) for four days protected mice against aneurysm development following peri-aortic application of $CaCl_2$ [15]. In that study, EPA reduced smooth muscle MMP-2 and macrophage MMP-9 expression, without effect on TIMP-1 or TIMP-2 gene expression. Whether similar findings can also be seen in other models of AAA, or with other n-3 PUFAs such as DHA, has not yet been investigated.

Subcutaneous infusion of angiotensin-II (AngII) promotes AAA formation in C57BL/6 mice [4]. Frequency and size of aneurysms greatly increases when AngII is infused into hyperlipidaemic mice, such as those deficient in the gene encoding apolipoprotein-E ($ApoE^{-/-}$) [4]. Medial degradation, luminal dilation and intraluminal thrombus formation are characteristic of AAA development in the $ApoE^{-/-}$ AngII-infused mouse model, and are all hallmarks of human AAAs [4]. This model of AAA is also similar to humans in that there is greater proclivity for aneurysm formation in males [4]. This model is not without limitations. Human AAA normally develops in the infrarenal aorta, and does not usually dissect [4]. In contrast, aneurysms in the $ApoE^{-/-}$ AngII-infused mouse model form in the suprarenal aorta, with dissection a common feature [4].

Studies using the $ApoE^{-/-}$ AngII-infused mouse as a model for AAA typically deliver AngII over 28 days [16]. However, initial changes to the aorta, including medial infiltration of macrophages and elastin degradation, are observed within the first four days of initiating AngII infusion [17]. Transmedial dissection and a resultant expanded lumen are evident within seven days of AngII infusion [16]. Haematoma development occurs between 4-10 days [17], during which time up to 10% of mice die due to ruptured AAA [17]. This suggests that two-day infusion of AngII into male $ApoE^{-/-}$ mice will be ideally suited to examine the effects of n-3 PUFAs on the early inflammatory response, prior to aneurysm development.

The aims of this study were to characterise MMP-2, MMP-9 and TIMP-1 immunoreactivity in endothelial cells lining the vessel lumen, smooth muscle cells of the media and adventitial inflammatory infiltrates of the abdominal aorta in $ApoE^{-/-}$ AngII-infused mice fed a diet with low (DHA, 0%; EPA, 0%; total n-3 PUFA, 0.14%) or high n-3 PUFA content (DHA, 0.3%; EPA, 0.07%; total n-3 PUFAs, 0.7%).

Materials and Methods

Dietary Supplementation of Animals

Three week old male $ApoE^{-/-}$ mice on a C57BL/6 background (n = 20) were housed in groups of five in a 12 h light/dark cycle, with *ad libitum* access to sterile water and feed. Mice were randomised to receive a meat-free rat and mouse chow diet containing low or high n-3 PUFA content (Table 1). Animal feed was autoclaved (121 °C, 25 min) to minimise risk of infection to the mice. Fatty-acid content of pre- and post-autoclaved feed has previously been analysed in this laboratory, with gas chromatography-mass spectrometry (GC-MS) analysis finding only a small diminution in n-3 PUFA content post-autoclaving. All animals received humane care in accordance with the 'Statement on Animal Experimentation' by the National Health and Medical Research Council of Australia. The protocol was approved by the Animal Ethics Committee of the University of the Sunshine Coast (Approval Number: AN/A/13/70).

Mice were fed the low or high n-3 PUFA diet for eight weeks, anaesthetised using sodium pentobarbital (32.5 μg/g body weight in 0.9% saline) prior to subcutaneous insertion