[課程-2]

審査の結果の要旨

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This study investigated the effects of the widely used ω-3 polyunsaturated fatty acid, EPA, on the formation of abdominal aortic aneurysms (AAAs). A mouse model of AAA, where $CaCl_2$ was applied to the infra-renal aorta directly, was used to elucidate both the effects and mechanism of action of EPA. The study yielded the following results:

1. Mice were fed a diet that was supplemented with or without 10% wt/wt EPA and induced to form AAAs. After 6-weeks, quantitative analysis of AAA diameters showed that the mice in the EPA diet group had significantly smaller AAAs compared to the control diet group, demonstrating that EPA effectively attenuates AAA formation.

2. The RNA from whole AAAs of the sham, control diet, and EPA diet groups were extracted, reverse transcribed to make cDNA, and analyzed using real-time PCR. This revealed that the expression levels of matrix metalloproteinases (MMPs) *Mmp2* and *Mmp9* were markedly reduced by 30% and 70%, respectively, in AAAs from mice treated with EPA, while there were no differences in the expression level of tissue inhibitors of such MMPs or other extracellular components. These results suggest that EPA shifted the AAA microenvironment to one that was relatively anti-proteolytic with respect to the balance between MMPs and its tissue inhibitors.

   Similarly, protein from whole AAAs of the sham, control diet, and EPA diet groups were extracted and analyzed using gelatin zymography to assay for MMP activity. This also showed reduced MMP2 and MMP9 activities at the protein level in AAAs after EPA treatment.

3. The ability of macrophages to express *Mmp9* was suppressed by EPA. This was demonstrated by extracting RNA from macrophages in AAAs of the control diet and EPA diet groups that were sorted by flow cytometry, and analyzing *Mmp9* expression by real-time PCR. In addition, this was further confirmed by *in vitro* experiments where the macrophage cell line, RAW264.7 cells, was used and treated with various doses of EPA and induced to express *Mmp9* with recombinant TNF-A protein.

Gene expression analysis with real-time PCR showed a dose-dependent reduction in *Mmp9* expression with increasing EPA concentration.

4. Using computed tomographic (CT) imaging of *in situ* AAAs in mice of the sham, control diet, and EPA diet groups, the level of AAA calcification was quantified and this showed that the volume of calcification was lower in the EPA treated group. Again, gene expression analysis of whole AAAs from sham, control diet, and EPA diet groups using real-time PCR also showed that up-regulation of the vascular calcification factor RANKL was significantly inhibited by EPA treatment. This may explain why reduced calcification in the AAAs of EPA-treated mice was observed.

5. Thioglycollate-elicited peritoneal macrophages were obtained by intraperitoneal injection of thioglycollate 4-days prior to harvest, and these cells were treated *in vitro* with recombinant RANKL protein. Analyzing their gene expression with real-time PCR clearly showed that *Mmp9* was clearly up-regulated in response to recombinant RANKL protein in a time-dependent manner. By assessing the expression level of RANKL in whole AAAs of wild-type mice on days 1, 3, and 5 after AAA surgery with real-time PCR, the expression of RANKL was seen to closely correlated with that of *Mmp9*. These results together support the possibility that RANKL can contribute to the production of MMP9 by stimulating aneurysmal macrophages *in vivo*.

6. While the dose of EPA used in mice is much higher than that used in humans, it appears that the protocol itself is not markedly different from that used in other reports and therefore can be considered to be satisfactory in terms of methodology.

7. As discussed in the study, blood pressure does not seem to be a major contributor to AAA formation in this mouse model, which is mainly driven by inflammatory pathways. Serum lipid/EPA concentrations after EPA feeding have been thoroughly investigated and confirmed in previous reports, and therefore it was not deemed to be necessary to repeat here.

Taken together, this thesis has demonstrated the novel effects of EPA in attenuating AAA formation and calcification, as well as shedding light on the possible direct involvement of vascular calcification factors in the formation of AAAs itself. Given the clinical significance and contributions to our basic understanding of AAA pathogenesis, this research is considered to qualify for the award of a doctoral degree.