BIOLOGICAL ACTIVITY OF HUMAN LIVER TYPE FATTY ACID BINDING PROTEIN & ITS SERUM CONCENTRATIONS IN PATIENTS WITH END-STAGE RENAL DISEASE

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BACKGROUNDS

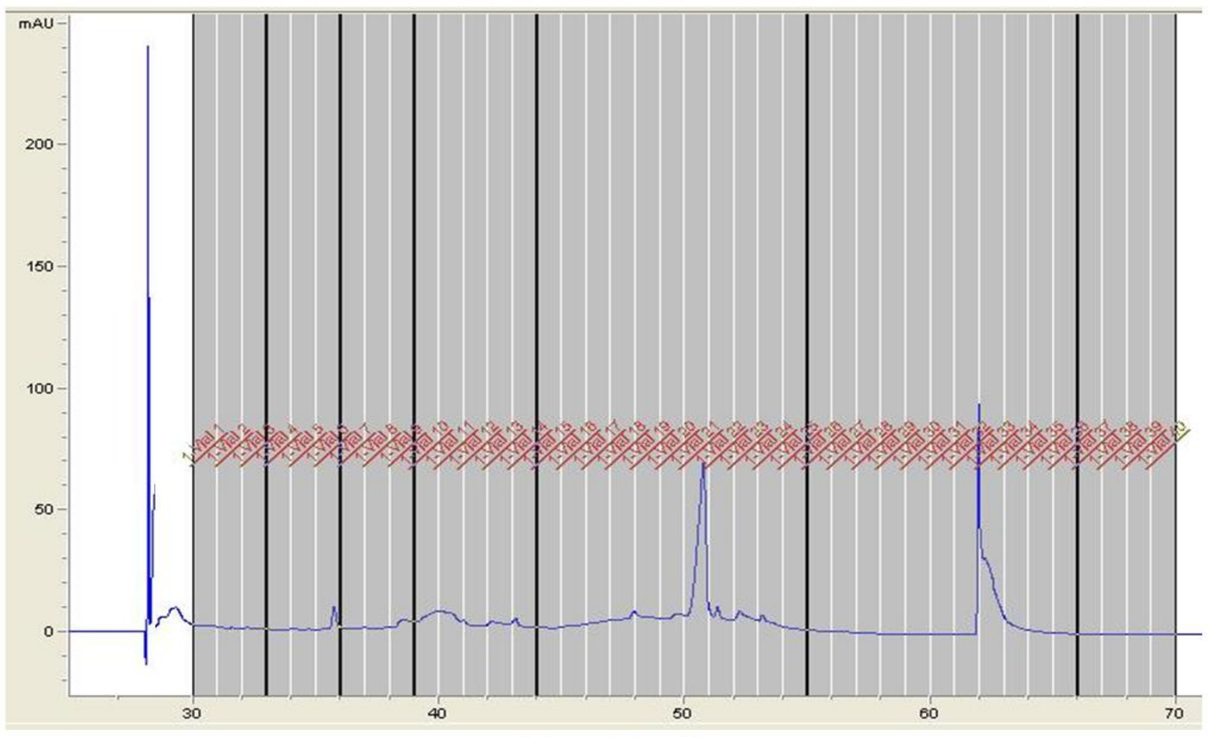
◆Fatty acid-binding proteins (FABPs) are a family of cytosolic proteins that are involved in the intracellular lipid responses in cells. However, it has been recently reported that these proteins do not act only as intracellular mediators but also have extracellular functions. This study aimed to investigate whether liver type (L)-FABP has a biological activity in the human blood and other tissues, and to determined plasma L-FABP levels in patients with end-stage renal disease (ESRD) on hemodialysis (HD).

METHODS

◆ We isolated L-FABP complementary deoxyribonucleic acid from the Huh7 human hepatocarcinoma cell line and expressed the recombinant L-FABP protein in Escherichia coli. High performance liquid chromatography was used to purify the recombinant protein. Blood samples of healthy volunteers and patients with ESRD on HD were taken after an overnight fast and collected in heparin- or ethylenediamine tetraacetic acid (EDTA)-coated tube. A549 lung carcinoma and THP-1 monocyte cells were stimulated with human recombinant L-FABP for 24 hr. Human whole blood cells were also treated with human recombinant L-FABP or interleukin (IL)-1 alpha (α). And then IL-6 levels were measured in cell culture supernatants by sandwich enzyme-linked immunosorbent assay (ELISA). Plasma L-FABP levels of healthy volunteers and ESRD patients were also measured with ELISA and compared.

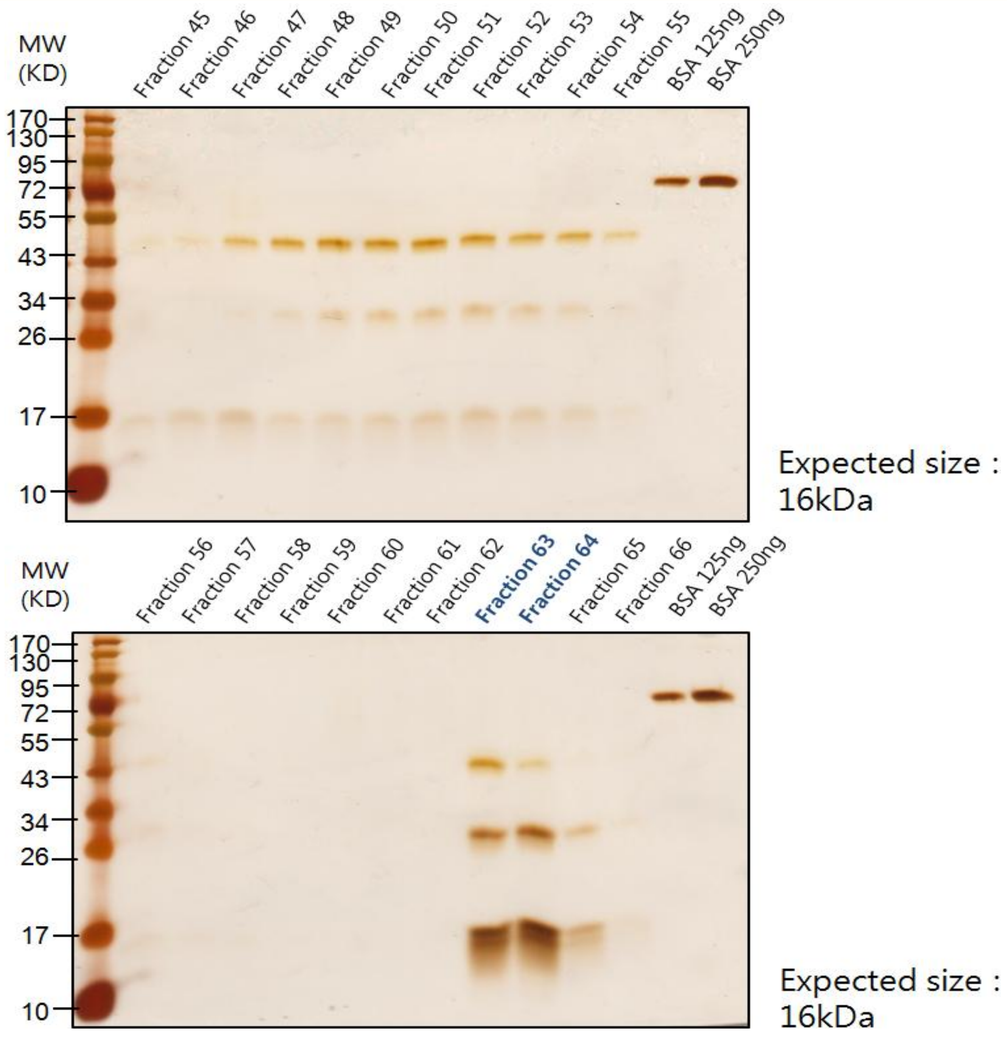
RESULTS

Fig. 1. Isolation of human recombinant liver-fatty acid binding protein by using high performance liquid chromatography.



Human recombinant liver-fatty acid binding protein (L-FABP) was expressed in *E. coli* and first purified by a Talon metal affinity chromatography. Then the protein was further purified by high performance liquid chromatography. The peaks corresponding to human recombinant L-FABP were observed right after 51 and 63 min. Units are in milli Absorbance Units (mAU) at 280 nm.

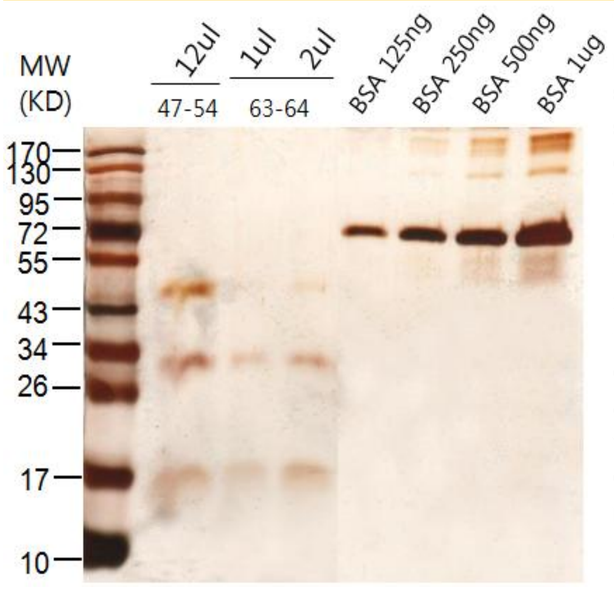
Fig. 2. Silver staining of human recombinant liver-fatty acid binding protein.



The purified human recombinant liver-fatty acid binding protein (L-FABP) was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and visualized by silver staining. (A) The eluants from fraction 45 to 55 were visualized by silver stain. A serious of protein bands with molecular weight of 16- (monomer form), 32- (dimer form), and 48 kDa (trimer form) were identified in nearly all fractions examined. (B) These protein bands were detected mainly in fractions 63 and 64 especially. The data represent one of three independent experiments. Molecular weight (MW) is indicated on the *left*.

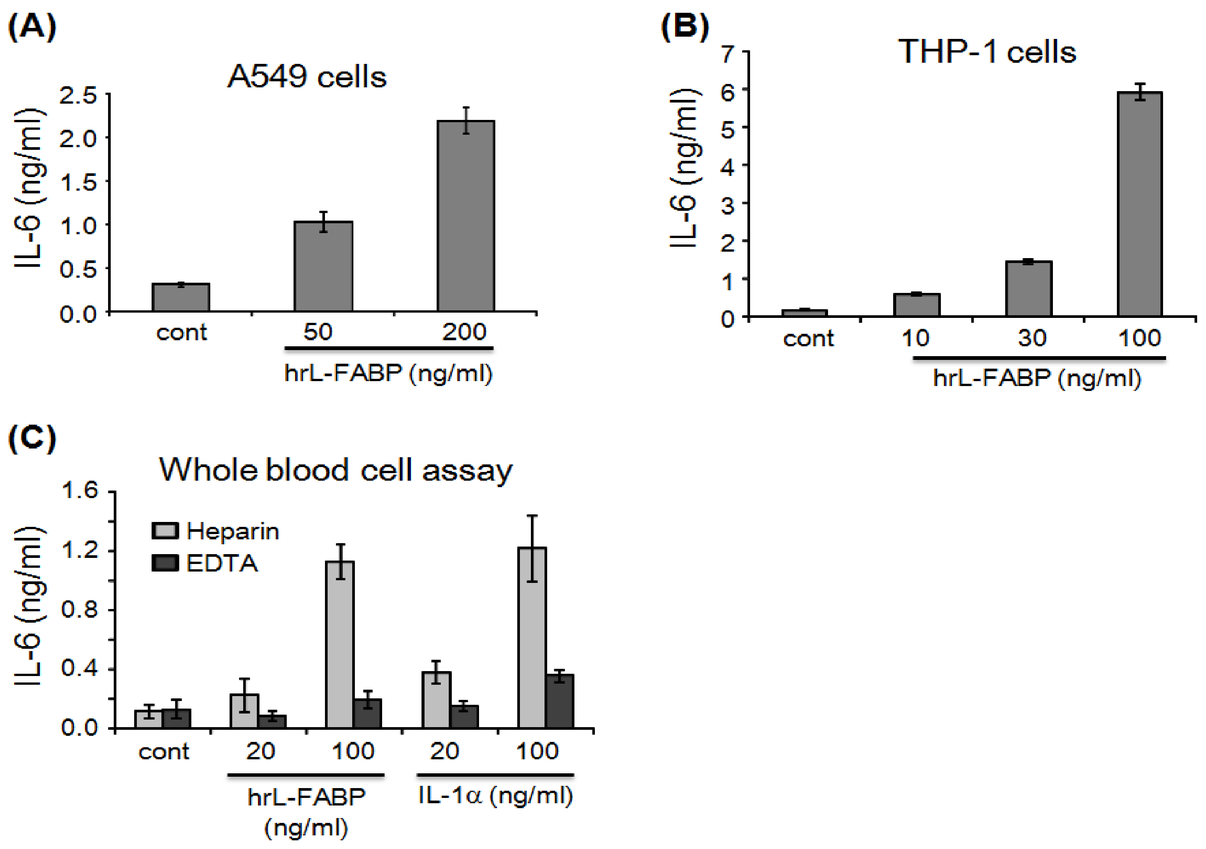
BSA; bovine serum albumin, KD; kilodalton.

Fig. 3. Quantification of human recombinant liver-fatty acid binding protein concentration.



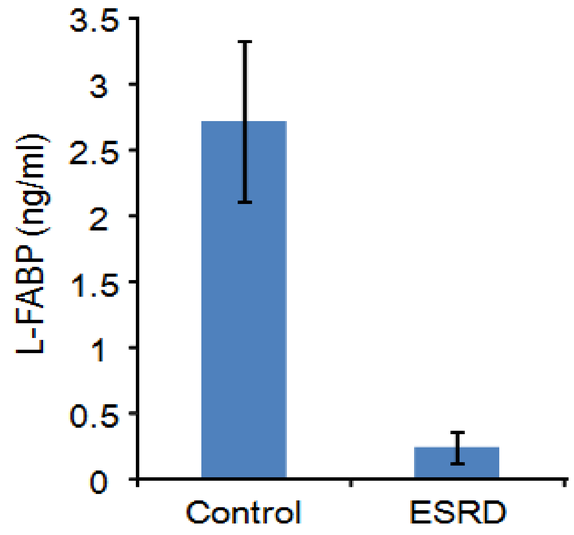
The purified human recombinant liver-fatty acid binding protein (L-FABP) was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, visualized by silver staining, and compared with various known amount of bovine serum albumin (BSA). Twelve µl of the eluants from fraction 47 to 54 was loaded in the second lane. One and 2 µl of the eluants from fraction 63 to 64 was loaded in the third and fourth lanes, respectively. Purified human recombinant L-FABP proteins from fraction 47 to 54 typically migrate as monomer, dimer, and trimer otherwise those from fraction 63 and 64 mainly migrate as monomer and dimer. A standard curve was created with density measurement of BSA standards and used to calculate estimates of human recombinant L-FABP protein concentration. The data represent one of three independent experiments. Molecular weight (MW) is indicated on the *left*.

Fig. 4. Biological activities of human recombinant liver-fatty acid binding protein



Human recombinant liver-fatty acid binding protein (L-FABP) was examined with A549 lung carcinoma and THP-1 monocytic and human blood cells. A549 cells (A) and THP-1 cells (B) were treated with various concentrations of human recombinant L-FABP as indicated under the horizontal axis for 24 h, and interleukin (IL)-6 levels were assessed by enzyme-linked immunosorbent assay (ELISA). Human recombinant L-FABP induced IL-6 in a dose-dependent manner in these cells. (C) Whole bloods from healthy volunteers were collected with heparin- or ethylenediamine tetraacetic acid (EDTA)-coated tube and IL-6 supernatant levels were measured with ELISA after 24 h of incubation with human recombinant L-FABP or IL-1 alpha (α) at concentrations indicated under the horizontal axis. Both the human recombinant L-FABP and IL-1α induced IL-6 in human blood cells compared with non-treated control cells. These effects were stronger in the blood cells collected with heparin-coated tube than in those collected with EDTA-coated tube. The data represent one of three independent experiments. *cont,* control; hr, human recombinant; L-FABP, liver-fatty acid binding protein

Fig. 5. Liver-fatty acid binding protein concentration in human serum



The serum liver-type fatty acid-binding protein (L-FABP) levels of the control group (n=a) and hemodialysis patients (n=b). Blood samples of healthy volunteers were taken after an overnight fast and those of end-stage renal disease (ESRD) patients were drawn before the start of a routine hemodialysis treatment. Levels were measured using our ELISA assay. The values of L-FABP in patients with ESRD were significantly lower than those in the control group. The plot shows the mean values, and the vertical lines indicate the standard errors.

ESRD, end-stage renal disease; L-FABP, liver-fatty acid binding protein

Conclusion

◆ We showed the biological activity of human L-FABP in human cell lines and whole blood cells by demonstrating that its human recombinant form induces IL-6 production. And the similar effect of L-FABP with IL-1α on whole blood cells indicates that circulating form of L-FABP can be a mediator of systemic inflammation. In addition, we also found that the serum levels of L-FABP in ESRD patients on HD were significantly lower than those in the healthy control group, suggesting that severe renal dysfunction may affect the induction of L-FABP in the liver.

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Hyun Woo
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21-24 2016
Held jointly with Österreichische Gesellschaft für Nephrologie
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