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Research Article

OxLDL-Induced Foam Cell Formation Inhibitory Activity of Pepsin Hydrolysate of Ark Shell (*Scapharca subcrenata* (Lischke, 1869)) in RAW264.7 Macrophages

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Inhibitory effect of ark shell (*Scapharca subcrenata* (Lischke, 1869)) proteolytic hydrolysates (ASHs) on oxidized low-density lipoprotein (oxLDL)-induced macrophage foam cell formation was investigated. Two types of ASHs were prepared by Alcalase® and pepsin, ASAH (ark shell-Alcalase® hydrolysates), and ASPH (ark shell-pepsin hydrolysate). Oil Red O staining results showed that ASPH suppressed foam cell formation and lipid accumulation more than ASAH in oxLDL-induced foam cell formation of RAW264.7 macrophages. ASPH reduced the levels of total cholesterol, cholesterol ester, and free cholesterol in oxLDL-treated RAW264.7 macrophages. It was found that ASPH increased cholesterol efflux and decreased cholesterol influx rate. In this regard, protein expressions of CD36 and scavenger receptor class A1 (SR-A1) for cholesterol influx and ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) for cholesterol efflux were investigated. ASPH treatment resulted in an increase of ABCA1 and ABCG1 expression but downregulated CD36 and SR-A1 expression. Furthermore, ASPH suppressed production of proinflammatory cytokines, including tumor necrosis factor-α and interleukin-6 and -1β, through regulating nuclear factor-kappa B (NF-κB) in oxLDL-induced foam cell formation of RAW264.7 macrophages. Taken together, our data indicate that ASPH might be a useful ingredient in functional foods for ameliorating atherosclerosis by preventing foam cell formation.

1. Introduction

Atherosclerosis, a progressive inflammatory disorder, is a major risk factor for the development of cardiovascular diseases, leading to the causes of morbidity and mortality [1]. Although the development of atherosclerosis consists of several steps, lipid dense macrophages (foam cells) and fat streak accumulation are two key events [2]. Foam cells play an important role in formation from the early-stage atherosclerotic lesions to the progression of advanced plaques [3]. Foam cell formation is normally induced by the dysregulation of lipid metabolic pathways including cholesterol influx, cholesterol esterification, and cholesterol efflux [4]. Abnormal uptake and accumulation of oxidized low-density lipoproteins (oxLDLs), which are mediated by excessive reactive oxygen species (ROS) in the subendothelial space, cause macrophage activation to form foam cells [5]. It is reported that scavenger receptors such as scavenger receptor class A1 (SR-A1) and CD36 play a pivotal role in the modified cholesterol uptake and foam cell formation [6]. On the other hand, ABCA1 and ABCG1 enhance cholesterol efflux from macrophages and play essential role in the reverse cholesterol transport [7, 8]. Thus, foam cell formation is promoted by the imbalance between oxLDL uptake via SR-A1 and CD36 and cholesterol efflux via ABCA1 and ABCG1. In line with this, a number of studies have been

trying to find a useful agent to prevent atherosclerosis through inhibiting foam cell formation in vitro and in vivo [6, 9, 9–15].

Protein hydrolysates or bioactive peptides (BAPs), which consist of 2 to 20 amino acids, are a group of biological molecules and are generally encoded in the sequence of parent proteins [16]. There are many approaches to produce BAPs, and the most common way is enzymatic hydrolysis with proteolytic enzymes such as pepsin, trypsin, and chymotrypsin as well as food-grade enzymes such as Alcalase®2.4L. So far, many BAPs possessing anti-inflammatory, antimicrobial, antiobesity, antiadipogenesis, and anticancer effects have been produced using the aforementioned enzymes by proteolytic hydrolysis from food, as well as from especially marine edible organisms [17–21]. The marine bivalve ark shell (*S. subcrenata*), is a popular food ingredient in Korea, Japan, and China with approximately 7,5000 tons produced annually in Korea. Recently, our research group found that ark shell is a good source for producing BAPs showing antioxidant, anti-adipogenesis, and osteogenesis [22–25]. These BAPs were produced by proteolytic enzymes such as pepsin and Alcalase®2.4L. According to these results, it is suggested that two proteolytic enzymes are useful for the production of BAPs with multifunctional effects. However, BAPs produced by pepsin and Alcalase®2.4L have not yet been evaluated for other biological effects, such as their antiatherosclerotic effect. Therefore, the objective of this study was to prepare BAPs using pepsin and Alcalase®2.4L and to evaluate antiatherosclerotic effect by measuring inhibition ability against oxLDL-induced macrophage foam cell formation. Underlying molecular mechanism was also investigated.

2. Materials and Methods

2.1. Materials. Ark shell was obtained from a local market (Sunchen, Korea). Alcalase®2.4.L.FG and pepsin (1 : 10,000) were purchased from Junsei Chemical Co. (Tokyo, Japan). LEE BioSolutions (360-10, Lee BioSolution, Maryland Heights, MO, USA) provided human plasma LDL. All cell culture reagents were purchased from Gibco BRL (Grand Island, NY, USA). Primary and secondary antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), NovusBio ®, USA, and Abcam (Dawinbio Inc). All other reagents utilized in this study were ordered from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of Ark Shell Hydrolysates. Ark shell hydrolysates (ASHs) were prepared by enzymatic hydrolysis using Alcalase®2.4L and pepsin separately following the protocol of Hyung et al. [26]. The prepared ark shell-Alcalase®2.4L hydrolysate (ASAH) and ark shell-pepsin hydrolysate (ASPH) were used to treat macrophages for the determination of foam cell formation inhibitory activities.

2.3. Oxidation of LDL and Determination of Thiobarbituric Acid-Reactive Substances (TBARS). LDL (1 mg/mL) was diluted in PBS and incubated with 10 μM $CuSO_4$ for 4 h at 37°C for oxidation and the TBARS assay was performed to determine the extent of LDL oxidation as described by Oh et al. [27]. Briefly, formed oxLDL was reacted with 25% w/v of TCA and 1% of TBA in 0.3% NaOH mixture in 1 : 1 ratio and boiled at 92°C for 40 min in dark condition. Absorbance was measured at 532 nm using a microplate reader (Multiskan™ GO, Thermo Fisher Scientific, Rockford, IL, USA). The extent of LDL modification was expressed using a standard curve of malondialdehyde (MDA) as nanomoles of MDA per milligram of LDL protein. TBARS value between 165 and 200 nM/MDA was used for all experiments.

2.4. Cell Culture and Treatment. Cells (RAW264.7 macrophages) were cultured in DMEM culture medium under standard conditions. Cell density of 1×10^5 or 1×10^6 cells/mL was used on 96-well plates or 6 cm culture dishes, respectively. ASAH or ASPH was added to the cells for 1 h followed by oxLDL exposure to another 24 h.

2.5. MTT Assay. Cytotoxicity of ASAH and ASPH in RAW264.7 murine macrophages was evaluated using the MTT assay as followed by the method of Hyung et al. [28]. Briefly, after the cell treatment with ASAH and ASPH (1–3 mg/mL) for 24 h, the cells were incubated with MTT working reagent for another 4 h. After the formazan crystal formation, DMSO was added to dissolve them and 540 nm wavelength was used to measure absorbance using a microplate reader (Multiskan™ GO, Thermo Scientific™, Waltham, MA, USA).

2.6. Oil Red O Staining Assay. Macrophages were treated as described previously, and Oil Red O staining assay was performed according to the method of Wu et al. [14]. After the treatment, 4% paraformaldehyde was added to each well for 1 h for the fixation of cells. After washed with 60% of isopropanol, freshly prepared Oil Red O working reagent was used to cell staining for 1 h in dark. Images were captured by an inverted microscope (DMI6000, Leica, Wetzlar, Germany) and absorbance was measured at 510 nm using a microplate reader (Multiskan™ GO, Thermo Scientific™, Waltham, MA, USA).

2.7. Cholesterol Influx and Efflux Assays. After the treatment of cells with ASPH as described previously in the 96-wellclear-bottom black plates, cholesterol influx and efflux assays were performed according to the methods of Wu et al. [14] and Lee et al. [29]. For cholesterol influx determination, after the cell treatment, 5 μg/mL of 25-NBD cholesterol was used to label the cells for 6 h and cholesterol levels in the cells were measured at 485 nm of excitation and 535 nm of emission wavelengths using a GENios microplate reader (GENios, TECAN, Männedorf, Switzerland).

For cholesterol efflux determination, the cells were labeled with 25-NBD cholesterol (1 μg/mL) for 6 h. After that, labeled cells were rinsed with PBS and incubated another 6 h in the DMEM medium. The amount of cholesterol in cells and released cholesterol in a culture medium were measured

at 485 nm of excitation and 535 nm of emission wavelengths using a GENios microplate reader (GENios, TECAN, Männedorf, Switzerland). Cholesterol efflux was shown as described by Lee et al. [29].

2.8. Determination of Intracellular Cholesterol Content. After cell treatment, intracellular cholesterol and cholesteryl ester contents were detected using a commercially available assay kit (Cholesterol/Cholesteryl Ester Quantitation Colorimetric Kit II; BioVision, Inc., Mountain View, CA, USA), according to the instructions of the manufacturer.

2.9. Determination of TNF-α, IL-1β, and IL-6 Levels. After cell treatment, proinflammatory cytokine (TNF-α, IL-1β, and IL-6) expression levels were detected using BioTrak™ ELISA kits (GE Healthcare) according to the instruction of the manufacturer.

2.10. Western Blot Analysis. Standard protocol was followed to perform the western blot analysis. Briefly, whole cell lysates were prepared using RIPA buffer in the presence of protease and phosphatase inhibitors (Roche Diagnostics, Seoul, Korea) after treated the cells as described previously. Nuclear fraction was extracted using the nuclear extraction kit (NE-PER Nuclear and Cytoplasmic Extraction Reagents, Thermo Scientific) according to the instructions of manufacturer. Chemiluminescence ECL assay kit (Life Technologies, Seoul, Korea) was used as the visualization agent of bands and the images were captured on Davinch-Chemi Imager™ (CAS400SM, Core Bio, Seoul, Korea).

2.11. Characterization of ASPH on Molecular Weight Distribution and Amino Acid Composition. The molecular weight distribution and amino acid composition of ASPH were determined as described by Marasinghe et al. [30]. Molecular weight distribution was analyzed using the HPLC system (Dionex UltiMateTM 3000, Thermo Scientific) equipped with a Superdex Peptide 10/300 GL column (No. 9611011, Pharmacia Biotech, Uppsala, Sweden) with loading 20 μL (5 mg/mL) onto the column, eluted at 0.5 ml/min and monitored at 214 nm.

Amino acid composition was determined by hydrolyzing ASPH (50 mg) with 6 N HCl at 110°C for 24 h in a sealed-vacuum ampoule. Then 50 mL of sodium citrate buffer (0.2 M, pH 2.2) was added to a volumetric flask after removing HCl, and amino acid composition was analyzed on an amino acid analyzer (Sykam DE/S 433D, Sykam, Eresing, Germany) equipped with a cation separation column (LCA K06/Na, 4.6 × 150 mm).

2.12. Determination of Proximate Composition of ASPH. Association of Official Analytical Chemist (AOAC) standard protocols were followed to analyze the proximate composition of ASPH according to the method of Marasinghe et al. [30]. Briefly, the method 988.12 (phenol-sulfuric acid method), method 922.06, method 923.03, and method 925.10 were used to determine crude carbohydrate content, crude lipid, crude ash, and moisture content, respectively.

Crude protein content was determined by subtracting all of the aforementioned compositions from 100.

2.13. Statistical Analysis. Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA) was used to do a one-way ANOVA analysis on the data, which were presented as means SD ($n = 3$). Values with $P < 0.05$ were regarded as statistically significant.

3. Results

3.1. Effect of ASAH and ASPH on oxLDL-Induced Foam Cell Formation. Cytotoxicity of ASAH and ASPH to macrophages was priorly determined and two samples showed no cytotoxicity in the tested concentrations (1~3 mg/mL) (Figure 1(a)). After pretreatment with ASAH or ASPH followed by exposure to oxLDL, Oil Red O staining was performed to determine foam cell formation. Figures 1(b) and 1(c) show that there are no cells stained with Oil Red O reagent in the control group (untreated), but the cells treated with oxLDL are heavily stained with Oil Red O reagent. The results of quantification analysis showed that ASAH and ASPH effectively inhibited foam cell formation of macrophages induced by exposure to oxLDL. There is no significant difference between ASAH and ASPH, but ASPH showed slightly higher inhibitory activity than ASAH. The inhibition rates were 71.09% for ASPH and 65.87% for ASAH at a concentration of 3.0 mg/mL. Based on these findings, ASPH was chosen for further analysis.

3.2. Effect of ASPH on Cholesterol Metabolism in oxLDL-Induced Foam Cell Formation. After treatment with oxLDL, there was a significant increase in total cholesterol, free cholesterol and, cholesterol ester in macrophages compared to those of the control group (Figures 2(a)~2(c)). However, the increase in cholesterol accumulation caused by exposure to oxLDL was significantly ameliorated by treatment with ASPH in a dose-dependent manner. At 3.0 mg/mL of ASPH, the inhibition rates are 43.77% for total cholesterol, 43.18% for free cholesterol, and 48.83% for cholesterol ester.

Since ASPH reduced cholesterol accumulation in oxLDL-induced foam cell formation, this study investigated the effects of ASPH treatment on cholesterol efflux and influx to elucidate how ASPH reduced the level of intracellular cholesterol. Figures 3(a) and 3(b) show that the cholesterol influx is significantly decreased dose-dependently and the inhibition rate is 25.42% at 3.0 mg/mL of ASPH. The activation of cholesterol efflux was also determined and the result showed that ASPH activated cholesterol efflux to 17.76% at 3.0 mg/mL of ASPH.

To further elucidate the role of ASPH in cholesterol metabolism, its effect on expression of proteins related to cholesterol influx and cholesterol efflux was investigated. Figure 3(c) shows that the expression of CD36 and SR-A1, two cholesterol influx-related proteins, is activated by

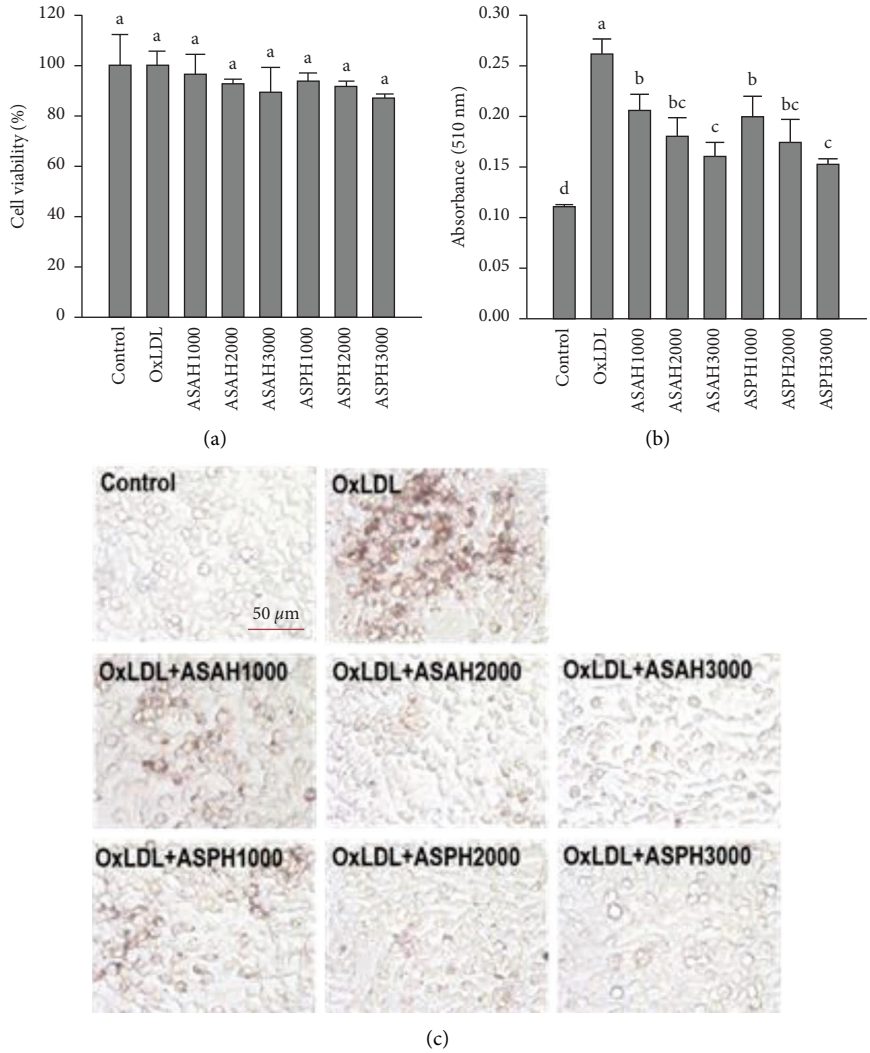

Figure 1: (a) Cell viability and (b) inhibition of foam cell formation of 1–3 mg/mL of ark shell-Alcalase® hydrolysates (ASAH) and ark shell-pepsin hydrolysates (ASPH) quantitatively, and (c) qualitatively in oxLDL-induced RAW264.7 murine macrophages. The macrophages were treated with ASAH (1–3 mg/mL) or ASPH (1–3 mg/mL) for 1 h and the MTT assay was performed to determine the cell viability. The experiments were conducted as three independent determinations ($n = 3$) with ±S.D., and error bars with different letters are significantly different ($P < 0.05$).

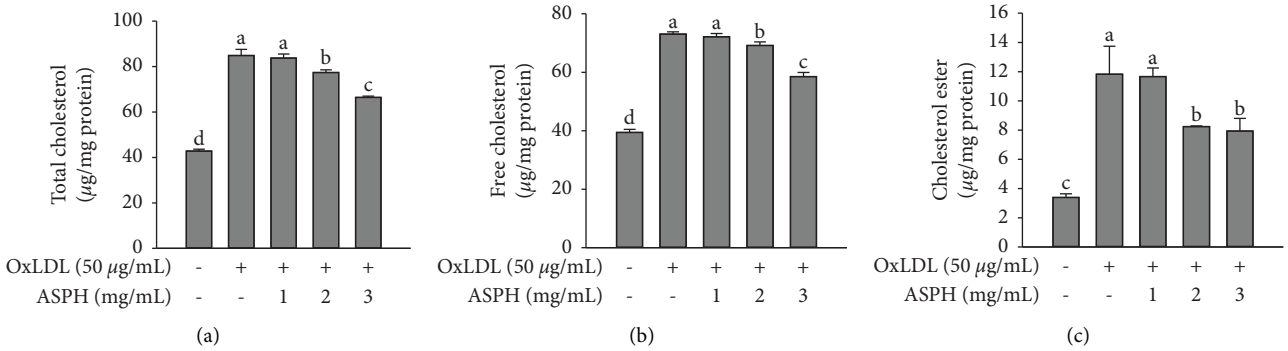

Figure 2: (a) Effect of 1–3 mg/mL of ark shell-pepsin hydrolysates (ASPH) on (A) total cholesterol, (b) free cholesterol, and (c) cholesteryl ester content in oxLDL-induced RAW264.7 murine macrophages. The macrophages were treated with ASPH (1–3 mg/mL) for 1 h followed by oxLDL treatment for 24 h. The experiments were conducted as three independent determinations ($n = 3$) with ±S.D., and error bars with different letters are significantly different ($P < 0.05$).

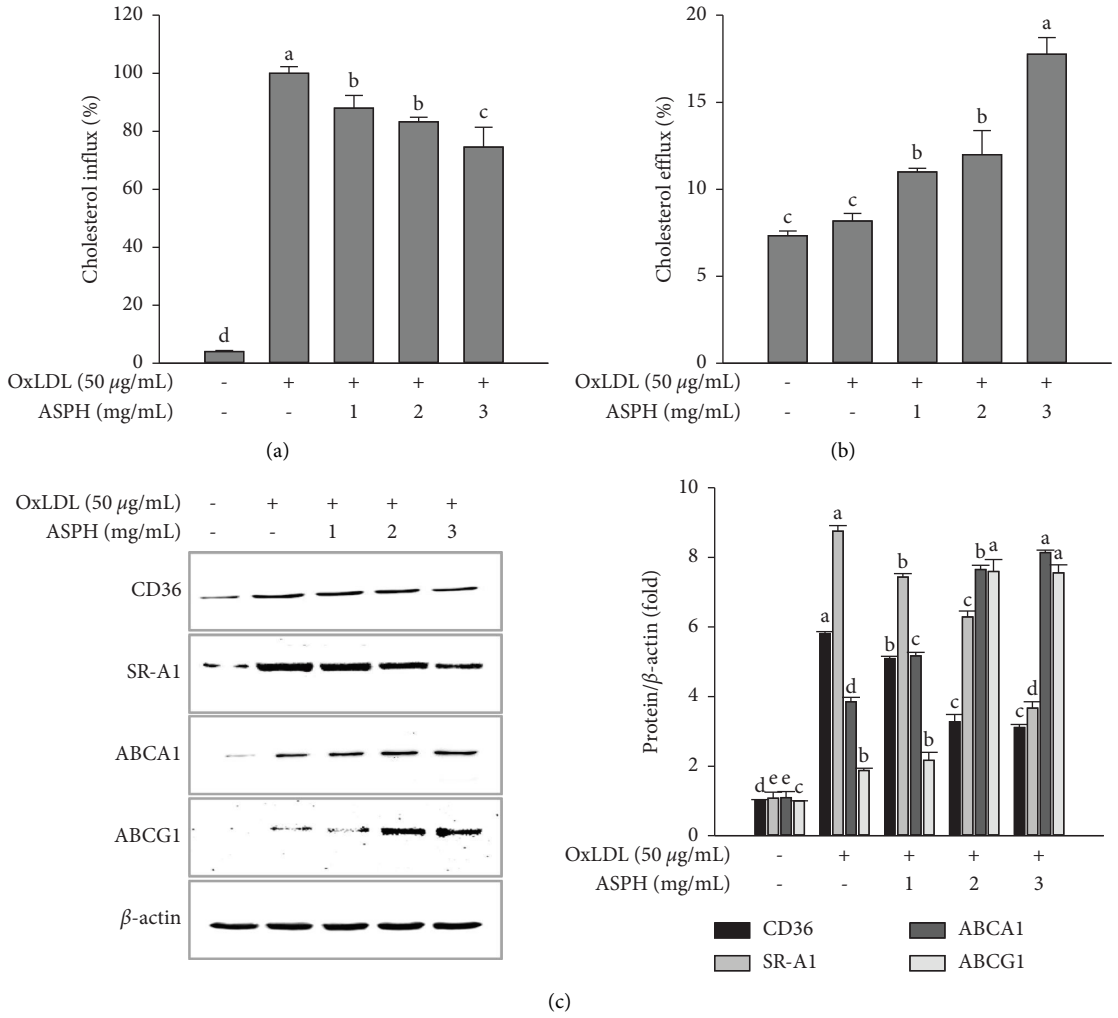

Figure 3: Effect of 1–3 mg/mL of ark shell-pepsin hydrolysates (ASPH) on (a) cholesterol influx (b) cholesterol efflux, and (c) CD36. SR-A1, ABCA1, and ABCG1 protein expression in oxLDL induced RAW264.7 murine macrophages. The macrophages were treated with ASPH (1–3 mg/mL) for 1 h followed by oxLDL treatment for 24 h. The experiments were conducted as three independent determinations ($n = 3$) with ±S.D., and error bars with different letters are significantly different ($P < 0.05$).

exposure to oxLDL compared to the control group. However, the increased expression of CD36 and SR-A1 was diminished by the treatment with ASPH. In addition, ASPH increased the expression of ABCA1 and ABCG1, cholesterol efflux related proteins, in a dose-dependent manner in macrophages.

3.3. ASPH Inhibits Proinflammatory Cytokine Production and NF-κb Nuclear Translocation. Atherosclerosis is a chronic inflammatory disease. Therefore, we examined the effect of ASPH on the inflammatory response during foam cell formation. The production of three proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and -1β (IL-1β), is significantly increased in oxLDL-exposed macrophages compared to the control group, as shown in Figures 4(a)~4(c). However, the ASPH treatment completely inhibited TNF-α, IL-6, and IL-1β production in macrophages.

Since NF-κB, a transcription factor, plays a key role in inflammatory reactions, the effect of ASPH on NF-κB nuclear translocation was investigated. Figure 4(d) shows that NF-κB expression in the nuclear fraction is increased in macrophages exposed to oxLDL compared to the control group. However, its nuclear translocation was inhibited by treatment with ASPH in a dose-dependent manner.

3.4. Characterization of ASPH. The chemical composition of ASPH was analyzed, and the results showed that ASPH was composed of 70.38% crude protein, 23.42% crude carbohydrate, 3.70% crude lipids, 2.45% moisture, and 0.05% crude ash.

GPC analysis in HPLC was performed to check molecular weight distribution of ASPH. Figure 5 shows that ASPH contained BAPs of various sizes and most of BAPs are less than 6,500 Da.

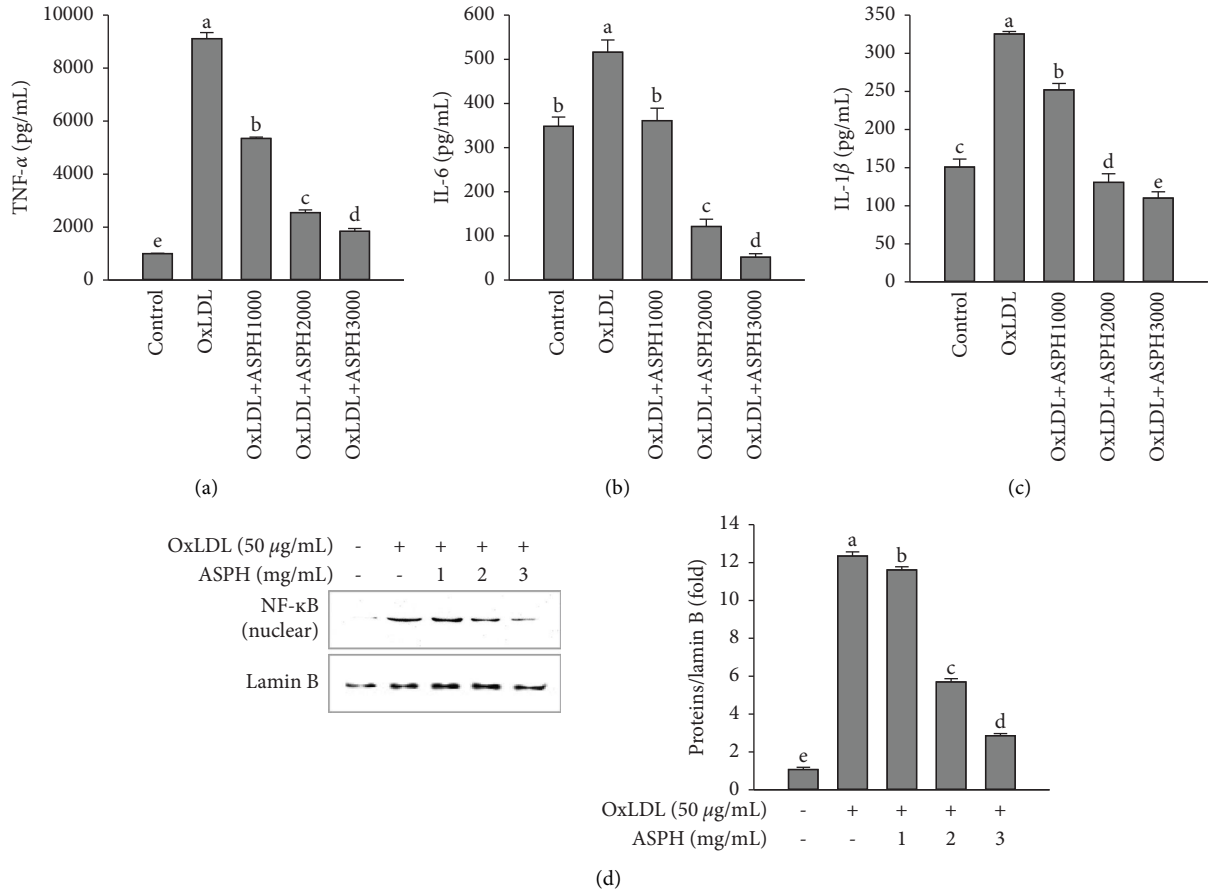

Figure 4: Effect of 1–3 mg/mL of ark shell-pepsin hydrolysates (ASPH) on proinflammatory cytokine production: (a) TNF-α, (b) IL-6, and (c) IL-1β in oxLDL stimulated RAW264.7 murine macrophages. (d) The effect of 1–3 mg/mL of ASPH on NF-kBp-65 molecule nuclear translocation in oxLDL stimulated RAW264.7 cells. The macrophages were treated with ASPH (1–3 mg/mL) for 1 h followed by oxLDL treatment for 24 h for proinflammatory cytokine detection and 2 h for NF-κB p-65 molecule nuclear translocation. The experiments were conducted as three independent determinations ($n = 3$) with ±S.D., and error bars with different letters are significantly different ($P < 0.05$).

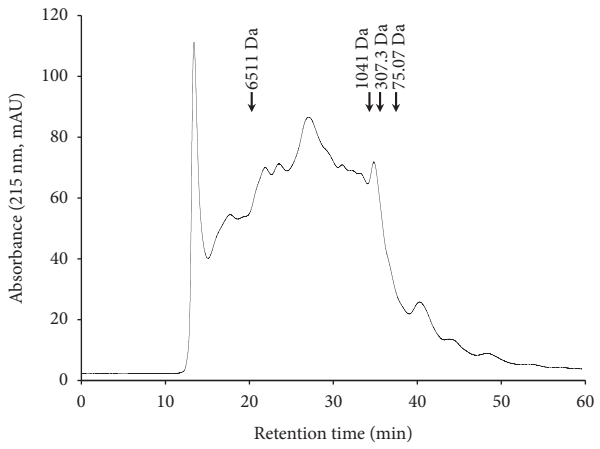

Figure 5: Molecular weight distribution of ark shell-pepsin hydrolysates (ASPH) on a GPC column by HPLC. The eluent was distilled water and the flow rate was 0.5 mL/min. The eluate was analyzed with a UV detector (Dionex UltiMate 3000) at 214 nm. The column was calibrated with a standard solution including aprotinin (6.5 kDa), angiotensin II (1041 Da), glutathione (307.32 Da), and glycine (75 Da) proteins.

Amino acid composition is summarized in Table 1. Glutamic acid was the highest in ASPH followed by aspartic acid, lysine, leucine, arginine, glycine, and alanine. Essential amino acid in ASPH was 38%.

4. Discussion

Protein hydrolysates or BAPs have received much attention due to their versatile bioactivities. In the present study, we firstly demonstrated that protein hydrolysates of ark shell by pepsin exhibited anti-atherosclerotic effect through inhibiting foam cell formation in macrophages induced by oxLDL. The uptake of oxLDL by macrophages is one of the crucial steps in promoting atherosclerosis, several studies have been analyzed intracellular cholesterol accumulation inhibitory effect using natural products such as Spiromastixones [14] and Mulberry leaf polyphenols [31] to determine their anti-atherosclerosis effect. Thus, as the foremost analysis, intracellular cholesterol accumulation was determined in the ASHs. OxLDL enormously induced the macrophages transformation into foam cells, and our ASPH treatment showed an effective inhibitory effect,

Table 1: Amino acid composition of ASPH.

Amino acids (AA)	AA (mg/100 g)	% AA
Aspartic acid	6563.06 ± 109.23	11.3
Threonine	2662.27 ± 42.23	4.6
Serine	2620.11 ± 52.86	4.5
Glutamic acid	8591.29 ± 121.30	14.7
Proline	2431.18 ± 15.72	4.2
Glycine	3608.36 ± 43.97	6.2
Alanine	3320.11 ± 37.89	5.7
Cystine	80.88 ± 7.12	0.1
Valine	2834.09 ± 23.64	4.9
Methionine	1149.94 ± 3.72	2.0
Isoleucine	2621.84 ± 30.34	4.5
Leucine	4353.07 ± 57.88	7.5
Tyrosine	1544.71 ± 23.86	2.6
Phenylalanine	2300.82 ± 32.04	3.9
Histidine	1826.17 ± 13.41	3.1
Lysine	4384.43 ± 66.42	7.5
Arginine	4038.54 ± 49.18	6.9
Total	54930.86 ± 716.55	100.0

indicating ASPH may have the potential to inhibit foam cell formation in oxLDL-induced macrophages.

Foam cell formation typically occurred due to the imbalance between cholesterol influx and efflux as uncontrolled cholesterol influx and impaired cholesterol efflux [6]. In the cholesterol influx process, macrophages were internalized modified LDL through scavenger receptors as SR-A1 and CD36. SR-A1 accumulate modified LDL by itself and CD36 consists of broad ligand specificity and higher oxLDL binding ability in human macrophages and peritoneal macrophages, respectively [8, 32]. On the contrary, promoting the cholesterol efflux, the first step of reverse cholesterol transport (RCT), reduced the risk of atherosclerosis. ABCA1 and ABCG1 are mediated the RCT mechanism though promoting cholesterol efflux to Apo-A1 and HDL, respectively [33, 34]. The imbalance cholesterol influx and efflux process lead to increase the total cholesterol, free cholesterol, and cholesterol ester level in macrophages [33]. Findings from other studies have been indicated the effect of foam cell formation inhibition through regulating cholesterol influx, efflux, and cholesterol accumulation using marine derived bioactive compounds [14, 35]). Our results also demonstrated that, ASPH attenuated the foam cell formation by reducing cholesterol influx and enhancing cholesterol efflux rates. To determine the cholesterol metabolic pathways, we examined the effect of ASPH on the protein expression of cholesterol influx and efflux and demonstrated that ASPH attenuated the protein expression of SR-A1 and CD36 but increased the protein expression of ABCA1 and ABCG1. In addition, ASPH effectively downregulated the levels of total cholesterol, free cholesterol, and cholesterol ester in oxLDL-induced macrophage foam cell formation.

Inflammation of macrophages is involved in all phases of atherosclerosis [36]. Macrophages have a higher ability to recognize and internalize oxLDL. Internalization of oxLDL leads to metabolic and functional changes in macrophages to become foam cells, following inflammatory mediator generations including proinflammatory cytokines [37]. NF-κB is a major player in inflammatory reactions and its signals can be seen in the whole process of atherosclerosis from beginning to plaque formation and eventual rupture of the atherosclerosis plaque. In normal unstimulated cells, NF-κB is inactivated in the cytoplasm by IκB proteins including IκBα. However, upon stimulation, several upstream molecules are activated in inflammatory responses, which in turn create phosphorylation of IκBα, and then result into ubiquitination and degradation of IκBα [38, 39]. Finally, NF-κB is released from the complex of IκBα/NF-κB and can translocate into the nucleus followed by stimulating the production of inflammatory mediators such as TNF-α, IL-6, and IL-1β [40]. Since, atherosclerosis is a chronic inflammatory disease, suppression of inflammatory responses or reactions in macrophages is considered to be a good strategy to prevent atherosclerosis. Recently, some natural products have been reported to inhibit the production of proinflammatory cytokines in foam cell formation of macrophage, endothelial cells, and vascular smooth muscle cells through inhibiting the nuclear translocation of NF-κB [41–44]. The current study found that the ASPH treatment inhibited the production of TNF-α, IL-6, and IL-1β, and that this effect was associated with the inhibition of NF-κB nuclear translocation.

So far, a wide range of studies have been attempted to develop natural products as an anti-atherosclerosis agent [14, 45–48]. However, there is scant information on protein hydrolysates or BAPs as an anti-atherosclerosis agent. Herewith, for the first time, we reported anti-atherosclerotic effect of protein hydrolysates from ark shell (*S. subcrenata*) by investigating the inhibitory effect on foam cell formation, which is a target for fighting atherosclerosis. However, there is a limit to this study, and in order to overcome it, research such as the purification and structural identification of BAPs responsible for foam cell formation inhibition activity is needed, and that work is in progress. Taken all together, it can be suggested that ASPH may be potentially used in functional foods for the prevention of foam cell formation by regulating inflammatory responses.

5. Conclusion

Bioactive peptides and/or protein hydrolysates gain a considerable attention recently due to their variety of biological activities. The present study clearly showed that ark shell protein hydrolysates inhibited oxLDL-induced foam cell formation. The mechanisms involved were to increase cholesterol efflux and inhibit cholesterol influx by upregulating the expression of ABCA1 and ABCG1 and downregulating the expression of CD36 and SR-A1. Our findings also suggested that ark shell protein hydrolysates ameliorated inflammatory responses in macrophage foam cell formation through blocking NF-κB nuclear translocation.

Data Availability

All data that support the findings of this study are available from the corresponding author upon request.

Ethical Approval

Not applicable.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Chathuri Kaushalya Marasinghe conceptualized the study, developed methodology, investigated the study, and curated the data. Won-Kyo Jung did funding acquisition and Jae-Young Je supervised and conceptualized the study, developed methodology, wrote the original draft, reviewed and edited the manuscript, and did funding acquisition.

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