ANTI-CANCER EFFECT OF ANTIOXIDANT PEPTIDE FROM HANWOO BEEF ROUND ON HCT116 HUMAN COLON CANCER CELLS

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I. INTRODUCTION

Hanwoo beef is a source of high-quality nutrients. Recently, interest on bioactive peptide extracted from beef has been growing. It was reported that peptide from beef sarcoplasmic protein hydrolysates had with anti-cancer properties in breast cancer cell [1]. Colorectal cancer is one of the most common malignancies and has the third highest and second highest incidence among men and women, respectively, worldwide [2]. Currently, studies on colorectal cancer are focused on identifying extracts and peptides from natural sources. Therefore, this study aimed to isolate antioxidant peptide from Hanwoo beef round and to determine its anti-cancer effect against HCT116 human colon cancer cell.

II. MATERIALS AND METHODS

Hanwoo beef round (*M. Semimembranosus*) was purchased from a local meat-packing center (Chuncheon, Korea) with 24 h post mortem. Hanwoo beef round (20 g) were homogenized with 200 mL of distilled water (1:10 dilution). The supernatant of Hanwoo beef extract (15 mL) was separated into a low-molecular-weight fraction (<3 kDa), using the centrifugal membrane filter. Antioxidant Hanwoo beef peptide (AHWP) was fractionated through RP-HPLC, using a Zorbax Eclipse C18 column (4.6 × 250 mm) using a linear gradient of acetonitrile (0–40%) with 0.1% trifluoroacetic acid. Antioxidant activities of the extracts and fractions were assessed using the oxygen radical absorbance capacity (ORAC) assay in accordance with the slightly modified method of Gillespie *et al.* [3]. The HCT116 cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum and 1% antibiotics at 37˚C in a 5% CO_2 humidified incubator. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and detected at 540 nm. The effect of AHWP on MAP kinase activation of HCT116 cells was determined through western blotting. Cells lysed with lysis buffer. Equivalent amounts of total cellular proteins (15 μg) were separated through sodium dodecyl sulphate polyacrylamide gel electrophoresis (10% running gel) and blotted onto polyvinylidene fluoride (PVDF) membranes. PVDF membranes were probed with primary antibodies (anti-ERK, -p-ERK, -JNK, -p-JNK, -p38, -p-p38, and β-actin antibodies at 1:200) at 4ºC overnight and horseradish peroxidase-conjugated secondary antibody (1:3,000-1:5,000) for 2 h at 20-25ºC. All experiments were performed in triplicate and results are presented as the mean and standard error of the mean (SEM) with Tukey's tests to determine significance at $p < 0.05$.

III. RESULTS AND DISCUSSION

As shown in Table 1, AHWP showed higher ORAC value at 202.66 μM TE/g of dry matter compared to the low-molecular-weight fraction (<3 kDa) and extracts ($p < 0.05$). When AHWP treated in HCT116, the growth of HCT116 cells was inhibited by AHWP in a dose-dependent manner (Table 2). Numerous studies have reported both antioxidant properties and anti-proliferative activity of protein extracts or peptides in cancer cell lines. The antioxidant peptide can induce apoptotic morphological changes and oxidative DNA damage in cancer cells [4] and a post-G1 cell cycle arrest [5]. Therefore, it suggested that antioxidant activity of AHWP can contributed to have anti-proliferative activity against HCT116 cells.

Table 1 Antioxidant activity of isolated extract and peptides from Hanwoo beef round

Samples	ORAC (μM TE/g of dry matter)
Hanwoo beef round extract	145.54^{C}
Low molecular weight peptide (<3 kDa)	167.38^{B}
Antioxidant Hanwoo beef peptide (AHWP)	202.66^{A}
SEM[1)]	4.662

[A-C] Means within a column with different superscript differ significantly at $p < 0.05$.
[1)] SEM, Standard error of means

Table 2 Effect of AHWP treatment on viability of HCT116 cells

Trait	CON	AHWP (μg/ml)			SEM[1)]
		50	100	250	
Cell viability (%)	100.00[a]	92.92[ab]	88.44[b]	74.76[c]	2.065

[a-c] Means within a row with different superscript differ significantly at $p < 0.05$.
[1)] SEM, Standard error of means

MAP kinase, such as extracellular-regulated protein kinase (ERK), c-Jun-N-terminal kinase (JNK) and p38 kinase play an important role in regulating cell differentiation, proliferation, and apoptosis [6]. Activation of MAP kinase can induce apoptosis in most cancer-cell lines [7]. As shown in Fig 1, the AHWP significantly activated phosphorylation of ERK, JNK, and p38 kinase at 100 and 250 μg/mL ($p < 0.05$). It suggested that the anti-proliferative effect of AHWP was resulted from increased phosphorylation of MAP kinases such as ERK, JNK and p38 kinase at 100 and 250 μg/mL.

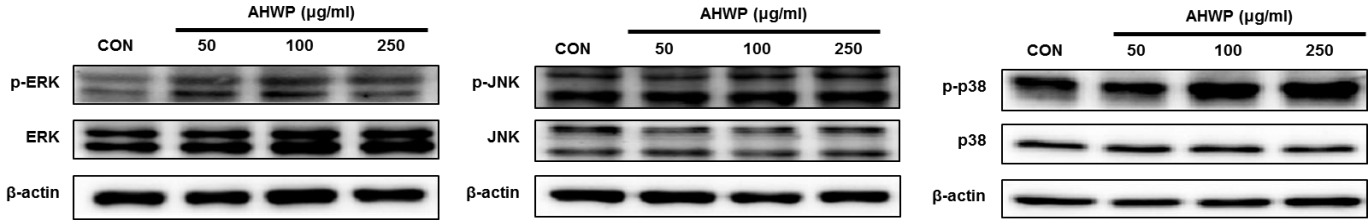

Figure 1. Effect of AHWP on phosphorylation of MAP kinases in HCT116 cells

IV. CONCLUSION

The antioxidant peptide was isolated from Hanwoo beef round. On the basis of its antioxidant activity, AHWP had anti-proliferative effects on HCT116 cells via activating of phosphorylation of ERK, JNK, and p38 kinase at 100 and 250 μg/mL. The present results suggest that AHWP can be useful as a potential nutraceutical and antioxidant-based anticancer agent.

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