ANTIOXIDATIVE AND ANTI-INFLAMMATORY EFFECT OF HORSE MEAT HYDROLYSATE ON C2C12 MYOBLAST

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I. INTRODUCTION

Oxidative stress, an increased production of reactive oxygen species (ROS), changes related to the aging process, and involvement in inflammation is a significant contributor to muscle loss and functionality, leading to symptoms of sarcopenia. Previous investigations have suggested protein supplementation can be a strategy for maintaining muscle mass [1], and horse meat is known for its high protein content [2]. In this study, we prepared three horse meat hydrolysates and separated them under 3kDa, which is known for its antioxidative activity and better absorption [3], with the aim of identifying their anti-inflammatory effect against C2C12 myoblast.

II. MATERIALS AND METHODS

Hydrolysates were prepared from horse meat (top round) using three food-grade enzymes - alcalase® (A4), papain (N4), and protamex® (M4). Controlled hydrolysis was carried out under specific conditions: pH 8.0 and 50°C for alcalase®, pH 8.0 and 37°C for papain, and pH 6.0 and 50°C for protamex® over a 4-hour period. The hydrolysates were separated into a low-molecular-weight fraction (<3kDa) using a centrifugal membrane filter, and the supernatants were freeze-dried. Antioxidant activities of the hydrolysates were assessed using the oxygen radical absorbance capability (ORAC) assay in accordance with method of Gillespie *et al*. [4]. The C2C12 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in a 5% CO_2 incubator. Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and detected at 540nm. Cellular ROS level was measured using the 2′,7′-dichlorofluorescin diacetate (DCF-DA) assay and detected at an excitation of 485nm and an emission of 535nm. The mRNA levels of IL-6 and TNF-α were measured using real-time PCR. Total RNA was extracted from the cells, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize. All data are presented as the standard error of the mean using the SAS (Statistical Analytical System) program (ver. 9.4).

III. RESULTS AND DISCUSSION

As shown in Table 1, A4 showed the significantly highest degree of hydrolysis at 37.67% compared to the other hydrolysates ($P < 0.01$) and an ORAC value of 848.05 μM TE/g dry matter, not significantly different from M4 (ORAC value of 886.13 μM TE/g dry matter). Additionally, application of A4 to C2C12 cells result in significant increase in cell growth up to 33.79% at the dose of 50-200 μg/mL (Table 2, $P < 0.01$), suggesting that A4 was non-cytotoxic to C2C12 cells.

Table 1 Degree of hydrolysis and antioxidant activity of horse meat hydrolysates

Samples	A4	M4	N4	SEM[1)]	P-value
Degree of hydrolysis (%)	37.7A	34.9B	26.9C	0.29	<0.01
ORAC (μM TE/g dry matter)	848AB	886A	823C	12.5	

[a-c] Means within a column with different superscript differ significantly at $P < 0.01$.

[1)] SEM, Standard error of means

Table 2 Effect of horse meat hydrolysate on viability of C2C12 myoblasts

Trait	CON	A4 (μg/mL)			SEM[1)]	P-value
		50	100	200		
Cell viability (%)	100C	134A	121B	117B	2.36	<0.01

[a-c] Means within a row with different superscript differ significantly between CON and A4 at P < 0.01.

[1)] SEM, Standard error of means

Oxidative stress, known to be one of the causes of muscle atrophy, increases cellular ROS and inflammation. Elevated cellular ROS levels and inflammatory cytokines can cause mitochondrial dysfunction and destruction of skeletal muscles [1]. As shown in Fig 1, A4 significantly increased the viability of C2C12 cells up to 55.55% at doses of 10-200 μg/mL (P < 0.01). Additionally, the increased production of ROS, IL-6, and TNF-α in LPS-treated C2C12 cells was attenuated by treating with A4 up to 58.92%, 24.07%, and 50.85%, respectively, at the dose of 10, 10, and 200 μg/mL (P < 0.01). This suggests that the increased cell viability and antioxidant activity of A4 were due to the decrease in ROS levels and inflammatory cytokines such as IL-6 and TNF-α in C2C12 cells.

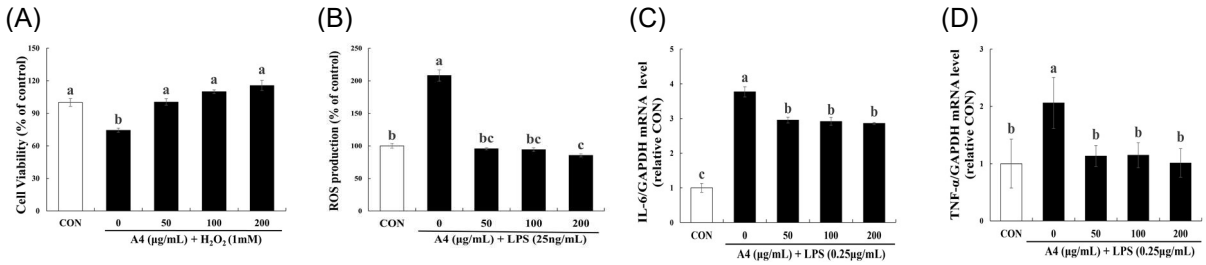

Figure 1. Cell viability of H_2O_2-inflicted C2C12 cells treated with A4(A), inhibitory effect of A4 on producing reactive oxygen species(B), mRNA of IL-6(C) and TNF-α(D) in LPS-treated C2C12 myoblasts

[a-c] Values of bar with different superscript among treatments differ significantly at P < 0.01.

IV. CONCLUSION

The antioxidant peptide was hydrolysed from horse meat round using alcalase®. Based on its antioxidant activity, A4 showed a protective effect against H_2O_2 on C2C12 cells and decreased ROS production in LPS-treated C2C12 cells. Furthermore, the mRNA levels of two inflammatory cytokines, IL-6 and TNF-α, were reduced in LPS-treated C2C12 cells. These findings suggest that A4 has the potential to be used as an antioxidant and cytokine-inhibiting product for diminishing inflammation.

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