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Investigation of the antioxidant potential of blue whiting protein hydrolysates in oxidatively-stressed 3T3-L1 adipocytes

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The prevalence of those who are both elderly and obese is increasing in many regions[(1)]. Obesity, which is closely associated with the generation of reactive oxygen species (ROS), is indeed a risk factor for vascular disease and associated complications[(2)]. Therefore, protection of adipocytes against oxidative stress may be beneficial in reducing obesity-related metabolic diseases. The murine 3T3-L1 cell line is a well-characterised, reliable model for the study of adipocyte biology. The objective of this study was to investigate the cellular antioxidant activity of six blue whiting soluble protein hydrolysates (BWSPH, BW-SPH-A-F), produced using a proprietary process, in 3T3-L1 adipocyte cells.

The antioxidant potential of the BWSPH was determined by investigating their ability to enhance the endogenous antioxidant glutathione (GSH) and catalase (CAT) enzyme activity, as well as inhibiting the production of ROS in oxidatively-stressed 3T3-L1 adipocytes. Statistical analysis was conducted using ANOVA followed by Dunnett's test (Prism 5.0, GraphPad Inc. San Diego, CA, USA). Results are expressed as the mean of at least three independent experiments ± SE.

In a first series of studies, 3T3-L1 preadipocyte cells were differentiated over a 12-day period in the presence of BWSPH (1.0% (w/v) dry weight) and then challenged with tertbutyl hydroperoxide (tBOOH) (1 mM) for 3 h prior to analysis of GSH concentration in the cell supernatant. In a second series of experiments, oxidative stress in 3T3-L1 fully differentiated adipocytes was induced with hydrogen peroxide (H2O2) (1 mM, 3 h) for subsequent CAT activity and ROS production analysis. All BWSPH at 1.0% (w/v) dry weight, were non-toxic to 3T3-L1 preadipocytes as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Preincubation with BW-SPH-A, BW-SPH-B and BW-SPH-C significantly increased GSH concentration in tBOOH-challenged 3T3-L1 adipocytes compared with tBOOH-stimulated cells which were differentiated in the absence of BWSPH (control) ($p < 0.05$). However, although exposure of 3T3-L1 cells to all BWSPH prior to treatment with H2O2 increased CAT activity compared to cells treated with H2O2 alone, these changes were not significant. Similarly, differentiation of 3T3-L1 cells in the presence of BW-SPH-A slightly reduced ROS production after treatment with H2O2 compared with the non-preconditioned H2O2 control, however, none of the BWSPH significantly altered ROS production compared with the oxidatively-stressed control.

Overall, it is possible that BW-SPH-A, BW-SPH-B, and BW-SPH-C has potential to modulate the endogenous non-enzymatic antioxidant defence system. Further research should investigate the effect of BWSPH on additional biomarkers of the intracellular enzymatic antioxidant defence system, such as superoxide dismutase, in order to obtain better knowledge on the overall antioxidant potential of BWSPH. Future experimentation will also investigate the immunomodulatory potential of these hydrolysates in adipocytes via examining their effect on pro-inflammatory cytokines interleukin-6, adiponectin and monocyte chemoattractant protein-1 (MCP-1).

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