Research Article

Evaluating the Effects of MKAVCFSL Derived from Bighead Carp (Hypophthalmichthys nobilis) Flesh on Antioxidant Activity in Caco-2 Cells In Vitro

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The effect of an antioxidative peptide Met-Lys-Ala-Val-Cys-Phe-Ser-Leu (MKAVCFSL) on oxidative stress in Caco-2 cell lines was investigated. Caco-2 cells exposed to excess oxidative stress could be restored when pretreated with the peptide. Reactive oxygen species (ROS) and malondialdehyde (MDA) within the cells could be scavenged by MKAVCFSL. The peptide could also enhance the activity of glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD), while catalase (CAT) activity did not show a significant difference between treatment and control samples. Meanwhile, it was observed that peptide treatment increased the concentration of glutathione (GSH). Yet the content of glutathion disulfide (GSSG) was hardly affected. The stability of MKAVCFSL was also assessed and an intact peptide was observed after simulated gastrointestinal digestion. Part of the peptide was hydrolyzed into fragments including MKA, FSL, AVCFSL, and MKAVCF. This study demonstrated that MKAVCFSL derived from bighead carp hydrolysates could ameliorate oxidative stress to protect the Caco-2 cells.

1. Introduction

Oxidative stress is defined as the potential biological damage caused by free radicals [1]; it demonstrates an excessive production of ROS that cannot be counteracted by the action of antioxidative protection systems of the cells. The imbalance between the oxidant species such as hydrogen peroxide (H$_2$O$_2$), superoxide (O$_2$–•), singlet oxygen (1/2 O$_2$), and the hydroxyl radical (•OH) was mainly produced from the mitochondrion. The oxidant species may trigger specific factors responsible for oxidative damage in the cells including cellular proteins, membrane lipids, nucleic acids, and eventually cell death. This damage has a correlation with several pathologies like cardiovascular, cancer, diabetes, and inflammatory diseases [2].

All cells possess elaborate antioxidant systems constitutive of interacting various weight components. Among them, SOD, GPx, and CAT play a significant role in scavenging ROS. Increased oxidative stress associated with the disease is often related to a depletion in enzymatic and nonenzymatic antioxidants [3], which reduced the ability to protect against excess ROS exposure. Therefore, extra antioxidants are supposed to be utilized as direct scavengers of free radicals, as inhibitors of lipid peroxidation, and so on [4]. Therefore, researchers’ interest in the identification, characterization, and application of antioxidants has increased to protect organisms from oxidative stress. Natural sources have been specifically considered because they appear to be safer for consumers than synthetic antioxidants, such as butylated hydroxytoluene (BHT), which have shown to be carcinogenic [5]. Food-derived antioxidant peptides have been purified from many protein hydrolysates such as sardinelle (Sardinella aurita) [6], chickpea (Cicer arietinum L.) protein [7], blue mussel (Mytilus edulis) protein [8], and
barley glutelin [9]. Yet, antioxidant peptide identified from freshwater fish was limited. In our previous study, we identified an antioxidant peptide MKAVCFSL from flesh hydrolysate of bighead carp (Hypophthalmichthys nobilis), which had effective DPPH radical scavenging activity, ferrous ions (Fe^{2+}) chelating activity, and reducing power [10]. However, there is a lack of research on the evaluation of antioxidant activity at the cellular level. In this study, the protective role of this antioxidant peptide against H_{2}O_{2}-induced oxidative stress was further evaluated in Caco-2 cell lines since the intestinal epithelium is the interface between the organism and its luminal environment, which is prone to suffer oxidative stress [11]. Contents of cellular ROS and MDA were assessed to study the antioxidant capacity at the cellular level. The enzyme activities, including SOD, GPx, GR, and CAT, and cellular antioxidant GSH were investigated to evaluate whether this peptide also initiated the cellular antioxidant system to protect Caco-2 cells against H_{2}O_{2}-mediated cell death. Besides, simulated gastrointestinal digestion was performed to evaluate the stability of the peptide before in vivo experiments.

2. Materials and Methods

2.1. Materials. Caco-2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco’s modified Eagle’s medium (DMEM) was the product of Hyclone (Logan, UT). Fetal bovine serum (FBS) was purchased from Gibco (New York, NY, USA). The flasks for growing cells were acquired from Corning Costar (New York, NY, USA). MKAVCFSL was synthesized by Jietai Synpeptide Co., Ltd. (Shanghai, China). H_{2}O_{2} and all other chemicals were purchased from China National Medicines Corporation Ltd. (Shanghai, China). Bicinchoninic acid (BCA) protein was from Solarbio (Beijing, China). Catalase, SOD, cellular GPx, GR, and GSH/GSSG assay kits were purchased from Beyotime Institute of Biotechnology (Jiangsu, China).

2.2. Simulated Gastrointestinal Digestion. In vitro pepsin-pancreatin-simulated digestion was performed according to the method described by Samaranayaka with slight modifications [12]. The peptide MKAVCFSL (50 μg/mL) was initially dissolved in distilled water, and the pH was adjusted to 2.0 using 5 M HCl. Pepsin was mixed with the peptide in a ratio of 1:100 w/w of the substrate, and the mixture was incubated at 37°C for 1 h. After that, the pH was adjusted to 5.3 using saturated NaHCO_{3} solution and was further augmented to 7.5 with 5 M NaOH. Subsequently, pancreatin (enzyme: substrate ratio, 1:50 w/w) was added to the mixture, which was incubated again at the temperature of 37°C for 2 h. Aliquots of the digested samples were collected at 1 h, 2 h, and 3 h. The enzymatic reaction was terminated by immersing the samples in a 95°C water bath for 10 min and cooling the samples to room temperature on ice. The digested samples were used to evaluate the stability utilizing LC-MS/MS analysis.

2.3. Determination of Amino Acid Sequence by LC-MS/MS. After simulated gastrointestinal digestion, the molecular mass and peptide sequencing analysis was performed using a Thermo Q-Exactive high-resolution mass spectrometer (Thermo Scientific, Waltham, MA, USA) in positive ion mode with a capillary temperature of 320°C and a 2 kV spray voltage applied to the emitter. The following electrospray ionization (ESI) conditions were utilized for MS analysis: a high resolution (70,000 FWHM) MS full scan (m/z 300–2000 Da) was performed to select the 10 most intense ions prior to MS/MS analysis using 10 eV energy collisional dissociation.

2.4. Induction of Oxidative Stress. The Caco-2 cells’ cultivation was conducted at the concentration of 3.2 × 10^{5} cells/mL in 24-well culture plates for 3 days and the fresh medium was replaced daily. Prior to oxidative induction, Caco-2 cells were incubated with antioxidant peptide for 24 h and then washed twice with phosphate-buffered saline (PBS), pH 7.2. After that, the cells were incubated for 2 h with 1 mM H_{2}O_{2} to induce oxidative stress. The cells were treated as follows: medium treatment without H_{2}O_{2} and peptide (control 1: C1), H_{2}O_{2} treated only (control 2: C2), peptide treated only (sample 1: S1), and medium treatment with H_{2}O_{2} and peptide (sample 2: S2). The cells were then lysed with cell lysis buffer (Beyotime, Jiangsu, China) and centrifuged at 10,000 g and 4°C for 10 min. The supernatant was collected and stored at –80°C until further experiment. The cell lysate protein concentration was measured using the Bicinchoninic Acid (BCA) Protein Assay Kit (Solarbio, Beijing, China).

2.5. Cytotoxicity Assay. Cell Counting Kit-8 (CCK-8) from Solarbio (Beijing, China) was used to measure the cytotoxicity of MKAVCFSL in Caco-2 cells. The peptide was dissolved in DMEM and then diluted in a culture medium and added to the cultures 24 h after cell seeding. Cells were treated as the method of 2.2, after which 10 μL of CCK-8 was added to each well, and the plates were further incubated for 4 h. Thereafter, the absorbance was read using a microplate reader (Bio-Tek, Synergy HT, USA) with 450 nm. The viability of cells was expressed as a percentage of the viability of cells in C1 treatment.

2.6. Determination of Intracellular ROS. The intracellular ROS contents inside the Caco-2 cells were measured according to the instruction of the ROS Assay Kit. Cells were seeded in 96-well plates at a concentration of 2.5 × 10^{5} cells/mL. Cultured cells were treated as the method of 2.4. After incubation, 10 μM DCFH-DA was added to the wells and then incubated at 37°C and 5% CO_{2} for 20 min, and cells were then washed with DMEM without FBS for three times. Fluorescence readings were taken at excitation and emission wavelengths of 488 and 525 nm using a Multi-Detection Microplate Reader (Bio-Tek, Synergy HT, USA), respectively. Cellular ROS levels were expressed as fluorescence intensity.
2.7. Measurement of Lipid Peroxidation. The cellular MDA content was evaluated to investigate the level of lipid peroxidation under oxidative stress according to the method of the MDA Assay Kit (Solarbio, Beijing). 100 μL cell lysate supernatant was mixed with 200 μL MDA working solution and heated at 100 °C for 30 min. After cooling the mixture to room temperature, samples were centrifuged at 10,000 g for 10 min. The absorbance at 532 nm and 600 nm was measured in a 96-well plate, respectively. The results of the MDA assay were expressed as nanomoles of MDA per milligram of protein.

2.8. Effect of MKAVCFSL on Endogenous Antioxidant Defence Systems in Caco-2 Cells under Oxidative Stress

2.8.1. Measurement of GSH and GSSG Concentration. Cellular GSH and GSSG were evaluated according to the direction of GSH and GSSG Assay Kit. Cell lysate supernatants were obtained by being rapidly freeze-thawed twice with liquid nitrogen and 37°C water baths and then centrifuged at 10,000 g for 10 min at 4°C. 10 μL total GSH and GSSG standards were mixed with 150 μL of 100 mM PBS containing 4 mM EDTA, 0.2 mM NADPH, 0.5 mM DTNB, and 100 units/mL glutathione reductase in a 96-well plate, respectively. Sample supernatants and supernatants which eliminated reduced GSH were mixed with the same PBS as well. The mixture was incubated for 5 min at room temperature and 0.5 mg/mL NADPH was added to each corresponding well. The absorbance was read at 412 nm. The concentration of total glutathione and GSSG in the cell lysate was calculated using a standard curve and GSH concentration was calculated from total glutathione contents deducted GSSG contents.

2.8.2. Glutathione Peroxidase (GPx) Activity. GPx was assayed according to the procedure of the Cellular Glutathione Peroxidase Assay Kit. Briefly, 10 μL cell lysate was mixed with 175 μL 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 11 μL of GR solution which contains 30 mM NADPH, 84 mM GSH, and 1 μL 5 U/μL GR. 4 μL 15 mM H2O2 was added to the solution mixture. The absorbance was monitored at 340 nm every 2 min for 10 min. The activity of GPx within the cell was calculated using a standard curve and expressed as milliunit of GPx per milligram of protein. One unit of activity was expressed as the conversion of 1 mM/min NADPH to NADP+.

2.8.3. Glutathione Reductase (GR) Activity. GR activity was determined using the method described by the Glutathione Reductase Assay Kit. In brief, 20 μL cell lysate was added to 100 mM PBS (0.85 mL, pH 7.5) containing 1 mM EDTA and 2.32 mM GSSG. NADPH (2 mM, 10 μL) was mixed with the solution and the absorbance of 340 nm was read every 1 min for 5 min at 25°C using a Multi-Detection Microplate Reader (Bio-Tek, Synergy HT, USA). Changes in the rate of absorbance were converted into units of GR per milligram of protein using a molar extinction coefficient of 6.22 mM⁻¹ cm⁻¹.

One unit of activity was defined as the reduction of 1 μmol/min GSSG.

2.8.4. Superoxide Dismutase Assay. SOD activity was investigated utilizing a Total Superoxide Dismutase Assay Kit according to the manufacture’s instruction. The water-soluble tetrazolium-8 (WST-8) method, which is more stable and sensitive, is currently used in the determination of SOD activity. Absorbance was determined at 450 nm. Results were expressed as units milligram of protein where protein content was determined by the BCA method. One unit of SOD is defined as the quantity of the enzyme in 20 μL of the sample solution that inhibits the reduction reaction of WST-8 with superoxide anion by 50%.

2.8.5. Catalase Assay. In order to measure the level of catalase in Caco-2 cell lysate, the Catalase Activity Assay Colorimetric Kit was used according to the manufacturer’s instructions. Absorbance was measured at 520 nm and results were expressed as units milligram of protein. One unit of catalase is defined as the amount of the enzyme that can catalyze 1 μmol H2O2 within 1 minute under the condition of pH 7.

2.9. Statistical Analysis. Statistical analysis was performed with SPSS software, version 20 (SPSS Inc., Chicago, IL, USA), using one-way ANOVA followed by Duncan’s multiple range test. Differences were considered at a significance level of p < 0.05.

3. Results and Discussion

3.1. Evaluating the Stability of MKAVCFSL after Simulated Gastrointestinal Digestion. To investigate the stability of the peptide, LC-MS/MS was performed to identify the variation of MKAVCFSL. According to the RP-HPLC chromatogram in Figure 1, undigested MKAVCFSL was eluted at 33–35 min. After pepsin-pancreatin digestion, several new peaks appeared such as fractions from 28.44 min, 30.13 min, and 37.55 min besides the MKAVCFSL peak. LC-MS/MS confirmed that new fragments MKA, FSL, AVCFSL, MKAVCF were released from MKAVCFSL after simulated gastrointestinal digestion. The majority of the peptide remained stable according to LC-MS/MS, which indicated the peptide had the ability to resist pepsin-pancreatin digestion. These results suggested MKAVCFSL was highly likely to successfully pass through the digestive tract and reach intestine to exert antioxidant activity pepsin-pancreatin.

3.2. Analysis of Effects of MKAVCFSL on Cellular Viability, ROS, and MDA. Our previous study has confirmed that the MKAVCFSL peptide showed effective antioxidant activity such as DPPH scavenging activity, reducing power, and ferrous ions (Fe2+) chelating activity [10]. Therefore, we highlighted if the antioxidant peptide could express good antioxidant activity at the cellular level. In our current study,
Caco-2 cells were induced by 1 mM H$_2$O$_2$, which was excessive and impaired the cell viability detected by cytotoxicity assay (data not shown). Figure 2 has shown that when cells are treated with H$_2$O$_2$, the cell activity was inhibited significantly compared with C1 samples suggesting that the cells’ ability to adapt to the addition of H$_2$O$_2$ in the media has been conquered. However, cell protection was achieved when cells were pretreated with 50 μg/mL MKAVCFSL (determined by cytotoxicity assay, data not shown) for 24 h since cell viability recovered from (72.06 ± 0.68)% to (89.18 ± 3.54)%. It was interesting that the viability of cells with peptide treated alone could also be improved. Thus, the concentration of ROS and MDA, which were biomarkers of oxidative stress in Caco-2 cells, was measured to investigate whether this phenomenon was associated with the peptide antioxidant activity or not. Figure 3 exhibited that intracellular ROS level expressed as fluorescence intensity has been increased after H$_2$O$_2$ treatment. While being pretreated with the peptide, ROS decreased to the untreated level. Meanwhile, peptide alone hardly influenced the ROS concentration, which indicated that the protective effect of the cells after H$_2$O$_2$ stimulation was related to antioxidant activity. MDA which was produced from lipid peroxidation could also further confirm the conclusion we have made. When Caco-2 cells were treated with 1 mM H$_2$O$_2$ for 2 hours, an almost 3-fold increase in lipid peroxidation
products was observed (from (0.40 ± 0.08) nmol/mg protein to (1.15 ± 0.08) nmol/mg protein) compared with the C1 sample. However, peptide significantly inhibited the formation of MDA to a concentration of (0.77 ± 0.06) nmol/mg protein in stressed cells (S2 sample) when compared with C2 sample. These results were in accordance with those observed with peptides isolated from Hoki fish on human embryonic lung fibroblasts [13] and silver carp peptides in human intestinal epithelial Caco-2 cells [14]. Besides antioxidant peptides, the antioxidant effect of other food compositions was also highlighted such as ascorbic acid, tocopherols, carotenoids, and other varieties of polyphenols and flavonoids [15]. However, the study on food-derived antioxidant peptides that was evaluated at the cellular level was still limited. The results for the protective effect of MKAVCFLS in Caco-2 cells completed the non-cellular antioxidant activity that we reported previously.

3.3. Analysis of Antioxidant Enzymes Activity Pretreated with MKAVCFLS. To investigate if the antioxidant peptide could also affect the internal antioxidant system of Caco-2 cells, various antioxidant enzymes including SOD, CAT, GPx, and GR as well as the endogenous antioxidant GSH were determined. SOD could contribute to converting superoxide to hydrogen peroxide, which is then decomposed by CAT or GPx into H2O2 [16]. Deleterious actions of H2O2 on Caco-2 cells were observed since SOD activity was inhibited for about 2-folds compared with C1. The reduction in SOD activity is deemed to be caused by an interaction between H2O2 and a copper moiety within the SOD molecule. Hydroxyl and copper-bound radicals could be produced during the process, which oxidized histidine residues resulting in the denaturation of the SOD protein [17, 18]. When preincubated with peptide for 24 h, SOD activity showed no significant difference in S2 compared with C2 treatment, which suggested that peptide had the ability to protect the SOD from the H2O2 toxicity. It is deduced that the SOD-protecting effects of MKAVCFLS may be due to their possible chelation of surplus copper ions in the SOD complex preventing the metal from reacting with H2O2. H2O2 stimulation, on the contrary, increased the catalase activities from (0.33 ± 0.06) units min−1 mg−1 protein to (0.55 ± 0.04) units min−1 mg−1 protein compared to the untreated baseline cells (Figure 4(b)). Catalase, as a primary defense enzyme against oxidative stress from exogenous, is a porphyrin-containing enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen [19]. Therefore, it is reasonable that the activity of catalase increased to scavenging excessive H2O2. On the contrary, Shi et al. [20] have reported that CAT activity decreased after treated by 1 mM H2O2 because it is a high dose for CAT to completely decompose H2O2 into H2O [21]. The addition of the peptide had no significant effect (p < 0.05) on the CAT activity of normal cells and H2O2-challenged cells. These results were similar to O’Sullivan et al. [22] and Kim et al. [23].

GPx also plays a critical role in protecting the cells from oxidative stress damage induced by a high concentration of H2O2 [21, 24]. GPx utilizes GSH to degrade H2O2 into H2O and converts GSH into GSSG. GR catalyzes the decrease of GSSG to the GSH in its sulphydryl form. Therefore, GSH can regenerate through the cyclic action between GPx and GR [25]. In normal cells without H2O2 stimulation, peptides did not enhance GPx and GR activity significantly (Figure 4). After the addition of MKAVCFLS, the activity of GPx, GR in the cells H2O2 treatment were increased with presented in Figures 4(c) and 4(d). This indicated that the peptide could not enhance the GPx and GR activity directly. The protective effect was involved in the removal of oxidative factors that occurred in the cellular metabolic environment. Mammalian GPx1-4 are selenoproteins with selenocysteine (Sec) in the catalytic center, of which GPx2 is mainly expressed in the intestinal epithelium [26]. The presence of Sec as the catalytic moiety of GPx was suggested to guarantee a fast reaction with the hydroperoxide and fast reducibility by GSH [27]. The selenol (-SeH) in SecGPxs reacts in form of a selenolate with H2O2 to selenenic acid (-SeOH) which is reduced back to -SeH by two GSH forming GSSG and H2O [28, 29]. Thus, MKAVCFLS might protect Sec in GPx from being attacked by H2O2. GR contains highly conserved domains, one of which can bind reduced nicotinamide adenine dinucleotide phosphate (NADPH) sustaining reduced GSH in cells. The antioxidant activity of MKAVCFLS may decrease the NAPDH converting into NADP⁺ contributing to the GR activity. Some studies have also verified the impact of oligopeptide and polyphenols on antioxidant enzyme activities in cells. It is demonstrated that the egg-derived peptide elevated GR, GST, and CAT enzymatic activity in human intestinal cells [30]. Other researchers have demonstrated phenolic compounds from olive oil significantly enhanced GR and GP activities in macrophages and verified that the impact of flavonoids on antioxidant protein expression was genus-specific in Caco-2 cells [31, 32].

3.4. Analysis of Cellular Antioxidants GSH. GSH is a tripeptide consisting of glutamine, cysteine, and glycine and is a key component in the nonenzymatic antioxidant mechanism of cells [33]. The present study has found that GSH level was significantly decreased in the cells treated with 1 mM H2O2 only (Figure 5) compared to untreated cells. However, pretreatment of peptide supplementation for 24 h before the addition of H2O2 restored GSH levels significantly. Meanwhile, MKAVCFLS alone could also elevate the GSH concentration in the cells. While GSSG, the oxidized state of glutathione, was not significantly influenced by the peptide without H2O2 stimulation. H2O2 alone augmented the concentration of GSSG from (3.06 ± 0.41) μM to (4.92 ± 0.63) μM compared to the C1 sample. Nevertheless, decreased GSSG concentration was observed in the S2 sample. Increased GSH concentration was unexpectedly in S1 since the activity of GPx and GR was not significantly different from untreated samples (C1). We inferred that the peptides may have a positive effect on GSH synthesis. Increased GSSG in C2 samples was also interesting because GPx and GR activity were both inhibited by excessive H2O2.
It could be speculated that the GPx showed effectively antioxidant activity to dispose oxidant stress initially. Thus, plenty of GSH was converted into GSSH. Besides, the toxicity of H₂O₂ decreased GR activity, which also prohibited the GSSH from reducing back to GSH, thus bringing about the accumulation of GSSH. The decreased GSSH in S2 was in accordance with the restored activity of GR, which could catalyze GSSG to form GSH at high efficiency to promote GSH level in the cells. MKAVCFSL had the potential for normal activity and functionality in the H₂O₂ model system to allow cells to maintain a balanced redox. These results validate the MKAVCFSL could protect the cells from oxidative stress damage.

Generally, MKAVCFSL enhanced GPx activity for subsequent lipid hydroperoxide and ROS induced detoxification using GSH and increased GR activity to regenerate GSH from GSSG. It also contributed to SOD activity without the addition of H₂O₂, but CAT was not influenced. The synergistic actions of cellular antioxidants, antioxidant enzymes, and the peptide efficiently ameliorated the damage of oxidative stress. The protective effect of MKAVCFSL against H₂O₂-induced injury may be due to two reasons correlated to its amino acids composition. On one hand, a peptide having radical scavenging activity can suppress free radical-mediated oxidation [34]. As a previous study earlier, MKAVCFSL contains more aromatic and hydrophobic amino acids and exhibited higher radical scavenging activity, which may be responsible for its protection [20]. In particular, phenylalanine and methionine residues in peptides and proteins were regarded as free radical scavengers in vivo [35]. On the other, some studies have exhibited that some peptides can prevent oxidative stress through reducing antioxidant enzyme capacity [30, 34], which concurred with our results. Indeed, the cellular regulation of the antioxidant system is complicated and involves intricate signaling networks which is not fully understood. Besides, different signaling networks enhance the complexity and increase difficulties to understand redox control and related processes. Although the novel peptide was proved to have effective antioxidant activity, it is necessary to conduct a profound investigation on the relationship between oxidative stress and the antioxidant activity in vivo.
Figure 4: Effects of MKAVCFSL on the cellular antioxidant enzyme. (a) SOD activity, (b) CAT activity, (c) GPx activity, and (d) GR activity. C1: control 1, medium treatment without H₂O₂ and peptide; S1: sample 1, peptide treated only; C2: control 2, H₂O₂ treated only; S2: sample 2, medium treatment with H₂O₂ and peptide. Results are the means ± SD from three independent experiments. Different small letters indicate significant differences between groups (p < 0.05).

Figure 5: Effects of MKAVCFSL on the cellular GSH and GSSG. (a) GSH concentration. (b) GSSG concentration. C1: control 1, medium treatment without H₂O₂ and peptide; S1: sample 1, peptide treated only; C2: control 2, H₂O₂ treated only; S2: sample 2, medium treatment with H₂O₂ and peptide. Results are the means ± SD from three independent experiments. Different small letters indicate significant differences between groups (p < 0.05).
4. Conclusion

This study demonstrated that MKAVCFSL derived from bighead carp protein hydrolysates can remain stable through simulated gastrointestinal digestion, although part of the peptides were degraded into smaller fragments. The peptide could effectively scavenge ROS and MDA within the Caco-2 cells. Meanwhile, after being pretreated with the peptide, upregulation of the activity of several cellular antioxidant enzymes was observed including GPx, GR, and SOD in H$_2$O$_2$-induced Caco-2 cells. However, CAT was not influenced significantly. In addition, the concentration of endogenous antioxidant GSH was also increased, led by MKAVCFSL. In summary, this octapeptide displayed protective effects in Caco-2 cells and may be a potential protective agent or food additives against oxidative stress.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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