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Antioxidative and anti-inflammatory potential with trans-epithelial transport of a buffalo casein-derived hexapeptide (YFYPQL)

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ABSTRACT

Inflammation and oxidative stress are two patho-physiological processes that go together. Anti-oxidants can be used as anti-inflammatory mediators to avoid the progression of many lifestyle associated diseases. The overlapping antioxidative and anti-inflammatory potentials of a buffalo casein-derived hexapeptide (YFYPQL) were studied with *in vitro* and *ex vivo* assays. An *in vitro* Caco-2 cell model suggested that pre-treatment with the hexapeptide showed protection against H_2O_2 induced oxidative cell death and inhibited ($p \leq 0.01$) ROS generation. Peptide treatment also reduced ($p \leq 0.01$) the levels of cellular oxidative products of lipids and proteins (MDA and protein carbonyls) and elevated ($p \leq 0.05$) the activities of the anti-oxidative enzymes (CAT, SOD and GP_X) by stimulating the nuclear response factor-2 (Nrf-2) stress signalling pathway. Moreover, peptide treatment reduced ($p \leq 0.01$) the Nrf-2 mRNA expression in Caco-2 cells even in the presence of H_2O_2. In addition to the antioxidative properties, this peptide also had a role in anti-inflammation with *ex vivo* conditions by suppressing ($p \leq 0.01$) mice splenocytes proliferation and enhancing the phagocytosis of murine peritoneal macrophages. Peptide treatment was also found to reduce the secretion of pro-inflammatory cytokine (IFN-γ) and elevated the levels of anti-inflammatory cytokine, IL-10 in splenocytes culture supernatants. Besides, the hexapeptide showed $0.91 \pm 0.04\%$ intact transport across the Caco-2 cell monolayer with an apparent permeability coefficient (P_{app}) of 4.75×10^{-5} cm min^{-1} using a transcytosis mediated mechanism. Thus, overlapping antioxidative and anti-inflammatory activities as well as transport across the epithelial membrane of the casein hexapeptide was established.

1. Introduction

Milk-borne bioactive peptides are regarded as potential active ingredient for preparation of various functional foods, nutraceuticals and pharmaceutical drugs due to their biological functions. Several bioactive peptides showed multifunctional properties. Some regions of the primary structures of caseins contained overlapping sequences that showed different activities. These regions have been considered as “strategic zones” that are partially protected from further proteolytic breakdown (Severin & Wenshui, 2005). Recently, active forms of the αs1-casein peptide ^{143}AYFYPEL^{149} were found in the casein jejunal digests at different sampling times in human volunteers (Sanchón et al., 2018). This anti-hypertensive peptide (Contreras, Carrón, Montero, Ramos, & Recio, 2009) had also been previously found in human gastric digests after milk ingestion (Chabance et al., 1998). Finding of this active sequence in human jejunum suggested its resistance to gastrointestinal digestion. Earlier the hexapeptide peptide, YFYPEL, isolated from αs1-casein showed good superoxide anion radical scavenging activity and the presence of a C-terminal dipeptide, Glu-Leu, was shown to be important for this activity (Suetsuna, Ukeda, & Ochi, 2000). Later, Martínez-Maqueda, Miralles, Cruz-Huerta, and Recio (2013) showed its mucin-stimulatory activity in human goblet cells and Fernández-Tomé et al. (2016) found opioid agonistic activity in guinea pig ileum. The presence of Tyr on the N-terminus for interaction with μ-opioid receptors was suggested to be important for various biological activities (Teschemacher, 2003). Similarly, the immunomodulatory potential of β-casomorphin-7 (YPFPGPI) and β-casomorphin-5 (YPFPG), having Tyr at the N-terminal was also established (Haq, Kapila, & Saliganti, 2014), although previously these peptides were predominantly known for opioid and mucin secretory activities. Peptides with multifunctional potentials have now been considered more useful than peptides with single activity as they affect multiple signalling pathways simultaneously to influence numerous biological processes in the cell (Aguilar-Toalá et al., 2017; Agyei, Potumarthi, & Danquath, 2015). The presence

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