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Bio-accessible milk casein derived tripeptide (LLY) mediates overlapping anti-inflammatory and anti-oxidative effects under cellular (Caco-2) and *in vivo* milieu

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Abstract

Inflammation and oxidative stress are closely linked patho-physiological processes which occur concurrently in many diseased conditions. Recently, interdependence between these two processes explains the antioxidant paradox associated with failure to select appropriate agents required for prevention of diseases known to be induced by oxidative stress. Present study established the overlapping anti-inflammatory and anti-oxidative potential along with bio-accessibility of milk casein derived tripeptide (LLY). Tripeptide exhibited anti-inflammatory response under *ex vivo* conditions by suppressing ($P<.01$) mice splenocytes proliferation and modulating their cytokines (IFN-γ, IL-10 and TGF-β) with improved phagocytosis of peritoneal macrophages. Conversely, tripeptide displayed extraordinary radical scavenging ability and cellular anti-oxidative potential using chemical assays and H_2O_2 induced oxidative stress model on Caco-2 cells. Under cellular assessment, on one hand tripeptide inhibited ($P<.01$) intracellular ROS generation and reduced MDA and protein carbonyls but on the other also increased ($P<.01$) the activity of anti-oxidative enzyme, catalase without much effect on SOD and GPx. This anti-oxidative potential was further established by studying relative expression of genes (*Nrf-2 and Keap1*) and Nrf-2 nuclear translocation associated with anti-oxidative signaling in Caco-2 cells. Bio-accessibility of tripeptide and its intact transport across Caco-2 cell monolayer was also found to be 1.72±0.22% through PepT1 mediated transport mechanism. Besides, tripeptide displayed strong anti-oxidative and anti-inflammatory potential under *in vivo* conditions in mice against ethanol induced oxidative stress by elevating ($P<.01$) liver GSH content and by decreasing ($P<.01$) the activities of anti-oxidative enzymes, MDA along with reduced expression of CYP2E1, PPAR-α, TNF-α and COX-2 genes than ethanol control.

Keywords: Bioactive peptide; Oxidative stress; Anti-oxidative enzyme; Nrf-2; Immuno-modulation; Bio-accessibility

1. Introduction

Research in last one decade has witnessed the production of various bioactive peptides from food sources after digestion which plays an important role in the nervous, cardiovascular, digestive and immune systems [1,2]. Initially most of the researchers explored various peptides hidden in the casein system with solo activity such as anti-oxidative, immunomodulatory, ACE inhibitory, antimicrobial and metal chelating properties [3–5]. Recently keen interest has emerged in identifying peptides with multifunctional or overlapping bioactivities over a particular bio-function because they can simultaneously trigger, modulate or inhibit multiple physiological pathways [6,7]. Such overlapping activities of peptides may play a great role in reversing the adverse health effects by synergistic and combinatorial influence on related physiological functions. In this context oxidative stress and inflammation are closely related patho-physiological processes, one of which can be easily induced by another. Both of the processes are simultaneously found in many pathological conditions [8]. If oxidative stress appears as the primary abnormality in an organ, inflammation will eventually develop and will further accentuate oxidative stress. Conversely, if inflammation is the primary event, oxidative stress will develop as a consequence which will further exaggerate inflammation [9,10]. Epidemiological and experimental studies strongly suggest a contribution of oxidative stress in many human diseases including cancer, atherosclerosis, hypertension, ischemia/perfusion, diabetes, asthma and alcoholism *etc.* [11,12]. Thus dietary supplements containing multiple natural antioxidants and/or anti-inflammatory agents may have several prophylactic advantages. At present, however, there is scarce information on the properties and potential of such supplements. To fill this gap, investigators used either combination of several components having anti-oxidative and anti-inflammatory properties [13] or focused their research on bioactive compounds having overlapping activities mostly derived from herbs such as Curcumin (diferuloylmethane: *Curcuma longa*), Magnolol (*Magnolia officinalis*), AS-IV (Saponin astragaloside-IV: *Astragalus sp.*) and other poly-phenolic rich food ingredients [14–18]. Nevertheless, the extraction of these components is laborious

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