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The role of milk protein-derived bioactive peptides in humans

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Abstract : The role of milk extends beyond simply providing nutrition to the suckled young. Milk has a comprehensive role in programming and regulating growth and development of the suckled young, and provides a number of potential autocrine factors so that the mammary gland functions appropriately during the lactation cycle. This central role of milk is best studied in animal models such as marsupials that have evolved a different lactation strategy to eutherians and allow researchers to more easily identify regulatory mechanisms that are not as readily apparent in eutherian species. Many studies have demonstrated that milk protein consumption has benefits in terms of promoting human health. This review assesses the intervention studies which have evaluated potential health enhancing effects in humans following the ingestion of milk proteins. The impact of milk protein ingestion has been studied to asses their satiating, hypotensive, antimicrobial, anti-inflammatory, anticancer, antioxidant and insulinotropic properties as well as their impact on morphological modifications (e.g., muscle and fat mass) in humans.
Key words: Milk proteins, Necrotizing enterocolitis, Nutrition, Bioactive peptides.

1. Introduction

Food protein-derived bioactive peptides (BAPs) have been extensively studied in relation to their potential health promoting effects in humans. A large number of studies have been conducted with milk protein-derived BAPs[1]. Despite major advances in medical and pharmaceutical sciences, as well as a broader access to health structures in certain countries, specific metabolic diseases now appear to be more prevalent worldwide. The metabolic syndrome (MetS) is defined as a combination of risk factors including abdominal obesity, insulin resistance along with high cholesterolemia and blood pressure (BP). It is estimated that ~25% of the world's population is affected by the MetS[2].

Over the past few decades, it has been shown that specific protein fragments, called bioactive peptides (BAPs), may beneficially modulate certain health related biomarkers, at least *in vitro*. To date, there have been many studies demonstrating the potentially beneficial effect *in vitro* of food protein-derived BAPs on biomarkers associated with the metabolic syndrome and bone health[3]. These include BAPs with antihypertensive, antidiabetic, antiobesity, antioxidant, immunomodulatory and mineral binding properties[4].

Several studies have suggested that milk protein-derived BAPs may be used as preventative/ prophylactic agents to alleviate symptoms of various diseases in humans. Although various drugs exist to cure/slow down the progress of specific diseases in humans, their side-effects may sometimes outweigh their benefits[5]. In this context, food protein-derived peptides and specifically specifically milk protein-derived BAPs have potential as natural alternatives to drugs for disease management[5]. For example, no cytotoxic effects have

been shown in small animals following the consumption of angiotensin converting enzyme (ACE) inhibitory peptides Ile-Pro-Pro and Val-Pro-Pro[6].

Milk protein-derived BAPs are therefore naturally present in a wide range of dairy products and foods containing dairy based ingredients. Depending on their sequence, these BAPs may reach the small intestine intact and be absorbed as is or they may be degraded by GI enzymes or serum peptidases in the circulation. Several studies have investigated the stability and bioavailability of milk BAPs, mainly following *in vitro* simulated gastrointestinal digestion (SGID) treatments[7] or permeation through Caco-2 cell monolayers[8]. However, only a limited number of studies have been carried out in relation to the stability and bioavailability of BAPs in humans[9]. This may, in some instances, be related to analytical limitations associated with the detection of peptides and notably short peptides[10] in relatively complex physiological fluids. In addition, because BAPs are naturally found within the body, notably in digestate fluids[11], the serum[9] and different tissues[12] at relatively low levels.

Although potential bioactive peptides have been identified from various animal or plant proteins belonging to current diet, milk proteins remain the major source of bioactive peptides and these peptides display a wide range of biological activities. Casein and whey proteins are sources of peptides with biological activity containing 2–50 residues, which are released only after hydrolysis of these proteins in vivo or in vitro. Their activity depends on both their residue composition and sequence, and some of them are known to display multi-functional properties. Bioactive peptides can display, inter alia, antioxidant, hypotensive, immunomodulatory, antithrombotic or opioid properties (Fig. 1) and, consequently, appear to be capable to exert beneficial health effects by acting on the nervous, digestive, cardiovascular and immune systems. Research concerning them has made the subject of several reviews[13].

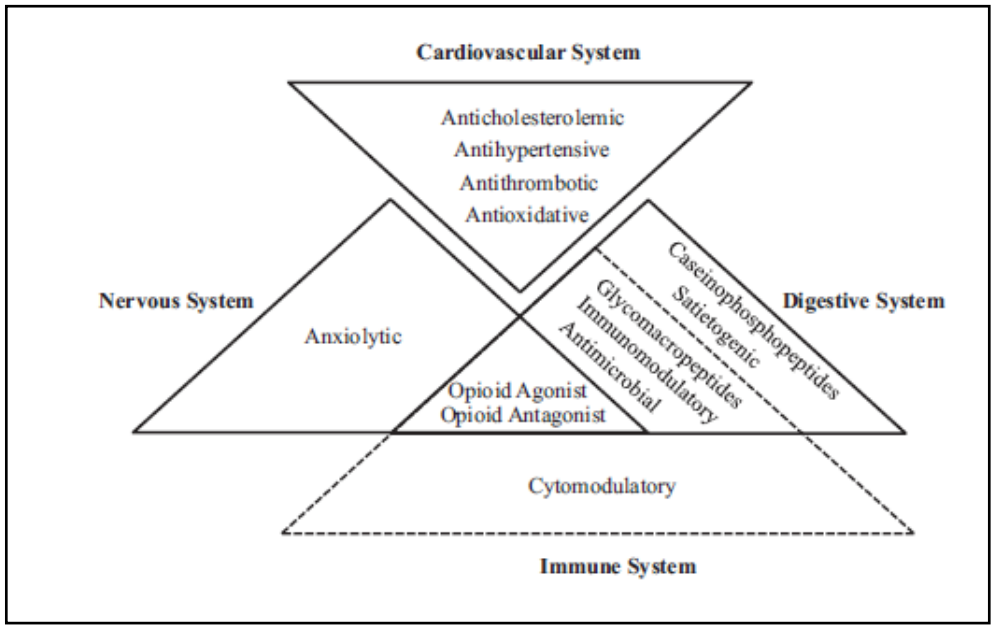

Fig. 1. Bioactivities of milk protein-derived peptides[13].

2. Effects of bioactive milk proteins on intestinal inflammation

2.1. Caseins (CN)

Upon feeding, buffer capacity of human milk rapidly increases gastric pH to ~pH 7. Depending on the feeding frequency, pH falls over the following 2–4 h, reaching ~pH 4, but as low as pH 3–3.5 in a minority of infants[14]. The buffering effect of human and bovine milk proteins prevents gastric pH from not reaching the optimum of enzymes such as pepsin A and pepsin C (gastricsin) (~pH 2). This results in only minor initial digestion of human milk proteins. However, pH is sufficient to release the C-terminal portion of K-CN (glycomacropeptide, GMP) into the whey. This, combined with gastric pH close to the isoelectric point (pI) of the CNs (pH 4.6), results in their precipitation.

2.2. Whey proteins

2.2.1. Alpha-lactalbumin

Human α-La protects soluble CD14 (sCD14) from proteolytic degradation, which could allow it to neutralize pathogens in the GIT lumen[15]. Direct effects of α-La occur at the GIT mucosa. In suckling rats, a hydrolysate of α-La increased the intestinotrophic hormone, GLP-2, enterocyte maturation and number, intestinal elongation, and the number of crypt-epithelial cells, compared to unhydrolyzed α-La[16]. α-La is a chemoattractant, stimulating the secretion of CXCL8, MIP-1 α and MIP-1β from human neutrophils. It also directs neutrophils, monocytes and lymphocytes to inflammatory sites[17]. α-La induced cell death in RAW 264.7 monocytic cell lines, by effects on caspase 3 and the active fragment of caspase 8, p18. Likewise, levels of anti-apoptotic Bcl-2 were reduced, and cytochrome C increased[18].

2.2.2. β-Lactoglobulin

β-Lg is one of the few bovine milk proteins which is not cleaved by gastric pepsin and therefore enters the upper intestine in intact form[19]. β-Lg peptidescan be further digested by intestinal commensal bacterial pro-teases releasing anti-inflammatory IL-10 from mouse splenocytes[20]. β-Lg abrogates IL-6 release following intestinal ischemia/reperfusion in rats[21]. Another key consideration is that β -Lg has a high (83%) amino acid homology with human glycodelin A, a protein involved in the maintenance of the feto-maternal immune system[22]. Glycodelin A suppresses all main immune cells, including both Th1 and Th2 responses[23]. Moreover, monoclonal antibodies raised against β -Lg cross-reactwith glycodelin A[24]. It is not known whether nat-ural antibodies to β -Lg cross-react with glycodelin A, nor whether β -Lg has similar activities as glycodelin A and this warrants further studies.

2.2.3. Lactoferrin

LF is present in many biological fluids and also in human neutrophils where it participates in the superoxide burst[25]. LF has both anti-inflammatory and pro-inflammatory effects in vivo[26].

2.2.5. Immunoglobulins

Newborn infants, and especially preterm infants, may have reduced levels of circulating Igs (IgA, IgM and IgE) and local Ig-related GIT protection against enteric antigens is facilitated by intake of mother's milk during breast feeding[27]. Igs also have more direct anti-inflammatory effects by directly chelating bacterial and viral antigens[28].

2.2.6. Enzymes

LP catalyzes the conversion of NO, and the co-substrate H_2O_2, together with thiocyanate into antimicrobial hypothiocyanate anions[29]. As mentioned earlier in Sections 2.3 and 2.4, both $H_2O_2$and NO are produced at the inflammatory site and their removal by LP and the concomitant generation of anti-microbial hypothiocyanate anions are indispensable anti-inflammatory mechanisms. Orally administered bovine LP has effects against DSS-induced colitis in mice, reducing IL-6 and intestinal crypt damage scores[30].

2.3. Milk fat globule membrane proteins

Eight principal MFGM proteins have been identified[31]. Many of these proteins are significantly glycosylated[32]. Several of these major MFGM proteins have no known effects against inflammatory processes, acting only indirectly via binding to bacteria and viruses[33].

3. Satiating and weight management effects of milk proteins in humans

It has been suggested that a significant increase in protein intake, over habitual intake, could help in weight management and positively alter body composition[34]. Significant differences in anthropometric parameters were reported in healthy and obese humans when the protein concentration in the test group was on average 58.4% (g/kg/day) higher than in the control group. In contrast, no effect on food intake was observed with differences of up to 38.8% (g/kg/day) between the test and control groups[35]. Milk proteins, which have been described for their satiating properties, may have potential for use as natural dietary components to reduce

food intake in humans[34]. Numerous human intervention studies have focused on the satiating properties of milk proteins and their effect on the reduction of food intake[36].

4. Antimicrobial role of milk proteins in humans

Particular attention has to date been given to lactoferrin (LF), a minor WP, as it is able in its intact format to display antimicrobial activities. The mechanisms involved are thought to relate to binding to the lipopolysaccharide membrane of microorganisms. LF has been shown to induce membrane disruption, to penetrate into dendritic cells, to sequester iron and to display prebiotic activities preventing pathogen growth[37]. Several human studies are found in the literature evaluating the antimicrobial properties of intact LF[38]. A human recom-binant LF solution (600 mL at 5 mg mL−1) was administered via gastrostomy tubes for 56 days to nursing-home residents at the start of an antibiotic treatment[39]. This resulted in a decreased incidence of antibiotic-associated diarrhea, which has in certain instances been linked with the presence of Clostridium difficile. Diar-rhea was seen with 44% of the subjects receiving the LF solution asopposed to 92% in the control group administered with a placebo. Bovine LF has also been used as an adjunct in the triple therapy against Helicobacter pylori in randomized control trials (RCTs)[40].

5. Antioxidant effects of milk proteins in humans

Antioxidant species (such as reactive nitrogen and oxygen species (ROS)) are naturally found within the human body. How-ever, high levels of antioxidant species are detrimental to human health as they may lead to cell damage[41]. The ingestion of milk proteins by humans has, in certain instances, been reported to reduce oxidative stress. Supplementation of healthy males participating in a resistance training program with WPI induced a significant increase in plasma total antioxidant capacity (TAC; +4%) and glutathione (GSH; +12%) level[42]. It was suggested that combining resistance training with WP consumption could help reduce the oxidant status in humans[42]. However, a different trend was reported in a similar study where WP supplementation combined with resistance training did not decrease the oxidant status in humans[43]. The impact of a Cys-rich WPI (20 g daily for 12 weeks) on plasma oxidative status (GSH and TAC) was evaluated in subjects with non-alcoholic steatohepatitis[44]. Along with an average weight reduction and other physiological improvements, the treatment led to an increased plasma TAC (+61%) and GSH (+28%) levels[44].

5. Conclusion

There is no doubt that the hydrolysis of milk proteins gives rise to a diversity of peptides, some of them displaying remarkable functionalities relevant to the maintenance of human health. The knowledge about new bioactive peptides from milk proteins and about their potent functionalities in the fermented milk products is consistently increasing. They offer an exciting opportunity in the area of the development of novel functional foods which in turn could contribute to the prevention and management of certain diseases such as hypertension, type 2 diabetes or obesity and more broadly metabolic syndrome.

The addition in the product of specific bioactive peptides isolated from milk protein hydrolysate seems to provide a practical approach to enhance the functionality of the fermented milk products. In this case, the initial concentration of the peptide added can be checked, but the peptide remains exposed to the proteolytic system of the starter LAB, except if extracellular protease/peptidase mutants are used during the fermentation step or peptides are encapsulated. Further, given the cost of purification of peptides, it is easier to add a hydrolysate keeping in mind that peptides with antagonist activities can be present in this hydrolysate.

The use of recombinant LAB seems to be a promising approach to increase the functionality of the fermented milk products and to deliver targeted health benefits to consumer. This also permits to produce into the fermented milk, bioactive peptides initially identified in food proteins not belonging to milk. As in the previous case, if the peptides are not produced directly into the milk product, but added to it, the problem of their susceptibility to hydrolysis by bacterial extracellular proteases/peptidases remains. Despite the risks of uncontrolled product expression and transfer of the transgene into a commensal bacterium, the production of bioactive peptides during milk fermentation using genetically modified organisms could be an area of interest for future research. Moreover, peptides with modified sequences can be designed in a view to increase their stability, functionality and duration of action.

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