IDENTIFICATION OF BIOACTIVE PEPTIDES IN A THERAPEUTIC PO-N-0464 INFANT FORMULA FOR THE NUTRITIONAL TREATMENT OF COW'S MILK ALLERGY

Matencio E¹, Gómez C², Recio I³, Gil A⁴, Romero F¹, Abellán P¹, Ros G²

1I+Q Department, Hero Spain, S.A. 2Human Nutrition and Food Science, Faculty of Veterinary Sciences, University of Murcia. 3Institute of Food Science Research (CIAL, CSIC-UAM) Madrid, Spain. 4Department of Biochemistry and Molecular Biology II. Institute of Nutrition and Food Technology. Centre of Biomedical Research University of Granada, Spain.

INTRODUCTION

Human milk is the gold standard of infant nutrition during the first months of life. It contains minor components such as bioactive peptides with activities such as antioxidant, antihypertensive, antibacterial and/or immunomodulator properties. These compounds seem to perform their functions when they are hydrolysed during the infant digestion and they have specific functions according with their amino acids sequence

The aim of this study was to identify bioactive peptides after an in vitro digestion in a new extensively hydrolysed formula, which may have a relevant role in the nutritional treatment of cow's milk allergy in infants.

METHODS

The hydrolysis procedure consisted on the simulation of gastric and intestinal digestion of the samples by in vitro enzymatic treatment. Soluble fraction was filtered and the water-soluble extract was subjected to ultrafiltration through a hydrophilic 3000 Da membrane. The hydrolysates were injected into an HPLC system connected on-line to an Esquire-LC quadrupole ion trap instrument for RP-HPLC-MS/MS analysis, according to the method of Hernández-Ledesma et al 2004¹. The m/z spectral data were processed using Data Analysis 3.0 (Bruker Daltonik) and transformed to spectra representing mass values. MS(n) spectra were processed in BioTools 2.1 (Bruker Daltonik) to perform peptide sequencing.

RESULTS

75 fragments, with a molecular weight lower than 1000 Da, were identified, 77% of them derived from whey (Figure 1). 14 fragments were identified which could have angiotensin converting enzyme (ACE) inhibitory activity (SLSQSK, EMPFPK, VVPPFLQ, VRGPFPI, LHLPLP, LHLPLPL, IIAEK, LDIQK, ALPMH, ALPMHI, VLDTDYK, GLDIQK, LDAQSAPL, VAGTWY). Other peptides with other possible functional effects were detected (Table 1)

CONCLUSION

 Some of the bioactive peptides found in this therapeutic formula could be isolated, produced and clinically tested with the aim to produce supplements for special medical purposes such as prevention or treatment of allergy

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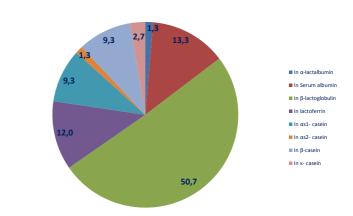


Figure 1. Bioactive peptides in the protein fractions of the extensively hydrolysed formula (%)

Table 1. Bioactive peptides identified in extensively hydrolysed formula and possible attributed effects.

| PEPTIDES | SOURCE / POSSIBLE EFFECTS |
|-------------------|--|
| ALPMH ALPMHI | Source: β-lactoglobulin • ACE-inhibitory activity ² • Antihypertensive propeties ^{3,4} • Hypocholesterolemic effects ⁵ |
| SLAM SLAMA | Source: β-lactoglobulin • Antioxidant ⁶ |
| LHLPLP LHLPLPL | Source: β-casein • Antihypertensive properties ⁷ |
| VLNENL | Source: αs1-casein • Immunomodulatory ⁸ • Actibacterial activity ⁸ |
| ISQPE | Source: Lactoferrin • Antibacterial effects ⁹ |
| IPAVF | Source: β-lactoglobulin • Antibacterial effects ¹⁰ |

REFERENCES

- 1. Hernández-Ledesma et al 2004 J. Chromatogr. A. 1049, 107-14.
- Mullally MM, Meisel H, FitzGerald RJ.(1996) Angiotensin-I-converting enzime inhibitory activities of gastric and pancreatic proteinase digests of whey proteins. Int Dairy J 7:299-303
 Murukami M. Tonourbi H. Takabashi B. Kitzawa H. kawai Y. Neeishi H and Saito T (2004). Structural analysis of a
- Murukami M, Tonouchi H, Takahashi R, Kitazawa H, kawai Y, Negishi H and Saito T (2004). Structural analysis of a new anti -hypertensive peptide (β-lactosin B) isolated from a commercial whey product. J Dairy Sci 87: 1967-1974
 Català-Clariana S, Benavente F, Giménez E, Barbosa J, Sanz-Nebot V (2010). Identification of bioactive peptides in
- Català-Clariana S, Benavente F, Giménez E, Barbosa J, Sanz-Nebot V (2010). Identification of bioactive peptides in hypoallergenic infant milk formulas by capillary electrophoresis-mass spectrometry. Analytica Chimica Acta 683 119–125
 Nagaoka, S., Y. Futamura, K. Miwa, T. Awano, K. Yamauchi, Y. Kanamaru, K. Tadashi, and T. Kuwata. (2001).
- Lidentification of novel hypocholesterolemic peptides derived from bovine milk betalactoglobulin. Biochem. Biophys. Res. Commun. 281:11–17.
- Hernández-Ledesma B, Quiros A, Amigo L, Recio I (2007). Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin. Int Dairy 117: 42-49
 Misruel MJ. Bergiel A. Barger M. Deleda MA. Algorada MA (2006). Artificiant programma of fact of pantides phtpiand
- Miguel M1, Recio I, Ramos M, Delgado MA, Aleixandre MA (2006). Antihypertensive effect of peptides obtained from Enterococcus faecalis-fermented milk in rats. J Dairy Sci. 89(9):3352-9.
 Lahov F. & Repelson W. (1996). Antihacterial and immunostimulating casein-derived substances from milk:
- Lahov, E., & Regelson, W. (1996). Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. Food and Chemical Toxicology, 3481):131-45
- Recio I, Visser S (1999). Two ion exchange chromatographic methods for the isolation of antibacterial peptides from lactoferrin In situ enzymatic hydrolysis on an ion-exchange membrane. Journal of Chromatography A, 831: 191–201
- Pellegrini A, Dettling C, Thomas U, Hunziker P (2001) Isolation and characterization of four bactericidal domains in the bovine b-lactoglobulin. Biochim Biophys Acta 1526: 131–140