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Potential bioactive properties of casein hydrolysates

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Casein constitutes approximately 80% of the total milk protein content. Bioactive casein peptides derived from the hydrolysis of casein directly influence numerous biological processes, evoking gastrointestinal, hormonal, immunological, neurological, and nutritional responses[(1)]. Previously, it has been reported that eight casein hydrolysate samples (A–H) exert varying effects on Jurkat cell viability and growth and that casein hydrolysate C enhances glutathione content in Jurkat cells[(2)]. Thus, the aim of the present study was to further assess the potential bioactive properties of the same casein hydrolysates by investigating their effects on cell membrane integrity, antioxidant enzyme activity and oxidant-induced DNA damage. In addition, the effects of these compounds on cytokine production in stimulated human Jurkat T cells were determined.

Cell membrane integrity was determined using the lactate dehydrogenase-release assay kit (Biogenesis, Poole, Dorset, UK). Supplementation with 0.01–1% (v/v) casein hydrolysates for 24 h had no effect on Jurkat cell membrane integrity. Cells were then treated with the casein hydrolysate samples (A-H) at a concentration of 0.05% (v/v) for 24 h. Catalase (CAT) and superoxide dismutase (SOD) activities were determined using Calbiochem catalase colorimetric kit and SOD assay kit II (Merck Chemicals Ltd, Nottingham, UK) respectively. Of the eight samples, casein hydrolysate A increased CAT activity ($P<0.05$) in Jurkat cells, whereas none of the samples affected SOD activity.

DNA damage was assessed using the comet assay by the method of Tice *et al.*[(3)]. Jurkat cells were too sensitive for this assay and as a result human colon adenocarcinoma Caco-2 cells were used. Caco-2 cells were pre-incubated with casein hydrolysate samples for 24 h followed by treatment with or without H_2O_2 (50 μM for 30 min). The presence of the casein hydrolysates alone did not affect DNA integrity. Exposure to H_2O_2 caused significant ($P<0.01$) DNA single-strand breaks and none of the casein hydrolysates protected against H_2O_2-induced DNA damage.

To assess potential immunomodulatory effects Jurkat cells were treated with concanavalin (Con) A (25 μg/ml) in the presence or absence of the casein hydrolysates for 24 h. Production of the cytokines IL-2 and IL-10 was determined by an eBioscience ELISA kit (Insight Biotechnology Ltd, Wembley, Middlesex, UK). Casein hydrolysates D-H significantly enhanced ($P<0.05$) ConA-induced IL-2 production but levels of ConA-stimulated IL-10 were not affected.

In conclusion, casein hydrolysate A exerted strong antioxidant effects in terms of CAT activity, and no casein hydrolysate exerted protection against oxidant-induced DNA damage. The bioactive properties of casein hydrolysates depend on the methods used for their preparation. Interestingly, casein hydrolysates D–H enhanced ConA-induced IL-2 production, which warrants further investigation.

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1. Shah NP (2000) *Br J Nutr* **84**, S3–S10.
2. Phelan M, Aherne SA, O'Brien NM, O'Sullivan D & Fitzgerald D (2008) *Proceedings of the 37th Annual Research Conference on Food, Nutrition and Consumer Sciences*, pp. 90–91. Cork, Republic of Ireland: UCC.
3. Tice RR, Andrews PW, Hirai O & Singh NP (1990) In *Biological Intermediates IV*, pp. 157–164 [CM Witmer, editor]. New York: Plenum Press.