Nano-encapsulation of tea polyphenols in casein micelles- The effect of EGCG-casein complex formation on the digestibility of caseins and the bio-accessibility of EGCG

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INTRODUCTION

Consumption of polyphenols has been associated with a reduction in the risk for several chronic diseases (Manach 2004). The biological activities of polyphenols are influenced by their absorption and metabolism, and their bioefficacy will depend on their release from the food matrix by the action of digestive enzymes and as a result of colonic bacterial fermentation (Cilla 2009).

Numerous studies have demonstrated that tea catechins form complexes with milk proteins, especially caseins (Jobstyl 2006; Papadopoulou 2004). Much less work has been conducted to understand the metabolic conversions of tea/milk complexes during gastro-duodenal digestion.

The objective of this study was to determine the significance of this association on the digestibility of the milk proteins and on the bioaccessibility of the tea polyphenol epigallocatechin gallate (EGCG).

MATERIALS AND METHODS

Quantification of EGCG binding to casein micelles
Green tea extract (minimum 90% Epi-gallocatechin Gallate (EGCG)) was mixed with skim milk. Dilutions were also carried out using permeate (milk serum), to obtain high EGCG/casein ratios. The mixtures were centrifuged, and the supernatant fraction (containing whey proteins) was analyzed by reverse phase HPLC (Ferruzzi 2006).

In vitro gastro-duodenal digestion
An *in vitro* digestion model mimicking the gastric stage and duodenal stage of human digestion (Malaki Nik 2010) was applied on isolated EGCG, skim milk and EGCG-milk mixture. The *in vitro* method included the human gastric stage, with simulated fluids containing salts, and pepsin, for a duration of 30 minutes, and a second duodenal stage, with fluids containing pancreatic juices and bile salts for 1hour.

SDS-PAGE analysis
During the gastric phase, samples were withdrawn from the mixture at 5, 10, 20, and 30 min for SDS-PAGE analysis. Proteolysis was terminated by raising the pH of the gastric mixture to 6.5 using 1M NaHCO3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was carried out using 15% resolving gels.

Bioefficacy tests
The anti-proliferative effect of EGCG-milk complexes on normal rat colonic epithelial cells (4D/WT) and those transformed by v-src (D/v-src) were studied using the Sulforhodamine B dye-binding assay (SRB) (Cha 2005). The cells were incubated for 24 h with different concentrations of isolated EGCG, or complexed with casein micelles, both before digestion and after digestion. The results are presented as percent change in cell proliferation with increasing polyphenol levels in the culture medium.

RESULTS AND DISCUSSION

Analysis of tea polyphenols by HPLC
Figure 1 illustrates the binding of EGCG to casein micelles quantified by HPLC. At low concentrations nearly all of the tea catechins were bound to the casein micelles. At about 2.5 mg/ml of milk the micelles reached a saturation point, after which, catechins were recovered in the serum phase.

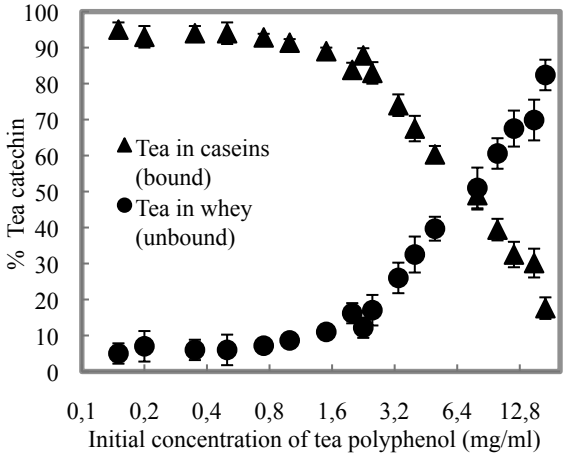

Figure 1: % Tea catechin bound with casein(▲) or recovered in the serum phase (●) as a function of concentration of tea polyphenol.

SDS-PAGE of milk and tea/milk complexes during the gastric phase
The electrophoretic analysis of milk during simulated gastric digestion demonstrated an extensive proteolysis of the caseins after 5 min of incubation. Only a faint band was recovered for undigested β-lactoglobulin. However, no significant difference was seen in the digestion patterns for skim milk with

or without EGCG. This shows that the complex formation between EGCG and milk caseins does not make the complex less susceptible to proteolysis (Figure 2).

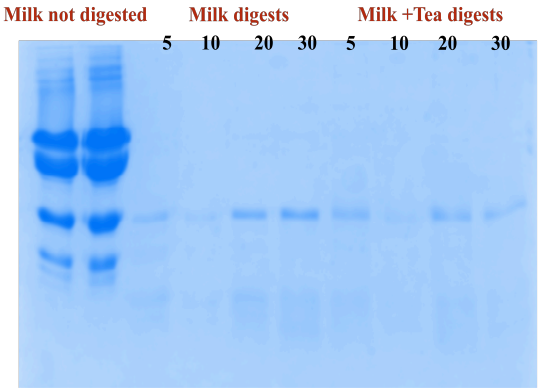

Figure 2: SDS-PAGE electrophoresis of the initial milk and of the digested samples after 5, 10, 20 and 30 min of incubation with simulated gastric fluid

Proliferation of normal (4D) and cancerous (v-src) rat colonic cells exposed to tea and tea /milk digests

The results for both normal and cancerous cell lines (Figure 3) showed that the EGCG complexed with milk, when compared to isolated EGCG, did not show a significant difference in cell proliferation. These results indicate that the strong binding of EGCG and milk casein does not affect the bio efficacy of the tea polyphenols. However, a selectivity of EGCG in inhibiting the proliferation of tumor cells rather than normal cells was seen in the results, whether in isolated form, or as complexed with milk. Other studies have shown that EGCG has different mechanisms in favouring normal cell survival and tumor cell destruction.

CONCLUSIONS

In spite of the complex formation of casein micelles and EGCG, the caseins are still susceptible to proteolysis during in vitro gastric digestion.

As casein micelles are recognized for their valuable functionality in the delivery of bioactives, the cell proliferation results demonstrate that EGCG-milk complexes after gastro-duodenal digestion have similar effects compared with isolated EGCG and their bioefficacy was not impacted by binding with milk caseins.

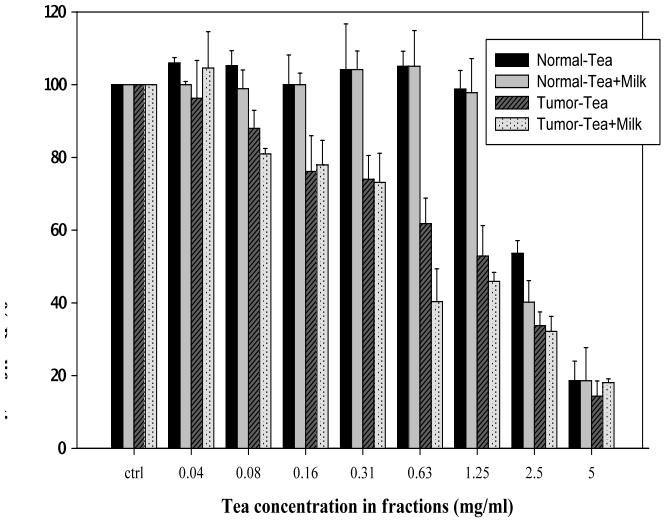

Figure 3: % Cell proliferation in different fractions

In addition, there were clear differences in the bioefficacy of EGCG for normal or cancer cells as there was a lower extent of inhibition of cancer cell proliferation by the fractions compared to normal cell lines. These results bring further evidence that casein micelles can be employed as an appropriate platform for delivery of bioactive compounds.

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