COMBINATORIAL ANTICANCER EFFECTS OF TEA POLYPHENOLS AND BOVINE LACTOFERRIN IN VITRO AND IN VIVO: MOLECULAR MECHANISTIC PATHWAYS

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Summary
Of late, chemoprevention by combination regimens that use tea polyphenols as one of the constituents has been found to be potentially effective. Since tea is consumed with milk in many parts of the world, we evaluated the antiproliferative and apoptosis inducing potential of green tea polyphenols (Polyphenon-E; P-E) and black tea polyphenols (Polyphenon-B; P-B) alone and in combination with bovine lactoferrin (bLF) on human oral cancer cell lines in vitro and in the well established hamster buccal pouch (HBP) carcinogenesis model in vivo. Polyphenon-E exerted more potent cytotoxic and apoptosis inducing effects in vitro, while Polyphenon-B was more effective in the HBP model. Although dietary administration of bLF and Polyphenon-B alone significantly reduced tumour incidence, combined administration of bLF and Polyphenon-B was more effective in inhibiting 7,12-dimethylbenz[a]anthracene (DMBA)-induced HBP carcinomas by modulating carcinogen-metabolizing enzymes, redox status, cell proliferation and apoptosis. These findings strengthen the observation that functional foods are promising candidates for chemoprevention because of their safety and the fact that they are not perceived as medicine.

Keywords
Anticancer, bovine lactoferrin, hamster buccal pouch carcinogenesis, tea polyphenols, apoptosis

Results and Discussion
Combination chemoprevention by tea polyphenols and bovine milk lactoferrin (bLF), which are rich in antioxidants is a promising strategy to control cancer incidence. We evaluated the anticancer effects of tea polyphenols (Polyphenon-E and Polyphenon-B) alone and in combination with bLF in the human tongue squamous cell carcinoma cell line CAL-27 and in the HBP model. Both Polyphenon-E and Polyphenon-B showed dose-dependent cytotoxic effects on CAL-27 cells with IC₅₀ values of 20 and 40μg/ml respectively. Cell death was mediated by apoptosis as revealed by nuclear fragmentation and condensation, with appearance of the Ao peak, generation of reactive oxygen species (ROS), decrease in the Bcl-2/Bax ratio, induction of mitochondrial permeability transition and activation of caspase-3 (figure 1). Overall, the potency of cytotoxic and apoptosis inducing effects of dietary agents on CAL-27 cells was in the order P-E and bLF combination (1:2 ratio)>P-E>P-B.
In the HBP model, topical application of DMBA induced squamous cell carcinomas that showed aberrant expression of cytokeratins, increased frequency of bone marrow micronuclei, decreased susceptibility to lipid peroxidation, and enhanced activities of glutathione redox cycle antioxidants as well as phase I (total cytochrome P450 as well as the expression of CYP1A1 and CYP1B1 isoforms) and phase II (GST and DT-diaphorase) xenobiotic-metabolizing enzymes. This was associated with increased cell proliferation and evasion of apoptosis as revealed by upregulation of proliferating cell nuclear antigen (PCNA), GST-π, NF-κB, mutant p53 and Bcl-2, with downregulation of Bax, Fas and caspase-3 expression. Although dietary administration of tea polyphenols and bLF alone significantly reduced tumour incidence, combined administration of bLF and Polyphenon-B was more effective in inhibiting DMBA-induced genotoxicity and HBP carcinomas by modulation of carcinogen-metabolizing enzymes, upregulation of antioxidant defences, inhibition of cell proliferation and apoptosis induction Figure 2 summarises the multifunctional inhibitory effects of the combination on DMBA-induced HBP carcinogenesis.

There could be several reasons for the conflicting data generated from in vitro and in vivo, most importantly bioavailability and host defence mechanisms that could impact chemoprevention in vivo. However, the results of the present study provide evidence that the HBP model is ideal for testing candidate agents before embarking on clinical trials. Based on the results, we advocate a designer item approach in which a combination of tea polyphenols and bLF could be added as active ingredients in chewing gums to increase the bioavailability of tea polyphenols in the oral cavity.

Figure 1: Induction of apoptosis in CAL-27 by dietary agents

Figure 2. Multiple pathways involved in DMBA-induced HBP carcinogenesis and intervention by bLF and P-B combination