

Pharmaceutical Regulatory Affairs 2012: High molecular weight (HMW) lactoferrin from bovine colostrum have anti-cancer Potentials- Deakin University, Australia

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Abstract

For the separation and purification of biomacromolecules such as proteins from natural or recombinant sources, the development and use of chromatographic methods have remained at the forefront of progress made, during the past five decades of research in molecular biology, biotechnology and biomedicine. With the recent aim of modern medicine to develop safe, effective drugs affordable to patients, there has been an increasing interest in milk and whey, as a potentially rich natural source of bioactive compounds. In particular, due to its numerous and diverse array of emerging therapeutic potentials, 76-80 kDa single-chain iron-binding glycoprotein lactoferrin (Lf), is considered the most valuable biomedical protein present in whey. With the successful clinical trials, multifunctional glycoprotein bovine lactoferrin is gaining attention as a safe nutraceutical and biologic drug targeting cancer, chronic-inflammatory, viral and microbial diseases. Interestingly, recent findings that human lactoferrin oligomerizes under simulated physiological conditions signify the possible role of oligomerization in the multifunctional activities of lactoferrin molecule during infections and in disease targeting signaling pathways. Here we report the purification and physicochemical characterization of high molecular weight biomacromolecular complex containing bovine lactoferrin (≥ 250 kDa), from bovine colostrum, a naturally enriched source of lactoferrin. It showed structural similarities to native monomeric iron free (Apo) lactoferrin ($\sim 78-80$ kDa), retained anti-bovine lactoferrin antibody specific binding and displayed potential receptor binding properties when tested for cellular internalization. It further displayed higher thermal stability and better resistance to gut enzyme digestion than native bLf monomer. High molecular weight bovine lactoferrin was functionally bioactive and inhibited significantly the cell proliferation ($p < 0.01$) of human breast and colon carcinoma derived cells. It induced significantly higher cancer cell death (apoptosis) and cytotoxicity in a dose-dependent manner in cancer cells than the normal intestinal cells. Upon cellular internalization, it led to the up-regulation of caspase-3 expression and degradation of actin. In order to identify the cutting edge future potential of this bio-macromolecule in medicine over the monomer, its in-depth structural and

functional properties need to be investigated further. Novel high molecular weight bovine lactoferrin from Australian bovine colostrum with anticancer properties. Bovine colostrum is a rich source of Lf than mature milk and among the low abundance colostrum whey proteins (representing 1% or less of the protein content of whey), Lf is found to be most abundant. By employing ultrafiltration and cation exchange chromatography, we have purified a high molecular weight (HMW) bioactive protein fraction with potent anti-cancer properties from Australian bovine colostrum. The purified 250 kDa HMW bioactive protein was further physico-chemically characterized as a trimeric bovine Lf (bLf) using SDS-PAGE, Western blotting, differential scanning calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). In order to evaluate the potential anti-cancer efficacy of HMW bLf, robust cell bioassays using human colon and breast cancer cell lines were employed. The novel findings of the study with promising results on anti-cancer efficacy will be presented in the meeting.