Utilization of solid lipid nanoparticles (SLN) in the delivery of curcumin in cocultures of HT-29-MTX and Caco-2 cells

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INTRODUCTION AND OBJECTIVES

The major barriers to the bioavailability of bioactive molecules include poor absorption, rapid metabolism and elimination (Anand et al. 2007). In order to achieve longer circulation durations in the body and resistance to metabolic processes, colloidal vectors are utilized in the delivery of such molecules.

Solid lipid nanoparticle (SLN) suspensions consist of submicron-sized crystalline lipid particles dispersed within an aqueous medium and have shown potential for encapsulation, protection and delivery of lipophilic functional components. For their utilization as a delivery vehicle in the food industry, optimization of the bioactivity in a safe manner is of paramount importance (Souto et al. 2009). However, there is a relatively poor understanding on the impact of physiological conditions of gastrointestinal tract on the behavior of solid nanoparticles (McClements & Xiao 2012).

One of the greatest challenges in developing an efficient nanocarrier for oral delivery is to overcome the absorption barrier of intestinal mucosa. Many of HT-29 subclones have been used in coculture with Caco-2 cells to design a model that more accurately mimcs the small intestinal epithelial layer (Pontier et al. 2001).

Curcumin extracted from the powdered rhizomes of turmeric is a lipophilic phytochemical which has been demonstrated to act as an antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, hepato- and nephro-protective, hypoglycemic, antirheumatic agent *in vivo* (Lin et al. 2000; Anand et al. 2007). According to Xiaoyong et al. (2008), hydroxyl groups of the benzene rings, double bonds in the alkene part, and the central β-diketone moiety are likely to be responsible for its bioactivity (Xiaoyong et al. 2008). Unfortunately, aqueous solubility of curcumin was estimated to be as low as 11 $ng.ml^{-1}$ (Kaminaga et al. (2003), which could severely limit its bioavailability.

Therefore, utilization of SLNs as a delivery tool for curcumin seems to be a viable option.

The objective of this work is to investigate the capabilities of SLN formulations to deliver a poorly absorbed lipophilic bioactive compound, namely curcumin using a co-culture system that closely mimics the human intestinal epithelium.

MATERIALS AND METHODS

A hot emulsification method based on high pressure homogenization was utilized to prepare curcumin loaded solid lipid nanoparticles. Curcumin was solubilized in the lipid phase (i.e., trimyristin) prior to homogenization. Two different SLN formulations were employed either stabilized with soy lecithin and sodium glycholate or only Poloxamer 407. Overnight refrigeration was utilized in order to ensure the crystallization of the molten lipid phase. Appropriate blanks were analyzed along with a liquid soy oil emulsion to investigate the influence of free vs. encapsulated curcumin and solidified fat particles vs. liquid oil droplets. Particle size and surface charge of the dispersions were characterized using light scattering techniques immediately prior to the transport experiment.

To perform the transport study a monolayer of absorptive Caco-2 cells and mucus secreting HT29-MTX cells growing on a transport filters was used.

Confluency of monolayers was monitored prior to and after the experiments by measuring the transepithelial resistance (TEER) of the monolayers. Fluorencence spectroscopy was employed to evaluate the changes in curcumin concentration in various compartments (i.e., apical, basolateral, mucus, and cell lysates) of the transport system. The apparent permeability (P_{app} in $cm.s^{-1}$) coefficients were calculated based on the permeation from the apical to the basolateral side.

RESULTS AND DISCUSSION

The mean size of SLN dispersions and soy emulsions were approximately 100 nm and they were found to be stable over the duration of the transport experiments. The addition of curcumin did not significantly affect the particle size distribution of the samples. The surface charge of Poloxamer 407 stabilized SLNs (-18.1± 1 mV) were significantly lower than that for lecithin and bile salt stabilized SLNs (-67± 2.3 mV).

The monolayer integrity was evaluated for HT-29-MTX, Caco-2 and the coculture HT29-MTX/Caco-2 for 21 days incubation on inserts (Figure1). Processing conditions induced considerable losses in the curcumin concentration of control samples, whereas only a small extent of SLN encapsulated curcumin was lost prior to the transport experiment. The transport study showed that depletion of solid

particles from the apical compartment was slow, which slowed down curcumin delivery reaching the equilibrium in about 1 h (Figure 2).

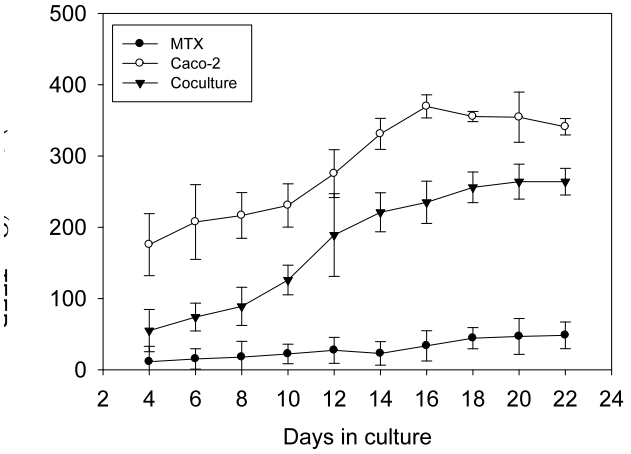

Fig. 1 TEER values were monitored for 21 days in culture for HT29 MTX, Caco-2 and coculture of HT29_MTX and Caco-2 cells seeded on 24-well Transwell® plates.

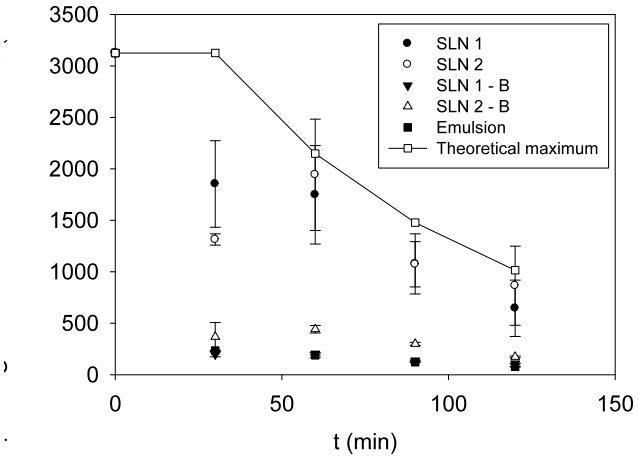

Figure 2: Depletion of curcumin from the apical compartment as a function of time. SLN 1 and 2 represent lecithin/sodium glycocholate and Poloxamer 407 stabilized SLN dispersions. B stands for the corresponding blank for each sample. Emulsion sample had a similar composition as SLN 1, whereas soy oil replaced trimyristin.

Approximately 1% of total curcumin was recovered in basolateral compartment, whereas curcumin found in mucus or cell lysates were both in the order of 2%. In the non-encapsulated samples, significant extents of losses were due to processing, storage losses, possibly including oxidation losses. The low basolateral concentrations of curcumin can be due to the rapid metabolism of of curcumin (Lin et al. 2000) which makes it difficult to monitor the uptake of the native molecule. The possible reasons for losses and the efficiency of the encapsulated and non-encapsulated curcumin will be further discussed.

CONCLUSIONS

SLN formulations increased the stability of curcumin during processing and storage and prior to the transport experiment, total curcumin concentration in the SLN samples were higher than that of the corresponding blanks and soy emulsion samples. The partitioning of curcumin in different compartments of the transport system was investigated. SLN dispersions can further be expected to enhance curcumin delivery through longer circulation durations and resistance to metabolic processes.

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