Cytotoxicity of conventional and PEGylated liposomes encapsulated with stigmasterol after gastrointestinal digestion

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Introduction: The bioavailability of many biologically active compounds in the digestive tract can be enhanced by encapsulating them into liposomes used as direct delivery system. Liposomes are a very attractive system for delivering drugs and bioactive substances into the human body, as they provide protection of these compounds from thermal and oxidative degradation as well as from the effects of light, pH, and enzymes. They are widely used in the pharmaceutical industry and their properties have been thoroughly studied and described. However, the use of liposomes in **food involves** research of a much broader type. The health and safety of foods is crucial for consumers and, in the case of **sterols**, it is prudent to establish a safe form of delivery that will prevent thermo-oxidative degradation and will heighten absorption in the gastrointestinal tract.
PEGylated liposomes show many positive properties over conventional liposomes: they have longer effects in the body, better accumulation in cells, reduced toxicity to healthy tissue, and increased stability. They also have some disadvantages: they reduce cellular uptake, they induce the so-called accelerated blood clearance (ABC), they can lead to pseudo-allergies, and they are unevenly distributed in tissues. These challenges indicate that further research and optimization is required to improve the safety and efficacy of PEGylated liposomes.
Despite many years of research into the properties of liposomes, results on their cytotoxicity remain limited. There is a lack of comparison in the literature of the effects of conventional and PEGylated liposomes **on normal cells** of the gastrointestinal tract. The use of such carriers in food requires very detailed studies.

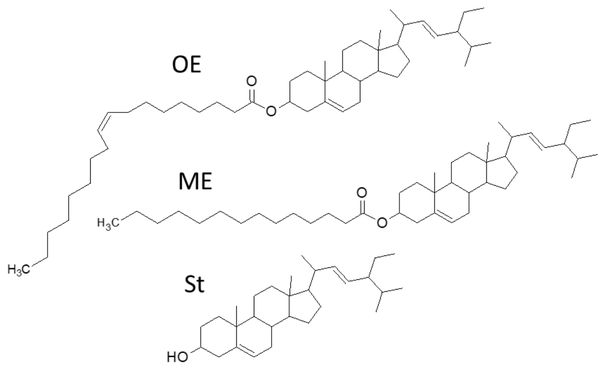

Figure 1 Chemical structure of stigmasterol (St), stigmasteryl myristate (ME) and stigmasteryl oleate (OE)

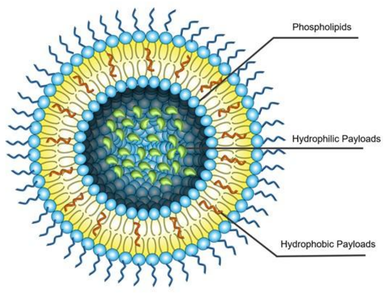

Figure 2 Structure of liposome

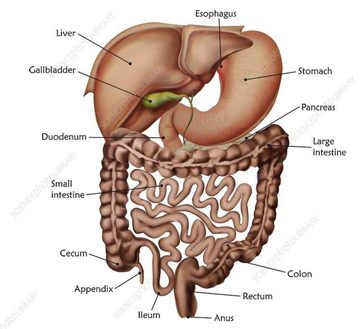

Figure 3 Gastrointestinal tract)

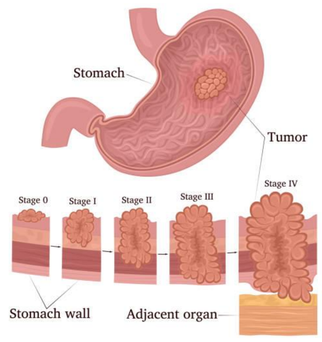

Figure 4 Stages of stomach cancer

Aim: The aim of this work is to investigate the effect of in vitro digestion on the cytotoxicity of liposomes containing stigmasterol and its esters with myristic and oleic acids. Two types of liposomes (conventional and PEGylated) and three types of normal gastrointestinal cells (hepatic, small intestine, and colon) were used.
Method: Chemical esterification was used to obtain the stigmasteryl esters with myristic and oleic acids. The standard of stigmasterol and its esters were encapsulated in liposomes. The cytotoxicity of conventional and PEGylated liposomes encapsulated with stigmasterol and its esters before and after their gastrointestinal digestion (both gastric and intestine stages) was assessed using human normal small intestinal HIEC-6 (ATCC CRL-3266) and colon mucosa CCD 841CoN (ATCC CRL-179) cells obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell lines were cultured under the conditions recommended by ATCC. In the cytotoxicity experiments, the cells were grown in 96-well plates at an initial density of 1.5 × 104 cells/cm^2. After 24 h, cell cultures were treated with liposomes encapsulated with stigmasterol and its esters for 48 h under standard culture conditions. Cell viability and metabolic activity were assessed using the MTT test following the procedure described.

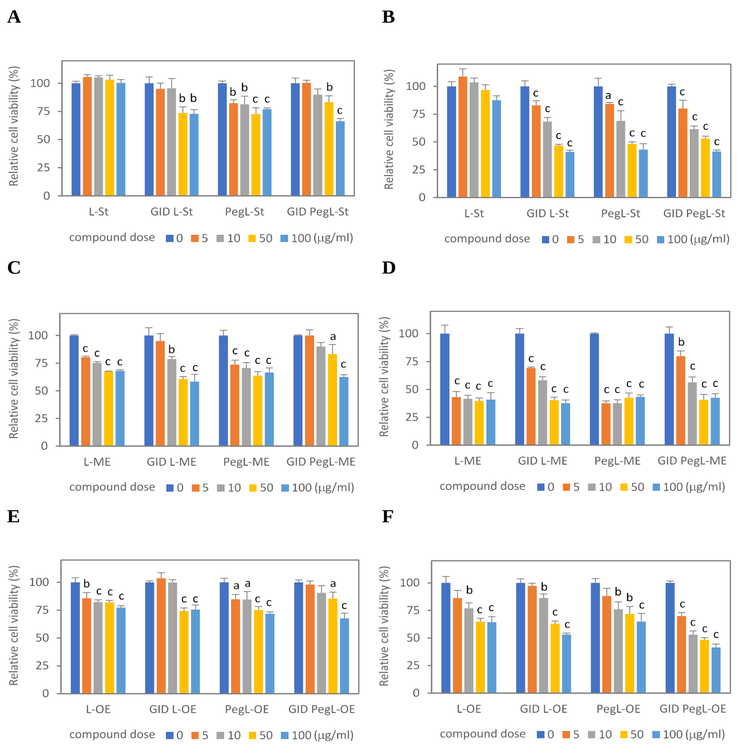

Figure 5 Cytotoxicity of conventional liposomes (L) and PEGylated liposomes (PegL) encapsulated with free stigmasterol (St) (A, B), stigmasterol mirystate (ME) (C, D), and stigmasterol oleate (OE) (E, F), both undigested and gastrointestinally digested (GID), to normal human small intestinal cells (HIEC-6 cells) (A, C, E) and colon mucosa cells (CCD 841CoN cells) (B, D, F). Values represent the means (n = 3) ± SD. The post hoc Tukey's post hoc test determined the significance of cytotoxic effects of liposome concentrations of 5, 10, 50, and 100 μg/ml (ap ≤ 0.05; bp ≤ 0.01; cp ≤ 0.001).

Results: The cytotoxicity of conventional liposomes can be ranked in the following order L-St < L-OE < L-ME, but the PEGylation of L-St significantly enhanced their cytotoxic activity. In contrast, PEGylation of liposomes encapsulated with stigmasterol esters (PegL-ME and PegL-OE) did not affect their cytotoxic potential.

Gastrointestinal digestion increased the cytotoxicity of L-St. In contrast, the cytotoxic potential of GID liposomes encapsulated with stigmasterol esters (GID L-ME and GID L-OE) was lower than that of undigested liposomes (L-ME and L-OE). The cytotoxicity to small intestinal cells was as follows: L-St = L-OE < L-ME, while the cytotoxicity to colon mucosal cells was slightly different: L-OE < L-St < GID L-ME. PEGylation of liposomes did not cause an increase in cytotoxicity to the small intestinal cells or colon mucosa cells. However, it should be highlighted that the cytotoxic effects of GID PEGylated liposomes were considerably higher in colon CCD 841CoN cells than in small intestinal HIEC-6 cells.

The use or nonuse of additional substances such as polyethylene glycol (PEG) in liposomes and the esterification of stigmasterol with saturated or unsaturated fatty acids both have significant impact on the stability of the encapsulated substances during gastrointestinal digestion and their cytotoxicity on cells in different parts of digestion tract.

The use of liposomes as carriers of plant sterols in foods still requires much research, including on their bioaccessibility and bioavailability.

Table: First cytotoxic doses (IC_{10}) of conventional liposomes (L) and PEGylated liposomes (PegL) encapsulated with free stigmasterol (St), stigmasterol mirystate (ME), and stigmasterol oleate (OE), both undigested and gastrointestinally digested (GID), determined in normal human small intestinal HIEC-6 and colon mucosa CCD 841CoN cell cultures.

Liposomes	Cytotoxic dose (IC_{10}) (μg/ml)	
	HIEC-6 cells	CCD 841CoN cells
L-St	>100	95.00 ± 10.77
GID L-St	25.40 ± 3.94***	3.02 ± 0.48***
PegL-St	3.01 ± 0.43###	3.38 ± 0.79###
GID PegL-St	9.28 ± 0.89***	2.80 ± 1.13
L-ME	1.06 ± 0.13	0.31 ± 0.05
GID L-ME	6.30 ± 1.38**	1.57 ± 0.52**
PegL-ME	0.82 ± 0.10	0.22 ± 0.03
GID PegL-ME	13.21 ± 4.97**	2.42 ± 0.47*
L-OE	2.51 ± 0.11	3.84 ± 0.59
GID L-OE	25.39 ± 3.12***	8.40 ± 1.42**
PegL-OE	3.56 ± 1.11	4.07 ± 0.55
GID PegL-OE	14.26 ± 6.66**	1.66 ± 0.21*

Values represent the means (n = 3) ± SD. Significant differences: *digested liposomes compared to undigested liposomes (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001); #PEGylated liposomes compared to non-PEGylated liposomes (#p ≤ 0.05; ##p ≤ 0.01; ###p ≤ 0.001).

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