BETA CASEIN NANOVEHICLES FOR TARGETED ORAL DRUG DELIVERY – TOWARDS TREATMENT OF GASTRIC CANCER

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Introduction

Beta-casein (β-CN), one of the four main caseins in bovine milk, has a pronounced amphiphilic structure (1), which enables it to self-associate under physiological conditions thereby forming stable micelle-like structures in aqueous solutions (2). Studies have shown the binding of lipophilic molecules, e.g. vitamin D3 (3) and vitamin A (4) to β-CN, suggesting that hydrophobic interactions are mainly responsible for the binding. Many antitumor agents are also hydrophobic, including *Vinca* alkaloids (e.g. vinblastine and vincristine) (5), epipodophyllotoxins (etoposide and teniposide) and taxenes (paclitaxel and docetaxel) (6). Many currently used chemotherapeutics are given intravenously (IV). The availability of suitable and effective oral therapeutic agents would make a significant contribution to patients' quality of life, may significantly reduce cost and may prove to be more effective than current modalities. Since caseins evolved to be easily digestible, β-CN nanocapsules are expected to readily release their cargo in the stomach, thus forming a target-activated release mechanism.

The major goal of the current research was to develop a rationally-designed drug delivery platform based on β-CN - nanoparticles. This drug delivery system would allow lipid-soluble drugs to be thermodynamically stable in aqueous solutions and to be orally delivered to the stomach for the treatment of gastric carcinoma, one of the leading causes of death among human malignancies Worldwide.

Experimental

Materials

Paclitaxel (Taxol) (T7191, purity >97%), vinblastine sulfate (V1377, purity >97%), β–CN from bovine milk (C6905, 90% purity), and pepsin from porcine gastric mucosa (P6887, 3260 unit/ mg protein, 0.92 mg protein/ mg solids) were purchased from Sigma-Aldrich Israel Ltd. (Rehovot, Israel). Irinotecan hydrochloride trihydrate and docetaxel trihydrate were purchased from Iffect Chemphar Co., Ltd. (Shenzehen, China). Cell proliferation kit (based on 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbox-anilide (XTT)) was from Biological Industries Ltd., Beth-Haemek, Israel.

Methods

Entrapment of five antitumor drugs (mitoxantrone, vinblastine, irinotecan, paclitaxel, docetaxel) in β-CN was performed by adding a drug-in-DMSO solution into a phosphate-buffered saline (PBS) containing β-CN while stirring. The association of drugs with β-CN was characterized by spectrophotometry and by Trp143 fluorescence quenching. Beta-CN-taxol nanovehicles were studied by cryogenic transmission electron microscopy (cryo-TEM). Simulated gastric digestion of taxol-loaded β-CN- nanoparticles was performed by continuously shaking for two hours at pH=2 and 37°C using the gastric protease, pepsin. The cytotoxic activity of encapsulated taxol at 1 mg/ml β-CN at a 6:1 taxol: β-CN molar ratio with and without a preceding optimal simulated gastric digestion (50:1 β-CN: pepsin w/w ratio, shaking at 37°C for 20 min) was compared to the cytotoxic activity of untreated taxol on human N-87 gastric cancer cells.

Results and Discussion

The maximal capacity and association constants of mitoxantrone, vinblastine, and taxol to β-CN were studied by performing a model fit to results of fluorescence quenching of Trp143 of β-CN, as presented in Figure 1. The optimal drug-to-β-CN molar loading-ratios for taxol and vinblastine at 1mg/ml β-CN were found to be

7.3±1.2 and 5.3±0.6 and the association constants were $(6.3\pm1.0)\cdot10^{3}M^{-1}$ and $(2.0\pm0.3)\cdot10^{4}M^{-1}$, respectively. Figure 2 presents a transmission electron micrograph of taxol-β-CN nanoparticles. It revealed that the drug formed nano-crystals to which β-CN is adsorbed, in addition to smaller micellar nanoparticles. Simulated gastric digestion was performed on taxol- β-CN nanoparticles. The cytotoxicity of encapsulated and digested taxol was compared to the cytotoxicity of the undigested encapsulated drug and to untreated free drugs on N-87 gastric carcinoma cell line and is presented in Figure 3. Following encapsulation and simulated digestion, taxol retained its cytotoxic activity. Taxol concentration required to inhibit cell growth by 50% (IC_{50}) following simulated digestion was similar (32.5±6.2) to that of unencapsulated taxol (25.4±2.6). Undigested β-CN-taxol nanoparticles did not show any cytotoxic effect on N-87 gastric cancer cells, suggesting the encapsulation may provide protection to mouth and esophagus during oral administration.

Our results suggest that Beta-CN is able to protect the upper GIT regions from the encapsulated taxol, and to achieve target-activated release of taxol in the stomach, hence it may serve as effective oral drug delivery vehicle for taxol and other drugs for treating malignant and non-malignant gastric disorders.

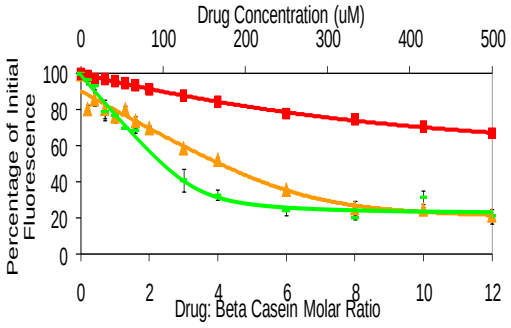

Figure 1 – Fluorescence quenching of β-CN's Trp143 Taxol (■), vinblastine (▲), and mitoxantrone (♦) entrapped in 1mg/ml β-CN (excitation: 287 nm, emission: 332 nm)

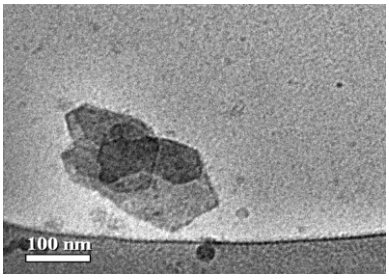

Figure 2 – TEM image of β-CN-taxol nanoparticles in PBS containing 2.5% DMSO (1mg/ml β-CN and 250μM taxol, 6:1 taxol:β-CN molar ratio)

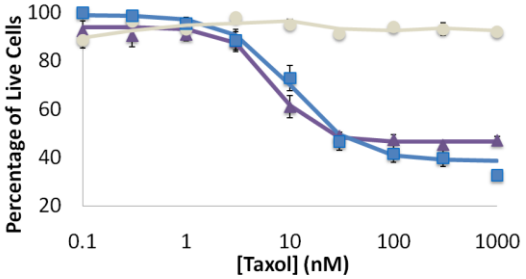

Figure 3 - Percentage of live cells as a function of taxol concentration: taxol - β-CN nanoparticles following simulated digestion▲, vs. undigested taxol - β-CN nanoparticles ■, and vs. untreated taxol ● (lines represent the model fit).

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