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Magnetic Solid Lipid Nanoparticles Loading 5-Fluorouracil to Colon Cancer Treatment

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Abstract: Magnetic solid lipid nanoparticles (MSLNs) made of iron oxide cores embedded within a glyceryltrimyristate solid matrix have demonstrated hem compatibility, appropriate magnetic response and adequate heating characteristics to be used in hyperthermia. We now tested the association of MSLNs to 5-fluorouracil (5-FU), a drug of chose in colon cancer treatment, to determine the ant proliferative effect modulation. We used HCT15 resistant and T-84colon cancer cells. The non-magnetic solid lipid nanoparticles (SLNs) were included as a control. In addition, we analyze the effect of both MSLNs and SLN in relation to the P-glycoprotein activity, a resistance mechanism implicated in colon cancer chemotherapy failure. Our results showed that both MSLNP and SLNP did not induce cell toxicity and that both NPs loading 5-FU (SLNP-5-FU and MSLNP-5-FU) did not modulate free 5-FU activity against T-84 and HCT15 cells. In addition, fluorescent microscopy analysis showed that none of the NPs was able to decrease rhodamine 123 into tumor cells indicating absence of activity in relation to P-glycoprotein pump. In conclusion, MSLNs could be used as an anticancer therapeutic system not only by their demonstrated hyperthermic properties but as a 5-FU transporter. Future studies will be necessary to know the applicability of this dual system (hyperthermia/drug) against colon cancer.
Keywords: Colon cancer, magnetic nanoparticles, 5-fluorouracil, cytotoxicity.

INTRODUCTION

Colorectal cancer is a major cause of morbidity and mortality throughout the world and represents for over 9% of all cancer incidence. In fact, colon cancer is the third most common cancer worldwide and the fourth most common cause of death [1]. Detection in early stages is associated to a good prognosis but 25% of colon cancer patients develop metastasis. Despite new advances in cancer colon treatment, these patients have a poor response to chemotherapy with a 5-year survival rate of 10% [2] 5-Fluorouracil (5-FU), alone or in association with others drugs such as irinotecan, oxiplatin, epidermal growth factor receptor inhibitors and angiogenesis inhibitors, continues in the first line of the antitumor molecules against colon cancer. However, its low accumulation in tumor tissues, systemic toxicity, and rapid drug metabolizationand drug resistance may be responsible to the treatment failure [3-5]. Nanoparticles (NPs) loaded 5-FU can help to solve drug limitations and thus improve colon cancer therapeutic results. In fact, Nano particulate systems can increase intratumor presence of the drug and its half-life and reduce drug toxicity [6]. In addition, Nano particulate systems may overcome drug resistance increasing the drug concentration in tumor cell [7]. Furthermore, NP surface functionalization with targeting molecules may increase tumor-selective of the drug [8]. The aim of this work is to incorporate the drug 5-FU to magnetic solid lipid nanoparticles (MSLNs) containing magnetite (γ-Fe_2O_3) [9] and to analyze the possible interference of the NPs-drug complex in drug activity against colon cancer cells. In addition we explore the possibility that MSLNs may interfere drug resistance molecules such as P-glycoprotein which are implicated in resistance of colon cancer and poor prognosis of these patients.

MATERIALS AND METHODS

Magnetic nanoparticles Solid lipid nanoparticles (SLNs) and magnetic solid lipid nanoparticles (MSLNs) containing maghemite (γ-Fe_2O_3) previously synthetized were used in the study [9]. Nanoparticles were loading with 5-FU following our protocol [10] to obtain SLNP-5-FU and MSLNP-5-FU. In addition, NPs associated to

rhodamine was synthetized (SLNP-rho, SLNP-5-FU-rho, MSLNP-rho, and MSLNP-5-FU-rho) following Zhang *et al*. to uptake studies [11].

In vitro cytotoxic studies Human colon cancer cell lines T84 and HCT-15 obtained from American Type Culture Collection (ATCC, USA) was used to determine the growth inhibition percentage (GI, %) by the sulforhodamine B (SRB) method. Cells were seeded in 24 well plates at a density of 8×10^3 cells/well for T84 cell line and 1×10^4 cells/well for HCT-15 in 400 µL per well of Dulbecco´s modified Eagle´s medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and 10% fetal bovine serum (Gibco®, Life Technologies, Madrid, Spain) and maintained in cell culture conditions of 37ºC of temperature and humidified atmosphere with 5% of CO_2. After 24 hr of incubation different treatments were added to cells: SLNP, SLNP-5-FU, MSLNP, MSLNP-5-FU and 5-FU, in increasing concentrations of drug ranging from 0.05 to 20 µM, for two exposure times of 72 and 120 hrs. After this time during 20 min at 4ºC, cells were fixed with 300 µL of 10 % trichloroacetich acid. Then, 300 µL of a solution of 0.4% SRB were added to cells for 20 min and after this, cells were washed three times with 1% acetic acid. A solution of 10 mM and pH 10.5 of Trizma® was added to cells stained with SRB, and it is resuspended to be measured in a Titertekmultiscan colorimeter (Flow, Irvine, California) at 492 nm. All the above reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The measured optical density was used to calculate the percentage of cellular growth (CG, %) and growth inhibition (GI, %):

Nanoparticle's cellular uptake study Tumoral cells T84 and HCT-15 were harvested in 6-well plates at a density per well of 1×10^5 cells in 2 mL of supplemented DMEM. After 24 hrs of incubation, cells were treated with free rhodamine 123 and loaded to NPs (SLNP-rho, SLNP-5-FU-rho, MSLNP-rho, MSLNP-5-FU-rho), and blank NPs as control. These treatments were applied at a rhodamine 123 dose of 1.3 µM for 0.5, 1, 2, and 4 hrs. HCT-15 cell line was pretreated for 1 hr with 14.3 µM verapamil hydrochloride (Sigma-Aldrich, St. Louis, MO, USA), to avoid expulsion of rhodamine 123 by P-glycoprotein, a transmembrane transporter involved in cell drug resistance which is over-expressed in this cell line [12]. Then, cells were washed three times with PBS, and observed by fluorescent microscopy in a Leica DM IL LED (Leica Microsystems S.L.U., Barcelona, Spain).

RESULTS AND DISCUSSION

In vitro cytotoxicity assay Blank SLNP and MSLNP show no toxicity in most of the concentrations tested. Only at the higher concentrations (10 µM) both T-84 and HCT15 cell lines showed some citotoxicity at 72 hrs. This toxicity remained at 120 hrs in HCT-15 (Fig. A). In addition, both SLNP-5-FU and MSLNP-5-FU did not affect free drug toxicity founding a similar percentage of cell death in all exposure times (Fig. A).

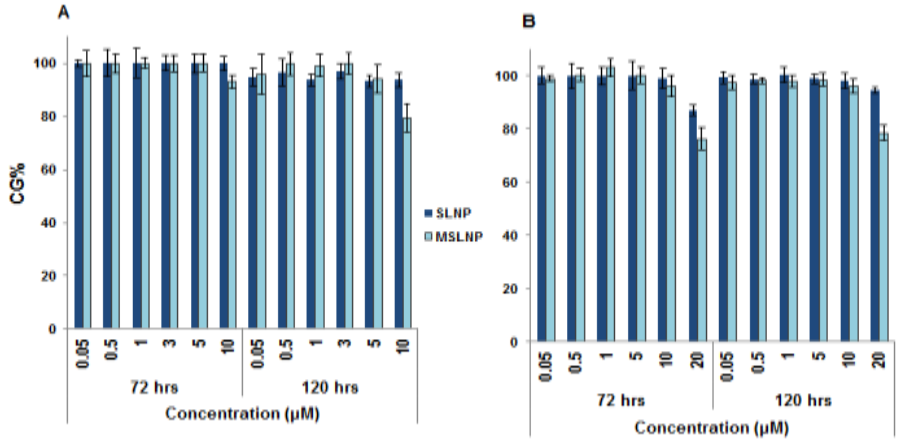

Fig-1: Cytotoxicity of blank NPs in T84 (A) and HCT-15 (B) human colon cancer cell lines. Results were expressed as percentage of growth inhibition (GI, %). Concentrations were set in relation to the drug (see Fig.2). The data represents the mean value ± SD of quadruplicate cultures

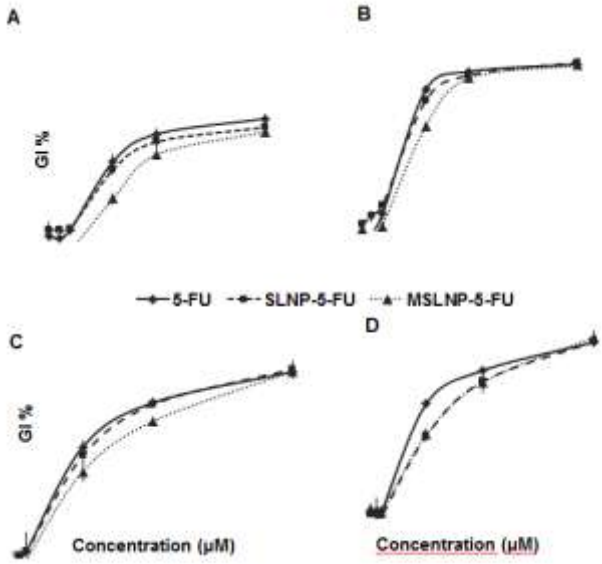

Fig-2: Cytotoxicity of 5-FU-loaded SLNP and MSLNP compared to 5-FU in T84 and HCT-15 cell lines after 72 hrs (A,C) and 120 hrs (B,D) of exposition. Results were expressed as percentage of growth inhibition (GI, %). The data represents the mean value ± SD of quadruplicate cultures

Uptake studies

Fluorescent microscopy images demonstrated that rhodamine 123 alone and rhodamine loading NPs (SLNP-rho, SLNP-5-FU-rho, MSLNP-rho, and MSLNP-5-FU-rho) induce a similar intensity indicating that our NP did not decrease the presence of drug into the tumor cells (Fig. 3 and 4). Moreover, SLNP-5-FU-rho and MSLNP-5-FU-rho did not induce any change of intensity (data not shown). Finally, the distribution pattern within the cell of rhodamine 123 loaded to NPs appears to be similar to the rhodamine alone in both HCT-15 and T-84 cells. This pattern is predominantly cytoplasmic, with no clear accumulation observed in any particular area (Fig. 3 and 4).

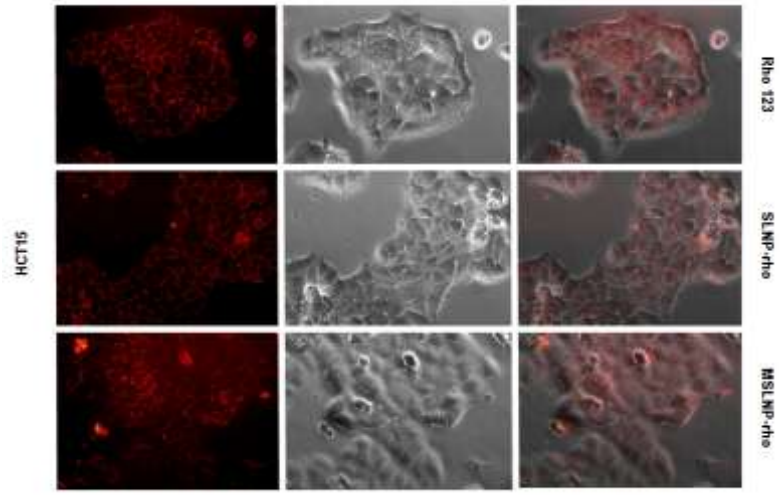

Fig-3: Representative fluorescent microscopy images of HCT15 cells exposed to magnetic and non-magnetic NPs loaded with rhodamine. HCT15 cells were treated for 4 hrs with rhodamine (rho) 123, SLNP-rho and MSLNP-rho. Images were taken at the magnification of 10×. Rhodamine (left), optical microscopy images of HCT15 cells (center) and merge (right)

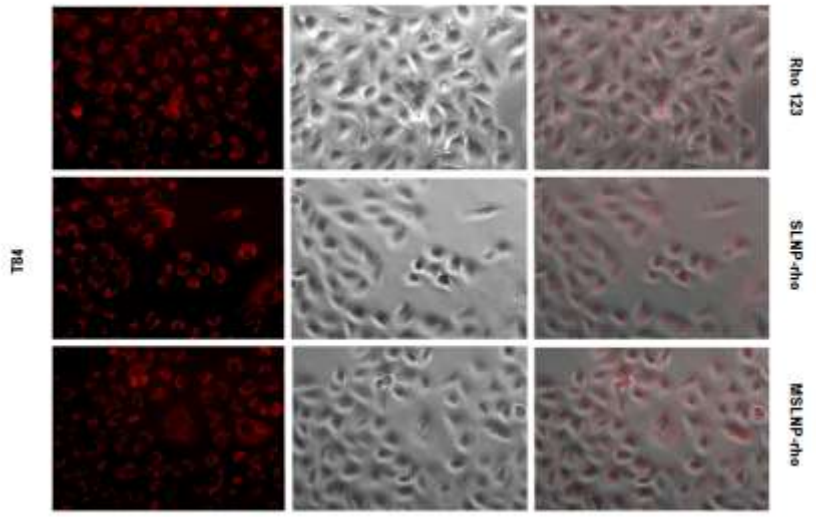

Fig-4: Representative fluorescent microscopy images of T84 cells exposed to magnetic and non-magnetic NPs loaded with rhodamine. T84 cells were treated for 4 hrs with rhodamine (rho) 123, SLNP-rho and MSLNP-rho. Images were taken at the magnification of 10×. Rhodamine (left), optical microscopy images of HCT15 cells (center) and merge (right)

SLNPs and MSLNPs were a biocompatible system which did not show toxicity in the cell lines assayed. In addition, both magnetic and non-magnetic 5-FU-loaded SLNPs did not decrease the toxicity of 5-FU and caused a concentration-dependent increase in the percentage of growth inhibition similar to those previously observed by Patel *et al.* [13] in the Caco-2 human colon cancer cell line exposed to 5-Fu-loaded SLNPs. These results indicate that the magnetic characteristics of our NPs system may be used to the treatment of the colon cancer treatment (thermotherapy) [9] associated to the cytotoxic drug activity and to localize tumor tissue through a system to recognize the magnetic material (i.e. MRI). On the other hand, SLNPs and MSLNPs did not affect the incorporation of the rhodamine into the cell, although their intracellular distribution was the same. It could be due to the physicochemical properties of SLNPs, as size, shape and surface charge which may change the cellular uptake efficiency. In fact, the surface charge is the one with the greatest impact on cellular uptake [14]. NPs with a positive surface charge enter more easily within cell due to the negative charge of cell membrane, compared to NPs with negative or neutral surface charge as in our case. Other authors have shown that the amount of NPS inside cells can be increased by adding a ligand to the surface of the SLNPs, such as ferritin, which increases their cell uptake in comparison with blank SLNPs in the breast cancer cell line MDA-MB-468 with ferritin receptors overexpressed [15]. In this context, the incorporation to our NPs of a colon cancer ligand could improve the applicability of our system.

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