Targeted Delivery of Anti-Cancer Drug Sorafenib through Magnetic Solid Lipid Nanoparticles

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ABSTRACT

Sorafenib is an anticancer drug approved by the Food and Drug Administration for the treatment of hepatocellular carcinoma and advanced renal carcinoma. The clinical application of sorafenib is promising, yet limited by its insolubility and severe toxic side-effects. The aim of this study is to develop and characterize sorafenib-loaded magnetic nanovectors to deliver and accumulate drug just to the disease site with the help of a remote magnetic field. Sorafenib and superparamagnetic iron oxide nanoparticles were encapsulated in solid lipid nanoparticles (SLNs) by a hot homogenization technique, by using cetyl palmitate as lipid matrix. Biological effects were evaluated *in vitro* on human hepatocarcinoma HepG2. Our results confirm the possibility to prepare stable SLNs able to kill cancer cells through sorafenib cytotoxic effect, and to enhance/localize this effect in a desired area thanks to the magnetically-driven accumulation of the drug.

Keywords: solid lipid nanoparticles, magnetic nanoparticles, sorafenib, HepG2

1 INTRODUCTION

The multi-kinase inhibitor (MKI) sorafenib (tradename Nexavar®, Bayer) has been recently approved by the FDA for the treatment of non-resectable hepatocarcinoma and advanced renal carcinoma (HCC) [1]. Preclinical studies show that sorafenib acts through several mechanisms to inhibit tumor angiogenesis and to induce tumor cell apoptosis [2]. Despite its proven survival benefit, sorafenib can lead to important side effects including hand and foot syndrome, diarrhea, and hypertension [3]. The aim of this study is the development of a magnetic nanovector able to efficiently and selectively deliver sorafenib toward cancer lesions thanks to a physical guidance mediated by magnetic nanoparticles.

The proposed system can selectively deliver sorafenib, by concentrating the drug in correspondence of a target site. Moreover, its use can increase the efficacy of the therapy avoiding the before mentioned side effects, such as drug aspecific biodistribution, which could expose healthy tissues to the drug action. The overcoming of this latter drawback could be one of the most important improvement

brought by our system, since lack of specificity in cell recognition represents the bottleneck of several traditional chemotherapy approaches. Solid lipid nanoparticles (SLNs) have been chosen to selectively target sorafenib. This nanovector is composed by a solid lipid matrix dispersed in aqueous solution and stabilized by surfactant, and incorporates the lipophilic drug inside its core. SLNs combine several benefits compared to other types of carriers. First of all, they ensure a controlled and sustained release of the drug by protecting the active molecule from degradation and metabolic inactivation thanks to the hard lipid matrix. Moreover, they allow a high loading of the drug, jointly to a low cellular and systemic toxicity [4].

Cetyl palmitate, an ester manufactured from raw materials of natural origin and used in several cosmetic formulations, has been chosen as the main component of SLNs core, because of its high biocompatibility [5].

To localize sorafenib action, magnetic targeting is ensured by encapsulation of superparamagnetic iron oxide nanoparticles (SPIONs) [6], that assure magnetic momentum and drivability to the lipid carriers.

2 MATERIALS AND METHODS

2.1 SLN preparation and characterization

Solid lipid nanoparticles were prepared by using an oil-in-water homogenization process at high temperature. In details, 5 mg of sorafenib tosylate (kindly provided by Bayer International) were dissolved in methanol at 65°C. 210 mg of cetyl palmitate (kindly provided by Gattefossé Italy) and 15 mg of SPIONs (average diameter of 10 nm; EMG1300 from Ferrotec) were dissolved in chloroform and thereafter mixed with the drug solution and gently stirred. This chloroform/methanol (2:1) mixture was then added into a vial containing Tween 80 (Sigma Aldrich) water solution (6%), and sonicated for 5 min with a probe-tip ultrasonicator (SONOPLUS mini20, Bandelin). The temperature of the emulsion was kept constant at 75°C to allow organic solvents evaporation. At the end of the procedure, the obtained dispersion was maintained at 4°C for 15 min to allow the formation of the SLNs. The final product (Sor-Mag-SLNs) was then purified by gel chromatography by using Sephadex G-25 pre-packed columns (GE Healthcare Life Sciences).

Plain magnetic SLNs (Mag-SLNs), *i.e.*, not loaded with sorafenib, were prepared following the same procedure, but omitting the drug, and were used as controls in biological experiments.

Several features of the obtained nanovectors were evaluated, including size distribution, zeta potential, and morphology through atomic force microscopy (AFM).

2.2 Biological experiments

Cell viability was assessed using the crystal violet nuclear staining assay. HepG2 cells were seeded in 96-well culture plates at a density of 5000 cells *per* well for 24 h, and then treated with Mag-SLNs (0-100 μg/ml) or Sor-Mag-SLNs (0-100 μg/ml) for 72 h. At the end of the treatment, the media were removed, and the cells were washed with PBS and stained for 10 min with 0.5% crystal violet (Sigma) dissolved in methanol/water. Subsequently, the dye was solubilized with 33% acetic acid and the absorbance was read at 570 nm on a microplate reader. Triplicates for each concentration were analyzed.

In vitro testing to assess targeting and biological effects has been performed on the hepatocellular carcinoma cell line HepG2 thanks to a dynamic culturing system based on a microfluidic set-up (Figure 1).
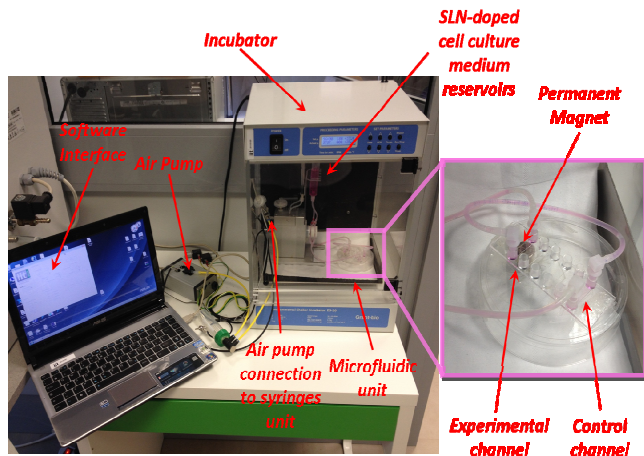

Figure 1: Photo of the set-up exploited for dynamic magnetic targeting experiments.

Accumulation of SLNs was obtained by means of an external permanent magnet (B_r = 1.32 T), and targeted internalization by cells was evaluated through confocal laser scanning microscopy (C2s, Nikon) by using fluorescent-labeled nanovectors.

Selective cytotoxic effects of Sor-Mag-SLNs (200 μg/ml) was investigated at 72 h after the targeted accumulation in the dynamic system with the Live/Dead® viability/cytotoxicity Kit (Molecular Probes), that allows live cells (stained in green by calcein) from death cells (stained in red by ethidium homodimer-1, EthD-1) to be discriminated.

3 RESULTS AND DISCUSSION

Results demonstrated the possibility to successfully fabricate stable magnetic SLNs encapsulating sorafenib. These nanovectors showed an average size of about 300 nm and a surface charge around -20 mV. AFM images of obtained drug-loaded SLNs are reported in Figure 2.
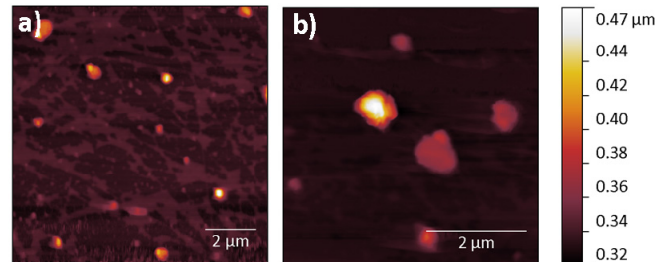

Figure 2: AFM topographical scans at low (a) and high (b) magnification of SLNs.

Antitumor activity of Sor-Mag-SLNs was investigated against HepG2 cells with the crystal violet proliferation assay and compared to that of the plain Mag-SLNs. As shown in Figure 3, Mag-SLNs did not significantly inhibit cell viability after 72 h of incubation (at all the investigated concentrations $p > 0.05$ with respect to the controls, ANOVA followed by Bonferroni *post-hoc* test). On the other hand, Sor-Mag-SLNs showed a strong cytotoxic effect at concentrations of 10, 25, 50 and 100 μg/ml, with a significant reduction of viability ($p < 0.05$), that reaches about 80% of the control for the 100 μg/ml Sor-Mag-SLN concentration (corresponding to about 3.20 μM of drug).
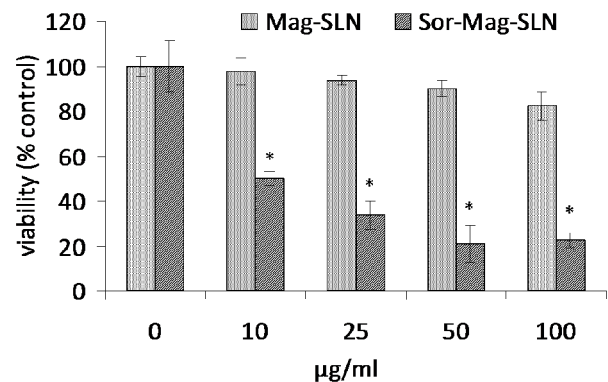

Figure 3: Crystal violet viability assay for increasing conentrations of Mag-SLNs and Sor-Mag-SLNs.

Magnetic targeting experiments were performed in dynamic conditions, and obtained results showed an enhanced Sor-Mag-SLN up-take by cells cultured in correspondence of the region of the microfluidic channel where the external permanent magnet was placed (Figure 4). Conversely, no significant nanoparticle internalization

could be appreciated in cells cultured in the control channel, thus demonstrating the possibility to actively targeting the nanoparticle up-take and, consequently to selectively localize the effect of the drug.
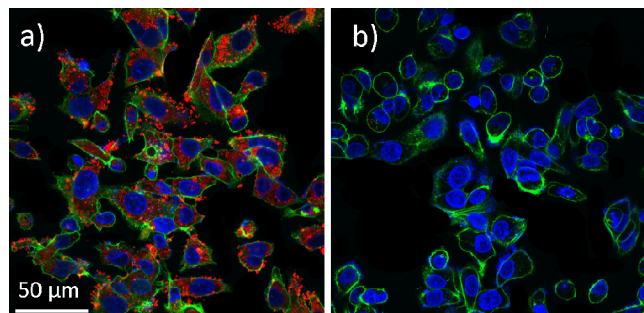

Figure 4: Magnetic targeting experiments show enhanced SLN internalization in correspondence of the region of the microfluidic channel where the external magnet was placed (a) with respect to the control channel (b). SLNs in red; f-actin in green; nuclei in blue.

The possibility of magnetically targeting the effects was demonstrated by the testing in dynamic conditions of the drug-loaded SLN efficiency, evaluated through the Live/Dead® viability/cytotoxicity assay after 72 h of incubation after the treatment. Quantitative evaluation of cell mortality, evaluated as percentage of cells positive for EthD-1, resulted of about 95% in the channel close to the magnet, while about 5 % in the control compartment.

Summarizing, in this work we have reported on a novel approach to actively target sorafenib own to its encapsulation in magnetic solid lipidic nanoparticles, prepared through a hot oil-in-water homogenization process. In particular, we focused on the problems related to the clinical use of sorafenib, related to the difficulty of localizing the drug effect, its poor solubility in aqueous environment, and its high toxicity for healthy tissues and organs. In this scenario, encapsulation of the drug into targetable nano-carriers could represent an advantageous approach to reduce undesired side-effects and to improve drug pharmacokinetics [7-9].

4 CONCLUSION

Our results confirm the possibility to prepare stable SLNs able to destroy HepG2 cancer cells through sorafenib cytotoxic effect, and to enhance this effect in a desired area thanks to the magnetically-driven accumulation of the drug.

Sorafenib-loaded magnetic solid lipid nanoparticles represent a good example of nanodevice in the field of oncology. Their potential dual function, as both contrast agent (data not shown) and therapeutic agent, make them a potential theranostic device for the treatment of cancer, and future experiments will be performed to extend analysis of the proposed nanoplatform *in vivo*.

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