Inhibition of ABCB1 (MDR1) Expression
by an siRNA Nanoparticulate Delivery System to Overcome Drug Resistance in Osteosarcoma

INTRODUCTION:

The use of neo-adjuvant chemotherapy in treating osteosarcoma has improved patients' average 5 year survival rate from 20% to 70% in the past 30 years. However, for patients who progress after chemotherapy, its effectiveness diminishes due to the emergence of multi-drug resistance (MDR) after prolonged therapy. In order to overcome both the dose-limiting side effects of conventional chemotherapeutic agents and the therapeutic failure resulting from MDR, we designed and evaluated a novel drug delivery system for MDR1 siRNA delivery. Novel biocompatible, lipid-modified dextran-based polymeric nanoparticles were used as the platform for MDR1 siRNA delivery; and the efficacy of combination therapy with this system was evaluated.

MATERIALS AND METHODS:

Preparation of MDR1 siRNA-containing nanoparticles

To a solution of MDR1 siRNA, 40μl of dextran-stearyl amine, 40μl dextran-thiol derivative, and 40μl of PEG-SH were added and incubated to form the hydrophilic shell of the nanoparticles (Fig 1).

Cell culture

Human osteosarcoma cell lines KHOS, U-2OS and the multidrug resistant cell lines KHOSR2, U-2OSR2 were cultured in RPMI1640 with 10% fetal bovine serum.

Assessment of MDR1 reversal

KHOSR2 and U2OSR2 treated with various concentrations of MDR1 siRNA nanoparticles were analyzed for P-gp expression. For comparison of the duration of MDR1 siRNA inhibition by either MDR1 siRNA alone or MDR1 siRNA loaded nanoparticles, 1×10^5 KHOSR2 cells/well were incubated with 100nM MDR1 siRNA alone and in nanoparticle form for 5 days. The expression of P-gp was determined by western blot analysis.

Fluorescence microscopy of cellular doxorubicin uptake

KHOS and KHOSR2 cells were seeded at densities of 5×10^4 cells / well in 6 well plates. MDR1 siRNA was applied and incubated for 48 h. Following the incubation, doxorubicin was added to each well and was incubated for additional 3 hours. The cells were visualized on a Nikon Eclipse Ti-U fluorescence microscope (Nikon Corp.).

In vitro cytotoxicity assay

In vitro cytotoxicity assays were performed by MTT assay. Briefly, 3 ×10^3 cells per well were plated in a 96-well plate. After 48 h of incubation with MDR1 siRNA-loaded nanoparticles or with medium alone, increasing concentrations of doxorubicin were applied. After 5 days, 10 μl of MTT (5mg/ml in PBS) was added to each well and incubated for 3 hr. The absorbance (A490) was read on a SPECTRA max Microplate Spectrophotometer (Molecular Devices).

RESULTS:

Stable suppression of P-gp

Western blotting was performed to estimate the effect of MDR1 siRNA loaded nanoparticle on P-gp expression. P-gp expression has been previously confirmed in the two drug resistant cell lines KHOSR2 and U-2OSR2. MDR1 siRNA loaded nanoparticle inhibited the expression of P-gp at a concentration of as low as 30 nM. Naked siRNA was able to suppress P-gp expression for 48 hours. siRNA loaded nanoparticles were slower in achieving the suppression of P-gp, but were able to maintain suppression for 96 hours (Fig 2).

Enhancement of intracellular doxorubicin accumulation

After a 3 hour incubation period with free doxorubicin in drug resistant osteosarcoma cells, the drug was primarily concentrated in the cytoplasm with a very low level of fluorescence observed in the nucleus (Fig 3A). When doxorubicin was administered after treatment with MDR1 siRNA loaded nanoparticle to drug resistant cell lines, an increase in fluorescence was observed in the nucleus and cytoplasm (Fig 3B). This subcellular distribution mimicked that of the drug sensitive variant when treated with doxorubicin (Fig 3C).

DISCUSSION:

The primary limitation for the successful treatment of osteosarcoma is the development of MDR against various chemotherapeutic agents. The objective of this study was to investigate the ability of MDR1 siRNA loaded dextran based nanoparticles to overcome P-gp mediated drug resistance in osteosarcoma. In this study, we showed that stable suppression of P-gp could be achieved using this system and that increased cytotoxicity of the nanoparticulate complex was due to increased accumulation of doxorubicin in the nucleus, emulating the behavior of doxorubicin treatment alone in drug sensitive cells. In conclusion, lipid-modified dextran-based polymeric nanoparticles are a promising platform for siRNA delivery. Nanocarriers loaded with MDR1 siRNA are a potential treatment strategy for reversing MDR in osteosarcoma.