Construction of Hyaluronic Acid-Coated Cisplatin-Loaded Hydrogel Nanoparticles for Targeted Chemotherapy.

Teppei Shirakura\textsuperscript{1,2}, Christof Smith\textsuperscript{2}, Yong-Eun Koo Lee\textsuperscript{2}, and Raoul Kopelman\textsuperscript{1,2}

\textsuperscript{1}Biophysics and \textsuperscript{2}Chemistry, The University of Michigan, 930 N. University Avenue, Ann Arbor, MI 48109
tshira@umich.edu

ABSTRACT SUMMARY

Hyaluronic acid coated, cisplatin-loaded polyacrylamide-based nanoparticles were constructed for targeted \textit{in vivo} chemotherapy. The cisplatin release profile was tuned to prevent side-effects. The surface coating of the nanoparticles by hyaluronic acid helped in actively targeting cancer cells.

INTRODUCTION

Chemotherapy of cancer is a non-invasive and powerful treatment, which is widely used in cancer treatment. On the other hand, it is known that many types of cancer have resistance against chemotherapy. In order to overcome the resistance, it is necessary to increase the dose of chemodrugs. However, such a higher global dose of chemodrugs causes side effects.\textsuperscript{1} Therefore, the targeted delivery of chemodrugs to the tumor is necessary so as to overcome the drug resistance, and avoiding the side effects at the same time, by increasing only the \textit{local} drug concentration.

A targeted delivery of chemodrugs to cancer tumors via targeted nanoparticles can achieve a locally high dose of chemodrugs in the tumor. Our group has invented various types of targeted and multifunctional “stealth” (PEG cloaked) polyacrylamide-based nanoparticles (PAA-NPs) for cancer diagnosis and therapy.\textsuperscript{2} PAA-NPs have been shown to be biocompatible both \textit{in vitro} and \textit{in vivo}. In addition, PAA-NPs are chemically highly engineerable by changing the type of acrylic monomers that are co-polymerized with acrylamide in the synthesis process.

In this work, we aimed to construct cisplatin-loaded PAA-NPs (Cis-NPs) that are coated with hyaluronic acid (HA) for the treatment of ovarian cancer. Cisplatin is one of the most widely used chemodrugs, and is especially used in breast, ovarian, bladder, lung, and in head and neck cancer.\textsuperscript{1} HA binds to CD44, which is strongly correlated with the survival rate of patients.\textsuperscript{3} We discuss the potential drug efficacy of Cis-NPs that are targeted with HA.

EXPERIMENTAL METHODS

PAA-NPs are prepared by the reverse-micelle microemulsion process. In the typical synthesis, 1.6 g of dioctylsulfosuccinate (AOT) and 3.3 g of Brij 30 were dissolved in 45 mL of argon-purged hexane. 0.711 g of acrylamide, 0.055 g of 3—aminopropyl methacrylamide, and 0.428 g of 3—(acryloyloxy)-2-hydroxypropylmethacrylate were dissolved in 1.3 mL of deionized water. Then, these two suspensions were mixed vigorously for 20 min under argon-purging. 100 µL of ammonium persulfate (10% w/v) and 100 µL of tetramethylethylenediamine were added to the mixture to initiate the polymerization while the system was purged with argon. 2 hours later, the hexane was removed from the system using a rotary evaporator, and the AOT and Brij 30 were removed by washing 5 times with 150 mL of ethanol and 5 times with 150 mL of water, using Amicon ultrafiltration cells with a 300 kDa molecular weight cutoff membrane. Cisplatin was loaded to the PAA-NPs either during the polymerization or after the water washing.

8.5 mg of HA was conjugated onto the surface of 10 mg of Cis-NPs in PBS (pH 7.4), using 6.3 mg of 1-Ethyl-3—(3-dimethylaminopropyl)carbodiimide as zero-length crosslinker.

Typical evaluation of the cisplatin release profile of Cis-NPs was conducted in the following way. 1 mg/mL Cis-NPs solution was prepared in PBS (pH 7.4), and placed in a 37°C
water bath. At specific time points, Cis-NPs were removed from 1 mL of the solution using a centrifugal filter with 100 kDa molecular weight cutoff membrane. The cisplatin concentrations in the filtrates were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES) with Optima 2000 DV (PerkinElmer).

Typically the targeting efficiency of HA was measured in vitro using cells with high expression of the targeted marker, or cells with low expression of the targeted marker, which were put on 96-well plates. The cells were mixed with fluorescently labeled PAA NPs whose surfaces were coated with HA. 4 hours later, the NPs were removed from the plate, and the fluorescence signals emanating from the wells were measured using Gemini SpectraMAX XS (Molecular Devices).

RESULTS AND DISCUSSION

The size of the synthesized Cis-NPs was measured to be 52.6 nm, determined by using dynamic light scattering with Delsa Nano C (Beckman Coulter). Cisplatin-content was measured to be 3.95 wt%, determined by using ICP-OES. Figure 1 is the typical result of the cisplatin-release analysis of the Cis-NPs. The NPs showed a slow release of cisplatin over 96 hours, which can avoid too fast release of cisplatin from Cis-NPs while circulating in the blood. Also, further adjustment of the release profile can be easily accomplished by changing the composition of the PAA-NPs.

![Figure 1. Cisplatin release profile from PAA-NPs in 96 hours in PBS (pH7.4).](image)

The synthesized HA-coated PAA-NPs showed a preferential binding to CD44\textsuperscript{high} cells over binding to CD44\textsuperscript{low} cells, as expected (See Figure 2).

![Figure 2. Cell-binding study of HA-coated PAA-NPs. HA-coated PAA-NPs (HA), or PAA-NPs without HA-coating (NP), were incubated with Hep3B, which is a CD44\textsuperscript{high} cell (3B), or with HepG2, which is a CD44\textsuperscript{low} cell (G2). Data for Hep3B and HepG2 were normalized independently.](image)

The cytotoxicity of the HA-coated Cis-NPs is under investigation.

CONCLUSIONS

Cisplatin was successfully loaded into PAA-NPs, and the nanoparticles were coated with hyaluronic acid. Cisplatin was released from PAA-NPs slowly enough so as to avoid unwanted toxicity, due to premature release. The cell-binding study showed that the hyaluronic acid-coated PAA-NPs had an ability to selectively deliver cisplatin to the cells with high expression of CD44. With the combination of targeting and controlled release, the constructed HA-coated Cis-NPs have the ability to treat cancer while reducing side effects, in contrast to what would occur under a globally high dose of cisplatin.

REFERENCES


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