Development of Temperature-Sensitive Hydrogel Nanoparticles for Targeted Chemotherapy

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ABSTRACT SUMMARY

Cisplatin-loaded poly(acrylic acid–co-acrylamide) nanoparticles (Cis-NPs) were developed for tumor-targeted and temperature-controlled chemotherapy, with the aim of reducing systemic side-effects. The uptake of the Cis-NPs into the late endosomes and lysosomes was observed using confocal microscopy. The modulation of the cisplatin release from the NPs was controlled by changing the temperature at lysosomal pH. In vitro result showed that the Cis-NPs can enhance the therapeutic efficacy.

INTRODUCTION

Chemodrug-loaded hydrogel nanoparticles (NPs) have been extensively applied towards improving therapeutic efficacy and reducing the systemic side-effects of cancer chemotherapy.¹ An important aspect of the development of these NPs is the control of the chemodrug release from the NPs. An ideal NP would not release chemodrugs while circulating in the blood stream, but only in the tumor tissue. To achieve this goal, environmental stimuli, such as temperature, pH have been studied as potential switch to control the release of chemodrugs.² Usually the temperature of the tumor is slightly higher than the normal body temperature, due to anomalous metabolic activities.² Thus, the higher temperature could be used as an ‘on’ switch for chemodrug release. Additionally, the local temperature in the tumor tissue can be further increased from outside the body.¹,²

In this work, the temperature sensitive NPs, Poly(acrylic acid-co-acrylamide), were synthesized and incorporated with cisplatin, which is widely used for treating breast, ovarian, bladder, lung, and head-and-neck cancers,³ with an aim to control the cisplatin release. The poly(acrylic acid-co-acrylamide) has been reported to act as a temperature sensitive co-polymer, and the volume of the NP matrix, increases with temperature.²

EXPERIMENTAL METHODS

Cis-NPs were prepared by the reverse-micelle microemulsion process. In the typical synthesis, 2.69 g of dioctylsulfosuccinate and 1.34 g of Brij 30 were dissolved in 38 mL of argon-purged hexane. Also, 0.12 g of acrylamide, 0.14 g of acrylic acid, 0.006 g of N,N-methylenebis(acrylamide), and 0.1 g of ammonium persulfate were dissolved in 1.5 mL of deionized water. Then, these two suspensions were mixed for 20 min, under argon-purging. 400 µL of tetramethylethylenediamine was added to initiate the polymerization, while the system was purged with argon. The NPs were thoroughly washed using our standard lab procedures.⁴ Cisplatin was incorporated by post-loading. The hydrodynamic size and ζ-potential was measured, using a Delsa Nano C system (Beckman Coulter). The cisplatin amount was quantified using an inductively coupled plasma optical emission spectroscopy (Optima 2000 DV, Perkin-Elmer).

Intracellular behavior of the Cis-NPs was investigated using fluorescence confocal microscopy (SP-5X, Leica). The NPs were labeled with fluorescein - 5 – thiosemicarbazide; lysosomes were stained with Lysotracker. The drug release profile of the Cis-NPs was investigated using a 1mg/ml Cis-NPs solution, which was prepared in a 50mM phthalate buffer (pH4) to mimic the lysosome environment. The NP solution was separately placed in 32 °C, 37°C, or 42 °C water baths for 6, 12, 24 and 48 hours each. The Cis-NPs were removed from the solution using a centrifugal filter, with 100 kDa MWCO membrane to quantify the released cisplatin at each experimental condition.
RESULTS AND DISCUSSION

The hydrodynamic size at room temperature was 132 (±3) nm and the ζ-potential was -56 (±5) mV. The Cisplatin content was measured to be 11 (±3) wt%.

![Figure 1. Fluorescence image showing colocalization of NPs (green) and lysosomes (red) in MDA cells. The colocalization is shown in yellow/orange.](image)

First, the uptake of the cisplatin NP's in the MDA-MB-435 cells was monitored, as shown in Fig. 1. These NP's were efficiently taken up by the cells; and there was a high degree of colocalization of the Cis-NPs with the lysosomes.

Since the NP's end up in the lysosomes, which are acidic in nature, we perform the drug release studies in similar conditions. Figure 2 shows the enhancement of cisplatin release at 37 °C and 42 °C, compared to 32 °C. The inset shows the overall cisplatin release at 37 °C and 42 °C. Initially, at higher temperature, the cisplatin release was significantly higher. This can be attributed to the relaxation of the matrix, the enhanced detachment of cisplatin from the Cis-NPs, and the higher diffusion rate of cisplatin when the Cis-NPs were heated. The amount of cisplatin released at both temperatures was similar after 48 hours. However, the initial release kinetics, between 6-12 hours, is most relevant due to clearance of the NP’s from the tumor tissues beyond this time period. Additionally, cell viability assays, on MDA cells, were performed to demonstrate the effectiveness of this technique. We observed up to 46% decrease in IC$_{50}$ value at 40 °C compared to 37 °C with this method. However, the free drug did not show any difference in the IC$_{50}$ value at these two temperatures.

![Figure 2. The percent enhancement of cisplatin release relative to the release at 32 °C. Overall release at three different temperatures (inset).](image)

CONCLUSIONS

Temperature-sensitive NPs was successfully developed with high cisplatin loading. The Cis-NPs were taken up into the cells by endocytosis, and transported into the lysosomes. At the lysosomal pH, the cisplatin release profile had a significant temperature dependence; consequently, showed enhanced therapeutic efficacy in vitro. The Cis-NPs has the potential for enhancing the chemodrug’s accumulation specifically in tumors and improve the therapeutic efficacy.

REFERENCES


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