6.0 Introduction

Efficient use of drugs requires their selective delivery at the site of action in a controlled rate especially in the case of potent drugs with strong side effects like cisplatin. Targeted delivery and controlled release of cisplatin from herceptin directed PAMAM cisplatin conjugates offer a new approach to improve the efficacy and tolerability of this drug, which are used to treat most common types of cancer.

The ultimate goal of cancer research is to translate scientific findings into practical clinical applications. Experimental discoveries are thought to begin at “the bench” with basic research, progress through pre-clinical animal studies, then show therapeutic efficacy in human clinical trials. Due to practical and ethical concerns associated with human experimentation, animal models have been essential in cancer research. The development of in vivo animal models that recapitulate the natural history of human cancers and their clinical response to therapy constitute a major prerequisite for rapid bench-to-bedside translation of investigational anticancer therapies and imaging agents that have shown promise in in vitro models, and thus serve as an important source of in vivo information [1-4].

Mouse cancer models are well known and are frequently used as models for cancer research. They have revolutionized our ability to study the fate of anticancer drugs in vivo and to better understand their pharmacokinetic and pharmacodynamic properties[5]. The most common rodent cancer models are xenografts and chemically or genetically induced cancers [1].

In the current study, a xenograft model of human ovarian cancer was established in SCID mice to evaluate the efficiency and toxicity of herceptin targeted dendrimer-cisplatin conjugates in comparison to free cisplatin. In addition, body weight changes and survival rate was also recorded as an indication of systemic toxicity [6].
6.1 Methodology

6.1.1 Animals

Female SCID mice were purchased at the age of 6 weeks and maintained in cages with paper filter covers under controlled atmospheric conditions. Cages, covers, bedding, food, and water were changed and sterilized weekly. Animals were handled in a sterile manner in a laminar down-flow hood. Ethical approval was obtained and the experiments were performed in compliance with guidelines approved by the IAEC (IAEC/XXXI/SRU/390/2014).

6.1.2 Antitumor activity of Herceptin-DGA-G4-cisplatin

A human ovarian cancer xenograft tumor model was developed by subcutaneous injection of SKOV-3 cells (7.5x10^6) into the right flank of each mouse using 50% matrigel. When the tumor volumes reached approximately 100mm^3 the animals were randomly divided into 3 groups. Animals in respective groups were treated with a single intraperitoneal (i.p) injection of PBS, cisplatin (8mg/kg) and herceptin-DGA-G4-cisplatin (8mg/kg) on days 0 and 7. Length and width of the tumors were measured using electronic calipers and the tumor volume (in mm^3) was calculated using the formula tumor volume (V) = 0.5 x length x width^2. Simultaneously changes in body weight and survival rates were also recorded.
6.2 Results

6.2.1 Antitumor activity of Herceptin-DGA-G4-cisplatin in SCID mouse tumor xenografts
The antitumor efficacy of herceptin-DGA-G4-cisplatin was investigated using a xenograft model of ovarian cancer generated by subcutaneous injection of SKOV-3 cells in the flanks of SCID mice. When tumors were about ~100mm³, the animals were randomly divided into three groups (two animals in each) in such a way that the differences among the groups were minimum. The treatments were done *i.p* every week for 2 weeks. Tumor volume, body weight changes and mortality rate were carefully and daily recorded during the study period. The antitumor efficacy and systemic toxicity of herceptin-DGA-G4-cisplatin and cisplatin were represented in Figure 6.1. Five days after treatment, tumor volumes in PBS treated mice increased rapidly whereas in mice treated with herceptin-DGA-G4-cisplatin and cisplatin, the tumor growth was inhibited. Notably, treatment with herceptin-DGA-G4-cisplatin was more effective in inducing tumor regression than free cisplatin, suggesting its superior anti-tumor activity. Body weight changes during the treatment period were recorded as an indication of systemic toxicity. In all the three groups i.e control, cisplatin and herceptin-DGA-G4-cisplatin, there was no significant difference in body weight.
Figure 6.1 Antitumor activity and systemic toxicity of cisplatin and HERceptin-DGAG4-cisplatin against SKOV-3 cancer bearing mice (a) images of excised tumors at the time of sacrifice, (b) tumor growth curves and (c) the body weight change curves. The results represent the mean ± SE. No. of tumors (n) = 4.
6.3 Discussion

The enhanced antitumor efficacy of the targeted conjugates might be attributed to herceptin mediated active targeting, receptor mediated endocytosis and sustained release properties of the DGA-dendrimer-drug conjugates, which would have increased cisplatin concentration preferentially in tumor tissues. Unfortunately, during the study period one animal in control group died on day 10 and hence the experiment was terminated and further data could not be obtained. While all the animals in both the treatment groups survived, mice in the control group probably could have died because of excessive tumor burden. Based on collective results, herceptin-DGA-G4-cisplatin demonstrated better antitumor activity in comparison with free cisplatin.

6.4 Conclusion

Herceptin-DGA-G4-cisplatin appeared as potent antitumor agents than the free drug in the developed ovarian cancer xenograft model. However, it is important to further increase the number of animals in each group and optimize the experimental conditions to take the study to the next level and which will be the focus of our future study. Nonetheless, our preliminary in vivo results suggest that herceptin-DGA-G4-cisplatin are capable enough to function as potent antitumor agents and justifies its further investigation in vivo.
6.5 References


