Developing novel anticancer drugs by targeting DNA

学位論文要旨

Cancer is characterized as rapid proliferation and uncontrolled growth of abnormal cells. In 2020, nearly 10 million individuals died from cancer in the world. Chemotherapy continues to be a mainstay of cancer treatment as a result of its high power to kill cancer cells despite the possible side effects. Platinum complexes and doxorubicin (DOX) are common anticancer drugs utilized in cancer treatment. Tissue-targeted therapy has been attracted as a promising strategy to improve specificity thereby enhance the opportunity to manage the disease. In this study, we developed novel cancer treatments by improving traditional chemotherapy. In chapter 1, platinum (II) complexes containing aromatic amine as ligands have been synthesized, characterized, and its inhibitory effect for 20S proteasome and in vitro cytotoxicity were evaluated, making the anticancer drugs to replace cisplatin. The new synthesized platinum complexes may be potential anti-cancer agents mainly due to their proteasome inhibition activity. In chapter 2, we developed thermosensitive liposomes (TSLs) encapsulating ^{125}I-labeled DOX derivatives and the TSLs used in combination with hyperthermia were evaluated to demonstrate effectiveness to Auger electron therapy. The combination of TSLs encapsulating ^{125}I-labeled DOX derivatives with hyperthermia would deliver ^{125}I to the nuclei and kill cancer cells.

Cancer is characterized as rapid proliferation and uncontrolled growth of abnormal cells. In 2020, nearly 10 million individuals died from cancer in the world, based on the world Health Organization (WHO). It has widely recognized that present cancer therapy methods call for significant improvements and new therapy approaches are urgently needed to reduce the risk of cancer disease. Chemotherapy is still a mainstay of cancer treatment due to its high ability to kill cancer cells in spite of the potential side effects. Platinum complexes and doxorubicin (DOX) are common anticancer drugs utilized in cancer treatment. A major hurdle of traditional chemotherapy is its relative nonspecificity for tumor cells. Tissue-targeted therapy has emerged as a promising strategy to improve specificity and thereby enhance the opportunity to manage the disease.

Cisplatin is one of the most effective anticancer drugs currently used in the treatment of cancer. However, due to its potent side effects such as nausea and nephrotoxicity, new drugs must be developed even though lots of effort has been put forth to achieve this particular aim. The interactions between cisplatin and nucleic acid have been reported to be mostly intrastrand in nature, with the drug coordinating with N7

(guanine (G)) in the –GG–or GXG–moieties of the nucleic acid strand, and also interstrand in nature, with coordination between the G and G or A between the DNA double strands. Intrastrand interactions, which account for a large proportion of the interactions, promote high anticancer activity and can be repaired by repair enzymes. These types of interactions, which are how cisplatin binds to DNA, block the replication of nucleic acids and are believed to be the main reason for the anticancer effects exhibited by cisplatin. However, it was reported that an adduct of the -GG-bound part of cisplatin with the protein HMGB1 would be crucial. Herein, the cisplatin metabolite adduct cisplatin GG (guanine (G)) phenylalanine (Phe) of HMGB1 was utilized as an adduct model to synthesize new platinum (II) complexes. In this study, we synthesize Pt(diamie)Cl_2 or Pt(bpy)(Pyrene-C3) which have ligand-ligand intramolecular aromatic ring stacking proximal metal ion. The possible contribution of Pt(diamine)(Pyrene-C3) or Pt(diamine)Cl_2 toward proteasome inhibition and cytotoxicity were studied; The results show that little proteasome inhibition was found for Pt(diamine)Cl_2 and the Pt(bpy)(Pyrene-C3) complex show strong proteasome inhibition (Table 1), making it ideal candidates for cancer treatment.

Table 1 Proteasome inhibition

Pt-Complexes	IC_{50} [μM]
Cisplatin	4.98 ±0.63
Pt(en)Cl_2	2.52 ±0.61
$Pt(Py)_2Cl_2$	4.76 ±0.67
Pt(BnC2)Cl_2	ND
Pt(NpC2)Cl_2	0.28 ±0.03
Pt(AtC2)Cl_2	1.19 ±0.19

ND = Not Determined

Auger electrons (AEs) are very low energy electrons that are emitted by radionuclides such as I-125 (^{125}I). This energy is deposited across a small distance (< 0.5 μm), resulting in high linear energy transfer that is potent for causing lethal damage in cancer cells. Thus, AE-emitting radiotherapeutic agents have great potential for the treatment of cancer. On the other hand, DOX is an anthracycline type of chemotherapy that is used to treat different types of cancer. DOX has a number of unwanted side effects, such as hematopoietic suppression and cardiac toxicity. In previous studies, a number of strategies have been reported to reduce toxicity and improve the activity of DOX. A liposome is one of the most famous drug delivery carriers to improve the biodistribution of low-molecular-weight drugs. Doxil is a typical liposomal formulation encapsulating DOX that highly accumulates in the nucleus. In addition, various types of stimuli-responsive liposomes have been developed to increase therapeutic efficacy by releasing drugs in cancer tissues. In this study, thermosensitive liposomes (TSLs) encapsulating ^{125}I-labeled doxorubicin (DOX) derivatives were developed for Auger electron therapy targeting the DNA of cancer cells. A radioiodinated DOX derivative [^{125}I]**5** highly accumulated into the nuclei of cancer cells and showed potent cytotoxicity to cancer cells by AEs. Then, [^{125}I]**5** was loaded in TSLs with high encapsulation efficiency. Potent release of [^{125}I]**5** from TSLs was achieved with heating, while the

decreased release was observed without heating. Furthermore, TSLs encapsulating [^{125}I]**5** showed a high uptake in the nuclei at 42 °C for 1 h (Figure 1). We supposed that [^{125}I]**5** was released by heating at 42 °C and accumulated in the nuclei in the cells. These results suggest that combining TSLs encapsulating [^{125}I]**5** with hyperthermia would be effective cancer therapy.

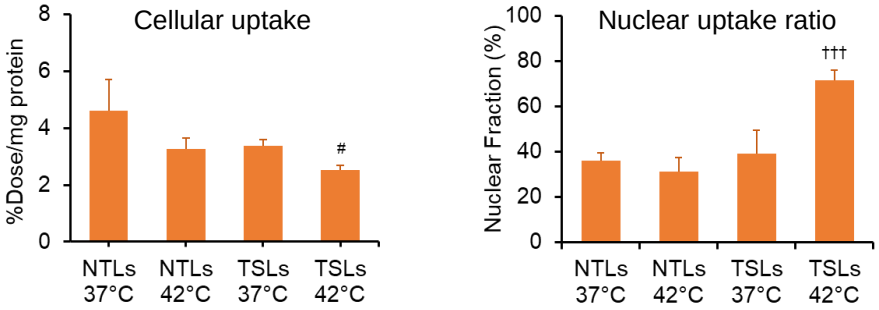

Figure 1 Cell uptake study using TSLs encapsulating [^{125}I]**5**. The cellular uptake and nuclear uptake ratio of NTLs and TSLs encapsulating [^{125}I]**5** at 37 or 42 °C for 1 h. Data were presented as mean ± SD for three samples. $^{\#}p < 0.05$ vs NTLs 37 °C, $^{\dagger\dagger\dagger}p < 0.001$ vs other conditions.

審査結果の要旨

化学療法は、様々ながん治療法が開発されている現代においても未だに使用されている優れたがん治療法であり、本研究では、化学療法で頻用されているシスプラチンとドキソルビシン (DOX) を用いた新たながん治療法の開発を行った。Pt 製剤の主なメカニズムは、がん細胞内の DNA への結合による DNA 合成およびそれに続くがん細胞の分裂阻害であるが、一部、タンパク質の分解を行う酵素複合体であるプロテアソームを阻害する効果が関与している。本研究では、DNA 結合能だけでなく高いプロテアソーム阻害活性を有する Pt 製剤として、複数の芳香環を配位子に有する Pt 製剤を開発し、高いがん細胞殺傷作用を有することを明らかにした。ドキソルビシンに関しては、その核移行性に着目し、非常に近接に存在する DNA のみに高い殺傷作用を有するオージェ電子を用いた新たな核医学治療法の開発を行った。オージェ電子放出核種で標識した DOX を、温度刺激により薬物を放出する温度応答性リポソームに内封したプローブを開発し、がん細胞内へ移行後、加温することにより放射性標識 DOX を放出し、放出された放射性標識 DOX が核内へ移行し、高い治療効果を示す可能性を示した。これら研究は、今後のがん治療戦略に有用な知見を与えるため、本論文が博士（創薬科学）に値すると判断した。