Synopsis

Use of metal based anticancer medication began with the clinical approval of cisplatin in 1978. Research led to the development of six platinum based drug candidates which are in use around the world. However there is a great need to develop better treatment strategies. The present work entitled “Cytotoxicity of Metal Based Anticancer Active Complexes and Their Targeted Delivery Using Nanoparticles” is an effort to prepare cytotoxic metal complexes based on platinum(IV) and copper(II) and deliver them selectively to cancer cells using a targeting ligand, biotin, with two different delivery vehicles, viz. PEGylated polyamidoamine dendrimer (PAMAM) and gold nanoparticles (AuNPs).

Chapter 1 provides a brief introduction to cancer and its characteristic features, followed by a short description about different treatment modalities in clinical practice. An account of the development of anticancer drugs starting from purely organic drugs to the field of metal based anticancer drugs is discussed. An overview of the available targeting strategies are discussed with specific examples. The section ends with the scope of the present work.

Platinum based anticancer drugs currently in use contain platinum in the +2 oxidation state. These drugs showed side effects and are often ineffective against resistant cells, especially in the latter stages of treatment. A recent focus of metal based anticancer drug research is the development of platinum(IV) systems which shows promise to have greater activity in cancer cells in a reducing environment. Reported platinum(IV) dual drugs contain the components of “cisplatin” or an analogue along with an active organic drug. But there are no known dual drugs based on platinum(IV) that would generate a cytotoxic metal complex along with cisplatin. In Chapter 2, a bimetallic dual drug (M4) (Figure 1), the first of its kind, with components of cisplatin and copper bis(thiosemicarbazone) has been prepared (Figure 1). The components and the bimetallic complex were characterized using several spectroscopic techniques. The dual drug M4 was found to be highly cytotoxic (IC₅₀ 1.3 μM) against HeLa cells and was better than cisplatin (IC₅₀ 6.8 μM). The bimetallic complex turned out to be better than the mixture (IC₅₀ 7.2 μM) of individual drugs which indicated possible synergism of the released cisplatin and the copper bis(thiosemicarbazone) from the dual drug.
A novel approach towards conjugation of platinum(IV) drugs to a carrier has been developed using a malonate moiety (Figure 2). The bis(butyric acid) complex, Pt(NH$_3$)$_2$(OCOC$_7$H$_7$)$_2$Cl$_2$ (M1), was taken as model complex to demonstrate the conjugation strategy. The complex M4 was also conjugated to the partially PEGylated 5$^{th}$ generation PAMAM dendrimers.

The cytotoxicity of M4 was reduced to a small extent on conjugation to the dendrimer. In the presence of 5 mM sodium ascorbate as a reducing agent, sustained release (40 %) of the drug was shown to occur over a period of 48 h by the drug release study. The reduction in cytotoxicity of the dendrimer conjugates could be due to incomplete release of the active drug. Unfortunately, no enhanced activity was observed with the additional targeting ligand, biotin. The drug uptake study revealed that the dendrimer conjugates were successful in entering cancer cells. There was no preferential uptake with biotin conjugated dendrimers which explained the similar cytotoxicity of dendrimer conjugates with and without biotin.

Different delivery vehicles showed varied efficiency in delivering the pay load (drugs) to the cancer site. In this connection, PEGylated gold nanoparticles have shown good promise as a drug
delivery vehicle. In Chapter 3, M1 and M4 are both conjugated to malonate functionalized PEGylated gold nanoparticles (30 nm). Biotin was also attached to the AuNPs for targeting HeLa cells.

![Figure 3: Schematic representation of the platinum(IV) drug and biotin conjugated AuNPs.](image)

The AuNPs were highly stable in water without agglomeration. There was no shift in the Surface Plasmon Resonance (SPR) band after conjugation of the drug molecules and targeting ligands. TEM images and DLS measurements showed there was no change in particle size. Drug conjugated AuNPs were also very stable in high salt concentrations as well as over a large range of pH. AuNPs with M1 were found to be less cytotoxic than the parent drug. Biotinylated AuNPs with M1 were more potent than non-biotinylated nanoparticles and increased cytotoxicity (35 %) was observed with biotin conjugation. Surprisingly, the enhanced activity of biotinylated AuNPs could not be correlated to the drug uptake study. The cytotoxicity of the bimetallic dual drug containing AuNPs were about 10-fold less and no increased activity was observed with the biotinylated conjugates. The reduced activity of AuNPs with the bimetallic drug was due to incomplete release from the AuNPs (20 % release after 48 h). But the release kinetics was very slow and sustained which might increase in vivo activity. The unexpected lower activity of biotinylated conjugates with copper bis(thiosemicarbazone) was suggestive of interference between bis(thiosemicarbazone) complex and the biotin receptor resulting in reduced drug uptake.

Copper bis(thiosemicarbazone) complexes hold very good promise as a class of non-platinum anticancer drug candidates. However, they lack selectivity towards malignant cells. Recently, CuATSM has shown hypoxia selectivity and very good cytotoxicity resulting in $^{64}$CuATSM being
used in advanced stages of clinical trials for imaging hypoxic cells. In **Chapter 4**, a copper bis(thiosemicarbazone) complex analogous to Cu(ATSM) with a redox active cleavable disulfide linker and a terminal carboxylic acid group (**CuATSM-SS-COOH**) was synthesised and characterised spectroscopically. The complex was highly cytotoxic and has an **IC$_{50}$** value (6.9 µM) similar to that of cisplatin against HeLa cells. The complex was conjugated to PEGylated gold nanoparticles by amide coupling between the acid group from the drug molecule and the amine on the AuNPs (20 nm) for smart drug delivery. The gold nanoparticles were decorated with biotin for targeted delivery to the HeLa cells.

**Figure 4:** *Schematic representation of the CuATSM-SS-COOH and biotin decorated AuNPs.*

The **CuATSM-SS-COOH** was insoluble in water but conjugation to PEGylated gold nanoparticles made it water soluble. The drug molecules and biotin conjugated AuNPs were highly stable which was confirmed by TEM and DLS measurements. Similar to the study described in the previous chapter, these AuNPs were also stable in a wide range of pH and salt concentrations. *In vitro* glutathione (GSH) triggered release study demonstrated substantial release of the cytotoxic agent from the AuNPs (60 %) over a period of 48 h. *In vitro* cell viability study with HeLa cells showed reduced cytotoxicity (**IC$_{50}$** 15 µM) of AuNPs with and without biotin containing drug conjugates relative to the parent copper complex (**IC$_{50}$** 6.9 µM). The reduction of the cytotoxicity correlated well with the released amount of the active drug from the nanoconjugates over the same time period. *In vivo* studies demonstrated the effectiveness of these nanoparticle carriers as suitable vehicles as they exhibited nearly four-fold reduction of tumor volume without significant loss in body weight. Moreover, the biotin targeted nanoparticle showed significant ($p < 0.5$) reduction in tumor volume compared to the non-targeted gold nanoparticles. Thus, this smart linking strategy
can be extended to other cytotoxic complexes that suffer from non-specificity, low aqueous solubility and toxicity.

Multinuclear anticancer active complexes do not act in the same way as that of their corresponding mononuclear analogues. In the case of multinuclear platinum complexes, the activity not only depends on the active moiety but also on the spacer length between the moieties. In Chapter 5, a series of multinuclear copper bis(thiosemicarbazone) complexes were prepared and characterised using different techniques.

![General structures of binuclear copper bis(thiosemicarbazone) complexes.](image)

Figure 5: General structures of binuclear copper bis(thiosemicarbazone) complexes.

All the complexes showed redox activity and have a very high negative reduction potential, i.e. these compounds would not be easily reduced in the biological medium and would remain as copper(II) species. As the concentration of the reducing agents are more within cancer cells, once these complexes are inside cells they would be reduced to Cu(I). These compounds were shown to be highly lipophilic from the large log P values. Unfortunately, these binuclear complexes were less active than similar mononuclear complexes. One possible reason for the reduced cytotoxicity of these complexes could be adherence of the complexes to the cell membrane due to the high lipophilicity of these complexes. Out of five different methylene spacers between two bis(thiosemicarbazone) moieties, the complex with a three carbon spacer was shown to be the most active against HeLa cells. The complexes with five and six methylene spacers turn out to be noncytotoxic. Further experiments are necessary to reveal the mechanism of action in these complexes.

In summary, bimetallic complexes can be very active and may be a way of overcoming drug resistance in platinum based therapy. A dual drug can be delivered using a malonate moiety and a disulfide linker. Gold nanoparticles are good delivery vehicles for these dual drugs and show great potential for improvement and translation to the next stage.