10 Abstract

This thesis deals with two ways for optimising the pharmacological properties of the cytostatic platinum lead compound **m-4F-Pt-Cl$_2$**. The first attempt was the use of the m-4F neutral ligand for the building of polynuclear alkylamin platinum complexes. Till now their pharmacology was rudimentally elucidated. In this work the cellular uptake by endocytosis after adsorption at the cellular membrane could be shown. The main uptake path was the macropinocytosis, an endocytotic path activated more rarely and shortly in normal tissue than in cancer cells. The adsorption to the cell membrane depended on charge and alkyl chain length of the bridging ligand. By increasing charge and increasing alkyl chain length a rise in cellular uptake was observed. The determination of platinum in the nuclei and DNA showed the escape from the endocytotic route. Thereby there was additionally a dependance on the leaving group at the platinum. Though capillary electrophoretic investigations showed a fast exchange of DMSO by chloride within two hours the compounds with chloride as leaving group caused a higher platinum content in the nuclei than the corresponding DMSO complexes.

The amount of platinum drug available for cellular uptake was reduced by a pronounced protein binding. Hence 10µM compound caused no antiproliferative effect at MCF-7 cancer cells when incubated in serum containing medium. In contrast there was a lasting (<200h) inhibition of nearly 50% when the cells were exposed to the same concentration of compound in serum free medium for 6h.

An outstanding cellular uptake and a particularly strong DNA-targeting were shown by **m-4F-Pt-DAB(PA)$_4$-DMSO,** a complex containing a DAB-PPI-dendrimer. Therefore the second attempt comprised the tethering of the platinum drug **m-4F-Pt-Cl$_2$** to this class of macromolecules. By designing the dendrimers the selectivity for tumor cells should be enhanced. The observed high cytotoxicity of the dendrimers was strongly reduced by the introduction of modifications on the endgroups (e.g. methionine and aspartic acid). Some of the modifications (methionine and phenylalanine) were releasable by enzymatic digest in modell experiments. The enzymatic hydrolysis found in the model incubations took place in intact MCF-7 cells with a remarkable velocity. Hence scarcely intermediates could be detected in cellular uptake experiments. There was a dependancy of the cellular uptake on the surface modification. Within a dendrimer generation higher cellular contents caused rising cytotoxicity. In contrast with rising dendrimer generation the cellular content decreased with an increasing cytotoxicity.

The cellular distribution was investigated by using dendrimers marked with the fluorescing dansyl group. For this compound group different surface modifications caused diverse patterns of distribution and cellular contents. The hydrophobic fully dansylated dendrimers showed a linear increase of cellular content. After 24 h precipitates appeared and were even visible by light microscopy. These aggregates didn´t hamper the cell proliferation of MCF-7 cells. Partial dansylation caused a strong cellular uptake with a saturation after 6 h. A perinuclear distribution was found by fluorescence microscopy. For these compounds the high cellular content correlated with the high cytotoxicity. The water well soluble pegylated and dansylated dendrimers showed punctual staining of the cytosol. Compared to the non pegylated dendrimers the cellular content was very low. Hence the cell proliferation was not affected except by **SM-G$_2$-(Dan)$_3$(NH$_2$)$_3$**.

After coordination of platinum to chelating surface groups the resulting dendrimers showed a higher cellular accumulation with respect to the non platinated dendrimers. At least small platinum-dendrimer-conjugates were able to escape from the endocytotic route, so that they were taken up in the nucleus and bound to DNA. The integrity of these complexes were shown by the use of fluorescence labelled platinated dendrimers. The cellular content of platinum and fluorophor did not differ significantly. These compounds in a concentration of 5µM caused up to 50 % inhibition of cellular growth of MCF-7 cells.

A distinct stronger antiproliferative effect was observed for the lead compound (**m-4F-Pt-H$_2$O-SO$_4$**) bound to dendrimers at dicarboxylic acids. The release of the drug was shown by fluorescence marked dendrimer platinates. For this compound the cellular platinum and fluorophor content was decoupled.

The discerning parameter for potency of dendrimer platinates was the binding behavior to serum albumins. Compared to antiproliferative active compounds the inactive compounds showed stronger accumulation in cells and higher amounts of DNA bound platinum. But those compounds were strongly bound to HSA. So only a small amount (<5%) was recovered and therefore available for cellular uptake.