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## Mechanism and circumvention of drugsresistance in tumor cells

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## SUMMARY

Treatment of cancer patients often fails because of development of resistance in the tumor against cytostatics: drugs effective early in the treatment finally do not damage for instance the DNA of the tumor cell anymore. This process of adaptation of the tumor and possible counter-measures are the subject of this thesis.

In order to do this research in the first place, the development of techniques to measure DNA damage was required, as well as drug resistant tumor cells to study the mechanisms.

Two types of measures can lead to the abolishment of the effects of resistance: changes in the resistant cells or extension and improvement of the cytostatic drug potential. Of both principles examples will be given: influence of the cell by offering unsaturated fatty acids, development of new drugs such as the cyclophosphazenes and enlargement of existing groups of drugs such as the anthracyclines.

In chapter 1 a review is given of a number of important cytostatics and the mechanisms responsible for resistance against those drugs.

In chapter 2, two techniques, the alkaline elution assay and the ethidiumbromide assay, are described. These assays make it possible to study the effect of cytostatics on one of the main targets of these drugs, the DNA. The ethidiumbromide assay is a technique that makes it possible to detect cross-links in whole non-dividing cells. Therefore this assay has the advantage that it is also applicable to (tumor) cells of patients.

In chapter 3 these techniques are used to evaluate the modes of action of a group of cytostatics under development, namely the cyclophosphazenes SOAz, AZP and AZM. As far as the effect on DNA is concerned, this appears to be a heterogenous group of cytostatics. SOAz gives no measurable damage, AZP leads to cross-links, and AZM results mainly in alkali-labile single strand breaks. Circumventing of resistance will to an important degree depend on introduction of new active cytostatics without cross resistance for established drugs. In view of the varying effects on tumor DNA, some of the compounds in this group will answer this demand.

In chapter 4 the development and characteristics of an adriamycin resistant human small cell lung carcinoma cell line is described. The morphological, biochemical and antigenical characteristics appear to be identical to the parent (sensitive) cell line, namely those of a variant

type human small cell lung carcinoma. There are differences in the membrane protein composition but no indications were found for a Pleiotropic Drug Resistance related glycoprotein. Also the cross resistance pattern for other cytostatics is different from that found in Pleiotropic Drug Resistance. After incubation with identical adriamycin concentrations the intracellular level of this drug is decreased 25-45% in the resistant cell line. However, this decreased level can not explain the resistance completely, because at identical intracellular levels there is a marked difference in cell kill. In this cell line several resistance mechanisms play a role. The genetic basis can be located in the increase in Double Minute chromosomes in the resistant compared to the sensitive cell line.

The investigation for other resistance mechanisms on DNA level in the same resistant cell line is described in chapter 5. DNA damage, consisting of single strand breaks, DNA-protein cross-links and double strand breaks, appear to be a possible cause for the cytostatic effect of adriamycin. This has been found in the resistant as well as in the sensitive cell line. The amount of DNA damage however is decreased in the resistant cell line. The ratio between DNA-protein cross-links, single strand breaks and double strand breaks, which is determined by the mode of action of adriamycin, is different in both cell lines. Possibly this is an effect of an altered effect of the drug on the DNA of the resistant cell line. When the number of double strand breaks is corrected for the intracellular adriamycin concentration there is still a decreased number of double strand breaks in the resistant cell line. This is probably based on an increase in the double strand break repair capacity in the resistant cell line after induction of double strand breaks with adriamycin and X-ray. Double strand breaks induced by adriamycin are repaired for 80% within 1 hour in the resistant cell line while there is no detectable repair in the sensitive cell line. X-ray induced double strand breaks are repaired in the resistant cell line with a  $t_{1/2}$  of 10 minutes and in the sensitive line with a  $t_{1/2}$  of 23 minutes. This mechanism will also attribute to the observed resistance.

In chapter 6 the effect on the resistant cell line of a number of adriamycin analogues is described. A lipophylic derivative like 4-demethoxydaunorubicin appears to have an increased cytotoxicity on weight base. Also the resistance factor is decreased compared to adriamycin. Other analogues have comparable resistance factors and cytotoxicity. The decreased clinical toxicity of some of the drugs can offer the possibility of

antagonizing the resistance cross-resistance is found.

In chapter 7 manipulation of the cell membrane is described. The incorporation of fatty acids (docosahexanoic acid) into the cell membrane at a concentration of adriamycin results in an increased cell kill in the resistant cell line. This method of cell membrane manipulation is of interest in the clinical situation.

Differences in the membrane and for a Pleiotropic Drug resistance pattern for other Pleiotropic Drug Resistance. At concentrations the intracellular resistant cell line. However, resistance completely, because at difference in cell kill. In a role. The genetic basis chromosomes in the resistant

mechanisms on DNA level in the 5. DNA damage, consisting and double strand breaks, effect of adriamycin. This sensitive cell line. The resistant cell line. The strand breaks and double action of adriamycin, is an effect of an altered cell line. When the number of intracellular adriamycin of double strand breaks in an increase in the double cell line after induction of double strand breaks induced in the resistant cell line sensitive cell line. X-ray resistant cell line with a  $t_{1/2}$  of 23 minutes. resistance.

cell line of a number of phylic derivative like decreased cytotoxicity on weight compared to adriamycin. Other cytotoxicity. The decreased offer the possibility of

antagonizing the resistance in vivo. Interestingly, for mitoxantrone no cross-resistance is found.

In chapter 7 manipulation of the diffusion of adriamycin through the cell membrane is described. In vitro incorporation of unsaturated fatty acids (docosahexanoic acid) in the cell membrane increases the intracellular concentration of adriamycin in the resistant and the non-resistant cell line. This results in an increased cytotoxicity in vitro for the resistant cell line. This method of circumvention of resistance is probably applicable in the clinical situation.