Mechanisms and circumvention of cellular resistance to cisplatin.
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SUMMARY.

Cisplatin (CDDP) is an active cytostatic agent. A limitation to its effectiveness initially or appearing during cytostatic treatment is the occurrence of resistance. This thesis describes mechanisms which are responsible for acquired cellular CDDP resistance. To investigate cellular CDDP resistance, a CDDP resistant subline (GLC₄-CDDP) is developed from a human small cell lung carcinoma cell line (GLC₄), after prolonged in vitro CDDP exposure.

In chapter 1 a review is given of cellular mechanisms which could be responsible for CDDP resistance. Mechanisms of resistance lead to avoid the formation of cytotoxic lesions or give rise to their rapid repair or tolerance. The CDDP induced DNA changes are considered to be the cytotoxic lesions. A decrease in the extend of these lesions can be caused by a change in the cytoplasmic and/or nuclear membrane, both could result in a lower cellular and/or nuclear CDDP concentration. Another possibility is a change in the toxicity of CDDP caused by an altered cytoplasmic and/or nuclear composition, for example by an increase in glutathione (GSH) or chloride content. A modification in a protein (for example a topoisomerase) or DNA can result in a decrease, change, tolerance or repair of the CDDP induced DNA changes. An altered DNA conformation can be the cause for example by a change in the intranuclear polyamine or topoisomerase concentration.

In the chapters 2, 3, 4, 5 and 6 the effect of CDDP on DNA has been studied. CDDP causes in the DNA the following platination products (the so called "adducts") : Pt-GG, Pt-AG, Pt-G-X-G, protein-Pt-DNA and the interstand crosslinks. To study the CDDP induced DNA damage, two methods have been used. One method has the ability to measure the Pt-GG, Pt-AG, G-Pt-G, Pt-GMP adduct content in digested platinated DNA in the fractions which arise after high
performance liquid chromatography separation. A low adduct concentration (< 15 ng Pt/ml) is measured by the use of polyclonal antibodies, higher concentrations are detected by atomic absorption. This method can not measure the interstrand crosslinks seperately from the Pt-G-X-G adducts. To detect the interstrand crosslinks the ethidium bromide assay is used.

Chapter 2 describes the development and characterization of a CDDP resistant human small cell lung carcinoma cell line. Compared with GLC4, GLC4-CDDP showed an increase in doubling time and a decrease in the cloning efficiency, the cellular size, the amount of double minutes per cell, the cellular and nuclear protein content, and the HLA-abc and HNK1 antigen expression. Cross resistance is found for Adriamycin, melphalan, cadmium chloride, carboplatin and cis-dichloro-trans-dihydroxo-cis-bis(isopropyl)amine platinum(IV), no cross resistance for vincristine is found. The platinum(Pt) concentration is the same for both cell lines. The amount of sulphydryl compounds is increased with a factor 2.5, this increment is caused by the increase of the GSH. A decrease in GLC4-CDDP compared with GLC4 is found in the amount of Pt bound to DNA and the interstrand crosslink formation. Under these circumstances no difference is found in the Pt-GG adduct content. Therefore various changes appeared to play a role in the development of CDDP resistance.

Chapter 3 describes the mechanisms which are important for the development of CDDP resistance in GLC4-CDDP. For these experiments GLC4 and the in vitro acquired CDDP resistant sublines GLC4-CDDP3 and GLC4-CDDP11 have been used, with a resistance factor of 3 and 11, respectively. These cell lines with the same origin but a quantitative difference in resistance, possess probably the same mechanism of resistance. No consistency is found between the resistance factor and the growth pattern, the cellular and nuclear Pt content, the differentiation antigens and the level of Pt-nonline histone DNA adducts: Pt-DNA is measurable, but no difference in the Pt-GG adduct content is found. Surprising is found after constant conversion. The removal of the Pt-GMP by alkaline phosphatase is not significant. No consistency is found between the Pt-GGM repair and the repair of non-repairable adducts.
histone chromatin protein binding. Also no difference is found in the interstrand crosslink formation caused by carboplatin, in spite of the existence of cross resistance for that drug. A correlation is found between the resistance factor and the GSH content, the amount of Pt bound to DNA, the Pt-GG adduct content and the amount of interstrand crosslinks. In these experiments a difference in Pt-GG adduct content is found, and not in the experiments described in chapter 2. This apparent contradiction is probably caused by a prolonged CDDP exposition time and the higher CDDP incubation concentrations used in chapter 3. In conclusion a reduced CDDP induced net DNA platination seems to be an important mechanism in CDDP resistance. This reduction in platination can be caused by a difference in formation and/or repair.

Chapter 4 describes the formation and repair of the following adducts: Pt-GG, Pt-AG, G-Pt-G and the Pt-GMP adduct. The total Pt bound to DNA is measured by atomic absorption. In GLC4 there is, 22 hour after the 2 hour 100 μM CDDP incubation compared with GLC4-CDDP, an increased net formation of the total Pt bound to DNA, Pt-GG and the Pt-AG adduct. No difference is found in the net formation of the Pt-GMP and the G-Pt-G adduct. Surprising is the slow Pt-AG adduct formation. In both cell lines a maximum is found after 10 hour. Quantitative this can not be explained by monoadduct conversion. In GLC4 there is 22 hour after the 2 hour 100 μM CDDP treatment a significant decrease in Pt-GG, Pt-AG and the Pt-GMP adduct. In GLC4-CDDP a significant decrease is measured in the total Pt bound to DNA, the Pt-AG and the Pt-GMP adduct. It is remarkable that the decrease in total Pt bound to DNA cannot be explained by any adduct measured by the polyclonal antibodies. The removal of the total Pt bound to DNA in GLC4-CDDP can be hampered by nalidixic acid, a topoisomerase inhibitor. It is possible that in GLC4-CDDP a repairable adduct is formed which cannot be detected by the polyclonal
antibodies. In conclusion, no evidence is found that repair of the Pt-GG, Pt-AG, G-Pt-G and the Pt-GMP adduct is an important mechanism in CDDP resistance.

Chapter 5 describes the comparison between an adriamycin (GLC4-ADR) and a CDDP resistant (GLC4-CDDP) cell line, both cell lines have been derived from the same parent cell line (GLC4), by in vitro incubation with respectively ADR and CDDP. In the clinic often combinations of drugs are used to prevent resistance. This strategy is based on the assumption that a close relation exists between the mode of action and the mechanism of resistance. In comparing the resistant cell lines with GLC4 no difference is found between the cell morphology, the doubling time, chromosomal damage, and the glutathione S-transferase. Compared with GLC4 in GLC4-ADR an increase is found in the amount of double minutes per cell, and in GLC4-CDDP a decrease. In GLC4-ADR and GLC4-CDDP compared with GLC4 a decrease is found in the cloning efficiency, the cellular drug level and the DNA damage. The sulphydryl and the GSH content is reduced in GLC4-ADR and increased in GLC4-CDDP. Glutathione reductase is increased in GLC4-ADR and equal in GLC4-ADR compared with GLC4. The protein content is equal in GLC4-ADR compared with GLC4, but reduced in GLC4-CDDP. In GLC4-ADR cross resistance is found for CDDP and collateral sensitivity for melphalan. In GLC4-CDDP cross resistance is found for adriamycin and melphalan. Concluding, in spite of the differences found in GLC4-ADR and GLC4-CDDP, which point out a different mechanism of action for adriamycin and CDDP, there is cross resistance for CDDP and adriamycine. Therefore on the basis of the mechanism of action no predictions can be made about the occurrence of cross resistance.

Chapter 6 describes the modulation of the cytotoxicity of the CDDP resistant cells with docosahexaenoic (DCHA), a poly unsaturated fatty acid. This fatty acid of the w-3 class, occurs in the diet primarily in fish oils.
Survival studies point out an increase in the sensitivity of the GLC₄-CDDP cells after DCHA incorporation, on the other hand the sensitivity of GLC₄ remains the same. DCHA incorporation has no effect on the GSH level, but results in both cell lines to an equal degree in an increased cellular Pt content and Pt-DNA binding. Also an increase is measured, in both cell lines, in the Pt-GG, Pt-AG, G-Pt-G and Pt-GMP adduct content, no change in adduct proportion is detected. DCHA incorporation results in an increased interstrand crosslink formation in GLC₄-CDDP, but not in GLC₄. In conclusion, DCHA addition results in GLC₄ in an increased tolerance of cellular Pt, Pt-DNA binding and the amount of the Pt-GG, Pt-AG, G-Pt-G and Pt-GMP adducts. In GLC₄-CDDP DCHA results in an increased sensitivity for CDDP. Striking is the fact that only the interstrand crosslink formation correlates with the cytotoxicity after DCHA incorporation. These findings make it interesting to investigate the effect of the clinical applicable fish oils in CDDP treated patients.

CONCLUSION.

This thesis describes how resistance against the chemotherapeutic drug CDDP can be raised in vitro in human tumor cell lines. Thereafter a number of experiments are reported that clarify the changes that can be responsible for resistance in the tumor cells.

The most important change in the resistant cells is a reduced amount of Pt DNA adducts compared to the sensitive mother cell line. This reduced number occurs because there are less interstrand as well as intrastrand crosslinks. This is not a consequence of a reduced Pt entrance into the cell, as cellular and nuclear Pt levels are the same in the resistant and sensitive