Understanding Cisplatin Resistance Using Cellular Models

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Summary

Many mechanisms of cisplatin resistance have been proposed from studies of cellular models of resistance including changes in cellular drug accumulation, detoxification of the drug, inhibition of apoptosis and repair of the DNA adducts. A series of resistant models were developed from CCRF-CEM leukaemia cells with increasing doses of cisplatin from 100 ng/ml. This produced increasing resistance up to 7-fold with a treatment dose of 1.6 μg/ml. Cisplatin resistance in these cells correlated with increases in the antioxidant glutathione, yet treatment with buthionine sulphoximine, an inhibitor of glutathione synthesis, had no effect on resistance, suggesting that the increase in glutathione was not directly involved in cisplatin resistance. Two models were developed from H69 SCLC cells, H69-CP and H69CIS200 using 100 ng/ml or 200 ng/ml cisplatin respectively. Both cell models were 2-4 fold resistant to cisplatin, and have decreased expression of p21 which may increase the cell's ability to progress through the cell cycle in the presence of DNA damage. Both the H69-CP and H69CIS200 cells showed no decrease in cellular cisplatin accumulation. However, the H69-CP cells have increased levels of cellular glutathione and are cross resistant to radiation whereas the H69CIS200 cells have neither of these changes. This suggests that increases in glutathione may contribute to cross-resistance to other drugs and radiation, but not directly to cisplatin resistance. There are multiple resistance mechanisms induced by cisplatin treatment, even in the same cell type. How then should cisplatin-resistant cancers be treated? Cisplatin-resistant cell lines are often more sensitive to another chemotherapeutic drug paclitaxel (H69CIS200), or are able to be sensitised to cisplatin with paclitaxel pre-treatment (H69-

CP). The understanding of this sensitisation by paclitaxel using cell models of cisplatin resistance will lead to improvements in the clinical treatment of cisplatin resistant tumours.

Keywords

Cisplatin, Platinum, Resistance, Chemotherapy, Glutathione, p21, Paclitaxel, SCLC, Leukaemia, Cell Models

Introduction

Cisplatin has been used in the treatment of cancer for over 30 years, and is highly successful for many cancers, including testicular, ovarian and lung cancer. Upon entering the cell, cisplatin becomes positively charged, and so is able to interact with nucleophilic molecules including DNA, RNA and proteins. Cytotoxicity is believed mainly due to interaction with DNA, forming inter- and intra-strand adducts, hindering both RNA transcription and DNA replication, leading to cell cycle arrest and apoptosis. Inevitably, the use of cisplatin is limited by the development of drug resistance. Numerous cellular mechanisms potentially contributing to clinical cisplatin resistance have been proposed (1,2) including changes in cellular drug accumulation, detoxification of the drug, inhibition of apoptosis and repair of the DNA adducts, as summarised in Fig. 1. Understanding these mechanisms and their role in resistance is important for the continued success of cancer treatment.

Cellular Models of Cisplatin Resistance

We have developed several cellular models to attempt to understand the cellular adaptations underlying cisplatin resistance mechanisms, and potential strategies to reverse this resistance. Small cell lung cancer (SCLC) is an aggressive form of lung disease, with treatment involving combination chemotherapy including cisplatin. While this produces 90% response in patients, relapse is rapid with patients developing resistant disease. We have treated H69 SCLC cells with 100 ng/ml cisplatin, to produce the H69-CP (3) or 200 ng/ml cisplatin to obtain the H69CIS200 cells (4). These doses are below an IC_{50} for cisplatin and are within the range achieved in the clinical use of cisplatin. The

cells were 2- to 4-fold resistant to cisplatin, but there was no decreased drug accumulation. To further identify molecular changes resulting from low, non-toxic doses of cisplatin, the model CCRF-CEM leukaemia cell was treated for 3-4 days with increasing doses of cisplatin from 100 ng/ml, a dose well below the IC_{50} for cisplatin (540 ± 30 ng/ml) for these cells. This produced a series of cells with increasing cisplatin resistance up to 7-fold resistance with a treatment dose of 1.6 μg/ml, after which resistance, as determined in a 4-day cytotoxicity assay, decreased (Figure 2). Resistance was associated with decreased cisplatin accumulation, although, there were no changes in expression of the multidrug transport protein MRP2 which transports cisplatin conjugated to glutathione to explain the decreased intracellular drug as increased drug efflux (5).

Detoxification mechanisms in cisplatin resistance

Cisplatin is very reactive towards the cellular antioxidant glutathione, readily forming complexes. Resistance in the CEM cells reflected changes in glutathione (Figure 2). However, treatment of these cells with buthionine sulphoximine (BSO), an inhibitor of glutathione synthesis, had no effect on cellular resistance. This suggests that although a cellular response to cisplatin treatment was to increase their glutathione levels, this was not directly involved in cisplatin resistance. Glutathione changes have frequently been reported in cells treated with cisplatin, and may contribute to cross-resistance to other drugs and radiation, but not necessarily directly to cisplatin resistance. This proposal is supported by the SCLC cells which, although 2 to 4-fold resistant to cisplatin, the H69-CP cells had increased glutathione and cross-resistance to radiation while the H69CIS200 cells had no change in glutathione and were not radiation resistant. This is also supported by the fact that radiation resistant H69 cells with increased glutathione are highly resistant to cisplatin (6).

However, glutathione is not the only thiol cellular redox system, and changes in the thioredoxin antioxidant system, thioredoxin reductase and thioredoxin, are also reported to confer cisplatin resistance (7). Increased thioredoxin reductase occurred in the cisplatin-resistant CEM cells, leading to cross- resistance to the thioredoxin reductase inhibitor auranofin, a gold compound clinically used as an antirheumatic drug. This

contrasts a recent report suggesting auranofin induces apoptosis in cisplatin resistant ovarian cancer cells, and so may be suitable to treat cisplatin resistant tumours (8). Again, the involvement of redox systems in cisplatin resistance is variable and may be dependent on cell type.

Cisplatin resistance and the cell cycle

In the CEM series of cisplatin-resistant cells, at higher levels of drug treatment the cells do not appear to be resistant as judged in a 4-day cytotoxicity assay. This is because cisplatin treatment causes the cells to stop growing. On removal of the drug, the cells then proliferate rapidly. While this resistance mechanism occurred at higher drug doses in the CEM cells, a similar response to cisplatin was evident after treatment with low levels of drug in the H69CIS200 cells (4), where cells rapidly grew on removal of drug. The contrast in resistant mechanisms developed in the H69CIS200 and H69-CP cells illustrates the diversity of mechanisms which may occur using similar treatment strategies even in the same cell line.

As well as alterations in the cell cycle allowing rapid proliferation post drug treatment, the H69CIS200 cells also have several chromosomal rearrangements which are not associated with the resistant phenotype, suggesting an increase in genomic instability in the resistant cell lines (9). We hypothesise that there is a deregulation between the cell cycle and DNA repair in the H69CIS200 cells allowing proliferation in the presence of DNA damage which has created an increase in genomic instability. The cellular response to DNA damage as a result of cisplatin treatment would be induction of p53, causing cells to arrest, by regulating the expression of cyclins and cyclin-dependent kinases. Cisplatin however does not induce the cyclin-dependent kinase-inhibitor p21 in 2780CP cisplatin resistant cells, supporting the disruption of the normal response pathway in resistant cells (10). Both the H69CIS200 cells and the H69-CP cells have decreased p21 expression, which may increase the cell's ability to progress through the cell cycle despite the presence of DNA damage. This not only provides a resistance mechanism, but will contribute to the genomic instability of the cells which in turn will increase the mutagenic potential of the cells in response to further drug treatment.

DNA Repair Mechanisms

Since the major effect of cisplatin is the formation of DNA adducts, increased DNA repair is a potential resistance mechanism. Nucleotide excision repair (NER) mainly repairs bulky DNA adducts such as those caused by interaction with cisplatin, and downregulation of ERCC1, a core protein required for NER, sensitised cells to cisplatin (11). We have found that the cisplatin-resistant H69CIS200 cells have no alteration in DNA repair capacity compared to the parental H69 cells. However, ERCC1 expression decreases in association with the cisplatin-induced cell cycle arrest in both sensitive and resistant cells rather than in association with any change in DNA repair. Both increased ERCC1 expression (12) and decreased ERCC1 expression (13,14) have been associated with sensitivity to cisplatin based combination chemotherapy. Cisplatin treatment may alter the expression of ERCC1 for reasons other than DNA repair. This may explain some of the contradictory results examining this gene as a marker for the clinical response to cisplatin therapy. The ability to differentiate between these different types of platinum resistance in the clinic will improve the choice of salvage chemotherapy in patients with cisplatin-resistant cancers.

Conclusions

It is apparent that there are multiple resistance mechanisms induced by cisplatin treatment, and as many of these are linked by the cellular stress response, it is difficult to determine which of these is more important in resistance. While many mechanisms have been identified, there is no consistent response, even in the same cell type to treatment with cisplatin. The question then is: how to treat cisplatin-resistant tumours. The cell models are useful not only for examining the potential of the new platinum drugs being developed, but also for looking for combinations of current drugs which may lead to improvements in response. A recent report demonstrated that combination of the cell cycle specific antagonist gemcitabine with cisplatin was more effective than either drug alone. This combination gave enhanced toxicity in cisplatin resistant cells, suggesting that gemcitabine reversed cisplatin resistance (15,16).

Of particular interest are the frequent reports of sensitivity to paclitaxel in cisplatin resistant cells. This was evident in the H69CIS200 cells which were 5-fold more sensitive to paclitaxel than the H69 cells. The other cisplatin resistant cells, although cross-resistant to many drugs, were not resistant to paclitaxel. However, treatment of these cisplatin resistant cells, but not the H69 cells, with non-cytotoxic doses of paclitaxel was able to sensitise the resistant cells not only to cisplatin, but to other drugs, and also to radiation (17,18,3) Paclitaxel sensitization occurred after at least a 12 hour pre-treatment of the cells, suggesting time is required for this response. Analysis of the protein profile of these cells showed that paclitaxel treatment reversed many of the cellular protein changes that accompanied the development of resistance (19). This activity of paclitaxel was independent of the cell cycle mediated effect of the drug, which suggests other signaling pathways are involved (18). Understanding this sensitization of cisplatin resistant cells would lead to improved treatment protocols for the treatment of all forms of cisplatin resistance, and suggests that while cisplatin resistance is multifactorial, the means to overcome resistance may lie in inhibition of one specific signaling pathway. Future studies using cell models of cisplatin resistance will lead to an understanding of ways to overcome cisplatin resistance and improve the treatment of cisplatin resistant tumours.

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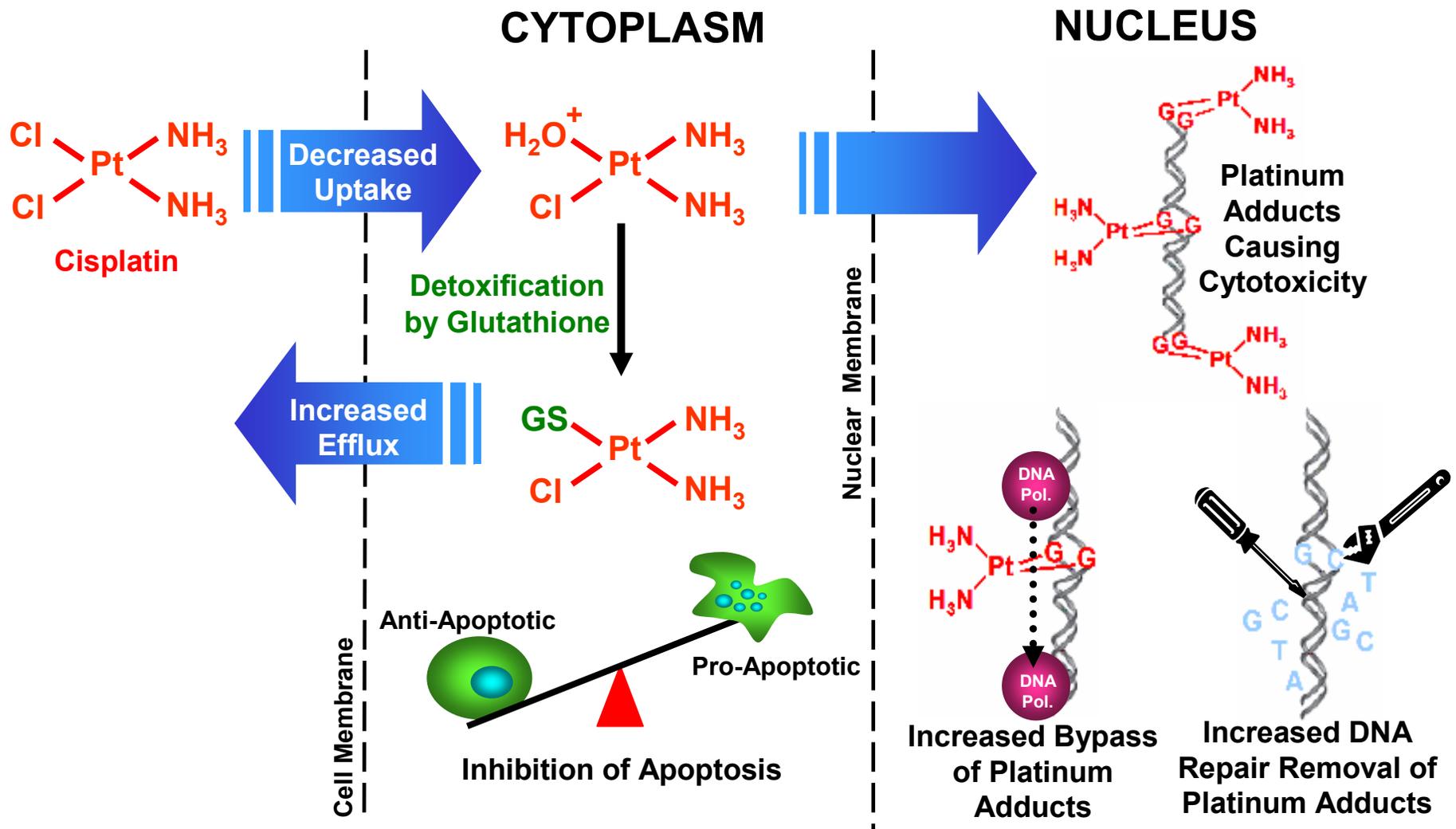
Figure 1. Cisplatin resistance mechanisms. Cisplatin is a neutral complex which on entering the cell becomes positively charged, and so able to interact with many molecules including DNA and proteins. Many mechanisms may contribute to cisplatin resistance including reduced uptake, increased efflux, increased detoxification, inhibition of apoptosis, increased ability to replicate DNA adducts and increased DNA repair. GS – Glutathione, Pol – Polymerase.
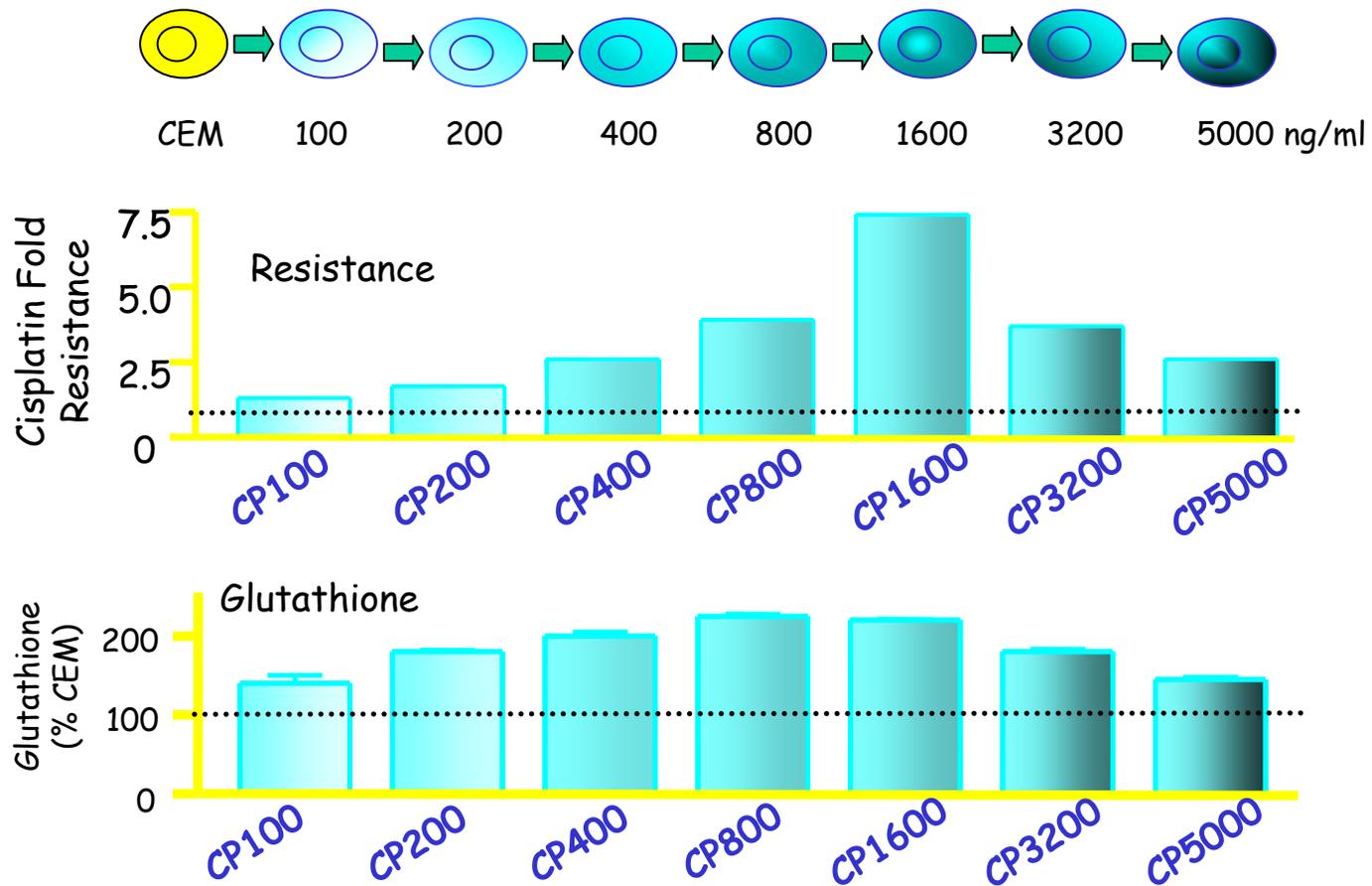
Figure 2. Cisplatin Resistance in CEM cells. CEM cells were treated for 3-4 days with cisplatin, commencing with 100 ng/ml. After 6 treatments, cells were stable to drug treatment and the doses increased. This developed a series of cisplatin-resistant cells. Cisplatin resistance (fold increase relative to the untreated CEM cells) is reflected in cellular glutathione levels.