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Commentary

Resistance to Cisplatin: How to Deal with the Problem?

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(A COMMENT ON: Andrews P.A., Murphy M.P., Howell S.B. Characterization of cisplatin-resistant colo 316 human ovarian carcinoma cells. *Eur J Cancer Clin Oncol* 1989, **25**, 619–625.)

SINCE its introduction in clinical practice, cisplatin [*cis*-diamminedichloroplatinum(II), CDDP] has become one of the most important drugs for treating solid tumors. CDDP-based chemotherapy has clearly improved the response rate for treatment of testicular, ovarian and small cell lung tumors. However, its impact on survival has only been marginal in most cases. The failure of cytotoxic regimens given with curative intent is believed to be linked, at least in part, to the development of drug resistant tumor cells.

To understand this phenomenon, resistant human and non-human tumor cell lines have been developed by repeated exposure to CDDP *in vitro* [1–5]. Several characteristic properties have been reported for these resistant cells:

1. The accumulation of CDDP in resistant cells can be lowered by decreasing the uptake of the drug. In contrast to multidrug resistance, the efflux is not increased. Reported data are in conflict about the mechanisms of CDDP membrane transport. Some authors claim there is passive transport, and others show evidence for active carrier dependent mechanisms which may be shared with neutral amino acids. Improvement of the transport defect has been described using liposome-trapped lipophilic platinum derivatives [6].
2. Several independent studies have found an association between acquired CDDP resistance and increased intracellular level of glutathione (GSH), the major cellular non-protein thiol. The exact mechanism by which GSH protects the cells is unknown. Some hypotheses have been put forward: (a) GSH could bind directly to CDDP and therefore inactivate the drug; (b) GSH could interfere with the formation of bidendate CDDP–DNA cross links; or (c) GSH could facilitate the repair of DNA lesions. Cellular GSH content can be reduced by treatment with buthionine-S-R-sulfoximine (BSO), a specific inhibitor of γ-glutamyl cysteine synthetase. This depletion is able to partially reverse the resistance of some CDDP cell lines, but is especially effective in increasing the cytotoxic action of CDDP on sensitive cells [7]. Resistant cell lines with high levels of glutathione-S-transferase (GST) have also been reported. This enzyme catalyzes the conjugation of electrophilic compounds such as CDDP at GSH, leading to the formation of an inactive product.
3. Metallothioneins (MTs) are cysteine-rich proteins and represent the major intracellular protein thiols. They play a role in Zn^{2+} and Cu^{2+} homeostasis and heavy metal detoxification. Data clearly show that acquisition of CDDP resistance is often accompanied with an increase in MTs and overexpression of MT mRNA [8]. Reversal of the CDDP resistance phenotype is associated with decreased MT content. Moreover, cells transfected with an expression vector containing DNA encoding for human metallothionein-IIA acquire resistance to CDDP and other alkylating agents.
4. CDDP forms covalent bounds with DNA, subsequently inhibiting its replication and/or tran-

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scription. Although there is no agreement on the exact nature of the critical cytotoxic lesions, different types of DNA lesion have been outlined. The major adducts produced are intrastrand cross-links formed by the binding of CDDP in the N7 position to neighboring guanines, adenine and guanine, or two guanines separated by one or more nucleotide(s). Interstrand cross-links of two guanines and DNA–protein cross-links have also been observed. Increased DNA repair activity have been reported for some resistant cell lines. Treatment with aphidicolin, an inhibitor of DNA polymerase alpha, decreases DNA repair activity and partially restores the sensitivity of CDDP-resistant cell lines [9]. Interestingly, GSH depletion by BSO treatment has the same effect [10]. These data suggest that enhanced DNA repair activity contributes to the CDDP-resistant phenotype.

The mechanisms involved in CDDP resistance have been studied in cells in culture and not all may be relevant in the clinical setting. Nevertheless, CDDP resistance appears to be a multi-factor process: the addition of multiple biological changes are responsible for decreased CDDP–DNA adduct formation which therefore lowers cytotoxicity. Different mechanisms can be induced, at various levels, from one cell line to another and thus their role in resistance can have a variable importance. Also, it makes sense to hypothesize that a solid tumor acquiring resistance to chemotherapy should contain several heterogeneous resistant clones that have preferentially induced different protective mechanisms in response to drug exposure. A recent report by Andrews *et al.* published in this Journal describes four CDDP-resistant cell lines arising from the same ovarian parental cell line but made resistant using different methods of selective pressure [11]. Although this study has not been completed, it is interesting to note that their cell lines express different characteristics depending upon the approach used to develop resistance.

Based on this knowledge, different ways to overcome CDDP resistance *in vitro* have already been described. These approaches do not take into consideration all the different aspects of the problem at the same time and, therefore, there is usually only partial reversal of resistance. If they prove to be clinically applicable, the best results will more likely arise from their combined rather than individual use. Among them, BSO is about to enter into clinical trials, although there is some concern about its effectiveness for the depletion of tissue GSH at non-toxic concentrations. So far, only high dose platinum compounds have been empirically used to overcome CDDP resistance with some success. It can be speculated that high dose CDDP can overcome defective membrane transport and saturate other mechanisms such as detoxification and increased DNA repair. Other *in vitro* data supporting this therapy include: (1) the description of dose–response curves for most alkylating agents, including CDDP; and (2) the difficulty of obtaining *in vitro* high levels of resistance with alkylating agents. This second argument is in contrast to other non-alkylating drugs like methotrexate or Adriamycin® where high resistance can be reached very easily. Ozols *et al.* [12] have shown that high dose CDDP (200 mg/m^2/cycle) in hypertonic saline can be administered with acceptable nephrotoxicity and is effective in tumors no longer responding to conventional doses. However, a high incidence of neurotoxicity limits the number of courses that can be given. Very high CDDP concentrations can also be reached with less toxicity by regional administration such as in the peritoneal cavity for ovarian carcinomas. Carboplatin (CBDCA), a platinum analog, has a spectrum of anti-tumor activity similar to that of CDDP and exhibits, at conventional doses, a wide cross-resistance with CDDP. In contrast to CDDP, the lack of non-hematological side-effects makes CBDCA a good candidate for use at high doses. In a phase I study, Shea *et al.* [13] have demonstrated that a six- to eight-fold dose escalation (maximal tolerable dose at 2000 mg/m^2/cycle given in 4-day continuous infusion) is possible without major side-effects. Dose up to 1600 mg/m^2/cycle can be given with acceptable hematotoxicity without autologous bone marrow transplantation [14]. Hematopoietic colony-stimulating factors, including GM-CSF, G-CSF and soon IL3, should reduce the severity of bone marrow suppression and facilitate the incorporation of high dose CBDCA into combination chemotherapy. High dose CBDCA (800 mg/m^2/cycle) has been used in refractory ovarian cancer with the same response rate and lower non-hematological toxicity than high dose CDDP. However, at this dosage, no response has been observed in tumor already resistant to CDDP-based regimens [15]. Very high dose CBDCA (up to 2000 mg/m^2/cycle) in combination with etoposide have been investigated in CDDP-refractory germ cell tumors [16]. The overall response rate was 44% (14 out of 32) including eight complete response, three being in unmaintained disease-free survival for more than 1 year. This study provides convincing evidence that resistance to CDDP can be overcome with massive doses of CBDCA. However, the exact role of such treatment and its real impact on survival needs to be established in randomized trials. Clearly, more knowledge about the fundamental processes involved in CDDP resistance are urgently needed in order to elaborate effective strategies to resolve this important problem.

REFERENCES

1. Teicher BA, Holden SA, Kelley MJ *et al.* Characterization of a human squamous carcinoma cell line resistant to *cis*-diamminedichloroplatinum(II). *Cancer Res* 1987, **47**, 388–393.
2. Behrens BC, Hamilton TC, Masuda H *et al.* Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987, **47**, 414–418.
3. Richon VM, Schulte N, Eastman A. Multiple mechanisms of resistance to *cis*-diamminedichloroplatinum(II) in murine leukemia L1210 cells. *Cancer Res* 1987, **47**, 2056–2061.
4. Sekiya S, Oosaki T, Andoh S, Suzuki N, Akaboshi M, Takamizawa H. Mechanisms of resistance to *cis*-diamminedichloroplatinum(II) in a rat ovarian carcinoma cell line. *Eur J Cancer Clin Oncol* 1989, **25**, 429–437.
5. Andrews PA, Velury S, Mann SC, Howell SB. *Cis*-diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Cancer Res* 1988, **48**, 68–73.
6. Perez-Soler R, Yang LY, Drewinko B, Louterzstain J, Khokkar AR. Increased cytotoxicity and reversal of resistance to *cis*-diamminedichloroplatinum(II) with entrapment of *cis*-bis-neodecanoato-*trans*-BR-1,2-diaminocyclohexaneplatinum(II) in multilamellar lipid vesicles. *Cancer Res* 1988, **48**, 4509–4512.
7. Hamilton TC, Winker MA, Louie KG *et al.* Augmentation of Adriamycin®, melphalan and cisplatin cytotoxicity in drug-resistant and sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. *Biochem Pharmacol* 1985, **34**, 2583–2586.
8. Kelley SL, Basu A, Teicher BA, Hacker MP, Hamer DH, Lazo JS. Overexpression of metallothionein confers resistance to anticancer drugs. *Science* 1988, **241**, 1813–1815.
9. Masuda H, Ozols RF, Lai G-M, Fojo A, Rothenberg M, Hamilton TC. Increased DNA repair as a mechanism of acquired resistance to *cis*-diamminedichloroplatinum(II) in human ovarian cancer cell lines. *Cancer Res* 1988, **48**, 5713–5716.
10. Laig GM, Ozols RF, Young RC, Hamilton TC. Effect of glutathione on DNA repair in cisplatin-resistant human ovarian cancer cell lines. *JNCI* 1989, **81**, 535–539.
11. Andrews PA, Murphy MP, Howell SB. Characterization of cisplatin-resistant colo 316 human ovarian carcinoma cells. *Eur J Cancer Clin Oncol* 1989, **25**, 619–626.
12. Ozols RF, Oscheya Y, Myers CE, Young RC. High-dose cisplatin in hypertonic saline in refractory ovarian cancer. *J Clin Oncol* 1985, **3**, 1246–1250.
13. Shea TC, Flaherty M, Elias A *et al.* A phase I clinical and pharmacokinetic study of carboplatin and autologous bone marrow support. *J Clin Oncol* 1989, **7**, 651–661.
14. Gore ME, Calvert AH, Smith IE. High dose carboplatin in the treatment of lung cancer and mesothelioma: a phase I dose escalation study. *Eur J Cancer Clin Oncol* 1987, **23**, 1391–1397.
15. Ozols RF, Oscheya Y, Myers CE, Young RC. High-dose carboplatin in refractory ovarian cancer patients. *J Clin Oncol* 1987, **5**, 197–201.
16. Nichols CR, Tricot G, Williams SD *et al.* Dose-intensive chemotherapy in refractory germ cell cancer. A phase I/II trial of high-dose carboplatin and etoposide with autologous bone marrow transplantation. *J Clin Oncol* 1989, **7**, 932–939.