Antidepressants and Platinum Drugs

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Abstract. *Background/Aim: Antidepressants are frequently prescribed concurrently with anti-cancer drugs and may have synergistic, additive or antagonistic effects. The present work investigated the effect of antidepressants on the cytotoxicity of platinum agents cisplatin, carboplatin and oxaliplatin. Materials and Methods: The cytotoxicity of platinum drugs alone or in combination with antidepressants was measured in HCT116 wild-type (wt), HCT116 (p53 –/–), HT-29, SKOV3 and A2780 cells using an apoptosis-based assay. Results: The effect of antidepressants on platinum cytotoxicity is both cell type- and drug dependent. Mostly additive effects were observed. Desipramine and fluoxetine caused the greatest effects, with cisplatin in general being most sensitive to their presence. There is little effect of p53 status on the drug-drug interaction while the calmodulin inhibitor W7 augmented cisplatin cytotoxicity relative to carboplatin and oxaliplatin. Conclusion: The drug-drug interaction between antidepressants and platinum anti-cancer agents requires detailed evaluation for optimization of patient care.*

Many cancer chemotherapeutic regimens include platinum-based agents. Patients with cancer also frequently receive adjuvant antidepressant therapy for depression, as well as to treat side-effects of cancer therapy, such as neuropathic pain. It is estimated that 25% of patients with cancer will experience major depression at some point during their illness (1). Given the frequency with which these drugs are prescribed, it is important to understand how antidepressants and anti-neoplastics interact – the combination may be synergistic, additive or antagonistic. The importance of this issue has been highlighted by recent discussion on the effects of selective serotonin re-uptake inhibitors on the efficacy of tamoxifen in breast cancer therapy (2, 3).

We have previously shown that the tricyclic antidepressant desipramine augments the cytotoxicity of cisplatin, oxaliplatin and the pre-clinical trinuclear platinum drug BBR3464 in HCT116 wild type (wt) cells (4). The mechanisms of such augmentation are complicated and may not be the same for all drugs. Desipramine was also subsequently shown to enhance cytotoxicity of carboplatin. These initial findings prompted us to survey the interaction between platinum drugs and antidepressants for cell type and drug specificity, and potential mechanisms. The structures of all the compounds investigated in this study are shown in Figure 1.

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Materials and Methods

Materials. HCT116 human colon carcinoma cells were a gift from Bert Vogelstein (Johns Hopkins University, Baltimore MD, USA). The A2780 cell line was a gift from Prof. S. Howell (UC San Diego). HT-29 and SKOV3 cells were purchased from ATCC (Manassas, VA 20110 USA). McCoy's 5a Medium Modified, RPMI, fetal bovine serum (FBS), L-glutamine, penicillin, streptomycin, HEPES buffer and sodium pyruvate were all purchased from Biofluids (Rockville, MD, USA). *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W7) was purchased from Axxora (Farmingdale, NY, USA). Desipramine, fluoxetine, citalopram, oxaliplatin and carboplatin were purchased from Sigma Aldrich (St. Louis, MO, USA). Cisplatin was synthesized as previously described (5).

Cell systems and culture conditions. HCT116(wt), HCT116 *p53* –/–, HT-29, A2780 and SKOV3 cells were used. The medium used for the HT-29 cells was McCoy's 5a Medium Modified with 10% FBS, 2 mmol/l L-glutamine, 100 U/ml penicillinin, 100 μg/ml streptomycin, 10 mmol/l HEPES buffer and 1 mmol/l sodium pyruvate (McCoy's; all from Biofluids). The medium used for all other cell types was RPMI-1640 with 10% FBS, 2 mmol/l L-glutamine, 100 U/ml penicillinin, 100 μg/ml streptomycin, 10 mmol/l HEPES buffer and 1 mmol/l sodium pyruvate (cRPMI; all from Biofluids). The cells were cultured in 175 cm^2 Cell Star tissue culture flasks from Greiner Bio-One (Frickenhausen, Germany) in an incubator with 5% CO_2 and humidified air.

Apoptosis studies. The present study broadly followed one previously published by our group (4). Cells were cultured in 6-well plates with 7.0×10^4 cells *per* well with 3 ml medium in each well. Cells were treated for 24 h, 48 h or 72 h with these conditions: untreated, platinum drug alone, antidepressant by itself, and platinum drug plus antidepressant. The platinum drugs were added to the medium after a

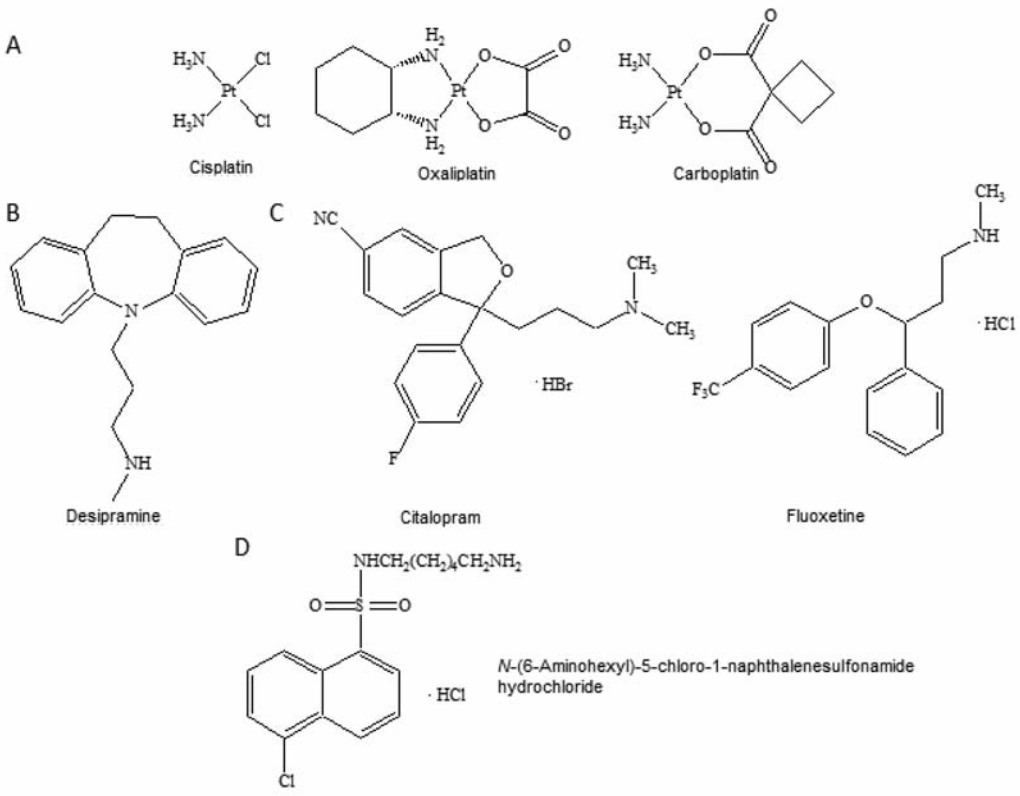


Figure 1. *Structures of the clinically used platinum drugs, antidepressants used in this study and the calmodulin inhibitor, W7. A: Structures of the three clinically used platinum anticancer drugs. B: Structure of the tricyclic desipramine. C: Structures of the selective serotonin re-uptake inhibitor antidepressants. D: Structure of W7, a calmodulin antagonist.*

1-h treatment period with the antidepressant. The specific concentrations used were chosen to give measurable apoptosis using individual platinum drugs after 48 h, and thus are dependent on the specific potency of each drug in the individual cell lines. Likewise, the concentrations of antidepressant used depended on the cell line used and varied to ensure the maximum observable response when combined with platinum drug. The concentrations of platinum drugs and antidepressants used for each individual cell line are indicated in the figures. At the defined time points, all cells (adherent and non-adherent) were collected. As described previously, the cells were fixed with an ethanol/FBS solution and stained with propidium iodide/RNAse A solution (4,6). The samples were analyzed for subdiploid DNA content using a Becton Dickinson FACScan flow cytometer (BD Biosciences, San Jose, CA, USA). This protocol allows the detection of intact *versus* fragmented DNA, which allows cell cycle analysis.

Results

Survey of cell lines. HT-29 Human Colon Carcinoma. The tricyclic antidepressant desipramine augmented the cytotoxicity of cisplatin and oxaliplatin in HCT116(wt) cells (4). Desipramine was also subsequently confirmed to enhance the cytotoxicity of carboplatin (Figure 2A). The interaction of desipramine with the three clinically-used platinum drugs was then investigated in HT-29 cells that respond differently to desipramine from HCT116(wt) (7, 8) (Figure 2). The inherent apoptosis caused by the drugs alone was significantly lower in HT-29 cells than that for HCT116 cells (4) but using a 72-h time point, as previously, the cisplatin/desipramine combination slightly reduced apoptosis compared to cisplatin alone (18% and 25%, respectively, Figure 2B). The case of oxaliplatin is the reverse – 23% and 9% for the combination and free oxaliplatin, respectively (Figure 2C). For carboplatin, the addition of desipramine had little effect (Figure 2D).

A2780 and SKOV3 human ovarian carcinomas. The A2780 cell line is generally considered to be sensitive to platinum drugs, as indicated by apoptosis induced by the drugs alone (Figure 3). Desipramine augmented the cytotoxicity of both cisplatin and carboplatin (Figure 3 A and C). In contrast, for oxaliplatin there was a significant increase in apoptosis at 48 h but at 72 h, there was no statistical difference between oxaliplatin and oxaliplatin plus desipramine (Figure 3B). The SKOV3 cell line is generally platinum-resistant and desipramine was unable to reverse resistance in the specific cell line, even at very high doses of platinum drugs (Figure 4).

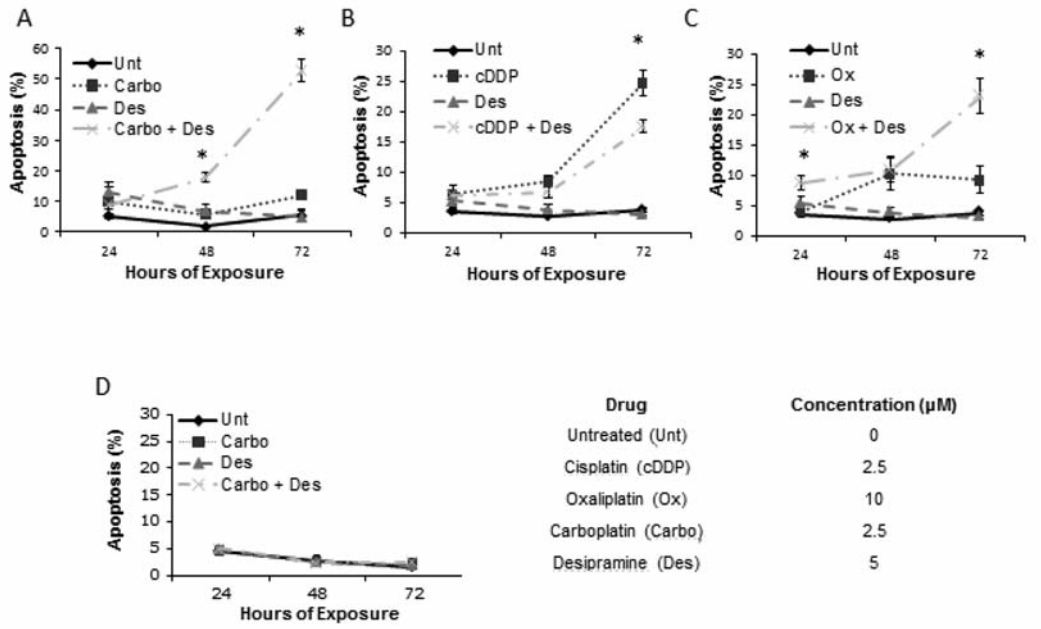


Figure 2. *Effects of the antidepressant desipramine on platinum drug cytotoxicity in colon cancer cells. A: Desipramine enhances the cytotoxicity of carboplatin in HCT116(wt) cells. Carboplatin and desipramine were 40 μM, see Materials and Methods. B, C and D: The effects of desipramine on platinum toxicity in HT-29 cells. The concentrations used are specified and were chosen to give measurable apoptosis using individual platinum drugs after 48 h, see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

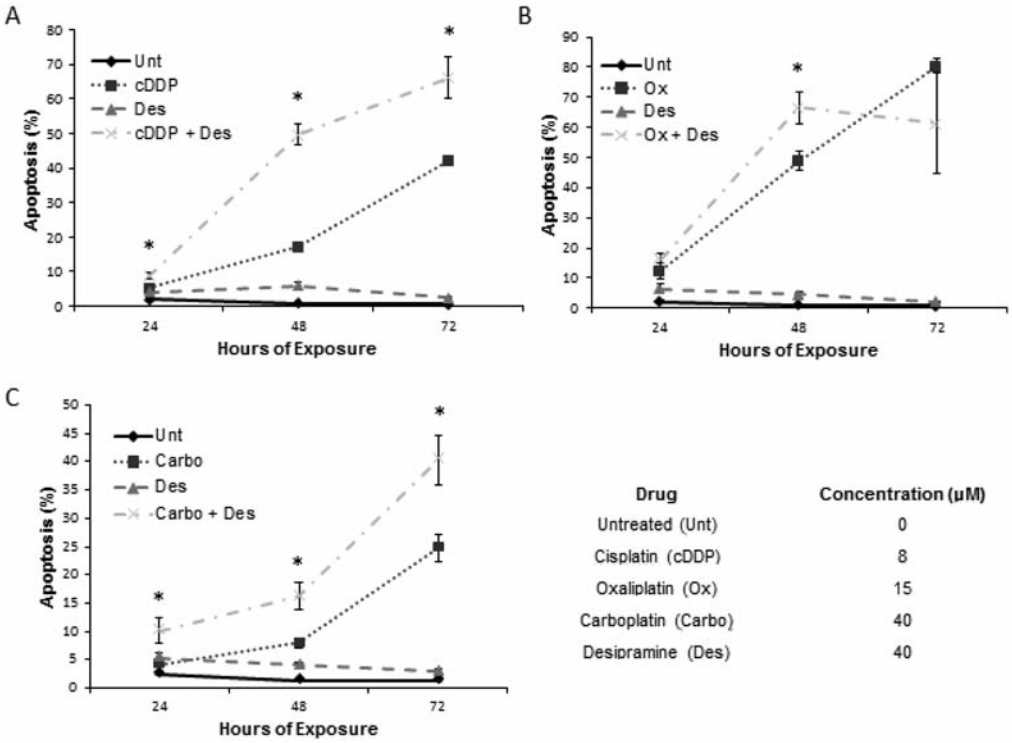


Figure 3. *Effects of desipramine on the cytotoxicity of cisplatin, oxaliplatin and carboplatin in A2780 cells. A: cisplatin plus desipramine. B: oxaliplatin plus desipramine. C: carboplatin plus desipramine. Concentrations indicated were chosen to give measurable apoptosis using individual platinum drugs after 48h, see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

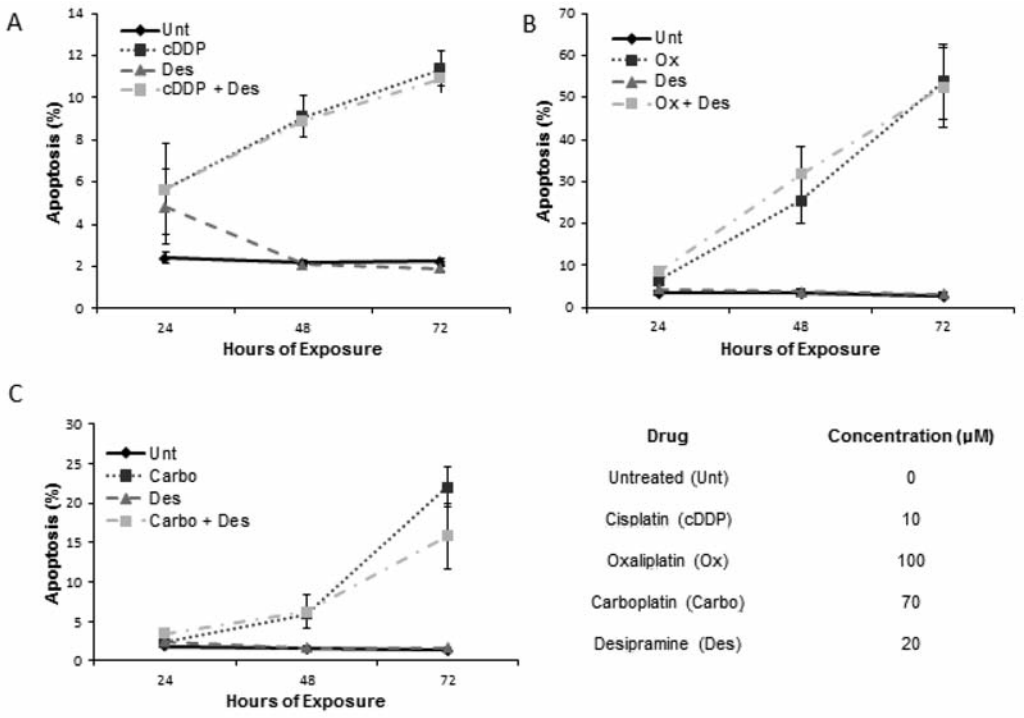


Figure 4. *Effect of desipramine on platinum toxicity in SKOV3 cells: A: cisplatin. B: oxaliplatin. C: carboplatin. Concentrations indicated were chosen to give measurable apoptosis using individual platinum drugs after 48 h, see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

Platinum drugs with other antidepressants. The other major class of clinically used antidepressants is that of selective serotonin re-uptake inhibitors (SSRIs), which are currently the ones most commonly prescribed. We, therefore, compared the effects of the tricyclics, as represented by desipramine, with selected SSRIs.

Citalopram in HCT116(wt) cells: In contrast to desipramine, time-course effects of citalopram appear to maximize after 48 h. The drug augments cisplatin cytotoxicity, but at 72 h, the apoptosis caused by the drug combination was not statistically different from that of the drug alone (Figure 5). In combination with citalopram, neither carboplatin nor oxaliplatin exhibited significant differences. If anything, the combination reduced the cytotoxicity of oxaliplatin.

Fluoxetine in HCT116(wt)cells: Fluoxetine (Prozac) augmented the cytotoxicity of both cisplatin and carboplatin in HCT116(wt) cells at all three time points investigated (Figure 6A and C). Fluoxetine has no effect on the cytotoxicity of oxaliplatin in HCT116(wt) cells (Figure 6 B).

In summary, the effects of the individual drugs as well as their combination with desipramine appear to be cell-line specific. Table I shows a summary of these data. There is also a significant difference between the individual SSRIs but fluoxetine had the most measureable effects on cisplatin cytotoxicity.

Table I. *Summary of cell line survey of desipramine effects on platinum drug cytotoxicity.*

	Drug combination		
Cell line	cDDP + Des	Ox + Des	Carbo + Des
HCT116 wt	++	++	++
HT-29	++	++	-
A2780	++	+	++
SKOV3	+	+	+

Note: + indicates that the percent apoptosis is greater than in the untreated control. ++ indicates that the combination is better than either drug alone. – indicates that the percent apoptosis is not significantly different from that observed in the untreated control. Concentrations used were as indicated in each Figure for each cell type.

Platinum drugs and desipramine in HCT116 p53 –/– cells. Our previous studies had shown that there was no direct relationship between the pharmacological factors affecting platinum drug cytotoxicity - such as plasma protein binding, cellular accumulation and/or the extent of cellular Pt-DNA binding - and the desipramine-mediated effects on platinum drug cytotoxicity (4). It is, therefore, necessary to examine the potential global biological responses that could be

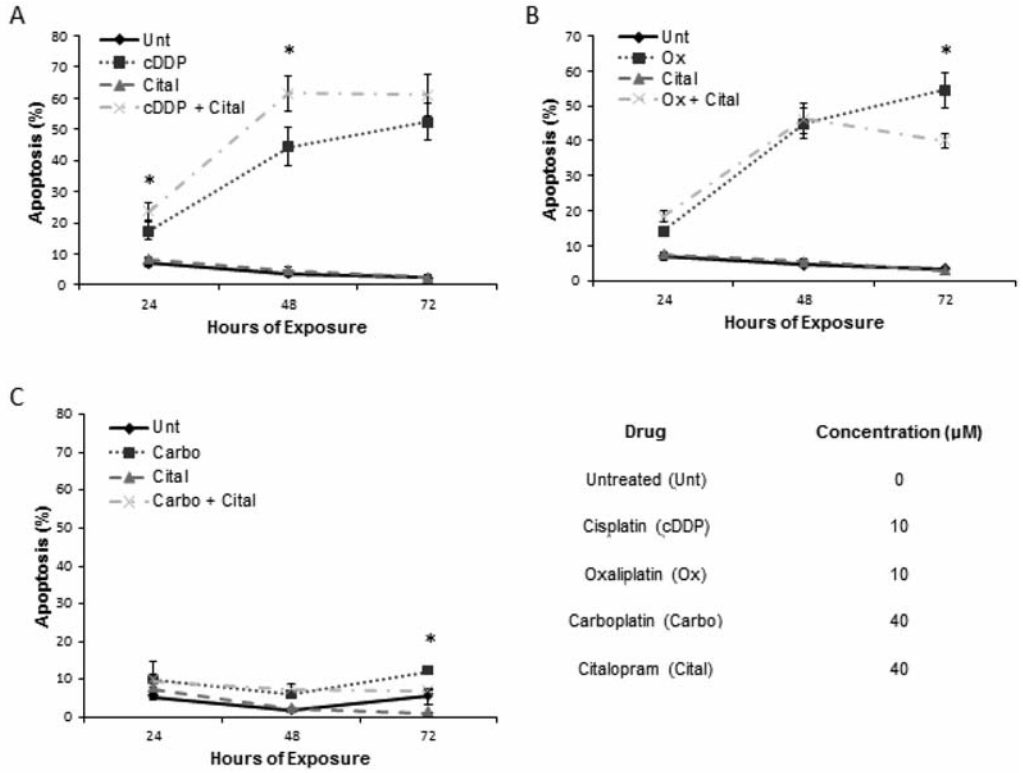


Figure 5. *Effects of citalopram on the cytotoxicity of platinum drugs in cisplatin, oxaliplatin and carboplatin in HCT116(wt) cells. A: cisplatin; B: oxaliplatin; C: carboplatin. Concentrations indicated were chosen to give measurable apoptosis using individual platinum drugs after 48 h, see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

augmented by desipramine. Platinum drugs are generally considered to elicit apoptotic responses *via* a p53-dependent pathway as a consequence of DNA modification (9). In contrast, while the molecular mechanism of desipramine apoptosis remains to be elucidated, it is unlikely to be a consequence of direct DNA targeting (7, 8). To investigate the role of p53, the interaction of tricyclic antidepressants on the cytotoxicity of platinum drugs was studied in the HCT116 *p53*–/– cell line. Desipramine augmented the cytotoxicity of all platinum drugs (Figure 7). However, the augmentation observed in the *p53*-knockout cell line was less than the one observed in the *p53* wild-type cell line.

Role of calmodulin inhibition. Global biological responses can be indirect, such as those of p53 elicited by platinum-drug DNA binding. The responses may also be direct where one or other drug is bound to a biomolecular target. In this sense, the role of calmodulin in possible mediation of cytotoxic effects of the platinum drug/antidepressant combination is of interest.

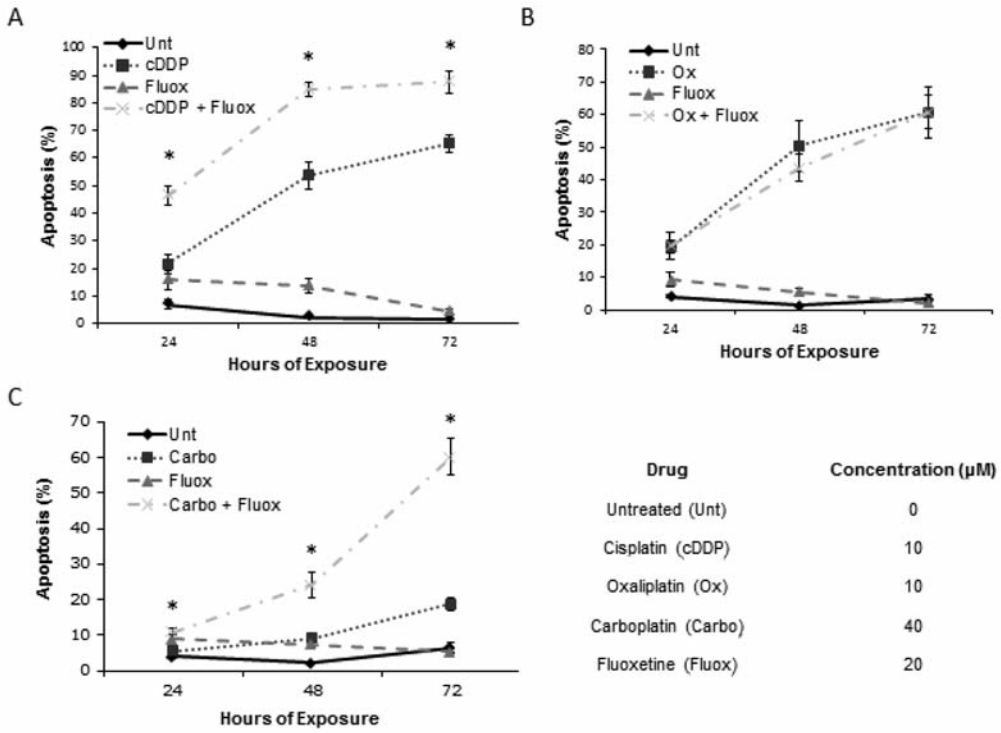
Cisplatin inhibits calmodulin conformational changes by direct interaction with the protein (10).

Cisplatin crosslinking may diminish the ability of calmodulin recognition of target proteins (11).

Early results also suggested that cisplatin cytotoxicity may be potentiated by trifluoperazine, a calmodulin inhibitor (12).

The tricyclic antidepressants (13) and fluoxetine (14) are also known to be calmodulin inhibitors. Furthermore, clomipramine, another tricyclic antidepressant and calmodulin inhibitor have been shown to increase the intracellular accumulation of vincristine and adriamycin in drug-resistant P388 (a murine leukemia cell line) tumor cells *in vitro*. This increased accumulation was associated with an increase in cytotoxicity, showing a partial reversal of resistance (15). More recently, a role for calmodulin-dependent protein kinases in the mechanism of action of desipramine and fluoxetine, at least in the hippocampus has been suggested (16).

Given this background, it was decided to investigate the role of calmodulin inhibition in the observed effects on cytotoxicity. To do this, apoptosis due to the platinum drugs was measured in the presence and absence of W7, a known calmodulin inhibitor (17), in both HCT116(wt) and *p53* –/– cells. In the HCT116(wt) cells, W7 augmented the



Drug	Concentration (μM)
Untreated (Unt)	0
Cisplatin (cDDP)	10
Oxaliplatin (Ox)	10
Carboplatin (Carbo)	40
Fluoxetine (Fluox)	20

Figure 6. *Effects of fluoxetine on cytotoxicity of platinum drugs in HCT116(wt) cells. A: cisplatin; B: oxaliplatin; C: carboplatin. Concentrations indicated were chosen to give measurable apoptosis using individual platinum drugs after 48 h, see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

cytotoxicity of cisplatin at all time points studied (Figure 8A). In contrast, W7 inhibited the cytotoxicity of oxaliplatin, while the effect of carboplatin plus W7 was not significantly different from that due to carboplatin alone at any of the three time points investigated. In the HCT116 *p53* –/– cells, the results are slightly different but again the combinations led to less apoptosis than in the wild-type line. W7 augmented the cytotoxicity of cisplatin, but not as much as in HCT116(wt) cells (Figure 8). Apoptosis due to the combination of oxaliplatin plus W7 was not significantly different from that due to oxaliplatin alone at any of the three time points investigated. It is interesting to note that W7 reduced the cytotoxicity of oxaliplatin in HCT116(wt) cells but not in HCT116 *p53* –/– cells. The combination of W7 plus carboplatin had little effect.

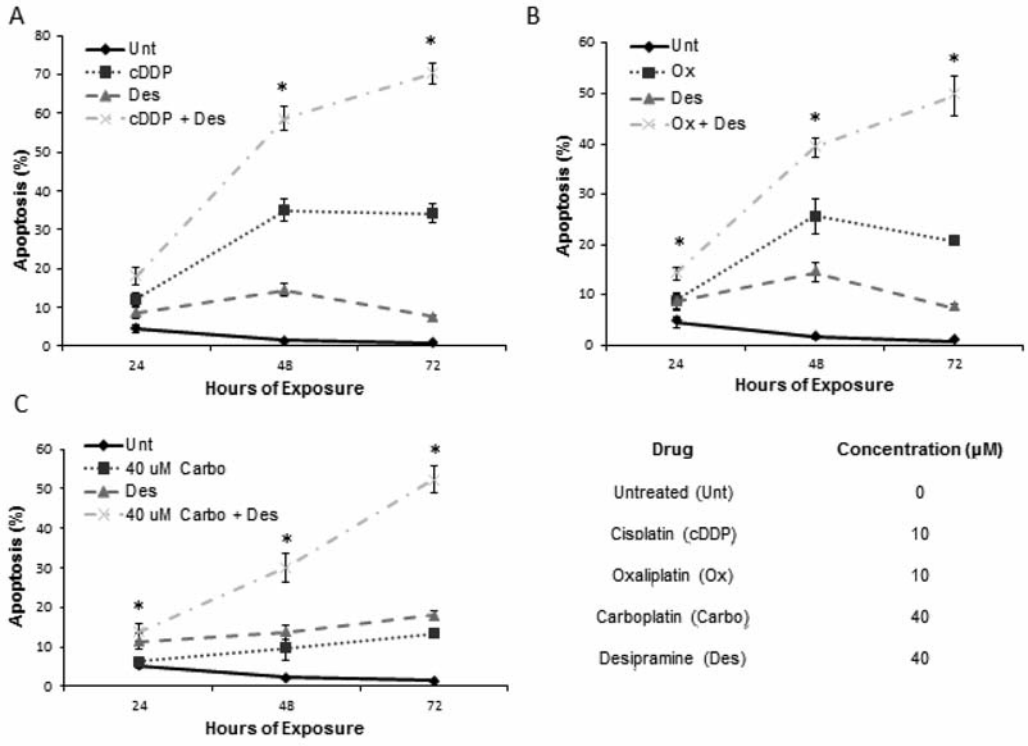
Discussion

The results presented here show that the effects of antidepressants and platinum drugs in combination are complicated but are clearly cell line- and platinum drug-specific. The HCT116(wt) and A2780 cells are clearly most sensitive to augmentation by desipramine. In HT-29 cells, however, the greatest effect of desipramine was on cisplatin. The platinum-resistant SKOV3 cell line is impervious to the effects of the antidepressant.

The observed effects are also drug-specific with regard to the antidepressants. Citalopram only enhanced cytotoxicity of cisplatin at the early time points, but even so, less than what was observed with desipramine. Fluoxetine on the other hand, strongly enhanced the cytotoxicity of cisplatin and carboplatin in HCT116(wt) cells. In contrast, fluoxetine had no effect on the cytotoxicity of oxaliplatin.

Detailed mechanistic investigations to explain these results at the molecular level are beyond the scope of this survey. Indeed, it is clear that drug and antidepressant combinations need to be examined on an individual basis. The presence or absence of p53 is not a major determining factor, although in general, the enhancement observed in *p53*-knock-out cells was less than what was seen in wild-type cells. This may suggest that the observed effect has two mechanisms at work: a p53-dependent mechanism and a p53-independent mechanism.

Calmodulin inhibition augmented the cytotoxicity of cisplatin in the HCT116(wt) and HCT116 *p53* –/– cells, with the enhancement again significantly less in the HCT116 *p53* –/– cells. Calmodulin inhibition by W7 resulted in reduction of oxaliplatin cytotoxicity in HCT116(wt) cells while having



Drug	Concentration (μM)
Untreated (Unt)	0
Cisplatin (cDDP)	10
Oxaliplatin (Ox)	10
Carboplatin (Carbo)	40
Desipramine (Des)	40

Figure 7. *Desipramine increases the cytotoxicity of platinum anti-cancer drugs in HCT116 p53–/– cells. A: cisplatin; B: oxaliplatin; C: carboplatin. Concentrations indicated were chosen to give measurable apoptosis using individual platinum drugs after 48 h, see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

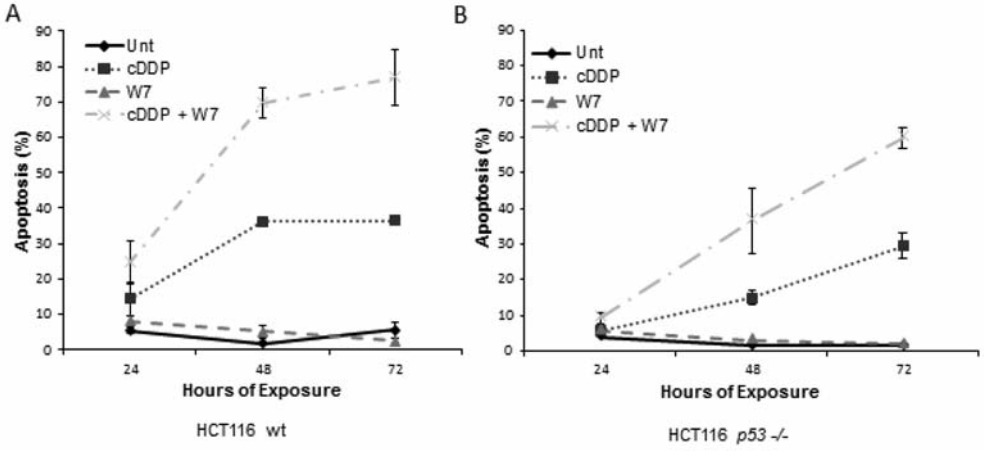


Figure 8. *Effects of the calmodulin inhibitor W7 on the cytotoxicity of cisplatin in colon cancer cells. A: HCT116(wt) cells. B: HCT116 p53–/– cells. Concentrations indicated were chosen to give measurable apoptosis by cisplatin after 48 h. see Materials and Methods. The data are means±SEM (n=9). *p-Value <0.05.*

little effect on HCT116 *p53* –/– cells. W7 had little or no effect on carboplatin cytotoxicity in either cell line.

In conclusion, these drug effects on the cytotoxicity of platinum drugs are both drug- and cell line-specific. The data suggest that there are multiple mechanisms involved in the observed effects. It is clear that there are both p53-dependent and -independent components to this mechanism, as well as a role for calmodulin inhibition. Furthermore, the platinum

drugs themselves may utilize these different mechanisms to different extents and the results may also be affected by the different pharmacokinetics of the three drugs. Nevertheless, we suggest that further research is necessary to elucidate the entire mechanism and discover what consequences this relevant drug-drug interaction may have on patient prognosis and quality of life.

Acknowledgements

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