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MODULATION OF CISPLATIN CYTOTOXICITY BY PERMEABILIZATION OF THE PLASMA MEMBRANE BY DIGITONIN *IN VITRO**

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Abstract—Killing of human ovarian carcinoma 2008 cells by cisplatin (DDP) is in direct proportion to the amount of drug entering the cell. DDP and its analogue [^{3}H]dichloro(ethylenediamine)platinum[II] ([^{3}H]-DEP) enter cells relatively slowly. We found that the uptake of [^{3}H]DEP into 2008 cells could be increased by treating the cells briefly with the plasma membrane-selective detergent digitonin. A similar effect was observed in an 11-fold DDP-resistant subline of 2008 cells, designated 2008/C13*5.25. A measurable effect was produced by concentrations as low as 5 μM, and 40 μM digitonin increased [^{3}H]-DEP accumulation at 1 hr by 4.4 ± 0.2- and 6.5 ± 0.7-fold (means ± SD) in 2008 and 2008/C13*5.25 cells, respectively. The effect was rapid, occurring within 1 min. Increased [^{3}H]DEP uptake was accompanied by increased platination of DNA (8.5-fold in 2008 cells and 18.5-fold in 2008/C13*5.25 cells), and by enhanced killing of both the DDP-sensitive and -resistant cells that was shown to be synergistic by median effect analysis. The combination index at 50% cell kill was 0.64 ± 0.14 (values <1 indicate synergy). We conclude that a brief exposure to digitonin can increase [^{3}H]DEP uptake *in vitro*, and can overcome the impaired [^{3}H]DEP accumulation associated with acquired DDP resistance. DDP and digitonin interact synergistically to increase tumor cell kill *in vitro*.

Decreased transport of cisplatin (DDP§) across the cell membrane plays an important role in the development of resistance to this agent [1, 2]. Many cell lines with acquired resistance to DDP have impaired cellular DDP accumulation (reviewed in Ref. 2), and this phenotypic change appears early during the development of resistance *in vivo* [3]. Because of its clinical importance, there has been interest in identifying agents that synergistically modulate the activity of DDP at the cellular level. Only a few such agents are known. A synergistic interaction between DDP and cytarabine has been reported [4–6]; however, this effect may be limited to a single cell line and its generality is controversial [7]. Anguidine [8] and forskolin [9] have been reported to enhance the cellular uptake of DDP, whereas uptake is blocked by aldehydes [10, 11] and ouabain [12]. Dipyridamole is also capable of enhancing the sensitivity of human ovarian carcinoma cells to DDP [13], and can markedly enhance the activity of DDP against human testicular and bladder cancers in xenograft models *in vivo* [14].

One strategy for restoring drug sensitivity is to increase the permeability of the cellular membrane in order to allow greater drug delivery into tumor cells. Melvik *et al.* [15] found that electropermeabilization of NHIK 3025 human uterine cells increased DDP accumulation and cytotoxicity. Other investigators have used detergents to enable exogenous substrates to enter cells [16, 17]. We have investigated the ability of one member of this class of drugs, digitonin, to permeabilize human ovarian carcinoma tumor cells to DDP and its radiolabeled analogue, [^{3}H]dichloro(ethylenediamine)platinum(II) ([^{3}H]DEP). Digitonin specifically binds to 3-11-α-hydroxysterol and permeabilizes cellular membranes in proportion to their cholesterol content. A potential advantage of using digitonin is that it has been reported to have no effect on cholesterol-poor membranes such as the endoplastic reticulum and the mitochondrial inner membrane when used at low concentrations [18]. Our results confirm that the cellular membrane is an effective barrier to DDP uptake, and demonstrate that permeabilization of this barrier with digitonin leads to both a marked increase in accumulation of DDP and its analogue, [^{3}H]DEP, and a synergistic enhancement of DDP cytotoxicity in the human ovarian carcinoma 2008 and 2008/C13*5.25 cell lines.

MATERIALS AND METHODS

Cell lines and clonogenic assay. Studies were conducted *in vitro* using the DDP-sensitive human ovarian cell line 2008 [19], and an 11-fold DDP-resistant subline of 2008 selected with DDP *in vitro* and designated 2008/C13*5.25 [12]. Cells were maintained in RPMI-1640 containing 5% bovine calf

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§ Abbreviations: CI, combination index; DDP, cisplatin; DEP, dichloro(ethylenediamine)platinum[II]; and PBS, phosphate-buffered saline, pH 7.9.

serum and 2 mM L-glutamine without antibiotics. Cultures were routinely tested for mycoplasma using the GenProbe™ kit (Gen Probe Inc., San Diego, CA), and found to be negative. Briefly, cells were harvested with trypsin, and seeded in triplicate in 60 mm plastic tissue culture dishes (Corning Glass Works, Corning, NY) at a density of 350 cells/dish. The cells were incubated under 5% CO_2 at 37°. After 10 days, the medium was aspirated from the dishes, and the resulting colonies were washed, fixed in methanol, and stained with Giemsa dye. Cell clusters containing 50 or more cells were scored as a colony.

Cellular accumulation of [^{3}H]*DEP.* [^{3}H]DEP was synthesized as previously described [20]. Cells (10^6) were seeded in 60 mm tissue culture dishes, and allowed to grow in log-phase until they became subconfluent. After 3 days, the dishes were exposed to heat at 65° for 15 min, or to various concentrations of digitonin for 10 min. The medium was aspirated and replaced by 2 mL of fresh 37° medium containing [^{3}H]DEP (0.2 μCi/mL, 5 μM). At appropriate time points, the medium was aspirated and plates were washed three times with ice-cold phosphate-buffered saline, pH 7.9 (PBS), at 0–4°. The cells were digested overnight in 1 mL of 1 N NaOH and a 100-μL aliquot was removed from protein determination [21]. The cellular [^{3}H]DEP accumulation was determined by liquid scintillation counting of the digested material after neutralization with an equal volume of 1 N HCl.

Effect of digitonin on cellular permeability. Kinetic analysis was done by flow cytometric measurement of forward light scatter and pulse width [22]. Log-phase cells were suspended at a concentration of 10^6 cells/mL in fresh medium, and kept at 37° in a water bath. The cells were excited with an argon laser in the UV move, at a wavelength of 350–360 nm. After a baseline observation period (10 sec), cell flow was interrupted to add the drug, and flow was restarted immediately. As controls, cells were lysed in 0.50 M NaOH or by making the medium 33% hypotonic by adding H_2O. Effects of 20 μM digitonin, as well as 20 and 100 μM dipyridamole were measured using a flow rate of 500 cells/sec for up to 100 sec.

The effect of digitonin on membrane permeability was further assessed by Trypan Blue staining. Log-phase cells were trypsinized and resuspended in fresh medium at a concentration of 500,000 cells/mL. After placing cells into Trypan Blue dye, digitonin was added. The cell-staining effect of 20 μM digitonin was followed by microscopy over 5 min.

Effect of digitonin on efflux of [^{3}H]*DEP.* Cells were loaded with [^{3}H]DEP by exposing them to 5 μM (5 μCi/mL) for 15 or 120 min. After the loading, cells were washed with cold PBS, and medium with or without 20 μM digitonin was replaced. The radioactivity remaining in the cells was determined at serial time points.

Median effect analysis. Median effect analysis was performed to formally assess the nature of the interaction between digitonin and DDP. Dose-response curves were determined for each agent alone, and in combination at a fixed ratio equivalent to the ratio of their IC_{50} values. Computer analysis of the dose–response curves was used to calculate the combination index (CI) at the level of 50% cell kill [23]. Values less than 1 indicate synergy, values equal to 1 indicate additivity, and values greater than 1 indicate antagonism.

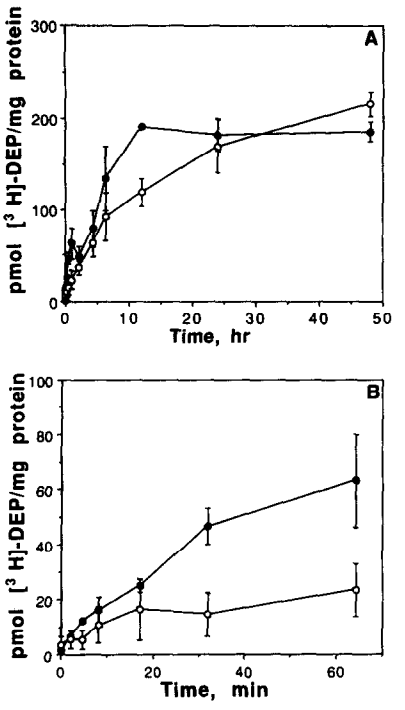

Fig. 1. Time course of [^{3}H]DEP uptake into 2008 (●) and 2008/C13*5.25 (○) cells. Panel A shows the uptake over the course of 48 hr, and panel B over the course of 65 min. Each point is the mean ± SD of at least 3 experiments performed in triplicate.

Measurement of DNA platination. Ovarian carcinoma cells (4×10^8) were treated with 3.3 mM DDP for 1 hr at 37° with or without concurrent exposure to 20 μM digitonin. DNA was extracted after digestion with proteinase K at 50° for 5 hr followed by phenol extraction, ethanol precipitation, and washing [24]. The level of DNA platination was determined by flameless atomic absorption spectroscopy with a graphite furnace [25].

RESULTS

Figure 1 shows the time course of [^{3}H]DEP uptake into 2008 human ovarian carcinoma cells, and the 11-fold DDP-resistant 2008/C13*5.25 subline. When the cells were incubated in 5 μM [^{3}H]DEP, uptake was relatively slow, and steady-state was not reached until 24 hr for the 2008 cells and 48 hr for the 2008/C13*5.25 cells. The rate constant determined from the initial uptake rate was 0.000331 ± 0.000047 min^{-1} (mean ± SEM) for the 2008 cells and 0.000082 ± 0.000023 min^{-1} (mean ± SEM) for the 2008/C13*5.25, reflecting the fact that the initial uptake into the DDP-resistant cells was only 25% of that in the sensitive 2008 cells. Since the plasma

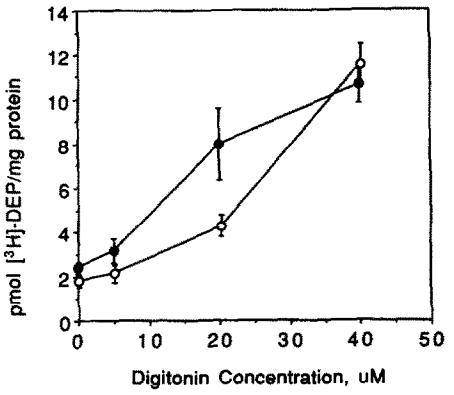

Fig. 2. Effect of digitonin on [^{3}H]DEP uptake as a function of concentration. Cells 2008 (●) and 2008/C13*5.25 (○) were exposed to various concentrations of digitonin for 10 min, then the medium was aspirated and [^{3}H]DEP was added for 60 min followed by washing. Each point is the mean ± SD of 3 experiments performed in triplicate.

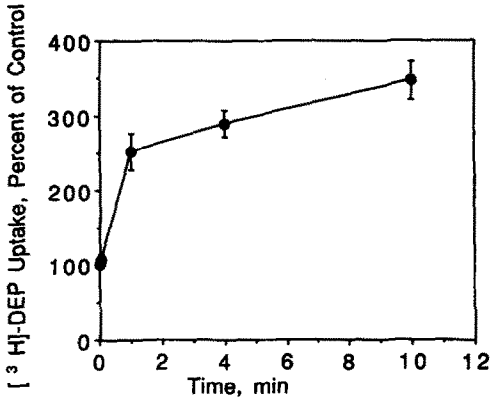

Fig. 3. Time course of digitonin effect on DEP uptake. The 2008 cells were exposed to 20 μM digitonin for various times, and then the accumulation of [^{3}H]DEP was determined over the ensuing 10 min. Each point is the mean ± SD of three experiments performed in triplicate. Control value (100%): 2.2 pmol [^{3}H]DEP/mg protein.

half-life of the free biologically active species of DDP is 26 ± 21 min (mean ± SD) in humans [26], it is the uptake that occurs over the first several hours that is clinically relevant. For purposes of comparison, the influx rate constant for 2008 cells for doxorubicin was 2.98 ± 0.51 min^{-1} (mean ± SD), whereas those for etoposide and vinblastine were 1.53 ± 0.15 and 8.22 ± 3.54 min^{-1} (mean ± SD), respectively [27]. Although all four drugs are thought to enter cells by passive diffusion, despite the smaller molecular weight of DDP (DDP, 300; doxorubicin, 543; etoposide, 588; and vinblastine, 927) some element of the cell constitutes a >4,600–24,800-fold greater barrier to its entry than to that of these other representative chemotherapeutic agents.

Digitonin was selected for use as a plasma membrane selective permeabilizing detergent, and Fig. 2 shows the ability of digitonin to enhance [^{3}H]-DEP uptake into both 2008 and 2008/C13*5.25 cells. A 10-min preincubation with digitonin was sufficient to increase accumulation of [^{3}H]DEP. Digitonin increased the uptake in a concentration-dependent manner; 20 and 40 μM increased [^{3}H]DEP uptake in the 2008 cells by 3.4 ± 0.6- and 4.4 ± 0.2-fold (mean ± SD), respectively. The effect of low concentrations (≤20 μM) was somewhat less in the 2008/C13*5.25 cells, but was equivalent in both cell types at 40 μM. Permeabilization of the plasma membrane by exposure to heat (65° for 15 min prior to initiation of [^{3}H]DEP exposure at 37°) produced an increase in the uptake of [^{3}H]DEP that was similar to that observed with 40 μM digitonin (results not shown). The effect of various durations of digitonin pretreatment on the [^{3}H]DEP uptake at 10 min into 2008 cells is shown in Fig. 3. An increase in [^{3}H]DEP uptake was evident by 1 min after the start of digitonin exposure, and permeabilization continued to increase for up to 10 min. Visual examination of the cells after exposure to 20 μM digitonin showed a dramatic increase in cell size over the first 2 min, consistent with the rapid membrane permeabilizing effect of this agent [28]. Furthermore, the effect of a 10-min digitonin treatment was assessed at 0, 1 hr, 4 hr, 8 hr, and 24 hr after digitonin exposure to determine how long the cells stayed permeable (data not shown). At 8 hr, approximately 50% of the cells could still be stained, but at 24 hr no cells were stainable by Trypan Blue.

The effect of detergents on membrane integrity can be quantitated with great sensitivity by flow cytometry as either a change in forward light scatter or pulse width due to osmotic swelling. Panel A in Fig. 4 shows that dipyridamole, a drug that increases DDP uptake without altering membrane permeability, caused no change in pulse width or light scatter. In contrast, 20 μM digitonin increased both parameters within 60 sec (panel B); a concentration of 80 μM lysed the cells (data not shown). Lysis of the cells with NaOH caused the expected complete loss of signal (panel C), and hypotonic lysis in water caused osmotic swelling followed by loss of signal (panel D). Thus, the time course of the effect of digitonin on [^{3}H]DEP uptake was consistent with a rapid loss of membrane integrity as measured by inability to maintain the osmotic equilibrium of the cell.

It was expected that if digitonin was non-specifically increasing membrane permeability, it should enhance [^{3}H]DEP efflux as well as influx. The data presented in Fig. 5 show that this was in fact the case. Digitonin enhanced efflux both when the cells had been loaded for only 15 min, at which time 53 ± 24% (mean ± SD) of the total intracellular [^{3}H] was ultrafiltrable, and when the cells have been loaded for 2 hr, at which time 27 ± 15% (mean ± SD) was ultrafiltrable. However, while 20 μM digitonin increased influx by 340% in 2008 cells, it enhanced efflux over the first 60 min by a maximum of only 30%.

The data in Table 1 show that digitonin also produced a large increase in DNA platination. When

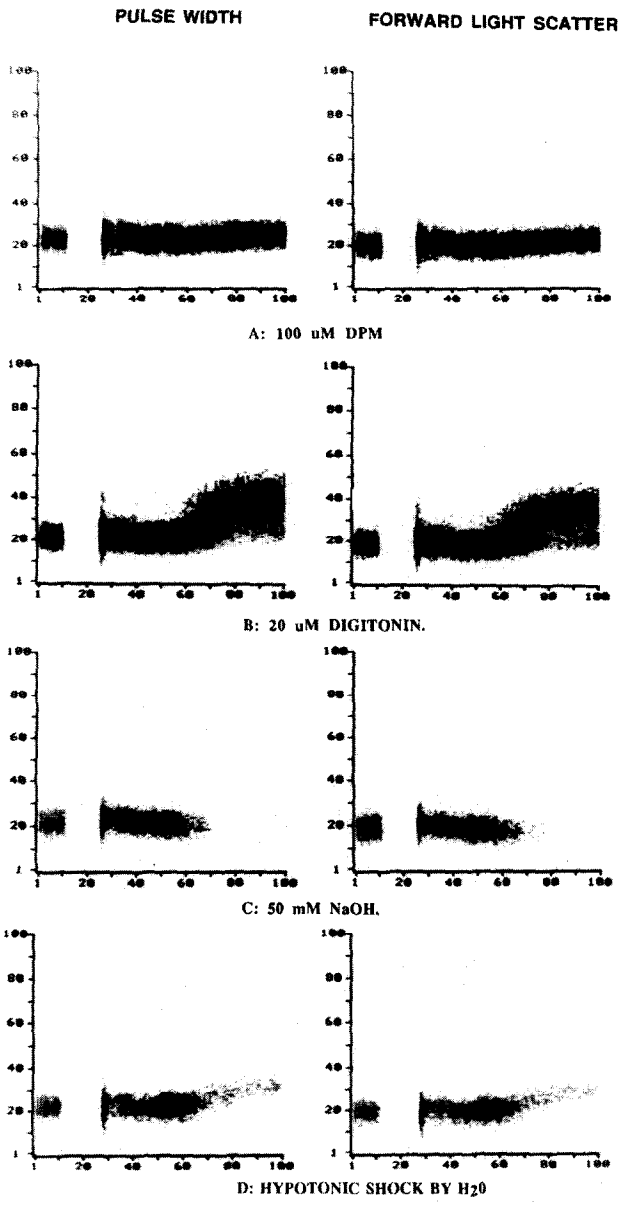

Fig. 4. Determination of cell permeability by forward light scatter and pulse width measurements. At the 10-sec time point, cell flow was interrupted to add the drug, and flow was restarted immediately. Panel A shows the effect of 100 μM dipyridamole, panel B the effect of 20 μM digitonin, panel C the effect of 50 mM NaOH, and panel D the effect of hypotonic lysis.

cells were treated with 20 μM digitonin for 10 min prior to the start of a 1-hr exposure to 3.3 mM DDP, there was a 8.5-fold increase in DNA platination in the 2008 cells, and a 18-fold increase in the 2008/C13*5.25 cells, indicating that the enhanced intracellular accumulation of DDP resulted in the increased delivery of DDP to at least one of its cytotoxic targets, DNA. However, it was noteworthy that while permeabilization of the plasma membrane restored the platination of DNA in the DDP-resistant cells to a level above that observed in the untreated DDP-sensitive cells, the absolute level of platination produced by digitonin pretreatment was still less in the resistant than in the sensitive cells.

The ability of digitonin to increase [^{3}H]DEP uptake and DNA platination led to the prediction that digitonin and DDP would produce synergistic killing of malignant cells. Since both agents are independently cytotoxic, median effect analysis was used to formally determine the nature of the interaction between these two drugs [29]. Median effect analysis permits calculation of the CI; CI

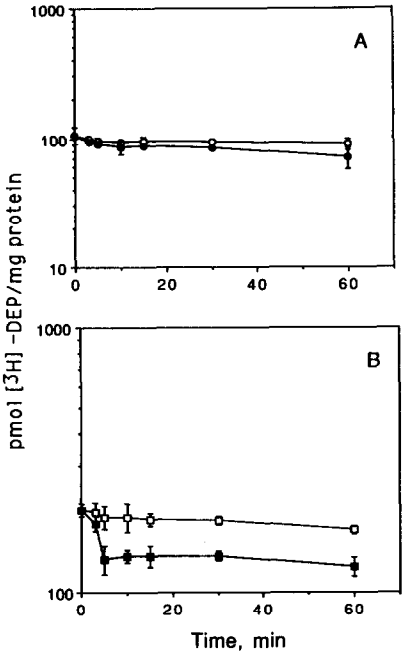

Fig. 5. Efflux of [^{3}H]DEP in 2008 cells. Cells were loaded by exposure to DEP for 15 min (panel A) or 120 min (panel B). After loading, cells were washed three times with cold PBS and medium containing no DEP was replaced. At the indicated times, cells were washed again and the remaining radioactivity was determined. Open symbols, no digitonin; closed symbols, 20 μM digitonin. Each point is the mean ± SD of 3 separate experiments performed with triplicate cultures.

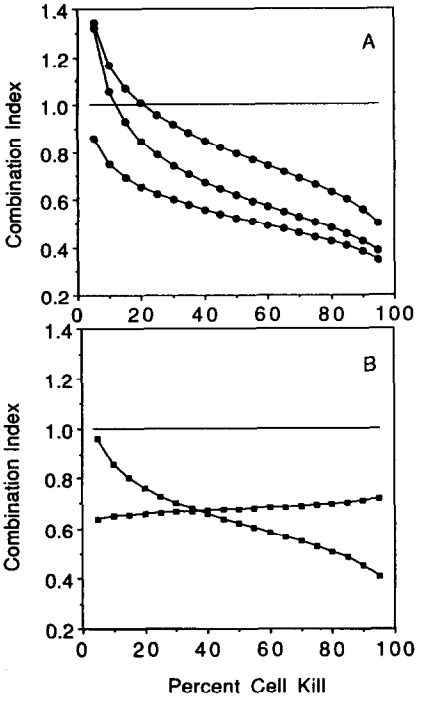

Fig. 6. Combination index for 2008 cells (panel A) and 2008/C13*5.25 cells (panel B) exposed to digitonin and DDP at a molar ratio of 1:7.5 as a function of the extent of cell kill. Each curve represents a separate experiment using triplicate cultures for each data point.

Table 1. Effect of digitonin on DNA platination

Cell line	Digitonin concentration (μM)	Adducts/kb
2008	0	10.72 ± 2.5*
	20	91.07 ± 3.8†
C13*5.25	0	3.43 ± 0.38
	20	61.85 ± 5.2†

* Mean ± SD, N = 3.
† $P < 0.005$, relative to control.

values of less than 1 indicate synergy, and the smaller the value of CI the greater the synergy. The IC_{50} for a 10-min exposure to digitonin was 3.4 μM and for a 1-hr exposure to DDP it was a 0.1 μM. Figure 6 shows that digitonin and DDP interacted in a highly synergistic manner in the killing of 2008 cells, with the magnitude of the synergy increasing with percent cell kill. The mean CI at the level of 50% cell kill was 0.64 ± 0.14 (SD) (panel A). In comparison, for the DDP-resistant 2008/C13*5.25 cells the CI at 50% cell kill was 0.65 ± 0.04 (panel B), indicating that exposure to these agents in combination was capable of producing synergy in both drug-sensitive and DDP-resistant tumor cells. The raw data from the different median effect experiments are shown as separate lines in Fig. 6. Such analyses often show a great deal of variance, particularly on both ends of the curves. The curves were statistically significantly different ($P < 0.05$) from 1.0 across 40–90% cell kill in 2008 cells, and across 10–95% in 2008/C13*5.25 cells.

DISCUSSION

The mechanism(s) by which DDP enters cells is not understood completely. Based on the fact that the initial uptake rate does not saturate with increasing DDP concentration, and that closely related analogues do not compete for uptake, many investigators have concluded that uptake occurs primarily by passive diffusion [30–32]. However, the uptake of DDP can be rapidly stimulated by activation of the protein kinase A signal transduction pathway [9], indicating that some component of uptake may be mediated by a regulatable channel or transporter.

The experiments reported here establish that some barrier, putatively the plasma membrane, constitutes a significant obstacle to DDP uptake even in DDP-sensitive cells. Cellular accumulation of [^{3}H]DEP was relatively slow compared to such natural products as doxorubicin, etoposide, and vinblastine, all of which have much higher uptake rate constants [27].

We have reported previously that, using ^{195m}Pt-DDP, the initial accumulation rate in the 11-fold DDP-resistant 2008/C13*5.25 cells was 11% of that in the parental 2008 cells [13]. The studies reported here confirm that the DDP analogue, [^{3}H]DEP, enters cells with similar kinetics and detects the same impairment of accumulation as observed with DDP itself.

Digitonin increased [^{3}H]DEP uptake very rapidly and in a concentration-dependent manner in both the 2008 and 2008/C13*5.25 cells. This increase of drug uptake occurred at the same time as the increase in permeability detected by flow cytometry. This confirms the work of Melvik *et al.* [15] who showed that DDP uptake could be increased by permeabilizing the membrane by electroporation, and is consistent with the work of Riehm and Biedler [33] who showed that the detergent polyoxyethylene-sorbitan monooleate (Tween 80) could sensitize resistant Chinese hamster ovary sublines to actinomycin D and daunomycin.

The increase in [^{3}H]DEP uptake was accompanied by a proportional increase in DNA platination, indicating that the extra [^{3}H]DEP entering the cell had ready access to the chromatin and was not just sequestered into subcellular compartments. Median effect analysis demonstrated that digitonin and DDP interacted in a truly synergistic manner with respect to cell killing in both the DDP-sensitive and -resistant cells, and that this synergy was of substantial magnitude. This result is entirely consistent with the observation that digitonin increased [^{3}H]DEP uptake and DNA platination, but it must be remembered that it may also result from an effect of DDP on the mechanism by which digitonin kills cells.

Comparison of the effect of digitonin on the DDP-sensitive 2008 and DDP-resistant 2008/C13*5.25 cells is noteworthy for several points. First, digitonin was able to overcome completely the impairment of drug accumulation observed in the 2008/C13*5.25 cells. However, despite the fact that 2008 and 2008/C13*5.25 cells do not differ with respect to the relative abundance of the major classes of membrane lipids [34], at digitonin concentrations of $\leq 20\ \mu M$, there was still a difference in the absolute amount of [^{3}H]DEP entering the cells. It required a higher concentration of digitonin (40 μM) to equalize uptake in the two cell types. This difference between the cell types was also reflected by the measurements of DNA platination. While 20 μM digitonin markedly increased the extent of platination in both cell types (from 11 to 91 adducts/kb in the 2008 cells, and 3 to 62 adducts/kb in the 2008/C13*5.25 cells), the extent of platination in the resistant cells was still only 68% of that observed in the sensitive cells.

While permeabilization of the plasma membrane with digitonin markedly increased [^{3}H]DEP uptake, it had a more modest effect on the efflux of [^{3}H]-DEP, despite the fact that a significant fraction of the total intracellular [^{3}H] remained ultrafiltrable following 15 min or 2 hr of [^{3}H]DEP exposure. The intracellular pharmacology of [^{3}H]DEP is not well understood, and the mechanism of this difference in effect on influx and efflux is unknown. However, this difference supports the concept of using digitonin to enhance drug delivery to tumor cells, since digitonin does not enhance washout of the drug to the same extent that it increases uptake.

In summary, it is clear that the plasma membrane acts as a barrier to DDP accumulation, and potentially limits the extent of DNA platination resulting from clinically relevant exposures to DDP. Brief exposure to digitonin increased [^{3}H]DEP uptake and synergistically enhanced the *in vitro* killing of both cells intrinsically sensitive to DDP and cells with acquired DDP resistance. These results are consistent with the hypothesis that increased drug delivery results in augmented tumor cell killing. Although digitonin is not currently used in the clinic, preliminary animal studies (Jekunen A and Howell SB, unpublished) indicated that C57Bl/6 mice tolerate doses of up to 40 mg/kg. The very rapid permeabilization produced by relatively low concentrations of digitonin suggests several therapeutic applications, the most important of which is intra-arterial chemotherapy. DDP is administered intra-arterially for the treatment of head and neck cancers and hepatocellular carcinoma [35, 36]. Because all of the advantage of an intra-arterial injection accrues to the tumor during the first passage of the drug through the tumor capillaries, injection of digitonin immediately before DDP may effectively increase drug delivery. Furthermore, it is diluted extensively as it enters the systemic circulation. *In vivo* studies with digitonin and other plasma membrane active agents are indicated in pursuit of this novel strategy.

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REFERENCES

1. Perez RP, Godwin AK, Hamilton TC and Ozols RF, Ovarian cancer biology. *Semin Oncol* **18**: 186–204, 1991.
2. Andrews PA and Howell SB, Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. *Cancer Cells* **2**: 35–43, 1990.
3. Andrews PA, Jones JA, Varki NM and Howell SB, Rapid emergence of acquired *cis*-diammine-dichloroplatinum(II) resistance in an *in vivo* model of human ovarian carcinoma. *Cancer Commun* **2**: 93–100, 1990.
4. Bergerat J-P, Drewinko B, Corry P, Barlogie B and Ho DH, Synergistic lethal effect of *cis*-dichlorodiammineplatinum and 1-β-D-arabinofuranosylcytosine. *Cancer Res* **41**: 25–30, 1981.
5. Bergerat J-P, Green C and Drewinko B, Combination chemotherapy *in vitro*. IV. Responses of human colon carcinoma cells to combinations using *Cis*-diamminedichloroplatinum. *Cancer Biochem Biophys* **3**: 173–180, 1979.
6. Drewinko B, Green C and Loo TL, Combination chemotherapy *in vitro* with *cis*-dichlorodiammineplatinum(II). *Cancer Treat Rep* **60**: 1619–1625, 1976.
7. Howell SB and Gill S, Lack of synergy between cisplatin and cytarabine against ovarian carcinoma *in vitro*. *Cancer Treat Rep* **70**: 409–410, 1985.

8. Hromas R, Meyn R, Jenkins S and Barlogie B, Anquidine enhances cis-platinum-induced DNA crosslinks in Chinese hamster ovary cells. *Proc Am Assoc Cancer Res* **25**: 370, 1984.
9. Mann SC, Andrews PA and Howell SB, Modulation of *cis*-diamminedichloroplatinum(II) accumulation and sensitivity by forskolin and 3-isobutyl-1-methylxanthine in sensitive and resistant human ovarian carcinoma cells. *Int J Cancer* **48**: 866–872, 1991.
10. Dornish JM, Melvik JE and Pettersen EO, Reduced cellular uptake of *cis*-dichlorodiammineplatinum by benzaldehyde. *Anticancer Res* **6**: 583–588, 1986.
11. Dornish JM and Pettersen EO, Protection from *cis*-dichlorodiammineplatinum-induced cell inactivation by aldehydes involves cell membrane amino groups. *Cancer Lett* **29**: 235–243, 1985.
12. Andrews PA, Velury S, Mann SC and Howell SB, *cis*-Diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Cancer Res* **48**: 68–73, 1988.
13. Howell SB, Vick J, Andrews PA, Velury S and Sanga R, Biochemical modulation of cisplatin. In: *Platinum and Other Metal Co-ordination Compounds in Cancer Chemotherapy* (Ed. Nicolini M.), pp. 228–234. Martinus Nijhoff, Boston, 1987.
14. Keane TE, Rosner G, Donaldson JT, Norwood DL, Poulton SH and Walther PJ, Dipyridamole-cisplatin potentiation: Enhanced *in vivo* cytotoxicity in xenograft models of human testicular and bladder cancers. *J Urol* **144**: 1004–1009, 1990.
15. Melvik JE, Pettersen EO, Gordon PB and Seglen PO, Increase in *cis*-dichlorodiammineplatinum(II) cytotoxicity upon reversible electropermeabilization of the plasma membrane in cultured human NHIK 3025 cells. *Eur J Cancer Clin Oncol* **22**: 1523–1530, 1986.
16. Keeney S and Linn S, A critical review of permeabilized cell systems for studying mammalian DNA repair. *Mutat Res* **236**: 239–252, 1990.
17. Lepers A, Cacan R and Verbert A, Permeabilized cells as a way of gaining access to intracellular organelles: An approach to glycosylation reactions. *Biochimie* **70**: 1–5, 1990.
18. Vercesi AE, Bernardes CF, Hoffmann ME, Gadelhat FR and Docampo R, Digitonin permeabilization does not affect mitochondrial function and allows the determination of the mitochondrial membrane potential of trypanosoma cruzi *in situ*. *J Biol Chem* **26**: 14431–14434, 1991.
19. Disaia PJ, Sinkovics JG, Rutledge FN and Smith JP, Cell-mediated immunity to malignant cells. *Am J Obstet Gynecol* **114**: 979–989, 1972.
20. Eastman A, Characterization of the adducts produced in DNA by *cis*-diamminedichloroplatinum(II) and *cis*-dichloro(ethylenediamine)platinum(II). *Biochemistry* **22**: 3927–3933, 1983.
21. Bradford MM, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
22. Shapiro HM, *Flow Cytometry*, Vol. 2. Alan R. Liss, New York, 1988.
23. Chou J and Chou T-C, *Dose–Effect Analysis with Microcomputers*. Elsevier Science Publishers BV, Amsterdam, 1986.
24. Ausubel MM, Preparation of genomic DNA from mammalian tissue. In: *Current Protocols in Molecular Biology* (Ed. Ausubel R), Vol. 1, Suppl. 9, pp. 2.2.1–2.2.3. John Wiley, New York, 1990.
25. Andrews PA, Wung WE and Howell SB, A high-performance liquid chromatographic assay with improved selectivity for cisplatin and active platinum (II) complexes in plasma ultrafiltrate. *Anal Biochem* **143**: 46–56, 1984.
26. Goel R, Cleary SM, Horton C, Kirmani S, Abramson I, Kelly C and Howell SB, Effect of sodium thiosulfate on the pharmacokinetics and toxicity of cisplatin. *J Natl Cancer Inst* **81**: 1552–1560, 1989.
27. Howell SB, Hom D, Sanga R, Vick JS and Abramson IS, Comparison of the synergism potentiation of etoposide, doxorubicin, and vinblastine cytotoxicity by dipyridamole. *Cancer Res* **49**: 3178–3183, 1989.
28. Schultz I, Permeabilizing cells: Some methods and applications for the study of intracellular processes. *Methods Enzymol* **192**: 280–300, 1990.
29. Chou T-C and Talalay P, Quantitative analysis of dose–effect relationships; The combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* **22**: 27–55, 1984.
30. Pinto AL and Lippard SJ, Binding of the antitumor drug *cis*-diamminedichloroplatinum(II) (cisplatin) to DNA. *Biochim Biophys Acta* **780**: 167–180, 1985.
31. Gale GR, Morris CR, Atkins LM and Smith AB, Binding of an antitumor compound to cells as influenced by physical factors and pharmacologically active agents. *Cancer Res* **33**: 813–818, 1973.
32. Binks SP and Dobrota M, Kinetics and mechanism of uptake of platinum-based pharmaceuticals by the rat small intestine. *Biochem Pharmacol* **40**: 1329–1336, 1990.
33. Reihm H and Biedler JL, Potential of drug effect by Tween 80 in Chinese hamster cells resistant to actinomycin D and daunomycin. *Cancer Res* **32**: 1195–1200, 1972.
34. Mann SC, Andrews PA and Howell SB, Comparison of lipid content, surface membrane fluidity, and temperature dependence of *cis*-diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian cells. *Anticancer Res* **8**: 1211–1216, 1988.
35. Cheung DK, Regan J, Savin M, Gibberman V and Woessner W, A pilot study of intra-arterial chemotherapy with cisplatin in locally advanced head and neck cancers. *Cancer* **61**: 903–908, 1988.
36. Kajanti M, Rissanen P, Virkkunen P, Franssila K and Mantyla M, Regional intra-arterial infusion of cisplatin in primary hepatocellular carcinoma: A phase II study. *Cancer* **58**: 2386–2388, 1986.