Pharmacological Basis for a Novel Therapeutic Strategy Based on the Use of Aquated Cisplatin

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ABSTRACT

In pursuit of a strategy for increasing delivery of platinum drugs to tumors, we compared the cytotoxicity, extent of cellular uptake, and DNA platination of native cisplatin (DDP) and aquated cisplatin (aqDDP) in human head and neck carcinoma UMSCC10b cells. AqDDP was 1.8-fold more toxic than DDP when tested against UMSCC10b cells in vitro. At high concentrations, aqDDP uptake was 3-fold more rapid than that of DDP; uptake of DDP and aqDDP was nonsaturable up to a concentration of 1600 μM. AqDDP produced 6.4-fold more platination of DNA than did DDP at the same concentration, suggesting that once inside the cell, aqDDP was 2-fold more effective at producing adducts in DNA than the native drug. Despite the paradox that aqDDP, which contains some charged species, entered the cell more rapidly than did neutral native DDP at high concentrations, studies on the effect of temperature, ATP depletion, and sulfhydryl group blockade did not provide evidence for uptake of aqDDP via a channel or transporter. AqDDP was more nephrotoxic to mice than DDP; however, s.c. administration of sodium thiosulfate protected against this toxicity and permitted a 7-fold escalation of aqDDP dose. These studies provide the preclinical basis for a novel therapeutic strategy based on the regional intraarterial or intracavitary administration of aqDDP in combination with a systemic neutralizing agent.

INTRODUCTION

DDP is a square planar coordination complex containing a central Pt atom surrounded by two chloride atoms and two ammine groups in the cis orientation. The mechanism by which DDP crosses the plasma membrane has not been clearly defined. It was originally proposed that DDP enters cells by passive diffusion (1, 2); however, DDP uptake can be modulated by a variety of factors, suggesting that the mechanism of uptake may also involve a carrier or channel (3–6). In comparison to most other clinically used chemotherapeutic agents, the cellular uptake of DDP is quite slow (7).

DNA has been recognized as the major molecular target for DDP, and its ability to platinate DNA is believed to be the main basis for its cytotoxicity. Once inside the cell, DDP undergoes aquation, aided by the low intracellular chloride concentration, to produce monoaquo and diaquo forms in which one or both of the chloride groups is replaced by a water molecule (8). Among the DDP analogues, potency is strongly related to the ability of water to displace the leaving groups. The aquation products are believed to be the forms of the drug that actually react with DNA, and aquation is the rate-limiting step (9). The cis configuration of DDP favors the formation of 1,2 guanine-guanine intrastrand cross-links as well as that of several other types of intrastrand and interstrand adducts that locally distort the DNA by unwinding and bending it (2). Platination interferes with a variety of DNA functions, including replication and transcription, and triggers a cellular injury response that involves activation of a G1 checkpoint and the apoptotic pathway, resulting in cell death. A good correlation between the extent of DNA platination and cytotoxicity has been well documented in cancer cell lines (10–15).

In an effort to increase DDP delivery to tumors, we have used the approach of producing extremely high local concentrations to drive the drug into the tumor down a steep concentration gradient by regionally administering DDP i.p. (16, 17) or intraarterially (18, 19) in very large doses (e.g., 200 mg/m2). To protect the systemic circulation from such large doses, we have developed the strategy of administering sodium thiosulfate i.v. at the same time, taking advantage of the fact that DDP reacts covalently with thiosulfate to produce a complex that remains soluble but is no longer toxic (16, 20).

It has been known for some time that aqDDP is more nephrotoxic than DDP due to its greater reactivity (21, 22). Based on the hypothesis that its increased reactivity may make aqDDP a better agent for regional drug delivery than DDP, we undertook a study to compare the toxicity of aqDDP and DDP against human UMSCC10b head and neck carcinoma cells in vitro and the relative nephrotoxicity of these two forms of the drug in a murine model with and without concurrent thiosulfate systemic protection.

MATERIALS AND METHODS

Chemicals and Reagents. Pure DDP powder was provided by Johnson-Matthey Inc. (Malvern, PA). 2-Deoxy-D-glucose, dinitrophenol, iodoacetic acid, mersalyl acid, ouabain oc-
tahydrate, sodium azide, sodium thiosulfate, and sulforhodamine B were obtained from Sigma Chemical Co. (St. Louis, MO). RPMI-1640 was obtained from Irvine Scientific (Santa Ana, CA), and fetal bovine serum was obtained from Life Technologies, Inc. (Gaithersburg, MD).

Drug Preparation. Stock solutions of DDP were prepared by dissolving the crystalline DDP powder in 0.9% NaCl at a concentration of 1 mg/ml. AqDDP was prepared by dissolving the pure DDP in water at the same concentration and incubating the solution in the dark for a minimum of 24 h (23).

Cell Cultures. The UMSCC10b cell line was derived from a human head and neck squamous cell carcinoma (24) and was generously provided by Dr. Gerrit Los of University of California San Diego Cancer Center. Cells were cultured in RPMI 1640 supplemented with 2 mM glutamine, 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 10% fetal bovine serum. Cells were maintained in a humidified incubator at 37°C in a 5% CO2 atmosphere. For the ATP depletion study, cells were incubated with glucose-free DMEM during the 1-h treatment with ATP-depleting agents.

Cytotoxicity Assay. Cells were seeded into 96-well plates at a density of 1000 cells/well in 100 μl of medium and allowed to grow for 24 h. Appropriate concentrations of drugs were then added in a final volume of 10 μl of medium/well. Control plates were fixed as described below to determine the cellular protein at the start of drug exposure. Immediately before treatment, DDP and aqDDP were diluted into the medium; cells were exposed for 1 h, after which the drug-containing medium was removed and replaced with the drug-free fresh medium, and the cultures were allowed to grow for an additional 48 h. Growth was stopped by adding 25 μl of 50% (w/v) trichloroacetic acid, and cellular protein was stained with sulforhodamine B and measured by spectrophotometry with an ELISA plate reader (25).

The relative growth rate (r) was calculated as previously reported (26). Each experiment was performed in triplicate. IC50 values were estimated by linear interpolation at r = 0.5.

Pt Accumulation. Cells were seeded in T-25 or T-75 cell culture flasks and grown until they approached confluence, at which point the medium was aspirated and replaced with RPMI 1640 containing the indicated concentrations of DDP or aqDDP. The flasks were immediately returned to the incubator, and exactly 1 h later, the cells were harvested by trypsinization, washed twice with PBS (Oxoid, Columbia, MD) at 4°C, and lysed with 1 N NaOH overnight. The cell lysate was centrifuged at 16,000 × g for 15 min to remove insoluble elements. DNA samples were obtained from the retro-orbital sinus 72 h after drug administration. BUN levels were measured with a BUN enzymatic rate assay kit (Sigma) according to the procedures provided by the manufacturer. Animals were sacrificed at 72 h, and the kidneys were removed, weighed, and fixed in buffered neutral formalin for histological examination.

Statistical Analysis. Comparison between control and experimental groups was made by Student's t test, assuming unequal variance. The 0.05 level of probability was used as the criterion of significance.

RESULTS

Under the conditions of aquation used in this study, the solution of DDP incubated in water for 24 h is expected to contain a mixture of approximately 60% dihydroxo and 38% monoqua/monohydroxo forms of the drug, with ∼<2% monoa-quo and diaquo forms (31). This mixture is identified as aqDDP in this report. The cytotoxicity of native DDP and aqDDP was compared against human UMSCC10b head and neck carcinoma cells in vitro. Fig. 1 shows the relative growth rate during the 48-h period after a 1-h exposure to drug as measured by the sulforhodamine B assay. Stock solutions of DDP and aqDDP were diluted into the medium at the start of the 1-h drug exposure. AqDDP was 1.8-fold more toxic than DDP against
Fig. 1 Relative cytotoxicity of DDP and aqDDP to UMSCC10b cells. Cytotoxicity was quantitated by sulforhodamine B assay after a 1-h exposure to the drug. Each data point and error bar, mean ± SD for three individual experiments, each conducted with triplicate cultures.

Fig. 2 Pt accumulation in UMSCC10b cells as a function of DDP and aqDDP concentration. Cells were exposed to drug for 1 h at 37°C. Each data point, mean ± SD of from four to seven individual experiments, each performed with duplicate cultures. * P < 0.001 for the difference in uptake between DDP and aqDDP.

Fig. 3 Pt accumulation in DNA isolated from UMSCC10b cells after a 1-h exposure to 800 μM DDP or aqDDP. Each bar, mean ± SD of four experiments performed with duplicate cultures. P < 0.01 for the difference in DNA platination by the two forms of the drug.

aqDDP was examined in cells exposed to 800 μM drug for 1 h, and the results are presented in Fig. 3. Compared to DDP-treated cells, aqDDP-exposed cells contained 6.4-fold greater amounts of Pt in their DNA.

We next sought to determine whether the uptake of aqDDP was any more or less energy-dependent than that of DDP by modifying the cellular ATP level. A panel of metabolic inhibitors and combinations of inhibitors was tested to identify a treatment that was effective in depleting ATP in UMSCC10b cells. Exposure to DNP, NaF, ouabain, or orthovanadate alone at concentrations up to those that caused substantial cytotoxicity reduced the ATP level by less than 50%, and none of these single-agent treatments altered the uptake of DDP or aqDDP (data not shown). Combinations of two agents resulted in more effective ATP depletion, and Fig. 4A shows that incubation of cells in glucose-containing medium with iodoacetic acid (300 μM) and DNP (250 μM) or in a glucose-free medium containing sodium azide (10 mM) and 2-deoxy-o-glucose (10 mM) decreased the cellular ATP level by more than 91 and 84%, respectively. Fig. 4B shows that pretreatment of UMSCC10b cells with sodium azide and 2-deoxy-o-glucose, although effective in depleting ATP by up to 84%, did not alter the uptake of either DDP or aqDDP. However, another combination treatment, DNP (250 μM) and iodoacetic acid (300 μM), which was capable of depleting ATP by 91%, produced a reduction in the uptake of both DDP and aqDDP. Uptake of DDP was reduced to 72% of control, whereas uptake of aqDDP was reduced to 48% of control. These results are consistent with the conclusion that neither DDP nor aqDDP uptake is highly sensitive to cellular ATP level. It should be noted that iodoacetate can react with a variety of targets in the cell and that the decrease in the uptake of aqDDP observed with the combination of DNP and iodoacetate may not be attributable solely to the reduction in ATP.

DDP is a neutral molecule, whereas aqDDP contains at least some charged species. If the uptake of either was mediated by a specific transporter, then one might expect a moderate to
high degree of temperature sensitivity. Cells were exposed to 800 μM DDP or aqDDP for 1 h at 4°C, 15°C, 22°C, and 37°C, and the total cellular uptake of Pt was determined. As shown in Fig. 5, the Pt uptake for both DDP and aqDDP was clearly temperature dependent, and over the temperature range examined, the uptake of aqDDP was consistently higher than that of DDP. Although the temperature coefficient (Q10) for aqDDP uptake was quite constant (Q10 = 2.5 between 4°C and 22°C, and Q10 = 2.8 between 22°C and 37°C), the temperature coefficient for the uptake of DDP varied more markedly. Over the lower temperature range (between 4°C and 22°C), it was relatively low (Q10 = 1.6), whereas over the physiologically relevant temperature range (22°C and 37°C) the Q10 value increased to a value of 6.7. These results are consistent with substantially different mechanisms of cellular uptake at normal body temperature for the two forms of the drug.

The integrity of several heavy metal transporters is dependent on the maintenance of reduced sulfhydryl groups in extracellular domains (32-34). Mersalyl acid, a membrane sulfhydryl-reactive agent to which cells are impermeable, has been reported to be an inhibitor of cadmium uptake in cadmium-sensitive Chinese hamster V79 cells (33). The effect of mersalyl acid on the uptake of DDP and aqDDP was examined in UMSCC10b cells. Mersalyl acid had no effect on the uptake of either DDP or aqDDP (data not shown); likewise, in these cells, there was no inhibition of the uptake of cadmium measured over 30 or 60 min at concentrations of 10, 50, and 250 μM using 1 μCi/ml 109Cd as the tracer by concentrations of mersalyl acid up to 400 μM on a schedule in which the cells were pretreated for 30 min, and mersalyl acid exposure was continued concurrent with the 1-h exposure to cadmium (data not shown).

On the basis of the more rapid cellular uptake of aqDDP and the increased extent of DNA platination, one would expect aqDDP to be more toxic than DDP in vivo, assuming that the pharmacokinetics of the two agents are reasonably similar. B6D2F1 mice were treated with increasing doses of either DDP or aqDDP, and the extent of renal damage was determined 72 h later by histopathological examination of the kidneys, the kidney:body weight ratio, and serum BUN level. Compared to the untreated control animals, there were no significant histological changes in the kidneys of animals given DDP at doses up to 10 mg/kg. However, 10 mg/kg aqDDP produced clear signs of histological damage manifested as congestion and characteristic proximal tubular necrosis (Fig. 6). A dose-related increase in kidney:body weight ratio was observed in aqDDP- but not in DDP-treated mice (Fig. 7A); this ratio was significantly greater for the mice given aqDDP at doses of 5 or 10 mg/kg than that
for untreated control animals and for mice treated with the same doses of DDP. In addition, aqDDP produced a dose-dependent increase in serum BUN level. As shown in Fig. 8A, mice treated with 10 mg/kg aqDDP sustained a 5.2-fold increase in BUN level compared to that of the control animals \( P < 0.001 \). This represents a 5.4-fold increase \( P < 0.001 \) over the change in BUN observed in mice given the same dose \( (10 \text{ mg/kg}) \) of DDP.

To determine whether sodium thiosulfate \( (\text{Na}_2\text{S}_2\text{O}_3) \) can confer protection against aqDDP-induced nephrotoxicity, we conducted a separate experiment in which B6D2F1 mice were given a fixed dose of \( \text{Na}_2\text{S}_2\text{O}_3 \) at 1000 mg/kg via the s.c. route 5 min before the i.p. injection of aqDDP at increasing doses of up to 24 mg/kg. It was found that this dose of \( \text{Na}_2\text{S}_2\text{O}_3 \) effectively reduced aqDDP-induced nephrotoxicity. In contrast to the marked proximal tubular necrosis produced by 10 mg/kg aqDDP in the absence of \( \text{Na}_2\text{S}_2\text{O}_3 \) pretreatment, there was less significant change in kidney histology even at the highest aqDDP dose of 24 mg/kg when given with \( \text{Na}_2\text{S}_2\text{O}_3 \) (Fig. 6). In addition, Fig. 7B shows that \( \text{Na}_2\text{S}_2\text{O}_3 \) prevented the aqDDP-induced increase in kidney:body weight ratio. Moreover, as shown in Fig. 8B, in the mice pretreated with \( \text{Na}_2\text{S}_2\text{O}_3 \), there was no increase in serum BUN up to an aqDDP dose of 18 mg/kg, and even at the highest dose of 24 mg/kg, the BUN was increased only 2-fold over baseline.

DISCUSSION

The major findings to emerge from the experiments reported here are that: (a) aqDDP was 1.8-fold more toxic than DDP when tested against UMSCC10b cells \textit{in vitro}; (b) at high concentrations, aqDDP entered UMSCC10b cells 3-fold more rapidly than DDP; (c) the uptake of DDP and aqDDP was nonsaturable up to a concentration of 1600 \( \mu \text{M} \); (d) based on the fact that aqDDP produced 6.4-fold more DNA platination than DDP at the same concentration, it seems that once inside the cell, aqDDP was 2-fold more effective at producing adducts in DNA than the native drug, but that these adducts were 3-fold less cytotoxic; (e) the effect of temperature on uptake differed for the two forms of the drug; and (f) ATP depletion had little effect on the uptake of either DDP or aqDDP. The results of this study pose somewhat of a paradox. DDP is a neutral molecule, and the lack of saturation and energy dependence of uptake are consistent with passive diffusion across the plasma membrane. In contrast, the monoaquo/monohydroxo form of the drug, estimated to constitute 38% of the Pt species present in aqDDP, carries a positive charge, and one would expect its ability to diffuse across the plasma membrane to be less than that of DDP. Nevertheless, the experimental results indicate that aqDDP enters UMSCC10b cells more readily than does DDP at high
Fig. 7  Kidney-body weight ratio 72 h after treatment with DDP or aqDDP. Each data point, mean ± SD of measurements made in three to four mice. A, mice received a single i.p. injection of DDP or aqDDP at the indicated doses. *, P < 0.05 between aqDDP-treated and untreated groups and between the aqDDP- and DDP-treated groups at the same doses. B, mice were pretreated with sodium thiosulfate (1000 mg/kg) by s.c. injection 5 min before administration of aqDDP via the i.p. route at the indicated doses. ◆, mice treated with DDP; ■, mice treated with aqDDP.

Fig. 8  Serum BUN 72 h after mice were treated with either DDP or aqDDP. Each data point, mean ± SD of samples obtained from three to four mice. A, mice received a single i.p. injection of DDP or aqDDP at the indicated doses. *, P < 0.001 between aqDDP-treated and nontreated mice and between aqDDP- and DDP-treated groups at the same doses. B, mice were pretreated with sodium thiosulfate (1000 mg/kg) by s.c. injection 5 min before administration of aqDDP via the i.p. route at the indicated doses. *, P < 0.01 between aqDDP-treated mice at 24 mg/kg and all the lower aqDDP dose groups. ◆, mice treated with DDP; ■, mice treated with aqDDP.

cellular concentrations. This suggests that either the monoaquo/monohydroxo form is entering the cell via a channel or transporter or that the dihydroxo form enters the cell sufficiently more rapidly than native DDP to offset any impairment in the uptake of the monoaquo/monohydroxo form due to its charge.

The fact that aqDDP is more toxic than native DDP to cells in vitro and in whole animal studies has been documented by several previous studies (21, 22, 35, 36). However, the monoaquo/monohydroxo species present in aqDDP is a highly reactive molecule, and it has not been clear whether its toxicity is a result of platination of elements of the plasma membrane accessible to extracellular drug or as a consequence of damage produced intracellularly after its entry into the cell. Although it does not exclude the possibility that aqDDP damages components of the plasma membrane, the data presented here argue cogently that aqDDP actually has greater access to the interior of the cell than DDP. The finding that, per unit of drug taken up, aqDDP formed approximately twice as many adducts in DNA as did native DDP is also of interest. Although increased reactivity with isolated DNA is expected of aqDDP, the aquated species are
also expected to react more readily with glutathione, sulfhydryl-containing proteins such as the metallothioneins and thioredoxin, and the nucleophilic sites on a variety of other molecules. One might have expected the enhanced reactivity of aqDDP to actually result in less platination of DNA, on the basis that the reactive species present in aqDDP are more reactive with intracellular thiols than with native DDP and thus at greater risk of being inactivated as they make their way through the cytoplasm before having a chance to react with DNA. AqDDP can be converted back to DDP if placed in a chloride-containing solution. Thus, some fraction of the aqDDP added to the tissue culture medium was probably converted back to native drug before it could enter the cell, and the actual difference in the initial rate of uptake is possibly greater than the 3-fold difference observed in these studies.

Studies on Pt accumulation and DNA platination in human ovarian 2008 cells exposed to aqDDP versus the native species of DDP have been reported by two other laboratories. Using aqDDP prepared by dissolving pure DDP in 150 mM NaNO₃ at a concentration of 5.58 mM for 24 h and then adding the drug to chloride-deficient medium (0.25 mM chloride), Jennerwein and Andrews (31) found that when cells were exposed to 100 μM total drug, none of the aqDDP species of DDP was preferentially transported but that species that had undergone extracellular aquation, once they entered the cell, were more reactive with DNA, producing a 1.9-fold increase in DNA adduct formation. Shirazi et al. (38) observed that when pure DDP was aquated by incubation in water and then added at a concentration of 1.6 mM to medium containing physiological concentrations of chloride concurrently with the start of drug exposure, the uptake of aqDDP was 1.2–6.7-fold greater than that of DDP. Our results are consistent with both reports in that no difference in uptake was observed at 100 μM, whereas enhanced uptake of aqDDP was readily apparent at 400 μM and above. Why differential uptake should become apparent at high drug concentrations is not clear. One possibility is that one of the species that has undergone extracellular aquation becomes a substrate for a transporter or channel only when present at high concentration. Another possibility is that such high concentrations produce membrane damage that differentially permits the entry of the species found in aqDDP.

We sought evidence for uptake of aqDDP via a channel or transporter by examining the effects of energy depletion, temperature reduction, and disruption of sulfhydryl groups on the outer leaflet of the plasma membrane on its cellular accumulation using native DDP as a control. Treatment of these cells with different single ATP-depleting agents (DNP, NaF, ouabain, or orthovanadate) reduced cellular ATP level by 40–50% but had no effect on the uptake of either form of the drug. Moreover, whereas combined treatment with sodium azide and 2-deoxy-D-glucose successfully depleted ATP by up to 84%, this also failed to reduce the cellular uptake of either DDP or aqDDP. These results are consistent with the conclusion that neither form of the drug was highly dependent on ATP for uptake and that accumulation of neither was likely to be limited under normal conditions by an ATP-requiring externally oriented transporter.

Consistent with results reported by other investigators (39), the uptake of DDP was significantly affected by lowering the temperature. Temperature reduction also affected the uptake of aqDDP; however, there were significant differences between the two forms of the drug. As the temperature was reduced below 37°C, the uptake of DDP was initially reduced to a greater extent (Qₘₚ 6.7) than that of aqDDP (Qₘₚ 2.8), indicating somewhat greater temperature sensitivity of the mechanism underlying DDP than that for aqDDP uptake. As the temperature was reduced below 22°C, the situation reversed. Although the temperature sensitivity of aqDDP uptake remained relatively constant (Qₘₚ 2.5), that of DDP fell (Qₘₚ 1.6). The biophysical significance of these results is difficult to interpret; however, they are not supportive of the concept that enhanced uptake of aqDDP is mediated by a channel or transporter, because these would reasonably be expected to demonstrate greater temperature sensitivity than the passive diffusion process that seems to account for a significant component of native DDP uptake.

Heavy metal transporters in several systems are dependent on the integrity of sulfhydryl groups in proteins on the outer part of the plasma membrane, as demonstrated by sensitivity to the membrane-impermeable sulfhydryl blocker mersalyl acid (32–34). In the case of DDP and aqDDP uptake in UMSCC1Ob cells, mersalyl acid at concentrations shown to be effective in other cell types had no effect on cellular accumulation. Because UMSCC1Ob cells do not express a mersalyl-sensitive cadmium transporter, the attempt to use cadmium uptake as a positive control for mersalyl effect failed, and no other mersalyl-sensitive transporter has been identified in these cells at present.

As a first step toward evaluating aqDDP as a candidate for this novel therapeutic strategy, we compared the toxicity of aqDDP to that of DDP. Our results are consistent with both reports in that no difference in uptake was observed at 100 μM, whereas enhanced uptake of aqDDP was readily apparent at 400 μM and above. Why differential uptake should become apparent at high drug concentrations is not clear. One possibility is that one of the species that has undergone extracellular aquation becomes a substrate for a transporter or channel only when present at high concentration. Another possibility is that such high concentrations produce membrane damage that differentially permits the entry of the species found in aqDDP.

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DDP and aqDDP to the dose-limiting organ in the mouse, which is the kidney. By all three measures of nephrotoxicity, including histological changes, kidney/body weight ratio, and BUN, aqDDP was more toxic than DDP: based on the latter two parameters, it was approximately 4-fold more toxic. On the other hand, s.c. injection of a modest dose of thiosulfate 5 min before i.p. administration of aqDDP provided clear protection against this toxicity. At 1000 mg/kg thiosulfate, administration permitted an approximate 7-fold increase in dose before an equivalent degree of nephrotoxicity was produced based on BUN measurement. In similar experiments done in the past (20), we demonstrated that this dose of thiosulfate was able to provide nearly complete protection against the nephrotoxicity produced by a single dose of 20 mg/kg DDP, but the relative ability of thiosulfate to protect against DDP- versus aqDDP-induced nephrotoxicity has not been compared directly. Further improvement in the therapeutic index of aqDDP given by regional administration may be attainable by optimizing the relative aqDDP and thiosulfate doses. Because aqDDP is more reactive and thus is expected to be inactivated more easily by sodium thiosulfate, it will be of importance in future studies to determine in an in vivo tumor model to what extent the systemic administration of sodium thiosulfate will decrease the uptake of aqDDP compared to that of DDP when the Pt drug is administered regionally, and the thiosulfate is administered systemically.

In summary, the studies reported here provide the preclinical basis for the development of a novel therapeutic strategy based on the local administration of aqDDP in combination with a systemic neutralizing agent. This strategy is likely to be of interest in the treatment of i.p. ovarian cancer, other tumors confined to a body cavity, and head and neck tumors with disease encompassed by the distribution of a single artery.

ACKNOWLEDGMENTS

We are grateful to Johnson-Matthey Inc. for providing pure DDP, to Dr. Gerrit Los for his generous support in providing the UMSCC10b cell line, and to Dennis Health for his technical assistance in this study.

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Pharmacological basis for a novel therapeutic strategy based on the use of aquated cisplatin.

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