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Dose schedule evaluation of metoclopramide as a potentiator of cisplatin and carboplatin treatments of xenografted squamous cell carcinomas of the head and neck

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The potentiating effect of metoclopramide on the tumor growth inhibition of cisplatin has been studied on human squamous cell carcinomas xenografted to nude mice. In this system, the optimal time interval for intraperitoneal administration of metoclopramide was 8 h after intraperitoneal administration of cisplatin. The optimal single dose level of metoclopramide in this study was 2 mg/kg. Metoclopramide enhanced the cytotoxic effect of cisplatin at all cisplatin doses tested between 2.5 and 7.5 mg/kg body weight. Under experimental conditions that gave optimal sensitization of cisplatin-induced cytotoxicity, there was no potentiation of the cytotoxic effect with metoclopramide in combination with carboplatin. There is great similarity in the cytotoxic action of cisplatin and carboplatin, with the main difference being a much slower rate of formation of DNA crosslink formation following carboplatin exposure. Hence the data reported here support an important role for the kinetics of formation and repairability of DNA damage as part of the mechanism of metoclopramide sensitization of platinum-containing drugs.

Key words: ADP-ribosylation, carboplatin, cisplatin, DNA repair, metoclopramide.

Introduction

Benzamide derivatives such as 3-aminobenzamide and nicotinamide have been shown to potentiate the effect of ionizing radiation[1] and alkylating agents.[2] The potentiating effect of these drugs are believed to be caused by their inhibiting effect on the DNA associated enzyme poly-adenosine-diphosphoribosyl-transferase (poly-ADPRT).[3,4] These drugs have not come to any clinical use since they are required in high and often toxic doses for potentiation.

Metoclopramide (MCA) is a *N*-carboxamide substituted benzamide derivative. These derivatives are not known to inhibit poly-ADPRT.[5] It is one of the most commonly used antiemetics for treatment of cytotoxic drug-induced emesis. Current dose recommendations for use as an antiemetic in cancer patients are 1–3 mg/kg body weight, given every 2 h up to a total of five doses.[6,7] MCA is well tolerated, and the side effects are rapidly reversible by stopping the drug, or with drugs such as diphenhydramine.[6]

The mechanism behind the antiemetic effect of MCA is not entirely understood. It has been classified as a dopamine$_2$ receptor antagonist.[8] The high dose treatment with MCA used to treat cisplatin-induced emesis is believed to be caused by antagonizing the 5-hydroxytryptamine$_3$ receptor.[9] This ability can explain the central antiemetic effect by the inhibition of input to the vomiting center from the chemoreceptor trigger zone. The peripheral effects of MCA are multifactorial and may include facilitation of acetylcholine release, as

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well as the sensitization of the muscarinic receptors of gastrointestinal smooth muscles, causing a constriction of the lower esophageal sphincter, promotion of gastric emptying and decreased bowl transit time.[10]

In previous papers, we have shown that MCA, at a dose of 2 mg/kg body weight, potentiates the effect of cisplatin[11] and ionizing radiation[12] without detectable increase of normal tissue toxicity. These effects were shown on human squamous cell carcinomas (SCC) xenografted to nude mice. The dose of MCA used in these studies was set at 2 mg/kg to equal the dose currently used in the clinic for the treatment of cisplatin-induced emesis.[6] MCA was given as a triple-dose regime: at the same time, 24 and 48 h after a single dose of cisplatin.

The aim of the present study was to examine the relationships between MCA and cisplatin important to the understanding of the function of MCA's sensitizing effect of cisplatin-induced cytotoxicity, and to collect data toward the development of a possible clinical use of MCA as a cisplatin sensitizer. We have examined the optimal timing of MCA in relation to cisplatin administration, and also in relation to the optimal MCA dose. The effect of MCA with increasing cisplatin doses has also been evaluated.

Cisplatin and carboplatin are two square planar heavy metal complexes containing a central platinum atom. They are thought to be converted intracellularly to the same active substance and therefore interact with DNA in the same way.[13] The reactive group of carboplatin (cyclobutane-di-carboxyl) is more stable than the two chlorine atoms present at the reactive sites of cisplatin. This difference leads to a slower formation of crosslinks in the DNA following carboplatin exposure. We have examined the possibility that carboplatin, with slower DNA binding kinetics, can be sensitized by MCA in the same *in vivo* system that was used to study the potentiating effect of MCA on cisplatin treatment.

Materials and methods

Mice

Five- to 8-week-old, male and female, BALB/c nude mice were used. Only animals showing progressive weight gain up to the start of treatment and weighing more than 18 g at the start of treatment were used.

Tumor

Two xenografted human squamous cell carcinomas originating from poorly differentiated tumors from the head and neck region were used (designated EH and AB). These tumor lines are the same as those used previously in our earlier studies which demonstrated metoclopramide sensitization of cisplatin- and radiotherapies.[11,12] They were serially transfered by subcutaneous inoculation of 2 × 2 × 2 mm pieces on both flanks of the animals. No tumor was less than 63 mm^3 at the start of treatment. Tumors that did not show growth from three days before treatment to the time of treatment were excluded. The range of tumor volumes at the start of treatment was 63–200 mm^3. The tumors were in their 101–105 passages.

Tumor volume measurements

Two orthogonal diameters of the tumors were measured with vernier calipers. The tumor volume was calculated according to the formula: volume = (length × $width^2$)/2.[14]

Toxicity

Drug toxicity was assessed as survival during the observation period and as the change in body weight of surviving animals.

Drugs

Metoclopramide (Primperan^R; H. Lundbeck, Copenhagen, Denmark), cisplatin and carboplatin (Bristol Laboratories, Syracuse, NY, USA) were obtained as commercially available preparations. Drugs were dissolved in isotonic saline and injected intraperitoneally in volumes of 0.01–0.02 ml/g body weight. In the experiments with carboplatin and MCA, the drugs were also given intravenously and intramuscularly. All animals in the control groups were given isotonic saline in volumes corresponding to those given to the treated groups.

Statistics

Data analysis was carried out using the RS/1 data analysis system (Bolt, Baranek and Newman Research Systems, Mass, USA). Differences within

treatment groups were tested with one-way analysis of variance (ANOVA)[15] and between different treatment groups with the Student's *t*-test.

Endpoints used for estimating tumor growth

Two different endpoints were used: the area under the growth curve (AUC), and specific growth delay (SGD).

AUC: Relative tumor size (RTS) (i.e. tumor volume at the time of measurement divided by tumor volume at the time of first treatment) was calculated for each individual tumor from the growth curves of ^{10}log (RTS) versus time. Such a measure accounts for both degree and duration of growth inhibition, and the daily fluctuations in size are smoothed out in this analysis.[16] All AUC estimations are based on an observation period of 21 days, and only animals surviving the 21-day period were included in the calculations.

SGD: SGD was calculated according to Berman and Steel.[17] From the growth curves of ^{10}log (RTS) versus time, the time (DT) taken for each tumor to grow to double the volume at the time of first treatment was obtained. Tumor growth delay was calculated as the difference between the DT of each individual tumor and the mean DT for the control tumors using the formula $SGD = (DT_t - DT_c)/DT_c$, where DT_t is the actual doubling time of the individual tumors, and DT_c is the mean doubling time for all the control tumors. Tumors that did not reach DT during the observation period were set to have reached DT at the end of the extended observation period (maximal 42 days). Tumors on animals that died before reaching their DT were excluded from calculations.

Experimental setup

Dose timing of MCA: The first set of experiments was set up to find the optimal time for MCA administration relative to the cisplatin administration. A single dose of cisplatin of 7.5 mg/kg body weight and a single dose of MCA of 2 mg/kg were used. MCA was given from 1 h before to 56 h after cisplatin administration. Several experiments were needed to cover this timespan due to limitations in the number of animals per experiment.

Dose response of cisplatin ± MCA: We have also looked at the potentiating effect of MCA at different was examined. The MCA was given as a single dose 8 h after cisplatin administration, which we have shown to be the optimal timing in the first part of this work. The dose of cisplatin used in these experiments was 5 mg/kg. Doses of MCA examined were 0.05, 0.1, 1, 2, 3, 5, and 10 mg/kg body weight.

Dose response of cisplatin ± MCA: We have also looked at the potentiating effect of MCA at different cisplatin doses. MCA was given as an optimal single dose of 2 mg/kg, 8 h after cisplatin administration. Cisplatin was given at doses of 2.5, 5.0, 7.5 and 10 mg/kg. The 10 mg/kg dose turned out to be toxic, with too many animals dying, and no meaningful evaluation could be undertaken.

Combinations of MCA and carboplatin: The combination of carboplatin and MCA was studied using a variety of experimental conditions relating to those used successfully for cisplatin + MCA. A dose response study of carboplatin was carried out. A dose of 60 mg/kg (corresponding to the maximal tolerated dose) was chosen for the later experiments. This dose gave a significant reduction of AUC. The time interval from 0 h to 48 h after carboplatin administration was tested for possible MCA potentiation, using both tumor lines EH and AB. Both carboplatin and MCA were administered by different routes in different experiments (intraperitoneal and intravenous), and MCA was tried in different time schedules including MCA at 0, 24 and 48 h after carboplatin administration, single doses from 0 to 48 h after carboplatin administration and 8 hourly doses throughout the 48 h period.

Results

The optimal time for MCA administration in all experiments was 8 h after cisplatin administration. The cisplatin dose used in the MCA timing experiment in Figure 1 gave a significant reduction of the AUC ($p = 0.011$). The addition of MCA to cisplatin treatment gave a further reduction of the AUC for the groups given MCA at 1, 8, and 16 h. Only the group given MCA 8 h after cisplatin had a significant reduction ($p = 0.007$) compared to the cisplatin-treated group. SGD values are shown in Table 1 and correspond therefore with the data obtained with AUC as endpoint. With SGD as endpoint we also obtained a significant difference

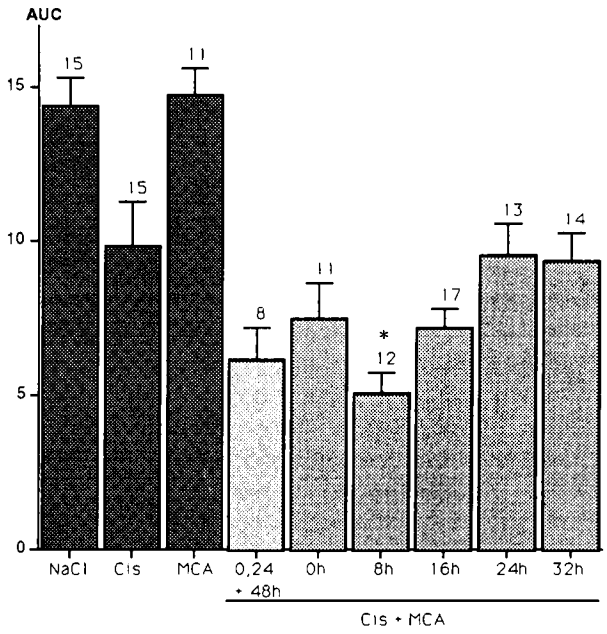

Figure 1. Bar graph of AUC values for an experiment with dose timing of MCA (2 mg/kg) with tumor line AB. The bars are the mean values of AUC, and the error bars are the standard error of the mean. Number of tumors in each group is indicated on top of all error bars. *Indicates the group of cisplatin + MCA that differed statistically from the cisplatin (7.5 mg/kg) group.

only for the group given MCA 8 h after cisplatin ($p = 0.03$). We have also compared and found this single-dose effect at 8 h after cisplatin administration at least as effective as the regime used in a previous study[11] where MCA was given at the same time as cisplatin and 24 and 48 h after cisplatin, but these two regimes did not differ significantly.

Table 1. SGD values from the same experiment as shown in Figure 1 evaluating the dose timing of MCA with tumor line AB

	Group	SGD ± SEM	Significance level
A	NaCl	0 ± 0.11	
B	Cisplatin (7.5 mg/kg)	1.43 ± 0.41	$p = 0.004$[a]
C	MCA (2.0 mg/kg)	0.13 ± 0.11	ns[a]
D	Cis + MCA 0, 24, 48 h	2.23 ± 0.75	ns[b]
E	Cis + MCA 0 h	1.94 ± 0.38	ns[b]
F	Cis + MCA 8 h	2.98 ± 0.54	$p = 0.03$[b]
G	Cis + MCA 16 h	1.77 ± 0.37	ns[b]
H	Cis + MCA 24 h	1.57 ± 0.82	ns[b]
I	Cis + MCA 32 h	1.22 ± 0.47	ns[b]

ns = not significant, defined as $p > 0.05$.
[a] Compared to NaCl treated controls (A).
[b] Compared to cisplatin (B).

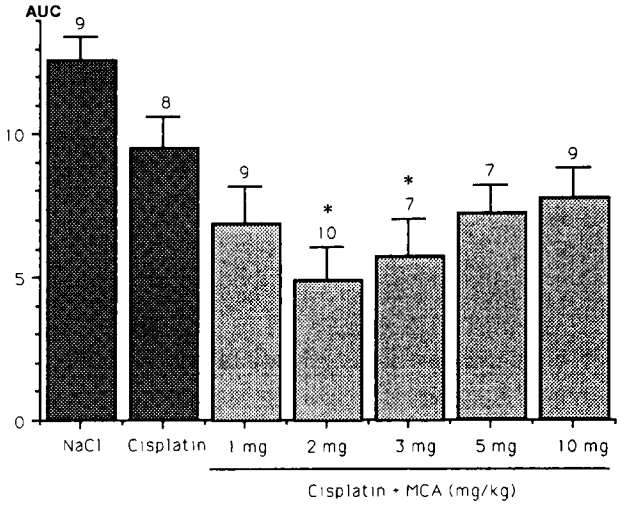

Figure 2. Bar graph, as Figure 1, from an experiment with dose response of MCA with tumor line AB. Number of tumors in each group is indicated on top of all error bars. *Indicates the groups that differed statistically from the cisplatin (5 mg/kg) group.

In the second experimental setup, evaluating the optimal single dose of MCA given 8 h after cisplatin administration (Figure 2), cisplatin on its own gave a significant reduction of the AUC when compared to the control group ($p = 0.04$). All doses of MCA from 1 to 10 mg/kg gave a reduction in the AUC values. The optimal potentiating dose of MCA was found to be 2 mg/kg. The AUC of this combination as well as that receiving 3 mg/kg MCA gave a significant reduction of AUC ($p = 0.012$ and $p = 0.044$ respectively). SGD values from this experiment are shown in Table 2 and correspond with the AUC data. Only the group given 2 mg/kg gave a significant increase of the SGD ($p = 0.009$). The lower doses of MCA (0.1 and 0.5 mg/kg) have

Table 2. SGD values from the experiment as shown in Figure 2 evaluating the dose response of MCA with tumor line AB

	Group	SGD ± SEM	Significance level
A	NaCl	0 ± 0.16	
B	Cisplatin (5.0 mg/kg)	0.42 ± 0.24	ns[a]
C	Cis + MCA 1 mg/kg	1.90 ± 0.67	ns[b]
D	Cis + MCA 2 mg/kg	2.66 ± 0.67	$p = 0.009$[b]
E	Cis + MCA 3 mg/kg	2.71 ± 0.97	ns[b]
F	Cis + MCA 5 mg/kg	1.50 ± 0.94	ns[b]
G	Cis + MCA 10 mg/kg	1.16 ± 0.64	ns[b]

[a] Compared to NaCl treated controls (A).
[b] Compared to cisplatin (B).

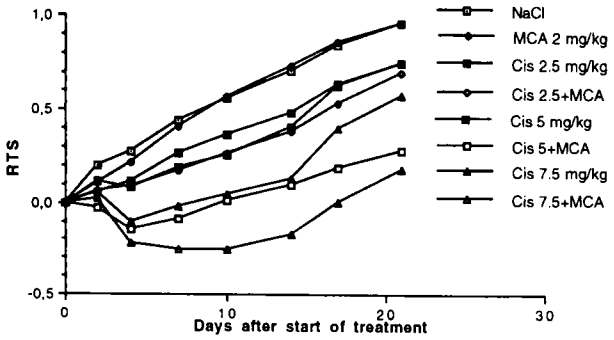

Figure 3. Growth curves of ^{10}log RTS (relative tumor size) from an experiment with increasing cisplatin doses ±MCA (2 mg/kg) with the tumor line EH.

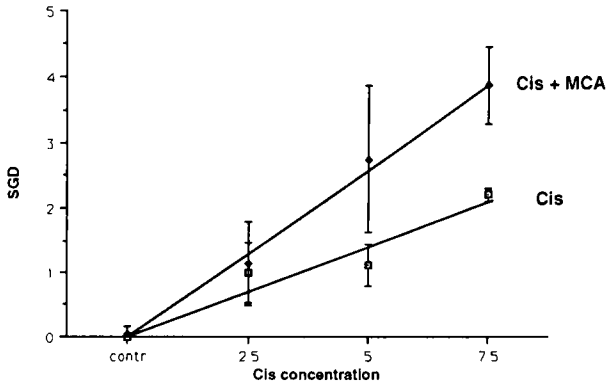

Figure 5. Graph with the SGD values from the experiment in Figure 3, indicating a linear dose–response relationship for both the groups treated with cisplatin alone, and the groups treated with the combination of cisplatin + MCA (mean + SEM).

also been evaluated in other experiments, but have not been found to show any potentiating effect. No increased toxicity was found between any of the groups treated with cisplatin + MCA compared to those treated with cisplatin only.

The growth curves for increasing doses of cisplatin ± 2 mg/kg MCA given 8 h after cisplatin administration are given in Figure 3. The AUC values from the same experiment are plotted in Figure 4. The cisplatin-treated groups receiving 5.0 and 7.5 mg/kg had a significant reduction of the AUC values compared to the controls ($p = 0.006$ and $p < 0.001$ respectively). MCA potentiated cisplatin at all tested doses, but in this experiment only the group receiving 7.5 mg/kg cisplatin was significantly potentiated by the addition of MCA. From the same experiment, we have plotted the SGD for the different doses with and without MCA. As can be seen in Figure 5, the dose–response data for the different cisplatin doses fall into a straight line, as do the data from the cisplatin plus MCA groups. These results

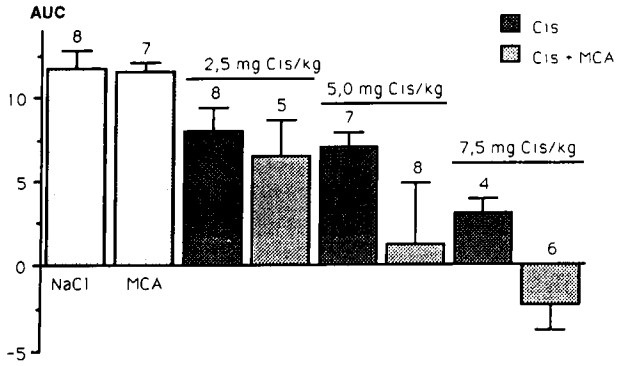

Figure 4. Bar graph with AUC values from the same experiment as in Figure 3. Number of tumors in each group is indicated on top of all error bars (mean + SEM).

indicate that MCA potentiates to the same extent at all tested cisplatin doses. Only the groups receiving 7.5 mg/kg cisplatin ± MCA differed significantly in SGD values (as for the AUC values). Increased mortality and weight loss was seen only in the groups treated with 7.5 mg/kg and 10 mg/kg cisplatin (mortality 40% and 80%). No difference was found between the groups treated with and without MCA even at these high and toxic cisplatin doses.

It should be noted that the two endpoints (AUC and SGD) used in all the above experiments gave essentially the same results for optimizing MCA dose and optimal time for MCA administration. Moreover, mortality increased with increasing cisplatin dose but was not further accentuated by MCA in any experiment. Weight loss did not differ significantly between groups receiving cisplatin and groups receiving the combination of cisplatin and MCA in any experiment. This is in accordance with previous work with this drug combination.[11]

In the dose response of carboplatin, the dose of 60 mg/kg gave a significant reduction of the AUC value compared to the NaCl-treated control group. The tumor growth inhibition of this carboplatin dose was comparable to the effect of the 7.5 mg/kg cisplatin dose, which we have shown in Figure 1 to be effectively sensitized by MCA. However, none of the tested combination regimes of carboplatin (60 mg/kg) and MCA (2 mg/kg) reported in the Materials and Methods section showed any potentiation of the carboplatin effect with either of the tumor lines AB or EH. This was true whether carboplatin and/or MCA were administered i.p. or i.v. The i.v. administration was tested to exclude

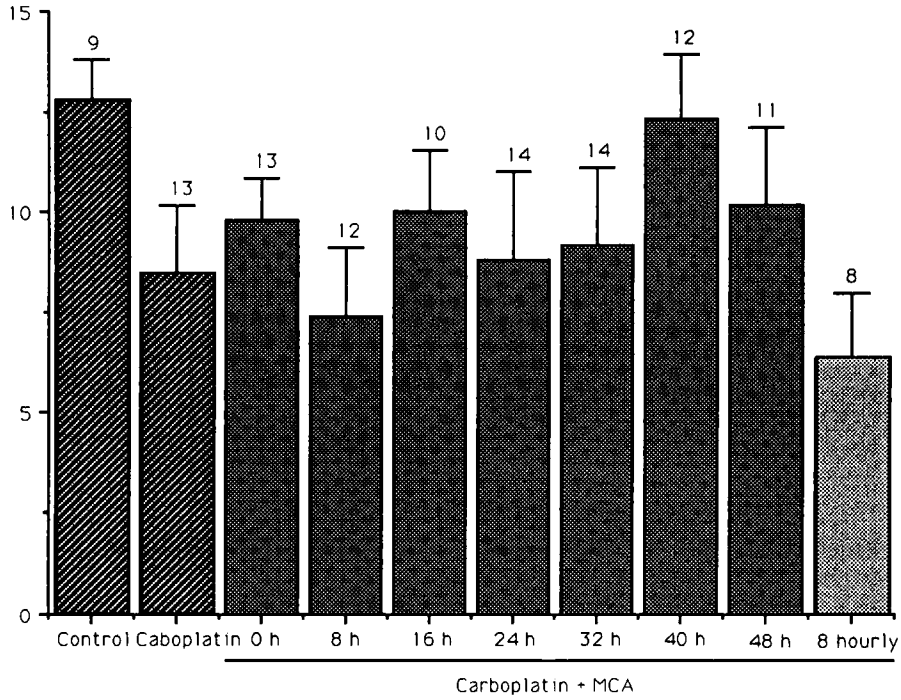

Figure 6. Bar graph with AUC values showing the effect of carboplatin (60 mg/kg) alone and in combination with single-dose MCA (2 mg/kg) treatment given at different time intervals after carboplatin administration (0–48 h) from an experiment with tumor line AB. The last bar represents one group of animals treated with carboplatin (60 mg/kg) and followed by MCA (2 mg/kg) at 0, 8, 16, 24, 32, 40 and 48 h. Number of tumors in each group is indicated on top of all ebars (mean + SEM).

any possible interaction of the carboplatin and MCA solutions intraperitoneally. In the experiment shown in Figure 6, carboplatin (60 mg/kg) was studied using tumor line AB in combination with MCA given as a single dose treatment from 0 to 48 h after carboplatin administration. No potentiating effect of MCA was seen in any of the groups. In this experiment, one of the groups also got MCA every 8 h from 0 to 48 h, but no statistical effect on the AUC value was seen in this group either.

Discussion

The time interval for the maximum potentiating effect of MCA on the cisplatin treatment in this *in-vivo* study was 8 h (Figure 1). Several authors have looked upon the time-relationship of the occurrence of DNA crosslinks in cells exposed to cisplatin,[13,18,19] and found maximal DNA crosslinks at 6 to 12 h after cisplatin administration. This time interval corresponds quite well with the time period for maximal MCA sensitization of the cisplatin effect on tumor growth. Although the mechanism of the potentiating effect of MCA is not known, one possible mechanism is that MCA interferes with the repair of DNA damage. Support for this mechanism is the fact that MCA is most potent as a potentiator of the cisplatin effect at the time of expected maximal DNA crosslinking, where repair inhibition is likely to be most critical. Structurally related compounds to MCA, such as 3-aminobenzamide and nicotinamide, are well characterized inhibitors of poly-ADPRT, and they are also effective sensitizers because they inhibit poly-ADPRT and thus DNA repair.[20–22] Preliminary *in vitro* results from our laboratory have shown inhibition of DNA repair in a whole cell system (human peripheral mononuclear leucocytes) at concentrations of MCA that parallel what we would expect to find in our nude mice system (unpublished data). There is also preliminary evidence that MCA alone can induce a dose-dependent increase in DNA damage, and a concomitant increase in cytotoxicity.[23] One possible explanation of these effects might relate to a mechanism whereby MCA imbalances Ca^{2+} homeostasis. Support for this line of reasoning comes from the fact that drugs that cause Ca^{2+} homeostasis imbalance by binding to calmodulin, have been shown to sensitize cells to cisplatin.[24]

Another possible mode of action relevant to the potentiating effect of MCA is an interaction with the uptake of nicotinamide. We have recently described an active transport system for nicotinamide in our laboratory,[23] and MCA can compete for the active transport of nicotinamide.[25] By this mechanism, MCA could interfere with the metabolism of NAD, the vital co-substrate for poly-ADPRT activity, and thereby inhibit DNA repair and enhance cytotoxicity.

The potentiating effect of MCA does not seem to be dependent on the cisplatin dose. In Figure 5, showing the SGD based on one tumor doubling, the cisplatin dose in the group getting cisplatin only must be doubled to accomplish the same effect as seen in the cisplatin + MCA group. In other words, one can significantly increase the antitumor effect of cisplatin with MCA in the group receiving 7.5 mg/kg of cisplatin without increasing the toxicity, while an increase in the cisplatin dose from 7.5 mg/kg to 10 mg/kg increases the mortality from 40% to 80%. The fact that the sensitizing effect of MCA is not dependent on the cisplatin dose (Figure 5) is also of importance to the lack of any potentiating effect of MCA on carboplatin treatment, since it makes it less likely that the carboplatin dose used is critical for showing a sensitization by MCA.

The carboplatin dose chosen from the dose–response experiment gave a significant effect on tumor growth, but none of the many dose regimes or different administration modes in combination with MCA gave any potentiating effect. Carboplatin is believed to act via the same active metabolites as cisplatin, and therefore it interacts with DNA causing identical DNA adducts.[13] Cisplatin and carboplatin do differ in their rates of intrastrand and interstrand adduct formation. Carboplatin gives a slower DNA crosslink formation that peaks at about 18 to 24 h after carboplatin administration, whereas the DNA crosslinking by cisplatin peaks at 6 to 12 h. As a consequence, the peak of DNA crosslinking formed from carboplatin exposure is less steep and more prolonged than with cisplatin. These results support an important role for the rate of production and level of DNA damage, as part of the molecular mechanism of MCA sensitization of platinum-containing drugs.

MCA has been used clinically as an antiemetic agent for many years in combination with cisplatin. No adverse effect of MCA on the cisplatin toxicity has to our knowledge been reported. MCA given as an antiemetic has not, based on the results of this study, been given at the most optimal time for potentiation of the cisplatin effect. It would be very interesting, nevertheless, to compare the antitumor effect of cisplatin with high dose MCA, to that of non-MCA containing cisplatin regimes, especially after optimizing the dose schedule for MCA potentiation. A clinical pilot study (phase I) of the combination MCA/radiation has been started in our clinics, and a clinical study to evaluate the potentiating effect of MCA on cisplatin treatment is now being prepared.

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