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4-METHYLTHIOBENZOIC ACID REDUCES CISPLATIN NEPHROTOXICITY IN RATS WITHOUT COMPROMISING ANTI-TUMOUR ACTIVITY

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Abstract— Administration of 4-methylthiobenzoic acid (MTBA) (100 mg/kg) strongly reduced cisplatin nephrotoxicity (7.5 mg/kg, 20 min after MTBA) in rats as determined by histopathology and blood urea nitrogen. Anti-tumour activity against a colonic adenocarcinoma, CC 531, that was implanted in rats, was unaffected by MTBA pretreatment. Studies with isolated renal proximal tubular cells (PTC) demonstrated that preincubation of the cells with MTBA diminished cisplatin nephrotoxicity *in vitro* as it did *in vivo*. Preincubation of the PTC with probenecid completely abolished the protective effect of MTBA against cisplatin toxicity. These data indicate that MTBA is actively transported into the PTC. The mechanism of action of MTBA was investigated by NMR studies which showed that cisplatin and *cis*-diamminediaquaplatinum(II), its hydrolysis product, reacted with the methylthio-sulphur. We suggest that MTBA after selective accumulation in the kidney inactivates cisplatin intracellularly by nucleophilic attack of the methylthio-sulphur to the Pt-moiety. Since MTBA shows no acute toxicity in the rat, even if administered at very high doses, it may be useful to suppress the nephrotoxic side effects of cisplatin anti-tumour therapy.

cis-Diamminedichloroplatinum(II) (CDDP, cisplatin)§ is one of the most potent cytotoxic drugs that is currently being used in the clinic. It has excellent anti-tumour effect on various malignancies, but shows a number of undesired side-effects [1]. The most serious and dose-limiting of these side-effects is a cumulative and dose-dependent nephrotoxicity. The mechanism of CDDP nephrotoxicity is still poorly understood [2, 3]. However, several attempts have been made to reduce it. Vigorous hydration and the use of diuretics are now included in most therapies since they attenuate nephrotoxicity [4]. These techniques, however, have proven to be only partially successful, since renal failure still occurs, especially after repeated administrations of CDDP. Therefore, several attempts have been made to reduce nephrotoxicity by co-administration of other compounds [5] like free radical scavengers [6, 7], selenium [8, 9], thiosulphate [10, 11], thiophosphonates [12], mercaptoethanesulphonate [13, 14] and dithiocarbamates [15, 16]. The dithiocarbamates and selenium seem successful [9, 17]. Many of the other compounds, however, seem not only to reduce the nephrotoxicity but the antitumour effect of CDDP as well [5, 18].

Most of the compounds that show an alleviation of CDDP-induced nephrotoxicity contain a nucleophilic sulphur atom that may form a coordinate bond to the Pt^{2+}-ion, that functions as a Lewis acid in this reaction. Platinum(II) compounds have recently been shown to react readily with the methionine–sulphur and other methylthioethers [19–21]. Our laboratory has previously reported that certain thioethers are potent scavengers of electrophilic reactive intermediates formed from *N*-hydroxyacetyl-2-aminofluorene [22, 23]. We have investigated in this study whether a methylthioether is able to reduce CDDP-induced nephrotoxicity in a rat model.

4-Methylthiobenzoic acid (MTBA) was chosen as model compound since it contains (i) a methylthio-group for complexation with platinum(II) species and (ii) an (aromatic) carboxylate group, which makes it prone to be accumulated by the renal proximal tubular cells (PTC) where CDDP-toxicity is located.

MATERIALS AND METHODS

Chemicals. CDDP was a gift from Pharmachemie B.V. (Haarlem, The Netherlands). *cis*-Diamminediaquaplatinum(II) (CDAP) was prepared from

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§ Abbreviations used: AAS, atomic absorption spectroscopy; AST, aspartate-aminotransaminase; ALT, alanine-aminotransaminase; BCS, bovine calf serum; BSA, bovine serum albumin; BUN, blood urea nitrogen; CDDP, *cis*-diamminedichloroplatinum(II); CDAP, *cis*-diamminediaquaplatinum(II); DMEM, Dulbecco's modified Eagle's Medium; γGT, γ-glutamyltranspeptidase; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; KW, kidney to body weight; α-MG, α-methylglucose; MTBA, 4-methylthiobenzoic acid; NMR, nuclear magnetic resonance; PTC, proximal tubular cell(s).

K_2PtCl_4 according to Lippert *et al.* [24]. 4-Methylthiobenzoic acid was purchased from Aldrich Chemical Co. (Brussels, Belgium). CDDP at a concentration of 2 mg/mL was dissolved in a sterile aqueous solution of 20 mg mannitol and 18 mg NaCl/mL. MTBA was obtained from Aldrich Chemical Co. and was dissolved in sterile, deionized water at a concentration of 50 mg/mL; the pH of the solution was adjusted to 7.4 with solid sodium bicarbonate and it was subsequently filtered (0.22 μM). Probenecid, Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F_{12} were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and defined bovine calf serum (BCS) from HyClone Labs Inc. (Logan, UT, U.S.A.).

Animals. Male SPF rats (180–220 g) were used in all experiments. WAG/Rij rats were obtained from the central Institute for the breeding of laboratory animals/Harlan Sprague–Dawley (CPB/HSB), Zeist, The Netherlands; Wistar rats were of the strain of the Sylvius Laboratory, University of Leiden (outbred with SPF Wistar rats from CPB/HSB). The animals had free access to a commercial diet (SRM-A, Hope Farms, Woerden, The Netherlands) and tap water and were kept on a 12 hr light/dark cycle in a temperature controlled room. For urine sampling, animals were placed in plastic metabolism cages 2 days before administration of compounds.

Tumour-model. A colonic adenocarcinoma, CC 531, was obtained from the Netherlands Cancer Institute. The tumour is well defined [25] and CDDP-sensitive [26]. The tumour grows both subcutaneously and *in vitro*. *In vitro* it is grown in a medium containing equal parts of DMEM and HAM's nutrient mixture F_{12}, supplemented with10% BCS, 100 mg/L streptomycin and 10^5 I.U./L benzylpenicillin.

WAG/Rij rats were inoculated subcutaneously in the left flank with 5×10^6 CC 531 tumour cells, harvested at the 72nd passage, in 300 μL phosphate buffered saline. Tumour growth was followed by estimating tumour size in three different directions using a micrometer.

In vivo *studies.* Rats were treated with 100 mg (0.60 mmol) MTBA/kg i.p., followed after 20 min by i.p. injection of 7.5 mg (25 μmol) CDDP/kg (group IV). The following control groups were used: (i) an i.p. injection of an aqueous solution of $NaHCO_3$ (50 mg/kg), adjusted to pH 7.4 with dilute HCl, followed by 7.5 mg CDDP/kg after 20 min (Group III), (ii) only MTBA treatment and, after 20 min, the vehicle (mannitol/saline) for CDDP (group II), or (iii) only both vehicles (group I).

In vitro *studies.* PTC were isolated from rat kidney by collagenase digestion and purified by isopycnic centrifugation as previously described [27]. Cells were incubated in Hank's buffer supplemented with 25 mM HEPES and 2.5% (w/v) BSA at a concentration of approximately 3×10^6 cells/mL, at 37° under 95% O_2/5% CO_2, on a rotatory shaker (160 cycles/min). After isolation, cells were preincubated for 15 min before the experiments were started. Probenecid and MTBA were dissolved at a high concentration in deionized water and the solutions were neutralized with solid sodium bicarbonate (to pH 7.40). They were added in a volume of 100 μL to the cell suspensions (final volume of 3 mL). Probenecid was added 5 min before addition of MTBA. After another 15 min preincubation, the cells were washed and resuspended in fresh, supplemented Hanks' buffer containing 250 μM CDDP. Control cells were treated equally with the appropriate vehicle solutions. Cytotoxicity was determined by the extent of inhibition of α-methylglucose (α-MG) uptake by the PTC as recently described [28, 29].

Kidney and liver function assessment in vivo. Daily blood samples (300 μL) were taken by orbital punction, using heparinized Pasteur pipettes, under light diethyl ether anaesthesia. The samples were centrifuged (10 min, 3000 *g*, 4°) and the plasma was stored at −20° until assayed. On day 4 the animals were anaesthetized with diethyl ether, weighed for determination of kidney to body weight (KW), and subsequently exsanguinated by collection of blood in heparinized syringes *via* the aorta. Aspartate-aminotransaminase (AST), alanine-aminotransaminase (ALT), blood urea nitrogen (BUN) and plasma creatinine (CREAT) were determined using commercial kits (Sigma Chemical Co.). The kidneys and spleen were removed and weighed.

Histopathology. From the rats the left kidney and the left large liver lobe were removed, cut longitudinally and immersed in 4% w/v formaldehyde in 0.1 M potassium phosphate buffer (pH 7.4) at 4° for 2 hr. After fixation, the tissues were dehydrated in a series of graded alcohols and embedded in polymethylmethacrylate (LKB 2218-501 mounting medium). Four series of sections of 4 μM were cut (Rotary one microtome, LKB) and two of these were stained with haematoxylin and eosin and the others for γ-glutamyltranspeptidase (γGT) activity and counterstained with haematoxylin [30]. The four series were coded and examined 'blindly' by an independent individual. The sections were examined by light microscopy at 40× and 100× magnification.

Platinum AAS. Part of the right kidney, liver and spleen were removed for determination of platinum content. Samples were weighed and analysed after enzymatic digestion and modification of the final matrix [31] by AAS with electrothermal atomization in a graphite furnace. The results are expressed as μg of platinum per g of protein.

NMR measurements. The ^1H NMR spectra were recorded with a Bruker WM 300 spectrometer. D_2O was used as a solvent; chemical shifts are relative to tetramethylammonium nitrate (TMA) which was added in trace amounts.

The reaction of MTBA with CDDP as well as the reaction of MTBA with CDAP were carried out in a NMR tube at 5 mM concentrations in 50 mM potassium buffer in D_2O (pH 7.20) at room temperature. Reactions were followed by ^1H NMR as a function of time. The extent of the reactions was measured by integration of the intensities of the methyl proton of both reaction products and starting compounds.

Statistical analysis. The data are presented as the arithmic mean ± standard error. Data were analysed with multivariate ANOVAs or Student's *t*-test. For

statistical analysis of histopathology data Wilcoxon's rank sum test was used. A probability $P < 0.05$ was considered to be statistically significant.

RESULTS

NMR measurements

In order to investigate the ability of MTBA to form a coordination complex with CDDP, a NMR study was performed. The reactions between MTBA and two platinum compounds, CDDP and its main hydrolysis product CDAP, were followed in time by proton NMR. CDDP and CDAP gave the same reaction products: three new signals arose, located at −0.40, −0.44 and −0.49 ppm. These signals can be assigned to methyl protons of platinated MTBA and are shifted downfield compared to those of free MTBA (−0.64 ppm). These downfield shifts indicate coordination to the sulphur atom. Since the coordination chemistry of platinum compounds to sulphur atoms is known to be very complicated, i.e. a number of complexes can be expected, including those in which the ammine ligands are released [21], no attempts have been made to characterize these products. The time required for 50% formation of the coordination complex for the reaction between MTBA and CDDP or CDAP was approximately 20 and 1.5 hr, respectively.

In vitro *experiments*

Previous studies in our laboratory showed that a concentration of 250 μM CDDP decreases α-MG uptake by isolated rat renal PTC by 50% in 2.5 hr [28, 29]. This concentration was chosen in all present experiments. Preincubation of the cells with 0.5 mM MTBA for 20 min, followed by washing of the cells to remove the MTBA, caused no significant decrease in α-MG uptake by the cells during the subsequent experiments as compared to controls. CDDP decreased α-MG uptake by 61 ± 4% in 3 hr in control PTC (Fig. 1). However, in PTC which were preincubated for 20 min with MTBA α-MG uptake was reduced by only 27 ± 6% upon incubation for 3 hr with CDDP.

Since at pH 7.40 MTBA is an organic anion, it might be accumulated in the PTC by the basolateral organic anion carrier. Probenecid can block efficiently the uptake of organic anions in the isolated PTC [27, 32]. When probenecid was present in the cell suspensions during the preincubation with MTBA, it completely prevented the protective effect of MTBA against CDDP cytotoxicity (Fig. 2). The combined pretreatment with probenecid and MTBA alone had no effect on α-MG uptake.

In vivo *experiments*

Liver function. Hepatotoxicity of MTBA given either alone or in combination with CDDP was determined by histopathology and by determination of AST and ALT. MTBA at a single or double dose of 100 mg/kg did not cause an elevated plasma ALT or AST activity in Wistar rats on any day after treatment. Coadministration of 7.5 mg/kg CDDP caused no increase in plasma transaminases either, nor did CDDP alone. Histopathology of the liver

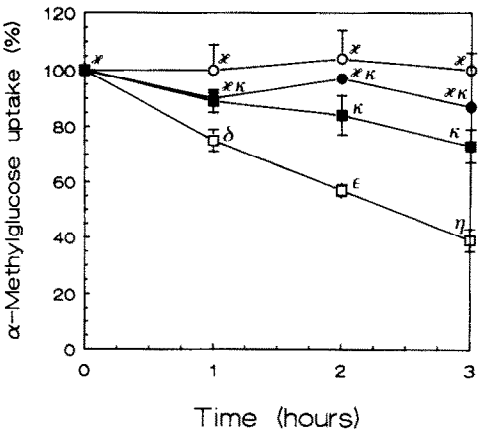

Fig. 1. Protection against CDDP-induced toxicity in isolated PTC by MTBA. Cells were preincubated for 15 min without (□) or with 0.5 mM MTBA (■); the cells were washed and subsequently incubated for the indicated time with 250 μM CDDP. Control cells, untreated (○) and treated with MTBA (●) were washed after 15 min incubation and subsequently incubated with supplemented Hank's buffer. Toxicity was determined by extent of α-MG uptake ($N = 3$). Results are the means ± SE of three separate experiments. Points with different superscripts are significantly different from one another (ANOVA, $P < 0.05$).

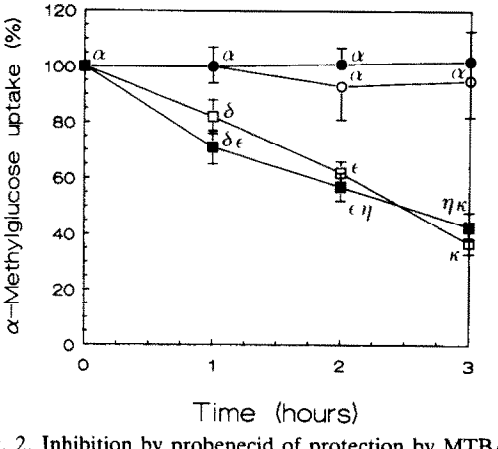

Fig. 2. Inhibition by probenecid of protection by MTBA against CDDP-induced toxicity in isolated PTC. Cells were preincubated with probenecid (5 mM), 5 min later, MTBA was added at a final concentration of 0.5 mM; after another 15 min cells were washed and subsequently incubated with fresh, supplemented Hank's-HEPES buffer (●) or with 250 μM CDDP (■). Control cells were incubated in supplemented Hanks'-HEPES buffer for 20 min; subsequently the cells were washed and incubated with fresh, supplemented Hanks' buffer (○) or with 250 μM CDDP (□). Toxicity was determined by α-MG uptake. All measurements were made in triplicate. Results are the means ± SE of three separate experiments. Points with different superscripts are significantly different from one another (ANOVA, $P < 0.05$).

Table 1. Effect of MTBA treatment on CDDP-induced nephrotoxicity in male Wistar rats

Treatment			Parameter		
CDDP (mg/kg)	MTBA (mg/kg)	Group	BUN (mM)	KW (% of body wt)	N
0	0	I	3.2 ± 0.2	0.40 ± 0.03	7
0	100	IIa	2.9 ± 0.4†	0.41 ± 0.01†	4
0	2 × 100	IIb	2.0 ± 0.3†	0.39 ± 0.01†	5
7.5	0	III	13.9 ± 2.1‡	0.55 ± 0.03†	13
7.5	100	IVa	5.1 ± 1.3§	0.43 ± 0.03‖	5
7.5	2 × 100	IVb	6.0 ± 1.4¶	0.47 ± 0.02¶	13

Rats were treated with MTBA once, 20 min before CDDP adminstration (groups IIa and IVa), or twice, 20 min before and 20 min after CDDP administration (groups IIb and IVb). Rats were killed on day 4; blood was collected for BUN measurements and the kidneys removed and weighed.

* All values are means ± SE. † Not significantly different from group I. ‡ Significantly different from groups I, II and IV. § Significantly different from groups IIb and III, not significantly different from groups I and IIa. ‖ Not significantly different from groups I and II, significantly different from group III. ¶ Significantly different from groups I, II and III. (Student's *t*-test, $P < 0.05$, unpaired, two-tailed).

showed no abnormalities or differences in any of the treated groups (II, III and IV) in comparison to the controls (group I).

Kidney function. Nephrotoxicity was assessed by histopathology, kidney to body weight (KW) and by several clinical chemical measurements. Loss of kidney function in rats treated with CDDP was demonstrated by a transient rise in urine volume, glucosuria and prolonged proteinuria. Glucosuria and proteinuria were less in rats pretreated with MTBA than in rats receiving CDDP alone (data not shown). Pilot-studies showed that BUN and KW were more sensitive parameter to assess kidney function than plasma creatinine, glucosuria or proteinuria. BUN increased steadily in the animals treated with CDDP with peak levels on the day of killing (day 4). Therefore, in all subsequent experiments BUN on day 4 was measured as parameter of kidney function. The increase in relative kidney weight correlated well with the extent of tubular necrosis as determined by histopathology. The kidney weights of animals that received CDDP without MTBA (Group III) had strongly increased but those that had been treated with MTBA before CDDP or with MTBA alone (Groups IV and II) did not differ significantly from controls. Animals that had been administered MTBA both before and after CDDP treatment showed a small increase in KW. The results are summarized in Table 1. An additional dose of MTBA, 20 min after the administration of CDDP did not improve the protective effect. Administration of multiple doses (200 mg/kg/day for 5 consecutive days) did not cause any overt acute toxicity.

Histopathology. Histopathologic evaluation of the sections stained for γGT activity of control animals, showed that γGT activity was almost exclusively present at the luminal side of the cells, with only very faint staining of the intracellular and basolateral membranes. In animals treated with CDDP, however, cells were swollen, the intracellular membranes showed γGT activity and the cells started to become detached from one another. Finally, the cells became completely detached from the basolateral membrane. In areas with the most extensive damage, an 'empty' tubule filled with cell debris was present. In the sections stained with haematoxylin and eosin, swollen cells and eosinophilic cells with fragmented or pycnotic nuclei were observed at this stage. The series of sections stained for γGT were used for ranking according to increasing area of damaged cells. After breaking the code, it turned out that no histological signs of cell damage were present in the sections of control animals that had received MTBA only ($N = 5$) and in sections of two rats that had received CDDP ($N = 8$) and two animals that had received CDDP and MTBA ($N = 8$). These non-responders were not included in the ranking of the sections of the twelve animals showing damage. The mean rank number of the CDDP treated animals was 9.2 while that for the CDDP and MTBA treated animals was 3.8. The difference was statistically significant ($P < 0.01$, Wilcoxon's rank sum test).

Platinum AAS. The platinum content of liver, spleen and kidney was determined (Table 2). A considerable variation in platinum content was found, especially in the kidney. Although the mean platinum concentrations were lower in all groups that had been administered MTBA together with CDDP, the differences found were not statistically significant for the kidney. However, the platinum content of the liver was lower in the group that had received MTBA before CDDP. The spleen contained less platinum in both groups that had been given MTBA and CDDP as compared to animals who received only CDDP.

Influence of MTBA on the oncolytic activity of CDDP

In order to assess the effect of MTBA co-administration on the anti-tumour activity of CDDP, rats were inoculated with a colonic adenocarcinoma and treated with 7.5 mg/kg CDDP alone or in

Table 2. Platinum content of kidney, liver and spleen of male Wistar rats after treatment with CDDP alone or with MTBA and CDDP

Treatment			Tissue platinum content			
CDDP (mg/kg)	MTBA (mg/kg)	Group	Kidney (μg/g)	Liver (μg/g)	Spleen (μg/g)	N
7.5	0	III	93 ± 13	31 ± 4	25 ± 3	13
7.5	100	IVa	72 ± 30	20 ± 7*	12 ± 4†	5
7.5	2 × 100	IVb	77 ± 20	28 ± 6	15 ± 4†	8

* Significantly different from group III: $P < 0.09$. † Significantly different from group III: $P < 0.025$.

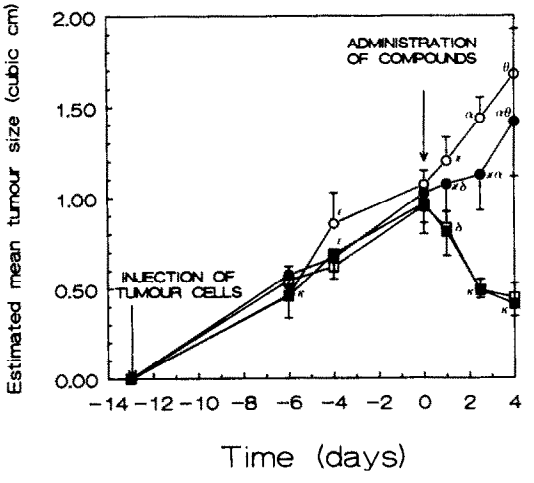

Fig. 3. Effect of CDDP and MTBA on tumour size (cm^3) in rats inoculated with CC 531 cells. Rats were treated as follows: (○) controls, receiving only the appropriate vehicles; (●) 100 mg/kg MTBA, followed after 20 min by mannitol/saline; (■) the MTBA-vehicle followed by 7.5 mg/kg CDDP; (□) 100 mg/kg MTBA followed by 7.5 mg/kg CDDP. Values are means ± SE. Points with different superscripts are significantly different from one another (ANOVA, $P < 0.05$).

combination with MTBA (administered 20 min before CDDP). On day 0 (13 days after inoculation with a cell suspension of CC 531) tumours had grown to a size of approximately 1 cm^3 in all rats. The animals were randomly divided into four groups and were treated with CDDP 20 min after having been administered MTBA or vehicle solution; control groups received only MTBA or the appropriate vehicles. In both control groups the tumour continued growing: no statistically significant effect of MTBA alone was observed (Fig. 3). CDDP was very effective: tumour growth was arrested and the size of the tumour rapidly declined. CDDP was equally effective if MTBA had been administered 20 min earlier. The data in Table 3 demonstrate that MTBA does not compromise the oncolytic activity of CDDP. In these animals too, the CDDP-induced nephrotoxicity is largely prevented by MTBA administration: BUN and kidney to body weight (KW) were significantly increased in the animals that had been treated with CDDP only, but in the animals that had received MTBA prior to CDDP no increase in BUN or KW was observed.

DISCUSSION

Both blood and urine analysis, as well as kidney to body weight ratio measurement demonstrated the protection by MTBA against CDDP-induced nephrotoxicity. MTBA itself showed no acute toxic effects on kidney or liver function. Renal histopathology confirmed the protective effect of MTBA. However, kidneys from rats receiving both MTBA and CDDP showed some damage. Thus, MTBA does not afford complete protection against CDDP-induced nephrotoxicity at this dose of CDDP. An additional dose of MTBA, 20 min after the administration of CDDP did not improve the protective effect by MTBA. The most important finding, however, was that the anti-tumour activity of CDDP against CC 531 adenocarcinoma, a CDDP-sensitive tumour, was not affected at all by treatment with MTBA. Similar results, at much lower doses of CDDP, are very recently reported by Basinger *et al.* [34] for methionine and some analogs.

We propose that MTBA is readily accumulated (by a probenecid sensitive carrier system) in the proximal tubular cells of the kidney. Subsequently, due to the methylthioether moiety, MTBA may coordinate to *cis*-Pt compounds. NMR studies confirmed that MTBA reacts readily with CDAP, the hydrolysis product of CDDP (which is the major product formed intracellularly) but more slowly with CDDP itself. Half of the amount of CDAP and CDDP had reacted with MTBA in 1.5 and 20 hr, respectively, to the coordination complex. However, these values are based on a 1:1 molar ratio; in the kidney much more favourable ratios may exist. The lipophilic CDDP can enter the cell easily and will be aquated rapidly due to the low intracellular chloride concentration ($T_{\frac{1}{2}} \approx 2$ hr, [33]); thus, the hydrolysis reaction will be rate-limiting rather than the reaction with MTBA.

The experiments with freshly isolated PTC clearly show that MTBA inactivates CDDP: pretreatment of the cells with MTBA diminishes toxicity of CDDP. Protection was not due to extracellular inactivation of CDDP by MTBA, because it persisted if the cells were washed to remove MTBA before CDDP was added. Moreover, preincubation of the cells with

Table 3. Effect of MTBA on oncolytic activity and nephrotoxicity of CDDP in male WAG/Rij rats, bearing an adenocarcinoma (CC 531)

Treatment			Parameter*			
CDDP (mg/kg)	MTBA (mg/kg)	Group	Tumour weight (g)	BUN (mM)	KW (% of body wt)	N
0	0	A	1.25 ± 0.22	2.8 ± 0.2	0.37 ± 0.01	4
0	100	B	1.58 ± 0.08†	2.9 ± 0.1	0.36 ± 0.01	3
7.5	0	C	0.66 ± 0.02‡	8.5 ± 3.0§	0.44 ± 0.03§	4
7.5	100	D	0.59 ± 0.07‡	3.5 ± 0.7‖	0.40 ± 0.01	5

Rats received i.p. 100 mg/kg MTBA followed by 7.5 mg/kg CDDP after 20 min; controls received only MTBA, CDDP or vehicles. Rats were killed on day 4, tumors and kidneys were removed and blood was collected for BUN measurement.

* All values are means ± SE. † Not significantly different from group A, significantly different from groups C and D. ‡ Significantly different from group A and B, not significantly different from group C or D. § Significantly different from group A and B. ‖ Not significantly different from group A and B, significantly different from group C.

probenecid, an inhibitor of organic anion transport, abolished the protective effect of MBTA against CDDP-induced toxicity. This may be explained by the fact that MTBA is an organic anion at physiological pH, and that probenecid prevents carrier mediated transport of MTBA. Probenecid itself, at concentration of 0.1 to 1.0 mM, showed no effect on the uptake or toxicity of CDDP in freshly isolated PTC (unpublished observation).

The data presented in this study clearly demonstrate that pretreatment with MBTA renders rats less susceptible to CDDP-induced nephrotoxicity. This pretreatment does not abolish the oncolytic activity of CDDP. The *in vitro* experiments suggest that MTBA is actively transported into the PTC of the kidney by means of a probenecid-sensitive carrier. Within the cell, MTBA most probably inactivates platinum species by nucleophilic attack of the sulphur atom on the platinum moiety.

Since MTBA shows no acute toxicity in the rat at the doses employed in this study, it is worthwhile to investigate whether it may be used in combination with CDDP in anti-tumour therapy in man.

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