Dithiocarbamates as Antagonists of Cisplatin Toxicity*

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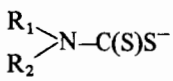
Abstract

New Pt–dithiocarbamate adducts were prepared and characterized in order to study the interaction cisplatin/dithiocarbamates, in relation to the lipophilic/hydrophilic character of the Pt-adducts. The effects of the dithiocarbamates on B16-F10 cell growth are reported. Changes in surface morphology were observed by scanning electron microscopy (SEM).

Introduction

Recently we synthesized and characterized various *N*,*N*-dialkyl-*S*-alkylcarbamodithioato complexes of platinum(II) and palladium(II) halides, which were tested for *in vitro* cytostatic activity against KB cells [1]. The complexes of general formula PtL_2X_2 and $PtLX_2$ ($L = R^1{}_2N{\cdot}CS_2R^2$; R^1 and R^2 = Me and Et; X = Cl, Br, I) have been obtained by reacting *S*-alkyl esters of dithiocarbamic acids (L) with PtX_2; the ligands act as monodentate through the thiocarbonyl group or bidentate through both the sulfur atoms, respectively. Unlike *S*-ethyl esters, *S*-methyl derivatives on reaction with PtX_2 give also *S*-demethylated species, together with PtL_2X_2 and $PtLX_2$. The crystal and molecular structure of $PtCl(S_2CNEt_2)(MeS_2CNEt_2)$, obtained by reacting $PtCl_2$ with Et_2NCS_2Me (DEDTM) in excess, has been solved by X-ray diffractometric methods [2]. For the importance of *S*-demethylation transfer in biological systems, we started *in vitro* and *in vivo* studies in relation to the *in vivo* hypothesized TDS–NaDEdtc–DEDTM≠ interconversion [3].

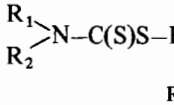
Moreover, in order to explain the synergistic antitumor action of NaDEdtc–cisplatin§ association [4–6], in relation to their metabolites and to the redistribution of the possible lipophilic adducts in the tissues, we extended the study to the following selected analogs of NaDEdtc containing polar or lipophilic groups:



	R_1	R_2
HDdtc	C_2H_4OH	C_2H_4OH
MAdtc	CH_3	CH_2COO^-
MGdtc	CH_3	$C_6H_{13}O_5$*

It is worth noting that, like NaDEdtc, the two last derivatives were found to be effective in reducing the cisplatin nephrotoxicity without loss of antitumor activity [7].

This paper reports the synthesis and characterization of the dithiocarbamates, their esters and adducts, and a study of the effects of Na_2MAdtc, NaMGdtc, NaHDdtc on B16-F10 cells, in the presence and absence of cisplatin.



	R_1	R_2	R_3
HDDTM	C_2H_4OH	C_2H_4OH	CH_3
HDDTE	C_2H_4OH	C_2H_4OH	C_2H_5
MADTM	CH_3	CH_2COOH	CH_3
MADTE	CH_3	CH_2COOH	C_2H_5
MGDTM	CH_3	$C_6H_{13}O_5$*	CH_3
MGDTE	CH_3	$C_6H_{13}O_5$*	C_2H_5

*Paper presented at the Symposium on Cisplatin and Inorganic Anticancer Drugs, Bari, Italy, November 6–7, 1986.

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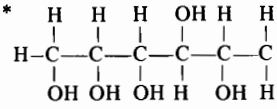
†L. Trincia was supported by a Recordati S.p.A. grant.

≠TDS = disulfiram = bis(diacetylthiocarbamoyl)disulfide; NaDEdtc = sodium diethyldithiocarbamate.

§Cisplatin = *cis*-diamminedichloroplatinum(II).



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Experimental

Reagents used were K_2MCl_4 (Johnson Matthey) where M = Pt or Pd. Dithiocarbamic sodium salts were prepared by reacting CS_2 with the appropriate amine in water/ethanol (Na_2MAdtc and NaMGdtc) and in BuONa–BuOH (NaHdtc), according to the general methods previously reported [8]. The corresponding methyl and ethyl *S*-esters were obtained by reacting the dithiocarbamic sodium salts with RI (R = Me, Et) in $EtOH/H_2O$ [9].

Preparation of the Complexes

$Pt(HDdtc)_2$ and $Pd(HDdtc)_2$ were prepared by adding K_2MCl_4 (0.2 mmol) to an aqueous solution of dithiocarbamate (0.44 mmol in 5 ml) under stirring at room temperature for 2 h. The separated solids were dried *in vacuo* over P_2O_5.

$Pt(MGdtc)_2$ and $Pd(MGdtc)_2$ were prepared under the same experimental conditions, but the reaction was carried out for 16 h.

The complexes were characterized by microanalysis and IR spectra. Melting points (uncorrected) were determined on a Büchi instrument. Elemental analyses (C, H, N) were made at the Microanalysis Laboratory of the Inorganic and Analytical Department of the University of Padua.

In Vitro Cytotoxic Activity

The experiments were performed using F10 metastatic cells of B16 murine melanoma (Fidler's source). The cells were seeded at 3×10^4 cells/ml in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 1% 200 mM glutamine, 1% Hepes buffer, 100 U/ml penicillin, 500 μg/ml kanamycin. Each experiment was set up in triplicate in Petri dishes. The cultures were exposed to Na_2MAdtc, NaMGdtc or NaHDdtc incubated in an atmosphere containing 5% CO_2 at 37 °C, recovered from culture dishes by 0.25% trypsin, neutralized by fetal calf serum, centrifuged at 1000 rpm for 10 min and counted in a hemocytometer.

The compounds, dissolved in saline and diluted medium in order to evaluate the cell growth, were tested at 10^{-4}, 10^{-5} and 10^{-6} M after 24 h exposure and at 10^{-3} M after both 24 and 48 h. Cell survival was evaluated in the culture exposed to cisplatin 10^{-5} M for 1 h, washed three times, incubated for 1.5 h with growth medium and then exposed to medium containing Na_2MAdtc, NaMGdtc or NaHDdtc (10^{-3} M) for 1 h [10].

All the results are reported as percent of controls and were statistically evaluated by Student's *t*-test.

Optical Microscopy

Controls and cells treated with Na_2MAdtc, NaMGdtc or NaHDdtc 10^{-5} M were cultured in duplicate on coverslips in Petri dishes and fixed in Bouin's fluid after 24 and 48 h, stained with hematoxylin–eosin, serially dehydrated in alcohol and cleared in xylene, mounted in Canada balsam and observed using optical microscopy. The same morphological research was performed on the cultures pretreated with cisplatin (10^{-5} M) and then exposed to Na_2MAdtc, NaMGdtc or NaHDdtc (10^{-3} M).

Scanning Electron Microscopy (SEM)

After 24 and 48 h incubation at 37 °C with the three compounds at 10^{-5} M the cells were washed in normal medium and fixed for 30 min at 37 °C, 2 h at room temperature and 2 days at 4 °C in 2.5% gluteraldehyde in 0.1 sodium cacodylate buffer with 0.1 M sucrose [11]. The fixed cells were then washed in medium for 30 min at room temperature. Finally the cells were postfixed in 1% OsO_4 in pH 7.2 buffer for 1 h, washed several times in distilled water, dehydrated through a graded ethanol series, dried by CO_2 critical point procedure in Balzers union, coated with gold using Edwards S150A Sputter Coater and examined using a scanning electron microscope (Cambridge Stereoscan 250).

Results

The prepared compounds, with their analytical data and IR frequencies in the 1400–1600 cm^{-1} region, are reported in Table I. Sodium dithiocarbamates were prepared by reacting CS_2 with the appropriate amine. The respective esters were obtained as reported in the experimental section by adding RI ($R = CH_3$, C_2H_5) to an alcoholic solution of the sodium salts. They were isolated as yellow oils or white solids. As reported in Table II, the sodium salts of the anions are very soluble in water; Na_2-MAdtc and NaHDdtc also dissolve in MeOH and in EtOH, whereas NaMGdtc is completely insoluble. The last compound also differs in its melting point (≃170 °C); Na_2MAdtc and NaHDdtc melt at 60 and 70 °C, respectively. Moreover the chemical reactivity seems quite different; in fact whereas Na_2MAdtc and NaHDdtc react easily with MCl_2 (M = Pt, Pd) to give the respective chelated compounds, NaMGdtc does not react. It is worth noting that $M(MAdtc)_2$ (where M = Pt or Pd) is very soluble in water, whereas $M(DEdtc)_2$ as well as $M(HDdtc)_2$ and $M(MGdtc)_2$ are completly insoluble. The IR spectra in the 1400–1600 cm^{-1} region, primarily associated with the ν(C–N) vibration, allow us to distinguish the various dithiocarbamate derivatives. In dithiocarbamic anions this band is present at very low frequency. Consequently it is covered by the alkyl group absorptions.

TABLE I. Analytical Data and Infrared Frequencies

Compound	Color	Melting point[a] (°C)	*Anal.* found (calc.) (%)			ν(C–N)[b] (cm^{-1})
			C	H	N	
NaHDdtc	white	60	28.88(29.54)	5.58(4.95)	6.90(6.86)	
$Na_2MAdtc \cdot 2H_2O$	white	70	19.57(19.59)	3.69(3.69)	5.69(5.70)	1460[c]
$NaMGdtc \cdot H_2O$	white	170	30.69(30.86)	6.18(5.82)	4.18(4.49)	1470[c]
$Pt(HDdtc)_2$	yellow	130	21.31(21.61)	3.37(3.62)	4.48(5.03)	1495
$Pd(HDdtc)_2$	yellow orange	200	25.82(25.72)	4.31(4.31)	5.29(5.99)	1485
$Pt(MAdtc)_2 \cdot 2H_2O$	yellow green	130	15.60(15.92)	2.34(2.33)	4.64(4.63)	1530
$Pd(MAdtc)_2 \cdot 2H_2O$	yellow orange	135	18.88(18.66)	2.91(2.74)	5.53(5.43)	1530
$Pt(MGdtc)_2$	pale yellow	160	26.11(26.11)	4.42(4.38)	3.79(3.80)	1550, 1530
$Pd(MGdtc)_2$	yellow	140	28.05(29.69)	4.70(4.98)	4.03(4.32)	1530, 1510
HDDTM	pale yellow oil		35.88(36.90)	5.35(5.06)	6.93(7.16)	1490
HDDTE	yellow oil		39.08(40.16)	5.66(5.74)	6.44(6.68)	1485
MADTM	white	122	33.76(33.50)	5.35(5.06)	7.70(7.81)	1470[c]
MADTE	white	110	37.48(37.28)	5.66(5.74)	7.25(7.24)	1470[c]
MGDTM	white	160	36.98(37.88)	6.75(6.71)	4.67(4.90)	
MGDTE	white	120	39.63(40.11)	7.14(7.07)	4.55(4.67)	

[a]With decomposition. [b]Medium strong bands. [c]The stretching may be confused with the δ(C–H): using Fluorolube mulls the same bands appeared.

TABLE II. Solubilities of the Compounds[a]

Compound	H_2O	MeOH	Acetone	CH_2Cl_2	Benzene	DMF	EtOH	n-Hexane
NaHDdtc	+ + +	+ +	–	–	–	+ +	+	–
Na_2MAdtc	+ + +	+ +	–	–	–	+ +	+	–
NaMGdtc	+ + +	+	–	–	–	+	–	–
$Pt(HDdtc)_2$	–	+ +	+	–	–	+ + +	+	–
$Pd(HDdtc)_2$	–	+	+ +	–	–	+ + +	–	–
$Pt(MAdtc)_2$	+ +	+	–	–	–	–	–	–
$Pd(MAdtc)_2$	+ +	+	–	–	–	–	–	–
$Pt(MGdtc)_2$	–	+	–	–	–	+	–	–
$Pd(MGdtc)_2$	–	+	–	–	–	+	–	–
HDDTM	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
HDDTE	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
MADTM	–	+ + +	+ + +	+	+	+ + +	+ +	–
MADTE	–	+ + +	+ + +	+ +	+	+ + +	+ + +	–
MGDTM	–	–	–	–	–	+ +	–	–
MGDTE	–	–	–	–	–	+ +	–	–

[a] + + + = very soluble, + + = soluble, + = slightly soluble, – = insoluble.

The band shifts in dithiocarbamic esters towards higher frequencies because of the increasing double-bond character of C–N [1]. A further shift towards much higher frequencies is observed in the complexes, caused by the chelation. The ν(C–N) values do not seem to be very sensitive to the nitrogen and sulfur substituents. On the contrary, the nature of the substituents is very important for other properties of the compounds, such as solubility.

In Vitro Cytotoxic Activity

The effects of the three dithiocarbamates on B16-F10 cell growth are reported in Figs. 1 and 2, and compared with those previously obtained in our laboratory with sodium diethyldithiocarbamate (NaDEdtc) [4, 12].

A statistically significant inhibition of growth was observed after 24 h exposure to Na_2MAdtc and NaHDdtc at 10^{-5} M and higher concentrations, while NaMGdtc caused no reduction of proliferation (Fig. 1). If the exposure to the three compounds at 10^{-3} M was prolonged for 48 h, the inhibition of cell growth was very significant for Na_2MAdtc and NaHDdtc (87% and 50%, respectively) while it was again insignificant for NaMGdtc (Fig. 2).

The exposure to cisplatin (10^{-5} M) alone for 1 h caused a slight inhibition of cell growth. When

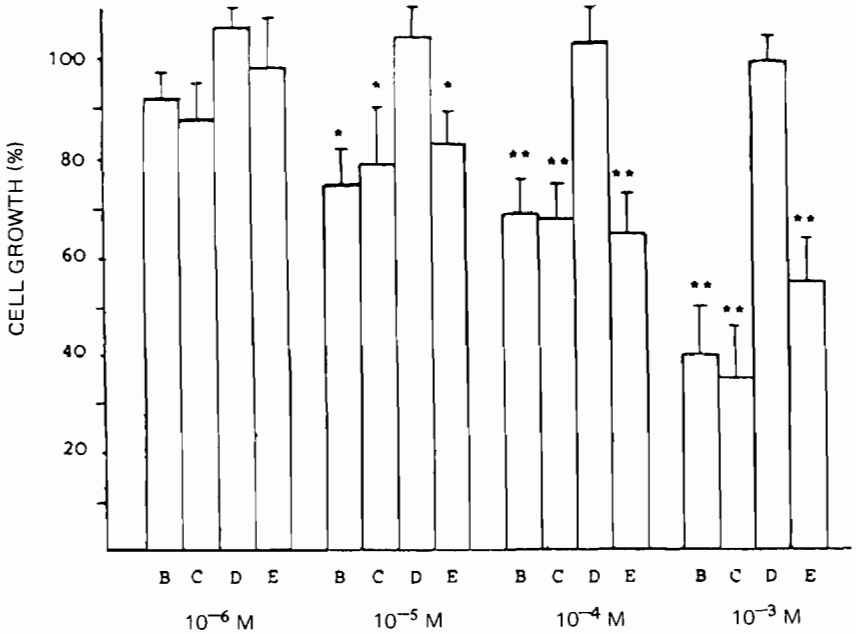

Fig. 1. Effects on cell growth of different concentrations of NaDEdtc (B), Na_2MAdtc (C), NaMGdtc (D) and NaHDdtc (E) after 24 h treatment. (*$p < 0.05$; **$p < 0.001$).

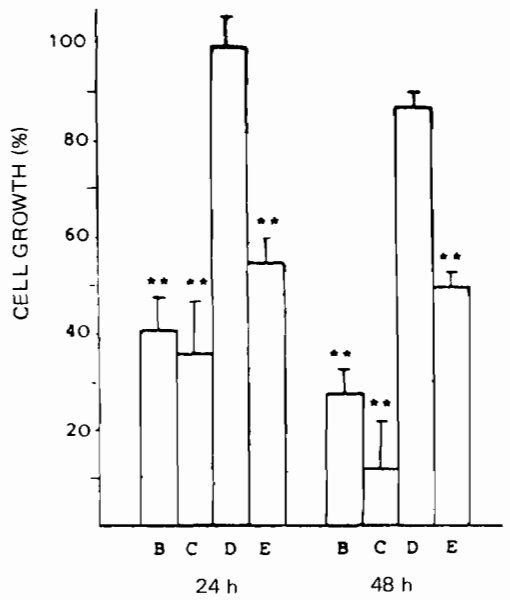

Fig. 2. Effects on cell growth of 10^{-3} M of NaDEdtc (B), Na_2MAdtc (C), NaMGdtc (D) and NaHDdtc (E) after 24 h and 48 h treatment. (*$p < 0.05$; **$p < 0.01$).

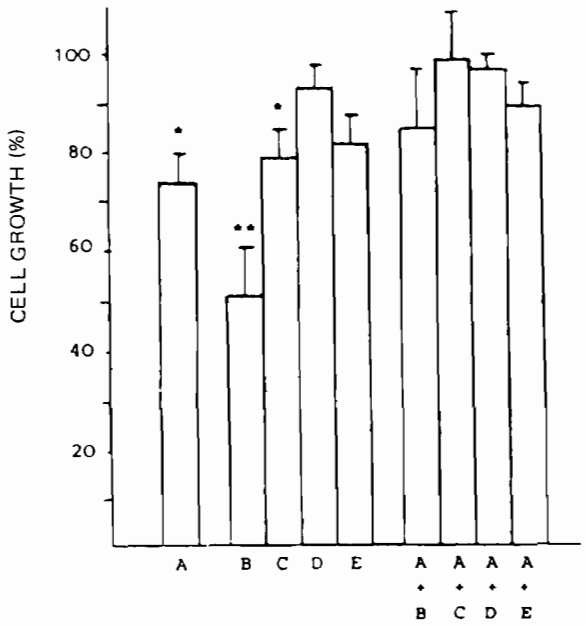

Fig. 3. Effects on cell growth of cells exposed to cisplatin (A) for 1 h, washed three times, incubated for 1.5 h with medium, then exposed to NaDEdtc (B), Na_2MAdtc (C), NaMGdtc (D) and NaHDdtc (E) for 1 h. (*$p < 0.05$; **$p < 0.01$).

the same cultures were afterwards treated with the three dithiocarbamates (10^{-3} M) for 1 h no cytotoxic effect was observed (Fig. 3).

Optical Microscopy

Following 24 h treatment with Na_2MAdtc, nuclear lesion and cytoplasmic membrane thickness were present in some cells. After 48 h exposure this membrane thickness was present in many cells. The cells exposed for 24 and 48 h to NaMGdtc or NaHDdtc

and the cultures pretreated with cisplatin and then exposed to the three dithiocarbamates showed no detectable modifications.

Scanning Electron Microscopy (SEM)

Changes in surface morphology were not observed by SEM in cells exposed for 24 h to the three dithiocarbamates in comparison with the control cultures. After 48 h, only the Na_2MAdtc induced a vacuolization of cell membrane.

Discussion

Both Na_2MAdtc and NaHDdtc, like the parent compound NaDEdtc, inhibit the growth of tumoral cells in a similar way but show a different cytotoxic effect. In fact, the cells treated with NaDEdtc are seriously damaged [12] even after 24 h exposure, while those treated with Na_2MAdtc show damage following 48 h exposure and those treated with NaMGdtc and NaHDdtc present no detectable modifications.

All the dithiocarbamates used, like NaDEdtc, develop an antitoxic effect probably as a consequence of the formation of platinum-chelated complexes, as indicated by the absence of any cytotoxic effect when the cells are pretreated with cisplatin.

Since Na_2MAdtc forms hydrosoluble adducts with Pt(II), while NaDEdtc and the other dithiocarbamates tested form insoluble Pt adducts (Table II), we suggest the use of Na_2MAdtc as an alternative drug for obtaining *in vivo* either a cytotoxic synergistic effect or an antagonism against cisplatin toxicity in combined treatment with cisplatin and chelating agents. This could lower the platinum distribution level in lipid-rich tissues. Moreover this study could allow a better understanding of the relationship between the effects and the different solubility properties of dithiocarbamates and/or their metabolites and platinum adducts.

Acknowledgement

This work was supported by a grant from C.N.R., Special Project 'Oncology' contract no. 840043444.

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