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ORIGINAL ARTICLE

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# Antitumoral activity of a sulphur-containing platinum complex with an acidic pH optimum

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**Abstract** Platinum complexes are essential tools for cancer treatment despite their toxic side effects. Here we describe a new platinum complex with sulphurs as complexing atoms (thioplatin). *Purpose*: To demonstrate that the antitumoral activity of a new sulphur-containing platinum compound (thioplatin) depends on a slightly acidic pH. *Methods*: Platinum uptake by tumour cells and interaction with DNA was determined at slightly acidic or alkaline pH. To demonstrate low in vivo toxicity the effects of thioplatin on body weight, blood urea nitrogen, white blood cell count and the histopathological appearance of small intestines and kidneys were evaluated at doses that displayed antitumoral effects against human small-cell lung cancer and human colorectal cancer xenotransplants in nude mice. *Results*: The slightly acidic pH optimum of thioplatin was proven by the altered electrophoretic mobility of plasmid DNA, quantitation of the platinum content in the DNA of tumour cells and cytotoxicity studies. Thioplatin displayed antitumoral activity without severe side effects such as weight loss, renal ischaemia, destruction of villi in the small intestine or leukopenia as observed at comparable doses of cisplatin. Furthermore, probably due to its lipophilic nature, thioplatin was taken up readily even by cisplatin-resistant cells. In vivo studies with human tumour xenografts in nude mice showed a therapeutic index of thioplatin five to ten times higher than that of cisplatin.

**Keywords** Platinum compound · Prodrug · Chemotherapy

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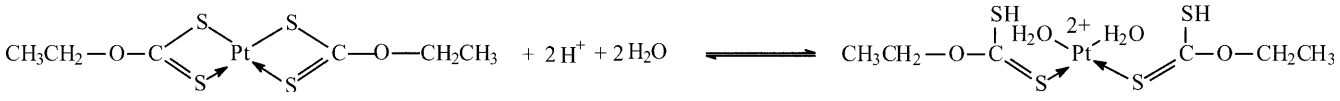
## Introduction

Cisplatin has been in use for cancer therapy for more than 25 years. It has proven to be one of the most active anticancer drugs and can even be curative in some tumour types. Several thousand new platinum coordination complexes have been synthesized which in general are derivatives of cisplatin containing two amino groups in the *cis*-position [1, 2]. Currently 28 platinum complexes, all of the diamino type, have entered clinical trials [2]. Therefore, it is not too surprising that new agents with widely different activity profiles have not been developed. It has been stated that the search for a platinum agent with increased activity still remains an elusive goal [2].

For more than 60 years it has been known that tumour cells frequently convert glucose anaerobically to lactic acid rather than aerobically to water and carbon dioxide [3]. As a consequence, lactic acid accumulates in solid tumours and the pH drops from 7.2–7.4 to values below 6.8 [4]. Therefore, it has been postulated that anticancer drugs with increased activity at slightly acidic pH should show improved therapeutic efficacy [4].

We synthesized bis-(*O*-ethyldithiocarbonato)platinum(II) (thioplatin), a platinum coordination complex in which platinum is complexed with sulphur atoms. Upon protonation, two sulphur ligands are reversibly opened so that the formation of a reactive aqua complex which is able to crosslink DNA becomes possible. With increasing pH, protons dissociate from the sulphur atoms and the inert molecule is reconstituted. We postulate the following pH-dependent reaction:



## Material and methods

### Platinum compounds

Thioplatin was synthesized by a ligand exchange reaction from *cis*-dichlorodiamminoplatinum and potassium ethylxanthate. *cis*-Dichlorodiamminoplatinum (1 mmol) was dissolved in 600 ml water. After the addition of 200 ml $CHCl_3$, 10 mmol potassium ethylxanthate was added. The reaction mixture was stirred for 6 h. After separation from the aqueous phase the organic phase was dried in a vacuum. The remaining solid was recrystallized from hexane. The structure of the thioplatin was verified by NMR spectroscopy and mass spectrometry. The purity was found to exceed 98%. For in vitro experiments thioplatin was dissolved in acetone (10 mg/ml stock solution). Thioplatin was used at concentrations below 100 μg/ml. Therefore the acetone concentrations in tissue culture medium never exceeded 1%. We found the growth of all tumour cell lines used by us to be unaffected by acetone at a concentration of 1%. For animal experiments, thioplatin was dissolved in Tween 80 (up to 66 mg/ml) followed by dilution in isotonic salt solution (final concentration 1 mg/ml). Cisplatin was used as a commercially obtained solution (Platinex, Bristol Myers).

### Interaction of platinum compounds with DNA

Plasmid pBR 322 DNA was diluted to a final concentration of 50 μg/ml in 100 m*M* Tris/HCl buffer of the desired pH. Platinum compounds were added to a final concentration of 200 μ*M* from 1 m*M* stock solutions. Samples were incubated at room temperature for 24 h under protection from light. Two volumes of ethanol were added and after incubation at –20°C for 1 h, DNA was collected by 10-min centrifugation at 15,000 *g*. The pellets were dissolved in 20 m*M* Tris and 1 m*M* EDTA, and 500 ng of each sample was applied to a 1.4% agarose gel. DNA was visualized under UV light after staining with ethidium bromide.

### Determination of platinum incorporation

HeLa cells were plated in petri dishes and 4 h later medium containing either 2.2 g/l (pH 7.4) or 0.85 g/l (pH 6.8) $NaHCO_3$ was added. After equilibration in an atmosphere containing 5% $CO_2$ at 37°C, cisplatin or thioplatin was added to a final concentration of 33 μ*M*, and 4 h later one dish of each treatment group was washed five times with 50 ml cold PBS, cells were scraped from the plates and the platinum content was determined by the neutron activation method. After freeze drying, samples and platinum standards were irradiated for 30 h with neutrons ($5\times10^{12}/cm^2/s$). After 1 week $\gamma$-radiation of 158.4 keV and 208.2 keV was determined and the platinum concentrations were calculated with the aid of standards. The limit of detection was 2 ng. All assays were done in quadruplicate.

After 16 h incubation at 37°C DNA was isolated from HeLa cells by phenol/chloroform extraction, followed by ethanol precipitation. The platinum content of each 100 μg DNA was determined as above. Standard deviations were found to be below 10% of the corresponding mean values.

### Cytotoxicity assay

Cells were plated in Linbro plates, and 4 h later medium containing either 2.2 g/l (pH 7.4) or 0.85 g/l (pH 6.8) $NaHCO_3$ was added. After equilibration in an atmosphere containing 5% $CO_2$, cisplatin or thioplatin was added at concentrations between 5 and 150 μ*M* in triplicate. The number of viable cells was determined 24 h later in a Neubauer haematocytometer after Trypan blue staining. From dose response curves, $IC_{50}$ (concentration at which 50% of cells were found to be dead) and $IC_{99}$ (concentration at which 99% of cells were found to be dead) values were determined (standard deviations were below 10% in all cases).

### Assessment of antitumoral activity in animal experiments

Human colorectal carcinoma cells (SW707) or human small-cell lung carcinoma cells (H16) were injected subcutaneously into nu/nu Swiss mice ($5\times10^6$ cells in 0.1 ml isotonic salt solution) and 12 days later, when the tumours had reached a diameter of 8–10 mm, groups of five animals received a single intraperitoneal injection of either 10 mg/kg thioplatin (dissolved in 10% Tween 80) or 10 mg/kg cisplatin. Thioplatin was dissolved less than 15 min before injection in Tween 80 (10 mg/ml) then diluted 1:10 in 0.9% NaCl. Under these conditions thioplatin stayed completely in solution for several hours. Tumour sizes were determined in two dimensions and relative tumour growth was calculated.

Blood urea nitrogen concentration was determined 5 days after treatment with 10 mg/kg cisplatin or 10 mg/ kg thioplatin. A commercial kit (Sigma, Munich) was used. White blood cell numbers were monitored individually in groups of four animals over a period of 15 days after injection. The mean values of the maximum changes occurring between days 10 and 14 are indicated.

## Results

It has been reported previously that platinum-initiated crosslinking of two adjacent guanine residues affects the tertiary structure of plasmid DNA. As a consequence the mobility of DNA is altered in agarose gels [5, 6, 7]. We sought to determine whether plasmid DNA would interact with thioplatin in buffers with pH between 6.0 and 8.0. At a pH between 6.0 and 6.5, all supercoiled form I DNA was converted to a structure that comigrated with relaxed form II and positively coiled DNA migrating between form I and II (Fig. 1, lanes 2–4). At pH 6.75, most of the DNA was still present as a form II-like molecule (Fig. 1, lane 5). At pH 7.0, some form I remained intact (Fig. 1, lane 6). At pH 7.25 and above, the mobility of the DNA remained unchanged (Fig. 1, lanes 7–10). In contrast, after incubation with cisplatin, plasmid DNA was present at all pH values as a form comigrating with positively coiled DNA (Fig. 1, lanes 11–19). This feature remained unaltered upon shorter incubation periods where conversion of plasmid DNA to a form II-like molecule was incomplete (data not shown).

Whether thioplatin interacts with live cells in a pH-dependent manner was also determined first with the human cervix carcinoma line HeLa. After incubation with thioplatin at the slightly acidic pH 6.8 almost eightfold more platinum was found to be bound to DNA as compared to the amount after incubation at

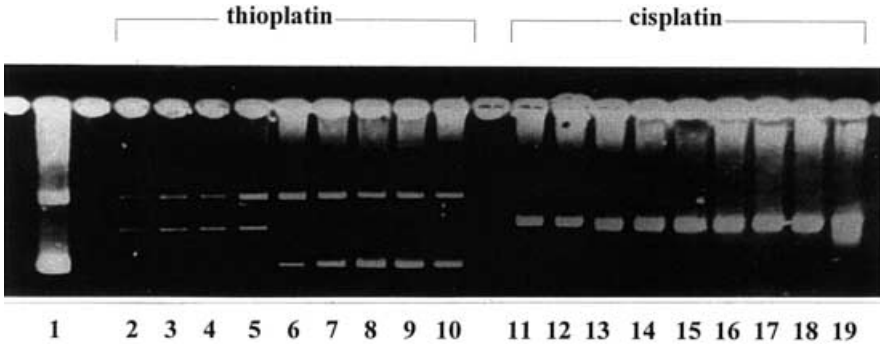



**Fig. 1** pH-dependent interaction of thioplatin with plasmid DNA (*lane 1* untreated control, *lanes 2, 11* pH 6.0, *lanes 3, 12* pH 6.25, *lanes 4, 13* pH 6.5, *lanes 5, 14* pH 6.75, *lanes 6, 15* pH 7.0, *lanes 7, 16* pH 7.25, *lanes 8, 17* pH 7.5, *lanes 9, 18* pH 7.75, *lanes 10, 19* pH 8.00)

pH 7.4 (Table 1). With cisplatin the opposite effect was observed, i.e. tenfold more DNA-bound platinum was recovered at pH 7.4. The uptake of platinum by the cells was pH-independent with both thioplatin and cisplatin (Table 1). However, 30–100-fold more platinum was found to be bound to cells after treatment with thioplatin than after treatment with cisplatin.

There is strong evidence that the cytotoxicity of cisplatin is due to its reaction with DNA [8]. At pH 6.8 the $IC_{50}$ of thioplatin in HeLa cells was 5 $\mu M$ , but at pH 7.4 the $IC_{50}$ was 9.2-fold higher at 51 $\mu M$ (Table 2). Opposite results were found with cisplatin: in the acidic medium the $IC_{50}$ was 86 $\mu M$ and at pH 7.4 the $IC_{50}$ was 3.4-fold lower (Table 2). With other tumour cell lines of human and mouse origin and with normal monkey kidney cells (CV1, Rita) similar results were obtained. In all cases thioplatin displayed a two- to eightfold lower $IC_{50}$ at pH 6.8 than at pH 7.4 (Table 2). With cisplatin the $IC_{50}$ was either lower at the alkaline pH or no significant pH-dependent difference was found.

**Table 1** pH-dependence of platinum incorporation

| Treatment | Total cellular platinum (ng/$10^7$ cells) | DNA-bound platinum (ng/mg) |
|---|---|---|
| Untreated, pH 6.8 | < 1 | < 10 |
| Untreated, pH 7.4 | < 1 | < 10 |
| Cisplatin, pH 6.8 | 140 | 14.6 |
| Cisplatin, pH 7.4 | 156 | 140 |
| Thioplatin, pH 6.8 | 4780 | 13,870 |
| Thioplatin, pH 7.4 | 3680 | 1,760 |

The in vivo activity of thioplatin was studied first with standard mouse tumour models, the leukaemia line L1210 and the sarcoma line S180 which do not grow as solid tumours. We found no increase in the survival time of L1210-bearing mice. In animals carrying S180 ascites, similar antitumoral activities of cisplatin and thioplatin were observed after a single intraperitoneal injection of 8 mg/kg of either compound (data not shown).

However, of interest was mainly the in vivo efficacy of thioplatin against solid tumours. This was evaluated with a human small-cell lung carcinoma (H16) and a colorectal carcinoma (SW707) xenografted into athymic nude mice. In line with published results [2], cisplatin was effective against H16 cells, while the colorectal carcinoma cell line SW707 was resistant at a dose of 10 mg/kg. At the same dose, thioplatin displayed a higher efficacy against both tumours. The mean tumour size of the thioplatin-treated group was less than that of the cisplatin-treated animals. Using the small-cell lung carcinoma line and cisplatin the tumour mass was reduced to 47% of the control group (T/C) after 3 days. With thioplatin a T/C of 23% was found (Fig. 2A). Cisplatin was ineffective against colorectal cancer cells, while tumour growth was prevented by a single injection of thioplatin at 10 mg/kg (Fig. 2B).

Loss of body weight is a rough measure of toxic side effects. While cisplatin-treated animals had lost 12% and 13% of their body weight by days 3 and 6 after treatment, no significant weight loss was recorded in the thioplatin-treated group (Fig. 2C). The dose-dependence

**Table 2** pH-dependent cytotoxicity

| Cell line | Cell type | Cisplatin | | | | Thioplatin | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $IC_{50}$ ($\mu M$) | | $IC_{99}$ ($\mu M$) | | $IC_{50}$ ($\mu M$) | | $IC_{99}$ ($\mu M$) | |
| | | pH 6.8 | pH 7.4 | pH 6.8 | pH 7.4 | pH 6.8 | pH 7.4 | pH 6.8 | pH 7.4 |
| HeLa | Human cervical cancer | 86 | 25 | 111 | 54 | 5 | 51 | 16.3 | > 55 |
| H10 | Human small-cell lung cancer | 72 | 42 | > 150 | 90 | 5.1 | 15.4 | 13.2 | 30.8 |
| SW707 | Human colorectal cancer | 9.6 | < 4.8 | 90 | 38.4 | 3.7 | 13.2 | 7.5 | 26.4 |
| CV1 | Monkey kidney | > 150 | > 150 | > 150 | > 150 | 3.9 | 23 | 13.2 | 106 |
| Capan2 | Human pancreas cancer | 143 | 139 | > 150 | > 150 | 17.3 | 50.6 | 40.9 | 99 |
| Dan-G | Human pancreas cancer | 45 | 36 | 78 | 72 | 15.4 | 52.8 | 20.9 | 64.5 |
| Jurkat | Human T-cell lymphoma | 52.5 | 54 | 120 | 126 | 6.8 | 21.1 | 13.6 | 132 |
| S180 | Mouse sarcoma | 63 | 24 | > 150 | 114 | 16.5 | 63.8 | 37.5 | > 110 |

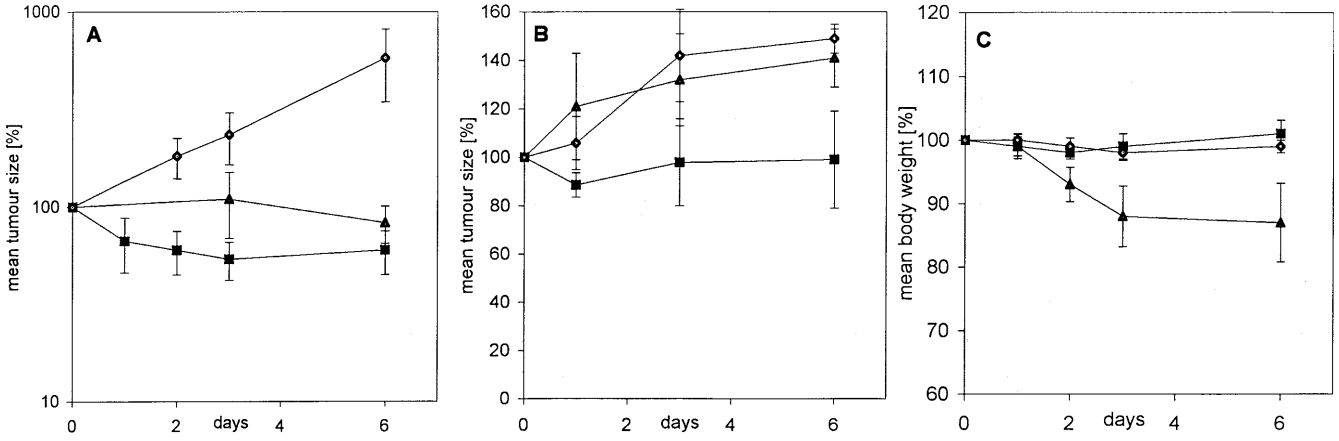



**Fig. 2A–C** Antitumoral effect of thioplatin on human xenotransplants. **A** Human H10 small-cell lung carcinoma, **B** human colorectal carcinoma cells (SW707), **C** mean body weights of the small-cell lung carcinoma groups (*diamonds* controls, *squares* thioplatin, *triangles* cisplatin; *error bars* SD)

of side effects was further evaluated in tumour-free animals. Up to 20 mg/kg thioplatin had no effect on body weight, whereas cisplatin caused a weight loss of 8% at 5 mg/ kg and 30% at 15 mg/kg. With thioplatin a slight decrease in body weight was seen only at 40 mg/kg (Fig. 3A).

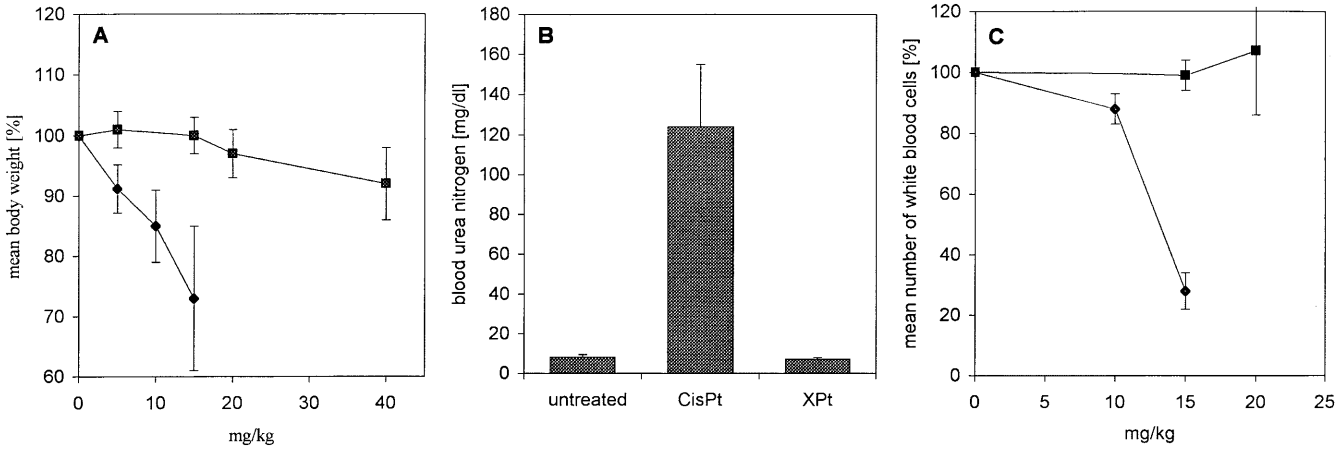
The main toxicity of cisplatin is directed against the kidneys. The resulting renal failure is mainly evaluated in terms of increase in blood urea nitrogen [9]. The average blood urea nitrogen level was 15-fold higher 5 days after the injection of 15 mg/kg cisplatin than in control mice. In contrast, after the administration of the same dose of thioplatin, nitrogen values were indistinguishable from the control levels (Fig. 3B). The high toxicity of cisplatin was also confirmed by histopathology. Histological sections of the kidneys and small intestines were prepared 4 days after cisplatin or thioplatin treatment. In cisplatin-treated animals, the kidneys showed severe degeneration and vacuolization of the tubuli (Fig. 4C). The villous structure of the small intestines was largely destroyed and large infiltrates were seen in the lamina propria (Fig. 4A). In contrast, after treatment with thioplatin even at 20 mg/kg, the structures of the kidneys (Fig. 4D) and small intestines remained unaffected (Fig. 4B).

Carboplatin, one of the second generation platinum complexes with reduced toxicity in the kidneys as compared to cisplatin, retains myelosuppressive activity [10]. Therefore myelosuppression by thioplatin was also of interest. As a consequence of myelosuppression a pronounced fall in white blood cell count was observed after the injection of cisplatin, but in groups of four animals monitored over a period of 15 days thioplatin at up to 20 mg/kg had no effect on white blood cells (Fig. 3C). Cisplatin caused a mean loss of 17% at 10 mg/kg and of 62% at 15 mg/kg.

## Discussion

These results demonstrate that, depending on pH, *O*-dithiocarbonic acid complexes with platinum can be reactive or show no reaction with DNA. The electrophoretic mobility of plasmid DNA was altered only after incubation with thioplatin at pH 7.0 and lower. As *O*-dithiocarbonic acids are very weak acids, protonation

**Fig. 3A–C** Comparison of the toxic effects of thioplatin and cisplatin. **A** Mean values of body weights, groups of four animals each, **B** blood urea nitrogen, **C** white blood cell counts (*squares*/*XPt* thioplatin, *triangles*/*CisPt* cisplatin; *error bars* SD)



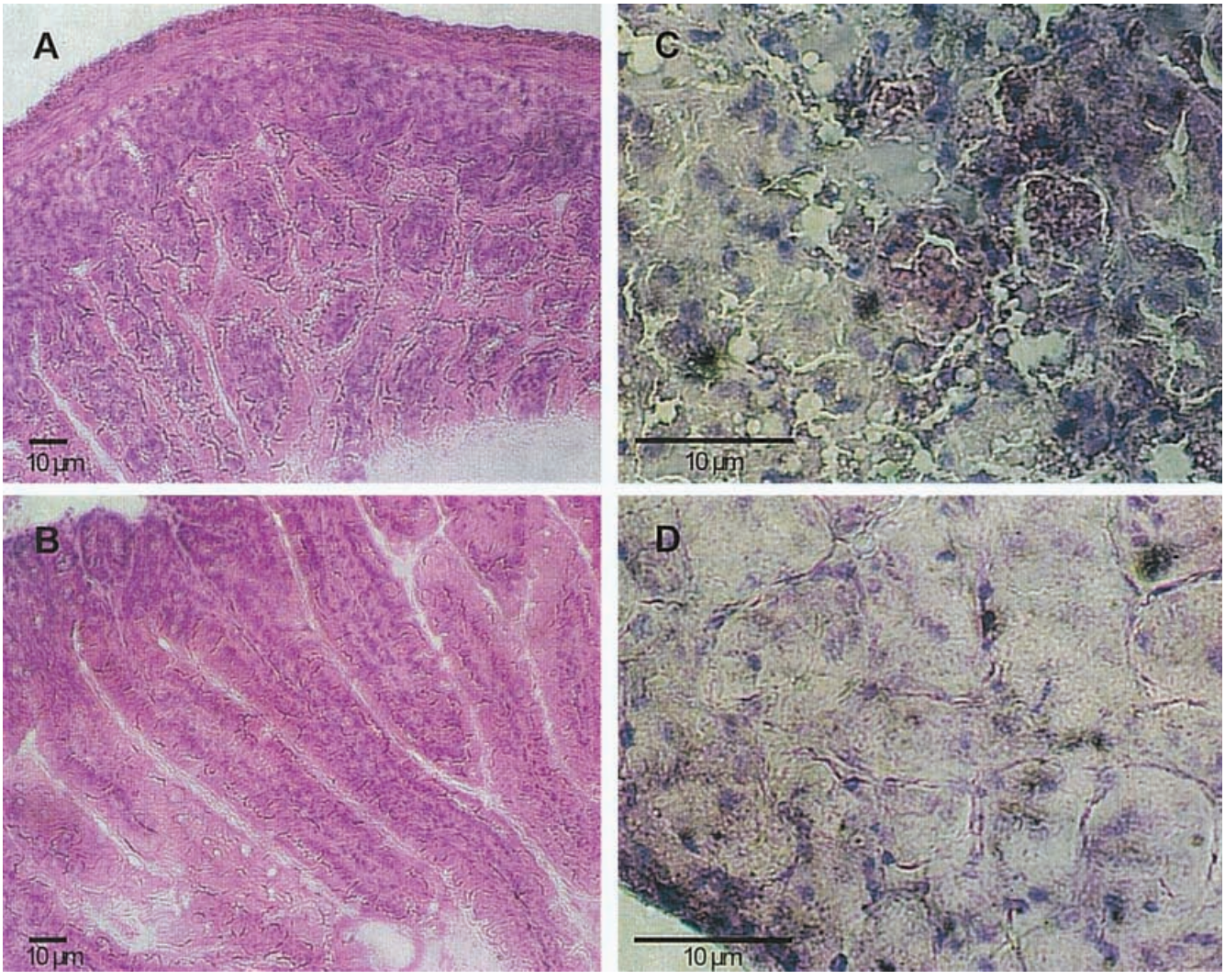



**Fig. 4A–D** Histopathology of kidney and small intestine after thioplatin and cisplatin treatment. Swiss mice were injected intraperitoneally with either 15 mg/kg cisplatin (**A**,**C**) or 20 mg/kg thioplatin (**B**,**D**). Histological sections were prepared 4 days later from small intestine (**A**,**B**) and kidney (**C**,**D**) and stained with haematoxylin and eosin (*scale bars* 10 µm)

of the dissociated form occurs at low $H^+$ concentrations, i.e. close to pH 7.0. Because sulphur has a high affinity for platinum, higher than amino groups which are the target of platinum in DNA, it is unlikely that the unprotonated form of thioplatin reacts with DNA. Upon protonation, two sulphur ligands are opened and reactive aqua complexes of platinum can be formed. It has been reported that diamminodichloroplatin is not reactive and that at least one of the chlorides has to be replaced by water to allow reactivity with DNA [11]. Yet the aquation and hence the reactivity of cisplatin with DNA is pH-independent.

Some efforts have been made to reduce the in vivo toxicity of cisplatin by coadministration of thio compounds such as biotin or methionine. Yet such treatment also abolishes the tumoricidal effect. Until now, this problem has been circumvented in selected situations such as ascitic tumour growth. In this particular case a window can be found where the tumour cells take up sufficient amounts of cisplatin before drug inactivation and serious damage to, for example, the renal tubuli. This approach allows blocking of the side effects via biotin, while the antitumoral activity remains largely unaffected [12].

Reactivity of thioplatin, in contrast to cisplatin, is pH-dependent. This allowed a four- to fivefold higher dose without severe side effects such as tubular or villous necrosis, weight loss or leukopenia. The pH-dependent cytotoxicity probably accounts for the lack of effects of thioplatin in mice injected with L1210 leukaemia cells. As L1210 cells do not form cell aggregates, the tumour cells grow in an environment of pH 7.4–7.6. At such pH values thioplatin displays only marginal cytotoxic effects. Thus solid tumours (with a pH below 7.0) are the potential targets for thioplatin.

In addition, thioplatin proved to be effective against cell lines resistant to cisplatin, the monkey kidney cell line CV1 and the human pancreas cancer cell line Capan2. Both cell lines were found to be as sensitive to thioplatin as cell lines that were not resistant to cisplatin (Table 2). A human colorectal carcinoma xenotrans-

plant, which was resistant to cisplatin also responded to thioplatin. There is strong evidence that in some cases resistance to cisplatin is due to the inability of cells to incorporate the drug [13]. Thioplatin is a highly lipophilic substance and thus binds with high affinity to the plasma membrane. Therefore a different mechanism of drug uptake is likely. The lipophilic nature of thioplatin could well be responsible for the about 30-fold higher uptake of thioplatin as compared to cisplatin by HeLa cells. Remarkably, platinum uptake was independent of pH while the interaction of thioplatin with DNA was almost eightfold higher at pH 6.8 than at pH 7.4.

As a consequence of both its lipophilic nature and its significantly increased reactivity at pH 6.8, thioplatin could provide a major breakthrough in chemotherapy since it may allow the administration of high doses, provide enhanced uptake even by cisplatin-resistant cells and show stronger tumoricidal activity with only minor side effects in normal tissue.

## References


1. Hollis LS (1991) In: Howell SB (ed) Platinum and other metal coordination compounds in cancer chemotherapy. Plenum Press, New York, p 115
2. Lebwohl D, Canetta R (1998) Clinical development of platinum complexes in cancer therapy: an historical perspective and an update. Eur J Cancer 34:1522–1534
3. Warburg O (1926) Über den Stoffwechsel der Tumoren. Springer, Berlin
4. Gerweck LE (1998) Tumor pH: implications for treatment and novel drug design. Semin Radiat Oncol 8:176–182
5. Knox RJ, Friedlos F, Lydall DA (1986) Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloroplatinum(II) and cis-diammine-(1,1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA. Cancer Res 46:1972–1979
6. Mong S, Daskal Y, Prestayko AW, Crooke ST (1981) DNA supercoiling, shortening, and induction of single-strand regions by cis-diamminedichloroplatinum(II). Cancer Res 41:4020–4026
7. Scovell WM, Kroos LR (1982) Cis- and trans-diamminedichloroplatinum(II) binding products different tertiary structural changes on SV40 DNA. Biochem Biophys Res Commun 104:1597–1603
8. Roberts JJ, Thomson AJ (1979) The mechanism of action of antitumor platinum compounds. Prog Nucleic Acids Res Mol Biol 22:71–133
9. von Hoff DD, Schilsky R, Reichert CM, Reddick RL, Rozencweig M, Young RC, Muggia FM (1979) Toxic effects of cis-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 63:1527–1531
10. Calvert AH, Harland SJ, Newell DR, Siddik ZH, Jones AC, McElwain TJ, Raju S, Wiltshaw E, Smith IE, Baker JM, Peckham MJ, Harrap KR (1982) Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum I. Cancer Chemother Pharmacol 9:140–147
11. Johnson NP, Hoeschele JD, Rahn RO (1980) Kinetic analysis of the in vitro binding of radioactive cis- and trans-dichlorodiammineplatinum(II) to DNA. Chem Biol Interact 30: 151–169
12. Jones MM, Basinger MA, Holscher MA (1992) Control of the nephrotoxicity of cisplatin by clinically used sulfur-containing compounds. Fundam Appl Toxicol 18:181–181
13. Mistry P, Kelland LR, Loh SY, Abel G, Murrer BA, Harrap KR (1992) Comparison of cellular accumulation and cytotoxicity of cisplatin with that of tetraplatin and amminedibutyratodichloro(cyclohexylamine)platinum(IV) (JM221) in human ovarian carcinoma cell lines. Cancer Res 52:6188–6193