**In vitro** formation of DNA adducts by cisplatin, lobaplatin and oxaliplatin in calf thymus DNA in solution and in cultured human cells

C.P.Saris, P.J.M. van de Vaart, R.C. Rietbroek and F.A. Blommaert

1Division of Molecular Carcinogenesis, 2Division of Experimental Therapy, The Netherlands Cancer Institute (Antoni van Leeuwenhoek Huis), Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands and 3Department of Medical Oncology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam

To whom correspondence should be addressed, care of R. Bernard

Two interesting representatives of a new generation of platinum-based cytostatic drugs that are currently being tested in clinical trials are lobaplatin [1,2-diaminomethyl-cyclobutane platinum(II) lactate] and oxaliplatin [1,2-diaminocyclohexane platinum(II) oxalate]. Since little is known about the DNA adduct formation of these compounds, we studied their formation in DNA in vitro in calf thymus DNA and in cells. The major adducts formed in vitro were the Pt-GG and Pt-AG intrastand crosslinks. The latter adducts could be detected using a recently developed 32P-postlabelling method. Using both this assay and atomic absorption spectrosocopy, it was shown that there is a substantially higher rate of the in vitro adduct formation by cisplatin, compared with lobaplatin and oxaliplatin. Platinum concentrations required to obtain 90% cell kill during a 2 h incubation of A2780 cells were 15 μM for cisplatin and oxaliplatin and 22 μM for lobaplatin. Using an antiserum originally raised against cisplatin-treated DNA, we were also able to detect platinum-DNA adducts induced by lobaplatin and oxaliplatin. Maximal nuclear staining for all three compounds was observed after a 4 h post-incubation period. The nuclear staining level induced by cisplatin was about 10-fold higher than after lobaplatin and oxaliplatin treatment. GG and AG adducts, measured by 32P-postlabelling, also showed maximum levels at about 4 h after treatment. Relative GG peak levels were 4:1:3 for cisplatin, lobaplatin and oxaliplatin, respectively. The ratios of GG over AG intrastrand crosslinks in the A2780 cells were not significantly different for the various compounds. In conclusion, the 32P-postlabelling technique has been shown to be appropriate for adduct analysis, not only for the classical Pt compounds cisplatin and carboplatin but also for novel platinum compounds like lobaplatin and oxaliplatin. Indicated large differences in reactivity of the latter compounds to DNA in vitro, compared with cisplatin. This difference was smaller in cells, suggesting enhancement of adduct formation by certain cellular mechanisms and/or compounds. From these studies, no conclusions can be drawn with respect to the cytotoxicity of the different Pt-GG and Pt-AG intrastand crosslinks formed by these compounds.

*Abbreviations: AAS, atomic absorption spectroscopy; carboplatin, cis-diamine(1,1-cyclobutane dicarboxylato)platinum(II); cisplatin, cis-diamine dichloroplatinum(II); DAB, 3,3'-diaminobenzidine-HCl; DACH, 1,2-diaminocyclohexane platinum(II) oxalate; DABC, 3,3'-diaminobenzidine-HCl; DACH, 1,2-diaminocyclohexane platinum(II) oxalate; d(GpG), 2'-deoxyguanylyl-(3'-5')-2'-deoxyguanosine; d(ApG), 2'-deoxycytidinyl-(3'-5')-2'-deoxycytidine; d(GpApG), cis-P(NH3)2 d(GpG); d(Pt-ApG), cis-P(NH3)2d(ApG); lobaplatin, 1,2-diaminomethyl-cyclobutane platinum(II) lactate; oxaliplatin, 1,2-diaminocyclohexane platinum(II) oxalate; PKN, T4 polynucleotide kinase; Rq, number of platinum atoms per nucleotide; SCX, strong cation exchange chromatography; TLC, thin layer chromatography; TU, thiourea.

Introduction

The platinum-based compounds cisplatin and carboplatin are among the most widely used and effective anticancer drugs. However, many tumours develop resistance during therapy, while others have intrinsic resistance to cis- and carboplatin. Another problem especially for cisplatin, is its severe neuro- and nephrotoxicity. Several platinum analogues that are in some way able to circumvent cis- or carboplatin resistance, and/or the severe side-effects of cisplatin, are currently under clinical evaluation (1). Lobaplatin is a relatively new compound, which has shown favourable activity in several preclinical models (2). It has shown complete or partial lack of cross-resistance with conventional platinum drugs in some testicular and ovarian carcinoma cell-lines and xenografts (3) and is currently being used in clinical trials (4,5). Oxaliplatin is one of the representatives of the DACH* carrier ligand based platinum drugs (6). Oxaliplatin has shown lack of cross-resistance in several cisplatin-resistant tumour cell-lines, like other DACH-based platinum compounds (7–9). Of further interest is the fact that oxaliplatin has shown activity in the treatment of colorectal cancer (10,11). This is of interest since colorectal tumours have intrinsic resistance to cisplatin (8).

Cytotoxicity of platinum compounds is believed to result from the formation of platinum-DNA adducts. Hence, for understanding (differences in) working mechanisms of platinum compounds, adduct formation studies are essential. Most of the sensitive assays for platinum-DNA adducts are based on the use of polyclonal antiseras raised against cisplatin-induced DNA damage (12–14). The specificity of the antiseras for the cis- and carboplatin derived bis-ammine adducts may cause decreased or lack of affinity for platinum adducts with other remaining ligands. For example, polyclonal antiseras raised against 1,2-diaminocyclohexane (DACH)–Pt adducts show substantial differences in affinity for the various stereoisomers of the DACH ligand (15). Both lobaplatin and oxaliplatin differ from cis- and carboplatin in the remaining ligands, and therefore also in adduct structure. Lobaplatin-derived adducts contain the 1,2-dimethylamino-cyclobutane ligand, whereas oxaliplatin analogues contain the 1,2-DACH ligand. There are no data available on Pt–GG and Pt–AG intrastand crosslink formation by lobaplatin, and only few data with respect to DACH-based platinum analogues. The ligand present in oxaliplatin, the trans-R,R-DACH isomer, (with sulphate rather than oxalate as leaving ligand), has been shown to induce a ratio of Pt–GG/Pt–AG ratio of 1.8 in vitro, as measured by atomic absorption spectroscopy (AAS), at a ratio of 0.01 Pt/nucleotide (16). In vitro studies, a more sensitive
method than AAS would be preferable. We recently described a highly sensitive $^{32}$P-postlabelling method for analysis of the intranast DNA adducts Pt–GG and Pt–AG (17). One of the steps of the assay is the removal of platinum (together with the remaining ligands) from the GG and AG adducts. This assay therefore completely bypasses any variation in the ligands that were originally present in the adducts and should therefore enable the detection of Pt–GG and Pt–AG intranast crosslinks of any origin. We present here data on DNA-adduct formation by cisplatin, lobaplatin and oxaliplatin in vitro and in A2780 cells.

**Materials and methods**

**Chemicals**

Cisplatin was obtained from Bristol Myers (Weesp, The Netherlands). Lobaplatin was a generous gift from ASTA Medica AG (Frankfurt Germany). It consisted of a 1:1 mixture of diastereoisomers. Oxaliplatin was a generous gift from Ciba-Geigy (Basel, Switzerland). $^{32}$P-ATP (3000 Ci/mmol) was obtained from Amersham (UK). PKN, kinase buffer, alkaline phosphatase and nucleas P1 were obtained from Boehringer (Mannheim, FRG). DNase I was obtained from Cooper Biomedical.

**In vitro platination**

Calf-thymus DNA solutions (final concentration 1.5 mM DNA, Boehringer, Mannheim, FRG) were incubated for 48 h at 37°C in the dark with equal volumes containing different concentrations of cisplatin, oxaliplatin or lobaplatin in a 10 mM Tris/0.1 mM EDTA (TE) buffer, pH 7.2. After incubation, DNA samples were precipitated with 100% ethanol, washed twice with 80% ethanol, redissolved in TE-buffer, and digested as described below.

For the kinetic study, 3 mM DNA was incubated for up to 48 h at 37°C with an equal volume of 30 μM cisplatin, oxaliplatin or lobaplatin. Samples were taken at time points 0, 1, 2 and 5.5 h in duplicate, and were immediately frozen on liquid nitrogen. The experiment was performed with and without blocking the conversion of mono- to intranast crosslinks by addition of TE. Immediately after thawing of one half of the samples, 10 mM TW was added and the mixture was incubated for 1 h at 37°C, or for 10 min at 24°C. Subsequently, the TU treated samples were precipitated with 100% ethanol, washed twice with 80% ethanol, and redissolved in 10 mM Tris/0.1 mM EDTA, pH 7.2 buffer. After this, the samples that were not treated with TU were precipitated, washed and redissolved in the same way. All samples were digested as described below.

**Preparation of the samples for $^{32}$P-postlabelling**

Pt–GG and Pt–AG levels were measured by $^{32}$P-postlabelling as described before (17) with some modifications.

DNA (10–50 μg) was digested in a mixture containing 1/10 volume of a 10X concentrated digestion buffer (500 mM sodium acetate, pH 5.5, 100 mM MgCl2), five units DNase I, and five units nuclease P1 per 100 μg, overnight at 37°C. After addition of 1/10 volume of 1 M Tris–HCl pH 9 and five units alkaline phosphatase the incubation was prolonged for 4 h. DNA digests were checked on HPLC as described previously (17).

Digested DNA samples were applied to cartridges (Sep-Pak light tC18 cartridges, part no. 36805, Waters) from which the original filling was replaced by strong cation exchange material (sulfonic acid group; Macherey Nagel, VYDAC 441040). Before use, the cartridges were subsequently washed with 2 ml 100% methanol, 3 ml of distilled water, 3 ml 5 M ammonium formate (pH 4.5), and 30 ml of distilled water. The SCX cartridges can be re-used. Unmodified nucleotides and nucleosides were eluted with 15 ml of 3 mM ammonium formate (pH 4.5). The platinum containing products were subsequently eluted with 2 ml of 5 M ammonium formate (pH 4.5). The pH of the eluted samples was raised above 7 by addition of 1/10 volume of ammonia solution (25%).

Platinum was removed from the bifunctional adducts during a 2 h incubation at 65°C after addition of 1/4 volume of 1 M NaCN. After deplatination the samples were neutralized with acetic acid. The deplatinated mono- and bifunctional adducts were purified using Sep-Pak light tC18 cartridges (pre-washed with 2 ml methanol and 10 ml distilled water) by elution of the inorganic compounds with 2 ml of distilled water. Subsequently, the dinucleoside monophosphates and nucleosides were eluted with 500 μl of methanol/H2O (20/80 v/v), from which the last 400 μl were collected. The samples were dried in vacuo, and redissolved in 15 μl of distilled water.

**Results**

**Detection of DNA-adducts**

Using a HPLC method described earlier by Jennerwein et al. (16) we determined the major products of the reaction of lobaplatin and oxaliplatin with calf thymus DNA (input ratio 1 part per 100 nucleotides). For both lobaplatin and oxaliplatin the adducts were assigned mainly as Pt–GG and Pt–AG, based on the reversed phase Supelco Supelcosil C18 column. The eluent was 5–25% methanol in 0.4 M ammonium acetate (pH 6) over 30 min at a flow rate of 1 ml/min. Fractions (0.5 ml) were collected and subjected to AAS.
Lobaplatin and oxaliplatin DNA adduct formation

Fig. 1. HPLC analysis of enzymatically digested samples of DNA treated with lobaplatin (A) or oxaliplatin (B) (input platinum/nucleotide ratio: 0.01). Shown are the platinum concentrations as measured by AAS (μM, light-gray bars), and the GG (dark-gray bars) and AG (black bars), as measured by the postlabelling assay (d.p.m. 32P, X = A,G).

on their elution pattern (Figure 1) when compared with the data of Jennerwein. The presence of platinum in the relevant HPLC fractions was confirmed by AAS. Furthermore, 32P-postlabelling analysis of the same fractions indicated the presence of GG and AG. The identities of the Pt-GG and Pt-AG adducts were additionally confirmed by the fact that treatment of these compounds with cyanide yielded the deplatinated dinucleoside monophosphates GG and AG, as was established using authentic marker compounds. These findings confirmed the expectation that these agents, like cisplatin, form substantial amounts of GG and AG intrastrand crosslinks. The lobaplatin-derived adducts appeared to be divided into partly overlapping sets of peaks. These peaks probably represent the two stereoisomeric forms of this adduct. This was confirmed by treatment of these adducts with cyanide, resulting in single GG or AG dinucleoside monophosphates.

Dose–response study

Using both AAS (Figure 2A) and 32P-postlabelling (Figure 2B), the in vitro dose–response relationship of lobaplatin and oxaliplatin was studied and compared with that of cisplatin (incubation conditions: 48 h at 37°C). With an input ratio of 1 part per 100 nucleotides, the AAS data showed that the relative (total) adduct levels were 3.1, 1.8 and 1 for cisplatin, lobaplatin and oxaliplatin, respectively. Using 32P-postlabelling, the number of GG + AG intrastrand adducts was detected by 32P-postlabelling relative to the expected amount of Pt–GG (60%) as measured by AAS, were 33%, 7.2% and 7% for cisplatin, lobaplatin and oxaliplatin, respectively.

Kinetic study

The in vitro kinetic behaviour of lobaplatin and oxaliplatin is shown in Figure 3. We studied the total adduct formation by AAS and the intrastrand GG and AG adduct formation by 32P-postlabelling. The experiment was performed with and without blocking the conversion of monoadducts to (intra)strand crosslinks by addition of TU. Clear differences were found in the rate of adduct formation by cisplatin compared with lobaplatin and oxaliplatin, both with AAS and 32P-postlabelling (no TU). While most of the cisplatin is bound after 6 h, there still seems to be an increase of both lobaplatin and oxaliplatin–DNA-adducts. The low GG and AG adduct levels detected by 32P-postlabelling indicate that a substantial amount of the adducts was still monofunctional. When TU was added immediately after the incubation (1 h, 37°C) (21), cisplatin still appeared to form adducts most quickly (data not shown). However, this experiment indicated that for cisplatin, the amount of Pt–GG + AG adducts was substantially lower after addition of thiourea, also at time points at which the conversion of mono- to di-adducts is known to be complete (4–6 h) (21).
Fig. 3. The in vitro formation of platinum-DNA adducts in time (input ratio: 0.01 pt/nucleotide), as measured by AAS (A, total platinum) and by postlabelling (B, GG plus AG) for cisplatin (●), lobaplatin (■) and oxaliplatin (▲).

This indicates that besides blocking the conversion of mono-adducts to bi-adducts, thiourea also affects bi-adducts in a way that they can no longer be detected by $^{32}$P-postlabelling, possibly by substitution of either (or both) of the N7-Pt bonds. Thiourea also affected the GG and AG adduct levels in the case of lobaplatin (to a smaller extent) and oxaliplatin (to a larger extent). A milder treatment (10 min at 24°C) as has been described before (22,23), resulted in efficient blocking of cisplatin derived mono-adducts and no loss of bifunctional adducts. However, this treatment was not sufficient to block mono-adducts of lobaplatin (data not shown). This suggests that each compound requires special incubation conditions to block the conversion of adducts correctly. We wanted to compare the compounds under identical conditions, we omitted the blocking of monofunctional adducts by thiourea under the conditions described.

With the $^{32}$P-postlabelling technique we could determine the ratio of GG over AG intrastrand crosslinks. These were $3.9 \pm 0.3$, $5.9 \pm 0.6$ and $3.4 \pm 0.3$ for cisplatin, lobaplatin and oxaliplatin, respectively (mean ± SEM).

Cell studies
After determining the reactivity of lobaplatin and oxaliplatin in vitro, we tested their reactivity in the human ovarian carcinoma cell line A2780. First, the cytotoxicity of all three compounds was tested in a colony forming assay. The resulting survival curves are shown in Figure 4, indicating an equal cytotoxicity for cis- and oxaliplatin, but a lower cytotoxicity for lobaplatin.

We studied the rate of adduct formation by immunocytochemistry and $^{32}$P-postlabelling. To this end, cells were treated with equimolar doses (15 μM, 2 h) of the three platinum compounds, followed by post-incubation in platinum-free medium for up to 24 h. For the immunocytochemical assay we used an antiserum (NKI-A59) that is believed to recognize intrastrand crosslinks or a conformational change in the DNA structure induced by cisplatin. In this experiment, lobaplatin and oxaliplatin induced comparable nuclear staining, whereas cisplatin showed substantially higher nuclear staining. Apparently, the antiserum is also able to recognize lobaplatin and oxaliplatin induced DNA damage. In the case of cisplatin, the staining seemed to be saturated, which was confirmed by a dose–response experiment (not shown). Therefore, the experiment was repeated with a ten times lower dose of cisplatin (1.5 μM), now resulting in comparable staining levels for all three compounds (Figure 5A). A maximum nuclear staining was observed after 4 h of postincubation, followed by a slow decrease. It has to be noted, however, that the ten-fold higher nuclear staining level caused by cisplatin, compared with lobaplatin and oxaliplatin, does not necessarily reflect actual differences in adduct levels, since the affinity of the antiserum towards adducts containing various carrier ligands might be different.

Pt–GG and Pt–AG adducts in drug-treated cells were analysed using $^{32}$P-postlabelling (2 h, 15 μM for all three compounds). As can be seen in Figure 5B, the overall ratio of adduct levels was about 20:3:1 for cisplatin, oxaliplatin and lobaplatin respectively. The high value, immediately after the 2 h treatment ($t = 0$) is an overestimation of the amount of bifunctional adducts due to the in vitro conversion of mono-
into bi-adducts during DNA isolation (21, Blommaert et al., submitted). Therefore, the actual maximum adduct levels were between 4 and 6 h. The ratio of GG over AG intrastrand crosslinks, as determined by \(^{32}\)P-postlabelling, were 3.7 ± 0.3, 4.5 ± 0.5 and 3.8 ± 0.6 for cisplatin, lobaplatin and oxaliplatin, respectively (mean ± SEM, Table I).

**Discussion**

We have confirmed the existence of the intrastrand crosslink adducts Pt–GG and Pt–AG for lobaplatin and oxaliplatin, using the \(^{32}\)P-postlabelling technique. Other platinum adducts, such as monoadducts or G–Pt–G, cannot be measured using this technique. Based on combined HPLC–AAS and \(^{32}\)P-postlabelling analysis of digested, highly platinated DNA, it was established that GG and AG intrastrand crosslinks are indeed the major bifunctional adducts.

There is a difference in the *in vitro* reactivity towards DNA between the three platinum compounds in the order cisplatin > lobaplatin > oxaliplatin. This was indicated *in vitro*, in both the kinetic and the dose–response experiments by \(^{32}\)P-postlabelling and AAS. The relative differences in the total number of platinum adducts between the three compounds as measured by AAS (3.1:1:8:1) were smaller than the differences in GG and AG intrastrand crosslinks as measured by \(^{32}\)P-postlabelling (14.8:1:8:1). After a 48 h incubation, the average recoveries for GG adducts after lobaplatin and oxaliplatin treatment were lower than for cisplatin. This can be explained by either differences in the contribution of GG and AG adducts to the total platination (such as higher levels of monoadducts), and/or by a different susceptibility of the different GG and AG adducts to the postlabelling assay. A likely explanation for a different susceptibility may be a less efficient enzymatic digestion of the bulky diaminocyclohexane/diaminomethylcyclobutane adducts. Peak areas of the parent nucleosides after digestion indicate no difference in digestion efficiency, but this does not allow firm conclusions on the efficiency of digestion/dephosphorylation of the GG and AG adducts, present in only minor amounts (Rb values < 0.01). We are currently investigating the digestion of DACH–platinum- and related adducts in more detail.

The blocking of the conversion of mono- into bi-functional adducts using thiourea under the conditions described (1 h, 37°C) (21) appeared to result also in the loss of intrastrand crosslinks GG and AG. The extent of the loss of adducts differed not only for the adducts GG and AG, but also from one compound to another. Although a milder treatment (10 min, 24°C) (22,23) appeared to be suitable for cisplatin and oxaliplatin, we found hardly any blocking in the case of lobaplatin. The requirement of different TU incubation conditions has been described before (21,22). Since we wanted to compare the compounds under identical conditions, we omitted the blocking of monofunctional adducts. As a consequence, the analysis of intrastrand crosslinks should take place after completion of mono- to bi-adduct conversion, in order to avoid overestimation of biadducts that were formed from monoadducts during DNA isolation.

The difference in reactivity *in vitro* towards DNA of cisplatin on the one hand and lobaplatin and oxaliplatin on the other hand is remarkable. The same phenomenon has been described for carboplatin, which contains, like lobaplatin and oxaliplatin, a carboxylic anion as leaving ligand. This difference *in vitro* has also been described when cisplatin is compared with carboplatin, showing a more than 100-fold difference in reactivity (24,25). This is probably caused by the low ‘leaving group’ ability of carboxylate ligands relative to the chloro ligands in cisplatin. The rate-limiting step of the interaction of carboplatin with DNA is thought to be the substitution of.

**Table I. The ratio of GG over AG adducts *in vitro* and in A2780 cells**

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<tr>
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<th><em>In vitro</em></th>
<th>In cells</th>
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<tbody>
<tr>
<td>Cisplatin</td>
<td>3.9 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Lobaplatin</td>
<td>5.9 ± 0.6*</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>3.4 ± 0.3</td>
<td>3.8 ± 0.6</td>
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*The GG/AG ratio for lobaplatin is significantly different from those of cisplatin (P = 0.007) and oxaliplatin (P = 0.0013). Other differences are statistically not significant.*
the first carboxy moiety by either water (24) or directly by the nucleophilic N7 of guanine (26). This may, to a lesser extent, also hold for both lobaplatin and oxaliplatin, and could explain the similarity in kinetic behaviour with respect to their reactivity towards DNA in vitro (6).

The antisemur NKI A59, initially raised against cisplatin treated DNA, was able to recognize DNA modification induced by all three compounds. In order to obtain comparable nuclear staining levels the dose of cisplatin had to be taken ten times lower than that of both lobaplatin and oxaliplatin. Since the adducts formed by all three compounds are recognized by the antisemur, it is likely that it recognizes DNA conformational alterations caused by platinum, rather than the molecular structure of the adduct itself. However, the differences in nuclear stain levels do not automatically reflect similar differences in adduct levels, since the presence of different carrier ligands may result in a difference in affinity of the antisemur towards the adducts. The nuclear staining levels, as a function of time, show similar patterns for all compounds. Maximum intranuclear crosslink (nuclear staining) levels after 4 h of post-incubation indicate that in these samples mono- to bifunctional adduct formation was completed. In this respect, lobaplatin and oxaliplatin differ from carboplatin, which shows maximum bifunctional adduct levels between 12 and 18 h (25).

GG and AG adduct levels were measured with 32P-postlabelling after treatment of the cells with equimolar concentrations of the different compounds. This experiment (Figure 5) indicated substantially higher GG and AG levels for cisplatin than for both other compounds. Even when the 4.7-fold difference in overall 'recovery' of GG and AG between cisplatin on the one hand and oxaliplatin and lobaplatin on the other hand, was taken into account, more cisplatin-DNA adducts were still present in the cells. This suggests that either less lobaplatin and oxaliplatin adducts were required to obtain a comparable toxicity, or that other types of platinum damage are involved. A comparable observation was made by Schmidt et al. (8) who found in A2780 cisplatin-sensitive cells that DACH-Pt adducts were 1.6-times more toxic than diamine-Pt–DNA adducts. With respect to the possible differences in cytotoxicity between the various adducts, we determined for each compound the relative occurrences of the Pt-GG and Pt-AG adducts (GG/AG ratios) in the cells; as explained earlier, other adducts cannot be detected with the postlabelling assay. As described previously, the GG/AG ratio decreased with the platinum/DNA input ratio (23). The data used for statistical analysis therefore only included samples with a P/nucleotide input ratio 0.01. Although the ratio of GG over AG in calf thymus DNA in solution was significantly higher for lobaplatin than for cis- and oxaliplatin, this difference was no longer significant in cells. No conclusions can be drawn at present with respect to the cytotoxicity of the individual adducts.

An interesting result of our studies is the difference in activity for both lobaplatin and oxaliplatin in cells compared with that in calf thymus DNA in solution. This phenomenon parallels the situation with carboplatin. Compared to cisplatin, a 5- to 20-fold higher concentration of carboplatin is needed to obtain equal cytotoxicity and adduct levels in cells, whilst in calf thymus DNA in solution a 100- to 230-fold higher concentration is needed to obtain equal platination (25). This suggests an 'activation enhancement' for carboplatin in cells. Possible explanations for the higher reactivity of carboplatin in cells are catalysis by substitution of the carboxy ligand by nucleophilic sites in the cellular environment (26), enzymatic cleavage (27), or the presence in the cell of oxygen free radicals (28). A comparable enhancement might exist for lobaplatin and oxaliplatin (6).

In conclusion, the 32P-postlabelling technique has been shown to be appropriate for adduct analysis, not only for the classical Pt compounds cis- and carboplatin but also for novel platinum compounds like lobaplatin and oxaliplatin. The reactivity of the latter compounds towards DNA in vitro indicated large differences, compared with cisplatin. This difference was smaller in cells, suggesting enhancement of adduct formation by certain cellular mechanisms and/or compounds. For more precise studies, for example in a clinical setting, it will be useful to examine in more detail differences between the various adducts in DNA digestion.

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