1300–1306 Nucleic Acids Research, 1995, Vol. 23, No. 8 © 1995 Oxford University Press

Detection of platinum–DNA adducts by ^{32}P-postlabelling

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Received January 23, 1995; Revised and Accepted March 10, 1995

ABSTRACT

We developed a sensitive ^{32}P-postlabeling method for the detection of bifunctional intrastrand crosslinks d(Pt-GpG) and d(Pt-ApG) in DNA *in vitro* and *in vivo*. After enzymatic digestion of DNA the positively charged platinum adducts were purified from unplatinated products, using strong cation exchange chromatography. Subsequently the samples were deplatinated with cyanide, because platinated dinucleotides are very poor substrates for polynucleotide kinase. The excess of cyanide was removed using Sep-pak C18 cartridges, and the resulting dinucleoside monophosphates d(GpG) and d(ApG) were subsequently postlabelled. Analysis of the postlabelling mixture was performed by a combined TLC and HPLC-procedure. Good correlations with existing methods (AAS, immunocytochemistry and ELISA) were found in DNA samples treated *in vitro* and *in vivo* with cis- or carboplatin. The detection limit of the assay was 1 adduct/10^7 nucleotides in a 10 μg DNA sample.

INTRODUCTION

The anti-tumor drugs *cis*-diamminedichloroplatinum(II) (cisplatin) and *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) are widely used in the clinic as chemotherapeutic agents against a broad range of tumors (1).

It has been generally accepted that the antitumor effect of platinum compounds is based on its interaction with DNA, leading to the formation of various types of platinum–DNA adducts (2). Reduced net drug uptake, high levels of scavenging compounds such as glutathione, and increased repair of DNA damage seem to be important factors involved in the cellular resistance to platinum drugs (3). Other mechanisms of resistance are related to changes in the response of cells to damaged DNA, such as the induction of cell cycle arrest and programmed cell death (4,5). The contribution of these mechanisms to clinical platinum resistance is not fully known (6). However, clinical studies indicate that a correlation exists between the tumor response and the extent of platinum–DNA damage in white blood cells (7) and buccal cells (8).

To study the formation and persistence of platinum–DNA adducts at pharmacologically relevant drug levels, a sensitive detection method is required. The most commonly used method, AAS (atomic absorption spectroscopy) of platinated DNA, has a limited sensitivity, and can not distinguish between the different adducts (9). The alkaline elution technique only recognizes interstrand cross links, representing only 1–2% of the adducts, and is not applicable in clinical studies, since it requires incorporation of radioactive compounds (10). Several immunochemical techniques have been developed, using polyclonal antibodies directed either against platinum adducts in high molecular weight DNA (11–13), or against the various platinum adducts in digested DNA (Pt-dG, d(Pt-ApG), d(Pt-GpG) and d(G-Pt-G)) (14,15). Another method using polyclonal antibodies enables recognition of platinum–DNA damage at the cellular level (8,16). A drawback of methods using polyclonal antibodies is, however, the limited availability of the antisera. Moreover, such specific antibodies may not be suitable to detect DNA adducts of novel platinum compounds with different ligands. Assays based on the polymerase chain reaction (PCR) (17), can be applied to study platinum–DNA damage as well as various other types of DNA damage (X-ray, UV) at the gene-level. Clinical application is, however, hampered by the relatively low sensitivity of the assay (18,19), although recently a new PCR based technique (single strand ligation PCR) has been reported which should be sensitive enough to measure platinum–DNA damage at the level of a single copy gene at physiologically relevant drug doses (20). Other assays that can detect platinum–DNA adducts at the gene level, use ABC excinuclease for the detection of intrastrand adducts and denaturation/reannealing reactions for the detection of interstrand crosslinks (21). However, the sensitivity of these assays is limited by the length of the target fragment, and clinical application seems unlikely. The postlabelling technique (22), widely used to detect and quantitate various types of DNA adducts (23) would seem an attractive alternative to detect and quantitate low levels of platinum–DNA damage. Unfortunately platinum–DNA adducts have proved to be poor substrates for PNK used for postlabelling (24). An alternative method is postlabelling of the unmodified nucleotide attached to the 5′ of the platinum–DNA adducts (25). However, the extra 5′ nucleotide probably complicates the discrimination between the various adducts. The problems with postlabelling of the platinated adducts can be overcome by removal of the platinum molecule with sodium cyanide (NaCN) prior to the postlabelling (26). In the assay described here, the platinum–DNA adducts Pt-dG, d(Pt-GpG), d(Pt-ApG) and d(G-Pt-G) were isolated from DNA digests by strong cation exchange chromatography (SCX). After deplatination of this mixture, the dinucleoside monophosphates d(GpG) and d(ApG) were ^{32}P-postlabelled. Detection of d(Pt-G) and

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d(G-Pt-G) adducts, however, was not possible since deoxyguanosine, resulting from deplatination of the latter adducts, lacks the 3′-phosphate, a prerequisite to act as a substrate for PNK.

MATERIALS AND METHODS

Chemicals

d(GpG) (ammonium salt) was obtained from Pharmacia (Pharmacia Biotech Benelux, Roosendaal, The Netherlands), d(ApG) (ammonium salt) was obtained from Sigma Chemical Company (St Louis, MO). [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from Amersham. Carboplatin was obtained from Bristol Meyers (Paraplatin, Bristol-Meyers Weesp, The Netherlands: each vial contained 150 mg carboplatin and 150 mg mannitol), and cisplatin from Lederle (Lederle Nederland BV, Etten-Leur, The Netherlands) and Bristol-Meyers (Platinol).

Enzymes and buffers

PNK, kinase buffer, alkaline phosphatase and nuclease P1 were obtained from Boehringer (Mannheim, Germany). DNAse I was obtained from Cooper Biomedical.

In vitro platination

d(GpG) was incubated for 48 h at 37°C in the dark with an equimolar concentration of cisplatin (Lederle). Rat kidney DNA was incubated overnight at 37°C with equal volumes containing different concentrations of cisplatin in a 10 mM Tris/1mM EDTA buffer, pH 7.2 (final concentration 0.5 mg DNA/ml). After incubation, DNA 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.

Rats and treatment

Inbred female Sprague–Dawley rats, 3–4 months old, from the SPF breeding colony of the Netherlands Cancer Institute, received a single i.p. dose of cisplatin or carboplatin. Cisplatin (Platinol) was administered in a solution of 0.14 M NaCl in a dose of 0, 2, 4, 6 or 8 mg/kg. The rats were sacrificed after 6 h. Carboplatin was given in doses of 0, 20, 40, 60 or 80 mg/kg, and rats were sacrificed after 20 (dose response), 8 or 48 h (comparison with ELISA). Carboplatin (Paraplatin) was dissolved in sterile water just before injection. Control animals were injected with 1 ml 0.15 M NaCl. Animals were kept under a 14–10 h light–dark cycle and fed with standard laboratory chow (Hope Farms, Woerden, The Netherlands) and water *ad libitum*. Rats were killed by exsanguination. Liver, kidneys, spleen and testes were quickly removed, frozen on dry ice, and stored at –80°C.

Isolation of DNA

DNA was isolated as described previously (15). No ammoniumbicarbonate or thiourea was added to block the conversion of monoadducts into bifunctional adducts, since isolation of the livers of the carboplatin- or cisplatin-treated rats took place at a time that the conversion of monofunctional into bifunctional adducts had come to completion (Blommaert *et al.*; unpublished data). The DNA samples from carboplatin-treated rats used for the comparison of competitive ELISA and postlabelling were isolated in the presence of 0.5 M ammoniumbicarbonate.

Analysis of total platinum–DNA binding

Total platinum–DNA levels of *in vitro* treated rat-kidney DNA were determined by AAS. The DNA concentration of these samples was measured by spectrophotometry (22 O.D. = 1 mg/ml at 260 nm).

Digestion of DNA

DNA (100 μg) was digested in a 100 μl mixture containing 1/10 vol. of a 10 × concentrated digestion buffer (500 mM bis-Tris, 20 mM $MgCl_2$, 10 mM $ZnCl_2$, pH 7.2), 5 U DNase I, 5 U nuclease P1 and 5 U alkaline phosphatase, at 37°C overnight. After addition of 1/10 vol. of 1 M Tris–HCl pH 9 the incubation was prolonged for 4 h. DNA digests were checked on HPLC using a Supelco Supercosil C18 (particle size 5 mm, length 25 cm) reverse phase column. The exact amounts of DNA were calculated from the deoxyadenosine peak (ε = 15 400). Eluent A: 40 mM KH_2PO_4, 1% methanol, pH 4. Eluent B: 100% methanol. Gradient: a linear gradient 10–30% B in 30 min.

Isolation of platinum adducts from digested DNA

Digested DNA samples (≤ 100 μg) were applied to cartridges (Sep-Pak light tC18 cartridges, part no. 36805, Waters) from which the original filling was replaced by strong anion exchange material (aromatic sulfonic acid; Macherey Nagel, VYDAC 401 SC). Before use, the cartridges were subsequently washed with 2 ml 100% methanol, and 3 ml of distilled water. Unmodified nucleotides and nucleosides were eluted with 15 ml of 3 mM ammonium formate (pH 6.0). The platinum containing products were subsequently eluted with 2 ml of 3 M ammonium formate (pH 6.0), from which the last 1600 μl were collected.

Deplatination of the digested platinum–DNA adducts

Platinum was removed from the bifunctional adducts during a 2 h incubation at 65°C after addition of ¼ vol. of 1 M NaCN. The deplatinated mono- and bi-functional adducts were purified using Sep-Pak light tC18 cartridges (previously 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 monophosphate and nucleosides were eluted with 500 μl of methanol–H_2O (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.

^{32}P-Postlabelling of the samples

To each sample 2.8 μl of water, 1 μl [γ-^{32}P]-ATP (3000 Ci/mmol, 3.3 pmol/ml, 1 μl kinase buffer and 0.2 μl (2 U) PNK were added, and the resulting mixture was incubated for 30 min at room temperature. When substrate levels were expected to exceed 1 pmol, they were diluted, prior to labelling, to a suitable concentration.

Combined TLC and HPLC analysis of the labelled dinucleotides

The excess of ATP and some non-platinum-related, unidentified, compounds were separated from the dinucleotides on TLC by

applying the reaction mixture on Polygram cel 300 PEI TLC sheets [Macherey-Nagel, 20 width × 40 (height) cm] and run overnight with 0.125 M KH_2PO_4 (pH 6.0) buffer. After autoradiography, the spots containing the compounds of interest were cut out and extracted with 5 M ammonium formate, pH 4.0 in two steps (1 × 1000 μl, 1 × 500 μl). After centrifugation, the supernatants were subjected to HPLC using Beckman System Gold equipment, consisting of a model 126 pump and a model 171 on-line radioisotope detector. The separation step was performed on a Supelco Supercosil C18 (particle size 5 mm, length 25 cm) reverse phase column run isocratically at a flow rate of 1 ml/min with 40 mM KH_2PO_4/5% methanol v/v, pH 4.0. The amount of dinucleotides (fmol) was calculated from the integrated d(^{32}pGpG) and d(^{32}pApG) peaks. (1 fmol = 6660 d.p.m., on the date of calibration). The original number of platinum–DNA adducts can be calculated after correction for the recovery, by using a standard platinated DNA-sample with known levels of d(Pt-GpG) and d(Pt-ApG).

Immunocytochemical analysis of platinum-induced modification

The immunoperoxidase staining procedure was carried out as described (8), with some modifications. The general outline of the method was as follows: cryostat sections were fixated with methanol–H_2O_2 (methanol, 0.3% H_2O_2 and 0.1% Na-azide to inactivate endogenous peroxidases). Sections were rehydrated via ethanol to water, incubated for 3 min with proteinase K buffer at 37°C (PK-buffer: 20 mM Tris–HCl; 5 mM EDTA, pH 7.4) and then incubated for 10 min at 37°C with proteinase K (50 μg/ml) in PK-buffer. The reaction was stopped by a 15 min incubation at room temperature in glycin (0.2% glycin; 0.04% Triton X-100 in PBS). Sections were transferred via water, 20 and 40% ethanol to ethanol–NaOH (40% ethanol, 0.05 N NaOH) and incubated for 10 min at room temperature and neutralized (5 s) in 1% acetic acid/40% ethanol (the incubations with NaOH and proteinase K were performed to denature DNA and to increase the accessibility of the cisplatin–DNA adducts for the antibodies). Sections were rehydrated to water and rinsed with wash buffer (50 mM Tris, 150 mM NaCl, 0.25% gelatin, 5 mM EDTA, 0.04% Triton X-100 pH 7.4) and PBS, incubated for 1 h with 5% human serum (to reduce non-specific antibody binding) and overnight with rabbit antiserum NKI-A59 against cisplatin-modified calf thymus DNA (27). Antibodies bound in the last step were visualized by double PAP staining i.e. sequential incubation of the cryostat sections with goat anti-rabbit immunoglobulin (GaR), peroxidase–(rabbit)antiperoxidase complex (PAP), GaR, PAP and finally, 3,3′-diaminobenzidine–HCl/H_2O_2, that served as a substrate. Each sample was stained in duplicate in separate stainings on different days. In each of two independently stained slides, the nuclear stain (defined as the sum of optical densities of the stained nuclear pixels (28) of 20 randomly selected nuclei was expressed in arbitrary units (a.u).

Immunochemical detection of d(Pt-pGpG) and d(Pt-pApG) adducts

The detection of d(Pt-pGpG) and d(Pt-pApG) was performed by competitive ELISA as described before (14,15).

RESULTS

Sample preparation

A complicating factor in the development of a ^{32}P-postlabelling assay for the detection of Pt-DNA adducts is the fact that, in contrast to their non-platinated analogs, the platinated dinucleoside monophosphates d(Pt-GpG) and d(Pt-ApG) are not good substrates for PNK (24). Therefore, a 'de-platination' step was included. For this purpose, cyanide, known for its strong affinity for platinum (II) ions by forming the $(Pt(CN)_4)^{2-}$ anion (26) can be used. As a consequence of this approach some additional purification steps are necessary.

The first one is necessary because d(GpG) and d(ApG), resulting from deplatination of the adducts, can not be discriminated from the unplatinated counterparts that may result from incomplete digestion of the total DNA. Consequently, a purification step is required. In the case of platinum-adducts, the purification is based on differences in net charge of the platinated and non-platinated digestion products. Therefore, DNA samples were digested to neutral nucleosides, which implicates a net positive charge of the platinated compounds, whereas the dinucleotides that resulted from incomplete digestion, are negatively charged. The purification was performed with a strong cation-exchange (SCX) column, from which all unmodified nucleosides and incompletely digested material were eluted with a low salt buffer. Subsequently, elution of the platinated products was performed with a high salt buffer.

A second purification step was needed to remove the excess of cyanide used for de-platination of the adducts which appeared to inhibit PNK activity (data not shown). For this purpose, the mixture of cyanide and the deplatinated adducts was applied to the Sep-pak C18 cartridge. Cyanide could be eluted with water, and subsequently the deplatinated adducts were eluted with methanol–water (20/80 v/v). The results of the various steps are shown in Figure 1 with d(Pt-GpG) as reference compound.

^{32}P-Phosphorylation of the dinucleoside monophosphates d(GpG) and d(ApG) and analysis of the labelled compounds

The eluted deplatinated adducts were dried *in vacuo*, redissolved in water and, after addition of a mixture of [γ-^{32}P]ATP , kinase buffer and PNK, phosphorylated during a 30 min incubation at room temperature. The phosphorylation was followed by a combined TLC and HPLC purification step. In Figure 2A an example is shown of a TLC chromatogram after ^{32}P-postlabelling. In Figure 2B the HPLC chromatogram is shown of the ^{32}P-containing material extracted from the d(pGpG) and d(pApG) spots of one of these samples. This figure shows that the HPLC step is required, because the ^{32}P-d(GpG)- and ^{32}P-d(ApG)-containing spots in the TLC chromatogram still contain other radioactive compounds. These compounds were not present in a control mixture containing the labelling mix and 15 ml of distilled water, but they were present in an untreated DNA sample. Since they did not correlate with platinum levels, the identity of these compounds was not studied.

^{32}P-Postlabelling of DNA treated *in vitro* with cisplatin

Application of the assay on DNA treated *in vitro* with cisplatin indicated linear correlations between the number of adducts per nucleotide and the cisplatin concentration used (Fig. 3). For comparison, the same samples were measured with AAS. As

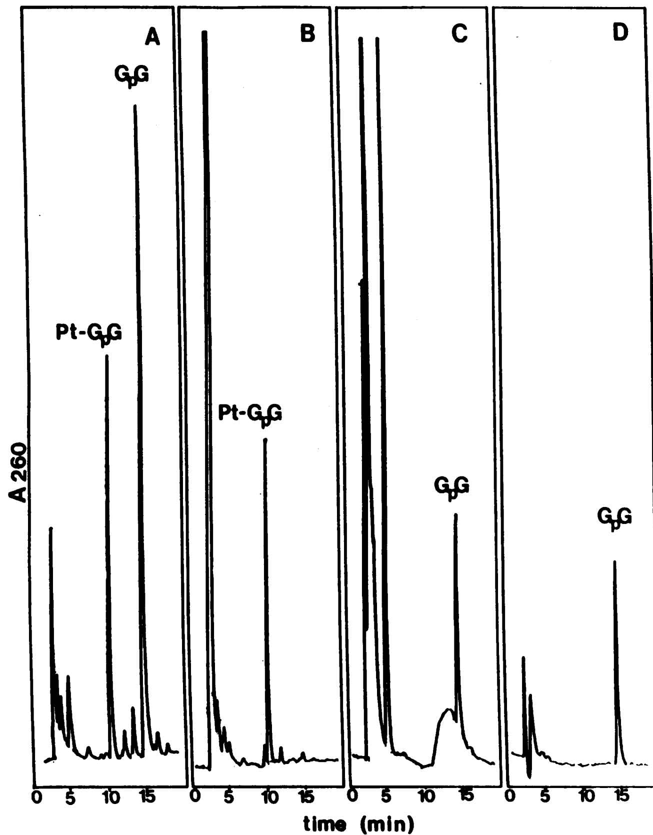


Figure 1. HPLC chromatograms of the samples taken during the successive steps of the sample preparation procedure. (**A**) Shows the presence of d(Pt-GpG) and d(GpG) peaks as present after a 48 h incubation at 37°C of a 1:1 mixture of cisplatin and d(GpG). (**B**) Removal of d(GpG) after SCX purification. (**C**) The d(GpG) peak after incubation of d(Pt-GpG) with sodium cyanide for 2 h at 37°C. (**D**) The deplatinated d(GpG) after purification on C18 Sep-pak. The HPLC conditions were identical to those used for analysis of the digestion conditions (see 'Materials and Methods').

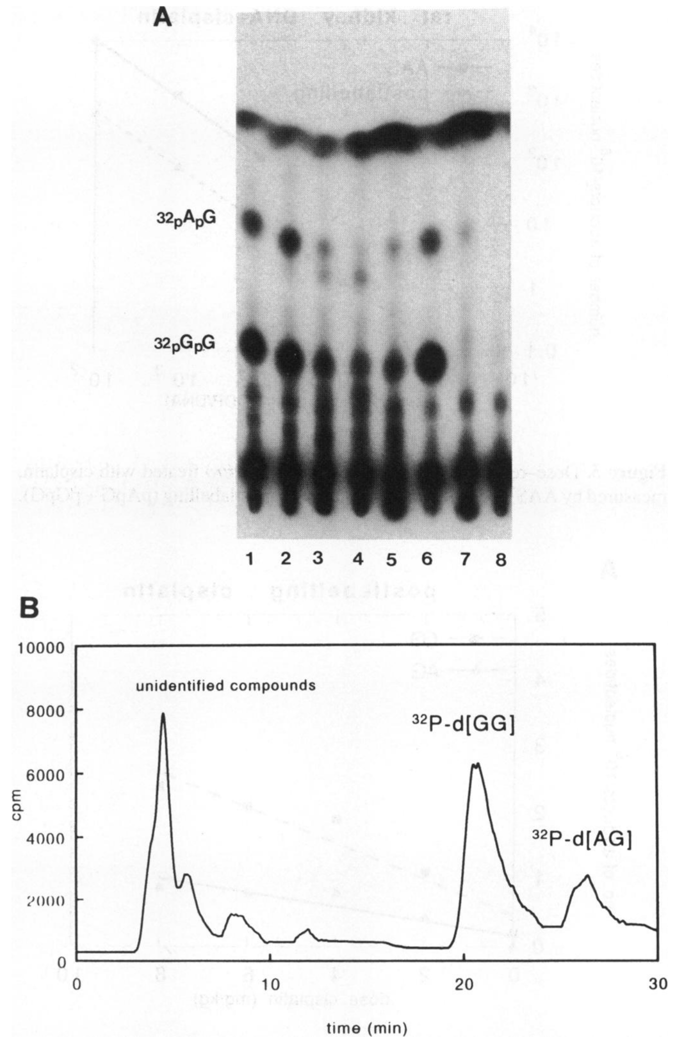


Figure 2. (**A**) TLC chromatogram of ^{32}P-postlabelling analysis of DNA from the following tissues of carboplatin treated rats (80 mg/kg): lane 1, liver (8 h after administration); lane 2, kidney (8 h); lane 3, spleen (8 h); lane 4, testis (8 h); lane 5, liver (48 h); lanes 6 and 7, cisplatin treated calf thymus DNA (cisplatin to DNA ratio: 10^{-5} and 10^{-7} respectively) and lane 8, negative control (liver DNA of untreated rat). (**B**) HPLC chromatogram of the spots cut from lane 3.

shown the detection limit of ^{32}P-postlabelling is about 100 times lower than that of AAS.

^{32}P-Postlabelling of *in vivo* platinated DNA

The method was also applied to various DNA samples isolated from the livers of rats treated with different doses of cis- or carboplatin. As shown in Figure 4, a linear relationship between dose and the ^{32}P-labelled d(GpG) and d(ApG) dinucleotides, derived from the d(Pt-GpG) and d(Pt-ApG) adducts was found.

For further validation, the ^{32}P-postlabelling assay was compared with two other sensitive methods for the detection of platinum–DNA adducts. Firstly, the immunocytochemical method performed with the NKI-A59 antiserum, that is believed to recognize the bifunctional intrastrand adduct d(Pt-GpG) and (probably) d(Pt-ApG), or a DNA conformational change induced by these adducts (unpublished results). Good correlations were observed between ^{32}P-postlabelling and the nuclear stain levels in livers from rats treated with carboplatin ($\chi^2 = 0.97$, $P = 0.04$) or cisplatin ($\chi^2 = 0.95$, $P = 0.02$) as shown in Figure 5.

Secondly, the ^{32}P-postlabelling data were compared with data obtained with competitive ELISA for the d(Pt-GpG) and d(Pt-ApG) adducts in digested and chromatographed DNA samples isolated from tissues of carboplatin-treated rats. The results of the comparison are shown in Figure 6. The slopes indicate linear correlations for the d(GpG) ($\chi^2 = 0.92$, $P = 0.01$) and for d(ApG) ($\chi^2 = 0.86$, $P = 0.03$).

DISCUSSION

We have developed a ^{32}P-postlabelling assay to quantitate the platinum–DNA adducts Pt-GpG and Pt-ApG, after removal of platinum with NaCN. A schematical representation of the assay is given in Scheme 1. After enzymatic digestion of DNA the positively charged platinum adducts were separated from unplatinated products, using strong cation exchange chromatography. After deplatination of the samples with cyanide, the excess of cyanide was removed using Sep-pak C18 cartridges. The resulting dinucleotides d(GpG) and d(ApG) were labelled, and the labelling mixture was purified and analyzed by a combined TLC and

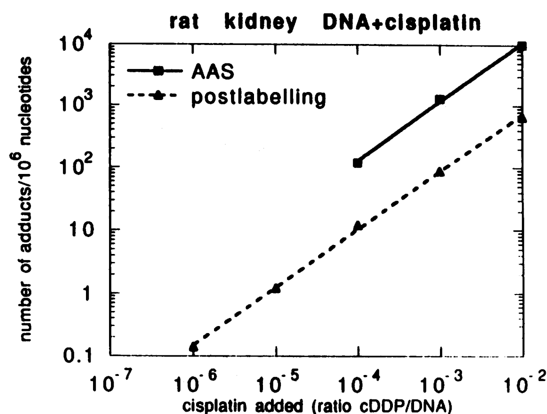


Figure 3. Dose–response relationship of DNA *in vitro* treated with cisplatin, measured by AAS (total platination) and by ^{32}P-postlabelling (pApG + pGpG).

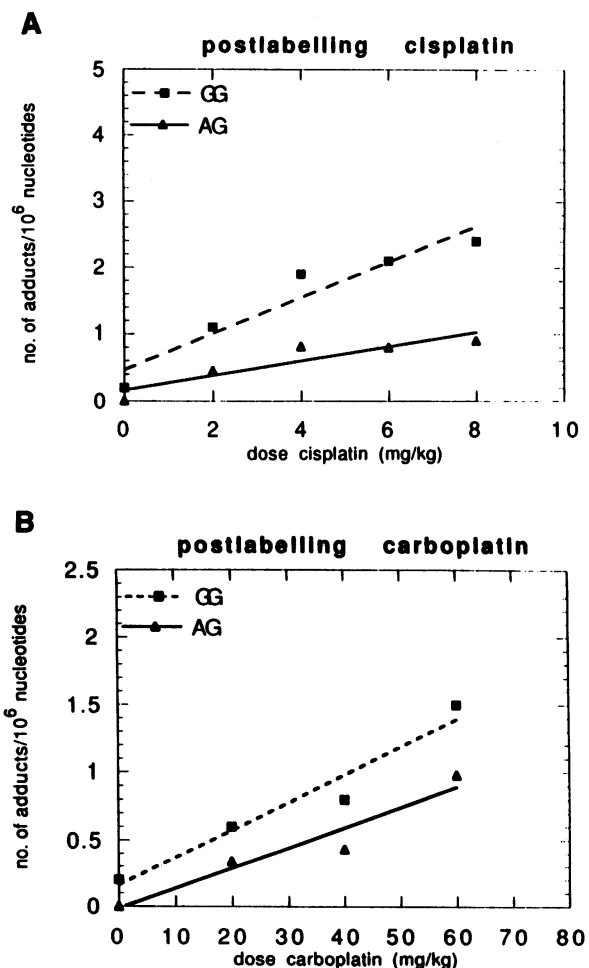


Figure 4. Dose-response relationship in the liver of rats treated with cisplatin (**A**) or carboplatin (**B**), measured with the ^{32}P-postlabelling assay.

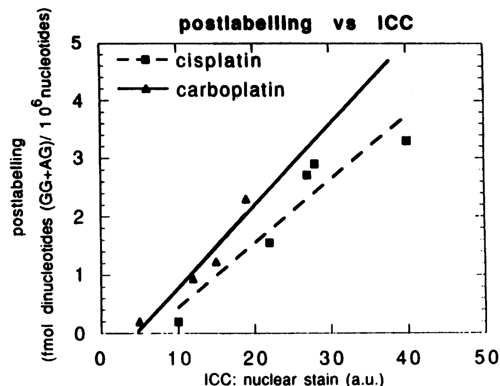


Figure 5. The correlation between ^{32}P-postlabelling and immunocytochemistry. Platinum–DNA damage, measured by the NKI-A59 antiserum, is expressed as nuclear stain in arbitrary units (carboplatin: $\chi^2 = 0.965, P = 0.036$; cisplatin: $\chi^2 = 0.945, P = 0.016$).

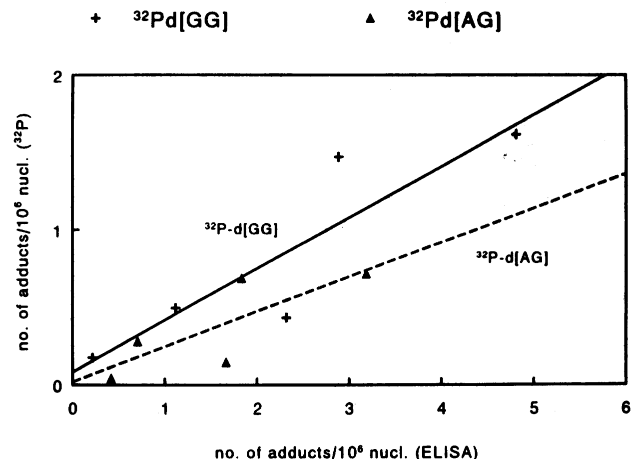
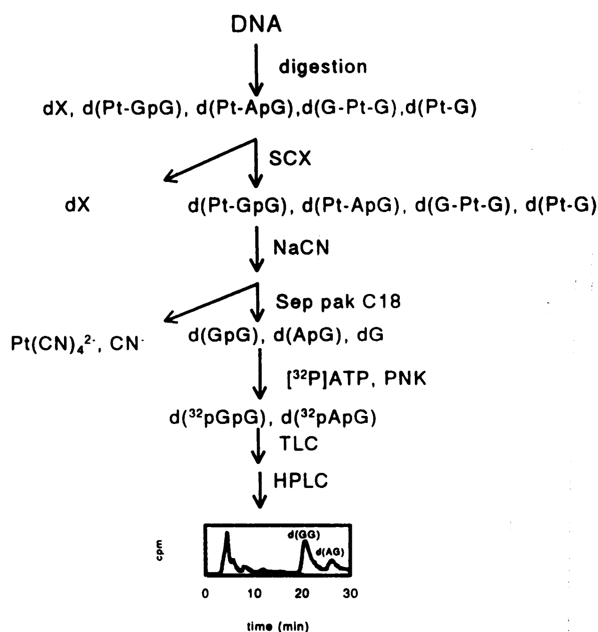


Figure 6. The correlation between ^{32}P-postlabelling and competitive ELISA [d(GpG) $\chi^2 = 0.92, P = 0.01$; d(ApG): $\chi^2 = 0.86, P = 0.03$].

HPLC-procedure. Linear correlations were observed between the number of labelled dinucleotides and the drug dose *in vitro* (cisplatin) and *in vivo* (cis- and carboplatin). Linearity of the postlabelling step under the described conditions was observed over 2 orders of magnitude; in the case of *in vivo* experiments, no dilution of samples was required. The adduct levels found in rat livers with the ^{32}P-postlabelling assay showed a good correlation with the nuclear stain level measured by immunocytochemistry, using an antiserum directed against bifunctional intrastrand DNA adducts (NKI-A59). d(Pt-GpG) and d(Pt-ApG) adduct levels in carboplatin-treated rats measured by postlabelling also correlated well to the levels measured by competitive ELISA. The relative occurrences of the d(Pt-GpG) and d(Pt-ApG) adducts after treatment of DNA or rats with cisplatin were 3:1 ($\chi^2 = 1.0, P = 0.0001$). This is in agreement with published data where relative occurrences of d(Pt-GpG) and d(Pt-ApG) in DNA treated with cisplatin were also reported to be 3:1 (2,29). In comparison, relative occurrences of the adducts in the DNA samples from various tissues of rats treated with carboplatin were somewhat different; relatively more d(Pt-ApG) adducts were formed: d(Pt-GpG):d(Pt-ApG) = 2.2:1 ($\chi^2 = 0.96, P = 0.001$). These results confirm data on the differences in carboplatin–DNA adduct formation in cells (Blommaert *et al.*, in press), rats and patients (unpublished data); the preference of cisplatin for pGpG sequences as observed both *in vitro* and *in vivo* is absent for carboplatin in

Scheme 1. Schematical representation of the ^{32}P–postlabelling assay.



cells and *in vivo*. In the latter case the relative occurrences of d(Pt-GpG) and d(Pt-ApG) are approximately 2:1. The platinum-related DNA damage in a TACT-sequence observed in cells by Grimaldi was not found in our assay (20). If this type of DNA damage actually contains platinum, it probably does not involve two crosslinked neighbouring nucleotides, since this type of adduct would have been detected by our assay.

The overall recovery of our assay was determined by using DNA samples with known levels of platination, as determined by AAS; the amount of Pt-(GpG) and Pt-(ApG) adducts present was calculated from their relative occurrences as described in literature; the average recovery was about 30%. The recovery values were the result of relatively small losses in the subsequent steps, rather than of a low efficiency in one particular step (data not shown). The overall efficiency of the postlabelling assay compared to the ELISA was found to be 33% for Pt-d(GpG) and 22% for Pt-d(ApG). The cause of the difference found in d(Pt-GpG)/d(Pt-ApG) ratio between postlabelling and ELISA is not clear, but may result from the difference in digestion conditions. However, these differences are relatively small when compared to differences described between other techniques (13,30).

The detection limit of our assay is equal to the amount of labelled dinucleotide, found in a negative control experiment (water subjected to all subsequent steps of the assay). This background value (as found in Fig. 4A and B at dose zero) is independent of the presence of DNA, or the amount of DNA that is subjected to the assay. Using the postlabelling assay we were able to detect about one d(Pt-GpG) adduct per 10^7 nucleotides (0.3 fmol/μg), using 10 μg of DNA, and by raising the amount of DNA the detection limit will be lowered proportionally. Using the postlabelling assay it is likely that platinum–DNA adducts in small clinical samples such as tumor biopsies can be measured. The postlabelling assay is much more sensitive ($\geq$ 100-fold) than AAS and PCR, and about five times more sensitive than immunocytochemistry and competitive ELISA. Although no data are yet available on the sensitivity of the postlabelling technique as described by Försti (25), their approach seems less preferable because of the presence of an additional nucleotide in the adducts, which complicates the identification of the adducts. An advantage of our method over the sensitive immuno-(cyto)chemical methods is that no antibodies are required. For the immunochemical detection of the various adducts, several specific (polyclonal) antisera (one for each type of adduct) are required; this makes the assay very laborious. Another disadvantage is the limited availability of the polyclonal antisera. Finally, because of their specificity, the presently available antibodies might be useless for the detection of adducts formed by novel platinum-drugs. If the latter have a different ligand, like DACH (diaminocyclohexane), the resulting adduct will be structurally different from adducts formed by cis- or carboplatin. With the postlabelling method, one can identify the dinucleotides that were the subject of platination. This approach allows the analysis of any platinated dinucleotide yielded by novel platinum-drugs. Preliminary results indicate that DNA adducts of novel platinum compounds such as oxaliplatin [l-OHP, oxalato (trans-1-1,2 dia minocyclohexane)] platinum (II) and lobaplatin [1,2-diaminomethyl-cyclobutaneplatin(II)-lactate] (31) can be detected using the postlabelling method. We are currently investigating the feasibility of the analysis of TLC chromatograms by using a phosphorimager. This would make the rather time-consuming HPLC step redundant. The first results look promising. In conclusion, the postlabelling assay offers a reliable and sensitive tool for the detection of platinum–DNA adducts both *in vitro* and *in vivo*, for the present and future generation platinum drugs.

ACKNOWLEDGEMENTS

This work was supported by the Dutch Cancer Society (grants NKB 90-16 and 90-17). The authors thank Dr A. M. Fichtinger-Schepman for the platinum–DNA adduct measurements with the competitive ELISA and Dr O. van Tellingen for the AAS measurements. Drs A. M. Fichtinger-Schepman, L. Smets and E. Kriek are acknowledged for critical reading of the manuscript.

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