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Treatment with Dilute Alkali-Nuclease S_1 Permits the Analysis of DNA Damage: Cells Treated with Platinum Analogues

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SUMMARY

We describe here an approach to characterize various lesions induced in DNA by drug treatments, using three parameters: (a) release of single-stranded DNA fragments by cell lysis in dilute alkali, which result from enzymatic strand scission during DNA repair or chemical alterations of DNA; (b) the presence of high molecular weight DNA in cells after lysis in dilute alkali followed by nuclease S_1 treatment which, due to drug-induced DNA cross-links and its level is a measure of the amount of DNA-containing cross-links; and (c) the appearance of small double-stranded DNA fragments, when the cell lysis is followed by digestion with nuclease S_1 to remove single-stranded DNA. This DNA shows the same characteristics as DNA of untreated cells, but it may contain monoadducts. By following the flow of label through the three parameters, one can characterize both the lesions induced in DNA and how the lesions are repaired. We report here results of three platinum analogues: cis-Pt(II), trans-Pt(II), and cis-FLAP(II). A large proportion of DNA in treated cells appears as fragments (parameter c). The *cis*- compounds and *trans*- compounds differ with regard to appearance of high molecular weight DNA (parameter b) and the initial release of fragments (parameter a).

Antineoplastic agents induce a variety of lesions in DNA of treated cells. The lesions are mainly due to either monoadduct formation (e.g., alkylation, methylation), or cross-linking or incorporation of drugs in the DNA strands (1). DNA of most cells contains a mixed population of lesions; e.g., cis-Pt(II) is an agent that forms various reaction products with DNA, such as DNA-protein cross-links, DNA inter- and intrastrand cross-links, or monofunctional cis-Pt(II)-DNA adducts (2).

The major intrastrand cross-link formed by cis-Pt(II) is the association of Pt to the N(7) positions of two adjacent guanosines (3). The three-dimensional structure of this adduct is known from X-ray crystallography (4). In contrast, trans-Pt(II) cannot form a similar adduct, indicating that this cross-link may be one possibility to explain the difference in biological activity between cis-Pt(II) and trans-Pt(II) (3, 5, 5a).

Size-distribution analysis of DNA is vital in studies of DNA damage and repair following treatment with cytotoxic drugs and irradiation. Because of the large size of mammalian DNA, no good analytical methods exist. Therefore, one often uses indirect methods; e.g., a major technique has been to measure the release of DNA strands from filters using alkaline solutions (alkaline elution) (6). Breaks and/or alkali-labile DNA regions arise either through enzymatic strand scission as part of a repair process or through direct chemical alterations in the DNA.

We wished to develop a method that allows analysis of the level of alkali-labile DNA and the level of cross-linked DNA. A possible new approach was indicated by our recent work on the mechanisms of DNA synthesis. To dissect DNA replication we lyse cells in dilute alkali to allow a partial DNA denaturation, neutralize the solution to allow renaturation of large DNA, and then introduce a treatment with nuclease S_1 to remove single-stranded DNA which has not renatured during the neutralization step (7, 8).

The main advantage of this approach is that the DNA of mature chromatin in cells not treated with drugs appears as short double-stranded DNA (8). These small DNA fragments are easily and reliably detected in gel electrophoresis. In contrast, DNA-containing (e.g., interstrand DNA) cross-links or DNA-protein cross-links appear as "HMW DNA". The amount of HMW DNA is a measure of the presence of cross-links. During the cell lysis (before treatment with nuclease S_1) there is also release of DNA fragments, due to enzymatic strand scission during DNA repair or chemical alterations of DNA.

We now describe this approach and present results obtained with three different drugs (platinum analogues) interfering with DNA: cis-Pt(II), trans-Pt(II), and cis-FLAP(II).

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ABBREVIATIONS: cis-Pt(II), *cis*-diamminedichloroplatinum(II); trans-Pt(II), *trans*-diamminedichloroplatinum(II); HMW, high molecular weight; cis-FLAP(II), *cis*-dichloro-di-(2-hydroxyethyl)-2-methyl-5-nitro-3-imidazolo) diamminedichloroplatinum(II); araC, cytosine β-D-arabinofuranoside; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate.

Materials and Methods

Cells, culture medium, and labeling with ^{3}H-thymidine. A human melanoma cell line, obtained from Flow Laboratories, U.K., was grown as monolayers at 37° in 5% CO_2 in air. The culture medium was Eagle's minimal essential medium with Earle's salts, containing 2 mM L-glutamine, 10% fetal calf serum, and antibiotics. The culture medium was routinely changed twice weekly and the cells were passaged every 4–6 days.

For experiments the cells were seeded in small culture dishes (35 × 10 mm) containing 3 ml of medium 24 hr before the addition of 50 μCi of tritiated thymidine (20 Ci/mmol; Amersham Center, U.K.), and the incubation was performed for the desired length of time (8).

araC, cis-Pt(II), and trans-Pt(II) were obtained from Sigma. cis-FLAP(II) was a gift from May & Baker Ltd, England.

Cell lysis. The incubation medium was drained from the culture dish and the cells were rinsed twice with cold phosphate-buffered saline. Cell lysis was performed in the dark at 0° by the addition of 2.25 ml of 0.03 M NaOH (pH 12.1). After 30 min the solution was neutralized by the addition of 0.9 ml of 0.067 M HCl, 0.02 M NaH_2PO_4. The sample was then either digested with nuclease S_1 (see below) or immediately made 0.5% with regard to SDS (8, 9).

Digestion with nuclease S_1. Immediately after neutralization of the dilute alkali used to lyse the cells, 300 μl of 300 mM sodium acetate (pH 4.6), 0.5 mM zinc acetate, 750 mM NaCl was added. Nuclease S_1 (200 IU/ml; Sigma) was then added and the mixture was incubated for 30 min at 37°. The digestion was stopped by making the solution 1% SDS/0.02 M EDTA.

Agarose gel electrophoresis. The labeled DNA was separated in 0.75% agarose gels using an LKB Multiphor electrophoretic system. The voltage gradient was 1 V/cm, using the following buffer system: 0.04 M Tris-HCl (pH 8.0), 0.04 M sodium acetate, 0.002 M Na_2EDTA, 0.2% SDS. The gels were cut in 1-mm-thick slices and the radioactivity was measured in a toluene-based scintillation fluid containing 3% Soluene 100, using a Packard scintillation counter.

Determination of platinum in DNA. Cells with steady state labeled DNA were treated with the platinum compounds. The DNA was isolated as described by Ciccarelli *et al.* (10) and the platinum content was determined by flameless atomic absorption spectroscopy with a Pye Unicam atomic absorption spectrophotometer PU 9000 at Analytica AB, Täby, Sweden.

Results

We use a procedure of alkaline cell lysis to partly denature the DNA (7–9). During the cell lysis the DNA starts to uncoil in the alkaline milieu. For the large DNA molecules of human cells, the time required for complete unwinding is long enough to be measured. In our experimental conditions the uncoiling, which is initiated at single-strand gaps and/or alkali-labile regions present in the DNA, results in denaturation of regions of DNA with the size up to 20 kb (11). When the solution is neutralized, the DNA larger than 20 kb renatures to form double-stranded DNA. If there exist small DNA replication intermediates (<20 kb), they appear as single-stranded DNA.

Experimental approach: three parameters of drug-DNA interaction. When cells treated with drugs are lysed in dilute alkali, single-stranded DNA fragments (<20 kb) are released from prelabeled DNA. Untreated cells do not release such fragments. The fragments are released because of the presence of alkali-labile regions and/or gaps induced by drugs in the DNA. The released DNA fragments represent one parameter of the interaction between drug and DNA ("alkali-released fragments"), and arise through enzymatic strand scission as part of a repair process or through direct chemical alterations in the DNA (9, 11–13). We have used this approach to show, for example, that 5-fluoropyrimidines induce lesions in DNA by two different mechanisms (12, 13).

The other two parameters depict the remaining DNA and are obtained in the following way. The cells are lysed in dilute alkali to detect alkali-released fragments. The DNA of cells lysed in dilute alkali is treated with nuclease S_1 after renaturation. Nuclease S_1 removes all the single-stranded DNA fragments ("alkaline fragments") as well as short single-stranded stretches of DNA present in the double-stranded DNA (8). The rate-limiting step in the digestion is the introduction of the first nick at the site of locally denatured DNA. When the molecule contains a single-stranded incision, it is quite efficiently cleaved by the nuclease (14). The enzyme needs at least three unpaired bases to recognize an incision site (15).

The prelabeled DNA of cells not treated with drugs appears after alkaline cell lysis and digestion with nuclease S_1 as small fragments of double-stranded DNA (Fig. 1) (8). This double-stranded DNA shows up in the gel as a rather broad peak migrating not far from the front. To obtain an approximate size determination of this DNA, the sample was treated with hot formamide immediately before gel electrophoresis (8). This converts double-stranded DNA to single-stranded DNA and, as well, prevents aggregation. The size of the single-stranded DNA turns out to be on the order of 70–200 bases. We refer to this DNA as "mature chromatin DNA."

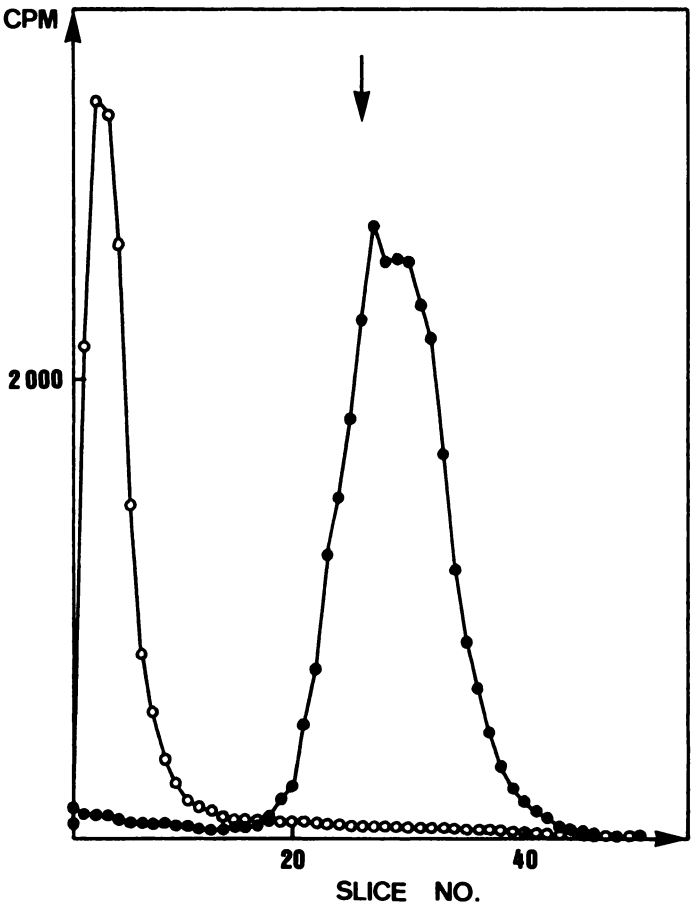

Fig. 1. Gel electrophoretic separation of prelabeled DNA. Two samples of cells were lysed in dilute alkali, and the solution was neutralized. One sample was then treated with detergent (○), whereas the other sample was first treated with nuclease S_1 before the addition of detergent (●). The DNA was then separated in 0.75% agarose gels. ↓, location of a 2-kb single-stranded DNA marker. The migration is from left to right.

Generation of the small double-stranded DNA fragments ("mature chromatin DNA") is the normal response of mature chromatin not affected by drugs. The reason for this is not clear as yet, but it is likely to be the result of topological constraints imposed by chromatin structure, rendering complete renaturation of mature chromatin difficult. The residual single-stranded regions offer sites for attack by nuclease S_1.

The presence of cross-links in DNA or between DNA-protein reduces the ability of dilute alkali to partially denature DNA. Furthermore, it is likely that there occurs snap back of DNA during renaturation. Also, it is possible that the presence of interstrand cross-links and DNA-protein cross-links induces topological changes, possibly reducing the generation of mature chromatin DNA. Therefore, if cross-links exist in the DNA, one would expect reduced amounts of mature chromatin DNA. Instead, the agarose gel separation should show HMW DNA.

Hence, the three parameters we use are: the fraction of DNA fragments released when cells are lysed in dilute alkali (alkali-released fragments), the fraction of mature chromatin DNA, and the fraction of HMW DNA. In cells not treated with drugs all DNA appears as mature chromatin DNA and there are no alkali-released fragments or HMW DNA.

Fig. 2 shows how we determine the proportion of labeled DNA in the three parameters. For each experiment two cultures are incubated in parallel. The proportion of alkali-released fragments is determined in the first agarose gel. From the second gel we determine the proportion of mature chromatin DNA and HMW DNA. The values obtained for mature chromatin DNA and HMW DNA are normalized with the value for large DNA determined in the first gel analyzing alkali-released fragments.

During the treatment with nuclease S_1 there is a small loss of label because of the removal of short single-stranded DNA stretches present in mature DNA. We have found earlier that this represents at most 2% of total label present in mature DNA (8). Therefore, we have not corrected for this effect of nuclease S_1.

Cells treated with araC. araC is an antimetabolite known to be incorporated into DNA (16–18). There is a correlation between the level of incorporation of araC into DNA and the cytotoxicity.

Cells with prelabeled DNA were incubated with araC (1 mM) for 60 min, then either immediately examined or examined after incubation in fresh medium for 24 or 48 hr. At each time-point three separate incubations were performed. The results show that there is no HMW DNA, which can be expected as araC does not induce DNA cross-links. There is a high proportion of alkali-released fragments, in agreement with work of others showing that DNA containing araC is alkali-labile (16–18). The level of alkali-released fragments is higher than the level of mature chromatin DNA up to 48 hr after drug treatment (Fig. 3). We have not performed incubations for time periods longer than 48 hr.

cis-Pt(II): different durations of the treatment. The three parameters described above were examined in cells treated with cis-Pt(II). cis-Pt(II) is known to induce DNA cross-linking as well as several different monoadducts (5, 10, 19, 20).

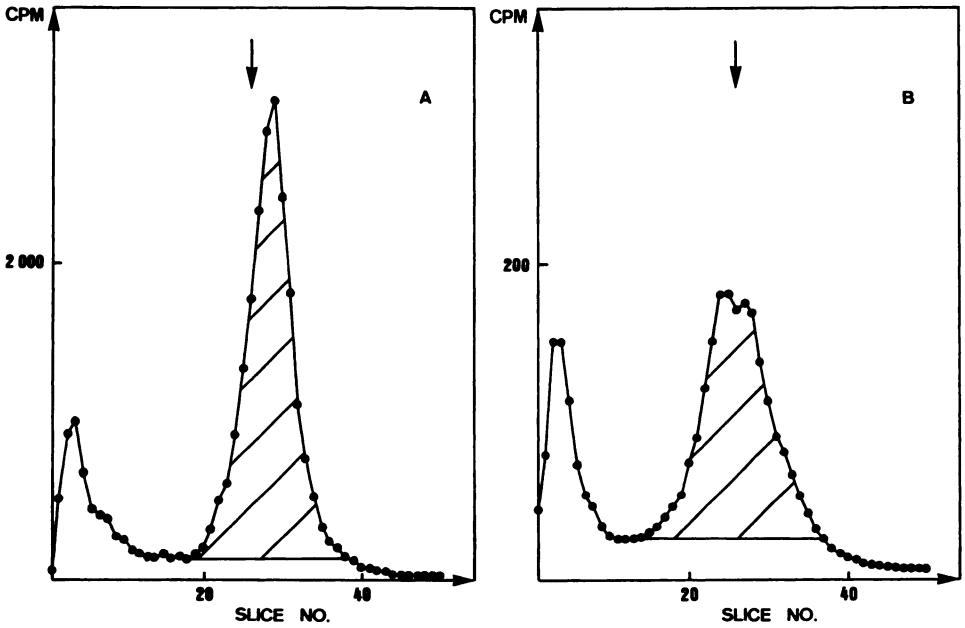

Fig. 2. Determination of labeled DNA in the three parameters. Equal amounts of cells with prelabeled DNA were incubated in two Petri dishes. The cells were treated with cis-Pt(II) (100 μg/ml) for 60 min. Forty-eight hr later the cells were lysed in dilute alkali and the solution was neutralized. A. The DNA from one Petri dish was separated in an 0.75% agarose gel to determine the relative proportion of single-stranded DNA fragments ("alkali-released fragments") released from large DNA. The fragments are located in the *hatched area* in A. ↓, location of a 2-kb single-stranded DNA marker. B. The DNA from the other Petri dish was treated with nuclease S_1 after cell lysis in dilute alkali and renaturation. This treatment removes the alkali-released fragments as well as short stretches of single-stranded DNA present in the HMW DNA. After this treatment, the DNA of cells not exposed to drugs appears in electrophoretic analysis in 0.75% agarose gels as short double-stranded DNA fragments ("mature chromatin DNA;" *hatched area* in B) (8). ↓, location of a 2-kb single-stranded DNA marker. The migration is from left to right. In cells treated with drugs inducing cross-links, like cis-Pt(II), "HMW DNA" still appears after the treatment with nuclease S_1. DNA appears at slices 2–6 in B. The percentage of total label in "alkali-released fragments" was determined in the gel by integration of peaks in A. The percentage of label in HMW DNA and mature chromatin DNA was determined from peaks in B. The two peaks in B represent a subdivision of the label appearing as a single peak of large DNA in A. Therefore, we normalized the values in B with the value of large DNA in A.

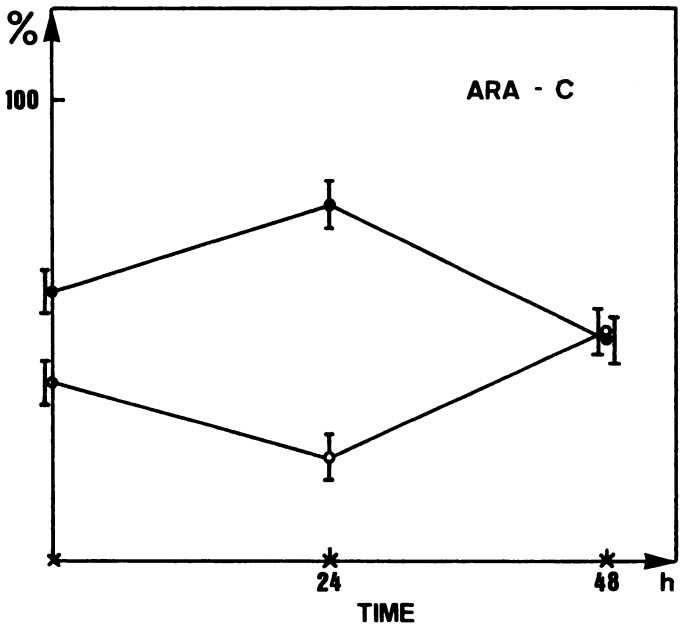

Fig. 3. Treatment with araC. Melanoma cells with prelabeled DNA were treated with araC (1 mM) for 60 min. The cells were then immediately lysed or lysed after cultivation in fresh medium for 24 or 48 hr. The distribution of label in alkali-released fragments (●), mature chromatin DNA (○), and HMW DNA (x) was then determined. *Bars* give standard error of three experiments.

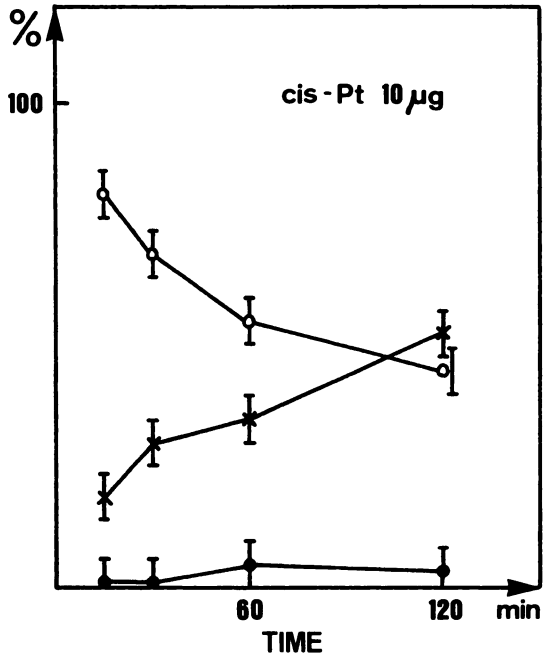

Fig. 4. Increasing duration of the treatment with cis-Pt(II). Melanoma cells with prelabeled DNA were treated with cis-Pt(II) at 10 μg/ml for 15 min, 30 min, 60 min, or 120 min. The cells were lysed immediately after the drug treatment. The distribution of label in alkali-released fragments (●), mature chromatin DNA (○), and HMW DNA (x) was then determined. *Bars* give standard error of three experiments.

Cells with prelabeled DNA were incubated for 15 min, 30 min, 60 min, or 2 hr with cis-Pt(II) at 10 μg/ml. The following was found when the percentage of label in the three parameters was determined (Fig. 4). A small proportion of DNA appears in alkali-released fragments. With increasing duration of the drug treatment, the amount of label in HMW DNA increases, reaching a value of > 50% in cells treated for 2 hr. This represents DNA with cross-links. Finally, with increasing duration of drug treatment there is a decrease in "mature chromatin DNA".

cis-Pt(II): different concentrations of drug. Cells with prelabeled DNA were treated with cis-Pt(II) for 60 min at either 10 μg/ml or 100 μg/ml. They were then either immediately analyzed or incubated in fresh medium for 24 or 48 hr.

When the percentage of label in the three DNA fractions was determined, the following was found (Fig. 5, A and B). In cells treated with cis-Pt(II) at 10 μg/ml there is a substantial amount of HMW DNA immediately after the drug treatment, which decreases to a low level after 24 hr. However, at 24 hr there is also a decrease in the amount of mature chromatin DNA, and a parallel increase in the amount of alkali-released fragments. At 48 hr there is a lower level of alkaline fragments. In parallel one can now detect an increasing level of mature chromatin DNA.

In cells treated with cis-Pt(II) at 100 μg/ml, the results are different. The initial amount of HMW DNA is higher, it decreases after 24 hr, and at 48 hr there is no detectable material. The mature chromatin DNA remains on a low level for 48 hr, never exceeding 20%. The amount of alkali-released fragments continuously increases with time. At 48 hr about 80% of the label appears as alkali-released fragments.

Cells treated with trans-Pt(II). trans-Pt(II) induces different monoadducts as well as types of cross-links (DNA-protein cross-links predominating) that differ from those induced by cis-Pt(II) (2). The treatment with trans-Pt(II) was for 60 min at either 10 μg/ml or 100 μg/ml. The cells were then either immediately analyzed or incubated in fresh medium for either 24 or 48 hr.

The results are similar at the two concentrations (Fig. 5, C and D). Initially there is a very low level of HMW DNA, which increases with the duration of the post-incubation in fresh medium, leveling off at 60–70%. In parallel there is a continuous decrease in the level of mature chromatin DNA. Throughout the experiment there is a very low, if detectable, level of alkali-released fragments.

Cells treated with cis-FLAP(II). cis-FLAP(II) is formed by the addition of cis-Pt(II) of two imidazole groups (21). The protocoll for cis-FLAP(II) was the same as for cis-Pt(II): treatment for 60 min at either 10 μg/ml or 100 μg/ml. The cells were then either immediately analyzed or incubated in fresh medium for either 24 or 48 hr.

The results are similar at the two concentrations (Fig. 5, E and F). Initially there is a high level of HMW DNA, which decreases with time. In parallel there is an increasing level of alkali-released fragments. The level of mature chromatin DNA is, throughout the experiment, always below 50%, with lower values at higher drug concentrations.

Hence the findings are reminiscent of those with cis-Pt(II) but not identical.

Treatment with thiourea. The formation of cross-links occurs in two steps. First there is a formation of a monoadduct followed by a reaction of the second group to form the cross-link. With regard to platinum compounds the second step is relatively slow and the conversion of monoadducts to cross-links can be blocked by treatment with thiourea (22). We have therefore examined the effect of thiourea.

In cells treated with cis-Pt(II), trans-Pt(II), or cis-FLAP(II)

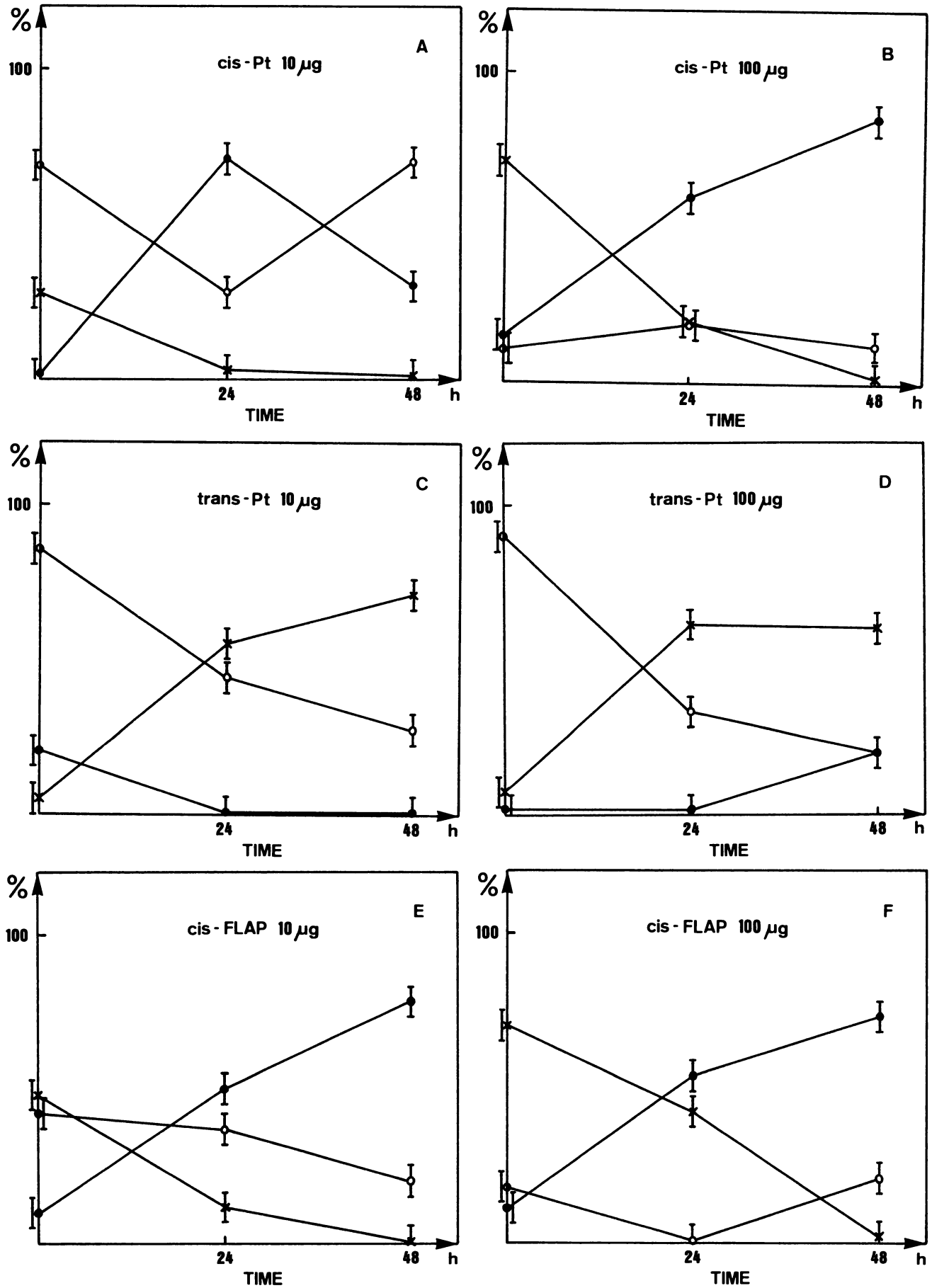

Fig. 5. Melanoma cells with prelabeled DNA were treated for 60 min with cis-Pt(II) (10 μg/ml) (A), cis-Pt(II) (100 μg/ml) (B), trans-Pt(II) (10 μg/ml) (C), trans-Pt(II) (100 μg/ml) (D), cis-FLAP(II) (10 μg/ml) (E), and cis-FLAP(II) (100 μg/ml) (F). The cells were then immediately lysed or lysed after cultivation in fresh medium for 24 or 48 hr. The distribution of label in alkali-released fragments (●), mature chromatin DNA (○), and HMW DNA (x) was then determined. *Bars* give standard error of three experiments.

and then incubated for 24 hr in medium with thiourea (10 mM), HMW DNA and alkali-released fragments are barely detectable (Fig. 6). More than 90% of the label is in mature chromatin DNA in cells treated with cis-Pt(II) or trans-Pt(II) and less in cells treated with cis-FLAP(II). The data support the interpretation that HMW DNA is formed due to the presence of cross-links. It does not discriminate, however, between DNA-DNA and DNA-protein cross-links.

Treatment with proteinase K. When the frequency of different cross-links has been estimated, it has been found that cis-Pt(II) induces a very low level (1%) of interstrand cross-links. The DNA-protein cross-links also represent about 1% of the DNA-cis-Pt(II) adducts. The intrastrand cross-links dominate. A bidentate, N^7-deoxy(GpG) intrastrand platinum adducts in DNA comprise up to 60% of the total cis-Pt(II)-DNA adducts (3).

As pioneered by Kohn *et al.* (6), one can use proteinase to distinguish between DNA-DNA and DNA-protein cross-links. We have therefore performed experiments with cis-Pt(II), trans-Pt(II), and cis-FLAP(II) and then lysed the cells in dilute alkali with proteinase K present. When the DNA was then examined, we found that this removed all HMW DNA from the trans-Pt(II)-treated cells and part of the HMW DNA of cis-Pt(II)- or cis-FLAP(II)-treated cells (Fig. 7). This makes it very likely that the HMW DNA is formed due to both DNA-DNA and DNA-protein interactions.

Removal of platinum bound to DNA. Cells were treated for 60 min with cis-Pt(II), trans-Pt(II), or cis-FLAP(II) at 100 μg/ml. The level of platinum bound to DNA was determined immediately after treatment and after incubation of the cells for 24 or 48 hr in fresh medium. The loss of platinum was corrected for the effects on DNA replication and cell cycle progression as outlined by Roberts and Friedlos (23). In agreement with their data, we have found that the rate of loss of platinum from DNA is similar with all three compounds (Fig. 8). Between 40 and 60% of the platinum is still present on the DNA 48 hr after treatment.

Next we examined cells treated with thiourea. Thiourea is known to block the appearance of platinum-containing cross-links (see Fig. 6). There is, however, at 24 hr, increased levels of alkali-released fragments, indicating the occurrence of repair of platinum-induced DNA lesions. Therefore, we measured directly the total level of platinum as well as the level of platinum present after digestion of the DNA with nuclease S_1 in cells treated with thiourea. The data showed that the treatment with nuclease S_1 removed 30% of the platinum. There remains in the mature chromatin DNA on the order of 20–40% of the platinum bound to DNA.

Discussion

Several methods allow the examination of various aspects of drug-DNA interaction, e.g., density-hybrid methods, alkaline elution, strand reassociation, and alkaline sedimentation. However, none of these approaches allows the analysis of all three parameters described here. The present approach represents a new possibility to examine drug-DNA interaction. It is of special interest in the situation where a drug induces both alkali-labile DNA and cross-linked DNA, either simultaneously or according to a sequential schedule, e.g., during DNA repair.

We describe here a new approach that allows the examination of three parameters of drug-DNA interaction in drug-treated cells. The three parameters we examined indicate the levels of: alkali-labile regions ("alkali-released fragments"), cross-linked DNA ("HMW DNA"), and "mature chromatin DNA."

cis-Pt(II) is known to form various products with DNA as DNA-protein cross-links, DNA inter- and intrastrand cross-links, and monofunctional cis-Pt(II)-DNA adducts (2). The monofunctional adducts may remain as monoadducts or they may give rise to cross-links.

Which of the platinum lesions are potentially lethal is, at present, not clearly established. Several reports indicate the interstrand cross-link which, however, is a relatively rare lesion amounting to about 1% of the total amount of cis-Pt(II) reacted with DNA (3).

When this drug is tested in our system, one can see that, with increasing duration of drug treatment (10 μg/ml), there is an increasing proportion of HMW DNA and a parallel decrease in mature chromatin DNA. When the drug treatment is limited to 60 min and the cells are then cultured for 24 or 48 hr in fresh medium, the amount of HMW DNA decreases gradually with time.

When cells are treated with cis-Pt(II) at 100 μg/ml, we can show that there is a higher level of HMW DNA and, in parallel, a decreasing level of mature chromatin DNA.

In cells treated with cis-FLAP(II), one can detect initially a high level of HMW DNA, which decreases with time. There is a parallel increase in the level of alkali-released fragments. The level of mature chromatin DNA is always below 50%.

The effects of trans-Pt(II) are very different. The trans-analogues are known to induce types of lesions in the chromatin

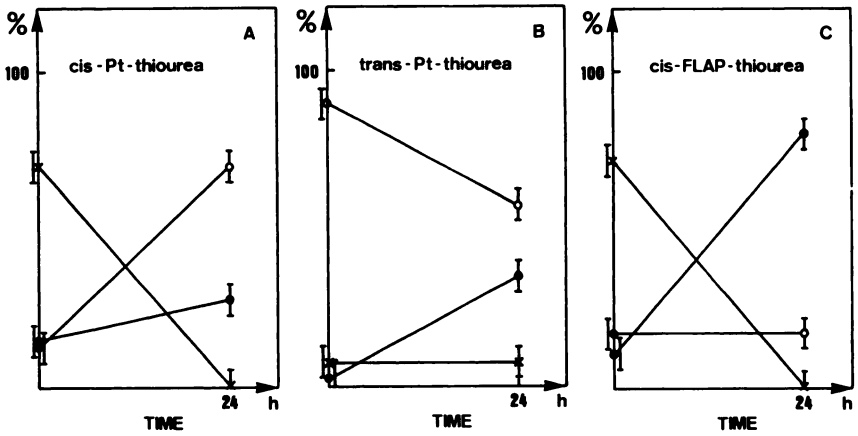

Fig. 6. Treatment with thiourea. Melanoma cells with prelabeled DNA were treated for 60 min with cis-Pt(II) (100 μg/ml) (A), trans-Pt(II) (100 μg/ml) (B), or cis-FLAP(II) (100 μg/ml) (C). The cells were then immediately lysed or lysed after 24 hr treatment with thiourea (10 mM). The distribution of label in alkali-released fragments (●), mature chromatin DNA (○), and HMW DNA (x) was then determined. *Bars* give standard error of three experiments.

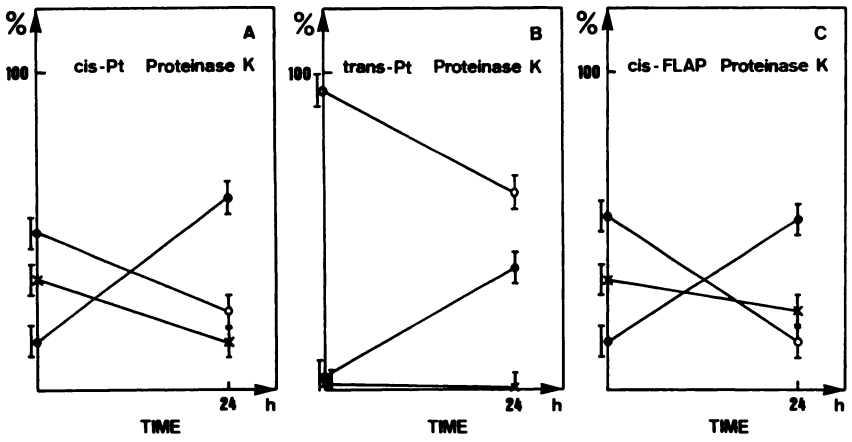

Fig. 7. Treatment of proteinase K. Cells with prelabeled DNA were treated for 60 min with cis-Pt(II) (100 μg/ml) (A), trans-Pt(II) (100 μg/ml) (B), or cis-FLAP(II) (100 μg/ml) (C). The cells were lysed in dilute alkali containing proteinase K (100 μg/ml) either immediately after drug treatment or 24 hr after the treatment. The distribution of label in alkali-released fragment (●), mature chromatin DNA (○), and HMW DNA (x) was then determined. *Bars* give standard error of three experiments.

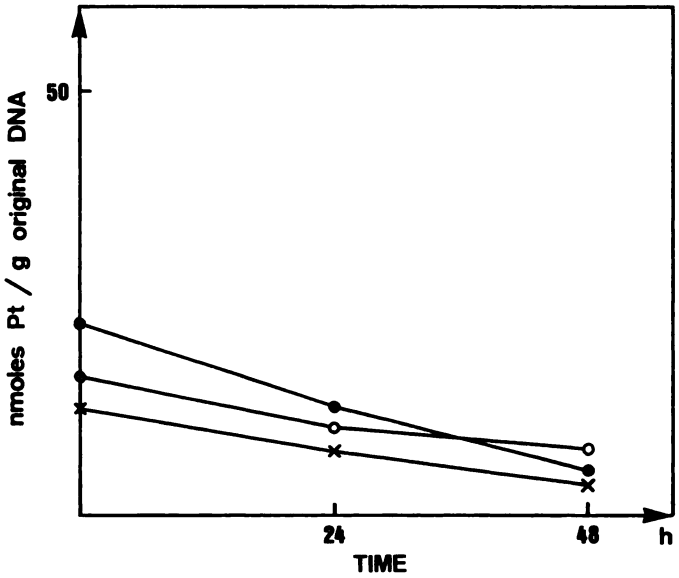

Fig. 8. Removal of platinum from DNA. Cells were treated for 60 min with either cis-Pt(II) (100 μg/ml) (●), trans-Pt(II) (100 μg/ml) (○), or cis-FLAP(II) (100 μg/ml) (x). The cells were then either immediately analyzed or analyzed after incubation in fresh medium for 24 or 48 hr. The platinum content of the DNA was determined by flameless atomic absorption spectroscopy. The binding of platinum to DNA was corrected for effects on DNA replication and cell cycle progression (23).

other than the cis- analogues, e.g., high levels of DNA-protein cross-links. Initially there is a rather low level of HMW DNA, which increases with time. In parallel, the level of mature chromatin DNA continuously decreases. Furthermore, alkali-released fragments remain at very low levels throughout the experiments.

Thiourea has been shown to prevent the conversion of platinum monoadducts to cross-links (22). When the effect of thiourea was tested on cells treated with platinum analogues, the results showed the disappearance of HMW DNA. Furthermore, the inclusion of proteinase K indicates the importance of proteins in the cross-links giving rise to HMW DNA.

The mature chromatin DNA represent DNA not undergoing DNA repair and showing the normal fragmentation of DNA during treatment with nuclease S_1. In this DNA there still exists a high level of bound platinum, as directly determined in thiourea experiments. There is no difference in the rate of disappearance of the three platinum compounds bound to DNA used in this study. The data argue that the effects we measure are due to differences in the DNA adducts formed by the platinum compounds, with some platinum adducts showing very little deleterious effects.

Little is known about the factors that may influence the effect of platinum analogues. It seems unlikely that histones per se are involved, since they are equally distributed in the chromatin. The non-histones are a more likely alternative. It is known that platinum compounds induce cross-links which mainly involve non-histone proteins (10). Furthermore, it has recently been claimed that differences in cytotoxicity of different platinum compounds are due to intrinsic differences in the DNA-bound adducts on DNA replication and not from differences in the rate of repair of the adducts (23).

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