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SHORT COMMUNICATION

Effect of DNA Conformation on Cisplatin Adduct Formation

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ABSTRACT. The anticancer drug *cis*-diamminedichloroplatinum(II) (cisplatin) has been shown previously to form adducts preferentially within internucleosomal or linker DNA rather than to DNA within the nucleosome. To determine whether other "open" regions of chromatin have an increased affinity for cisplatin, adduct formation within specific chromatin domains was analyzed. There was a significant increase in cisplatin–DNA adduct formation for DNA associated with the nuclear matrix (NM) compared with other chromatin domains and total unfractionated DNA. In contrast, treatment of the same cells with *trans*-diamminedichloroplatinum(II) (transplatin) did not result in preferential adduct formation. These findings led to the hypothesis that it might be possible to alter DNA to make it a more favorable target for cisplatin. The effect of arginine butyrate on cisplatin–DNA adduct formation was analyzed in human cancer cells. The combination of arginine butyrate and cisplatin resulted in a concentration-responsive increase in cisplatin–DNA adduct formation in PC-3 cells and an overall increase in cisplatin–DNA adduct formation in three other human cancer cell lines. The same combination also resulted in a significant increase in drug-induced cytotoxicity at a low concentration of cisplatin. These results suggest that chromatin configuration can affect cisplatin adduct formation. BIOCHEM PHARMACOL 51;5:717–721, 1996.

KEY WORDS. cisplatin; chromatin; arginine butyrate

The chemotherapeutic agent *cis*-diamminedichloroplatinum(II) (cisplatin) mediates cytotoxicity as a direct result of DNA adducts [1]. Despite the knowledge that ApG and GpG intrastrand sites are the predominant target for this agent [2, 3], it is likely that the chromatin structure imposes an additional level of specificity on adduct formation. For example, this [4] and other laboratories [5, 6] have shown that compared to DNA within the nucleosome, internucleosomal (linker) DNA is a preferred site for cisplatin–DNA adduct formation. Despite this finding, there has not been an investigation into whether non-random cisplatin adduct formation occurs in higher order chromatin structures such as DNA associated with the NM.¶ The NM is a scaffold or matrix of proteins that anchor DNA into 50–100 kb loops called bulk DNA [7]. NM-DNA is known to be a critical domain for transcription and replication [7], so that adducts in this area may significantly impact on cytotoxicity. In addition to possibly providing a better understanding of cisplatin-mediated cytotoxicity, investigating how the chromatin structure influences adduct formation may lead to strategies for altering chromatin to enhance platinum uptake.

MATERIALS AND METHODS

Cell Lines

The human tumor cell lines used in this study, PC-3 and DU-145 (prostate cancer), OVCAR (ovarian cancer), and A-549 (lung cancer), were obtained from the ATCC (Rockville, MD) and propagated in 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and H-Minimum Essential Medium (H-MEM) (Gibco, BRL). The human foreskin fibroblasts were obtained by primary culture of human foreskins obtained from the GWU Hospital and propagated in H-MEM supplemented with 15% FBS, 2.5 mM HEPES, with essential amino acids, 0.1 mM sodium pyruvate and 1% gentamycin.

Drugs

Cisplatin and *trans*-diamminedichloroplatinum(II) (transplatin) were obtained from the Sigma Chemical Co. (St. Louis,

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¶ *Abbreviations:* NM, nuclear matrix; AB, arginine butyrate; 1SF, first soluble fraction; TM, low salt fraction; HS, high salt fraction; and AA, alkylating agent.

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MO). Freshly prepared stocks dissolved in PBS were used in each experiment. AB (Greenpark, Houston, TX) was dissolved in water as a 10% (352 mM) stock solution.

Chromatin Subfractionation Assay

Subconfluent human foreskin fibroblast cells (10^6) were treated with 150 μM cisplatin or transplatin for 1 hr. Immediately after drug exposure, DNA was harvested [10] from a portion of these cells to determine total DNA adduct formation, and the remainder was used for isolating nuclei for chromatin subfractionation using previously described methods [8]. Nuclear subfractionation was performed essentially as described by Obi *et al.* [9], using previously described minor modifications [8]. Subfractions were purified by phenol/chloroform extraction and ethanol precipitation [10], and the platinum content in each was determined by flameless atomic absorption spectroscopy (FAAS) [11]. FAAS values in each fraction were normalized to DNA concentration using UV spectrophotometry [10].

Effect of AB on Cisplatin Adduct Formation

Subconfluent human tumor cells were pretreated with various concentrations of AB or mock medium prior to the addition of cisplatin or transplatin for 1 hr. DNA was extracted and purified, and RNA was removed immediately after drug treatment [10]. Overall cisplatin- and transplatin–DNA adduct formation was measured by FAAS [11].

Effect of AB on Cisplatin Cytotoxicity

PC-3 cells (5×10^3) were plated in triplicate dishes 1 day prior to treatment in the presence or absence of 0.5 mM AB for 30 min. Cells were then exposed to cisplatin for 1 hr prior to washing the cells in PBS and adding growth medium. Colony survival was assayed 6–8 days later.

Statistical Analysis

Statistical evaluation of chromatin fractionation data was performed using a one-factor ANOVA. Analysis of differences in the effects of AB on cisplatin–DNA adduct formation and colony survival was performed using a two-sided *t*-test.

RESULTS AND DISCUSSION

Adduct Formation within Specific Chromatin Domains

Using human foreskin fibroblast cells, the subnuclear fractionation procedure was used to determine if chromatin exhibits preferential DNA adduct formation within NM-DNA. The initial treatment [9] with micrococcal nuclease preferentially digests linker DNA, resulting in the isolation of the 1SF, comprising lower-molecular weight mononucleosomal DNA [8, 9]. Following isolation of the 1SF, chromatin fractions soluble in low and high salt buffers (TM and HS, respectively) were isolated. The TM and HS fractions together represent bulk or loop-associated DNA, and in these and other experiments [8, 9] they always contain the largest fraction of DNA. After removal of these fractions, the remaining component represents DNA associated with the NM. We [8] and others [9] have shown previously that NM-associated DNA derived by this method is enriched in actively transcribed sequences.

In six separate cisplatin and three separate transplatin experiments, the 1SF, bulk chromatin (TM and HS), and the NM fractions represented 12.5, 73, and 14.5% of the total DNA recovered from the nuclei, respectively. Cisplatin- and transplatin-treated cells had approximately the same fraction of DNA within specific domains (data not shown), and these fractions did not differ appreciably from previous experiments on chromium-treated CHO cells [8].

Following a 1-hr cisplatin exposure, there was a 6-fold increase in adduct formation within NM-DNA compared with the bulk DNA (the sum of the TM and HS) (Fig. 1A). The increase in cisplatin in NM-DNA was also statistically significant compared with the 1SF (5-fold) and the total unfractionated DNA (3-fold) ($P < 0.01$). This finding is more striking considering that NM-DNA is AT rich [7] and, therefore, is not an obvious target for the primarily GpG and ApG intrastrand cisplatin adducts [2, 3].

One explanation for the increase in cisplatin adduct formation within NM-DNA is related to the "open" DNA conformation that this region must assume for active transcription and replication [12, 13]. The possibility that a relief in superhelical strain within NM-DNA might result in a more attractive target for cisplatin has also been offered as an explanation for the preference that some other guanine-specific agents, such as chromium [8] and benzo[*a*]pyrene [9], have for this region.

Some of the other agents that display a preference for adduct formation within NM-DNA also exhibit an affinity for adduct formation within the 1SF [8, 9]. Presumably, this is because a higher percentage of the 1SF is transcriptionally active, and therefore in a more open conformation. Cisplatin did not display an affinity for adduct formation within the 1SF (Fig. 1A). This may be because isolation of this fraction results in the loss of linker DNA, a preferred site for cisplatin adduct formation [4–6].

It is possible that preferential adduct formation within NM-associated DNA partially mediates platinum cytotoxicity, particularly because of the critical functions of NM-DNA. Nonuniform DNA damage has been shown to influence chemotherapeutic agent toxicity for other agents. For example, cytotoxicity induced by the topoisomerase II inhibitor VM-26 has been shown to correlate with damage only within specific genomic regions, but not with damage within bulk DNA [14].

Effect of AB on Cisplatin DNA Adduct Formation

To determine whether short-term exposure to AB affects adduct formation, subconfluent PC-3 cells were exposed in triplicate with 0.1 to 1.0 mM AB for 30 min before, and during a 1-hr exposure, to 100 μM cisplatin. Treatment with escalating levels of AB resulted in a concentration-dependent increase in

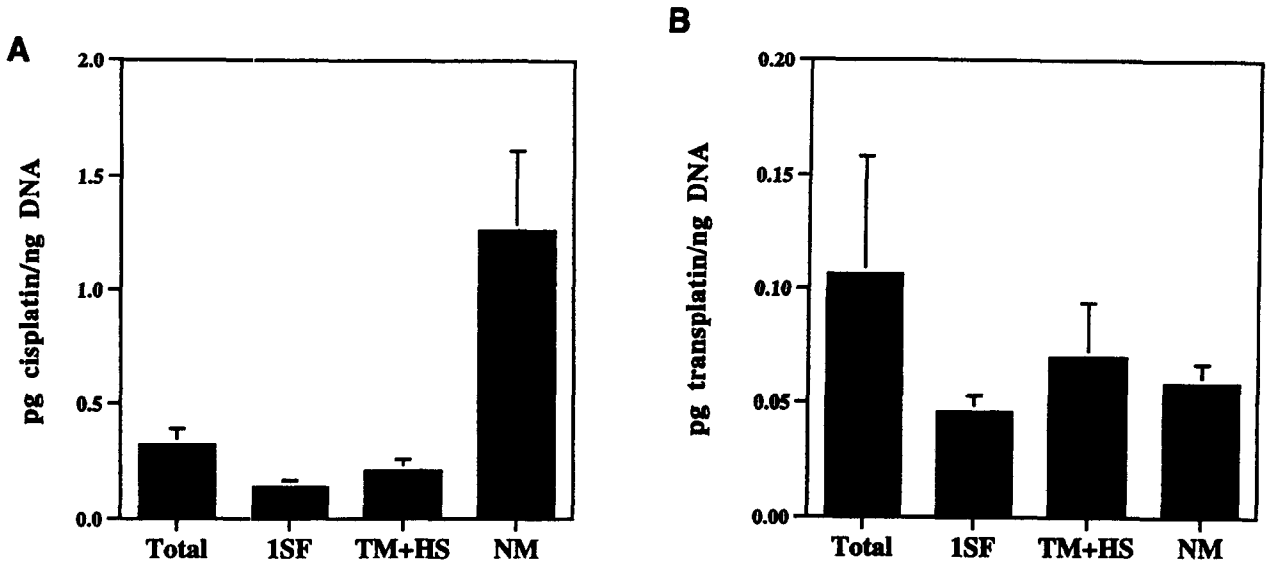

FIG. 1. Distribution of cisplatin- and transplatin–DNA adducts within chromatin domains. Nuclear subfractionation was performed according to the conditions described in Materials and Methods following treatment of human foreskin fibroblasts with 150 μM cisplatin (A) or transplatin (B) for 1 hr. DNA adduct formation was measured within the 1SF or first soluble fraction, the TM and HS fractions representing the bulk or loop-associated DNA, and the NM or nuclear matrix domain. Total represents non-fractionated chromatin. Cisplatin adduct formation (A) was increased significantly within the NM compared with the other fractions ($P < 0.01$). In contrast, there were no statistical differences comparing adduct formation within the chromatin domains of transplatin (B) treated cells. Values are means ± one SD; N = 6 for panel A and N = 3 for panel B.

cisplatin–DNA adduct formation compared with cells treated with cisplatin alone (Fig. 2). Results without AB (see figure legend) were significantly ($P < 0.05$) different from results obtained after exposures to 0.2, 0.5 and 1.0 mM AB concentrations. The increase in adduct formation at 0.1 mM AB was not significant.

Since plasma butyrate levels of at least 0.6 mM have been achieved in patients using AB [15], we investigated the effect of 0.5 mM on cisplatin adduct formation in other human tumor cell lines. Using the same treatment schedule (a 30-min pretreatment with AB prior to the addition of cisplatin for 1 hr), we detected a variable but statistically significant increase in cisplatin adduct formation in DU-145, PC-3, OVCAR, and A-549 lines treated with AB and cisplatin compared with cells treated with cisplatin alone (Fig. 3).

Although not known with certainty, the capacity of AB to increase cisplatin–DNA adduct formation may be mediated by an effect on DNA conformation. Although AB affects multiple cellular processes, its most direct and principal effect results from rapid inhibition of histone deacetylase and a resulting hyperacetylation of lysines on histones H3 and H4 [16, 17]. Histone hyperacetylation has been postulated to lead to conformational changes [17, 18], including those associated with transcribed DNA [18]. For example, an overall increase in gene transcription has been observed following treatment with a specific histone deacetylase inhibitor derived from fungi, trapoxin [19]. The "open" conformation associated with histone hyperacetylation may permit a more favorable interaction with transcription factors [17]. The time course of these experiments provides further proof that AB mediates enhanced cisplatin adduct formation by affecting chromatin configuration. The effects of AB on histone are immediate, whereas the effect of subsequent enhanced gene transcription and protein formation would not be expected to be manifest for hours post exposure.

Effect of AB on Cisplatin-Induced Cytotoxicity

The above studies focused only on adduct formation immediately after drug exposure. To determine if AB treatment affects cisplatin cytotoxicity, PC-3 colony survival was analyzed comparing combined treatment to treatment with cisplatin alone. Figure 4 demonstrates that the same schedule of AB treatment (30 min prior to, and in combination with, cisplatin) resulted in enhanced cytotoxicity compared to treatment with cisplatin alone. This effect was significant at the lowest concentration used in this study (5 μM). Although enhanced cytotoxicity was also observed for 25 and 50 μM cisplatin, these results were not significant. A similar pattern of cytotoxicity has been observed for other human cancer cell lines treated with this combination.* The implication of these findings is that the increase in adduct formation observed in cell culture immediately following AB treatment (Figs. 2 and 3) is sufficient to result in enhanced cell toxicity, and that not all of the increased adducts induced by AB are repaired immediately.

In these experiments, AB acts as a modulator of cisplatin cytotoxicity. Modulators of AAs tend to have their greatest effect at lower AA concentrations [20], as if their moderate effects are overwhelmed by the relatively large number of adducts produced at higher AA exposures. Importantly, how-

* Foss *et al.*, manuscript in preparation.

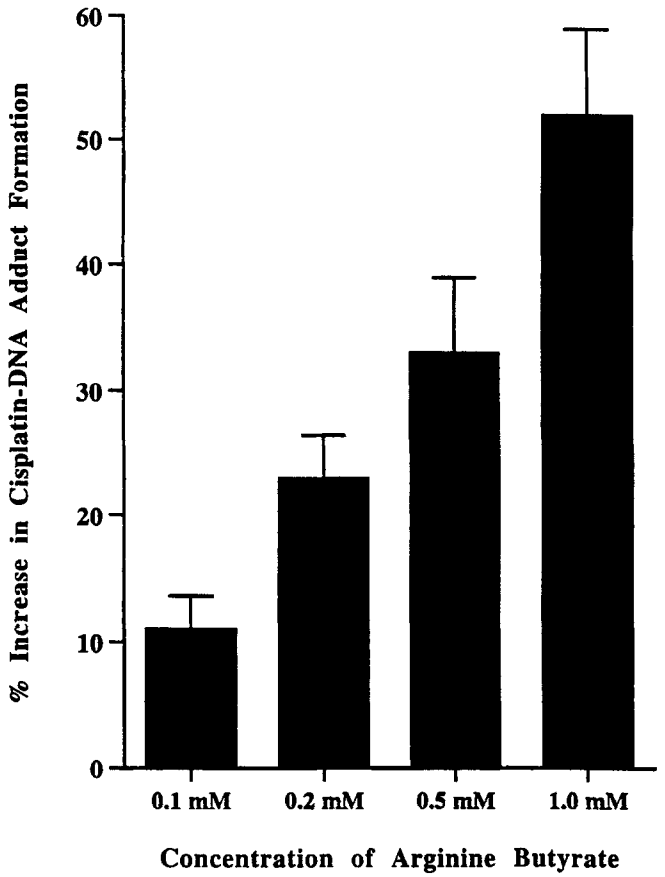

FIG. 2. Increase in cisplatin–DNA adduct formation in the presence of AB. The average percent increase in cisplatin–DNA adduct formation in PC-3 cells treated with concentrations of AB ranging from 0.1 to 1.0 mM was compared with adduct formation in cells treated with cisplatin (100 μM) alone, according to the conditions described in Materials and Methods. Mean cisplatin–DNA adduct formation in the absence of AB was 0.39 pg platinum/ng DNA. Results from treatments with all concentrations of AB were significantly ($P < 0.05$) different from control except for treatment with 0.1 mM AB. Values are means ± one SD for 4 independent experiments.

ever, plasma cisplatin levels achievable in human therapy are closer to that obtained by the 5 μM concentration [20].

Although it is conceivable that the combination of cisplatin and a butyrate analog such as AB may be clinically useful, it is not known if this combination would have a favorable therapeutic index for cancer compared with normal cells. However, this possibility exists since chromatin and NM proteins may be altered in cancer compared with normal cells [21, 22].

Chromatin Configuration and Transplatin Adduct Formation

In contrast to results with cisplatin, there was no specificity for adduct formation within any chromatin domain following treatment of foreskin fibroblasts with transplatin (Fig. 1B). Treatment of these cells did result in less DNA adduct formation than exposure to an equimolar concentration of cisplatin. This is probably related to the known propensity of transplatin to form more protein-adducts, especially to histones [23]. Also in contrast to its effects on cisplatin adduct formation, AB treatment did not result in an increase in the amount of transplatin in PC-3 DNA (data not shown). These results suggest that the conformational properties that favor cisplatin adduct formation are not those that favor transplatin adduct formation. However, it is not possible to conclude that the difference in cisplatin and transplatin cytotoxicities is due solely to the propensity of cisplatin for adduct formation within NM-DNA, because multiple other explanations have been proposed for the lack of cytotoxicity exhibited by this compound [24–27].

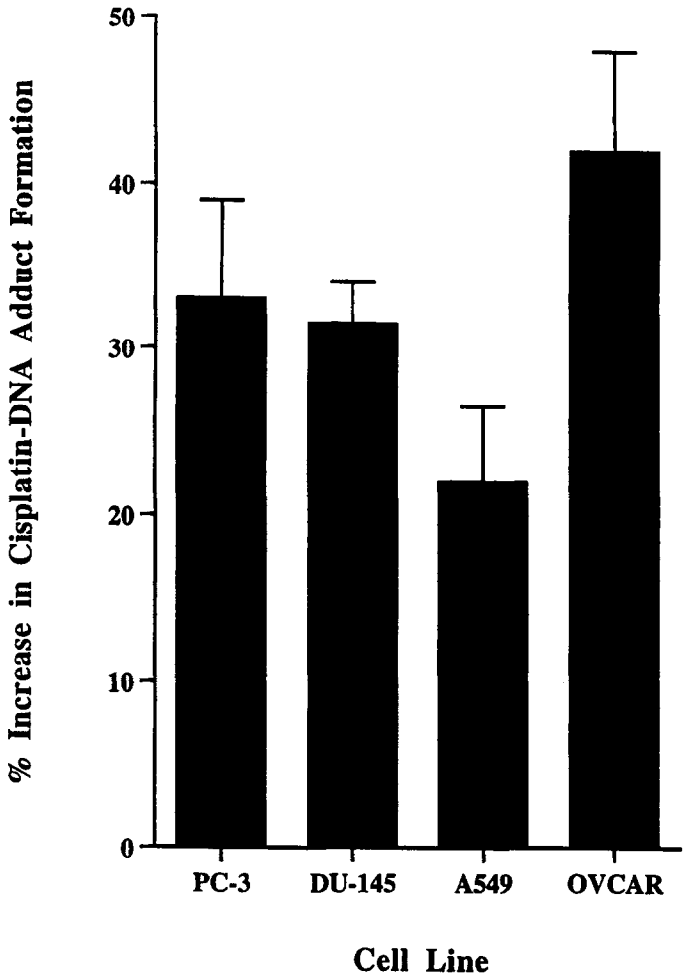

FIG. 3. Increase in cisplatin–DNA adduct formation in human cancer cell lines treated with AB. The percent increase in cisplatin–DNA adduct formation for cells treated with the combination of 0.5 mM AB and 100 μM cisplatin compared with cisplatin alone was measured in the depicted cell lines according to conditions described in Materials and Methods. Mean cisplatin–DNA adduct formation values in the absence of AB, represented as pg platinum/ng DNA were 0.38 pg/ng for PC-3 cells, 0.56 pg/ng for DU-145 cells, 0.24 pg/ng for A549 cells, and 0.38 pg/ng for OVCAR cells. AB resulted in a significant ($P < 0.05$) increase in cisplatin–DNA adduct formation for all cell lines. Values are means ± one SD for 4 separate determinations.

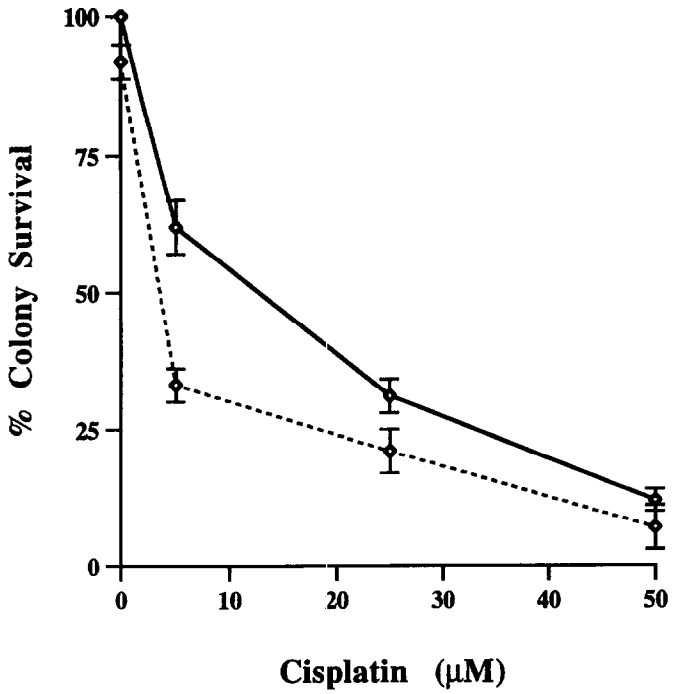

FIG. 4. Effect of AB on cisplatin-induced colony survival. PC-3 cells were treated with increasing concentrations of cisplatin in the absence (—) or presence (---) of 0.5 mM AB according to conditions described in Materials and Methods. AB significantly increased cisplatin cytotoxicity only at the 5 μM cisplatin concentration ($P < 0.05$). Values are means ± one SD for 4 independent experiments.

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