Biochemical Pharmacology, Vol. 37, No. 9, pp. 1835–1836, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

DNA INTERSTRAND CROSS-LINKS INDUCED BY CIS-DICHLORODIAMMINE PLATINUM IN OVARIAN CANCER CELLS GROWING IN PRIMARY CULTURE

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Cis-dichlorodiammine platinum (DDP) is widely used for the therapy of many human tumors. Testicular and ovarian tumors are particularly susceptible (1,2,3) but it is not known why. DDP causes DNA-intrastrand, DNA-interstrand (DNA-ISC) and DNA-protein cross-links (4,5). In some cell lines the number of DNA-ISC correlates well with DDP cytotoxicity (6,7). However, freshly obtained cancer cells growing in primary culture probably better reproduce the drug susceptibility of the original tumor they derive from, so we developed a system for growing highly purified, well characterized human ovarian tumor cells suitable for molecular pharmacology studies. We report here the initial studies on DNA damage and cell cycle perturbation induced by DDP in these cells.

MATERIALS AND METHODS

Biopsies of human tumors or metastasis were freed as much as possible from hemoragic, necrotic and connective tissue and minced with opposing scalpels. The minced tissue was incubated with 0.2% collagenase and 100 U/ml hyaluronidase for 60 min at 37°C. DNase I, 25 μg/ml, was added for the last 3 min of incubation. Digestion yielded a suspension of single cells, small clumps of 3 to 30 cells, and larger, partially digested tissue fragments. This suspension was filtered once through 250 μm mesh nylon gauze to remove the undigested bits, then through 30 μm mesh nylon gauze to collect small cell clumps. The small cell clumps were "pre-plated" on plastic culture dishes for 6-16 hours. Unattached cell clumps were collected by centrifugation and viability was determined by a dye exclusion test. Primary cultures were started with an inoculum of 3×10^4 viable cells/cm^2. Cells from ascitic fluids were collected after two discontinuous gradients of 100% Ficoll-Hypaque and filtered through 30 μm mesh nylon gauze. Viability was assessed by a dye exclusion test. Purity of cells was confirmed by flow cytometry analysis of DNA content and using specific monoclonal antibodies (OC 125, MOv2 and MOv19). Cells from primary tumor, metastasis of ascitic fluid were labeled for 48 h with ^{14}C-thymidine (0.05 μCi/ml). After 16 hours of postlabeling chasing in ^{14}C-thymidine-free medium, cells were treated with DDP for 1 hour. At the end of treatment and at different post-incubation times in drug-free medium, cells were scraped from culture flasks and processed for DNA-ISC measurement by the Kohn et al. alkaline elution method (8). DDP-induced cell cycle perturbation was determined by flow cytometry using propidium iodide as staining material (9).

RESULTS

DDP caused DNA-ISC in all the ovarian tumor cells as assessed by the alkaline elution method. The kinetics of DNA-ISC production is shown in Table 1. DNA-ISC were undetectable at the end of 1 hour exposure to DDP, but became evident after some hours of recovery in drug-free medium. The number of DNA-ISC was maximum between 9 and 24 h and differed in individual cases. At 48 h some repair of DNA-ISC was evident in the majority of cases, there being fewer DNA-ISC than at 24 h.

Table 1. DDP-Induced DNA-ISC in Human Ovarian Cancer Cells Growing in Primary Culture.

Cells derived from	DNA-ISC index[a] after different recovery times[b]			
	6 h	9 h	24 h	48 h[c]
PRIMARY TUMOR (n=22)	92 (39-158)	108 (16-313)	109 (15-219)	83 (23-194)
METASTASIS (n=4)	140 (42-205)	201 (53-358)	211 (52-349)	---
ASCITES (n=9)	62 (21-144)	63 (9-150)	74 (9-162)	28 (17-39)

[a] Expressed in rad equivalents: mean (range)

[b] Cells were exposed to 40 μM DDP for 1 hour then washed and maintained in drug-free medium for 0, 6,9,24 or 48 hours (recovery time). At time 0 (end of treatment) no DNA-ISC were detectable.

[c] At 48 h the number of cultures (n) was 11 for primary tumor and 2 for ascites.

The DNA-ISC index was higher in cells derived from metastases and lower in cells from ascitic fluid. However, analysis on two different samples (e.g. primary tumors vs metastasis or primary tumor vs ascitic fluid) from the same patient showed up no significant differences. In ten cases the cell cycle perturbation caused by DDP was investigated by flow cytometry. The proportion of cells in S phase (with a parallel decrease in G2M phases) was markedly raised in all cases.

DISCUSSION

Using cell lines in vitro DDP was found to cause DNA-protein cross-links, DNA inter and intra-strand cross-links and monofunctional adducts (4,5). DNA is the most likely target for the cytotoxic action of DDP and several studies reported good correlations between DDP induced DNA-ISC and cytotoxicity (6,7). Thus, it was worth investigating the formation and repair of these lesions in well characterized cultures of human ovarian tumors. In our conditions, as previously described in established cell lines (4,6), at the end of 1 hour exposure to DDP no DNA-ISC were detectable. DNA-ISC arose after some hours of recovery in drug-free medium. The explanation has been proposed that some of the DDP monoadducts to DNA, rapidly formed during drug exposure (not detectable by alkaline elution methods) are then converted to DNA-ISC, unless repaired, by a second, slower reaction which takes several hours (10). The varying amounts of DNA-ISC in the different cases might reflect different efficiency of repair of the potentially crosslinkable monoadducts.
In cancer cell lines (4,6,11) including some from human ovarian carcinoma (unpublished data), after short drug treatment, DDP-induced DNA-ISC were repaired almost completely within 24 hours, whereas in primary cultures of ovarian cancer cells the number of DNA-ISC declined only to a limited extent at 48 h, and at 72 h the DNA lesions were still not completely repaired (data not shown). Perhaps related to this is the finding that 72 h after DDP treatment ovarian cancer cells in primary cultures are still arrested in S phase, whereas established cell lines exposed to the drug are blocked in G2 phase (12). At present we are studying larger numbers of primary cultures of human ovarian cancers to ascertain the relationship between DDP-induced DNA damage (and repair) and the drug-induced cell cycle perturbation and cytotoxicity.

ACKNOWLEDGEMENTS

This work was partially supported by CNR (Italian National Research Council) Progetto Finalizzato "Oncologia" No. 86.00653.44 and by the Italian Association for Cancer Research.

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