Elevated mitochondrial cisplatin–DNA adduct levels in rat tissues after transplacental cisplatin exposure

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Introduction
cis-Diamminedichloroplatinum(II) (cisplatin*), given to pregnant rats at 5 mg/kg body wt, is a transplacental carcinogen for fetal liver, kidney, nervous system and lung, resulting in tumor incidences of 22.5, 10.5, 6.1 and 7.5% respectively in offspring grown to adulthood (1). Nonetheless, women discovered to have ovarian cancer during pregnancy are currently treated with single and multiple agent chemotherapy protocols that include cisplatin or diamminecyclobutane dicarboxylato platinum(II) (carboplatin) (2). Since the offspring of these pregnancies have not shown anatomic abnormalities and the mothers are typically cancer-free at the time of delivery (3), the treatment is considered successful. However, because a single transplacental cisplatin exposure is tumorigenic in rodents (4–6) and because the drug has been shown to cross the placenta in patas monkeys (7), the possibility exists that the children born to cisplatin-treated mothers may have sustained genotoxicity and may later encounter adverse health problems related to cisplatin exposure.

This study was originally designed to document cisplatin–DNA adduct formation in genomic DNA of maternal and fetal rat tissues after exposure to a single tumorigenic dose of cisplatin administered on day 18 of gestation. The major results, published elsewhere (8), have demonstrated that the drug crosses the placenta in rats; adducts are widespread in multiple maternal and fetal tissues; the extent of genomic cisplatin–DNA adduct formation is essentially related to dose. In conjunction with the same experiment, a limited number of maternal and fetal tissues were examined for mitochondrial cisplatin–DNA adduct formation, and the results are reported here.

Materials and methods

Chemicals, animals and exposures
Cisplatin, purchased from Sigma Chemical Co. (St Louis, MO), was dissolved in 2.5% sterile aqueous sodium chloride immediately prior to use.

Nine pregnant F344/NCr rats were provided by the Animal Production Area of the NCI–Frederick Cancer Research and Development Center and treated according to the Guide for the Care and Use of Laboratory Animals (NIH Publications, 86.23, 1995). For the experiment (see 8) three animals per group were given a single i.p. injection of either 2.5% sterile saline or 5 or 15 mg cisplatin/kg body wt at 18 days of gestation and killed 24 h later. Cisplatin–DNA adducts were determined by dissociation-enhanced lanthanide fluoroimmunoassay using a cisplatin–DNA standard modified in the same range as the biological samples.

Values for genomic cisplatin–DNA adducts in multiple maternal and fetal tissues have been presented elsewhere. Here, genomic DNA adduct levels for liver, brain, kidney and placenta are reported again for comparison with mitochondrial DNA adduct levels in the same tissues. In maternal and fetal brain, mitochondrial DNA adduct levels were ~7- to 50-fold higher than genomic DNA adduct levels, and in fetal liver they were ~2- to 16-fold higher than genomic DNA adduct levels. These studies demonstrate extensive cisplatin–DNA adduct formation in brain and liver mitochondria of fetal rats exposed transplacentally and suggest that mitochondrial DNA in some organs may be a particular target for cisplatin genotoxicity.

Abbreviations: cisplatin, cis-diamminedichloroplatinum (II); DELPHA, dissociation-enhanced lanthanide fluoroimmunoassay.
a supercoiled molecular marker to determine the purity and integrity of the sample.

Cisplatin–DNA adduct measurement by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA)

Cisplatin–DNA adducts were determined by DELFIA, an adaptation of the enzyme-linked immunosorbent assay (7,11,12) that has been previously described (8). Briefly, microtiter plates were coated with 0.36 ng cisplatin–DNA modified to 3% with the modification level determined by atomic absorbance spectrometry. Plates were blocked with fetal calf serum. Rabbit antiserum elicited against cisplatin–DNA (4.3% modified) was used as primary antibody (13,14) at a dilution of 1:120 000 in 2% fetal calf serum. Antibody was incubated with 35 μg sample or standard cisplatin-modified DNA and samples were assayed three times using triplicate experimental and control wells. Biotinylated goat anti-rabbit IgG (Vector, Burlingame, CA), at a dilution of 1:20 000 in 1% fetal calf serum, was used as secondary antibody. Subsequent incubations were with europium-labeled streptavidin, diluted 1:2500 in Wallac Assay Buffer (Wallac, Gathersburg, MD) and Wallac Enhancement Solution (Wallac). Fluorescence was read with a DELFIA Research Fluorometer (Wallac) at 614 nm. The DELFIA standard curve comprised calf thymus DNA modified with cisplatin to 0.5 pmol Pt/μg DNA, determined by atomic absorbance spectrometry. The 50% inhibition for 4 standards was 0.59±0.04 pmol Pt/μg DNA. The detection limit was 2 fmol Pt/μg DNA.

Results

This study compares cisplatin–DNA adduct formation in genomic and mitochondrial compartments from pregnant rats (n = 3) exposed perinatally to a single dose of 5 or 15 mg cisplatin/kg body wt. The drug was given on day 18 of gestation and maternal and fetal tissues were obtained 24 h later. Cisplatin–DNA adduct levels were determined by DELFIA in pooled tissues from the fetal rats of each litter and in the corresponding maternal tissues. Fetal rat tissues were pooled because each fetus provided too little tissue for individual assay. However, with each tissue combined from an average of seven fetuses per litter, there was sufficient DNA to assay each tissue two or three times and consider each litter as a separate data point.

Values for the maternal tissues, presented in Table I, demonstrate that the kidney did not have high mitochondrial cisplatin–DNA adduct levels, liver had essentially similar adduct levels in both compartments and the brain had much higher levels in mitochondrial DNA as compared with genomic DNA, at both doses assayed.

Values for the fetal tissues, shown in Table II, demonstrate that placenta and kidneys did not give measurable adduct levels in mitochondrial DNA, although low levels of adducts were measurable in genomic DNA. In contrast, cisplatin mitochondrial DNA adduct levels in brain and liver were higher than the corresponding genomic DNA adduct levels.

Discussion

The data presented here demonstrate increased levels of cisplatin mitochondrial DNA adducts, as compared with genomic DNA adducts, in livers and brains of maternal and fetal rats given one exposure to cisplatin at a tumorigenic level (5 mg/kg body wt) and a toxic level (15 mg/kg body wt) of drug. The genome DNA adduct data presented here have been previously published (8) along with genomic DNA adduct values from many other maternal and fetal tissues. The purpose of this presentation is to underscore the novel observation that highly elevated mitochondrial DNA adduct levels occur in rat brain and liver, tissues that are targets for tumorigenesis. It is interesting to note that the two tissues accumulating cisplatin mitochondrial DNA adducts, liver and brain, are relatively more quiescent at the late stage of gestation, as compared with kidney and placenta. One could postulate that replication of mitochondria accomplishes adduct dilution. Any functional impairment that may have been produced in the mitochondria by these high adduct quantities is currently unknown. Future studies will focus on the functional effects of cisplatin exposure in this organelle.

Mitochondria contain double-stranded circular DNA of ~16 000 bp that has no histones (15). The lack of histones results in accessibility of mitochondrial DNA to damage from outside influences and is generally considered to contribute substantially to the enhanced binding of carcinogenic chemicals to mitochondrial DNA as compared with genomic DNA. In addition to studies with cisplatin (16,17), benzo[a]pyrene...
bound to DNA are due both to higher initial cisplatin binding and to inefficient DNA adduct removal.

The transplacental tumorigenicity observed in livers and brains of rats (1) and mice (25) is supported by observations of direct genomic DNA adduction (8). In addition, transplacental cisplatin exposure is associated with ras activation in mouse skin at GpG sites in codons 12 and 13 (26), suggesting that fetuses may be susceptible to initiation by this drug. The functional consequences of the high levels of mitochondrial cisplatin adduction observed in brain and liver in these experiments and any possible associations with tumorigenesis have not been assessed. Compared with muscle and some other organs, these are not tissues that have high energy requirements. However, there is ample evidence in the literature that exposure to cisplatin can reduce mitochondrial respiratory function and adenosine triphosphate production as well as membrane-associated calcium in rat kidney (27–30) and in kidneys of cancer patients (31). In addition, cultured cells made resistant by continued exposure to cisplatin respond by increasing mitochondrial size and function (32,33). Moorehead et al. (34) measured cisplatin–DNA adduct levels in sensitive and resistant variants of a fibrosarcoma cell line and showed that the resistant cells had half as many DNA adducts and decreased mitochondrial membrane potentials as compared with the parental cells. Both of these responses improve the survival capabilities of the resistant cells (34). Taken together, all of these studies suggest that high levels of cisplatin adduction in mitochondrial DNA may be responsible for impairment of mitochondrial function. Investigation of mitochondrial integrity in transplacently exposed rats will be a focus for future investigations.

Acknowledgements

Appreciation is extended to Margaret Taylor for editorial assistance. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

References

5. Leopold,W.R., Miller,E.C. and Miller,J.A. (1979) Carcinogenicity of N-nitrosodimethylamine (21) and 7H-dibenzo[c,g]carbazole (22) have all exhibited preferential mitochondrial DNA binding. Cisplatin studies were performed with nude mice carrying a human gingival melanoma (16) and with cultured Chinese hamster ovary cells (17); in both cases the cisplatin binding to mitochondrial DNA was ~5-fold higher than that observed in genomic DNA.

Issues surrounding the binding of carcinogens to mitochondrial DNA must necessarily consider the ability of mitochondria to remove or repair such damage; evidence from the literature suggests that mitochondria do not perform nucleotide excision repair but do remove adducts using a process similar to base excision repair (23). In the case of cisplatin, only minimal repair of the intrastrand cis-diamminediamineplatinum-\(\text{N}^2\)-d(GpG) and -d(ApG) intrastrand adducts was observed, but there was efficient repair of the interstrand cross-links (23). In livers of rats given the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine adduct levels were similar in both genomic and mitochondrial DNA after one dose of compound, but after several doses there were higher adduct levels in the mitochondrial DNA, suggesting an inability to remove the accumulating DNA damage (24). Cell culture studies by Olivero et al. (unpublished observations) have also suggested that accumulating mitochondrial levels of cisplatin


