

Interaction of Cisplatin with Cellular Macromolecules: A Fourier Transform Infrared Spectroscopy Study

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Abstract

Platinum is a metallic element, which may react with our cellular component through its involvement in cancer chemotherapy medications. Cisplatin is one of the most useful antineoplastic drugs against human ovarian carcinoma, which has the central element of platinum in its structure. The nature of chemical interaction between platinum and cellular macromolecules is yet to be understood. We examined the interactions of platinum with human ovarian OV 2008 cancer cells by infrared spectroscopy, through the exposure of this cell line to cisplatin. These studies showed that there was an interaction between cisplatin and DNA and proteins. Nature of these interactions and their possible effects are discussed in this paper. In summary, it has caused denaturation of proteins and modification of the interchain packing of the DNA. Our results show that infrared spectroscopy is a potentially useful technique for monitoring the interaction of elements and drugs with cell components.

Keywords: Cisplatin; Fourier Transform; Infrared Spectroscopy; Ovarian carcinoma; DNA;RNA; Cell membrane.

Introduction

The study of the interaction of metal molecules and complexes with biological macromolecules is of special biological interest. Study of these complexes is especially important in research on antitumor drugs and the environmental hazards by heavy metals (1). Cisplatin is a cancer chemotherapeutic agent which is used in a variety of tumours, including lung cancer, head and neck cancer, testicular cancer, ovarian cancer and bladder cancer. The mechanism of cytotoxicity of cisplatin has not been well defined. However, it has been suggested that cytotoxicity is due to DNA alkylation, or due to interactions with cell proteins (2-3). Therefore, it is interesting to study the interaction of cisplatin with DNA, protein and

other important cellular components. Figure 1 presents the chemical structures of cisplatin (5).

Infrared (IR) spectroscopy is a powerful method for the study of chemical compounds (6). In the study of biological samples, this method can be used to determine the relationship of different peaks to specific bimolecular structures in cells or tissues. The modification of the pattern and intensities of different IR spectral regions in cancerous cells has provided some information regarding the application of this technique for diagnostic purposes (7).

The aim of this study is to investigate the nature of intracellular interaction of cisplatin with

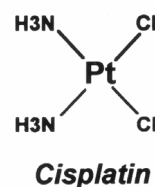


Figure 1. Molecular structure of cisplatin

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some macromolecules using IR spectroscopy. We are reporting the structural modifications of nucleic acids and proteins of human ovarian OV 2008 cells after the exposure to cisplatin.

Experimental

Cisplatin was the clinical formulation of 1 mg/ml in 0.9% saline solution supplied by Horner Laboratories (Montreal, Canada). This formulation was diluted in additional 0.9% saline solution to the appropriate concentrations and incubated for one hour at 37°C before use. The human ovarian cell line (OV 2008) was a donated from Dr. Rakesh Goel, Ottawa, Canada. The cells were cultured following the standard methodology of the American Type Culture Collection (ATCC, Rockville, MD). These cells were grown on tissue culture dishes in a humidified incubator at 37°C with a 5% CO₂ atmosphere. They were maintained in complete medium consisting of RPMI 1640, supplemented with 10% heat-inactivated fetal calf serum. RPMI media, fetal calf serum and glutamine were purchased from Gibco BRL laboratories. Glutamine (300 ng/l) was added to the media before its usage.

Cells were exposed to 0.33 mM of cisplatin in serum-free media for 1 h. Control cells were exposed to the equivalent volumes of sterile saline solution. Cells were harvested by gentle scraping off the tissue culture plates in 10ml saline solution. The experiments were performed in quadruplicate and the pellets were stored at -110°C until analysis. Repetition of experiments using fresh or previously frozen samples revealed that IR spectra of these cells were not affected by storage at -110°C (data not shown).

IR spectra were obtained by placing small amounts (about 0.01 mg) of cell pellets at room temperature between two AgCl windows of an IR adsorption spectra sample holder. Fourier

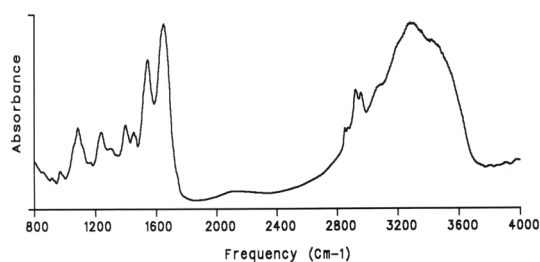


Figure 2. Whole region human ovarian OV-2008 IR spectra.

transform-IR spectra at the region of 400-4000 cm⁻¹ were measured with a Bomem Model Michelson 110 Fourier-transform spectro-photometer with a liquid nitrogen cooled mercury cadmium telluride detector. For each spectrum, 512 scans were co-added at a spectral resolution of 4 cm⁻¹. Infrared spectroscopy was conducted on each sample four times. Data were processed using the software developed at the National Research Council of Canada, Ottawa, to plot the graphs and to derivatize or deconvolute specific regions of spectra (8-9).

The mean and standard errors of the peaks' frequencies were calculated for each sample. The significance of the peak frequency shift for all samples, in comparison to control samples, was measured using alternate t-test with Welch's approximation.

Results and Discussion

Figure 2 shows the IR spectra of the OV 2008 cell line. This study focused on IR regions related to three macromolecules (DNA and proteins) and the lipid bilayer matrix of cell membrane. Table 1 lists the spectral frequencies in these regions for OV 2008 cells exposed to cisplatin and saline solution. The last three columns of this table summarises the results of alternate t-test with Welch's approximation.

The interaction of cisplatin with DNA has been reviewed by a number of investigators. The intra-strand binding of platinum compounds to DNA produces a severe local distortion in the DNA double helix, leading to unwinding and kinking (10). The results of IR spectroscopy results on the interaction of cisplatin with cellular DNA are described below.

Table 1. Important infrared peaks of different regions of OV-2008 cells exposed to saline and 0.33 mM cisplatin(CP). First two columns list the maximum peak frequencies related to cellular DNA, proteins, carbohydrates and the lipid bilayer of cell membrane (see text for details). The three last columns summarise the degrees of freedom (f), Welch's approximate t, and the two-tailed p value of alternate t-test. P values lower than 0.05 were considered as significant.

Saline	0.33 mM CP	f	T	p value
971.19±0.08	969.55±0.17	4	8.729	0.009
1059.61±0.02	1058.55±0.05	3	19.684	0.0003
1087.50±0.10	1085.86±0.11	5	11.032	0.0001
1159.29±0.14	1158.26±0.13	5	5.391	0.003
1173.93±0.80	1173.50±0.30	3	0.5033	0.6494
1242.81±0.13	1241.88±0.23	4	3.520	0.0244
1632.81±0.10	1632.63±0.25	3	0.6685	0.5517
1652.15±0.26	1653.13±0.40	5	2.054	0.0951
2852.69±0.07	2852.87±0.20	3	0.8495	0.4580
2872.91±0.09	2873.16±0.18	4	1.242	0.2820

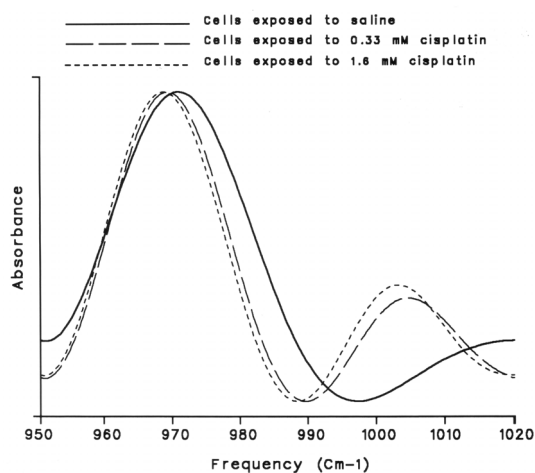


Figure 3. Region 950-1020 cm^{-1} of OV 2008 cells exposed to cisplatin (solid line: control cells, dashed line: platinum treated cells).

As shown in figure 3, there are two bands in this region of the IR spectra of OV 2008 cell line: one at $971.19 \pm 0.08 \text{ cm}^{-1}$ and the other at $1012 \pm 0.91 \text{ cm}^{-1}$. This figure originates from the third power derivative of the original spectrum with a breakpoint of 0.15. The band at 971 cm^{-1} is due to nucleic acids and the symmetrical stretching mode of dianionic phosphate monoesters of phosphorylated proteins, whereas the next band has not yet been exactly assigned (11). Figure 3 shows the shift of these two bands as a result of cell exposure to 0.33 mM of cisplatin and its generated metabolites, in comparison to the control cells. The frequencies of maximum peak absorbance show extremely significant differences between cells exposed to platinum compounds and controls ($p=0.009$).

Figure 4 is resulted from third power derivatization of the original spectra with the breakpoint of 0.3. The band at 1087 cm^{-1} in this region is mostly related to the DNA packing condition. This band is usually at 1082 cm^{-1} in normal cells, and will shift to about 1086 cm^{-1} in malignant cells. It is mainly due to the symmetric phosphate (PO_2^-) stretching mode of the phosphodiester backbone of nucleic acids (12). Degree of interchain interactions in the tertiary structure of DNA double strand is reflected by the frequency shift of this band. A weaker interchain packing will result in frequency shift of this band to a lower frequency; a pattern which is shown after 1 h exposure of OV 2008 cells to different concentrations of cisplatin. Therefore, from the IR spectroscopy point of view, platinum

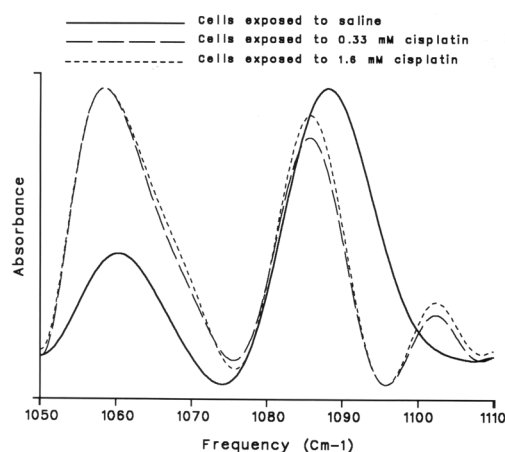


Figure 4. Region 1050-1110 cm^{-1} ; IR spectra of the symmetric phosphate stretching mode of the phosphodiester backbone of nucleic acid of OV 2008 (solid line: control cells, dashed line: platinum treated cells).

interaction with cellular DNA decreased its packing, closer to what is usually seen in normal cells. Statistical analysis showed a very significant difference between control cells and those exposed to the platinum compounds ($df = 5$, $p=0.003$).

Figure 5 represents a broad region and consists of two overlapping bands with maximums near $1221.56 \pm 0.15 \text{ cm}^{-1}$ and $1242.81 \pm 0.13 \text{ cm}^{-1}$, -respectively. The component band at 1242 cm^{-1} is mainly due to nonhydrogen-bonded phosphodiester groups and that at 1221 cm^{-1} is due to hydrogen-bonded phosphodiester groups (12). This graph indicates that the hydrogen bonding of the phosphodiester groups of nucleic acids increases with the

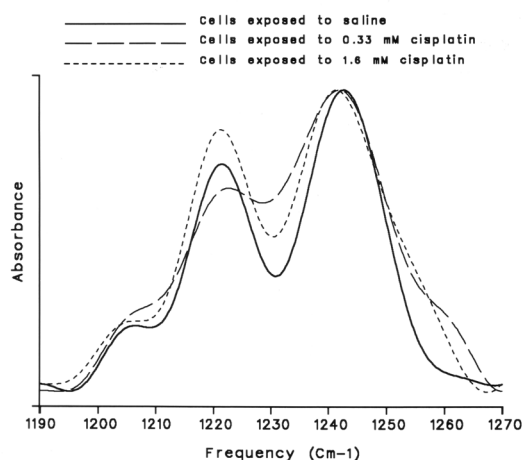


Figure 5. Region 1190-1270 cm^{-1} ; IR spectra of OV 2008 cells resulted from the vibrational mode of phosphodiester group of nucleic acids (solid line: control cells, dashed line: platinum treated cells).

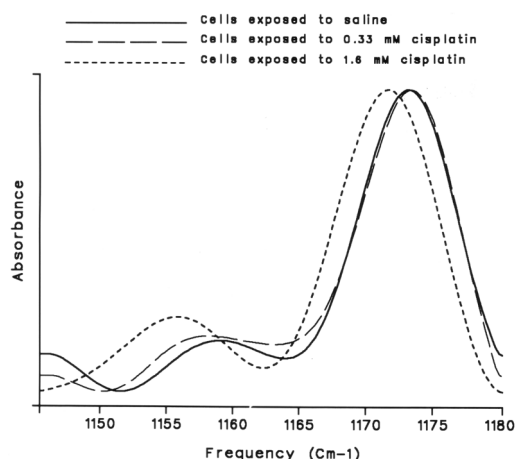


Figure 6. Region 1145-1180 cm^{-1} ; this region of the IR spectra of OV 2008 cells is mainly related to the stretching mode of C-O groups of cell proteins (solid line: control cells, dashed line: platinum treated cells).

addition of platinum compounds. A 1.4 cm^{-1} shift to the left is also apparent in the nonhydrogen-bonded phosphodiester related band, compared to the control cells ($\text{df}=4$, $P<0.05$). This shift indicates that the nonhydrogen-bonded phosphodiester groups of nucleic acids are bonded to a heavy group in cisplatin exposed cells.

Platinum compounds are irreversibly bound to plasma proteins (13). The band at $F-1173.93 \pm 0.80 \text{ cm}^{-1}$ (Figure 6) is due to the stretching mode of C-O groups of cell proteins. A shift of this band to a lower frequency indicates a decrease in the vibration of C-OH groups due to binding with another group. The C-O band in this region originates mainly from the C-OH group of

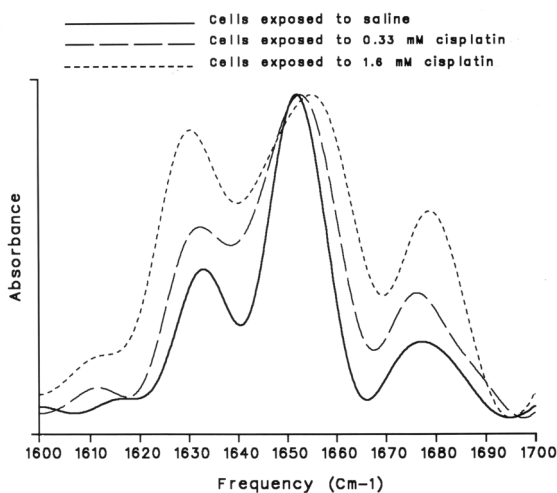


Figure 7. Region 1600-1700 cm^{-1} ; IR spectra of Amide I band of OV 2008 cells (solid line: control cells, dashed line: platinum treated cells).

serine, threonine and tyrosine in cell proteins (14). Statistical analysis of this band failed to show any significant differences between two group of cells under investigation ($\text{df}=3$, $p=0.6494$). Therefore, it is concluded that at this concentration, platinum compounds do not make a strong and critical binding with serine, threonine and tyrosine amino acids.

Figure 7 shows the deconvoluted amide I band spectra of OV 2008 cells (solid line) and of the cells exposed to cisplatin (dashed line). Amide I band in this region is due to the in-plane C=O stretching vibration, weakly coupled with C-N stretching and in-plane N-H bending of the amide groups in proteins. Peak maximum of the groups in this region is sensitive to the secondary structure of proteins in the cells (15). The α -helical structure peak maximum comes near $1652.15 \pm 0.26 \text{ cm}^{-1}$, the parallel β -sheet near 1632 cm^{-1} , and that of antiparallel β -sheet near 1677 cm^{-1} (7). The spectra of the control cells in figure 7 shows that the cellular proteins are to a large extent α -helices in OV 2008 cells. The relative amount of β -sheet segment in comparison to that of the α -helical segments is increased in cells exposed to cisplatin. On the other hand the amide I band shifts slightly towards higher frequencies, indicating that hydrogen bonds in the α -helical segments of the proteins are weaker as a result of exposure to cisplatin. The width of the whole spectrum is also an indicator of some degree of denaturation in the whole proteins of cells exposed to cisplatin.

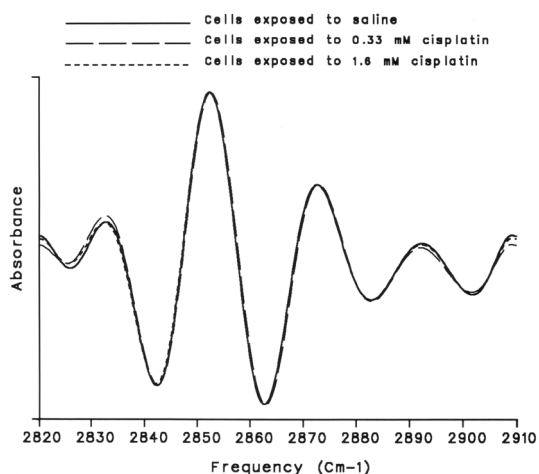


Figure 8. Region 2820-2910 cm^{-1} ; this region of IR spectra of OV 2008 cells is related to the CH_2 stretching mode of methylene chains in membrane lipids (lines of treated and control cells are overlapped. solid line: control cells, dashed line: platinum treated cells).

In spite of all alterations in the IR spectra of nucleic acids and proteins in OV-02008 cells exposed to cisplatin, there is no difference between spectra shown in figure 8. This figure represents the region of 2820-2910 cm^{-1} of OV 2008 cells, with the third power derivatization of the original spectra and a breakpoint of 0.3. The dominant infrared band at $2852.69 \pm 0.07 \text{ cm}^{-1}$ arises from the CH_2 asymmetric stretching mode of the acyl chain methylene groups, while the weaker band at $2872.91 \pm 0.09 \text{ cm}^{-1}$ is due to the symmetric stretching mode of CH_3 (16). As is shown in this figure, there is no discernible effect of cisplatin on the methylene chains of the membrane lipids.

As a conclusion, our work with human ovarian cancer OV 2008 cells exposed to cisplatin provides potentially valuable information about the nature of the interactions of platinum compounds with cellular macromolecules. We conclude that the biochemical groups most susceptible to the interaction by cisplatin are the phosphodiester group and the phosphate backbone of nucleic acids and the amide group of proteins. Our studies suggest that IR spectroscopy may be a useful tool to examine the intracellular effects of platinum.

Acknowledgments

This research was supported by the National Cancer Institute of Canada with funds from the Canadian Cancer Society and in part by support from Shaheed Beheshti University of Medical Sciences in Iran.

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