SR FTIR spectroscopy investigation of Pd@S-CD nanocomposite system effects on biomolecules in cervical carcinoma cells

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Abstract

Nanocomposite system formulated from surface-modified S-doped carbon dot (S-CD) nanoparticle with a potential metallodrug, palladium(II) complex, dichloro(1,2-diaminocyclohexane)palladium(II), [Pd(dach)Cl_2] (Pd@S-CD), was investigated as a model system for the treatment of cervical carcinoma (HeLa) cells. To examine the intracellular biochemical effects induced by the Pd@S-CD, we used Synchrotron Radiation-based Fourier-transform infrared spectroscopy (SR FTIR). SR FTIR spectroscopy was employed to investigate the alterations in cellular components' biochemical composition and secondary structure upon exposure to Pd@S-CD. Spectral analysis, complemented by statistical techniques, revealed changes in biomolecules, lipids, proteins, nucleic acids, and carbohydrates caused by the treatment with Pd@CDs. These results and the increased cytotoxicity of the system demonstrate its high anti-cervical cancer therapeutic potential .

Keywords: transition metal complex, Synchrotron Radiation, Fourier Transform Infrared Spectroscopy, nanocomposite

1. Introduction

Palladium(II) complexes are one of the leading contenders for replacing cisplatin in cancer treatment due to the similarity of their metal center to platinum(II) complexes. The Pd(II) complexes with different ligands can interact with biomolecular targets in diverse ways, thus providing a high potential for developing new and more efficient anticancer drugs. Substituted polyamines have been shown to coordinate efficiently with both Pd(II) and to act as modulators of the hydrophilic/lipophilic properties of the resulting chelates, thus improving their administration and bioavailability [1]. To improve the efficacy of applied metallodrug, they can be combined with various nanocarriers, which preserve the activity of a drug or can affect the intracellular target molecules [2]. As nanocarriers, various nanoparticles are tested. Among them, those based on carbon, such as carbon dots (CDs), are good candidates because of their physicochemical properties and biocompatibility, which can increase the cellular uptake of a drug. For instance, cisplatin combined with S- or N-doped CDs (cis-Pt@S, N-CDs) was demonstrated to overcome cisplatin resistance in cis-Pt-resistant ovarian cancer cells [3]. The intracellular effects that transition metal complexes induce in cells, which lead to their cytostatic effects, which include the entire cellular regulatory system, have yet to be fully elucidated. Synchrotron Radiation Fourier Transform Infrared Spectroscopy (SR FTIR) has a high potential, spatial resolution, and sensitivity, a highly brilliant infrared light source that provides higher quality spectra at a cellular level [4]. Therefore, SR FTIR can provide information about the drug's effect on individual cellular biomolecules without their isolation from the cells, and we have applied this approach to elucidate biochemical changes in cervical cancer, HeLa, cells induced by Pd@S-CD.

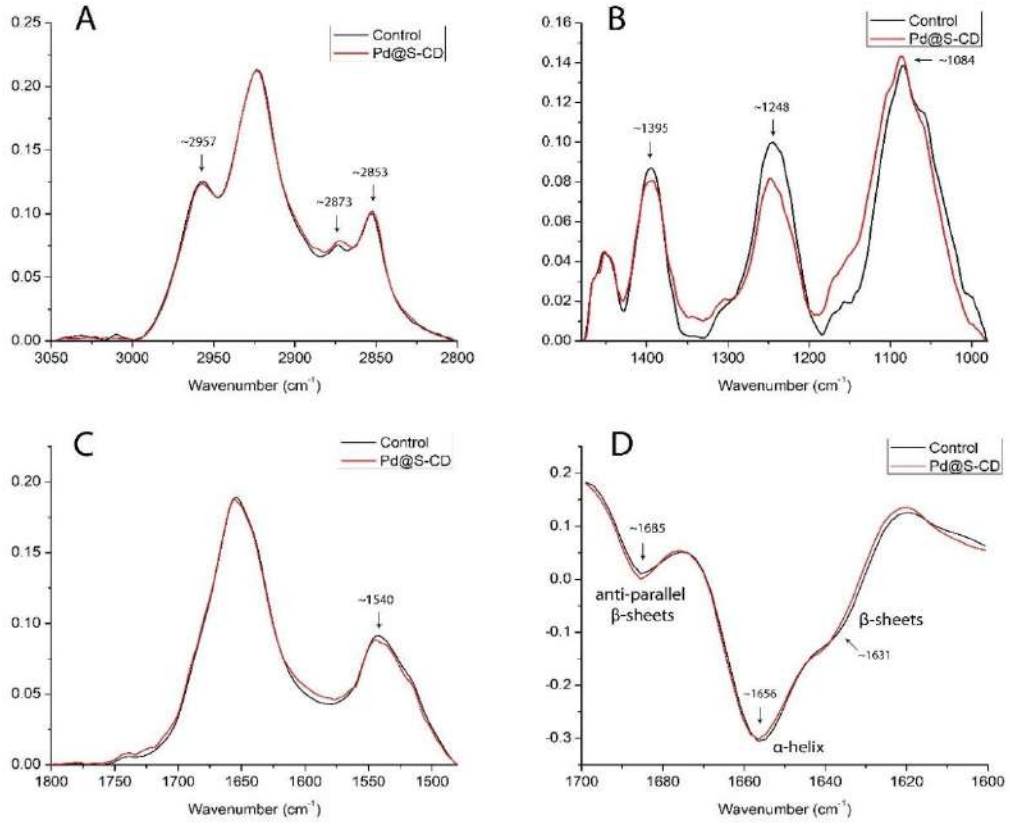
2. Methods

HeLa cells were treated with Pd@S-CD for 48 h. Pd(II) complex concentration was 85 μM, and S-CD 20 μg/mL. After the treatment, cell cytotoxicity was determined by the Sulforhodamine B (SRB) assay. Changes in cell biomolecules were analyzed by SR FTIR spectroscopy (Synchrotron ALBA, MIRAS beamline, Barcelona, Spain) with cells seeded and lyophilized on CaF_2 glass support [5]. Spectra for treated and control cells were collected in the 4000–900 cm^{-1} mid-infrared range. Spectral analysis, including rubber band baseline correction and vector normalization for every single cell, was implemented for three different areas: 3050−2800 cm^{-1} lipid area, 1800−1480 cm^{-1} proteins and esters, and 1480−900 cm^{-1} nucleic acids and carbohydrate region. The second derivative (17 smoothing points, third polynomial order, and vector normalization) was determined for the Amide I protein region (1700–1600 cm^{-1}). The principal component analysis (PCA) for each data set was performed.

3. Results and discussion

Pd@SCD treatment of HeLa cells left 70% viable cells after 48 h. This viability was targeted to enable the study of intracellular biochemical changes in viable cells.

Significant spectral differences were present in all inspected areas: lipids, proteins, nucleic acids, and carbohydrates. The average spectra of those regions and the second derivative of the Amide I average spectra are shown in Figure 1, whereas the most affected signals are listed in Table 1. The lipid region is affected in CH_2 and CH_3 stretching assigned bands, while the protein region differs in all main secondary



structure bands, as indicated in Table 1.

Figure 1. The average FTIR spectra of Pd (II) treated (red) and control (black) HeLa cells in the lipid (A), nucleic acid and carbohydrate (B), and protein (C) regions are presented. The second derivative and vector-normalized spectra of the averaged FTIR spectra of Amide I in the 1700-1600 cm^{-1} region is shown (D).

The most remarkable differences can be seen in the carbohydrate and nucleic acid region (1480–980 cm^{-1}), implying that Pd@S-CD affects these macromolecules the most. In this region, bands that contributed the most to the overall spectral differences were assigned to phosphate and P-O-C stretching and COO^- symmetric stretching. These changes could be a consequence of direct nanocomposite-nucleic acid interactions, or a result of downstream changes caused by nanocomposite-protein interactions.

Table 1. Vibration frequencies and band assignments of the regions with the most significant differences between Pd@S-CD treated and control HeLa cells.

Region	Wavenumber (cm^{-1})	Band assignment
Lipid	2957	asymmetric CH_3 stretching
	2873	symmetric CH_3 stretching
	2853	symmetric CH_2 stretching
Protein	1685	anti-parallel β-sheets
	1656	α-helix
	1631	β-sheets
	1540	Amide II α-helix
Carbohydrates and nucleic acids	1395	COO^- symmetric stretching
	1248	asymmetric phosphate stretching
	1106	symmetric P-O-C stretching
	1084	symmetric phosphate stretching

3. Conclusions

In this work, we have emphasized the complexity of the intracellular interactions of the transition metal complexes and identified intracellular biochemical processes that might lead to tumor elimination. Conformational modifications in the main classes of macromolecules were experimentally documented.

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