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Short Communication

Investigating the Apoptosis Ability of Ethylenediamine 8-Hydroxyquinolinato Palladium (II) Complex

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Abstract

Purpose: High solubility, low renal toxicity and apoptosis-inducing ability of palladium complexes are the reasons for their synthesis.

Methods: *In vitro* cytotoxic study of previously synthesized $[Pd(en)(8HQ)]NO_3$, was carried out on breast cancer MCF-7 cell lines and prostate cancer DU145 cell lines. DNA fragmentation indicative of apoptotic was also evaluated by TUNEL assay on DU145 cell line.

Results: FT-IR spectra of final complex confirmed the existence of chelating ligands. The DU145 cells unlike the MCF-7 cells, demonstrated the significant influence of the Pd (II) complex. The IC_{50} values of $[Pd(en)(8HQ)]NO_3$ and cisplatin on DU145 cells were 27 and 8.3 µM, respectively. Moreover, nearly 38% apoptosis was evident in DU145 cells after treatment with $[Pd(en)(8HQ)]NO_3$.

Conclusion: $[Pd(en)(8HQ)]NO_3$ has great potential in DNA binding and induction of apoptosis; thus it can be used in the future against prostate cancer.

Introduction

Although cisplatin is mainly utilized for treatment of various cancers such as ovarian, breast, stomach and prostate, its side effects and its drug resistance have led to more research for synthesis of new and nontoxic derivatives of platinum-group metals.[1] Among the close elements of platinum in the periodic table, palladium complexes have high solubility and low renal toxicity; so several palladium complexes with different ligands such as phenanthroline derivatives, N-heterocyclic carbenes, Schiff bases etc were synthesized.[2-4] One of the donor and lipophilic chelating ligands is 8-Hydroxyquinoline (8HQ), a multifunctional ligand, that possess therapeutic and antioxidant properties. It can act as a potent chelator for restoring metal balance, when the diseases are related to metal imbalance.[5] Moreover, it can form a planar complex with palladium and thus complex can intercalate between DNA strands. In another mechanism, 8HQ metal complexes can facilitate the membrane damage, DNA cleavage and apoptotic cell death in tumors.[6]

The synthesis and theoretical investigations of ethylenediamine 8-hydroxyquinolinato palladium (II) complex have been previously reported. We have also evaluated the DNA binding and cytotoxic effect of ethylenediamine 8-hydroxyquinolinato palladium (II) complex on leukemia cell lines (K562) that did not elicit noticeable effect in comparison with cisplatin. In this study, the *in vitro* cytotoxic effect of 8HQ-palladium (II) complex on breast cancer MCF-7 cell lines and prostate cancer DU145 cell lines was reported.

Materials and Methods

8-hydroxyquinoline (8HQ), ethylenediamine (en), potassium tetrachloropalladate(II) (K_2PdCl_4), sodium bicarbonate, (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and sodium chloride were purchased from Merck (Germany). MCF-7 and DU145 cell lines were obtained from Pasture Institute (Tehran, Iran). TdT-mediated dUTP Nick-End Labeling (TUNEL) kit was purchased from Roche Applied Science (Germany). t-Octylphenoxypolyethoxyethanol (Triton X-100), phosphate buffered saline powder, sodium citrate and paraformaldehyde were obtained from Sigma (USA). All media and cell culture components were obtained from Life Technologies (USA).

Synthesis of ethylenediamine 8-hydroxyquinolinato palladium (II) complex

The preparation of ethylenediamine palladium (II) dichloride $[Pd(en)Cl_2]$ was carried out using previously reported method.[7] Briefly, in ice bath, K_2PdCl_4 (1.63 g, 4.99 mmol) was dissolved in 200 mL water and ethylenediamine (0.34 mL, 5 mmol) was added dropwise

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to the mixture. After 2 h stirring at RT, the yellowish brown precipitate was filtered and then washed with water, ethyl alcohol and diethyl ether (Figure 1 panel A). Yield was 89%.

Thereafter, same amounts of [Pd(en)Cl_2] (1 mmol), $NaHCO_3$ and 8-hydroxyquinoline were dissolved in 20 mL water and mixture was stirred for 2 h at 50°C.[7,8] Volume of the solution was increased to 60 ml, and then $AgNO_3$ (0.34 g, 2.0 mmol) was added to the solution and mixture was stirred at 50°C under dark condition for 7 h. Stirring was continued at RT and dark condition for 10 h. The AgCl precipitate was filtered and the obtained yellow solution was evaporated to dryness by rotary evaporator. Yield was 78%. ^{1}H NMR chemical shifts data of final complex have been reported in our previous study.[7] Figure 1 (Panel B) illustrates the molecular structure of ethylendiamine 8-hydroxyquinolinato palladium (II) complex.

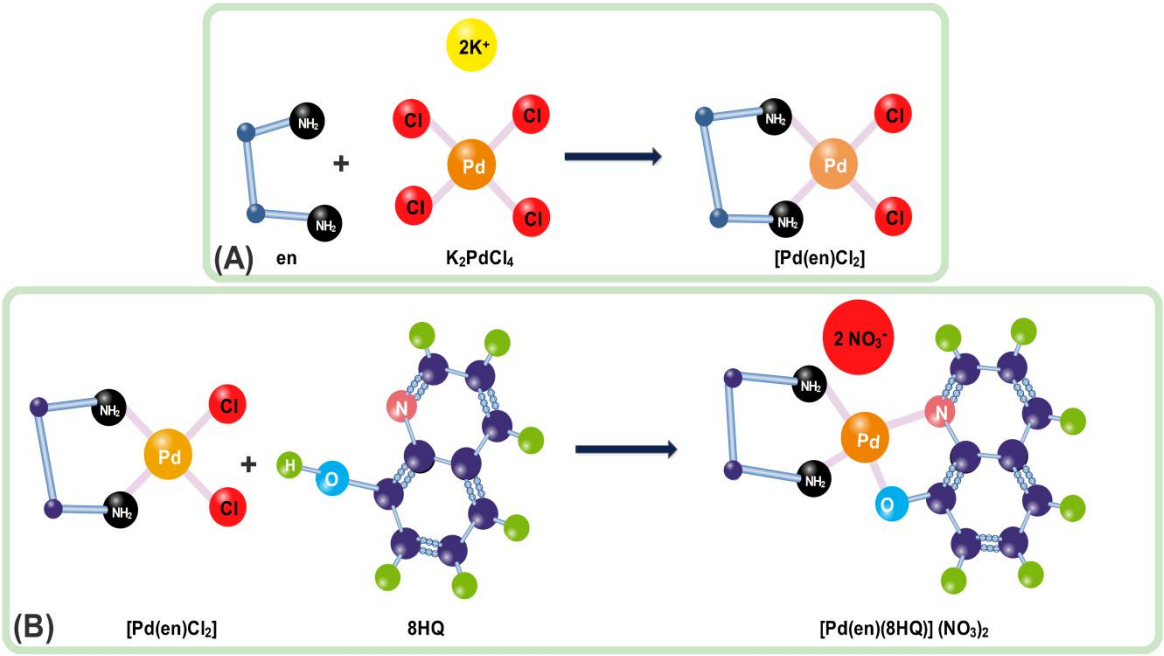

Figure 1. Schematic structure of (A) ethylenediamine palladium(II) dichloride [Pd(en)Cl_2] and (B) ethylenediamine 8-hydroxyquinoline palladium (II) complex [Pd(en)(8HQ)]NO_3. The planar structure is obvious.

Cell culture and in vitro cytotoxicity analysis: MTT assay

Prostate cancer DU145 cells and breast cancer MCF-7 cells were cultured according to existing standards.[9,10] Thereafter, both DU145 cells and MCF-7 cells (1×10^4 cells/well) were transferred onto 96-well plates and at 24 h post-seeding, different concentrations (i.e. ranging from 12.5 to 200 µM) of Pd(II) complex and cisplatin were added to them. The treated cells were incubated for 24, 48, and 72 h. At appropriate times, the media was removed and cells were subjected to (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). Thereafter, plates were incubated for 4 h at 37°C in a CO_2 incubator. Finally, the media was removed and absorbance of formed formazan crystals was read at 570 nm by adding DMSO (200 µL) and Sorenson's buffer (25 µL) to each well.

Apoptotic study by TUNEL assay

Based on the amount of IC_{50} obtained, DU145 cells were incubated with 27 µM of prepared palladium for 72 h. After washing with phosphate buffered saline (PBS), control and treated cells were immersed in 4% paraformaldehyde solution for 1 h and then rinsed with PBS. To permeabilize the cells, a solution of Triton X-100 and sodium citrate (0.1 g in 100 mL H_2O) was added for 15 min and were washed with PBS. Finally, the cells were incubated with TUNEL reaction mixture for 1 h in optimum conditions and were rinsed with PBS. For quantification of apoptotic cells, a total of 400 cells were counted utilizing fluorescent microscope (Nikon Eclipse Ti-U, Japan). Apoptotic cells exhibited intense nuclear staining when compared to diffuse staining in non-apoptotic cells.[11]

Results and Discussion

Characterization of palladium (II) complex

Palladium complex was synthesized by the displacement of chelating ligands (8HQ and en) with the chlorine in K_2PdCl_4. In the reaction of palladium (II) complex with $AgNO_3$, nitrate can acts as a counter ion and can help to complex solubility.[12]

In the FT-IR spectrum of [Pd(en)(8HQ)], absorption peaks at 1110, 2920, 3020, and 3414 cm^{-1} are related to ν (C–O stretching), ν (C–H aromatic), ν (C–H aliphatic) and (N–H stretching), respectively. In addition, absorption peaks at 1500 and 1600 are related to C=C aromatic and C=N aromatic, respectively. Figure 2 illustrates the FTIR spectra of Pd (II) complex.[7]

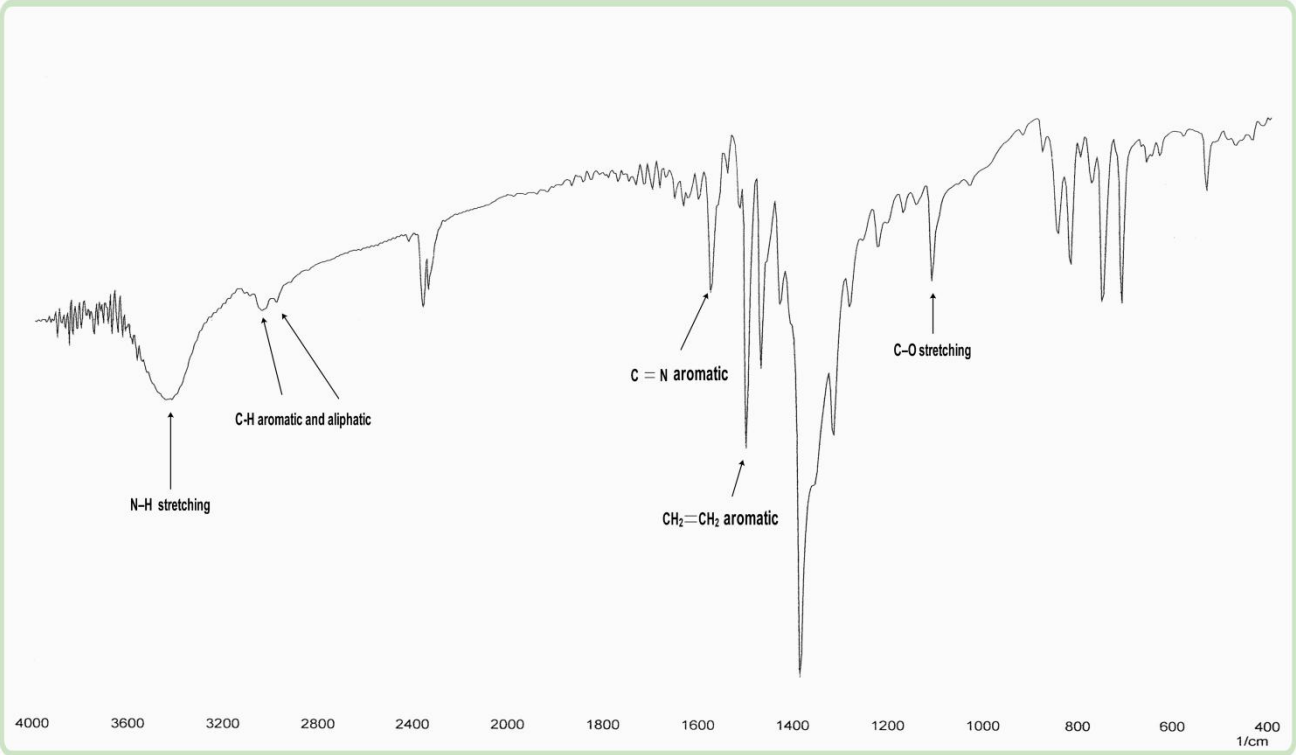

Figure 2. The FTIR spectra of Pd (II) complex. The main absorption peaks are related to: C–O stretching, C=C aromatic and C=N aromatic.

In vitro effects of Pd complex and cisplatin on MCF-7 and DU145 cell lines

Forasmuch as the cytotoxicity of Pd complexes against human breast cancer cell lines and prostate cancer cell lines has been confirmed,[3,13] the MCF-7 and DU145 cell lines were selected for cytotoxicity evaluation of [Pd(en)(8HQ)]NO_3 in comparison with cisplatin. Figure 3 illustrates the impact of [Pd(en)(8HQ)]NO_3 in the breast cancer MCF-7 cells (Figure 3, panel A–C) and prostate cancer DU145 cells (Figure 3, panel D–F). In DU145 cells, Pd(II) complex led to prolonged inhibitory up to 72 h in sustained manner when compared to cisplatin (about 80% toxicity for Pd(II) complex). In MCF-7 cells, the cytotoxic effects of [Pd(en)(8HQ)]NO_3 were partly low after 72 h when compared to DU145 (i.e. ~60–65% toxicity). According to Table 1, the cytotoxic effect of [Pd(en)(8HQ)]NO_3 on breast cancer cells seems to be only time dependent, while it displays a trend of time and concentration dependent inhibitory effects on prostate cancer cells. The cytotoxic effect of cisplatin on both breast and prostate cells was time and dose dependent. In line with our results, Khan et al.[13] reported that their newly synthesized Pd(II) complexes are highly effective against DU145 cells.

TUNEL assay

Platinum-group drugs have the ability to induce apoptosis in tumor cells through DNA binding and also triggering cellular processes.[14] Our previous studies have confirmed that the binding of [Pd(en)(8HQ)]NO_3 can cause some changes in the stability of DNA. This water soluble complex may interact with DNA as an intercalator, which may interfere with DNA replication and cell proliferation. On the other hand, 8HQ can form chelate complexes with metal ion of ribonucleotide reductase which is an important enzyme for DNA synthesis.[15] This affinity to DNA may increase for 8HQ metal complexes in comparison to free ligands.[16] According to IC_{50} values in Table 1, prepared Pd (II) complex has shown good inhibitory effects on DU145 prostate cancer cells. To determine whether the cell death was attributable to [Pd(en)(8HQ)]NO_3-induced apoptosis, TUNEL assays were carried out during the course of 72 h treatment with 27 µM [Pd(en)(8HQ)]NO_3 (IC_{50} value of Pd(II) complex).[17] As illustrated in Figure 4, a detectable level of apoptosis was evident in DU145 cells after treatment with prepared Pd(II) complex when compared to untreated cells. After counting the apoptotic cells, nearly 38% of apoptosis was evident after 72 h of treatment of DU145 cells with 27 µM of prepared palladium complex. According to similar work carried out by Ulukaya et al.,[18] palladium complex has demonstrated extensive growth-inhibitory effect against prostate cancer cells. They claimed that the Pd complex induced DNA damage and also cell death in prostate cancer cells. In another study, Chen et al.[19] reported that clioquinol, an 8-hydroxyquinoline derivative, after binding to copper can suppress androgen receptor (AR) protein expression, and induce apoptotic cell death in human prostate cancer. So, based on all the evidence and also on the TUNEL results, induction of apoptosis after treatment with [Pd(en)(8HQ)]NO_3 is obvious in prostate cancer DU145 cells.

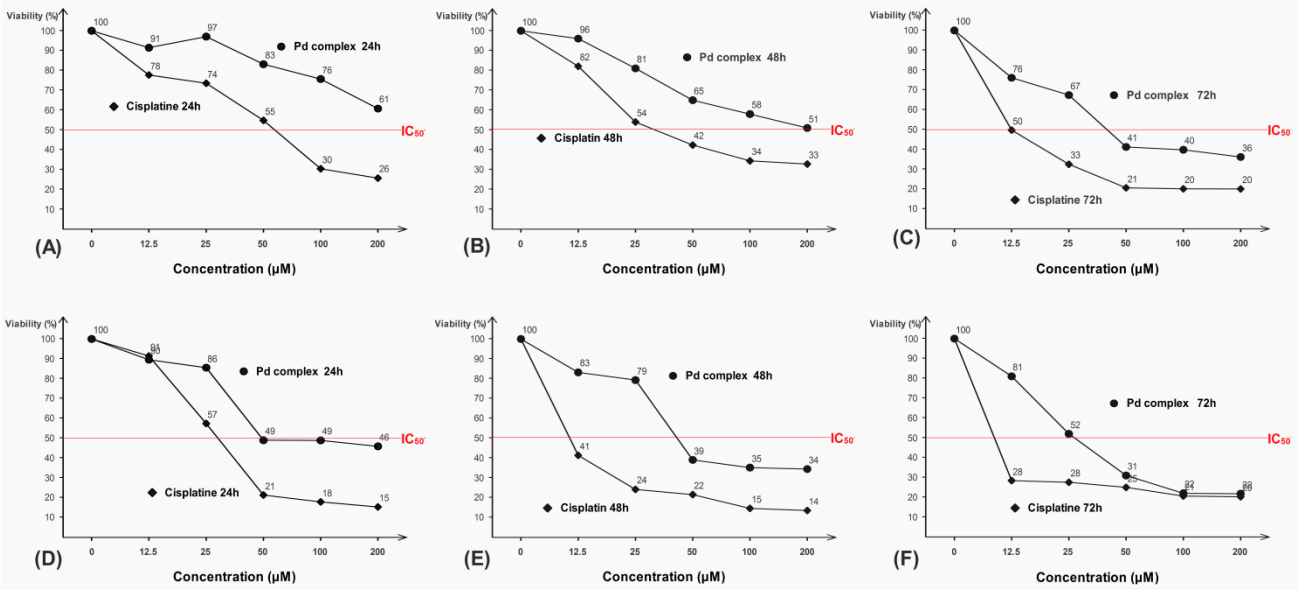

Figure 3. Cytotoxic study (MTT assay) of Pd(II) complex and cisplatin in the MCF-7 and DU145 cells. (A-D) Treated breast cancer MCF-7 cells after 24, 48 and 72h. (D-F) Treated prostate cancer DU145 cells after 24, 48 and 72h. The cytotoxic effect of $[Pd(en)(8HQ)]NO_3$ on prostate cancer cells display a trend of time and dose dependent, similar to cisplatin.

Table 1. The IC_{50} values of $[Pd(en)(8HQ)]NO_3$ and cisplatin in two different cell lines and three different times.

	MCF-7 (µM)			DU145 (µM)		
	24h	48h	72h	24h	48h	72h
Cisplatin	61	35	12.5	37.5	10	8.3
Pd (II) Complex	----	----	41	53	40	27

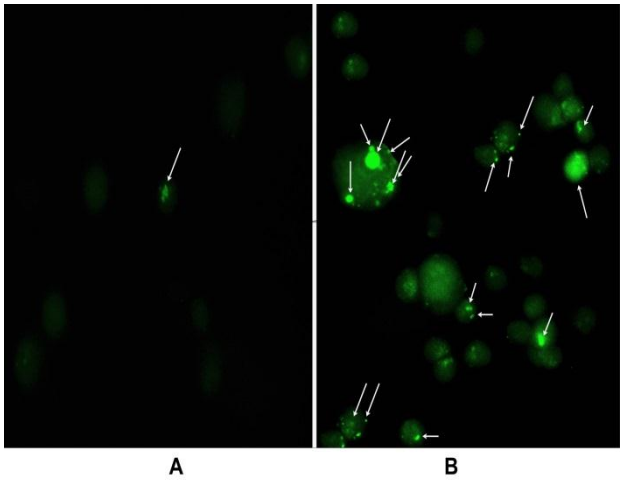

Figure 4. DNA fragmentation of DU145 cells. A) Untreated cells. B) Treatment cells with 27 µM $[Pd(en)(8HQ)]NO_3$ (TUNEL assays).

Conclusion

It has been confirmed that palladium complexes could induce apoptosis in tumor cells by binding with DNA. In this study, we have shown that $[Pd(en)(8HQ)]NO_3$ is able to affect DU145 cell lines in a time and dose dependent manner. Moreover, with fluorescence staining, we suggest that $[Pd(en)(8HQ)]NO_3$ has the capacity to interact with DNA and induce apoptosis in prostate cancer cells. In conclusion, we propose that this palladium complex can be utilized against prostate cancer.

Acknowledgments

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Ethical Issues

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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