Extremely low frequency electromagnetic field sensitizes cisplatin-resistant human ovarian adenocarcinoma cells via P53 activation

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Abstract In the following study, extremely low frequency electromagnetic fields (EL-EMF) radiation was used to restore sensitivity in the cisplatin-resistant A2780 ovarian cancer cells. For this purpose A2780 cells were treated with different doses of cisplatin and EL-EMF (50 Hz, 200 gauss, and 2 h) alone. Cytotoxicity was the measurement using MTT assay. After calculating IC₅₀ for cisplatin (90 µg/ml) a lower concentration from IC₅₀ (30 and 60 µg/ml) was used to be combined with EL-EMF. We compare the effects of each cisplatin, EL-EMF and combination groups using acridine orange–propidium iodide (AO/PI) and DAPI staining, caspase 3/9 activation assay and Annexin/PI assay. We also assessed changes in P53 and Matrix metalloproteinases 2 (MMPs) gene expression with

semi-quantitative RT-PCR. Results indicated an EL-EMF-dependent proliferative decrease which was found <10 %, and occurred independently of cisplatin. The decreased proliferation rate for 30 and 60 µg/ml cisplatin was about 20 and 40 %, respectively, while for synergistic groups 30 and 60 µg/ml cisplatin with 2 h EL-EMF exposer, showed 47 and 71 % decrease in viability in rats. DAPI staining indicated that chromatin break down significantly increased in synergistic groups. Acridine orange staining also confirmed MTT assay results. Caspase activity significantly increased in the combined groups. Semi-quantitative RT-PCR showed that in synergistic groups of cisplatin and EL-EMF, expression of P53 was increased but the expression level of MMP-2 gene decreased. Results from this study showed that changes generated by the non-invasive EL-EMF can make resistant cells sensitive to cisplatin.

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Introduction

Cancer is one of the most destructive diseases in the world. Current cancer treatments including surgical intervention, chemotherapy, and radiation therapy often kill healthy cells and causes toxicity to the patient (Sudhakar 2009). In addition, possibility of establishing resistance to treatment strategy and tumor

growth is enhanced, since the vast majority of the deaths occur after medical intervention with anti-cancer therapy, both conventional chemotherapy and novel targeted therapy. It can be concluded that these patients die from drug resistant cancers and tumors start growing again (Trédan et al. 2007; Thomas and Coley 2003). A number of studies have revealed that mechanisms underlying the development of drug resistance in cancer cells are manifold, complex and are very likely dependent on cell and microenvironment context. For example the chemotherapy drug cisplatin has been one of the doctors' first lines of defense against tumors, especially those of the lung, ovary and testes. While cisplatin is often effective when first given, its use represents a major drawback. Hence, the new therapeutic method should be produced with greater influence and fewer side effects or in combined with conventional chemotherapy drug.

Electromagnetic waves can be very promising in this area. Non-ionizing electromagnetic fields, from extremely-low frequency to radiofrequency, have been shown to cause biological effects even at low intensity. Some of these effects may be applied to medical treatments (Galluzzi et al. 2012). A recent example is that, several reports have evaluated the effects of EL-EMF on tissue repair (Lai and Singh 2010). Reported clinical benefits from using the specific EMF signals to treat advanced hepatocellular carcinoma, stabilizes the disease and even produces partial responses in a subset of the patients (Pesce et al. 2013). The basic mechanisms by which this weak EL-EMF interacts with living matter are currently unknown, although several mechanisms have been proposed to account for the initial interactions with cells (Costa et al. 2001). The basic mechanism is the forced-vibration of all free ions on the surface of a cell membrane that is caused by an external oscillating field and it has been shown that this coherent vibration of electric charge is able to irregular gate electro-sensitive channels on the plasma membrane and cause disruption of the cells' normal electrochemical balance (Costa et al. 2001). It seems that this is able to change numerous pathways lead to various cell responses. Many studies show that electromagnetic field, through change in function or cells' functional processes, induces cell proliferation and differentiation, disorder in cell cycle, induction of programmed death, disorder in intracellular interactions, DNA transcription, gene expression and free radical

production (Hardell and Sage 2008). Also Electromagnetic waves in different frequencies could be applied for cancer therapy, because cancer cells use high amount of iron and are more susceptible to magnetic fields (Lai and Singh 2010). It is generally accepted that 50/60 Hz EL-EMF does not transfer sufficient amounts of energy to the cells in order to damage DNA directly and cause genotoxic effects (Simko and Mattsson 2004). Altered expression of regulatory proteins involved in signal transduction pathways that control the apoptotic pathway can also affect sensitivity to the drug (Galluzzi et al. 2012).

The aim of this study was to assess the effect of the simultaneous use of cisplatin and EL-EMF from a cisplatin and EL-EMF to overcome cisplatin resistance and initiate cell death at low concentrations of cisplatin. In this study, we used the A2780 cell line to assess the biological effects of short-term EL-EMF exposure in cisplatin resistance. Such cells are considered to be a reliable *in vitro* model of cisplatin resistance and are widely used in the removal of drug resistance studies.

Material and method

Reagents and media

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], acridine orange, propidium iodide and DAPI (4',6-diamidino-2-phenylindole) were obtained from Sigma-Aldrich Company, Ltd. (Poole, United Kingdom)). Fetal bovine Serum (FBS), RPMI1640 culture medium were purchased from Sigma-Aldrich Company, Ltd. (Poole, United Kingdom). The high Pure RNA Isolation kits were purchased from Roche (Mannheim, Germany) and cDNA synthesis kit from Fermentas Inc. (Vilnius, Lithuania). RT-PCR kit was purchased from Pars Tous (Mashhad, Iran) and primers were obtained from Bioneer (Daejeon, Korea). All solutions were prepared with double distilled water and other chemicals were of analytical grade. Also commercial cisplatin was purchased from drug store.

Cell culture

A2780 was purchased from Iran Pasteur Institute (Tehran, Iran) and cultured in RPMI1640 with 10 %

FBS. Cells were incubated at 37 °C in 5 % humidified CO₂. When cells reached 90 % confluency, they were trypsinized. With 0.25 % trypsin for 5 min at 37 °C. After centrifugation, cells were re-suspended and used for further analysis.

Cisplatin treatment

The cells were seeded in either 6- or 96-well tissue culture plates. Then the cells were treated with 0 to 100 µg/ml cisplatin and were further analysed.

Exposing cells with electromagnetic fields and treatment with cisplatin

Cells were seeded in either 6- or 96-well plates and were divided into two groups. After 24 h of successful cell culture, two groups of cells were treated with cisplatin (30 or 60 µg/ml) and EL-EMF, one group was treated with EL-EMF alone. All groups were placed in an electromagnetic field (50 Hz frequency, 200 G) for 2 h. Electromagnetic field generator was assembled in the animal developmental research laboratory of Islamic Azad University of Mashhad. Then the cells were returned to the incubator for additional 46 h. Further tests were done using these cells.

MTT assay

Cells were seeded into 96 well dishes (5×10^4 cells/well) and were treated cisplatin and EL-EMF for 24 or 48 h as it was described before. At the end of the incubations 50 µl of MTT solution (5 mg/ml in PBS) was added to each well and left for 4 h in dark conditions. Subsequently, the insoluble formazan produced was dissolved in a solution containing 10 µl of DMSO and left for 20 min at room temperature. Finally absorbance was read at 570 nm with plate reader spectrophotometer (Epoch, Biotek, Winooski, VT, USA). The cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = (A_{\text{treated}}/A_{\text{control}}) \times 100$$

where A_{treated} and A_{control} is the absorbance of the treated and untreated cells, respectively.

AO/PI staining

For AO/PI staining and viability determination, 20 µl of cell sample and 20 µl of AO/PI staining solution were combined. 20 µl of stained sample was analyzed by fluorescent microscopy.

DAPI staining

Cultures were prepared by adding approximately 5000 cells to each well of a cell culture plate containing gelatin-coated coverslips. When cells reached the desired density, the culture medium from each well was removed and the cells were washed twice with PBS. The cells were treated with cisplatin (30 µl/ml), EMF (200 G, 2 h) and EL-EMF in the presence of cisplatin (as describe above). After that the wells were washed with PBS, cold paraformaldehyde (4 %) was added for 20 min at 4 °C and 5 min at RT and again the wells were washed with cold PBS twice. For nuclear staining, DAPI solution (1 µg/ml) was added to each well and was left for 10–60 s. Then wells were washed with cold PBS twice. Finally they were observed under fluorescent microscopy.

Caspase 3 and 9 activity assay

In summary, A2789 cells were treated with cisplatin, EL-EMF and a combination of these factors for 24 h. The parallel controls did not receive any treatment. After this, the pellets were suspended in 50 µl of chilled cell lysis buffer and cells were incubated on ice for 10 minutes then the cells were centrifuged for 1 minute using a micro centrifuge (10,000 g). Protein concentration was determined with the biuret method. 50–200 µg protein was diluted with 50 µl cell lysis buffer for each assay. DTT and DEVD-p-NA substrate were added. Finally absorbance of samples was read at 405 nm in a micro plate reader. Fold-increase in caspase 3 activity was determined by comparing these results with the level of the un-induced control.

Semi-quantitative RT-PCR

P53 and MMP-2 gene expression were analyzed with RT-PCR. For this purpose RNA was extracted from approximately 30×10^5 A2780 cells, that have been treated with cisplatin (30 µg/ml), EL-EMF (200 G) and combination of them for 24 h using the RNA extraction kit according to the manufacturer's instructions. The mRNA was reverse transcribed to cDNA with the Advantage RT-PCR kit using the manufacturer's instruction, briefly. cDNA was amplified using a thermocycler at 94 °C for 40 seconds, 56 °C for 50 seconds and 72 °C for 60 seconds (35 cycles). Specific primers used for amplifying cDNA are presented in Table 1. The PCR products were analyzed by the electrophoresis of samples in 1.5 % agarose gels stained with fluorescent dye, green viewer. Resulting density bands were compared with control using Image J software.

Annexin V/propidium iodide assay

Percentage of early and late apoptotic cells was determined by Annexin-V-FITC/PI staining of cells that were treated with cisplatin, EL-EMF and a combination of both. According to the manufacturer's instructions, the cells were treated for 48 h with cisplatin or (and) EMF or both of them. Then the harvested cells were centrifuged at 200 g, and re-suspended in the appropriate buffer. In the following 5 µl Annexin-V-FITC labeling and 5 µl PI solution were added to the cells and they were incubated for 5 minutes at 25 °C. Finally the cells were analyzed with flow cytometer (Bd, Franklin Lakes, NJ, USA).

Result and discussion

Platinum-based drugs are used for the treatment of a wide variety of solid tumors, including testicular,

ovarian, bladder, colorectal and lung tumors (Mazzeo et al. 2003). Cisplatin exerts anticancer effects via multiple mechanisms. The most prominent mode of action involves the generation of DNA lesions followed by the activation of the DNA damage response. Cisplatin binds to the DNA strand and hinders both DNA replication and RNA translation and eventually trigger mitochondrial apoptosis (Kelland 2007). Despite a consistent rate of initial responses, cisplatin treatment often results in therapeutic failure because of development of chemo resistance (Galluzzi et al. 2012). Therefore, vast researches are in progress for introduce new therapeutic agents to restore the sensitivity of resistant cancer cells. Benefits include low-cost and ready availability, ease of localized application, few if any side-effects, and indefinite shelf life (Ross and Harrison 2015) leads to a rapid increased interest in electromagnetic field treatments in recent years.

Cytotoxicity measurement

The cytotoxicity of cisplatin, EL-EMF and a combination of them was measured using MTT assay. MTT is commonly used to monitor the response and health of cells in culture after treatment with various stimuli. The results from the current study showed that cisplatin can reduce proliferation of A2780 cells and the calculated IC₅₀ for treated cells with cisplatin was 90 µg/ml at 48 h. 50 Hz frequencies EL-EMF with 200 g intensity has no significant effects on viability rate. It is important to mention that the chosen concentrations of cisplatin were lower than the IC₅₀ of A2780 cells. Hence 30 and 60 µg/ml doses of cisplatin were selected to investigation synergistic effects. Using these concentrations less than 20 and 40 % cells death occurred, respectively. The result indicated that EL-EMF can increase A2780 sensitivity to cisplatin. Using the 30 µg/ml cisplatin in combination with EL-EMF led to decreased viability

Table 1 Primer sequence

Gene	Forward primer	Reverse primers
Beta actin	5'CCC GCC GCC AGC TCA CCA TGG 3'	5'AAG GTC TCA AAC ATG ATC TGG GTC 3'
MMP-2	5'CTG CAT CCT CAG CAG GTTG 3'	5'GTC TCG GAT AGT CTT TAT CC 3'
P53	5'TTG CCG TCC CAA GCA ATG GA 3'	5'TCTGGGAAGGGACAGAAGATGAC 3'

percentage from 90 % (cisplatin alone) to 60 % (combining cisplatin and EL-EMF) and applying 60 $\mu\text{g/ml}$ cisplatin with EL-EMF with 200 G caused decreased viability percentage from 70 to 40 %. The results of this section was that EL-EMF at an intensity of 200 G for 2 h increased cisplatin sensitivity in the A2780 cisplatin resistance cell line (Fig. 1).

DAPI staining results

DAPI is a blue fluorescent probe that fluoresces brightly upon selective binding to the minor groove of double stranded DNA, where its fluorescence is approximately 20-fold greater than in the non-bound state. The result from this staining reveals that cisplatin in combination with EL-EMF can induce nuclear fragmentation in A2780 cells and has the ability to enhance the cell killing effect, that is the hallmark of apoptosis. Also this effect was more effective than when using one of them alone. Such percentages of lost DNA integrity were greater in the combination group. It is generally accepted that DNA damage and subsequent induction of apoptosis may be the primary cytotoxic mechanism of cisplatin and other DNA-binding antitumor drugs (Siddik 2003). Moreover, resistance to cisplatin can result from the defects in the DNA damage-induced apoptotic signal transduction pathways. As it was reported previously extremely low frequency electromagnetic fields (50 Hz EMF at a flux density of 1 mT) can affect DNA integrity in human cells (Mihai et al. 2014). EL-EMF can promote elevated DNA fragmentations in combination with cisplatin (Fig. 2). It might be thought that these two factors reinforce each other's

effect to destroy DNA, thus act stronger when combined together. This observation reveals good agreement with MTT assay studies as mentioned above.

AO/PI staining assay

AO and PI are nuclear staining (nucleic acid binding) dyes. AO is permeable to both live and dead cells and stains all nucleated cells to generate green fluorescence. PI enters dead cells with compromised membranes and stains all dead nucleated cells to generate red fluorescence. Cells stained with both AO and PI fluoresce red due to quenching, so all live nucleated cells fluoresce green and all dead nucleated cells fluoresce red. Percentages of green cells (live cells) decreased in cells that were exposed to EL-EMF and cisplatin together (Figs. 3 and 4). The cells stained with AO and PI showed typical apoptotic changes whose nuclei were highly condensed or fragmented, whereas cell nuclei from EMF or cisplatin groups were stained green and showed a fine reticular pattern.

Caspase 3 and 9 activity assay results

A group of intracellular proteases called caspases are responsible for the deliberate disassembly of the cell into apoptotic bodies during apoptosis. Caspase 9 activates disassembly in response to agents or insults that trigger the release of cytochrome *c* from mitochondria. Caspase 9 was activated when completed with apoptotic protease activating factor 1 (APAF-1) and extra-mitochondrial cytochrome *c* (Vanden

Fig. 1 MTT assay results. Percentage survival of cells after exposure to cisplatin for 48 h (a), the combination of cisplatin and EL-EMF for 48 h, Data are presented as mean ($\pm$ SD), $P < 0.05$

Fig. 2 Nuclear DAPI staining results. DNA and nuclear fragmentation shown by DAPI staining in A2780 cells. Control of chromatin in normal mode (a), Cells were treated with

electromagnetic field (b), Cells were treated with cisplatin (c), Cells were treated with cisplatin and electromagnetic field combined (d)

Berghe et al. 2013). The activated caspase-9 then cleaves and activates the effector caspases like caspase-3 and caspase-7, which play roles in mediating PARP cleavage and DNA digestion: caspase-dependent apoptosis is the best-known modality of programmed cell death (Lakhani et al. 2006). Caspase activation was assessed in cells that were treated with cisplatin, EL-EMF and cisplatin + EL-EMF for 24 h. Caspase-3 and caspase-9 activation in cells treated with EL-EMF + 30 µg/ml cisplatin was higher than when applied one of them alone. It was known that the caspase cascade is activated in response to cisplatin insult; this activation leads to an irreversible commitment to apoptotic cell death. Results from this study were that cisplatin cannot induce caspase activation significantly in A2780 cells due to their resistance to cisplatin. But when cisplatin was applied together with EL-EMF caspase 3/9 activation was increased significantly in comparison with the single treatment of cisplatin and EL-EMF alone. It should be noted that the EL-EMF alone treatment did not show a significant effect on caspase activation (Fig. 5). Other researches also confirmed that EL-EMF cannot promote caspase

activation and cannot trigger the apoptosis pathway (Akdag et al. 2010) (Jiménez-García et al. 2010). Pirozzoli et al. (2003) finding confirmed our results. They have not found alterations in the cellular ability to undergo programmed cell death, after exposure of the neuroblastoma cell line to 50 Hz electromagnetic field (Pirozzoli et al. 2003).

Annexin V/propidium iodide assay results

One of the early events that take place in the programming cell death is the loss of plasma membrane integrity. The membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane; thereby PS is exposed to the external cellular environment (Vanden Berghe et al. 2013). Annexin V when labeled with a fluorescent tag, such as FITC, can be used with flow cytometry to measure this event. Staining with Annexin V-FITC is typically used in conjunction with a live/dead dye such as propidium iodide to detect necrotic cells. Cells were treated with cisplatin, EMF or both of them, stained with Annexin-V/PI and

Fig. 3 Nuclear acridine orange/propidium iodide (AO/PI) staining results. Untreated cells fluoresced in *green* (a). On the contrary, cells treated with cisplatin at 30 $\mu\text{g/ml}$ fluoresced in *green* and *yellow* (b), cells treated with EL-EMF fluoresced in *green* and a few in *yellow* (c), cells treated with cisplatin and EL-EMF together fluoresced in *green*, *yellow*, and *red* in the cytoplasm (d), in this group dead (*red*, *yellow*) cells significantly in comparison to cisplatin and EL-EMF alone

Fig. 4 Histogram from percentage of live and dead cells from AO/PI staining for A2780 cells, data are presented as means ($\pm$ SD)

analyzed using flow cytometer. The result charts were divided into four parts. In the necrotic area cells are characterized negative for Annexin-V and positive for PI, late apoptotic cells characterized positive for Annexin-V and positive for PI, intact cells

characterized negative for Annexin-V and negative for PI and early apoptotic cells characterized positive for Annexin-V and negative for PI. As it was shown in Fig. 7, the percentage of apoptotic cells increased in

Fig. 5 Histogram from caspase activity assay compared to control. Results are expressed as mean of three different experiments; data are presented as means ($\pm$ SD), * $P < 0.05$, ** $P < 0.01$

the combined group compared with the use of cisplatin or EMF alone (Fig. 6).

Possible mechanism

Previous studies have reported some electrical properties in cells such as membrane surface charge. They are especially influenced by ELF-EMF (Ross and Harrison 2015). The flow of electrical current increases permeability of cells for charged drug molecules. The electrical field imposes a force on the charged drug. This electrical force drives the charged drug molecules through the membrane far more efficiently than in the case of pure diffusion or

passive transdermal drug delivery (Zhou and Uesaka 2006). It can be assumed that the electric component of the electromagnetic field increases intracellular accumulation of cisplatin and in this way efficiency and cisplatin sensitivity was increased. An increased free radical production is demonstrated in macrophages after EL-EMF exposure. In addition, it is believed that EL-EMFs may prolong the lifetime of super oxides and increase their concentration in living cells. Also EL-EMFs induce an “activated state” of the cells like in the phagocytic activity, which enhances the release of free radicals, which lead to a genotoxic event following chronic exposure (Consales et al. 2012). Since free radicals are highly reactive,

Fig. 6 Annexin V/propidium iodide assay results: determination of the percentage of apoptotic cells in a cell population treated with cisplatin, EMF and cisplatin + EMF in comparison to control cells

they may interact with DNA. P53 activation activates caspase 9, which then activates caspase 3 to execute the death program. Free radicals are known to directly activate multiple proteins involved in signaling pathways that regulate cell function. Free radicals responsive MAP kinases are known to control a wide range of cellular processes including: cellular differentiation, cell cycle control, cytokine levels and apoptosis (Steinbeck et al. 2013). These effects may be enhanced when EL-EMFs and cisplatin are applied in combination and in this way also intensify the effects of cisplatin to cell death induction. It was reported that EL-EMF was associated with a reduction of cell proliferation and this suggests that EL-EMF protects hepatocytes from the increased proliferation induced by treatment with carcinogene (Jiménez-García et al. 2010). This effect of EL-EMF in combination with cisplatin may affect viability rate. In addition, these waves can increase sensitivity to cisplatin by removing cells from the proliferative phase.

Semi-quantitative RT-PCR Results

P53 is considered as a “guardian of the genome” and is a certain DNA repair before DNA replication. DNA damage through cisplatin may cause to P53 gene expression which subsequently leads to both cell cycle arrest in G1 phase and induction of apoptosis. Also some genes that regulate sensitivity to apoptosis are regulated by the P53 molecule. In addition, P53 protein affects enhanced transcription of pro-apoptotic Bax directly while it decreased transcription of anti-apoptotic Bcl-2 proteins (Ventura et al. 2007). However P53 gene mutation is the most common cause of abnormalities identified in different cancers. Inactivation of P53 is one of the most predominant mechanisms of resistance (Ventura et al. 2007). In vitro and in vivo studies showed that increased P53 activity leads to enhanced apoptosis in some cancers cells (Speidel 2010). It has been reported that high levels of P53 expression and DNA-damaging agents like cisplatin and radiation work synergistically to induce apoptosis in cancer cells (Chen et al. 2005). As it can be seen in Fig. 6, cisplatin had no significant effect in the expression of P53 genes in cisplatin-resistant cells although it was to be expected. But when cisplatin was combined with EL-EMF exposure, P53 expression

was increased significantly. It can be suggested that EL-EMF and cisplatin together increased P53 activity, resulting in increased sensitivity of A2780 cells to cisplatin.

MMPs are secreted by cervical and ovarian cancers, especially MMP-2 and MMP-9, play crucial roles in tumor invasion and metastasis (Roomi et al. 2010). It has been shown that many human cancers are associated with increased expression of MMP-2 and MMP-9 (Misra et al. 2014). The results from this study indicated that MMP-2 gene expression was decreased in the combination group of cisplatin and EL-EMF in comparison with control group. In cisplatin groups MMP-2 expression had no significant increase, because this cell was cisplatin resistant. In the EL-EMF exposure group, MMP-2 expression increased. Other studies showed that an increasing MMP-2 gene expression took place when SK-OV-3 ovarian cancer cells were exposed to EL-EMF (Patruno et al. 2012). But these two factors act together to reduce the expression of these genes. The results of this study indicated that the increase of the expression of P53 gene and the down-regulation of the MMP-2 gene can be an important option in the cancers therapy strategy (Fig. 7).

Conclusion

In the current study it was assumed that changes generated by EL-EMF can render resistant cells sensitive to cisplatin. This study was designed to assess the efficacy of EL-EMF (200 G, 2 h) in combination with cisplatin to increase sensitizes in resistance cancer cells. For this purpose the toxicity of cisplatin and EL-EMF was measured alone and inhibitory concentration (IC_{50}) was calculated. Then concentrations less than IC_{50} were used for a combination applied with EL-EMF. A statistically significant increase of apoptotic rate was found when cells were exposed to 50 Hz EL-EMF in the presence of cisplatin. These results showed that EL-EMF has the ability to make A2780 resistant cells sensitive to cisplatin. In the current investigation, EL-EMF potentiated cisplatin-induced apoptosis. In a P53 dependent manner cisplatin in combination with EL-EMF has been suggested as a potential way to improve cancer

Fig. 7 Scanning densitometry (**a**, **b**) of semi quantitative RT-PCR products for P53 gene (**a**) and MMP-2 (**b**) ($P > 0.05$). The control group was assumed to represent one number greater than represent more expression compared control group (P53)

numbers less than one represents lower expression compared to control (MMP-2). Data are presented as means ($\pm$ SD), * $P < 0.05$, ** $P < 0.01$

therapy. Reduced DNA repair increased the damage of nucleic acids and cell cycle arrest is the possible mechanism involved in the cells sensitive to cisplatin.

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