Synergetic effects of silver and gold nanoparticles in the presence of radiofrequency radiation on human kidney cells

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Abstract

Objective: The aim of this study was to compare the effects of radiofrequency radiation (RF) in synergism with gold (Au) and silver (Ag) nanoparticles (NPs) on the survival fraction of human normal kidney (HNK) and human embryonic kidney (HEK) cells. Materials and Methods: HNK and HEK cells were divided into three groups as control, 1 and 2 h/day-irradiated groups for 8 days. To compare the effects of RF in the presence of Au-NPs and Ag-NPs, the cells were incubated with NPs during the irradiation. In other words, six other groups were designed for the cell incubated with Au-NPs and Ag-NPs including control, 1 and 2 h/day-irradiated groups for 8 days. Generalized estimating equation model was applied to consider the natural correlation of repeated measurements over the time. Results: The mean survival fractions of HNK + Ag-NPs and HEK + Au-NPs were 0.098 less, 0.184 and 0.055 more than HEK cells, respectively. Along with the time, the mean fraction in HEK + Ag-NPs and HEK + Au-NPs groups in comparison with the HEK increased by the rate of 0.005 and decreased by the rates of 0.01 and 0.005, respectively. The mean survival fractions in HEK + Ag-NPs and HEK + Au-NPs were significantly less than that of HEK cells (P < 0.05). Conclusions: RF radiation can affect both HNK and HEK cells when irradiated for 2 h/day for 8 days. The results showed that the Ag-NPs do not increase the synergistic effects of RF compared to the Au-NPs. RF radiation at the presence of Au-NPs can be used as an efficient treatment for melanoma.

Key words: Biological effect, gold nanoparticle, hyperthermia, in vitro study, radiofrequency radiation, silver nanoparticle

INTRODUCTION

In the recent decade, with the increased use of devices which produce radiofrequency radiation (RF), controversial reports over the effects of such devices on human health have been presented.[1-4] Although the effects of these radiations on human cells are not completely understood, it has been stated that RF radiation at 300 MHz to several GHz can induce torques on cell’s molecules which results in a displacement of ions from unperturbed positions, vibrations inbound charges of both ions and electrons, and rotation and reorientation of dipolar molecules.[5] Moreover, RF radiation may cause thermal effects on cells by thermal denaturation of critical targets.[6]

These effects might cause substantial damages in normal cells; even so, it can be employed in radiation oncology for cancer treatment.[7] Some studies have demonstrated that tumor cells are more sensitive than healthy cells to RF microwave (MW)
radiation.\[8-10\] Therefore, the MW radiation can be used in partial necrosis of cancer cells, though this may be dangerous for normal surrounding tissues.\[11-13\] In this regard, clinical studies have shown the difficulty of RF energy deposition in malignant tissue, deep within the body, without damaging neighboring healthy tissues.\[14\]

Over the past decade, nanotechnology is widely used to transport chemotherapeutic and biologic agents into the malignant tissue while sparing normal organs.\[15\] In addition, a significant number of experimental and theoretical researches have demonstrated high MW absorption cross section of living cells in the presence of nanoparticles (NPs).\[16,17\]

The efficiency of MW radiation for therapy can be significantly enhanced by using nano-sized absorbing agents, which are targeted into a tumor area to absorb the radiation for the treatment of cancer cells as a selective destruction method.\[18,19\] Such an effect can be achieved by using conductive nano-sized agents such as gold (Au) and silver (Ag) NPs.\[18,19\]

The basic mechanism for MW energy absorption is related to the coupling of the magnetic moment of the magnetic NPs (MNs) and the external MW field.\[20\] The efficiency of the energy absorption is mainly related to the size, size distribution, and magnetic anisotropy of the NPs and the amplitude and frequency of the external radiation field.\[20\]

The aim of the present study was to investigate whether the 900 MHz MW radiation can affect the survival of human normal kidney (HNK) and human embryonic kidney (HEK) cells. The focus of this work was to evaluate the synergistic effects of Au-NPs and Ag-NPs with MW radiation on HNK and HEK cell viability.

**MATERIALS AND METHODS**

**Synthesis of the gold nanoparticles**

Au-NPs were synthesized according to standard wet chemical methods using sodium borohydride.\[21\] “Fifty ml of an aqueous solution containing 4.3 mg of solid sodium borohydride was added to 100 ml of a 100 µmol/L aqueous solution of tetrachloroauric acid. The solution was kept under vigorous stirring overnight.”\[21\] Then, the Au-NPs were filtered through 0.22 µm paper filter. The size of NP was investigated and calculated by the use of an electron microscopy. The average size of Au-NPs was 20–50 nm.

**Synthesis of the silver nanoparticles**

To synthesize the Ag-NPs, 0.001–0.01 M of Ag nitrate solution was diluted in a vessel containing two platinum electrodes. Particle size was depended on the electrical current and voltage which fed into the system and was determined after reducing the transparent Ag nitrate solution following its conversion into a brown colloid system.\[21\] The size of NP was investigated and calculated by the use of electron microscopy. The average size of Ag-NPs was 25–40 nm.

**Cell culture**

Experiments were carried out on HNK, provided from Isfahan University of Medical Science Laboratory (Isfahan, Iran) and HEK cells provided from Iran Cell Bank of Pasteur Institute (Tehran, Iran). The cells were cultured in 25 ml culture flasks in Dulbecco’s modified Eagle’s medium (DMEM Gibco Laboratories, Cergy Pontoise, France) supplemented with 10% fetal bovine serum (FBS Gibco Laboratories, Cergy Pontoise, France), 2 mM glutamine, 100 U penicillin per ml, and 100 µg streptomycin per ml (Gibco Laboratories, Cergy Pontoise, France). Cells were grown in a humidified cell incubator at 37°C under 5% CO₂ atmosphere and 95% air.

**Experimental design**

To perform the experiment, 96-well plates were used. Cells were seeded, and the density of 5000 cells/well was put in each well. They were allowed to adhere and grown overnight in 200 µl mediums.

At first, to determine the optimum NP concentration, the cells were incubated with fresh medium containing serial concentrations (0–80 µM) of NPs for 2 h.\[21\] Then, the survival of the cells was investigated after 24 h and was compared with the control group.

To investigate the effects of RF radiation on the cell proliferation, both the two cell lines of HNK and HEK were irradiated for 1 and 2 h/day for 8 days. For RF radiation, an RF simulator was used. The simulator was adjusted on 1.0 W and 900 MHz frequency for the exposure. The distance between the simulator antenna and the wells was kept at 2.5 cm.

To investigate the effect of RF radiation on the HNK cells, the cells were divided into three different groups. For the first group (Group 1-a), as control one, no radiation and NPs were applied. For the 2nd and 3rd groups (Groups 2-a and 3-a), the cells were exposed to RF simulator for 1 and 2 h/day, respectively, for 8 days.

The HEK cells were divided into nine groups. For the first group (Group 1-b), as control one, no radiation and NPs were applied. For the 2nd and 3rd groups (Groups 2-b and 3-b), the cells were exposed to an RF simulator for 1 and 2 h/day, respectively, for 8 days. For these two groups, NPs were not used. Moreover, to investigate the effects of NPs on the cell proliferation in the presence the RF radiation, six groups were designed. For the 4th group (Group 4-b), no radiation was applied and the cells were incubated with Au-NPs for 2 h/day for 8 days. The 5th and 6th groups (Groups 5-b and 6-b) were exposed to the RF simulator for 1 and 2 h/day, respectively, for 8 days. Both Groups 5-b and 6-b were incubated with Au-NPs during the exposition. For the 7th group, no radiation was applied and the cells were incubated with Ag-NPs for 2 h. The 8th and 9th groups (Groups 8-b and 9-b) were exposed to the RF simulator for 1 and 2 h/day, respectively, for 8 days. Both 8-b and 9-b groups were incubated with Ag-NPs during the...
exposure time. Table 1 shows the studied groups and design of the experiment.

To investigate the radiation effects on the cells’ survival, (3-[4,5-dimethylthiazol-2-yl]-2,5-phenyltetrazolium bromide) (MTT) assay was performed on different days during and postirradiation period. Since the cell lines were adhesive, their media could simply be renewed without making any damage to the cells.

To avoid the variability inherent to the assay used, all tests were performed for three independent experiments.

**Statistical analysis**

A population average method, generalized estimating equation (GEE) model, was applied to consider the natural correlation of repeated measurements over the time. “There are two steps in GEE models including covariance analysis of repeated measurements over the time (across the cases) and definition of a link function between the linear predictors and the mean response. The most important feature of GEE models is the population average interpretation of the results where according to multiple cases in the data, the trend over the time is an average of multiple trends.”[22] Using this method, one can evaluate the effect of different factors and covariates on the response variable. One can replace the covariate or factor by its actual measure and multiply it to the estimated coefficient, which results in a predicted value of the response. In this work for the studied groups, the synergic effects were evaluated using GEE as one unit increase in time. The results are presented as estimate of covariate/factor coefficient in the model, its standard error, and the $P$ value. Statistical analysis was performed using SPSS software version 16.0 (Chicago, IL, USA) and R.3.1.0 software (Vienna, Austria) (an open source programing environment for data analysis). For the analysis, one-way analysis of variance and multiple comparisons were applied. A significant level of 0.05 was considered for the tests.

**RESULTS AND DISCUSSION**

### The size and concentrations of gold nanoparticles and silver nanoparticles

A size histogram curve of the gold-NPs, by counting at least 300 particles, showed that 45.5% of the Au-NPs were in the 20–30 nm range. In addition, it was found that the best concentration for the used Au-NPs was 50 µM. The size histogram curve of the Ag-NPs showed that 50% of the NPs were in the 25–40 nm range. For the used Ag-NPs, the best concentration was found to be 40 µM.

### 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyltetrazolium bromide assay results

Figure 1 shows the results of the MTT assay of HNK and HEK cells in the absence and presence of Au-NPs and Ag-NPs and also the irradiation. As stated earlier, for the control group (A), no radiation was applied, and groups B and C were irradiated to the RF simulator for 1 and 2 h/day, respectively. The comparison of curves in each figure shows the proliferation and variation rates of HNK and HEK cells in each step and also illustrates the effects of Au- and Ag-NPs on HEK cells in the absence of irradiation.

Figure 2 shows the rate of differences of proliferation for each cell line. The effects of control, 1 and 2 times irradiation/day on each cell line can be found in these figures. Figure 2a and b show the mean optical density (OD) of HNK and HEK cells in various days for the control, 1 and 2 irradiation/day groups. In Figure 2c and d, the effects of 1 and 2 irradiation/day on HEK cells in the presence of Au- and Ag-NPs are shown.

The mean ± standard deviation of fraction along with time in different radiation time and groups is shown in Table 2.

The effects of all the affective parameters on the cells such as the irradiation, time, and NPs are analyzed using GEE model. The results of GEE analysis are shown in Table 3. This table includes the estimated coefficient, its standard deviation, and the $P$ value.

The table can be summarized in four different formulas for each of the groups where a significant difference was found between HNK and HEK groups. The HEK + AuNPs and HEK + Ag-NPs were statistically the same as HEK.

The four formulas for HEK, HNK, HEK + AuNPs, and HEK + Ag-NPs are as follows, respectively:

**HEK as reference:**

$OD = 0.022 \times day - 0.022 \times radiation + 0.022 \times day \times radiation$

**HNK:**

$OD = -0.098 + 0.005 \times day + 0.024 \times radiation - 0.009 \times day \times radiation$

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**Table 1: The studied groups and design of the experiment**

| Group | Cell   | Irradiation | Nanoparticle |
|-------|--------|-------------|--------------|
|       | HNK    | HEK         | 1 h/day      | 2 h/day      | Au-NPs | Ag-NPs |
| 1-a   | ✓      | -           | -            | -            | -       | -       |
| 2-a   | ✓      | -           | -            | -            | -       | -       |
| 3-a   | ✓      | -           | -            | -            | -       | -       |
| 1-b   | ✓      | -           | -            | -            | -       | -       |
| 2-b   | ✓      | ✓           | -            | -            | -       | -       |
| 3-b   | ✓      | ✓           | ✓            | -            | -       | -       |
| 4-b   | ✓      | ✓           | ✓            | ✓            | -       | -       |
| 5-b   | ✓      | ✓           | ✓            | ✓            | -       | -       |
| 6-b   | ✓      | ✓           | ✓            | ✓            | -       | -       |
| 7-b   | ✓      | ✓           | ✓            | ✓            | -       | -       |
| 8-b   | ✓      | ✓           | ✓            | ✓            | -       | ✓       |
| 9-b   | ✓      | ✓           | ✓            | ✓            | ✓       | ✓       |

**Legend:**

HNK: Human normal kidney, HEK: Human embryonic kidney, Au-NPs: Gold nanoparticles, Ag-NPs: Silver nanoparticles

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Figure 1: The optical density of cells after 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyltetrazolium bromide assay in different days for control (a), 1 (b) and 2 (c) h irradiation/day for kidney cells (groups 1-a, 2-a, and 3-a), human embryonic kidney cells (groups 1-b, 2-b, and 3-b), and human embryonic kidney cells in the presence of gold nanoparticles (groups 4-b, 5-b, and 6-b) and silver nanoparticles (groups 7-b, 8-b, and 9-b). Error bars indicate the standard deviation of the mean optical density of cells in wells in each group.

Figure 2: Optical density of cells derived from the 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyltetrazolium bromide assay for human normal kidney and human embryonic kidney cells among different exposed group (Subgroups 1-a, 2-a and 3-a [a], 1-b, 2-b and 3-b [b], 4-b, 5-b and 6-b [c] and 7-b, 8-b and 9-b [d]). Error bars indicate the standard deviation of the optical density of cells in wells for each group.
Table 2: The mean±standard deviation of survival fraction along with time in different radiation time and groups

| Cell     | Group          | Radiation (h/day) | Time (day) |        |        |        |
|----------|----------------|------------------|------------|--------|--------|--------|
| HNK      | 1-a            | 0.74±0.225       | 0.01       | 0.18   | 0.133  | 0.81±0.133 |
|          | 2-a            | 0.82±0.168       | 0.15       | 0.21   | 0.055  | 0.82±0.081 |
|          | 3-a            | 0.68±0.183       | 0.20       | 0.63   | 0.082  | 0.83±0.075 |
| HEK      | 1-b            | 0.91±0.110       | 0.19       | 1.28   | 0.119  | 0.55±0.091 |
|          | 2-b            | 0.54±0.103       | 0.21       | 0.51   | 0.042  | 0.52±0.049 |
|          | 3-b            | 0.52±0.015       | 0.25       | 0.50   | 0.048  | 0.48±0.117 |
| HEK + 4  | 4-b            | 0.41±0.084       | 0.23       | 1.16   | 0.205  | 0.99±0.165 |
| Au-NPs   | 5-b            | 0.36±0.031       | 0.24       | 0.43   | 0.182  | 0.61±0.202 |
| 6-b      | 2               | 0.41±0.117       | 0.25       | 0.41   | 0.074  | 0.81±0.084 |
| HEK + 7  | 7-b            | 0.91±0.414       | 0.26       | 0.77   | 0.231  | 0.87±0.292 |
| Ag-NPs   | 8-b            | 0.76±0.066       | 0.28       | 0.69   | 0.186  | 0.81±0.183 |
| 9-b      | 2               | 0.41±0.107       | 0.30       | 0.60   | 0.108  | 0.71±0.131 |

HNK: Human normal kidney, HEK: Human embryonic kidney, Au-NPs: Gold nanoparticles, Ag-NPs: Silver nanoparticles

Table 3: The results of generalized estimating equation analysis

| Parameter                  | B   | SE   | Significant |
|----------------------------|-----|------|-------------|
| Day                        | 0.022 | 0.003 | 0.026       |
| Radiation                  | -0.222 | 0.028 | 0.005       |
| HEK+GNPs                   | 0.055 | 0.053 | 0.001       |
| HEK+SNPs                   | 0.184 | 0.062 | 0.000       |
| HNK                        | -0.098 | 0.098 | 0.309       |
| HEK                        | Reference |      |             |
| Day×radiation              | 0.002 | 0.002 | 0.338       |
| (HEK+GNPs)×day            | -0.027 | 0.005 | 0.004       |
| (HEK+SNPs)×day            | -0.032 | 0.004 | 0.004       |
| (HNK)×day                 | -0.017 | 0.006 | 0.015       |
| (HEK)×day                 | Reference |      |             |
| (HEK+GNPs)×radiation      | 0.055 | 0.048 | 0.253       |
| (HEK+SNPs)×radiation      | 0.033 | 0.041 | 0.418       |
| (HNK)×radiation           | 0.246 | 0.062 | 0.005       |
| (HEK)×radiation           | Reference |      |             |
| (HEK+GNPs)×day×radiation | 0.004 | 0.003 | 0.235       |
| (HEK+SNPs)×day×radiation | 0.006 | 0.003 | 0.08        |
| (HNK)×day×radiation       | -0.011 | 0.004 | 0.015       |
| (HEK)×day×radiation       | Reference |      |             |

The table includes the estimated coefficient, its SE, and the P value. SE: Standard of error, HNK: Human normal kidney, HEK: Human embryonic kidney, SNPs: Silver Nano Particles, GNPs: Gold Nano Particles, SD: Standard deviation.

At the baseline with no radiation, a significant difference was found between the HEK and HEK + Ag-NPs groups (P = 0.015) and along with the time, the groups of HNK, HEK + Ag-NPs, and HEK + Au-NPs were statistically developing in different shapes (P < 0.001). In other words, the mean OD in HNK, HEK + Ag-NPs, and HEK + Au-NPs was 0.098 less, 0.184 and 0.055 more than HEK, respectively, where these differences were significant only by comparing HEK to the HEK + Ag-NPs groups. Along with the time, the mean OD in HNK, HEK + Ag-NPs, and HEK + Au-NPs groups in comparison to the HEK increased by the rate of 0.005 and decreased by the rates of 0.01 and 0.005, respectively.

At the baseline and along with the time with irradiating the groups for 1 h, a significant difference in mean fraction was found between the HNK and HEK groups. The mean fraction in HNK, HEK + Ag-NPs, and HEK + Au-NPs was 0.074, 0.005, and 0.112 less than HEK at the baseline, while longitudinally comparing to the HEK group, the mean fraction decreased by the rates of 0.004, 0.002, and 0.001, respectively.

Considering the irradiated groups for 2 h, a significant difference in the proliferation of the HNK and HEK compared to the baseline as well as along with the days was found. The fraction mean at the baseline in HNK, HEK + Ag-NPs, and HEK + Au-NPs groups was 0.046, 0.19, and 0.275 less compared to the HEK. Along with 1 day passing the time, the mean OD in HNK, HEK + Ag-NPs, and HEK + Au-NPs decrease by 0.017 and increase by 0.002 and 0.003, respectively.

The effects of RF on human health are not completely understood, and there is some controversy about them. One idea is that their biological outcomes are due to their thermal effects. Blank and Goodman assumed that the mechanism of EM signal transduction in the cell membrane may be explained by direct interaction of electric and magnetic fields with mobile charges within enzymes. Cellular response to EM fields is activation of the same stress response system seen in heating, but at very much lower energy than the response to heat shock. In this study, we have investigated the effect of RFR on the proliferation...
and survival fraction of two cells of HNK and HEK. According to statistical analysis for the HNK cells, the mean cell death in three groups of radiation (1-a, 2-a, and 3-a) is the same and does not show significant differences ($P > 0.05$). Although the average cell death in various days is different, these differences are similar. Therefore, it can be concluded that the RFR exposed to 1 and 2 h/day for 8 days did not affect the HNK cells.

For HEK cells, the average cell death in various days and exposed groups (1-b, 2-b, and 3-b) was significantly different. The results of statistical analysis showed that the mean cell death in group 3-b was significantly higher ($P < 0.05$) compared to groups 1-b and 2-b. It can be understood that RFR can damage the HEK cells’ exposition which is performed for 2 h/day for 8 days. Therefore, there are different results for these two cells. Investigating the previous studies shows that some researchers such as Phillips et al. reported DNA single-strand breaks exposed to cellular telephone frequencies; also, they proposed that DNA repair rates may be affected by exposure to RF.$^{[26]}$ Lai and Singh reported DNA strand breaks from RF at low-intensity levels. In addition, they reported a dose-dependent increase in DNA single- and double-strand breaks in brain cells after 2 h of exposure to 2450 MHz RFR.$^{[27]}$ Hence, large parts of the DNA damages can redound to cell death; it can be concluded that the cell death or repair after exposure is due to the ability to repair after damage. As the rate of cell proliferation in HNK cells is lower than the HEK cells, it can be concluded that for the high-rate proliferation cells, RFR can affect the cells and their death rate. Some studies indicated that the cells’ growth rate represented a curve with an exponential reduction in clonogenic survival as a function of time at the temperature range of 43°C–47°C.$^{[26,27]}$ These results are in a good agreement with our findings.

As the tumor cells have a more rapid proliferation cycle than the normal healthy cell, the RF ablation method results in successful partial necrosis of tumor, but turns out to be dangerous in many situations as the improvement of blood circulation under RFR can provoke a further development of tumors, and it is applicable only for a few organ sites (liver, kidney, breast, lung, and bone).$^{[28,29]}$

Hence, if the thermal energy of RF could be localized in malignant tissue deep within the body without damaging the surrounding healthy tissues, it could be used to increase the tumor cells’ death.$^{[14]}$ One proposed method is the use of some metallic or semiconducting NPs which heat in an electromagnetic field and can significantly enhance the cells’ temperature.$^{[30,31]}$

To investigate the effects of MNPs in increasing the effects of RF radiations, two NPs of Au-NPs and Ag-NPs were used. The effects of 1 and 2 h/day RF radiation were investigated in the presence of these NPs.

Absorption of infrared light induces surface plasmon resonance that is converted to heat.$^{[32]}$ Therefore, Au-NPs can be used widely in hyperthermia procedures. The Ag-NPs have the same properties as mentioned for Au-NPs, and it was predicted that these NPs can be useful in increasing the hyperthermia effects of RF. Resonant absorption peak and cross section of nanostructures can vary based on their size and shape.$^{[13–15]}$

The results of using RF radiations when the HEK cells are in proximity of Au-NPs showed that the differences between groups 4-b, 5-b, and 6-b are statistically significant ($P < 0.05$). The cell death in group 6-b was higher than 5-b and both of them were significantly higher than 4-b. Therefore, the use of Au-NPs can increase the effect of RF radiation on HEK cells.

However, the results of the use of Ag-NPs did not show significant effects as the Au-NPs. Although the average cell death in groups 7-b, 8-b, and 9-b was different, this difference was not statistically significant ($P > 0.05$). Therefore, it can be concluded that, in term of this work, the Ag-NPs do not increase the effect of RF radiations as same as the Au-NPs.

**CONCLUSIONS**

RF radiation can affect both HNK and HEK cells when irradiated for 2 h/day for 8 days. The results showed that the Ag-NPs do not increase the synergetic effects of RF compared to the Au-NPs. RF radiation in the presence of Au-NPs can be used as an efficient treatment for melanoma cancer.

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**Conflicts of interest**

There are no conflicts of interest.

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