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Original Article

Evaluating the Radiosensitization Effect of Hydroxyapatite Nanoparticles on Human Breast Adenocarcinoma Cell Line and Fibroblast

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What's Known

- Only a few studies have investigated the radiosensitization effect of Nanohydroxyapatite (nHAP). Hydroxyapatite nanoparticles have indicated anti-proliferation properties on the human breast adenocarcinoma cell line (MCF-7).
- Many cancer cells show resistance to common treatments. Researchers are still seeking new drugs that can reduce cancer cell growth while keeping side effects at minimum.

What's New

- The first report on the radiosensitization effect of hydroxyapatite nanoparticles on the MCF-7 cell line.
- Hydroxyapatite nanoparticles showed anti-proliferation properties and selective radiosensitization effect against MCF-7 cells with a radiosensitization enhancement factor of 1.87 at a dose of 3.5 Gy and 100 µg/mL concentration.

Abstract

Background: Nanohydroxyapatite (nHAP) exhibit anti-proliferative effects on various cancer cells. However, to date, there are only a few studies on the radiosensitization effect of nHAP. The present study aimed to investigate the possible enhancement of the radiosensitization effect of nHAP on human breast adenocarcinoma cancer (MCF-7) and fibroblast.

Methods: nHAP was extracted from fish scales using the thermal alkaline method and characterized at Babol University of Medical Sciences (Babol, Iran) in 2017. The anti-proliferative and the radiosensitization effects of nHAP were investigated by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT), clonogenic assay, and apoptosis assay. MCF-7 cells and fibroblasts were incubated with different concentrations of nHAP and at different periods. The MTT solution was added and the absorbance was measured at 570 nm. The MCF-7 cells were exposed to 0, 1.5, 3.5, and 5 Gy X-ray irradiation and incubated for 10-14 days. The data were compared using the one-way analysis of variance (ANOVA) followed by the *post hoc* tests (Tukey's method).

Results: The results showed that nHAP significantly inhibited the growth of MCF-7 cells compared with controls ($P<0.001$), but the difference was not statistically significant for fibroblasts ($P=0.686$ at 400 µg/mL at 72 hours). After 48 hours, the proliferation of MCF-7 cells and fibroblasts was inhibited by about 81% and 34% at 400 µg/mL concentration, respectively. The radiosensitization enhancement factor for MCF-7 cells and fibroblasts at a dose of 3.5 Gy and 100 µg/mL concentration were 1.87 and 1.3, respectively.

Conclusion: nHAP can be considered as a breast cancer radiosensitization agent with limited damage to the surrounding healthy tissue.

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Keywords ● Radiation-sensitizing agents ● Radiotherapy ● Nanoparticles ● Hydroxyapatites ● MCF-7 cells

Introduction

Breast cancer is the most common type of cancer in women. Currently, common treatments for breast cancer are associated with adverse side effects. In addition, many cancer cells can exhibit drug resistance.[1] Researchers are still seeking new drugs that can reduce cancer cell growth while keeping side effects at a minimum. Radiosensitizers are developed to improve

cancer treatment by making tumor cells more sensitive to radiation therapy.[2] However, one of the main limiting factors for the implementation of radiosensitizers in clinical care has been their selectivity and specificity to cancer cells. Therefore, there is a serious need to use improved radiosensitizers in combination with ionizing radiation to selectively kill cancer cells while minimally affecting healthy cells. Nanotechnology has the potential to overcome this challenge.[3] One such nanoparticles are hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which has great compatibility with the body.[4] Research studies have shown that hydroxyapatite nanoparticles (nHAP) can prevent the proliferation of cancer cells and have the potential to be used as a treatment for cancer.[5, 6]

Several studies have evaluated the anti-proliferative activity of nHAP on different types of human cancer cells. Tang and colleagues investigated the cytotoxic effect of nHAP on gastric cancer cells (MGC80-3), cervical adenocarcinoma epithelial cells (HeLa), HepG2, and normal human hepatocyte cells (L-02).[7] They reported that nHAP could prevent the growth of cancer cells with an arrangement of MGC80>HepG2>Hela, but did not affect on L-02. They also investigated the effect of nHAP on osteosarcoma cells and osteoblasts, and demonstrated the selective effect of nHAP on osteosarcoma cells.[8] They also reported that the proliferation of osteoblast was enhanced, but induced apoptosis in osteosarcoma cells. Another study reported that nHAP not only had an anti-neoplastic function on glioma cells in a culture medium, but also reduced the toxicity of chemotherapy drugs through the inhibition of DNA repair.[9] Meena and colleagues reported that nHAP could prevent the growth of MCF-7 cells and induce cell apoptosis. They showed that nHAP induced the production of intracellular reactive oxygen species and activated p53, which may cause DNA damage and cell apoptosis.[10] Despite the cytotoxic effect of nHAP, only a few studies have addressed the radiosensitization effect of nHAP. One recent study has demonstrated the enhanced radiosensitization effect of nHAP on glioblastoma U251, breast tumor, and brain metastatic tumor MDA-MB-231BR cells through the inhibition of DNA repair.[9] However, tumors arising in different organs show varied responses to radiation and radiosensitization, i.e., treatment may suit a certain type of cancer but no other types. The objective of the present study was to investigate the effect of nHAP on the radiosensitization of MCF-7 cells and human fibroblasts.

Materials and Methods

Chemicals and Reagents

Fish scales (*Rutilus Frisii Kutum* from the Caspian Sea) were purchased from a local fish market. All reagents were of analytical grade and purchased from Merck, Germany. Additional procurements were: MTT (Merck, Germany), Dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany), penicillin (Sigma-Aldrich, Germany), and streptomycin (Sigma-Aldrich, St. Louis, MO, USA).

Cell Lines

MCF-7 cells and human fibroblasts were obtained from the Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran. The cell lines were cultured in a Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich, Germany), Dulbecco's Modified Eagle *Medium* (DMEM, Sigma-Aldrich, Germany), and Grand Island Biological Company (GIBCO). The cell culture (Life Biosciences, USA) was added to 10% fetal bovine serum (Gibco, Life Technologies, Carlsbad, CA), 100 U/mL of streptomycin (Gibco, Life Technologies, Carlsbad, CA), and 100 U/mL penicillin. They were incubated in a CO_2 humidified incubator at 37 °C. 3-4 passages were applied before subsiding in the assay.

Isolation and Characterization of nHAP

The alkaline heat treatment method, with a slight modification, was used to isolate nHAP.[11] The fish scales were treated with 5% (w/v) NaOH and stirred for 5 hours at 70 °C. The product was dried at 60 °C for 72 hours, then, 5% (w/v) NaOH was added, and heated up to 100 °C for 1 hour. The isolated nHAP was washed until the pH reached 7.0±0.1 and then dried at 70 °C. To characterize nHAP, Fourier-transform infrared (FT-IR) spectroscopy was performed using a Bruker Tensor 27 FT-IR-spectrophotometer (Bruker, Germany) in the frequency range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. The spectra were recorded in transmittance mode. Scanning electron microscopy (MIRA3T ESCAN- XMU, Czechia), was used to characterize the size and the morphology of nHAP using a VEGA/TESCAN KYKY-EM3200 microscope coupled with an energy dispersive X-ray spectrometer (EDX) for elemental analysis (XFlash 6130, TESCAN, Germany).

MTT Assay

MTT colorimetric assay was performed to determine cell viability. MCF-7 cells (6×10^3 cells/well) and fibroblasts (12×10^3 cells/well)

were seeded in a 96-well flat-bottom microplate. After 24 hours, the cultured media were exchanged with fresh media in different nHAP concentrations (12.5, 25, 50, 100, 200, and 400 µg/mL). They were incubated for 24, 48, and 72 hours at 37 °C, after which 50 µL of MTT solution (5 mg/mL in *phosphate-buffered* saline (Sigma, USA)) was added to each well. After 4 hours at 37 °C, the formazan precipitate was solubilized with isopropanol (150 µL) and the absorbance was measured by an ELISA plate reader(Bio Tek, USA) at 570 nm.[12] The proliferation inhibition ratio was calculated using the following formula:

$$\text{Inhibition of cell growth } (\%) = \frac{(A - B)}{A} \times 100$$

A: Control absorbance (without treatment),
B: Absorbance of the treated sample at 570 nm

Irradiation Procedure

The fibroblasts and MCF-7 cells were seeded in a 6-well plate, 96-well-plate, or T-25 flask and treated with 100 µg/mL nHAP. Radiation doses (0, 1.5, 3.5, and 5 Gy of 6-MV X-ray) were applied as single fractions. The treated and non-treated cells were irradiated simultaneously. The cells were exposed to a dose rate of 1.04 mU/min using a linear X-ray accelerator (Primus, Siemens, 6-MV) with a vertical beam at 58 cm source to surface distance (SSD) and a field size of 30×30 cm^2 located at central axis with a dose rate of approximately 200 cGy min^{-1} at 180 degrees gantry angle. Subsequently, the cell viability was measured using clonogenic and MTT assays. Cell apoptotic assay was also carried out.

MTT Assay after Irradiation

After irradiation, the cells were incubated at 37 °C for 24 hours. Next, the culture media was changed and the cells were incubated at 37 °C for another 24 hours after which the MTT assay was carried out. The radiosensitization enhancement factor was calculated as the inhibitory percentage of the irradiated cells treated with nHAP divided by the inhibitory percentage of irradiated cells only (without nHAP).

Clonogenic Assay

The MCF-7 cells (100, 400, 600, and 1000 cells/well) were cultured in a 6-well plate and assayed with four X-ray exposures (0, 1.5, 3.5, and 5 Gy), respectively. The cells were then incubated at 37 °C for 10 to 14 days, after which they were fixed with acetic acid-methanol (1:4) and stained with diluted crystal violet (1:30). The colonies with more than 50 cells were counted and the surviving fraction was estimated using the following formula.[13]

$$(\%) = \frac{\text{Number of colonies counted}}{\text{Number of cells plated} \times \text{PE}} \times 100$$

PE: Plate efficiency

$$\text{PE } (\%) = \frac{\text{Number of colonies counted}}{\text{Number of cells plated without any treatment}} \times 100$$

Apoptosis Assay

The MCF-7 cells and fibroblasts were treated with 100 µg/mL concentration for 2 hours exposed to 3.5 Gy X-ray and incubated at 37 °C for 48 hours. The cells were then centrifuged (3000 ×g) and the pellets were stained with fluorescent dye Acridine orange/ethidium bromide (AO/EB); 100 µL/mL AO and 100 µL/mL EB in PBS. The cell morphology was observed under a fluorescence microscope (Nikon, Japan) and the images were recorded.

Statistical Analysis

The data were analyzed using SPSS software, version 19.0 (IBM, USA), and the results were presented as mean±SD. Normal distribution of the variables was examined using the Kolmogorov-Smirnov test. The difference in continuous variables between the groups was compared using the one-way analysis of variance (ANOVA) followed by the *post hoc* tests. Statistical significance was based on probability values of 0.05 or less. The experiment was repeated three times.

Results

Isolation and Characterization of nHAP

The nHAP was isolated from the scales of *Rutilus Frisii Kutum* (Caspian white fish).[14] FT-IR spectroscopy was performed to characterize the absorption peaks of nHAP. The phosphate bands observed in 565, 603, 605, and 1026 cm^{-1} were attributed to the bending and the asymmetric stretching modes (figure 1a). The scanning electron micrographs (SEM) showed that the nHAP were spherical in shape with a diameter of approximately 11.6 nm (figure 1b). The elemental analysis confirmed that nHAP comprised of calcium (Ca), phosphorus (P), and oxygen (O) with a Ca/P molar ratio of 1.51 (figure 1c).

Inhibitory Effect of nHAP

The treatment of MCF-7 cells with nHAP showed a significant reduction in proliferation after treatment with 12.5 µg/mL concentration of nHAP. It remained constant up to 50 µg/mL and then followed a decreasing trend depending on the level of concentration (figure 2a). The results

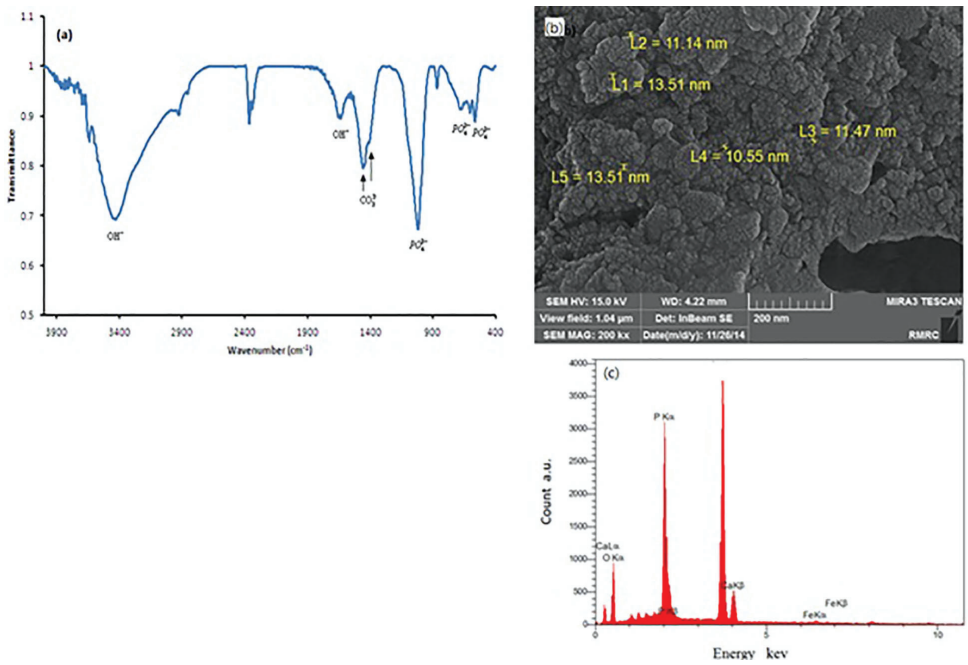

Figure 1: Characterization of nHAP isolated from Caspian Sea fish scales is illustrated. (a) The Fourier-transform infrared spectra were taken in the range of 400-4000 cm^{-1}. (b) The Scanning Electron Microscopy - Energy Dispersive X-rays Spectroscopy (SEM-EDX) images show a spherical shape with a diameter of approximately 11.6 nm. (c) The elemental analysis confirmed nHAP with a Ca/P molar ratio of 1.51.

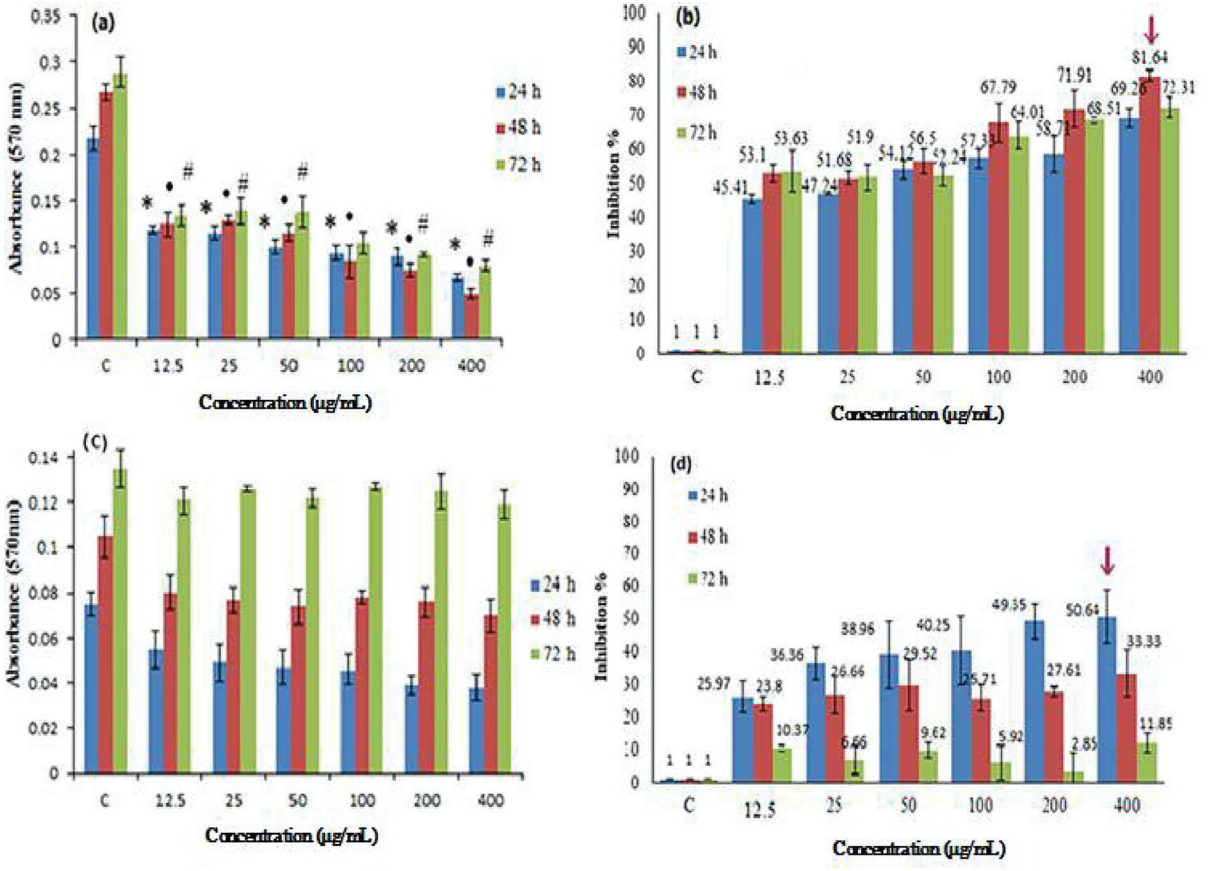

Figure 2: The cytotoxic effect of nHAP at different concentrations is shown. The cytotoxic effect of nHAP on (a) MCF-7 cells and (c) fibroblasts is illustrated. Mean±SEM values were obtained from three independent experiments. The symbols *, ·, and # indicate significance at different concentrations compared with the controls. (c) The ANOVA test followed by a *post hoc* test was performed at 24 (*), 48 (·), 72 (#) hours (P<0.001). MTT assay was used to determine the percentage of nHAP inhibition effect on (b) MCF-7 cells and (d) fibroblasts. The arrow indicates the highest inhibitory effect.

showed a statistically significant difference (P<0.001) between the MCF-7 cells treated with nHAP and those untreated (controls) at all concentrations (table 1). The maximum inhibition of 81.64% was detected in 400 µg/mL concentration after 48 hours (figure 2b).

The treatment of fibroblasts with nHAP indicated a slight decrease in cell growth (figure 2c), but no significant reduction was observed compared to the controls (P=0.111 in 400 µg/mL after 24 hours) (table 2). The highest inhibitory effect (50.64%) was observed in 400 µg/mL concentration after 24 hours (figure 2d).

Radiosensitization Effect of nHAP

The effect of combined nHAP and radiation on MCF-7 cells and fibroblasts was examined. The results showed that the effect of radiation on MCF-7 cells reduced in a dose-dependent manner (figure 3a). Moreover, the anti-proliferation effect

Table 1: The P values for the effect of different concentrations of nHAP on MCF-7 cells after 24, 48, and 72 hours

Concentration (µg/mL)	12.5	25	50	100	200	400
			Time: 24 hours			
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12.5		1.000	0.786	0.267	0.142	<0.001
25			0.948	0.501	0.307	<0.001
50				1.000	1.000	0.050
100					1.000	0.307
200						0.501
			Time: 48 hours			
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12.5		1.000	1.000	0.003	<0.001	<0.001
25			0.992	0.001	<0.001	<0.001
50				0.062	0.003	<0.001
100					1.000	0.02
200						0.267
			Time: 72 hours			
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12.5		1.000	1.000	0.010	0.001	<0.001
25			1.000	0.023	<0.001	<0.001
50				0.034	<0.001	<0.001
100					0.986	0.424
200						1.000

Table 2: The P values for the effect of the different concentration of nHAP on fibroblasts after 24, 48, and 72 hours

Concentration (µg/mL)	12.5	25	50	100	200	400
			Time: 24 hours			
0	0.664	0.411	0.346	0.311	0.133	0.111
12.5		0.999	0.996	0.993	0.878	0.833
25			1.000	1.000	0.985	0.971
50				1.000	0.994	0.987
100					0.997	0.993
200						1.000
			Time: 48 hours			
0	0.425	0.306	0.213	0.331	0.272	0.115
12.5		0.272	0.999	1.000	1.000	0.970
25			0.995	1.000	1.000	0.971
50				1.000	1.000	1.000
100					1.000	0.991
200						0.997
			Time: 72 hours			
0	1.000	1.000	1.000	1.000	1.000	0.686
12.5		1.000	1.000	1.000	1.000	0.864
25			1.000	1.000	1.000	0.805
50				1.000	1.000	0.850
100					1.000	0.788
200						0.821

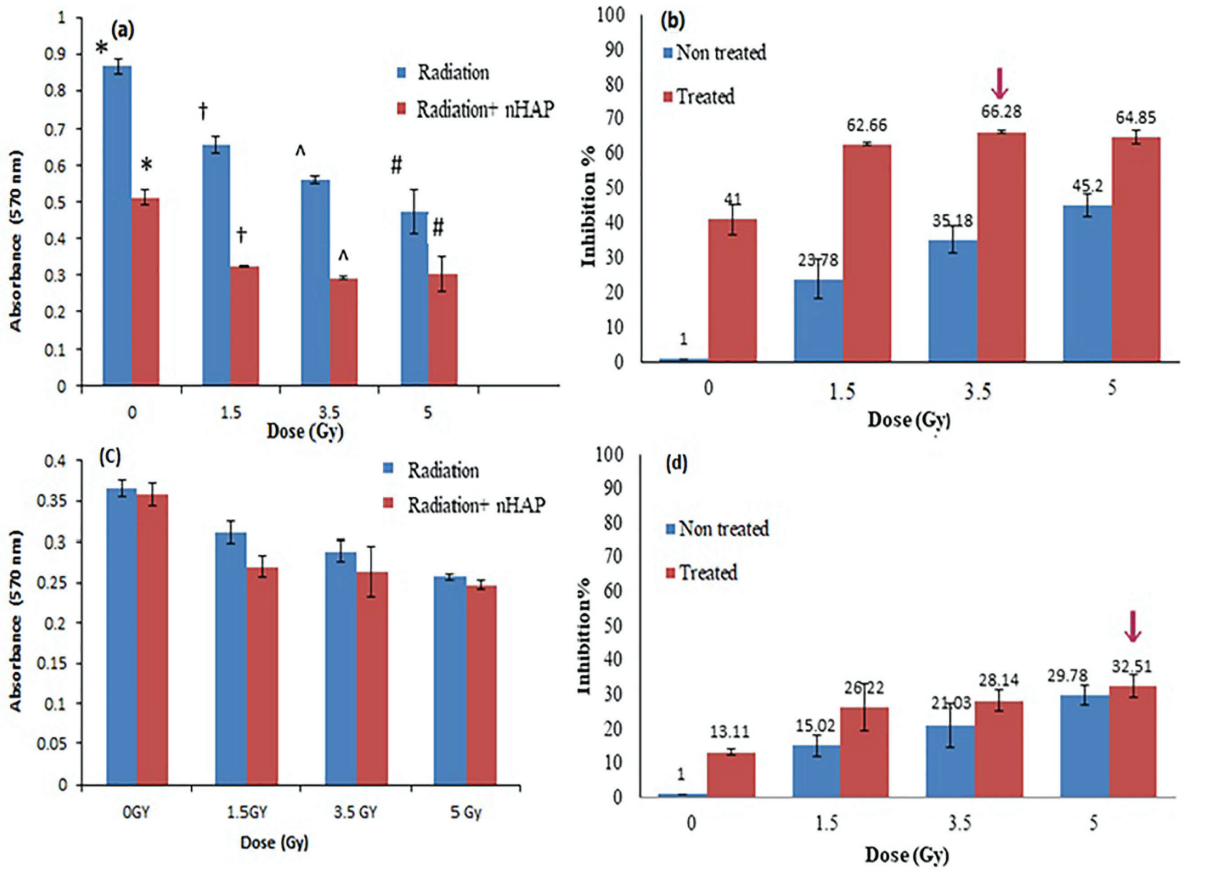

Figure 3: The effect of Nanohydroxyapatite on the proliferation of (a) MCF-7 cells and (c) fibroblasts at different radiation doses isare shown. The symbols *, †, ^, and # indicate significance at different doses of radiation compared with the controls (P<0.05). (c) The proliferation of fibroblasts was not significant at all doses. Cells were precultured for 2 hours in 100 µg/mL nHAP and exposed to 1.5, 3.5, and 5 Gy X-ray radiation, after which the culture media was changed. Cell proliferation was determined after 48 hours by MTT assay. The inhibition percentage of 100 µg/mL of nHAP and X-ray radiation on MCF-7 cells (b) and fibroblasts (d) was obtained from the MTT assay. The arrow indicates the highest inhibitory effect.

of radiation was clearly enhanced by the addition of nHAP. As a result, inhibition after exposure to 3.5 Gy changed from 35.33% in non-treated cells to 66.28% in treated ones (figure 3b). The radiosensitization enhancement factor value at 3.5 Gy was 1.87±0.02. The results showed that when the cells were exposed to radiation only, the decrease in cell survival at 1.5 and 3.5 Gy doses was not significant compared to the controls (P=0.723 and 0.123, respectively), but was significant at 5 Gy (P=0.003). However, when radiation was combined with nHAP, the difference was significant (P<0.001) at all doses compared with the controls. Furthermore, there were significant differences (P<0.001) at all radiation doses (e.g., 1.5 Gy and 1.5 Gy+nHAP, P<0.001).

The radiation on fibroblasts resulted in reduced cell proliferation (figure 3c). The results showed a significant effect at 1.5, 3.5, and 5 Gy compared with the controls (P=0.048, 0.002, and <0.001, respectively). Similarly, it was significant at 1.5, 3.5, and 5 Gy in combination with nHAP compared with the controls (P=0.007, 0.003, and <0.001, respectively). The results also showed that there was no significant difference between the cells treated with radiation and those with radiation in combination with nHAP (1.5 Gy vs. 1.5 Gy+nHAP: P=0.184, 3.5 Gy vs. 3.5 Gy+nHAP: P=0.999, and 5 Gy vs. 5 Gy+nHAP: P=1.000). The maximum inhibition at a dose of 5 Gy was 29.78% and increased to 32.51% when combined with nHAP. In each dose, the percentage of fibroblast inhibition did not reach 50% (figure 3d).

The radiosensitization effect of nHAP on MCF-7 cells was assessed using the clonogenic assay. The survival curve (figure 4) illustrates cell survival at different radiation doses. As shown, nHAP treatment with 100 µg/mL concentration shifted the survival curve downward compared with that of the irradiated cells alone, indicating the anti-proliferation effect of nHAP.

Cell apoptosis assay was performed through morphological analysis using fluorescence microscopy. In this method, live cells are green, survival apoptotic cells are yellow, and dead cells are red. When cells were treated with

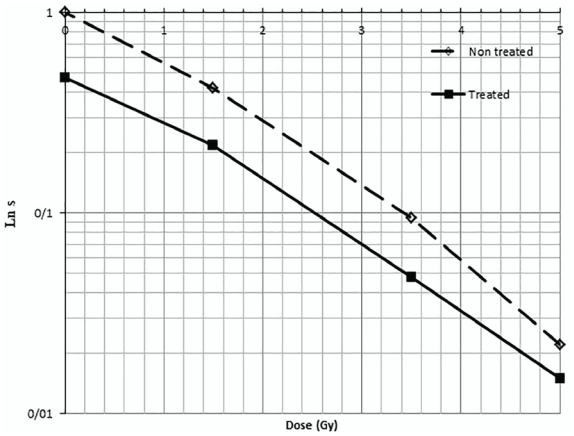

Figure 4: Cell survival curves for MCF-7 cells are illustrated. Cells were precultured for 2 hours in 100 µg/mL nHAP and exposed to 1.5, 3.5, and 5 Gy X-ray radiation for 2 hours, after which the culture media was changed. Clonogenic assay was performed after 10 days of cell incubation.

radiation or radiation combined with nHAP, the morphological features of apoptotic cells were identified through AO/EB staining. The nHAP induced the apoptosis of MCF-7 cells.

Discussion

The present study is the first report on the radiosensitization effect of nHAP on the MCF-7 cell line. The results showed that nHAP selectively affected MCF-7 cells, but the effect was much less on fibroblasts. After the addition of nHAP, there was an initial large drop in MCF-7 cell growth. The cytotoxic effect of the nanoparticles showed a concentration-dependent trend above 50 µg/mL. Furthermore, the effect due to combined radiation and nHAP showed a significant increase of MCF-7 cell sensitivity to radiation while this effect was lower on fibroblasts. The radiosensitization enhancement factor for MCF-7 cells was 1.87 at a dose of 3.5 Gy and a concentration of 100 µg/mL. We isolated nHAP from fish scales with an approximate size of 11.6 nm. A previous study investigated the effect of different sizes of nHAP and reported that the smallest particle size (20 nm) was more effective on the proliferation and the apoptosis of osteoblast-like MG-63 cells.[15] Note that small particles can penetrate cancer cells more quickly and uniformly than larger particles.[16]

A previous study on the cytotoxic effects of nHAP against MCF-7 cells showed that nHAP caused inhibition of cell growth in a dose-dependent manner with IC_{50}=31.43 µg/mL after 48 hours.[10] Our results showed that nHAP could significantly inhibit the proliferation of MCF-7 cells. The inhibitory effect was about 50% at 12.5 µg/mL and remained constant up to 50 µg/mL, after which the effect was concentration-dependent. A similar effect was observed by Zhi-Liu and colleagues in their study on the cytotoxic effect of nHAP on liver cancer cells (BEL-7402) at various concentrations (0, 12.5, 25, 50, 75, 100, 150 and 200 µg/mL). They showed that the inhibitory effect at 25 µg/mL concentration was more than 50% compared with the control. The cytotoxic effect remained constant up to 100 µg/mL and then followed a concentration-dependent pattern.[17] These observations suggested that nHAP may affect MCF-7 cells through at least two mechanisms. The main mechanism of growth inhibition by nHAP was related to increased calcium concentration and nuclear localization in cancer cells.[16] The second mechanism was associated with the fluctuations in intracellular Ca^{2+} homeostasis, often in the form of cytoplasmic increase, which can activate cytotoxic mechanisms associated with the reversible or irreversible injury. It has been shown that nHAP might interact with cytoplasmic organelles (e.g., intracellular vesicles, mitochondria, and lysosomes) and thus interfere with cell function and survival.[18] Based on our results, it seems that the main mechanism of growth-inhibitory at low concentrations of nHAP is likely due to elevated calcium concentrations in cancer cells. Whereas in high concentrations of nHAP, the main mechanism may be the accumulation of nHAP in the nucleus of cancer cells. It has been reported that intracellular Ca^{2+} accumulation may stimulate lethal processes through mechanisms such as programmed cell death, mitochondrial dysfunction, enzymatic processes, and DNA damage.[19]

The results of the present study showed the inhibition of MCF-7 cell proliferation was significantly higher than that of fibroblasts at all nHAP concentrations; inhibition of MCF-7 cells and fibroblast growth at 100 µg/mL were 67.79% and 25.71%, respectively. In line with our study, a previous study investigated the inhibitory effects of nHAP on the cell proliferation of three normal cells (hepatocytes, lung fibroblast, keratinocyte) and three cancer cells (MGC-803, Os-732, Bel-7402) using the MTT assay.[5] They also reported that the inhibitory effects on human cancer cells were much greater than on normal cells. The differences in the degree of inhibition depended on the cell type. In their study, the inhibitory effects of nHAP on cell proliferation were dependent on the treatment time. In contrast, we did not find any dependency on treatment time. It has been hypothesized that nHAP has a higher inhibitory effect on cancer cells than on normal cells. Cancer cells have a higher metabolic activity than normal cells; therefore, nanoparticles can enter

the cancer cells through endocytosis more readily than normal cells.[20] Additionally, the presence of many negatively charged groups on the surface of cancer cells than the normal cells has been shown. Moreover, because of the electrostatic interactions, nHAP with positive charge could have a higher adhering quality to cancer cells than the normal cells.

To date, there are only a few studies on the radiosensitization effect of nHAP. This effect on glioblastoma cells U251 and the breast tumor brain metastatic MDA-MB-231BR was evaluated using the clonogenic assay. The results showed that nHAP increased the radiosensitivity of tumor cells. Similar to our findings, Chu and colleagues[9] reported a higher reduction in the number of colonies when radiation was used in combination with nHAP compared with radiation alone. The mechanism of action for nHAP and cell growth inhibition was examined using the apoptosis method. Previous studies have indicated that nHAP could induce apoptosis.[8-10] Tang and colleagues reported that nHAP could significantly inhibit cell proliferation and could further induce apoptosis in HepG2 cancer cells, but it had no effect on normal hepatic cells (L-02).[7] Our results, however, showed that nHAP could induce higher apoptosis by MCF-7 cells than by fibroblasts. It is known that radiation adversely affects intracellular components, especially DNA. The DNA double-strand break (DSB) is important in biological lesions caused by ionizing radiation, especially in higher doses. It has been suggested that nHAP can increase the radiosensitivity of cancer cells due to the inhibition of DNA DSB repair caused by radiation.[9]

As the main limitation of the present study, we did not investigate the mechanism of the effect of nHAP and its radiosensitization effect on MCF-7 cells. It is recommended to investigate this in future studies.

Conclusion

The cytotoxic effects of radiation were enhanced when combined with nHAP, suggesting that nHAP sensitized cancer cells to radiation. The results showed that nHAP exerted lower toxicity effect on fibroblasts than on MCF-7 cells. Therefore, it can be concluded that nHAP selectively enters tumor cells and reduces damage to the surrounding tissue during irradiation therapy. Further research is required to demonstrate these effects on different cell lines.

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Conflict of Interests: None declared.

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