Original research

Apoptosis induction in the cancerous myeloid cells by folic acid-functionalized gold & silver nanoparticles with and without microwave

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Introduction

Metal nanoparticles, such as gold and silver nanoparticles have attracted much interest during the last decades for their special chemical & physical properties. Recently, gold nanoparticles were studies for diagnosis and treatment of cancers. On the other hand, silver nanoparticles have been in use for biological purposes because of their easy & cheap synthesis and application (1).

Nanoparticles of gold and silver can be functionalized with active biologic moieties like antibodies, drugs and chemicals, enabling them to react with specific cells. Since some antigens have higher expression on some cancer cells, they may be used for identification of cancer cells. One of the molecules which shows increased expression on neoplastic myeloid cells is folic acid receptor, which may be helpful for detection and apoptosis induction in those cells. Of course, there are still other molecules and antigens on the surface of cancerous myeloid cells which can probably be of similar utility (2).

Past studies showed that penetration and cytotoxic effects of nanoparticles can be increased by electromagnetic waves (such as infrared, ultraviolet, radiofrequency and microwave) and magnetic fields, which cause local hyperthermia. There are some limitations to the use of these waves; for example, magnetic fields affect circulation and heart impulses, infrared wavelengths have low tissue penetration, and application

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of radiofrequency wave needs special needles which are hard to tolerate by patients and cause severe complications in about 10% of cases, although they have good tissue penetration. Microwave can penetrate tissues deeply and do not invade tissues despite their local effects when used in conjunction with nanoparticles (3).

The aim of this study was to evaluate the effects of folic acid-functionalized gold & silver nanoparticles on cancer cells from 4 patients with acute myeloid leukemia (AML). We measured the rate of cell cytotoxicity by nanoparticles with and without use of microwave.

Materials & methods

Preparation of cells:

Four patients with known AML (M1, M2, M3 and M4), all recently diagnosed by histopathology, special stains and immunohistochemistry, were enrolled in the study before initiation of any treatment and after signing an informed consent form. Five mL of heparinized venous blood were drawn from each patient and centrifuged at 800 g for five minutes. White blood cells and plasma were transferred into another propylene plastic tube. The approximate number of cancerous cells in each sample was determined by examination of blood smear and also hematology analyzer. The final concentration of neoplastic myeloid cells in each tube was adjusted to about 400 cells/µL.

Preparation of folic acid-functionalized gold and silver nanoparticles:

Twelve grams of folic acid was dissolved in 10 mL of deionized water, with added 100 microliter of 1 M NaOH for complete dissolution. Five mL of 1 mM $HAuCl_4$ and also 5 mL of 1 mM $AgNO_3$ were prepared, and the above folic acid solution was gradually added to each of them. For synthesis of nanoparticles of gold and silver, each tube was incubated at $50^{\circ C}$ for 8 hours. Scanning electron microscope (SEM) (Hitachi) images and UV-visible spectrophotometer were used for confirmation of the synthesis of nanoparticles.

Attachment of nanoparticles to neoplastic cells and apoptosis induction:

In separate wells of a 96-well ELISA microplate, 50 µL of the cell suspension was added to 50 µL of each of gold and silver nanoparticles and incubated at $37^{\circ C}$ for one hour. Another 2 wells were treated the same way but with exposure to microwave (Asus) for 10 seconds so that its temperature reached at $50^{\circ C}$, and then incubated at $37^{\circ C}$ for one hour to permit completion of apoptosis. All of the tests were duplicated for quality control purposes.

The MTT test:

We added 20 µL of 5 mg/mL MTT salt to each well and incubated them at 37$^{\circ c}$ for 3 hours. After adding 50 µL of 70% isopropanol to each well, its absorbance was read at 490 nm.

Statistical analysis:

Paired t-test was used to compare the absorbance read-out in each of the above-mentioned 6 groups of wells: group 1= cells + gold nanoparticles + microwave; group 2= cells + silver nanoparticles + microwave; group 3= cells + gold nanoparticles; group 4= cells + silver nanoparticles; group 5= cells + microwave; group 6= cells without nanoparticle or microwave.

Results

After synthesis of functionalized nanoparticles of gold and silver with incubation at 50$^{\circ c}$, the tubes containing $HAuCl_4$ turned to red color, while the tubes with $AgNO_3$ showed a yellow color. The UV-visible spectrum absorbance of functionalized nanoparticles shows the peak absorbance for gold nanoparticles at 450 nm, and for silver nanoparticles at 420 nm. The atomic force microscopy (AFM) revealed that the size of gold nanoparticles was 50 nm, and that of silver nanoparticles between30. nm (figure 2).

The MTT test showed the maximum amount of apoptosis in the AML-M1, AML-M2, AML-M3 and AML-M4 cells to be 98.4%, 33.5%, 90% and 69%, respectively, as shown in table 1.

Table 1. The highest amount of apoptosis in the cells.

	With microwave		Without microwave	
	Maximum apoptosis with gold nanoparticles (p-value)	Maximum apoptosis with silver nanoparticles (p-value)	Maximum apoptosis with gold nanoparticles (p-value)	Maximum apoptosis with silver nanoparticles (p-value)
AML-M1	٩٨.٤% (٠.٠٠١)	٦٠.٧ % (٠.٠٠٦)	٩١.٢ % (٠.٠٠١)	٥٧.٨ % (٠.٠٠٣)
AML-M2	٣١.٣% (٠.٠٠٦)	٣٣.٥ % (٠.٠٠٧)	١٧.٢ % (٠.٠١)	١٨.٣ % (٠.٠١٤)
AML-M3	٦٤ % (٠.٠٠٣)	٩٠ % (٠.٠٠١)	٥٢.١ % (٠.٠١٢)	٦١.٧ % (٠.٠٠١)
AML-M4	٦٩ % (٠.٠٠٧)	٦١.٦ % (٠.٠٠١)	٦٦.٦ % (٠.٠٢٧)	٥٣.٨ % (٠.٠١)

Discussion

This study showed that the highest rate of apoptosis of AML cells (seen in AML-M1 type) was 98.4% when exposed to gold nanoparticles plus microwave, whilst 91.2% when only gold nanoparticles were applied. It also demonstrated that the highest amount of apoptosis of AML cells (seen in AML-M3 type) was 90% when exposed

to silver nanoparticles plus microwave, whilst 57.8% when only silver nanoparticles were used (seen in AML-M1 type). There was a significant (p-value= 0.001) difference between usage of microwave alone and microwave plus nanoparticles.

The lack of similarity in the rates of apoptosis among these 4 types of neoplastic cells may be due to difference in number of folic acid receptors in them. Overall, the best apoptosis induction was in AML-M1, and the least apoptosis was in AML-M2.

As is seen in the table, microwave alone induces some apoptosis. Gold nanoparticles were more potent in inducing apoptosis compared with silver nanoparticles, probably due to its greater load of folic acid or its smaller size.

The method of synthesis of gold and silver nanoparticles in this study was according to Jou and coworkers, and showed good properties. The size of nanoparticles has been 30 nm, which can be changed by modifying the different factors affecting the method. It is obvious that the particular amount of apoptosis seen in this study can be related to only this size, because the size of each nanoparticle greatly affects its properties.

Since the microwave used in this study had a high power, the length of exposure was very short. Microwave can penetrate the body deeply. The disadvantage of using microwave with 2.45 GHz frequency is that it increases the temperature in normal cells as in neoplastic cells, because this wavelength affects the water molecules not

nanoparticles.used microwave which had a frequency of 4.5 MG which likely had no effect on normal cells. The advantage of microwave is that its use is simple and cheap.

The notable limitation of this study was using MTT test alone for the evaluation of apoptosis. It might be more accurate if future studies would include other methods of apoptosis analysis, such as staining with annexin V, lactate dehydrogenase measurement and assessment of involved genes. Also, the future research on this field should address cytotoxicity issues. It is obvious that in-vivo studies for evaluation of the efficacy of this new method are needed, too.

References: