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Review Article

A systematic review of gold nanoparticles as novel cancer therapeutics

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Abstract

***Objective(s)*:** The current systematic study has reviewed the therapeutic potential of gold nanoparticles as nano radiosensitizers for cancer radiation therapy.
***Materials and Methods*:** This study was done to review nano radiosensitizers. PubMed, Ovid Medline, Science Direct, SCOPUS, ISI web of knowledge, Springer databases were searched from 2000 to September 2013 to identify appropriate studies.
Any study that assessed nanoparticles, candidate of radio enhancement at radiotherapy on animals or cell lines was included by two independent reviewers.
***Results*:** Gold nanoparticles can enhance radiosenstivity of tumor cells. This effect is shown in vivo and in vitro, at kilovoltage or megavoltage energies, in 15 reviewed studies. Emphasis of studies was on gold nanoparticles. Radiosensitization of nanoparticles depend on nanoparticles' size, type, concentration, intracellular localization, used irradiation energy and tested cell line.
***Conclusion*:** Study outcomes have showed that gold nanoparticles have been beneficial at cancer radiation therapy.

Keywords: Gold nanoparticles, Radio sensitizer, Radiation therapy, Systematic review

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Introduction

Nanoparticles can improve cancer diagnosis, imaging and therapy at the cellular and molecular levels (1). Gold as a drug and medicinal agent have been used for disease treatment since long time ago (2). Primitive application of gold for medicinal purposes returned to Alexandria, Egypt, Over 5000 years ago. It was used for mental, bodily and spiritual purification (3). Today gold, especially gold nanoparticles (GNPs) have become an interesting research area in cancer diagnosis, imaging and especially treatment. This is due to biocompatible properties of GNPs (4). Also, trustworthy methods exist for economical GNPs synthesis with different sizes (2–500 nm) and shapes (spheres, rods, tubes, wires, ribbons, plate, cubic, hexagonal and triangular).Also, Organic and inorganic molecules can be attached to GNPs' surface (5).

Radiotherapy is one of the key modalities for treatment of cancer. Radiotherapy is the most common cancer treatment (6). Almost 52% of cancer patients undergoes radiotherapy at least once during their treatment course (7). One of the greatest challenges in radiotherapy is that ionizing radiation cannot differentiate between healthy tissue and solid tumors. Tissue around tumor is also affected by radiation. Therefore, healthy tissue benefits from less radiation dose. Radiotherapy requires for development on radiation delivery techniques to reduce injury to surrounding tissues. To overcome this problem, radio sensitizers are one of the right solution. Radio sensitizers are adjunctive treatments which make tumor cells more vulnerable to radiation. Radio sensitizers are planned and designed to improve tumor cell killing while having much less effect on normal tissues (8).

Recent progresses have been made towards gold nanoparticles to suggest them as novel radio sensitizers. Application of GNPs as radio sensitizer is a promising strategy to increase efficiency of radiotherapy. This is the first systematic review of literature to assess the application of GNPs in radiotherapy as radio sensitizer.

Materials and Methods

Search strategy

Our systematic review was compatible with the PRISMA guidelines (9). A systematic search was done in the databases of PubMed, Ovid Medline, Science Direct, SCOPUS, ISI web of knowledge and Springer from 2000 to September 2013. Searches were limited to English language. The following search terms were used:

("gold nano particles"/gnp) AND (radio sensitizer or radiosensitization or "radiation dose-enhancing" or "radiation sensitizing agents" or " enhanced X-ray therapy " or "enhancement of radiation sensitivity") OR (radiation therapy or radiotherapy). Also synonyms and derivate of the terms were used for finding more articles.

To have a wide-ranging search and to find possible relevant articles, manual search was done on reference list of articles. We included articles*:*

1- Studying GNPs as the volunteer of a radio sensitizer substance.

2- Ionizing radiation has been used at the study.

3- Aim of study has been on the cancer radiotherapy.

4- Cell lines /animals should have been tested.

Thesis, meetings and other unpublished data were excluded.

First, titles and abstracts of the searched studies were read to determine their potential eligibility for the review. Article which met our inclusion criteria were included .Then full text of each possibly relevant study was retrieved and assessed independently by authors. After the

assessment, the authors agreed on the reporting of 15 Articles in a meeting selection. For assessing agreement between authors Cohen's kappa statistic was used (Cohen's kappa =0.9).

Independent extraction of articles was performed .Following data were extracted: papers cite, publication year, type of nanoparticle, radiation dose and type, NPs size. We also noted outcomes of studies regardless of author, affiliation and journal. We gathered data from all studies identified irrespective of nanoparticle synthesis method.

Due to heterogeneous nature of the studies identified, the data available did not allow us to use formal statistical techniques such as meta-analysis.

Heterogeneity results from variations in studies method, outcome measures, sizes and types of NPs and cell line types.

Results

The search of databases yielded 65 publications. 52 of articles were excluded due to inclusion criteria.2 articles were added after checking the references list of included articles.

Finally, 15 articles were reviewed. Figure 1 shows the algorithm of the study selection procedure.

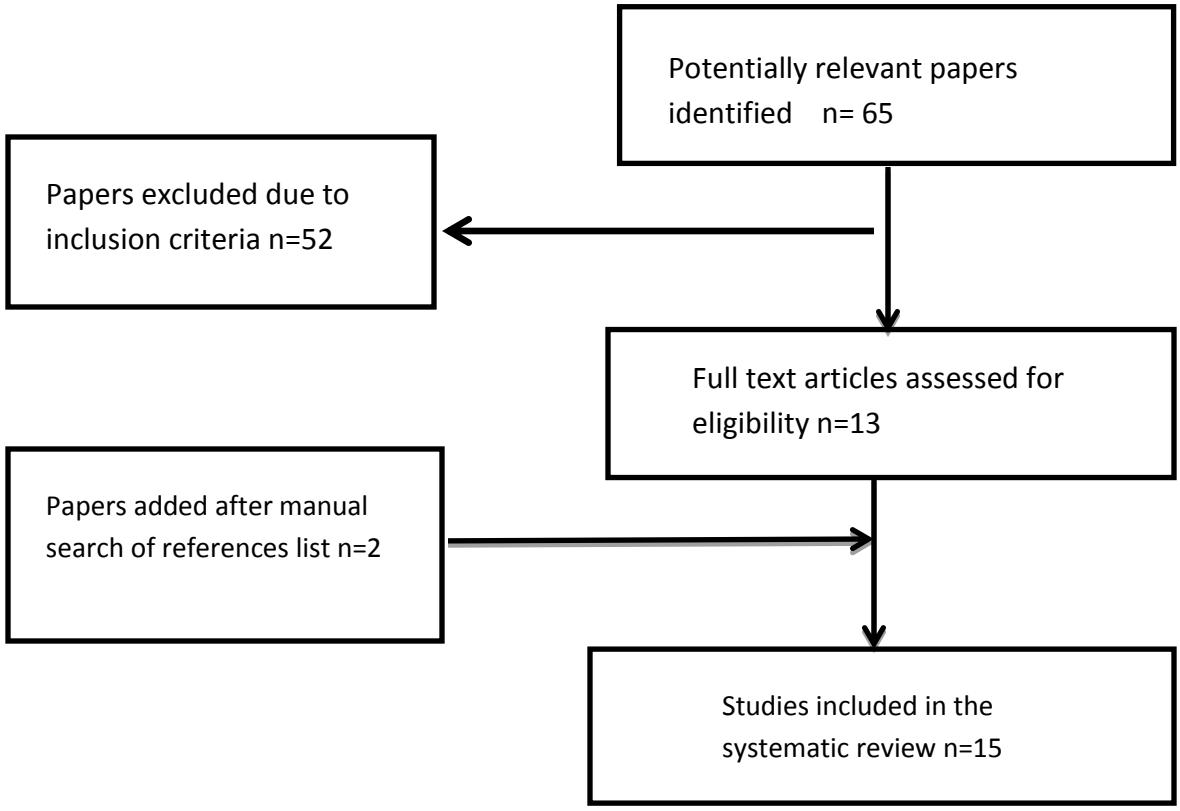

Figure 1: Stages of the search strategy for Identification of relevant literature.

15 Papers were carried out using GNPs. Remarkable studies have been done to show GNPs' radio sensitization effect. GNPs have received special attention during last decade (10). It has been shown that by using GNPs less radiation dose is needed (11). Table 1 shows GNPs sizes, cell types and radiation doses and types used for each study.

Table 1. Gold nanoparticles size and types of radiation and dose.

First author	Gold Nanoparticle size	Cell type	Type of radiation and dose
Joh D Y	approximately 12 nm	human U251 glioblastoma cells (ATCC),	*in vitro* 4 Gy (150 kVp), *in vivo* 20 Gy (175 kVp) to the brain
Jain S	1.9 nm	human prostate cancer cells (DU145) breast cancer cells(MDA-MB231) lung epithelial cells (L132)	X-ray (6 MV, 15 MV) and electron (6 MeV, 16 MeV) Varian 2100CD linear accelerator 3.55 Gy/min and 3.85 Gy/min, for 6 MV and 15 MV(respectively) 4.0 Gy/min for both 6 MeV and 16 MeV
Wang C	13 nm	lung-cancer cells (A549)	X-rays(6 MV) linear accelerator 10Gy
Roa W	15 nm	human prostate carcinoma cell (DU-145)	cesium-137 2 Gy (single dose)
Chang M.Y	Approximatel y 13 nm	melanoma cells (B16F10)	Electron (6 MeV) Varian 2100C linear accelerator 25 Gy
Kaur H	ranging from 5-9 nm	HeLa cell line (human cervix cancer cells)	γ-radiation and carbon ion irradiation 62 MeV 12C6 LET of 290 keV/μm. 0.9, 1.9, 2.8 and 3.7 Gy
Chattopadhyay N	30 nm	MDA-MB-361	X-rays(100 kVp) In vivo : 0.5 Gy In vitro : 11 Gy
Lechtman E	30 nm	human prostate adenocarcinoma (PC-3)	X-ray (300 kVp) 0, 1, 2, 4, and 8 Gy
Zhang X	30 nm	Human prostate carcinoma cells (DU-145)	X-rays(200-kVp) 2 Gy
Kong T	10.8 nm	breast-cancer cells (MCF-7) nonmalignant breast-cells (MCF-10A)	X-ray(200-kVp), γ -rays caesium-137 or cobalt-60 radiation 2 Gy
Rahman W N	1.9 nm	bovine aortic endothelial cells	X-ray (80 kV and 150 kV) 0, 1, 2, 3, 4, and 5 Gy Electron (6 MeV and 12 MeV) linear accelerator (Clinac 2100C Varian) 1 Gy/min
Liu CJ	6.1 nm	EMT-6 cell CT26 cell	10 Gy X-ray(8.048 keV) commercial biological irradiator (E(average) = 73 keV), a Cu-Kalpha(1) , Electon (6.5 keV),

A pioneering study was done on mice bearing subcutaneous EMT-6 mammary carcinomas by Hainfeld (12). Mice were divided into two groups: treated with either GNPs and radiation or radiation alone. These two groups had had 86% and 20% one year survival respectively. Another in vivo study was done by Hainfeld recently. He used the same size used in the previous study (1.9 nm GNPs).

Mice bearing murine squamous cell carcinoma (SCCVII) were radiated with X-ray (68 keVp, 42 and 30 Gy). Significant tumor growth delay and long-term tumor control was seen with 42 Gy but not with 30 Gy (13) Also mice were radiated with 157-keV photons; more tumor radiosenstivity was seen with GNPs accompanied by 50.6 Gy than 44 Gy.

Another animal study was done at 2008 (14). They injected melanoma cells (B16F10) to mice. After GNP injection, mice were irradiated with electron (25 Gy). They showed that GNPs radiosensitized melanoma cells. In comparison with control group, tumor growth rate was decreased; apoptotic signals and survival rates were increased.

It is demonstrated that radio sensitization is cell line-dependent, as Jain s et al. showed that GNPs radio sensitization occurred in MDA-MB-231 cell line but not in DU145 or L132 cell line despite GNP uptake (15).

Bonded GNPs

Different functional groups can be attached to GNPs such as PEG, thiol, peptides and antibodies. Binding ligands and molecules bestows several characteristics to the particle. Daniel Y. Joh *et al*. after in vitro experiments showed that intravenously injected PEGylated-GNPs radiosensitized human glioblastoma cells to radiotherapy and increased mice survival (16). Another study about PEGylated-GNPs showed that in the presence of this nanoparticle, EMT-6 and CT26 cell survival rates were decreased (17).

A recent study has assessed effects of targeted GNPs on tumor radiation sensitivity (15). This study had two parts: in vivo and in vitro. At the in vivo part, athymic mice bore subcutaneous MDA-MB-361 xeno-grafts. Mice were injected Human Epidermal Growth Factor Receptor-2 targeted GNPs or saline intratumorally. After 24 hours, mice received a single dose radiation of X-rays (100 kVp, 11 Gy). These mice had slower growth rate than control mice (which were only radiated). Remark-ably, in vivo results were in agreement with in vitro. Survival curve of cells exposed to targeted GNPs and radiation was significantly lower than cells exposed to x-radiation alone. But, survival curves for cells exposed to GNPs and radiation versus radiation alone were not significantly different. Thus, targeted GNPs cause more radio sensitivity than neutral GNPs.

Glucose capped GNPs (Glu-GNPs) enhanced radiation sensitivity in radiation-resistant human prostate cancer cells study is another bonded GNPs study (18). It is shown that Glu-GNPs trigger cell cycle acceleration in the G0/G1 phase and restrain cell in the G2/M phase. This activation occurs with sensitivity to ionizing radiation. Similar studies about Glu-GNPs showed that irradiation of HeLa cells with Glu-GNPs outcomes in enhanced radiation sensitivity (19). Also similar effects are seen on lung cancer cells and ovarian cancer cells (20) (21). Another study showed that Glu-GNPs have a greater decrease in cellular proliferation than neutral GNPs (22).

Binding groups bring about changes in GNPs location. Kong et al compared thioglucose and cysteamine-capped GNPs in breast-cancer cell line (MCF-7) versus a nonmalignant breast-cell line (MCF-10A) (23). This study showed that Cysteamine-capped GNPs were mostly bound to the

MCF-7 cell membrane, but thioglucose – capped GNPs enter the cells and were dispersed in the cytoplasm.

Discussion

Reviewed studies have demonstrated radio enhancement effect of GNPs. They have unique properties like bio compatibility and modifiable surfaces that make them great volunteer to be radio sensitizer. The sensitizing characteristics of NPs have been tested on various cell lines and animals. Different sizes, concentrations, cell lines, radiation sources and doses have been used at the reviewed studies. Radiation sensitivity using NPs depends on nanoparticle type, cell line, irradiation energy, nanoparticle size, concentration and intracellular localization.

In vitro radio sensitization and in vivo tumor growth retardation accompanied by longer survival give researchers the proof of using GNPs. All reviewed studies showed consistency of their result and confirm enhancement of radiotherapy by using GNPs. Such enhancement takes place as long as GNPs accompanied by radiation. GNPs without radiation result are similar to no treatment (12).

Probable mechanism involved in GNP radio enhancement is cell cycle changes and elevated reactive oxygen species production (18, 21). In the presence of GNPs, more radicals electrons are produced. It is suggested that radio sensitivity of GNP's can be attributed to enhanced localized absorption of X-rays, release of low-energy electrons from GNPs and efficient deposition of energy in the form of radicals and electrons (24). Most of studies compared using GNPs with not using them.

Five factors affecting GNPs as radio sensitizer

Concentration

The effect of GNPs concentrations on dose enhancement is much greater than GNPs size. Increasing GNPs concentration decrease cells growth rate (15). It seems rational as increasing the concentration of GNPS causes number of GNPs increase, consequently, the number of gold atoms. Therefore, more photoelectric interactions between photons and gold atoms occur (25). Higher GNPs concentrations have higher risk of toxicity. Therefore, the balance between dose enhancement effect and toxicity should be set.

Size

GNPs can be produced over a wide range of sizes (0.4–5000 nm). Some of GNPs properties are attributed to size. Size is a strong factor in existence time in blood. Smaller GNPs are filtrated through kidneys quickly, while larger ones avoid clearing. GNPs size affects cellular uptake. Since only GNPs of size 1-100 nm can enter cells, optimal size design can increase cell internalization (24). Large-sized GNPs have the most efficient dose enhancement effect (DEF) (26, 27). This diameter has also the highest cellular uptake (28).

Modifying GNP's surface

A 0.8-nm GNP has seven ligand sites, a 2-nm has ~100, and a 15-nm has approximately 4000. PEG, carboxyl or amino groups, thiol derivative drugs, DNA, lipids, carbohydrates, antibodies, peptides or organic moiety can be attached to GNPs. Any of these bindings confers beneficial properties to GNP.

As an example, PEG binding helps GNPs to avoid reticuloendothelial system uptake (29). Glucose binding GNPs enter the cells and spread in the cytoplasm more than neutral GNPs (23) , as it was shown in 5 out of 15 review study (18-22). Cancer cells have more metabolisms than normal cells, which create a greater need to glucose. Therefore, when glucose is coated on surface of GNPs, cancer cells take up the

glucose with GNPs attached to it. Glucose increases cell internalization and afterwards increases radio sensitivity.
GNPs' surface can be modified for targeting of cancer cells by antibodies or hormones (30). If GNPs can be localized in cancerous cells, cancerous tissue receive higher dose compared with normal tissue during a radiotherapy treatment. Also, less radiation dose is needed.

Intracellular localization

Gathering of GNPs inside the cells and intracellular localization improve the radiation effects as photon and electron interaction increase. Study of Kong et al, Chattopadhyay et al. suggested that localization of GNPs within the cells is chief factor in increasing radiation cytotoxicity (15, 23).

Radiation dose

Several reports have shown GNPs' radio sensitization with kV (proton and X-ray) and KeV. Also such radio enhancement is shown at MV X-rays (15, 11) and MeV energies (14, 19, 24, 31). Dose enhancement factor (DEF) depends on radiation energy and amount of GNPs (29).

Cell type

Cytotoxicity of GNPs alters in different cell types (23, 32). GNPs could enhance the sensitivity of some cells to irradiation but not all cells, as glucose capped GNPs did not radio sensitized human diploid fibroblast cells but did enhance human prostate carcinoma cells (18). Another proof, despite cellular uptake in human prostate cancer cells and lung epithelial cells, radio sensitization was not observed in neither of them (15).
GNPs cellular uptake levels and cell cycle phases might justify it. Metallic materials block cells at the G2/M phase, the most radiosensitive phase of the cell cycle; therefore augment cell radio sensitivity (33).

Conclusion

Literature supports using GNPs as radio sensitizer for radiation therapy. Desired and anticipated outcomes would be reached by changing factors affecting radio sensitivity. These results demonstrate signs of forth-coming success of the GNPs in cancer treatment.

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