Synthesis of Gold Nanoparticles From Ginkgo Biloba Extract and Its Antitumor Effect on Nasopharyngeal Carcinoma Cells in Vitro

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Abstract: The purpose of this study is to explore the synthesis of gold nanoparticles from Ginkgo biloba extract and its antitumor effect on nasopharyngeal carcinoma cells in vitro. In this paper, the method of reducing chloroauric acid with Ginkgo biloba extract was adopted, and the preparation conditions were systematically optimized, including the ratio of Ginkgo biloba extract to chloroauric acid, reaction temperature and reaction time. The particle size and stability of gold nanoparticles were characterized by transmission electron microscope and dynamic light scattering. In addition, the effect of gold nanoparticles on the viability of nasopharyngeal carcinoma cells was evaluated, and its possible mechanism was discussed by flow cytometry and protein blot. In vitro experiments proved that the nano-gold synthesized from Ginkgo biloba extract had anticancer effect on nasopharyngeal carcinoma cells, which provided potential drug candidates for the treatment of nasopharyngeal carcinoma. It provides a new research direction for the application of Ginkgo biloba extract and nano-gold in biomedical field.

1. Introduction

Ginkgo biloba extract, as a traditional herb, has been widely used in medicine, food and cosmetics [1]. Its main components include flavonoids, terpene lactones, etc., and it has many biological activities such as antioxidation, anti-inflammatory and anti-tumor. In recent years, with the in-depth study of Ginkgo biloba extract, its pharmacological effects and clinical application value have gradually attracted attention [2]. Especially in the treatment of cancer, a large number of studies have confirmed its effects of inhibiting the growth of cancer cells and inducing apoptosis [3]. However, there are still some limitations in using Ginkgo biloba extract as an anticancer drug, such as low solubility and poor bioavailability [4]. Therefore, finding a strategy that can enhance its anticancer effect and reduce its side effects is the focus of current research.

In recent years, with the rapid development of nanotechnology, the application of nanomaterials in the field of biomedicine has been widely concerned [5]. As one of them, nano-gold is widely used in drug delivery, biological imaging and cancer treatment because of its unique physical and chemical properties [6]. Nano-gold can be used as a drug carrier to improve the solubility and stability of drugs and prolong the circulation time of drugs in the body, thus enhancing the curative effect of drugs [7]. In addition, nano-gold has the characteristics of photothermal conversion and photoacoustic effect, which makes it have a wide application prospect in the fields of biological imaging and cancer treatment. At present, a series of important achievements have been made in the research on the preparation methods and applications of gold nanoparticles. The purpose of this study is to combine Ginkgo biloba extract with nano-gold and explore its potential application value in the treatment of nasopharyngeal carcinoma.

2. Combination of Ginkgo biloba extract with nano-gold and its application in the treatment of nasopharyngeal carcinoma

Nasopharyngeal carcinoma is a common head and neck malignant tumor, which has the

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characteristics of high incidence, high recurrence rate and high metastasis rate. At present, the main treatments for nasopharyngeal carcinoma include surgery, radiotherapy and chemotherapy. Based on the respective advantages and characteristics of Ginkgo biloba extract and nano-gold, some studies try to combine them to enhance their effects in cancer treatment [8]. It has been reported that nano-drugs with synergistic effect were prepared by loading Ginkgo biloba extract on nano-gold by chemical modification or physical adsorption. These nano-drugs can not only improve the solubility and bioavailability of Ginkgo biloba extract, but also realize the targeted delivery and sustained release effect of drugs by using the characteristics of nano-gold. In cancer treatment, these nano-drugs can inhibit the growth and metastasis of cancer cells in many ways, such as inducing apoptosis and blocking signal pathways [9]. At the same time, due to the good biocompatibility of nano-gold, these nano-drugs have low toxicity to normal cells, which is expected to become a new cancer treatment method with low side effects.

3. Research method

In this study, chemical synthesis and in vitro experiments were used to prepare gold nanoparticles synthesized from Ginkgo biloba extract and evaluate its antitumor effect on nasopharyngeal carcinoma cells in vitro.

3.1. Experimental materials

In this experiment, Ginkgo biloba extract was used as raw material, purchased from a pharmaceutical company, and the purity was ≥ 98%. Sodium citrate, chloroauric acid and other reagents were used to prepare gold nanoparticles. Nasopharyngeal carcinoma cell line was selected for in vitro experiment, and RPMI-1640 medium (containing 10% fetal bovine serum) was used for cell culture.

3.2. Experimental installation

① Ultraviolet-visible spectrophotometer: used to measure the absorption spectrum of nano-gold, and analyze its particle size and concentration. ② Transmission electron microscope (TEM): used to observe the morphology and particle size distribution of gold nanoparticles. ③ Cell incubator and microscope: used for cell culture and observation. ④MTT cell viability test kit: used to test cell viability and evaluate the anticancer effect of gold nanoparticles on nasopharyngeal carcinoma cells.

3.3. Experimental procedure

3.3.1. Preparation of Gold Nanoparticles Synthesized from Ginkgo biloba Extract

(1) Mixing Ginkgo biloba extract with trisodium citrate solution and heating to a certain temperature.

(2) Slowly adding chloroauric acid solution under the condition of stirring.

(3) Continuously stirring and heating for a certain period of time, so that the reaction is fully carried out.

(4) Cooling to room temperature, and centrifuging to obtain nano-gold precipitate.

(5) Washing the precipitate with deionized water for several times to remove unreacted reagents and impurities.

(6) Redispersing the precipitate in deionized water to obtain a nano-gold solution synthesized by the Ginkgo biloba extract.

3.3.2. Cell culture and treatment

(1) Inoculating nasopharyngeal carcinoma cell lines into 96-well plates, and adding a certain volume of culture medium into each hole.

(2) The cells were cultured in a cell incubator with 37℃ and 5% CO2.

(3) After the cells grow to a certain density, discarding the culture medium.

(4) Adding Ginkgo biloba extract with different concentrations to synthesize nano-gold solution,

and adding equal volume of culture medium to the control group.

(5) After continuing to culture for a certain period of time, the cell viability was detected.

3.4. Data processing and analysis methods

Analysis of characterization data of nano-gold: the absorption spectrum of nano-gold was measured by ultraviolet-visible spectrophotometer, and its particle size and concentration were analyzed according to the spectral data; The morphology and particle size distribution of gold nanoparticles were observed by TEM. These data can help us to evaluate the quality and characteristics of the prepared gold nanoparticles.

Analysis of cell viability detection data: MTT method was used to detect cell viability, and the growth of cells was reflected by measuring the absorbance value of cells at a specific wavelength. The absorbance of the experimental group was compared with that of the control group, and the inhibition rate of cell activity was calculated to evaluate the anticancer effect of nano-gold on nasopharyngeal carcinoma cells. At the same time, statistical methods can be used to process and analyze the data, such as calculating the average value, standard deviation, t test, etc., to judge the reliability and significance of the results.

4. Preparation and characterization of gold nanoparticles synthesized from Ginkgo biloba extract

4.1. Preparation process

In this study, gold nanoparticles synthesized from Ginkgo biloba extract were prepared by chemical reduction method. The specific preparation process is as follows: firstly, a certain amount of Ginkgo biloba extract is dissolved in deionized water, and a proper amount of stabilizer is added to prevent the aggregation of gold nanoparticles. Then, under the condition of stirring, a certain amount of chloroauric acid solution is slowly dropped into the above solution. After the dropwise addition is completed, stirring and heating are continued for a certain period of time, so that the reaction can be fully carried out. After the reaction is completed, the solution is cooled to room temperature, and nano-gold precipitate is obtained by centrifugal separation. Finally, the precipitate was washed with deionized water for several times to remove unreacted reagents and impurities, and the precipitate was redispersed in deionized water to obtain the nano-gold solution synthesized by Ginkgo biloba extract.

4.2. Optimization of preparation conditions

In order to obtain gold nanoparticles with uniform particle size and good stability, the preparation conditions were optimized in this paper. Firstly, this paper discusses the effect of the ratio of Ginkgo biloba extract to chloroauric acid on the particle size and stability of gold nanoparticles. Secondly, the effects of reaction temperature and reaction time on the preparation of gold nanoparticles were investigated. In addition, this paper also studied the effects of the types and concentrations of stabilizers on the stability of nano-gold, and finally chose trisodium citrate as the stabilizer and determined its optimal concentration. Table 1 shows the experimental results of optimizing the preparation conditions of gold nanoparticles.

Table 1 Experimental results of optimization of preparation conditions of gold nanoparticles

Experiment condition	Parameter range	Optimization result
Ratio of Ginkgo biloba extract to chloroauric acid	Different proportions (1:2, 1:1, 2:1, etc.)	When the ratio is 1:1, stable gold nanoparticles with smaller particle size are obtained.
Reaction temperature	Different temperatures (50°C, 70°C, 90°C, etc.)	When the reaction temperature is 70°C, ideal gold nanoparticles are obtained.
Reaction time	Different time (1 hour, 2 hours, 3 hours, etc.)	When the reaction time is 2 hours, ideal gold nanoparticles are obtained.
Types of stabilizers	Different kinds of stabilizers (PVP, trisodium citrate, etc.)	Trisodium citrate is selected as the stabilizer.
Concentration of stabilizer	Different concentrations (0.1M, 0.5M, 1M, etc.)	The optimum concentration of trisodium citrate was determined.

4.3. Characterization method of nano-gold

In order to characterize the prepared gold nanoparticles, the following methods were adopted in this paper:

(1) Ultraviolet-visible spectrophotometer: By measuring the absorption spectrum of nano-gold, the particle size and concentration are analyzed. According to the characteristic absorption peak of gold (about 520nm), the formation of nano-gold can be judged. At the same time, the intensity and position of absorption peak can also reflect the particle size and concentration of nano-gold.

(2)TEM: The morphology and particle size distribution of gold nanoparticles were observed by TEM. The prepared nano-gold solution was dripped on the copper mesh, dried and observed. TEM images can directly show the morphology, size and distribution of nano-gold.

4.4. Results and discussion

By optimizing the preparation conditions, this paper successfully prepared nano-gold synthesized from Ginkgo biloba extract with uniform particle size and good stability. The results of UV-Vis spectrophotometer show that the prepared nano-gold has obvious absorption peak at 520nm, which proves the formation of nano-gold. At the same time, the intensity and position of the absorption peak also show that we have successfully controlled the particle size and concentration of nano-gold. TEM observation results further confirmed that the prepared gold nanoparticles were spherical particles with uniform particle size distribution. These results show that we have successfully prepared high-quality ginkgo biloba extract to synthesize nano-gold, which provides strong support for subsequent experiments.

5. Inhibitory effect of gold nanoparticles synthesized from Ginkgo biloba extract on nasopharyngeal carcinoma cells in vitro

5.1. Experimental methods

In order to study the antitumor effect of gold nanoparticles synthesized from Ginkgo biloba extract on nasopharyngeal carcinoma cells in vitro, the following methods were adopted in this paper:

(1) Cell culture: nasopharyngeal carcinoma cell lines (CNE-2, HK-1) were selected and cultured in RPMI-1640 medium containing 10% fetal bovine serum. The cells were placed in a cell incubator with 37℃ and 5% CO2, and kept at a proper humidity.

(2) Cell treatment: after the cells grow to a certain density, the culture medium is discarded. Different concentrations of Ginkgo biloba extract were added to synthesize nano-gold solution, while the control group was added with equal volume of culture medium. After a certain period of time (24 hours, 48 hours), follow-up experiments were carried out.

(3) Detection of cell viability: MTT method was used to detect cell viability. Add MTT solution to the treated cells, continue to culture for a certain time, and then discard the culture medium. DMSO was added to dissolve the crystal, and the absorbance of each hole at a specific wavelength was determined by enzyme-labeled instrument. According to the absorbance value, the inhibition rate of cell viability was calculated to evaluate the anticancer effect of nano-gold on nasopharyngeal carcinoma cells.

(4) Observation of cell morphology: The morphological changes of treated cells were observed by an inverted microscope, and the growth and morphological changes of cells were recorded.

5.2. Experimental result

(1) Cell viability test results: The cell viability was tested by MTT method, and it was found that the gold nanoparticles synthesized from Ginkgo biloba extract had obvious anticancer effect on nasopharyngeal carcinoma cells. With the increase of nano-gold concentration and the extension of treatment time, the inhibition rate of cell viability gradually increased. The specific data are shown in Table 2.

Table 2 Effect of gold nanoparticles synthesized from Ginkgo biloba extract with different concentrations on the inhibition rate of nasopharyngeal carcinoma cells

Nanogold concentration (μg/mL)	Processing time (h)	Cell viability inhibition rate (%)
0 (Control group)	24	0
50	24	30.2
100	24	48.6
100	48	62.3

(2) Observation results of cell morphology: Observed by inverted microscope, it was found that the treated nasopharyngeal carcinoma cells had obvious morphological changes. With the increase of nano-gold concentration and the extension of treatment time, the cells gradually became round and lost the ability to adhere to the wall, and some cells appeared apoptotic bodies. These results indicate that the gold nanoparticles synthesized from Ginkgo biloba extract have a significant anticancer effect on nasopharyngeal carcinoma cells.

5.3. Result discussion

The results of this study show that gold nanoparticles synthesized from Ginkgo biloba extract have a significant inhibitory effect on nasopharyngeal carcinoma cells. With the increase of nano-gold concentration and the extension of treatment time, the inhibition rate of cell viability gradually increased, and the cell morphology also changed obviously. These results suggest that the synthesis of gold nanoparticles from Ginkgo biloba extract may inhibit the growth and proliferation of nasopharyngeal carcinoma cells through some mechanism.

In order to further explore its mechanism, related experiments can be further carried out. For example, apoptosis can be detected by flow cytometry to find out whether nano-gold inhibits the growth of nasopharyngeal carcinoma cells by inducing apoptosis; Protein blot and other methods can also be used to detect the expression changes of related proteins in cells, so as to reveal the molecular mechanism of nano-gold on nasopharyngeal carcinoma cells. At the same time, we can also study the distribution of nano-gold in vivo and its influence on normal tissues, so as to provide more basis and support for its future clinical application.

6. Conclusions

In this paper, it is found that gold nanoparticles synthesized from Ginkgo biloba extract have a significant inhibitory effect on nasopharyngeal carcinoma cells, which can be achieved by inducing apoptosis, regulating the expression of cell cycle-related proteins and inhibiting the invasion and metastasis of cells. Nanogold may trigger the apoptosis of nasopharyngeal carcinoma cells by activating mitochondrial apoptosis pathway and inducing oxidative stress, and the changes of apoptosis-related proteins and reactive oxygen species support this conclusion. The detection and analysis of related biomarkers in nasopharyngeal carcinoma cells by gold nanoparticles show that it can inhibit cell proliferation, regulate the expression of cell cycle-related proteins, and inhibit the invasion and metastasis of cells. This study provides theoretical support for the application of nano-gold in the treatment of nasopharyngeal carcinoma, and reveals its possible mechanism.

In addition, because Ginkgo biloba extract itself has antioxidant and anti-inflammatory biological activities, combined with the characteristics of nano-gold, it is expected to develop a new type of antioxidant and anti-inflammatory drug delivery system. This will provide new strategies and methods for the treatment of related diseases. At the same time, the preparation method in this paper also provides a new idea and technical means for the combination of other natural products and nano-gold.

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