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Effect of DMSO@Γ-Fe2O3 Nanomagnetic Fluid Thermotherapy Combined with Docetaxel on the Biological Behavior of Human Gastric Cancer AGS Cells under the Alternating Magnetic Field

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Keywords:

Gastric cancer; Nanomagnetic fluid thermotherapy; Alternating magnetic field; AGS; Docetaxel

1. Abstract

1.1. Objective: To study the effects of DMSO@γ-Fe_2O_3 nanomagnetic fluid thermotherapy combined with the chemotherapeutic drug docetaxel on the biological behavior of human gastric cancer AGS cells under an alternating magnetic field of a certain intensity.

1.2. Methods: In vitro warming experiments were performed on DMSO@γ-Fe_2O_3 nanomagnetic fluid under an alternating magnetic field to determine the optimal temperature (43.5°C) required for nanomagnetic fluid thermotherapy. Human gastric cancer AGS cells were divided into control, nanomagnetic fluid thermotherapy, chemotherapy and nanomagnetic fluid thermotherapy combined with chemotherapy groups (referred to as the thermo-chemotherapy group). The CCK8 assay was used to evaluate the inhibitory effect of DMSO@γ-Fe_2O_3 nanomagnetic fluid on AGS cells and to determine the working concentration of docetaxel on AGS cells; A cell scratch assay was performed to explored the cell migratory movements of cell groups, and a transwell assay was performed to test the invasion of each group of cells and flow cytometry to detect apoptosis of cells in each group.

1.3. Results: The warming effect of DMSO@γ-Fe_2O_3 nanomagnetic fluid was good, and when the concentration of DMSO@γ-Fe_2O_3 nanomagnetic fluid was ≥ 7gL^{-1}, the temperature could reach over 43.5°C. The drug concentration at 24h IC50 was used as the working concentration for the experiment, and the working concentration of docetaxel was determined to be 0.20 mM. Both nanomagnetic fluid thermotherapy alone at 43.5 °C and chemotherapy alone had inhibitory and killing effects on AGS cells ($P < 0.05$), and the flow cytometry results showed that the apoptosis rates of the control group, the nanomagnetic fluid thermotherapy group, the chemotherapy group, and the thermochemotherapy group were 6.11 %, 17.67 %, 20.21 % and 43.80 %.

1.4. Conclusion: Under the effect of alternating magnetic field, DMSO@γ-Fe_2O_3 nanomagnetic fluid thermotherapy (43.5°C) could significantly enhance the inhibitory effect of the chemotherapeutic drug docetaxel on AGS cells.

2. Introduction

Gastric cancer, one of the most common malignant tumors, currently ranks 5th in incidence and 4th in mortality worldwide [1].

The exploration of cancer treatment, such as the novel treatment modality of nanomagnetic fluid thermotherapy [2], which injects magnetic nanofluids into tumor tissues, induces magnetic nanoparticles into an alternating magnetic field to generate heat, which rises the temperature of the tumor tissues and then kills the cancer cells. On the basis of the advantages of traditional thermotherapy, nanomagnetic fluid thermotherapy has better specificity and targeting, which reduces the probability of damage tothe normal tissues around the tumor being damaged by thermotherapy. The temperature of the tumor tissue increases during thermotherapy, which can simultaneously promote blood circulation in the tumor area and improve the permeability of blood vessels, which is conducive to the treatment of tumors that reach the tumor-targeting area. Some scholars [3] have combined anti-cancer substances to achieve the dual effect of thermotherapy and drug therapy, and this process of nanomagnetic fluids both enhances the dual role of heat production and targeted drug delivery. Literature shows [4] that the ability of tumor cells to absorb magnetic nanoparticles is 8~400 times that of normal cells. After research, nanomagnetic fluid thermotherapy has made good progress in the study of glioma [5], esophageal cancer [6], lung cancer [7-9], hepatocellular carcinoma [10-11], pancreatic cancer [12-14] and breast cancer [15-17] cancers, which have not been reported in the treatment of gastric cancer. In this study, we used in vitro cultured gastric cancer cell AGS to investigate the effect of DMSO@γ-Fe_2O_3 nanomagnetic fluid thermotherapy combined with docetaxel on the biological behavior of human gastric cancer cell AGS under alternating magnetic field, which provides experimental basis for the role of nanomagnetic fluid thermotherapy combined with chemotherapy in gastric cancer treatment.

3. Materials and methods

3.1. Reagents, materials and experimental apparatus

DMSO@γ-Fe_2O_3 nanomagnetic fluid was purchased from Qinghe County Ruijiang Metal Materials Company at a concentration of 10 g/L, and the magnetic nanoparticles were 10 nm in diameter, and the main reagents used in the experiments were docetaxel (DTX), CCK8 reagent, trypsin, dimethyl sulfoxide (DMSO), 1640 medium and fetal bovine serum. The UHF electromagnetic induction heater, automatic enzyme labeling detector and flow cytometer were provided by the laboratory of the Institute of Clinical Research and Translational Medicine of Gansu Provincial Hospital, of which the frequency of the UHF electromagnetic induction heater was 1M Hz and the power was 5kW.

3.2. Experimental method

3.2.1. DMSO@γ-Fe_2O_3 nanomagnetic fluid in vitro warming experiment: DMSO@γ-Fe_2O_3 nanomagnetic fluid with a concentration of 10 g/L was diluted into nanomagnetic fluid with a concentration of 2, 3, 4, 5, 6, 7, 8 g/L in 1640 medium containing 10% fetal bovine serum, and 4 ml of each of them was added to a 25 ml culture flask with the bottom of the flask facing the center of the ultra-high-frequency magnetic induction coil, which keep 0.2 cm away from the center of the coil. The mixture was heated for 1h and the temperature was measured once every 10 min. The heating curve of the DMSO@γ-Fe_2O_3 nanomagnetic fluid was plotted with time as the horizontal coordinate and the temperature as the vertical coordinate.

3.2.2. Cell lines and cell culture: Human gastric cancer AGS cells were provided by the Laboratory of Clinical Research and Translational Medicine Institute of Gansu Provincial Hospital. AGS cells were cultured in RPMI 1640 medium (containing 10% fetal bovine serum) and placed in 5% CO2, 37℃ incubator, and passaged every 48 h. AGS cells in the growth phase were randomly divided into control group, nanomagnetic fluid hyperthermia group, chemotherapy group, and nanomagnetic fluid hyperthermia combined with chemotherapy group (referred to as thermo-chemotherapy group). The control group continued to be cultured after replacing the culture medium, the nanomagnetic fluid hyperthermia group continued to be cultured after adding different concentrations of DMSO@γ-Fe_2O_3 nanomagnetic fluid extracts and placed in a magnetic field for 1 h at 43.5 ℃ for hyperthermia and the chemotherapy group continued to be cultured for 24 h after adding the working concentration of DTX. In the thermo-chemotherapy group, the same concentration of DTX as that in the chemotherapy group was added and incubated for 1 h at 43.5°C under an alternating magnetic field.

3.2.3. CCK8 assay for cell proliferation-toxicity detection:

i. Cytotoxicity assay with different concentrations of DTX: Inoculate 2×10^3 cells per well in 96-well culture plates and culture for 24 h. Group A (drug-added): 4 different concentrations of DTX (0.05, 0.10, 0.20, 0.40 mM and the plates were placed in an incubator to continue to cultivation for 48 h. Cell viability was then measured by the CCK8 assay and the IC50 value was determined, Group A (without drug): cells were present without DTX and the cell viability was determined by the CCK8 method after incubation for 48 h. Group A (blank): there was only medium without cells and drug, and incubation was continued for 48 h. Finally, the absorbance of each group at 450 nm was determined after adding CCK8.

ii. Cytotoxicity experiments with different concentrations of DMSO@γ-Fe_2O_3 nanomagnetic fluid: 2×10^3 cells were inoculated into each well of 96-well culture plates and cultured for 24 h. Group A (drug-added): 100 μl of 2, 3, 4, 5, 6, and 7 g/L DMSO@γ-Fe_2O_3 nanomagnetic fluid was reintroduced respectively and the incubation was continued for 48 h after 1 h of inductive heating in an alternating magnetic field. Group A (without drug): Only cells continued to incubate for 48 h. Group A (blank): there was only medium without cells and drug and also continued to incubate for 48 h. Eventually, the absorbance of each group at 450 nm was determined after the addition of CCK8.

The absorbance (A value) of 96 wells was measured at 450 nm using an automatic enzyme labeling detector, and calculated according to the formula: Cell inhibition rate* (%) = [A (blank) - A (drug-added)] / [A (blank) - A (without drug)] × 100%.

3.2.4. Scratch test: The inoculated cells were grown to more than 90% of the field of view of the medium and scratched vertically. PBS was used to wash off the scratched cells and the cells were incubated with 1640 medium containing 10% fetal bovine serum for 48 h, and then photographed under a microscope.

3.2.5. Transwell invasion assay: Single-cell suspensions were prepared from logarithmic growth phase cells, counted and added into the inner chamber of the Transwell and the lower chamber was filled with complete medium. After 24 h, the Transwell was removed and the culture medium in the chamber was discarded. Washing the inner chamber of the Transwell with PBS and fixing the cells with 4% paraformaldehyde.Then wiping off the cells in the inner chamber with a cotton swab. The cells were stained with 0.1% crystalline violet, washed with PBS and observed under a microscope.

3.2.6. Detection of apoptosis by flow cytometry: Four groups of cells were selected in the logarithmic growth phase: control, nanomagnetic fluid thermotherapy, chemotherapy, thermo-chemotherapy groups. The corresponding treatment was carried out and then cultivated them in an incubator and standby, and the apoptosis of the cells in each group was observed using flow cytometry.

3.3. Statistical Processing

SPSS 21. 0 software was used to process data. Measurement data were expressed as x ± s. Comparisons between multiple groups were analyzed by one-way ANOVA, and comparisons between two groups were peerformed by t-test.

4. Results

4.1. DMSO@γ-Fe_2O_3 Nanomagnetic Fluid Warming Experiment in Vitro

The rate and peak temperature of warming of DMSO@γ-Fe_2O_3 nanomagnetic fluid with different concentrations were positively correlated with the concentration (Figure 1) under the effect of electromagnetic induction. When the concentration of DMSO@γ-Fe_2O_3 nanomagnetic fluid was ≥7 g/L, the temperature of the cell culture solution could reach more than 43.5°C. The temperature of the nanomagnetic fluid rose rapidly in the first 25 min and then increased gently between 25 and 45 minutes under an induction of alternating magnetic field, and ultimately, stabilized after the temperature peaked at 45 minutes.

4.2. Cell Culture

AGS cells were cultured in DMSO@γ-Fe_2O_3 nanomagnetic fluid at a concentration of 2 g/L for 24 h in a 37°C, 5% CO2 incubator. AGS cells were obdseved to grow well under an optical microscope (10×20) and rust-colored magnetic nanoparticles wereobdseved in the cells (Figure 2), which proved that the AGS cells phagocytosed of γ-Fe_2O_3 magnetic nanoparticles and that the magnetic nanofluid not induced by the alternating magnetic field has no toxic effect on gastric cancer cells.

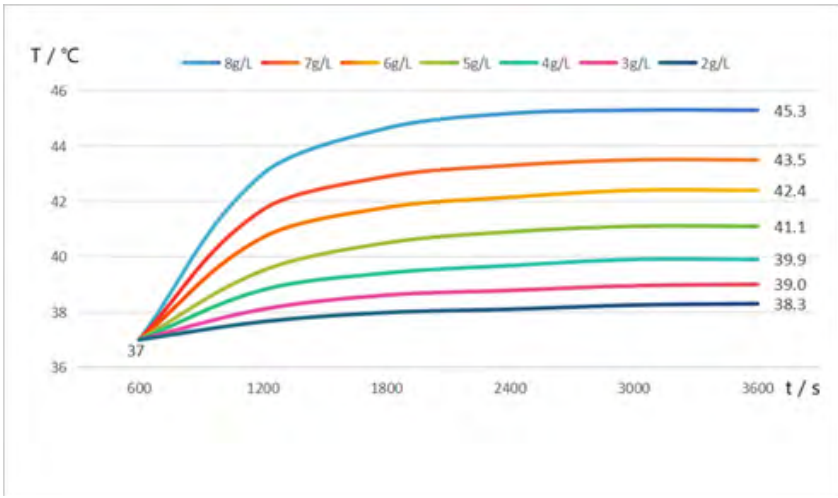

Figure 1: In vitro warming experiments with different concentrations of DMSO@γ-Fe_2O_3 nanomagnetic fluids

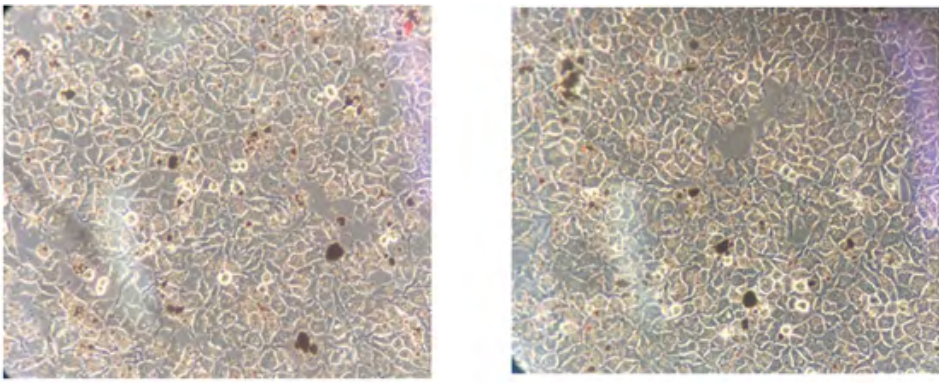

Figure 2: Phagocytosis of γ-Fe2O3 magnetic nanoparticles by AGS cells

4.3. CCK8 Experimental Results

Within a certain concentration range, the higher the drug concentration, the stronger inhibitory effect on AGS cell proliferation. When the concentration of DMSO@γ-Fe_2O_3 nanomagnetic fluid was 7 g/L, the inhibition rate of AGS cell growth could reach 58.86% after induction heating (43.5°C, 1 h) by alternating magnetic field (Figure 3a); when the concentration of DTX was 0.2 mM, the inhibition rate of AGS cell growth was 45.04% (Figure 3b).

4.4. Scratch Test

The results of the scratch tests revealed that the wound healing ability was reduced in all groups, except in the control group (Figure 4). The three experimental groups with drug addition were observed to have sparse and detached cell growth under the microscope after 48 hours of incubation, and the cell density was significantly lower than that at the very beginning, whereas the control group showed the evidently opposite trend.

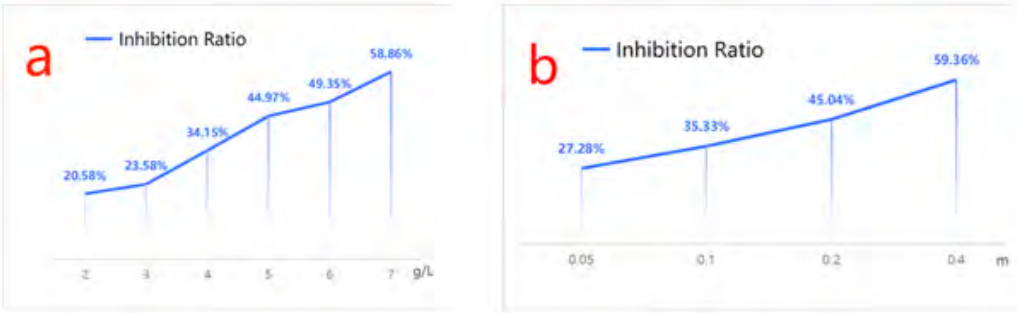

Figure 3a: Inhibition of AGS cells proliferation with a diverse range of concentrations of DMSO@γ-Fe2O3 nanomagnetic fluids in the presence of the alternating magnetic field

Figure 3b: Inhibitory effects of different concentrations of DTX on AGS cells proliferation

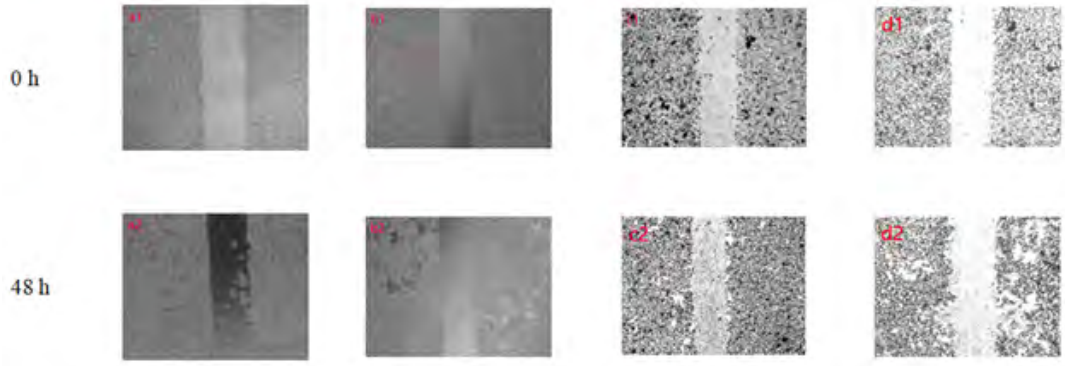

Figure 4: a. Control group; b. Chemotherapy group (DTX concentration of 0.2 mM); c. Nanomagnetic fluid hyperthermia group (DMSO@γ-Fe2O3 concentration of 7 g/L); d.Thermo-chemotherapy group (0.2 mM DTX combine with 7 g/L DMSO@γ-Fe2O3)

4.5. Transwell Invasion Assay

After AGS cells were inoculated in Transwell for 48h, it was observed (Figure 5) that the average number of membrane-penetrating cells (423.33 ± 32.58) in blank control group, (267.00 ± 31.95) in docetaxel group, (399.33 ± 17.24) in nano-magnetic fluid hyperthermia group, and (137.00 ± 33.41) in thermo-chemotherapy group, and the average average number of membrane-penetrating cells in experimental group was number of perforated cells was significantly lower than that of the control group, and the difference was statistically significant ($P < 0.05$).

4.6. Flow Cytometry Detection of Cell Apoptosis

The results detected by flow cytometry (Figure 6) showed that the apoptosis rates in the control group, chemotherapy group, nano-magnetic fluid thermotherapy group, and thermo-chemotherapy group were 6.11 %, 20.21 %, 17.67 %, and 43.80 % respectively with significant differences ($P < 0.05$).

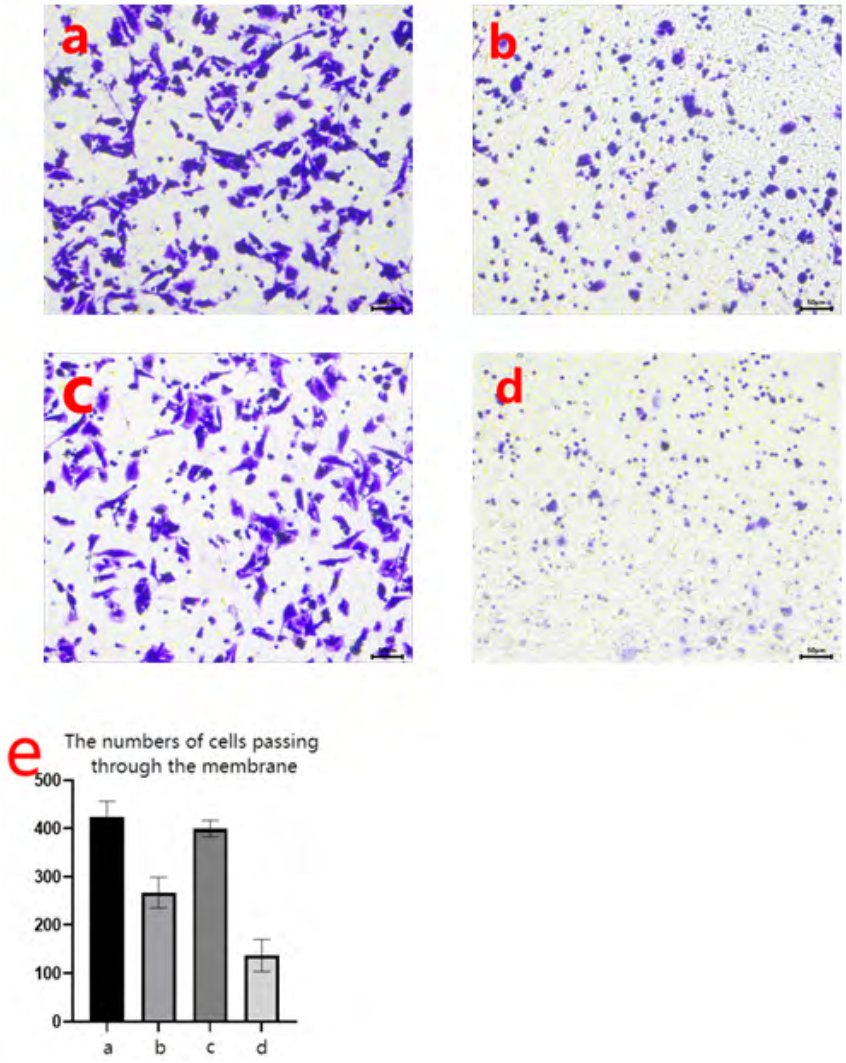

Figure 5: Results of Transwell invasion assay

a. Blank control group

b. DTX chemotherapy group

c. Nano-magnetic fluid thermotherapy group

d. Thermo-chemotherapy group

e. Statistical results of Transwell invasion experiment

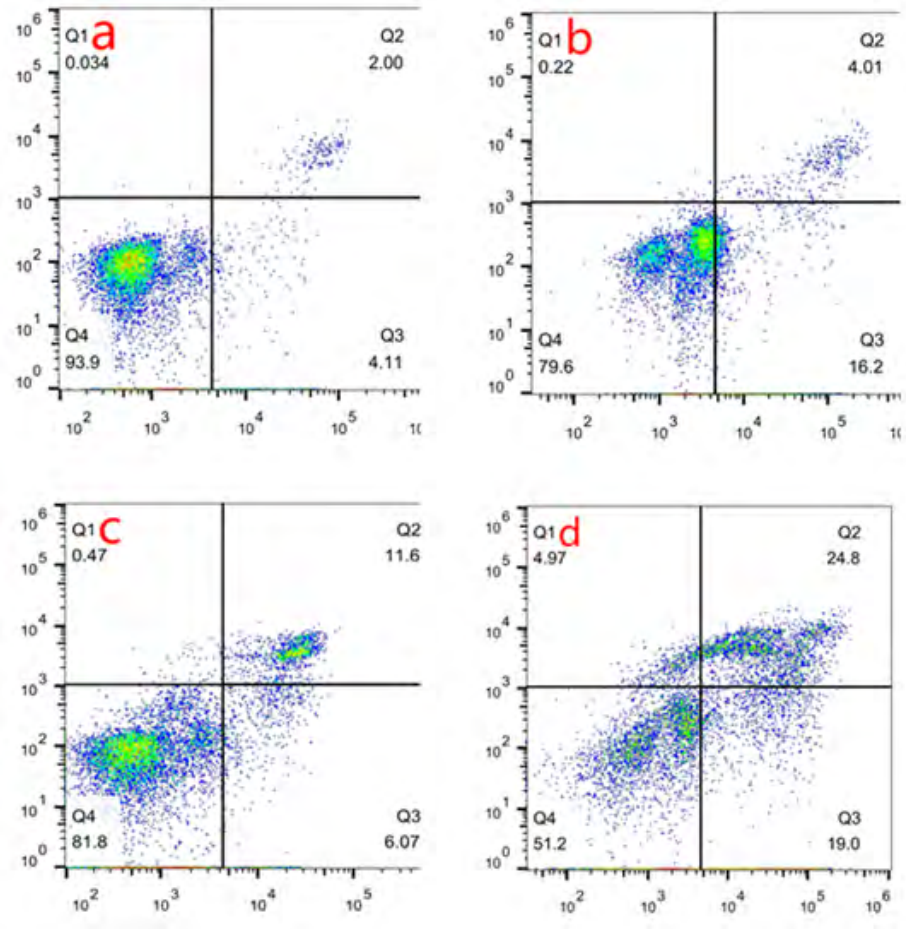

Figure 6: Results of apoptosis detection in each group by flow cytometry

a. Blank control group

b. Nano-magnetic fluid thermotherapy group

c. DTX chemotherapy group

d. Thermo-chemotherapy group

5. Discussion

Cancer cells are generally more heat-sensitive than normal cells, the temperature of the tumor tissue rises during thermotherapy and tumor cells can be killed when the treatment temperature is reached. While conventional thermotherapy may damage normal tissues and cells which surrounding tumors when the time comes to thermotherapy, however, the nanomagnetic fluid thermotherapy without this disadvantage. Nanomagnetic fluid thermotherapy induces high temperatures through colloidal suspensions of magnetic nanoparticles dispersed in a nonpolar medium [18] which are injected intravenously and then targeted to the tumor cells by magnetic localization. At the same time, magnetic particles have high microvascular permeability and interstitial diffusion effects in tumor tissues, they penetrating tumor cells and subsequently placing the tumor in a high-frequency alternating magnetic field. This induces magnetohydrothermal heating to cause thermal necrosis of the encapsulated tissues, whereas normal tissues and cells without magnetic nanoparticles do not warm up. Briefly, it destroys tumor cells by increasing their temperature while maintaining the temperature of the surrounding normal tissue cells at a reasonable level [19].

Therefore, magnetic nanoparticles can be used as thermotherapeutic agents, chemotherapeutic agents and radiation therapy enhancers. Iron oxide nanoparticles are often used as thermotherapeutic agents for magnetic fluid thermotherapy owing to their various advantages such as simplicity of preparation, variety of preparation methods and low cost. Moreover, biocompatible polymers such as DMSO are often used to prevent their aggregation or biodegradation in living organisms. DMSO is a superior solvent with high cell membrane affinity. Meanwhile, DMSO is not only a common freezing agent for freezing and storing cells during cell culture, but also a solubilization carrier for many interventional drugs [20]. Studies have shown that DMSO itself has the ability to induce differentiation, inhibit growth and promote apoptosis in cancer cells.

In this study, magnetic nanoparticles γ-Fe_2O_3 were used as a thermotherapeutic agent at a temperature of 43.5℃ with a concentration of 7 g/L, and DMSO was used as a biocompatible material to prepare the nanomagnetic fluids, magnetic nanoparticles were

embedded in the tumor site, which was inductively heated up by an alternating magnetic field to induce apoptosis of the tumor cells. At the same time, the chemotherapeutic drug DTX has been used in conjunction with enhanced therapeutic effect. The experimental results of this study show that DMSO@γ-Fe_2O_3 nanomagnetic fluid thermotherapy combined with DTX is an effective anti-tumor modality that exerts anti-tumor effects by inhibiting tumor cells proliferation and promoting apoptosis. However, the molecular mechanisms underlying its effects on anti-tumor cell proliferation and apoptosis remain to be elucidated. The synergistic effect of the two can be considered to effectively reduce the dosage of chemotherapeutic drugs, thus alleviating their toxic side effects brought about by them and improving to improve the quality of survival of cancer patients.

6. Conclusion

In this study, the effects of DMSO@γ-Fe_2O_3 nanomagnetic fluid thermotherapy combined with the chemotherapeutic drug docetaxel on the biological behavior of gastric cancer cells were analyzed by cellular experiments. These results indicate that thermotherapy combined with chemotherapy is more effective and safer than nanomagnetic fluid thermotherapy or chemotherapy alone for the treatment of gastric cancer. Therefore, this study provides a scientific basis for the clinical trials of gastric cancer treatment and a new method for the treatment of gastric cancer.

7. Funding

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