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Analysis of Mechanism of Action of Cuprous Oxide Nanoparticles in Treatment of Cervical Cancer under Real-time Ultrasound Elastography

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ABSTRACT

It aimed to explore the adoption value of cuprous oxide nanoparticles (Cu_2O NPs) in the clinical treatment of cervical cancer under the evaluation of real-time ultrasound elastography. In this experiment, the solution of Cu_2O NPs was synthesized and used in 90 selected mouse models of cervical cancer. It was found that Cu_2O NPs can significantly control the reproduction of various types of cervical cancer cells, effectively stopping the cycle of cervical cancer cell lines in the G1/G0 phase. In addition, the tumor weight of 0.25g in the Cu_2O NPs group was notably lighter than that of 1.1g in the control group. The weight change of the mouse was 21g compared with 15g of the cis-dichlorodiamine platinum (CDDP) group, and it was proved that Cu_2O NPs were less cytotoxic to the body and have few side effects. Moreover, real-time ultrasound elastography showed that there were 26 cases with a tumor elasticity score no less than 2 in the Cu_2O NPs group, accounting for 87%, which was better than that of the CDDP group (17 cases, 57%), and the difference was substantial (P<0.05). The shear wave velocity of Cu_2O NPs (1.46±0.48 m/s) was also lower than that (1.73±0.62 m/s) of the CDDP group, which suggested that the tumor body hardness of mice in the Cu_2O NPs group was lower, and the difference was considerable (P<0.05). In short, Cu_2O NPs had good functions such as inhibiting the proliferation and spread of tumor cells and blocking the cell cycle. Moreover, the toxic and side effects of the drug were slight, and it was an ideal new type of treatment for cervical cancer.

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Introduction

Cervical cancer is a malignant tumor occurring in the cervix, which ranks first in the incidence of female cancers and is extremely harmful to women's health.It is mainly caused by human papilloma virus (HPV) infection. Random sex, heredity, and premature sexual life are also important factors for the occurrence of cervical cancer (1,2). Surgical treatment is suitable for early or isolated recurrence of cervical lesions, which can be removed at one time. However, the scope of operation is large, the duration of treatment is short, and the postoperative recurrence rate is high (3). The combination of radiotherapy and chemotherapy is mostly used in middle and advanced cervical cancer, but the commonly used chemotherapy drugs often have adverse effects such as renal toxicity and bone marrow transplantation (4). Radiotherapy will cause permanent damage to the ovaries of patients (5), which can't satisfy the desire of young patients to continue to have children, affects the quality of life of patients to a certain extent, and aggravates the physical and mental burden of patients and their families. Therefore, it is necessary to find new drugs and therapies as a new treatment for cervical cancer. Nowadays, many studies found that the biological activity of nanomaterials has considerable application potential in the aspect of tumor cytotoxicity (6,7). Tumor tissue is characterized by high permeability and retention. Cuprous oxide (Cu_2O) NPs are an inorganic nano-drug that can pass through the cracks in the tumor endothelial system and accumulate the drug in the tissue (8). Aided by this, drugs can be intelligently transported in the body, with functions of targeting positioning and drug release control (9), and the toxic and side effects of drugs are also reduced at the same time. Moreover, it has good functions of inhibiting the growth and metastasis of tumor cells and blocking the cell cycle (10), which is an ideal new drug for the treatment of cervical cancer.

Once cervical cancer is diagnosed, imaging examinations should be carried out immediately to determine the stage and shape of the tumor, as well as the range and location of metastasis, so as to implement the corresponding treatment plan and not

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delay the disease. In general clinics, transvaginal ultrasound is used as the first choice of imaging examination. Ultrasonic elastography is a new type of ultrasonic examination in recent years. It uses ultrasound imaging combined with digital signal processing or digital image processing technology to apply an external force to the mass. Under the action of physical laws such as elasticity and biomechanics, biological tissues will respond and change to a certain extent, and the nature and extent of disease can be judged according to the elastic information of biological tissues (11). Ultrasound elastography has new functions that traditional ultrasound does not have, and can display and locate lesions effectively and vividly (12). It has improved the current conventional ultrasound technology and has important adoption value in the diagnosis of cervical cancer.

In this experiment, the synthetic Cu_2O NPs solution was prepared and applied to the constructed 90 cases of cervical cancer mouse models. The adoption value of Cu_2O NPs in the treatment of cervical cancer was assessed by observing the growth inhibition rate of Cu_2O NPs on tumor cell lines HeLa, SiHa, Caski, and C-33A. and the effect on cell line cycle, as well as the degree of elasticity and shear wave velocity of mouse subcutaneous tumors under ultrasound elastography.

Materials and Methods

Research objects and model construction

A total of 90 male non-obese diabetic/severe immunodeficiency mice (6-8 weeks) were selected, which were provided by The First People's Hospital of Wenling Animal Experiment Center. This experiment had been approved by the Shanghai SLAC Laboratory Animal Co., Ltd. This experiment had been approved by the Shanghai Laboratory Animal Center, Chinese Academy of Science and conformed to the standards for the feeding, management, and use of experimental animals.

Human cervical epidermal cancer cells with good growth were selected and digested with 0.25% trypsin cell digestion solution. A medium containing 10% fetal bovine serum, various amino acids, and glucose was used to stop digestion. Then, the cells were centrifuged at 1,200 rpm for five minutes, and the supernatant was removed. The human cervical epidermal cancer cells were resuspended using a blank culture medium containing various amino acids and glucose, and the total number of cells was counted. The terminal density of cells was modulated to 2×10^6 cells /mL. When the cell suspension was injected, the left thumb and forefinger were used to lift the posterior skin of the mouse's neck. Then, the little finger pressed the lower limbs and tail of the mouse, and the syringe was held in the right hand to inoculate the 100μL cell suspension subcutaneously into the left lower abdomen of the mouse to make it grow. Cu_2O NPs solution was synthesized (section 2.4), so did cis-dichlorodiamine platinum (CDDP) solution. CDDP at low temperature was added to 5% glucose injection and placed in a thermostat at a specific temperature until it was fully dissolved.

After tumor implantation, the length and short diameter of tumor body were measured weekly, and the volume of tumor was calculated. When the tumor diameter reached 6-8mm, the mice were randomly divided into three groups with equal number. The mice in experimental group were injected with 5mg/kg Cu_2O NPs solution, once a day. The mice in CDDP group were given abdominal injection of 3mg/kg CDDP solution, once every other day. Those in control group were given 5% glucose injection percutaneous, once a day. The above methods were used for five weeks.

Materials and reagents

Cervical cancer cell lines HeLa, SiHa, Caski, and C-33A were purchased from Wuhan Procell Life Science and Technology Co., Ltd.Medium containing various amino acids and glucose was purchased from Wuhan Procell Life Science and Technology Co., Ltd.Fetal bovine serum was purchased from Shanghai Kamaishu Biotechnology Co., Ltd.Phosphoric acid buffer salt solution was purchased from Hubei Wanye Pharmaceutical Co., Ltd.0.25% trypsin cell digestive liquid was purchased from Nanjing BioChannel Biotechnology Co., Ltd.Propidium iodide dyeing reagent was purchased from Fuzhou Aoyan Experimental Equipment Co., Ltd.Dimethyl sulfoxide was purchased from Shandong chuxin chemical co., LTD.

Instrument

Room temperature centrifuge was purchased from Beijing Haitian Youcheng Technology Co., Ltd. Constant temperature water bath was purchased from Jinan OLABO Scientific Instrument Co., Ltd. Vacuum drying oven was purchased from Shanghai

Dengsheng Instrument Manufacturing Co., Ltd. 4℃ refrigerator was purchased from Beijing Fuyilian Electric Co., Ltd. Liquid Nitrogen Storage Tank was purchased from Jinan OLABO Scientific Instrument Co., Ltd. Biological microscope was purchased from Jinan OLABO Scientific Instrument Co., Ltd. Hemocytometer was purchased from Yixing Xinyi Instrument Co., Ltd.

Synthesis of Cu_2O NPs

Cetane trimethylammonium bromide dissolved in water at 50℃ at 0.1mol/L and copper sulfate solution at 0.1mol/L were prepared. The 0.35mL copper sulfate solution was injected into a test tube containing 3mL cetacean trimethylammonium bromide using a micropipette and was thoroughly stirred and mixed in a constant temperature water bath. Then, it was added into 10mL 0.04mol/L sodium borohydride solution for secondary stirring. After about 18-20 hours, yellow clarified nanocrystal Cu_2O was obtained. It was placed in an ultrasonic centrifuge tube, centrifuged at 12,000g centrifugal force for 20 minutes, then the supernatant was removed and 40mL anhydrous ethanol was added. After ultrasonic blending treatment for 25 minutes, the supernatant was removed after centrifugation with 12,000g centrifugal force for 20 minutes. Then, 40mL pure water was injected, and the supernatant was removed by centrifugation at 12,000g for 20 minutes. Finally, they were put into a vacuum-drying oven to dry overnight. Wet dry Cu_2O NPs of 3mg were weighed and completely dissolved in 10% medium containing various amino acids and glucose. After being filtered through a filter membrane, it was stored in a refrigerator at 4℃.

Cell culture and observation

The thermostatic water bath was set to the standard 37℃, and the freeze tubes containing the four cell lines were taken out of the liquid nitrogen storage tank, inserted into the foam plate, and placed in the thermostatic water bath tank constantly shaken. When completely dissolved, they were placed in a centrifuge, centrifuged at 1,200 rpm for 5 minutes, and the supernatant was then discarded. Then, 5mL medium cells containing various amino acids and glucose and 10% fetal bovine serum were injected into the culture dish and gently shaken to make them fully mixed. The petri dishes were cultured in 5% carbon dioxide cells at 37℃ for 24 hours, and the liquid was changed every other day. The liquid change meant that the old medium was absorbed and discarded, and the cells were cleaned with phosphate buffer solution. Then, the phosphate buffer solution was absorbed and discarded. The above operations were repeated twice, and a new medium was added to continue the culture. When the cell density increased to 80%-90%, 0.25% trypsin cell digestion solution was applied to the above carbon dioxide incubator for digestion for about 5 minutes, and the cells were placed under a biological microscope. When most of the cells were observed to be rounded off the wall of the chamber, the digestion process was halted by a medium containing various amino acids and glucose and 10% fetal bovine serum. Then, the culture was diluted proportionally and sub-cultured. The cells were then re-suspended and prepared as single-cell suspension. 20μL cell solution was added into 180μL culture solution containing various amino acids and glucose, then one drop of the suspension was slowly dropped from the side of the blood cell counting board. After 2 minutes of reaction time, the number of cells in each cell in the four corners of the counting cell was observed and counted under a biological microscope (inverted).

For cells with good growth, 0.25% trypsin cell digestion solution was used to prepare single-cell suspension when its density increased to about 80%-90%. The cells were centrifuged for 5 minutes at 1,200 rpm to remove the supernatant. Then, they were added to the cryopreservation solution made with 10% dimethyl sulfoxide, 20% phosphate buffer solution, and 70% medium, standing at 4℃ for 30 minutes, -20℃ for 30 minutes. After the cells were left in a liquid nitrogen storage tank mouth suspended for 30 minutes, they were put into the liquid nitrogen storage.

Effects of Cu_2O NPs nanoparticles on the proliferation of cervical cancer cells

Some cells with good growth conditions were absorbed and prepared into cell suspension after digestion, centrifugation, and dilution. A completely new well plate was wet with phosphoric acid buffer solution and then added with 100μL cell suspension to each well, shaken gently to mix well and placed in a

CO2 cell incubator. Then, 60μg/mL Cu_2O NPs medium was taken and diluted into 1μg/mL, 2μg/mL, 4μg/mL, and 8μg/mL according to a certain proportion. After culture for 24 hours, the old medium was removed, and each well of the control group was injected with a medium containing various amino acids and glucose and 10% fetal bovine serum. Each well of the test group was given 100μL of 1μg/mL, 2μg/mL, 4μg/mL, and 8μg/mL Cu_2O NPs medium, respectively. After the mixed sample was left at a specific temperature for 48 hours and 72 hours, the medium was removed. 100μL of the mixture containing 10% cell counting kit-8 (CCK-8) reagent was added to the wells, and the plate was placed at 37°C, 5% carbon dioxide cell incubator at a specific temperature and left for 1 hour. Then, it was taken out and placed in a dark place for 10 minutes. After the temperature of the orifice was equal to room temperature, the cell absorbance at 450nm was measured. The professional software was employed to calculate the control rate and semi-inhibitory concentration of 1μg/mL, 2μg/mL, 4μg/mL, and 8μg/mL Cu_2O NPs on the proliferation of cervical cancer cells.

Effect of Cu_2O NPs on the cycle of cervical cancer cells

After digestion, centrifugation, and dilution, some cells with good growth conditions were absorbed and prepared into cell suspension. A new 6-well plate was taken and added with 2mL cell suspension in each well, shaken gently to mix well, then placed in a CO2 cell incubator. After 24 h of culture, the cells were attached to the wall and injected with 1μg/mL, 2μg/mL, 4μg/mL, and 8μg/mL Cu_2O NPs medium. After standing at a specific temperature for 48 hours, the supernatant was removed. Then, the cells were washed with phosphoric acid buffer solution, and trypsin cell digestive juice was added. When cells were fully digested, the digestion process was stopped with a 10% medium containing various amino acids and glucose. Centrifuged at 1,200 rpm for 5 min, the supernatant was removed and the cells were washed with phosphate buffer solution. Then, the cells were resuspended and centrifuged at 1,200 rpm for five minutes, and the supernatant was removed. After cells were left for 30 minutes at a specific temperature in a dark place, a machine was used to detect the liquid.

Ultrasound and elastography

French Supersonic Aixplorer color Doppler ultrasound diagnostic system equipped with shear wave elastography was adopted, with linear array probe, frequency of 6-10MHz, and elastography software. Before the examination, the hair of the tumor site mice was shaved, the mice were fixed on a clean experimental bench, and the tumor site was scanned in multiple sections. The size, shape, internal structure, echo, and boundary of the lesion were observed and recorded. The probe was gently touched against the surface of the skin where the tumor was, without exerting any force. When the ultrasonic diagnostic instrument was set to shear wave elastography mode, the 2D gray scale and elastic images of the tumor can also be displayed. The color-coded category was set to 0-180kPa and the probe was placed for 3-6 seconds. When the color-filled the whole sampling box, the detection was stopped. The shear wave velocity value was measured and calculated by an imaging physician with rich experience in elastic ultrasound examination. The same part was repeatedly measured three times, and the average value was taken and recorded. The criteria for elasticity score (from soft to hard) are shown in Table 1.

Table 1. Standards for shear wave elastography scoring

1 score	The lesions and surrounding tissues are uniformly blue.
2 scores	The lesions are mainly blue and green with occasional red.
3 scores	The lesions are doped with blue, green, and red in similar proportions.
4 scores	The lesions were mainly red, mixed with a little green.
5 scores	The lesions are mostly red with no or only a little green.

Statistical analysis

SPSS 24.0 was used for statistical analysis of all data in this experiment, which was expressed as mean ± standard deviation ($\bar{x}\pm s$). One-way ANOVA was used for the comparison of multiple mean values, and *t*-test was used for the comparison between groups. $P<0.05$ was statistically significant.

Results and discussion

Inhibition of different concentrations of Cu_2O NPs on cervical cancer cell lines

The CCK-8 method was used to study the inhibition of cell proliferation of HeLa, SiHa, Caski, and C-33A of cervical cancer by Cu_2O NPs after 48 hours and 72 hours at concentrations of 1μg/mL,

2μg/mL, 4μg/mL, and 8μg/mL, respectively. Figures 1 and 2 showed that Cu_2O NPs can control the reproductive ability of various types of cervical cancer cells, and it was directly proportional to the action time and concentration.

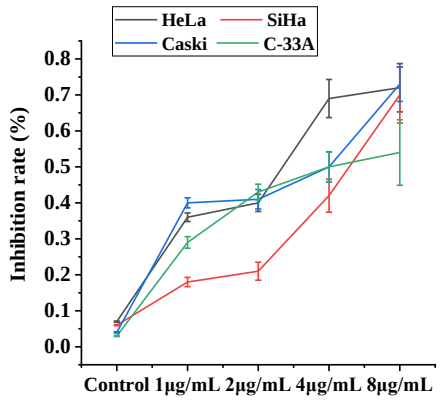

Figure 1. Inhibition rate after Cu_2O NPs treatment of 48h.

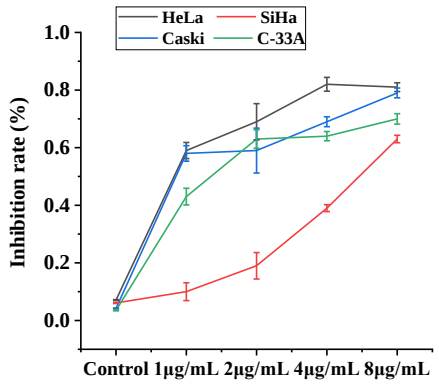

Figure 2. Inhibition rate after Cu_2O NPs treatment of 72h.

Effect of Cu_2O NPs on the cycle of cervical cancer cell lines

Given that cell cycle arrest will control the reproduction of cells, experiments were implemented to test the effects of Cu_2O NPs at concentrations of 1μg/mL, 2μg/mL, 4μg/mL, and 8μg/mL on cell cycle arrest of cervical cancer cell lines of HeLa, SiHa, Caski, and C-33A. The early stage of DNA synthesis is the G1 stage, the DNA synthesis stage is the S stage, the late DNA synthesis stage is the G2 stage, and the M stage is the cell division stage. Cells that temporarily leave the cell cycle, stop cell division and perform certain biological functions are in the G0 phase. In Figures 3-6, Cu_2O NPs can effectively arrest the cycle of various types of cervical cancer cells in the G1/G0 phase, and at the same time had the characteristics of arresting effect and concentration and content.

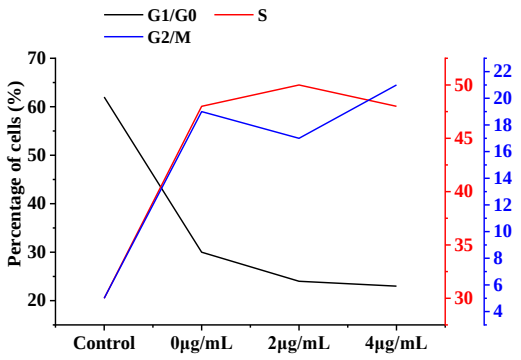

Figure 3. The effect of Cu_2O NPs on the cell cycle of cervical cancer cell HeLa.

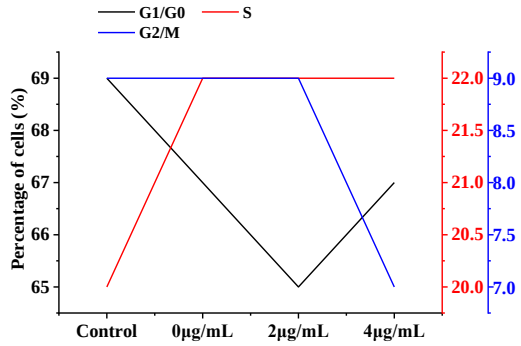

Figure 4. The effect of Cu_2O NPs on the cell cycle of cervical cancer cell SiHa.

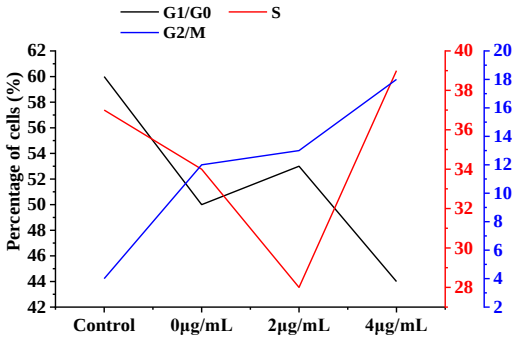

Figure 5. The effect of Cu_2O NPs on the cell cycle of cervical cancer cell Caski.

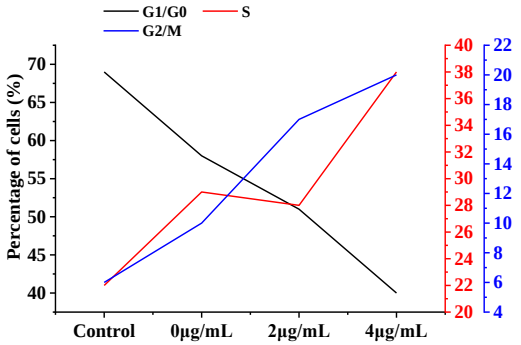

Figure 6. The effect of Cu_2O NPs on the cell cycle of cervical cancer cell C-33A.

Inhibition of tumor growth by Cu_2O NPs

CDDP has the characteristics of a broad anti-cancer spectrum, strong effect, and no cross-resistance. It is one of the most commonly used drugs in combined chemotherapy today. However, traditional chemotherapy drugs have some adverse effects such as weight loss, which affects the quality of life of the users. In Figures 7 and 8, through testing, the weight of mouse tumors was significantly lighter than that of the control group after injection of Cu_2O NPs, which inhibited tumor growth, but there was little difference from the CDDP group. However, there was almost no weight loss in mice after the usage of Cu_2O NPs. Compared with the CDDP group, Cu_2O NPs were more friendly to clinical treatment.

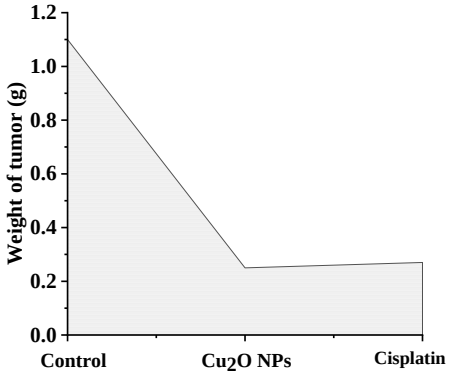

Figure 7. Tumor weights of three groups of mice.

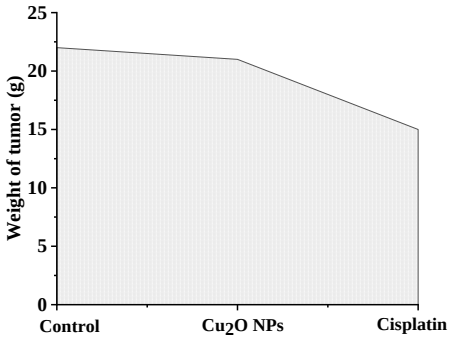

Figure 8. Body weight changes of the three groups of mice after tumor formation.

Comparison of tumor elasticity scores of three groups of mice

In Figure 9, real-time ultrasound elastography showed that there were 26 cases with an elasticity score of no more than 2 in the Cu_2O NPs group, accounting for 87% of all tumor-bearing mice. In the CDDP group, 17 cases had an elasticity score of no more than 2, accounting for 57% of all tumor-bearing mice. The tumor elasticity score of the Cu_2O NPs group was significantly better than that of the CDDP group, and the difference was great (P<0.05).

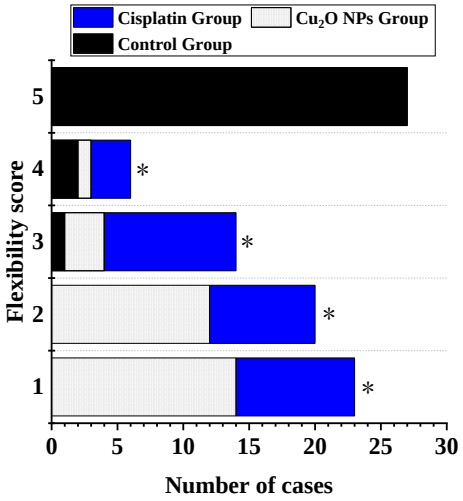

Figure 9. Comparison of tumor elasticity scores of the three groups of mice. Note: * represented that the elasticity score of the tumor body in the Cu_2O NPs group was significantly different compared with the CDDP group (P<0.05).

Comparison of the shear wave velocity of the three groups of mice

In Figure 10, real-time ultrasound elastography showed that the shear wave velocity of the Cu_2O NPs group was 1.46±0.48m/s, while the shear wave velocity of the CDDP group was 1.73±0.62m/s. The shear wave velocity of the Cu_2O NPs group was lower than that of the CDDP group, indicating that the tumor body hardness of the Cu_2O NPs group was lower than that of the CDDP group, and the difference was substantial (P<0.05).

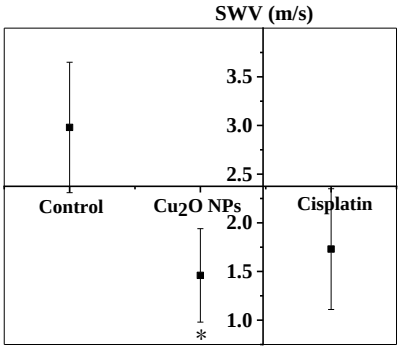

Figure 10. Comparison of the shear wave velocity of the three groups of mice. Note: * represented that the tumor hardness of the mice in the Cu_2O NPs group had a statistically significant difference compared with the CDDP group (P<0.05).

Cervical cancer is currently the only clear cause of gynecological cancer, mostly in the cervical scale column junction. The disease is caused by continuous infection of high-risk type HPV virus, which is closely related to sexual life and is the most prevalent female tumor disease (13,14). Early cervical cancer can be treated by surgery, but the duration of treatment is relatively short, and the probability of postoperative complications is high (15). Patients with advanced cervical cancer are usually treated by radiotherapy and chemotherapy. However, these traditional treatment methods have obvious deficiencies in improving the prognosis of patients and are not capable of improving the long-term survival rate of patients (16). Therefore, it is necessary to explore and study new anti-tumor drugs for cervical cancer. Inorganic nanomaterials are one of the leading research objects of anti-tumor nanomaterials. Cu_2O NP is a new type of inorganic nanomaterials. It can selectively inhibit the growth and proliferation of tumor cell lines by inhibiting the Wnt signaling pathway, inducing apoptosis of tumor cell lines, and also blocking the cycle of tumor cell lines (17,18). In addition, it has a broad clinical application prospect in the field of anti-tumor. In recent years, a new type of ultrasonic technology has gradually entered the public's vision. Real-time ultrasonic elastography converts the elastic information of biological tissue measured by an ultrasonic probe into visible images. In this way, clinicians can judge the possible pathological changes of corresponding tissues or organs, as well as the location, shape, and size of the lesions more intuitively based on the soft and hard conditions of biological tissues (19). Real-time ultrasound elastography can detect areas that traditional ultrasound can't, as well as imaging that can show the spread of disease, showing superior characteristics in the clinical diagnosis of disease.

In this study, 90 immunodeficient mice were implanted with human cervical epidermal cancer cells in their lower left abdomen. After tumor growth and maturation, mice were randomly divided into the experimental group (5mg/kg Cu_2O NPs), cisplatin group (3mg/kg CDDP), and control group (5% glucose injection). The above methods were used for five weeks, followed by a series of observational cultures and then determinations. The experimental results showed that Cu_2O NPs could significantly control the reproductive capacity of various types of cervical cancer cells, and effectively stagnated the cycle of various types of cervical cancer cells in the G1/G0 phase. Its control ability was proportional to the action time and concentration. The tumor weight of mice in the Cu_2O NPs group was significantly lighter than that in the control group, and the body weight of mice in the Cu_2O NPs group was less than that in the CDDP group. Real-time ultrasound elastography showed that the tumor elasticity score of the Cu_2O NPs group was better than that of the CDDP group, with remarkable differences ($P<0.05$). The shear wave velocity was also lower than that of the CDDP group, indicating that the tumor body hardness was lower, and the difference was significant ($P<0.05$). This result was consistent with the discussion in the article by Wang et al. (2017) (20). With Cu_2O NPs treatment, the elasticity score and shear wave velocity of the tumor were lower than that treated with traditional chemotherapy drugs, and the stiffness of the tumor tissue was not high. Moreover, the adverse side effects to the body were smaller, which was of great significance in the clinical treatment of cervical cancer.

Conclusions

In this experiment, a synthetic Cu_2O NPs solution was used. Ninety cervical cancer mouse models were divided into Cu_2O NPs group, 5% glucose control group, and CDDP group for the experiment. The experimental results showed that Cu_2O NPs could significantly control the proliferation of cervical cancer cells and inhibit the cell cycle in the G1/G0 phase, with little toxicity and side effects. Real-time ultrasound elastography showed that the Cu_2O NPs group had a relatively better tumor elasticity score and lower shear wave velocity, which meant that the tumor hardness was low. Cu_2O NP is friendly in clinical treatment and is a new treatment drug for cervical cancer that is superior to traditional chemotherapy drugs. However, the sample size collected in this experiment is small, and both observation and detection have certain limitations and one-sidedness. It is expected that further research in this direction will be carried out in the future to provide a more reliable basis for the clinical treatment of cervical cancer.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

References

1. Berman TA, Schiller JT. Human papillomavirus in cervical cancer and oropharyngeal cancer: One cause, two diseases. Cancer. 2017 Jun 15;123(12):2219-2229. doi: 10.1002/cncr.30588. Epub 2017 Mar 27. PMID: 28346680.
2. Bishop JA, Andreasen S, Hang JF, Bullock MJ, Chen TY, Franchi A, Garcia JJ, Gnepp DR, Gomez-Fernandez CR, Ihrler S, Kuo YJ, Lewis JS Jr, Magliocca KR, Pambuccian S, Sandison A, Uro-Coste E, Stelow E, Kiss K, Westra WH. HPV-related Multiphenotypic Sinonasal Carcinoma: An Expanded Series of 49 Cases of the Tumor Formerly Known as HPV-related Carcinoma With Adenoid Cystic Carcinoma-like Features. Am J Surg Pathol. 2017 Dec;41(12):1690-1701. doi: 10.1097/PAS.0000000000000944. PMID: 28877065; PMCID: PMC5680105.
3. Ge J, Sun J, Li J, Zhang Q, Lv X, Chen B. Operation for locally advanced cervical cancer after concurrent chemoradiotherapy. Int J Clin Oncol. 2020 May;25(5):948-954. doi: 10.1007/s10147-019-01585-w. Epub 2020 Mar 31. PMID: 32236755.
4. Annede P, Gouy S, Haie-Meder C, Morice P, Chargari C. Places respectives de la radiothérapie et de la chirurgie dans les cancers du col utérin [Place of radiotherapy and surgery in the treatment of cervical cancer patients]. Cancer Radiother. 2019 Oct;23(6-7):737-744. French. doi: 10.1016/j.canrad.2019.07.151. Epub 2019 Aug 24. PMID: 31455591.
5. Tan Mbbs Mrcp Frcr Md LT, Tanderup PhD K, Kirisits PhD C, de Leeuw PhD A, Nout Md PhD R, Duke Mbbs Frcr S, Seppenwoolde PhD Y, Nesvacil PhD N, Georg PhD D, Kirchheiner PhD K, Fokdal Md PhD L, Sturdza Md Frcpc A, Schmid Md M, Swamidas PhD J, van Limbergen Md PhD E, Haie-Meder Md C, Mahantshetty Md U, Jürgenliemk-Schulz Md PhD I, Lindegaard Dm DMSc JC, Pötter Md R. Image-guided Adaptive Radiotherapy in Cervical Cancer. Semin Radiat Oncol. 2019 Jul;29(3):284-298. doi: 10.1016/j.semradonc.2019.02.010. PMID: 31027645.
6. Choi D, Montermini L, Jeong H, Sharma S, Meehan B, Rak J. Mapping Subpopulations of Cancer Cell-Derived Extracellular Vesicles and Particles by Nano-Flow Cytometry. ACS Nano. 2019 Sep 24;13(9):10499-10511. doi: 10.1021/acsnano.9b04480. Epub 2019 Sep 10. PMID: 31469961.
7. Raza A, Hayat U, Rasheed T, Bilal M, Iqbal HMN. Redox-responsive nano-carriers as tumor-targeted drug delivery systems. Eur J Med Chem. 2018 Sep 5;157:705-715. doi: 10.1016/j.ejmech.2018.08.034. Epub 2018 Aug 13. PMID: 30138802.
8. Zheng F, Luo Z, Zheng C, Li J, Zeng J, Yang H, Chen J, Jin Y, Aschner M, Wu S, Zhang Q, Li H. Comparison of the neurotoxicity associated with cobalt nanoparticles and cobalt chloride in Wistar rats. Toxicol Appl Pharmacol. 2019 Apr 15;369:90-99. doi: 10.1016/j.taap.2019.03.003. Epub 2019 Mar 5. PMID: 30849457.
9. Chen X, Zhang C, Tan L, Wang J. Toxicity of Co nanoparticles on three species of marine microalgae. Environ Pollut. 2018 May;236:454-461. doi: 10.1016/j.envpol.2018.01.081. PMID: 29414370.
10. Cruz JC, Nascimento MA, Amaral HAV, Lima DSD, Teixeira APC, Lopes RP. Synthesis and characterization of cobalt nanoparticles for application in the removal of textile dye. J Environ Manage. 2019 Jul 15;242:220-228. doi: 10.1016/j.jenvman.2019.04.059. Epub 2019 May 3. PMID: 31048227.
11. Tuzun D, Ersoy R, Kilicyazgan A, Kiyak G, Yalcin S, Cakir B. Elastosonography scoring and strain index of thyroid nodules with Hurthle cells. Minerva Endocrinol. 2016 Jun;41(2):157-65. Epub 2014 Oct 13. PMID: 25310014.
12. Prada F, Del Bene M, Rampini A, Mattei L, Casali C, Vetrano IG, Gennari AG, Sdao S, Saini M, Sconfienza LM, DiMeco F. Intraoperative Strain Elastosonography in Brain Tumor Surgery. Oper Neurosurg (Hagerstown). 2019 Aug 1;17(2):227-236. doi: 10.1093/ons/opy323. PMID: 30496587.
13. Abudula A, Rouzi N, Xu L, Yang Y, Hasimu A. Tissue-based metabolomics reveals potential biomarkers for cervical carcinoma and HPV infection. Bosn J Basic Med Sci. 2020 Feb 5;20(1):78-87. doi: 10.17305/bjbms.2019.4359. PMID: 31465717; PMCID: PMC7029203.
14. Li P, Ma J, Zhang X, Guo Y, Liu Y, Li X, Zhao D, Wang Z. Cervical small cell carcinoma frequently presented in multiple high risk HPV infection and often associated with other type of epithelial tumors. Diagn Pathol. 2018 May 22;13(1):31. doi: 10.1186/s13000-018-0709-9. PMID: 29788963; PMCID: PMC5964718.
15. Nezhat C, Roman RA, Rambhatla A, Nezhat F. Reproductive and oncologic outcomes after fertility-sparing surgery for early stage cervical cancer: a systematic review. Fertil Steril. 2020 Apr;113(4):685-703. doi: 10.1016/j.fertnstert.2020.02.003. PMID: 32228873.
16. Gadducci A, Cosio S. Neoadjuvant Chemotherapy in Locally Advanced Cervical Cancer: Review of the Literature and Perspectives of Clinical Research. Anticancer Res. 2020 Sep;40(9):4819-4828. doi: 10.21873/anticanres.14485. PMID: 32878770.
17. Dong H, Li Y, Liu Y, Wen Y, Zou Z, Yang T, Cui Z, Shi D, Li Y. A nano-immunotraining strategy to enhance the tumor targeting of neutrophils via in vivo

pathogen-mimicking stimulation. Biomater Sci. 2019 Dec 1;7(12):5238-5246. doi: 10.1039/c9bm01278h. Epub 2019 Oct 11. PMID: 31602440.

18. Ertuğrul H, Yalçın B, Güneş M, Kaya B. Ameliorative effects of melatonin against nano and ionic cobalt induced genotoxicity in two *in vivo* Drosophila assays. Drug Chem Toxicol. 2020 May;43(3):279-286. doi: 10.1080/01480545.2019.1585444. Epub 2019 Mar 18. PMID: 30880493.
19. Ding D, Chen Y, Liu X, Jiang Z, Cai X, Guo SW. Diagnosing Deep Endometriosis Using Transvaginal Elastosonography. Reprod Sci. 2020 Jul;27(7):1411-1422. doi: 10.1007/s43032-019-00108-2. Epub 2020 Apr 24. PMID: 32333226.
20. Wang Y, Yang QW, Yang Q, Zhou T, Shi MF, Sun CX, Gao XX, Cheng YQ, Cui XG, Sun YH. Cuprous oxide nanoparticles inhibit prostate cancer by attenuating the stemness of cancer cells via inhibition of the Wnt signaling pathway. Int J Nanomedicine. 2017 Mar 31;12:2569-2579. doi: 10.2147/IJN.S130537. PMID: 28408824; PMCID: PMC5383080.