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The effect of ginsenoside Rg3 combined with doxorubicin on the proliferation of MHCC97-L liver cancer cells

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Abstract. Purpose: MHCC97-L liver cancer cells, as one of the common liver cancer cells, have 40% metastasis rate of lung cancer. As a commonly used and widely used drug for the treatment of various cancers on the market, doxorubicin has also shown significant effects in treating MHCC97-L cells in previous studies. Meanwhile, research has also shown that ginsenoside Rg3 can play a significant role in the treatment of MHCC97-L liver cancer cells. This study explores the therapeutic effect of the combination of ginsenoside Rg3 and doxorubicin on MHCC97-L liver cancer cells, in both vitro and vivo. Methods: The experiment will use MHCC97-L human liver cancer cell and xenograft mouse model. The cell proliferation is measured by using the CCK-8 detection method and the tumor inhibition rate of the drug in vivo experiments can be calculated formula. Possible results: There are 3 most possible results: (1)The combination of doxorubicin and ginsenoside Rg3 has stronger inhibitory effect than doxorubicin only on MHCC97-L cells, both in vitro and in vivo; (2) The combination of doxorubicin and ginsenoside Rg3 has a strong inhibitory effect in vitro experiments, but the effect is not significant in vivo experiments; (3) The combination of doxorubicin and ginsenoside Rg3 has similar inhibitory effect than doxorubicin only on MHCC97-L cells, both in vitro and in vivo. Conclusion: Our research results will provide important information for the clinical treatment of chemotherapy for lung cancer. Future research should focus on improving drug interactions in vivo and drug metabolism in vivo.

Keywords: ginsenoside Rg3, doxorubicin, MHCC97-L liver cancer cell.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common malignant tumor of the liver in adults, with an incidence of about 0. 8% in China, accounting for 55% of the global total number of patients, and the related mortality rate is second only to lung cancer[1]. At present, it is widely believed that hepatitis B or C virus infection, alcohol related cirrhosis, and non alcoholic steatohepatitis are the main risk factors for HCC4-6[2]. Among them, HCCLM3, a liver cancer cell line, has a 100% lung metastasis rate. As a liver cancer cell line with the same genetic background but different metastasis potential, MHCC97-L has a lung metastasis rate of only 40%, and is commonly used for joint research with HCCLM3 as a control group[3].

The commonly used chemotherapy drugs currently include doxorubicin, bleomycin, and mitomycin. Doxorubicin, also known as adriamycin, has significant effects on lung cancer[4]. Research has shown

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that doxorubicin can inhibit the growth of liver cancer cells, but it can stimulate cancer cells and cause a series of stress protective reactions, leading to drug resistance, and has significant cardiac toxicity. And as the drug dosage accumulates during use, heart damage continues to worsen, ultimately leading to heart failure[2].

At the same time, ginsenoside Rg3 can inhibit the growth of MHCC97-L liver cancer cells by increasing the expression of protein ARHGAP9, which can inhibit the migration and invasion of hepatocellular carcinoma cells by regulating FOXJ2/E-cadherin and its target gene CDH1[5]. It has a significant inhibitory effect on the migration and invasion of human liver cancer cells HepG2 and MHCC97-L in vitro, as well as on the growth of BABL/c nude mice HepG2 and MHCC97-L tumors[4].

Therefore, this article aims to investigate whether the simultaneous action of ginsenoside Rg3 and doxorubicin can achieve better inhibitory effects on MHCC97-L liver cancer cells in both vitro and vivo.

Hypothesis: I predict that increasing concentrations and treatment durations with Ginsenoid Rg3 combined with a fixed amount of adriamycin kills MHCC97-L HCC liver cancer cells both in vitro and in vivo in MHCC97-L xenograft mice better than adriamycin alone

2. Methods

2.1. Reagents and materials

Human liver cancer cell line MHCC97-L, DMEM medium and fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), tetramethylazolamide (MTT), Annexin V/PI apoptosis reagent, sodium dodecyl sulfate (SDS), Tris base, polyacrylamide,CCK-9 reagents, doxorubicin(ADM), and ginsenoside Rg3[1,4]. BALB/c nude mice, 6 weeks old, male, weighing 19-21 g, kept at constant temperature (25 ℃ -27 ℃), constant humidity (45% -50%), high dust and bacteria removal in fresh air, and free from specific pathogens (SPF)[6].

2.2. Equipments

Constant temperature carbon dioxide incubator, desktop high-speed freezing centrifuge, vertical pressure steam sterilizer, biosafety cabinet, inverted microscope, electric constant temperature water bath, flow cytometry, enzyme marker (450nm filter)[1,4].

2.3. Cell culture

The human liver cancer cell line MHCC97-L was cultured in a high sugar DMEM medium containing a mixture of 10% FBS, 100 U/ml penicillin, and streptomycin in a 5% carbon dioxide incubator at 37 ℃.

2.4. Establishment of subcutaneous transplanted tumors in nude mice

Take logarithmic long term MHCC97-L cells as 7x10^6 cells per nude mouse were inoculated subcutaneously in the anterior axilla[6]. When the tumor volume reached 50 mm^3, the experimental group and the control group were injected with PBS containing doxorubicin and ginsenoside Rg3, and PBS containing only doxorubicin via tail vein, respectively. Blank group only injected with PBS. PBS was injected into the blank group once every 4 days, a total of 6 times[7]. After the injection is completed, the mice are euthanized and the tumor is removed to observe the volume of the tumor in the mice.

2.5. Observing the number and size of intrahepatic metastatic tumors

After the treatment of 3 reagents(ADM, ADM+Rg3, and normal saline), the mice were euthanized using the neck removal method, and the liver of the mice was removed[7,8]. Use a vernier caliper to measure the maximum diameter *a* and minimum diameter *b* of the tumor, and calculate the tumor volume (V=*ab* ^ 2/2). Calculate the tumor inhibition rate, tumor inhibition rate (%)=(control group tumor weight - experimental group tumor weight)/control group tumor weight × 100%[6].

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2.6. CCK-8 method for detecting the effect of ginsenoside Rg3+ADM on the proliferation of MHCC97-L cells

Select MHCC97-L cells with logarithmic growth phase, centrifuge at 1000 r/min for 5 minutes, stain with trypan blue, and count on a counting plate[2]. By cell weight 1 × 10^4/well inoculated on 96 well plates, with 100 per well μ L. Experimental groups: MHCC97-L cell control group, ADM experimental group, and ADM+Rg3 experimental group, with an additional reagent control group. The MHCC97-L cell control group was cultured without any other drugs, only MHCC97-L cells. The ADM+Rg3 experimental group adds 120 μ g/mL to each well on top of the ADM experimental group of ginsenoside Rg3. Each group was equipped with 3 compound wells and incubated in an incubator for 48 hours. Add 10μ L CCK-8 solution to each hole, continue to incubate in the incubator for 4 hours[1]. The absorbance values of each well at 450 nm were detected using an enzyme-linked immunosorbent assay.

2.7. Treatment, concentrations, durations, positive and negative controls-details

2.7.1. In vitro

The concentration of ginsenoside Rg3 in the experimental group is 120 μ G/mL; The cell control group was cultured without any drugs and only contained MHCC97-L cells; The reagent control group only contains culture medium and drugs, without cells. ADM experimental group added ADM concentration of 8 μ Mol/L; The ADM+Rg3 experimental group added 120 to each well on the basis of the ADR experimental group μ G/mL of ginsenoside Rg3. Each group has 3 compound holes. After 24 and 48 hours of cultivation in the incubator, add 10 to each well μ L CCK-8 solution, continue to incubate in the incubator for 4 hours. Detect the absorbance values of each well at 450 nm using an enzyme-linked immunosorbent assay and calculate the cell proliferation inhibition rate. Cell proliferation inhibition rate=*1- [(As-Ab)/(Ac-Ab)]×100%*. Among them, *As* represents the absorbance of the experimental well (including cells, culture medium, CCK-8 solution, and drug solution); *Ac* is the absorbance of the cell control well (including cells, culture medium, CCK-8 solution, without drugs); *Ab* is the absorbance of the reagent control well (including culture medium, CCK-8 solution, drug, not cells)

2.7.2. In vivo

The experimental group received tail vein injection of PBS containing Rg3+ADM, the control group received tail vein injection of PBS containing only doxorubicin, and the blank group received tail vein injection of PBS at a dosage of 5 mg/kg doxorubicin and 120 mg/kg Rg3. Treat once every 4 days, a total of 6 times.

All experiments are repeated with at least 3 parallel groups, represented in the form of x ± s. The statistical differences between the groups were calculated using SPSS 22.0 software. $P<0.05$ indicates a significant difference between the two groups of data, while $P<0.01$ and $P<0.001$ indicate a significant difference between the two groups of data.

3. Results

Table 1. Possible results on MHCC97-L cell proliferation.

	Result1	Result2	Result3	Result4	Rsult5	Result6	Result7	Result8
Rg3+ADM using CCK-8 method	++	++	+	+	+	++	-	-
ADM only using CCK-8 method	+	+	-	-	-	+	-	-
PBS only using CCK-8 method	-	-	-	-	-	-	-	-
Rg3+ADM in mice	++	+	+	+	-	-	+	+
ADM only in mice	+	-	+	-	-	-	+	-

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Table 1. (continued).

PBS only in mice	-	-	-	-	-	-	-	-

"+" represents a significant decrease in cell proliferation, the number of "+" represent the degree of reduction in cell proliferation. "-" represent not significantly different from negative control.

Possible result 1: The combination of doxorubicin and ginsenoside Rg3 has stronger inhibitory effect than doxorubicin only on MHCC97-L cells, both in vitro and in vivo.

Possible result 2: The combination of doxorubicin and ginsenoside Rg3 has a strong inhibitory effect in vitro experiments, but the effect is not significant in vivo experiments.

Possible result 3: The combination of doxorubicin and ginsenoside Rg3 has similar inhibitory effect than doxorubicin only on MHCC97-L cells in vitro, but, in vivo, Rg3+ADM have similar effect with ADM only.

Possible result 4: Both in vivo and in vitro, there is an inhibitory effect for ADM+Rg3 on cell growth, but it is not very obvious. And for ADM only group, there is no inhibitory effect on cell growth.

Possible result 5: ADM+Rg3 can affect the cell proliferation in vitro, but it does not work well in vivo. For ADM only group, both in vivo and vitro, it has no effect.

Possible result 6: ADM+Rg3 can significantly affect the cell proliferation in vitro, compared with ADM only. But in vivo, both treatments have no effect.

Possible result 7: In vivo, ADM+Rg3 have similar effect with ADM only, but, in vitro, both treatment does not work.

Possible result 8: Rg3+ADM treatment only works at in vivo experiment.

4. Discussion

Previous studies have shown that ginsenoside Rg3 itself has inhibitory effects on the growth and metastasis of MHCC97-L liver cancer cells. However, the cost of ginsenoside Rg3 is too high, and direct purchase of ginseng can also result in minimal therapeutic effect due to insufficient concentration of ginsenoside Rg3. As an anticancer drug widely used in the treatment of various cancers, doxorubicin has certain toxicity and can cause irreversible damage to the heart[8]. This experiment combines the advantages of both, removes the dross of both, and uses doxorubicin in combination with ginsenoside Rg3 to save the cost of ginsenoside Rg3 and reduce the dosage of doxorubicin to achieve the same therapeutic effect.

Technically, under the dual mechanisms of doxorubicin embedding DNA to inhibit cell growth and ginsenoside Rg3 upregulating the expression of protein ARHGAP9 to inhibit MHCC97-L cell growth, combination therapy has effectively played an ideal role in both in vivo and in vitro experiments. And possible result 1 is the prediction for this ideal situation. However, cells may develop resistance to doxorubicin, resulting in a less significant inhibitory effect of ginsenoside Rg3 on MHCC97-L cell growth, which will lead to many different situations like possible results 2-6. In addition, the interaction between drugs cannot be ruled out, and results 7 and 8 are predictions for this mechanism.

In order to achieve the ideal state like possible result 1, in future research, it is necessary to first optimize the prevention of doxorubicin resistance. At present, the combination of doxorubicin and nanocarriers for chemotherapy has achieved initial results. By using nanocarried targeting therapy and controlling drug dosage, free drugs in other areas are minimized to avoid receptor mutations in cells after receiving doxorubicin, leading to doxorubicin resistance.

5. Conclusion

Overall, this experiment explores the feasibility of combining doxorubicin with ginsenoside Rg3 to inhibit the proliferation of MHCC97-L cells, which is theoretically extremely significant. The actual results of this experiment can not only alleviate the resistance of cancer cells to doxorubicin through multi drug therapy, but also better cure MHCC97-L liver cancer cells. Combining existing targeted cancer drugs, implementing precise targeting of cancer cells through nanocarriers is no longer a

challenge. Therefore, combining extracted components from natural plants with chemical drugs for combined treatment will gradually overcome the side effect mechanism of drug interactions and implement more precise and fierce strikes on cancer cells.

References

[1] Y. Zheng, Y. Pan, Y. Hong, M. Xiang, H. Wang, Y. Wang& T. Chen.(2012). The effect of doxorubicin on the expression of Wnt5b and Nanog in liver cancer cell line MHCC97-L. Guiyang yi xue yuan xue bao, 37(3), 235–237. https://doi.org/10.3969/j.issn.1000-2707.2012.03.005

[2] Z. Tang, M. Li, C. Zhang, et. The effect of ginsenoside Rg1 combined with doxorubicin on the proliferation and drug resistance of K562/ADR cells [J] Medical research and education, 2023, 40(1): 1 9. DOI: 10. 3969/ j. issn. 1674490X. 2023. 01. 001.

[3] Liu, Xu, Jia, Li, Ma&Ge. (2010). Expression of angiogenic mimicry genes in human liver cancer HCCLM3 and MHCC97-L cell lines with different metastatic potentials. In Shandong yi yao (Vol. 50, Issue 42, pp. 16–18). Anhui province hospital. https://doi.org/10.3969/j.issn.1002-266X.2010.42.008

[4] Sun, Song, Y.-N., Zhang, M., Zhang, C.-Y., Zhang, L.-J., & Zhang, H. (2019). Ginsenoside Rg3 inhibits the migration and invasion of liver cancer cells by increasing the protein expression of ARHGAP9. Oncology Letters, 17(1), 965–973. https://doi.org/10.3892/ol.2018.9701

[5] Zhang, Tang, Q.-F., Sun, M.-Y., Zhang, C.-Y., Zhu, J.-Y., Shen, Y.-L., Zhao, B., Shao, Z.-Y., Zhang, L.-J., & Zhang, H. (2018). ARHGAP9 suppresses the migration and invasion of hepatocellular carcinoma cells through up-regulating FOXJ2/E-cadherin. Cell Death & Disease, 9(9), 916–10. https://doi.org/10.1038/s41419-018-0976-0

[6] Zhao, Liu, Yin, Jiang, Shi, Wang& Sun. (2010). Fluid dynamics method transfection IκBαSR Inhibition of SR on Orthotopic Transplantation of Human Liver Cancer in Nude Mice. Nanjing yi ke da xue xue bao, 6, 736–740.

[7] Li, Cui, & Zhang. (2019). Chitosan nanoparticles loaded with doxorubicin can inhibit mouse osteosarcoma. Zhongguo zu zhi gong cheng yan jiu, 23(26), 4194–4199. https://doi.org/10.3969/j.issn.2095-4344.1359

[8] Luo, Li, M.-S., Teng, J.-S., Dai, X.-M., Tang, X.-X., Xing, Y., & Lü, X.-X. (2020). Adriamycin promotes the secretion of cardiac fibroblasts IL-1β and research on the mechanism of type I collagen. Zhongguo bing li sheng li za zhi, 36(9), 1543–1550. https://doi.org/10.3969/j.issn.1000-4718.2020.09.002