

How does Matrine Affect the Survival Rate of Tumor Cell Lymphocytic Leukemia Tumor Cell and its Pathway

Honghan Xu

Shenzhen Middle School, Shenzhen, Guangdong, 518023, China.

Abstract

In recent years, traditional Chinese medicine has shown an important and indispensable role in the treatment of cancer due to its small side effects and low prices. Matrine, a chemical component extracted from plants in the legume plant, has been proved to have potential therapeutic value on lymphocytic cancer. The purpose of this experiment is to study the effect of matrine on promoting tumor cells apoptosis by using Jurkat cells and MOLT-4 cells and to detect the pathways of matrine in nude mouse's Jurkat cell tumor. Thus, it can provide theoretical basis for future study and clinical treatment. In this experiment, the Annexin V-FITC / PI staining was used to detect the apoptosis ratio of tumor cells and Western blot was used to detect the pathway of matrine molecules in nude mouse's tumor cell. This proposal includes 6 possible research results, 4 of them are the possible result in survival rate of tumor cells in both in-vivo study and in-vitro study: 1) Matrine induces both cancer cells apoptosis and has the optimal concentration; 2) Matrine induces only in-vitro cancer cells apoptosis and has the optimal concentration; 3) Matrine induces only in-vivo tumor cells apoptosis and has the optimal concentration; 4) Matrine successfully induces tumor cell apoptosis, but without accurate optimal concentration. The other two results are about study in pathway: 5) p38 MAPK is activated in the matrine-induced apoptosis pathway; 6) p38 MAPK is not activated in the matrine-induced apoptosis pathway.

Keywords

Matrine, Lymphocytic leukemia tumor, Apoptosis, Pathway.

1. Introduction

Lymphocytic leukemia is a malignant tumor. Lymphoma originates in lymph nodes or lymphocytes, which is divided into two types: Non-Hodgkin Lymphoma (NHL) and Hodgkin Lymphoma (HL). Lymphoma can cause a series of symptom such as low immunity and anemia. As a plethora of research has established, malignant tumors are extremely difficult to cure. According to data from the China Cancer Registry (CCR), lymphoma has become the ninth highest incidence tumors for men. The mainstream treatments for lymphocytic leukemia are chemotherapy, targeted therapy, and hematopoietic stem cell transplantation. These treatments are expensive, causing patient suffering, and having low possibility for stem cell matching. However, the treatment of new medicine, such as traditional Chinese medicine, have the potential to greatly reduce the treatment cost and alleviate the suffering of patients.

Traditional Chinese medicine has always been thought that can only treat self-healing diseases and chronic like colds and hypertension. However, in deeper study of traditional Chinese medicine in recent years, people have discovered that traditional Chinese medicine may have the potential to treat cancer.

With the development of extraction methods, it had been found that there are a variety of active ingredients exists in traditional Chinese medicine. The matrine family is a typical active substance in

traditional Chinese medicine that has anti-cancer ability. Matrine family anti-cancer active substances includes matrine, oxymatrine, sophoridine and sophoramine. Matrine (MAT) is the main active substance isolated from the legume plant such as *Sophora flavescens*, *Sophora alopecuroides*. Matrine can be prepared by solvent extraction, ion exchange and resin adsorption. [1] People have conducted a lot of investigation and research on the anti-cancer ability of matrine. Some scientists have proved that it has the ability to cause lymphoma cell apoptosis, [2] and matrine can also reduce the adhesion rate of lymphoid tumor cells, which in turn delays cancer metastasis.[3] From this, it can be found that, matrine, an active substance, has excellent potential in the treatment of lymphocytic leukemia

P38 MAPK is a protein kinase widely presents in mammalian cells. This protein has five isomers, p38 α (p38), p38 β 1, p38 β 2, p38 γ , p38 δ ; and among them, p38 α , p38 β 1, p38 β 2 are widely present in various tissue cells. P38 MAPK protein is closely related to cell apoptosis in various tumors (such as cervical cancer, ovarian cancer, liver cancer and lymphoma). Studies have indicated that matrine can promote the apoptosis of raji cells by activating p38 MAPK protein.

This experiment is going to focus on whether matrine can cause apoptosis in two types of cancer cells (Jurkat cells and MOLT-4 cells) and the optimal concentration of apoptosis. If matrine does has the ability to cause apoptosis in these two types of cancer cells, it will be needed to investigate whether p38 MAPK is involved in the matrine-induced apoptosis pathway.[2]

According to papers on matrine and lymphoma, it can be hypothesized that matrine will reduce the survival rate and adhesion rate of tumor cells and increase the early apoptosis of tumor cells and p38 MAPK protein is involved in the regulation of matrine on lymphoma cells.

2. Materials and Methods

2.1 Chemicals, cells and animals

2.1.1 Chemicals

Matrine is prepared for this study. High purity matrine can be purchased. In the laboratory, matrine needs to be solute into an appropriate concentration. In order to determine the optimal concentration of matrine for apoptosis of lymphoma cells, solution of matrine is needed to be prepared at 0.1g/L, 0.15g/L, 0.2g/L. [3]

2.1.2 Cells

In order to test the apoptosis rate of lymphocytic leukemia tumor cell, Jurkat cell, a kind of suspension T cell leukemia cell, will be used [3]; and MOLT-4, a kind of human acute lymphoblastic leukemia cell, which will be transplanted into nude mice. [4]

2.1.3 Animals

40 3-week-old nude mouse. The mouse will be used in the transplanting of MOLT-4 cancer cell.

2.2 The in-vitro study

2.2.1 The in-vitro cell culture

Jurkat cells should be cultured overnight in a 37 ° C incubator. Ensure there is sufficient oxygen in the incubator.

2.2.2 Cell proliferation stage

In the experiment, the Jurkat cells is divided into four groups. The negative control group will be soak in 10 mL of PBS buffer. The experimental group will soak in 10 mL PBS buffer with the concentration of matrine at 0.1g/L, 0.15g/L and 0.2g/L respectively. The cells will continue to grow in the incubator for two weeks. After two weeks, the cells will be taken out and calculate the apoptosis rate of these tumor cells by Annexin V-FITC / PI staining. This experiment will be repeated at least three times.

2.2.3 Detection of apoptosis rate

Annexin V-FITC / PI staining is used in the experiment in order to detect the survival rate of Jurkat cells so that it can the apoptosis rate of cells could be calculated. With the apoptosis rate, if matrine treatment is effective can be found.

2.3 The in-vivo study

2.3.1 Transplantation of tumor cells

Culture the cell in 20% FCS (calf serum) RPMI medium with a constant temperature incubator which contain 5% carbon dioxide and 95% oxygen. [5]

Treated nude mice three times with 200 rad X-rays, after that, Molt-4 cells were injected subcutaneously. This method can greatly improve the success rate of tumor transplantation in nude mice. [6] If the success rate of tumor transplantation is too low, tumor cells in the inoculated mice can be used for transplanting again by making the tumor into new cell suspension.

2.3.2 Medicine intervention

Detect the growth of tumor with MRI, when the radius of tumor is more than 10mm, divided the mice into 5 groups. The mice were injected with saline containing 0 mg/kg, 0.01 mg/kg, 0.1 mg/kg, 1 mg/kg, and 2 mg/kg matrine every two days, and measure the radius continuously in order to determine the situation of the disease of the mice with MRI. [6]

2.4 The Study in Pathway

2.4.1 Cell culture

Jurkat cells will be used in this experiment. Jurkat cells should be cultured overnight in a 37 ° C incubator. Ensure there is sufficient oxygen in the incubator.

2.4.2 Detection of protein expression

In this experiment, Western blot will be used to detect whether p38 MAPK protein is involved in the regulation of matrine on apoptosis of Jurkat cells. The activity of p38 MAPK protein was detected before and after the involved of matrine, and whether the average activity of p38 MAPK was improved. This experiment needs to be repeated at least three times.

3. Possible Result

3.1 Result 1: Matrine induces both cancer cells apoptosis and has the optimal concentration

3.1.1 In-vitro study

In the experiment of matrine inducing apoptosis of Jurkat cells, the apoptosis rate of jurkat cells increased and showed a bell-shaped distribution. From the experimental data, it can be known that the concentration of matrine corresponding to the maximum apoptosis rate is the optimal concentration of matrine inducing apoptosis of Jurkat cells.

3.1.2 In-vivo study

In the experiment of matrine inducing the apoptosis of MOLT-4 tumors in nude mouse model, the radius of MOLT-4 tumors decreases significantly. Moreover, the decrease in tumor mass can be distributed in a bell-shape graph. According to the experimental data, it can be known that the concentration of matrine corresponding to the minimum value of tumor mass is the optimal concentration of matrine for the induction of apoptosis of MOLT-4 tumor in the nude mouse model.

3.2 Result 2: Matrine induces only in-vitro cancer cells apoptosis and has the optimal concentration

3.2.1 In-vitro study

The same as 3.1.1

3.2.2 In-vivo study

In the experiment of matrine inducing apoptosis of MOLT-4 tumors in nude mice, there was no significant change in the mass, volume and metastasis rate of MOLT-4 tumors. It requires further experimental data in determining whether matrine can induce apoptosis of nude mice's MOLT-4 tumors.

3.3 Result 3: Matrine induces only in-vivo tumor cells apoptosis and has the optimal concentration

3.3.1 In-vitro study

In the experiment of matrine inducing apoptosis of Jurkat cells, the apoptosis rate of Jurkat cells has not significantly changed. It requires further experimental data in determining whether matrine can induce apoptosis of Jurkat cancer cells.

3.3.2 In-vivo study

The same as 3.1.2

3.4 Result 4: Matrine successfully induces tumor cell apoptosis, but without accurate optimal concentration

3.4.1 In-vitro study

In the experiment of matrine inducing apoptosis of Jurkat cells, all medicine treatment groups have shown an increasing in apoptosis rate in a degree. It still requires further study in order to determine the optimal concentration of matrine that induce Jurkat cells apoptosis.

3.4.2 In-vivo study

In the experiment of matrine inducing the apoptosis of MOLT-4 tumors in nude mouse model, the mass, volume and metastasis rate of MOLT-4 tumors in all medicine treatment groups decreases significantly. But it still requires further study in order to determine the optimal concentration of matrine that induce MOLT-4 tumor cells apoptosis.

3.5 p38 MAPK is activated in the matrine-induced apoptosis pathway

P38 MAPK is activated in the matrine-induced apoptosis pathway and participates in regulating apoptosis. As p38 MAPK is the junction of apoptosis signal transduction pathways, it may be involved in the activation and regulation of multiple downstream proteins.

3.6 p38 MAPK is not activated in the matrine-induced apoptosis pathway

P38 MAPK is not activated in the matrine-induced apoptosis pathway. However, it does not represent that p38 MAPK is not involving in the regulation of apoptosis of tumor cells or the matrine pathway, and the study of specific pathway of matrine require further research.

4. Conclusion

The latest research shows that matrine not only has strong medicinal potential in the field of tumor, but also has great medicinal potential in epidemics and skin diseases. [7,8] In recent years, people have paid more and more attention to the broad anti-infection, anti-tumor and enhancement of immunity of matrine.

In recent years, many studies on the anti-tumor performance of matrine have gradually find out the pharmacological properties of matrine. Studies have shown that FAS / FASL protein plays an important role in the cell pathway of matrine-induced apoptosis.[9] Studies have also pointed out that matrine can inhibit the expression of Bcl-2 protein in cells and shift the Bax protein to mitochondria, which can promote the apoptosis of tumor cells.[10] Some studies have successfully found the optimal concentration of matrine in MDS lymphoma cell lines, and the highest apoptosis rate of SKM-1 cells induced by matrine in this research can reach 27.44%. [11]

Through the results obtained in this study, researchers can have a better understanding of the anti-tumor properties of matrine. If the result is consistent with Result 1, it can be found that matrine have good performance in both in-vivo and in-vitro experiments, and the results of best concentration are distributed in a bell-shape. This result proves that matrine has great potential in medicine. If the result is consistent with Result 2, it can be known that although matrine works well in in-vitro experiments, it has no good therapeutic effects in in-vivo experiments. It may be due to improper experimental design in the choice of concentration of medicine or other reasons, which needs further study to

confirm. If the result is consistent with Result 3, it can be found that matrine has achieved good results in-vivo experiments, but in in-vitro experiments it cannot have a good treating effect. This may be due to matrine's pathway or some other reasons. If the result is consistent with Result 4, it can be found that matrine has medical effect in each treatment group and there is no specific optimal concentration for the drug. It still needs further studies adjusting a more precise concentration range in the experiment to assure the optimal therapeutic drug concentration. In the study of the cellular pathway of matrine, if the p38 MAPK protein is involved in matrine pathway, it will confirm p38 MAPK protein's participation in the anti-tumor effect of matrine. If the p38 MAPK protein is not involved in the pathway, then it is necessary to do further studies on the effect of on other protein pathways of matrine.

References

- [1] Lai, J.P., et al., Preparative separation and determination of matrine from the Chinese medicinal plant *Sophora flavescens* Ait by molecularly imprinted solid-phase extraction. *Anal Bioanal Chem*, 2003. 375(2): p. 264-9.
- [2] Liu, Z.S., et al., [Effect and mechanism of matrine on apoptosis of Raji cells]. *Zhong Yao Cai*, 2011. 34(4): p. 576-9.
- [3] Zhang, W., B.T. Dai, and Y.H. Xu, [Effects of matrine on invasion and metastasis of leukemia cell line Jurkat]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*, 2008. 28(10): p. 907-11.
- [4] Ziegler, H.W., G. Frizzera, and F.H. Bach, Successful transplantation of a human leukemia cell line into nude mice: conditions optimizing graft acceptance. *J Natl Cancer Inst*, 1982. 68(1): p. 15-8.
- [5] Hudson, W.A., et al., Xenotransplantation of human lymphoid malignancies is optimized in mice with multiple immunologic defects. *Leukemia*, 1998. 12(12): p. 2029-33.
- [6] Hu, Z.L., et al., [Effect of matrine on mouse hepatitis and tumor necrosis factor production induced by *Propionibacterium acnes*/lipopolysaccharides]. *Yao Xue Xue Bao*, 1996. 31(9): p. 662-5.
- [7] Liu, J.Y., et al., Effect of matrine on the expression of substance P receptor and inflammatory cytokines production in human skin keratinocytes and fibroblasts. *Int Immunopharmacol*, 2007. 7(6): p. 816-23.
- [8] Zhang, Y.B., et al., Matrine-Type Alkaloids from the Roots of *Sophora flavescens* and Their Antiviral Activities against the Hepatitis B Virus. *J Nat Prod*, 2018. 81(10): p. 2259-2265.
- [9] Liang, C.Z., et al., Matrine induces caspase-dependent apoptosis in human osteosarcoma cells in vitro and in vivo through the upregulation of Bax and Fas/FasL and downregulation of Bcl-2. *Cancer Chemother Pharmacol*, 2012. 69(2): p. 317-31.
- [10] Yang, J. and S. Yao, JNK-Bcl-2/Bcl-xL-Bax/Bak Pathway Mediates the Crosstalk between Matrine-Induced Autophagy and Apoptosis via Interplay with Beclin 1. *Int J Mol Sci*, 2015. 16(10): p. 25744-58.
- [11] Gu, Y.Y., et al., Matrine induces apoptosis in multiple colorectal cancer cell lines in vitro and inhibits tumour growth with minimum side effects in vivo via Bcl-2 and caspase-3. *Phytomedicine*, 2018. 51: p. 214-225.