学 位 論 文 の 要 旨

氏名　CHEN　ZHI

学 位 論 文 名	Molecular Mechanism of Matrine From *Sophora Alopecuroides* in the Reversing Effect of Multi-anticancer Drug Resistance in K562/ADR Cells
発 表 雑 誌 名 （巻，初頁～終頁，年）	BioMed Research International (in press)
著 者 名	Zhi Chen, Nobuhiro Nishimura, Takayuki Okamoto, Koichiro Wada, and Kohji Naora

論 文 内 容 の 要 旨

INTRODUCTION

Matrine is the main alkaloid derived from herbal medicine *Sophora alopecuroides*. Its anti-tumor effect has been widely concerned in recent years. Many researchers have revealed that matrine has a cytotoxic effect on cancer cells due to inhibiting the proliferation of the cells and induce apoptosis. Now an anti-cancer injection containing matrine named "Compound Kushen Injection" has been applied clinically in China. Recently, some researches indicated that matrine might have more valuable properties that sensitize resistant cancer cells to chemotherapeutic agents. However, there is still no systematic research illustrating the mechanism for suppressing the effect of matrine on drug resistance.

Multidrug resistance is a notorious mechanism that induces cancer cells to develop resistance against chemotherapies. Drug transport associated with multidrug resistance is mainly the export of anticancer drugs from cells inside by adenosine triphosphate-binding cassette (ABC) transporters. Especially, activation of ABCB1 is a common mechanism for cellular resistance to doxorubicin (DOX), paclitaxel (PTX), and vinblastine. It is noticeable that a relevant research had found that silencing NF-kappa B can attenuate activation of ABCB1. Deregulated apoptosis may be responsible for multidrug resistance in cancer therapy. Intrinsic apoptosis is involved in the cleavage of caspase-3 and -9. B cell lymphoma 2 (Bcl-2) proteins family is playing critical roles in regulating intrinsic

apoptosis. Therefore, NF-kappa B, as an up-stream protein of Bcl-2 proteins, can inhibit the intrinsic apoptotic pathway. Collectively, activation of NF-kappa B can probably simultaneously induce drug export and hamper apoptosis, leading to drug resistance.

In this study, we aimed at clarifying if the non-toxic concentration of matrine could enhance the anticancer drugs in multidrug resistant cells. Moreover, we also attempt to figure out the mechanism by which matrine restores multidrug resistance. Finally, the effects of matrine on the expression and function of drug exporting transporters and apoptosis-relating proteins were examined.

MATERIALS AND METHODS

We evaluated the reversing effect of matrine on chemo-resistant leukemia K562/ADR cells and its intact K562 cells. Matrine in a range of the non-toxic concentration was employed in the whole study. IC_{50}s of cancer medicines were tested using WST-8 assay. Drug export and apoptotic rates were examined using flow cytometry. The mRNA and protein expressions of ABC transporters and apoptosis-relating proteins were quantified by quantitative real-time PCR and Western blotting, respectively.

RESULTS AND DISCUSSION

To test the non-toxic concentration range in K562 cells and its chemoresistant subtype K562/ADR cells, firstly, we examined the cytotoxicity of matrine for 48 h by WST-8 assay. IC_{50} of matrine on K562 cells was 3.14 folds higher than on K562/ADR cells. IC_5s of matrine on the two kinds of cells were regarded as the maximum value of the non-toxic concentration. Therefore, we set 300 μmol/L as the maximum concentration of matrine, which ensures that over 95% of the two kinds of cells would be viable at the same time, in all the following experiments.

Based on the non-toxic concentration range of matrine, cytotoxicity of DOX or PTX in the combination of matrine for 48 h were tested. The reversal fold value represents how much resistance in K562/ADR cells was reversed. In contrast to K562 cells, K562/ADR cells had exhibited much stronger resistance against DOX. In the combination of 200 and 300 μmol/L matrine, reversal fold values in K562/ADR cells were 2.30 and 2.88, respectively, indicating more than doubled sensitization to DOX. However, no effect of matrine on the IC_{50}s of DOX in the parental cells was observed. This trend was also found in the IC_{50}s of PTX.

Apoptotic rate of cells induced by DOX for 16 h in K562/ADR cells was severely prohibited compared to K562 cells. Though matrine alone had no significant effect on neither K562 cells or K562/ADR cells, it could dramatically enhance both the early and

late apoptotic rates of cells in the combination of DOX.

To examine whether matrine could reverse resistance of DOX and PTX in K562/ADR cells through inhibiting the function of ABCB1, we measured the intracellular levels of rhodamine 123, an ABCB1 substrate, in the presence or absence of matrine. The accumulation of rhodamine 123 in K562/ADR cells was much lower than that in K562 cells. Furthermore, matrine improved the intracellular levels of rhodamine 123 in both cell lines. However, improvement in K562/ADR cells (increased by 133.6%) was much more significant than in K562 cells (increased by 9.6%).

Expression of ABCB1 mRNA in K562/ADR cells was 6.3 folds higher than that in K562 cells, while increased expression was not found in ABCC1 and ABCG2. The ABCB1 mRNA level in K562/ADR cells was decreased to 87.5% by exposure to matrine. Protein expression of ABCB1 in K562/ADR cells was 4.85 folds high as that in K562 cells, which was decreased by 25.2% under the treatment of matrine. Under the treatment of DOX for 48 h, NF-kappa B expression was 25% higher and phosphorylated NF-kappa B was 30% (though not statistically significant) higher in K562/ADR cells in comparison to K562 cells. In the combination of matrine, phosphorylated NF-kappa B was decreased to 75% in the resistant cells, which recovered to the level in the parental cells.

As an executor of apoptosis, protein expression of cleaved caspase-3 in K562/ADR cells was approximately one-third of that in K562 cells. With the treatment of matrine, the expression in the resistant cells was improved by 32%. As for an intrinsic apoptotic factor caspase-9, in K562 cells, the protein expression level of caspase-9 was 15% lower and cleaved caspase-9 was 85% higher than that in K562/ADR cells. In the combination of matrine, the expression of caspase-9 and cleaved caspase-9 in resistant cells were improved by 19% and 42%, respectively. Survivin, which can inhibit the caspase-3 and caspase-9 activation, was significantly suppressed by matrine in resistant cells. Expression of Bcl-xL, a suppressor of caspase-9 activation, was lower in K562 cells than in K562/ADR cells. Matrine down-regulated the expression of Bcl-xL into 69% in the resistant cells.

CONCLUSION

In conclusion, our study demonstrated that matrine resensitized multidrug resistant K562/ADR cells through two ways: re-activating apoptosis and inhibiting drug efflux. Matrine can down-regulate phosphorylation of NF-kappa B to recover pro-apoptotic factor and suppress anti-apoptotic factors, leading to facilitated intrinsic apoptosis. In addition, matrine can down-regulate ABCB1 expression to induce diminished drug efflux, which may be also related to the suppressed NF-kappa B.