論文の内容の要旨

論文題目

Traditional Chinese medicine and its active compounds perform inhibitory effect and synergic effect with chemotherapeutics in hepatocellular carcinoma

（漢方薬とその有効成分の肝細胞がんに対する抑制効果及び化学療法併用によるシナジー効果）

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HCC is one of the most severe cancers worldwide with high morbidity and mortality. Surgery and chemotherapy are the preferred therapeutic strategies for advanced HCC. However, for metastasis and the drug resistant nature of terminal HCC, patients with advanced HCC are not suitable for surgery and there have been few effective chemotherapeutic agents for HCC treatment. Thus, exploration of combination chemotherapeutic strategies is considered to contribute to the development of chemotherapy for HCC. The previous studies of our laboratory illuminated that cinobufacini and its compounds bufalin and cinobufagin could inhibit HCC growth by inducing Fas- and mitochondria-mediated apoptosis pathways. So we wondered whether combination of cinobufacini and doxorubicin could achieve better antitumor effects than monotherapy.

In the beginning, doxorubicin was used in combination with cinobufacini, bufalin, and cinobufagin respectively. The experimental results suggested that combination of cinobufacini and doxorubicin showed a stronger growth inhibition ability than either cinobufacini or doxorubicin alone, and the results had significant differences (p<0.05). Meanwhile, bufalin and cinobufagin have been proven to have weaker inhibitory activity than cinobufacini on HCC cells, and the combination of them with doxorubicin had a similar suppressive effect compared to doxorubicin and cinobufacini.

In comparison, there was an interesting result that cinobufacini had a better anti-proliferation activity than peer-to-peer concentrations of bufalin and cinobufagin, and cinobufacini combined with doxorubicin also did a better job than bufalin or cinobufagin combined with doxorubicin. As we know, cinobufacini contains a number of bufadienolide cardiotonic steroids, such as bufalin, cinobufagin,

resibufogenin, and telocinobufagin, which play a role as tumor inhibitors together. Thus, we supposed that cinobufacini as a mixture could cause a better anti-tumor effect than particular components alone and even a simple mixture of two components such as mixture of bufalin and cinobufagin.

Going a step further, we added a mixture of bufalin and cinobufagin to our project. The experimental results exhibited that the inhibitory effect of cinobufacini was stronger than the mixture of components, and the mixture of components caused a better effect than one component alone. In addition, the suppressive activity of the mixture could not compare to all of the components. This means the effect of bufalin and cinobufagin could not reach two fold of bufalin or cinobufagin separately. Because bufalin and cinobufagin target the same pathway, it may be that a limited number of targets limit the effect of their mixture.

Previous research by our laboratory proved that cinobufacini, bufalin and cinobufagin all could induce apoptosis by activating mitochondria-mediated and Fas-mediated apoptosis pathways. In our previous research we focused on expression of protein but not on RNA expression, and we were wondering whether cinobufacini and its components could affect the transcription of apoptosis-related genes. Thus, we picked up four apoptosis-related genes and their corresponding proteins, Bcl-2, Bid, Bax, and cytochrome c which are major elements in apoptotic pathways. The real-time PCR assay and Western blot assays were performed synchronously. In the results of the present study, the RNA expression level of Bcl-2 decreased, but that of Bax, Bid, and cytochrome c increased. The protein expression level of Bcl-2 and Bid decreased, but that of Bax and cytochrome c increased. Bid should transfer to t-Bid molecules to get into mitochondria, and the decreasing amount of Bid suggested that an increasing amount of it penetrated as the t-Bid form. The low level of Bcl-2 attenuated its inhibitory effect on apoptosis, and meanwhile the high level of Bax further antagonized the Bcl-2 and promoted the apoptosis pathway in mitochondria. Cytochrome c was released by mitochondria into cytoplasm. In the cytoplasm, it activated downstream elements of caspase-9, caspase 3, and PARP, and then caused DNA fragmentation. Based on the previous studies of our laboratory, the change of Bid was associated with activation of the Fas pathway, and this pathway also could activate a caspase-8/10/3-related cascade reaction. Through both Fas- and mitochondria-mediated pathways, combination reagents induced apoptosis of HCC cells. As a result, these reagents could not only affect protein expression but also interrupt RNA expression. To do both things simultaneously made the anti-tumor activity more efficient.

Above all, our research suggests that cinobufacini and its active compounds could inhibit HCC cells by activating mitochondrial and Fas-related signaling pathways, the combination of cinobufacini and doxorubicin could reach a better inhibitory effect than any other reagents respectively and the apoptotic effect of cinobufacini was stronger than particular bufalin, cinobufagin, or a mixture of them alone. Thus, the combination of cinobufacini with the chemotherapeutic drug could be a better way to improve the treatment effect and life quality of HCC patients. In the future, more traditional medicines and related active compounds would involve in the combination therapy for various cancers and other diseases.