

Abstract

The overall objective of this research is to understand the formation and principle of Chinese medicine formulae “Yanhusuo San” (YHSS), as well as the anticancer effect and mechanism of YHSS. Currently, the results about the integral effects of Chinese medicine formulae on cancer cell proliferation and metastasis are usually controversial. The effects of the whole Chinese medicine formulae vs. single herbs on the various periods of cancer metastasis were not well evaluated. The combinatorial effects of the components of Chinese medicine formulae were investigated to seek the methods to calculate the synergistic antitumor effect and the potential application of these methods to evaluate the cancer therapy with Chinese medicine.

Another goal of this research project is to identify the active compounds in YHSS and to investigate their anti-metastasis and anti-angiogenesis mechanism. In this thesis, the following questions have been addressed: the anti-proliferation effect of Ezhu and Yanhusuo (chapter 2), the anti-angiogenic effect of Yanhusuo (chapter 6), the antimetastatic effect of both Yanhusuo and Ezhu (chapter 4 and 5), and the mechanism of YHSS on cancer suppression.

The results showed that both Ezhu and Yanhusuo could induce the cell death and inhibit the cell proliferation on five cancer cell lines. In cell invasion assay section, we found that Yanhusuo extract inhibited the migration and invasion of MDA-MB-231 cells *in vitro*. In addition, Yanhusuo extract inhibited the mRNA expression and activity of metalloproteinase 9 (MMP9). The anti-cancer metastasis effect of Yanhusuo involves in activation of p38 and inhibition of ERK1/2 and SAPK/JNK mitogen-activated protein kinases (MAPKs) signaling. Our experiments have demonstrated the biological activity of Yanhusuo against cancer metastasis *in vitro* and provided a rationale for its further investigation.

In the anti-angiogenic study, in order to determine the active anti-angiogenic compounds in Yanhusuo, we studied the antiproliferative activities of several main constituents of Yanhusuo, which belong to a group of protoberberine alkaloids, on HUVECs and identified berberine as a powerful angiogenesis inhibitor in Yanhusuo. Both Yanhusuo extract and its active compound berberine significantly suppressed the VEGF-induced up regulation of matrix metalloproteinase 2 (MMP2) at both mRNA and protein levels. Their

functional effects, including the inhibition of MMP2, were shown to be mediated through VEGF-triggered ERK1/2 pathways. Our findings have provided novel insights into the antiangiogenic effects of Yanhusuo and berberine, and offered scientific basis for their traditional clinical application as a cancer treatment.

In vitro synergy analysis of anticancer agents is a useful method to determine the ratio and/or dose of drugs in clinical combination therapy. But these methods are hardly used to evaluate the composition of traditional Chinese medicine formulations. Isobologram and combination index (CI) analyses were used for evaluation the combination effect of Yanhusuo and Ezhu in different ratios. Our results indicated that the combination of two herbal extracts exhibited the strongest anti-cancer cell proliferation effect at the ratio of 3:2 (Ezhu to Yanhusuo). Furthermore, Boyden chamber assay, flowcytometry, and fluorescent microscopy were used to study the cell invasion ability, cell cycle distribution and cytochrome c release of the combination in the ratio of 3:2. Measuring the ERK1/2 (extracellular signal-regulated kinase 1/2), p-ERK1/2 and p-Rb by western blotting analysis showed that the combination effect of Yanhusuo and Ezhu was resulted from suppressing the expression of p-ERK.

As neither isobologram nor CI analyses provide output of concentration-effect curves for investigator evaluation, our third objective was to develop a method to calculate the combinations not just in a single level. A modified coefficient of drug interaction (CDI) was used to evaluate the combinatorial anticancer effect between the two pure compounds from Yanhusuo. In comparison with isobologram and CI methods, the results from CDI methods for synergetic efficacy evaluation between two compounds on cancer cell in vitro was in accordant with other two methods, and CDI is applicable to evaluate the synergetic effects of drugs by more than two different sites. The results implied potential benefits for applying the modified CDI method to calculate the combinations for more than two elements.

In summary, our studies have elucidated the antitumor mechanism of the Yanhusuo San in vitro and provided helpful information to explain the anticancer metastasis effects of Yanhusuo San. In addition, drug combination analysis and synergism have dealt with methods that are closely tied to the definition of additivity and the departures from additivity that are consequences of this definition. Our studies were intended to investigate the drug combination analysis and synergism of anticancer CMs, which involved in the dose response of the individual agents. Ultimately, our results could offer the reference for the clinical use of YHSS and provide a suitable method to the synergism investigation of a Chinese medicinal formula in anticancer research.