学 位 論 文 の 要 旨

氏名　DAXIA（ダシャ）

学 位 論 文 名	Antifungal Activity and Mechanism of Action of Ou-gon (*Scutellaria* root extract) Components Against Pathogenic Fungi
発 表 雑 誌 名 （巻，初頁～終頁，年）	Scientific Reports (In press)
著 者 名	Xia Da, Yayoi Nishiyama, Duerna Tie, Kyaw Zaw Hein, Osamu Yamamoto, Eishin Morita

論 文 内 容 の 要 旨

INTRODUCTION

Fungal infections are potentially fatal complications associated with the use of popular immunosuppressive drugs, including anticancer drugs, and hence, the development of safe antifungal drugs for clinical use would greatly benefit patients. The rise in the incidence of fungal infections has exacerbated the need for next-generation antifungal agents, as many of the currently available drugs have undesirable side effects, are ineffective against new or re-emerging fungal strains, or lead to rapid development of resistance. Moreover, fungal infections can be dangerous for patients who receive anticancer or immunosuppressive agents, including immunocompromised patients, because immunosuppression renders the patient vulnerable to viral, bacterial, and fungal infections. Therefore, the discovery of antifungal drugs with low toxicity, broad spectrum of activity, and a new mode of action is becoming increasingly important.

We previously evaluated the antifungal activity of 61 commercially available Kampo medicines towards *Trichophyton rubrum* using the microbroth dilution assay. Seven of these medicines exhibited antifungal activity, with six containing Ou-gon, an extract from the roots of *Scutellaria baicalensis* Georgi. Furthermore, crude Ou-gon extract exhibited pronounced antifungal activity. Ou-gon is one of the popular crude drugs in Kampo medicine, traditionally used in the Far East because of its anti-inflammatory, antimicrobial, and anti-allergic activities. The identification of the active constituents of Ou-gon would facilitate their synthesis and structural modification to obtain active components with enhanced efficacy and potential

therapeutic usefulness. The current study aimed to identify antifungal components in Ou-gon and to determine their mechanism of action against pathogenic fungi.

MATERIALS AND METHOD

Antifungal activity was assessed by the microbroth dilution method using four common human pathogenic fungi, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, and *Candida albicans*. Components of crude Ou-gon extract were separated by reversed-phase high-performance liquid chromatography. Active antifungal components were identified by liquid chromatography-electrospray ionization tandem mass spectrometry. Terminal deoxynucleotidyl transferase dUTP nick end-labelling assay (TUNEL), SYTOX® green uptake assay, determination of intracellular reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) as well as microscopy (confocal laser microscopy, scanning and transmission electron microscopy (SEM, TEM)) were used to probe the mode of action.

RESULTS AND DISCUSSION

The current study demonstrated that baicalein and wogonin, the major flavones in Ou-gon extracts, possess potent antifungal activity against pathogenic fungi, as determined by the microbroth dilution assay. The SYTOX® green uptake assay was used to detect cell damage, revealing structural disintegration of the plasma membrane. Upon exposure to baicalein and wogonin, the clear SYTOX® green staining of fungal cells indicated a loss of cell membrane integrity. The TUNEL assay was used to determine the induction of apoptotic DNA fragmentation. TUNEL staining of baicalein- and wogonin-treated fungal cells suggested the presence of apoptotic DNA breaks. Apoptosis as a mechanism of baicalein- and wogonin-induced cell death was supported by the finding that the fungal cells produced ROS upon exposure to these compounds. ROS accumulation is considered one of the primary biochemical causes of apoptosis. Inappropriate regulation of ROS levels can damage cells, leading to abnormal fungal growth and consequential apoptotic-like cell death. Changes in the MMP, indicating the opening of transition pores in the mitochondrial membrane and release of apoptogenic factors into the cytosol, are considered another characteristic of apoptosis. Intracellular ROS accumulation and MMP reduction can be regarded as the keys to the antifungal activity of baicalein and wogonin. Apoptosis-like programmed cell death thus likely constitutes the antifungal mechanism of baicalein and wogonin.

Further evidence of ultrastructural changes of fungal cells associated with baicalein and wogonin exposure was obtained from SEM and TEM analyses. The two compounds induced different ultrastructural changes. The degeneration of cytoplasmic organelles and efflux of cytosolic contents in baicalein-treated hyphae as indicated by SEM and TEM suggest that baicalein possibly induced disturbance of the plasma and intracytoplasmic membrane synthesis,

thereby impairing membrane function and leading to morphological changes. The partial swelling, and shrinkage or cracking of the cell wall found in wogonin-treated fungal cells suggest that wogonin perturbs the biosynthesis of cell wall components, thus inhibiting cell wall synthesis.

Importantly, baicalein and wogonin exert nearly no or minor cytotoxic effect on healthy human cells. We therefore suggest that baicalein and wogonin are attractive candidates that might replace, or be used synergistically with, antimicrobial drugs, including anticancer drugs or immunosuppressive agents, to treat the ensuing fungal infections.

In future, it will be necessary to test the antifungal activities of baicalein and wogonin against other pathomycetes and to evaluate the effects of baicalein and wogonin against fungal infections, e.g., tinea pedis, candidiasis, and aspergillosis, *in vivo*. Furthermore, investigation of the effects of baicalein and wogonin on fungal gene expression may improve our understanding of their structural differences, specificity, and antifungal mechanisms of action. Finally, further identification and enhancement of additional biological activities of baicalein and wogonin would expand their application potential.

CONCLUSION

In summary, our findings suggest that baicalein and wogonin are major compounds with antifungal activity in Kampo medicine that elicit apoptosis-like programmed cell death in pathogenic fungi. The antifungal effects of baicalein and wogonin may lead to the development of new and safe treatment strategies, especially for the clinical treatment of infections caused by pathogenic filamentous fungi.