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Abstract of Thesis/Dissertation

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Title :

Efficacy of piperaceae plant extracts and microorganism-derived compounds: metacytofilin and kijimicin against *Toxoplasma gondii*

(トキソプラズマ・ゴンディに対するコショウ科植物抽出物、微生物由来化合物メタサイトフィリンおよびキジマイシンの効果)

Abstract

Toxoplasmosis is one of the most important diseases in public health causing the severe life-threatening particularly in immunocompromised patients and in congenital infection. The treatments are required for the severe clinical signs or congenital toxoplasmosis. The current treatment regiments using the combination of pyrimethamine and sulfadiazine are limited because of the lower efficacy and the side effects. Therefore, searching the new substances is required for the treatment. Natural products such as plants and microorganism are the major sources of drug discovery because the secondary metabolites have diversity of bioactive and pharmacological properties. The aim of my thesis is to evaluate the crude extracts from piperaceae plants as plant source, kijimicin isolated from bacteria *Actinomadura* sp. and metacytofilin isolated from fungi *Metarhizium* spp. on anti-*Toxoplasma* activity in an *in vitro* and *in vivo*.

Herbal medicines and natural herb extracts are widely used as alternative treatments for various parasitic diseases, and such extracts may also have potential to decrease the side effects of the standard drugs used to treat toxoplasmosis (sulfadiazine-pyrimethamine combination). In chapter 1, I evaluated how effective the Thai piperaceae plants, *Piper betle*, *P. nigrum* and *P. sarmentosum*, are against *Toxoplasma gondii* infection *in vitro* and *in vivo*.

At first, I extracted the piperaceae plants with ethanol, passed them through a rotary evaporator and then lyophilized them to obtain crude extracts. The *in vitro* study based on half maximal inhibitory concentration (IC_{50}) indicated that the *P. betle* extract was the most effective on inhibition of parasite growth in human foreskin fibroblast (HFF) cells (IC_{50} on RH-GFP strain of *T. gondii*: 23.2 μg/ml, IC_{50} on PLK-GFP strain: 21.4 μg/ml). Furthermore, treatment of experimental mice with the *P. betle* extract for 7 days after infection with 1,000 tachyzoites of the *T. gondii* PLK strain increased mouse survival (survival rates: 100% in 400 mg/kg-treated, 83.3% in 100 mg/kg-treated, 33.3% in 25 mg/kg-treated, 33.3% in untreated mice). Furthermore, treatment with 400 mg/kg of the *P. betle* extract resulted in 100% mouse survival following infection with 100,000 tachyzoites. The present study shows that *P. betle* extract has the potential to act as a medical plant for the treatment of toxoplasmosis.

In chapter 2, kijimicin obtained from bacterium *Actinomadura* sp. MI215-NF3 was tested. Kijimicin, which represents an important type of ionophore group, has antibacterial activity against Gram-positive bacteria. Kijimicin inhibits human immunodeficiency virus replication in both acute and chronic infections. Additionally, kijimicin inhibits *Eimeria tenella* efficiently, compared with monensin or salinomycin. This study was carried out to evaluate the anti-*Toxoplasma* activity of kijimicin in an *in vitro* and *in vivo*. *In vitro*, the cytotoxicity on HFF cells and anti-*Toxoplasma* activity of kijimicin were compared with monensin, which is representative of ionophore, and anti-*Toxoplasma* drugs such as azithromycin, clindamycin, spiramycin and sulfadiazine. IC_{50} on anti-*Toxoplasma* activity of kijimicin, monensin and clindamycin was 45.6 nM, 1.3 nM and 238.5 nM, respectively. Furthermore, kijimicin and monensin inhibited the invasion of parasites (IC_{50} = 216.6 pM and 513.1 pM, respectively). The results from transmission electron microscopy showed that kijimicin-treated parasites found the swollen of the parasites and several vacuole-like structures. These appearance of ultrastructure changings are also observed in monensin-treated *T. gondii*. *In vivo*, the *Toxoplasma*-infected mice with 5,000 tachyzoites of the *T. gondii* PLK strain were treated with kijimicin or PBS by intraperitoneal injection for 7 days, and then morbidity and mortality for 30 days post infection (dpi) were observed. Kijimicin-treated groups at 10 and 3 mg/kg/day showed 91.7% and 66.7% mouse survival, respectively. On the other hand, all mice died within 18 dpi in PBS-treated group. Additionally, kijimicin-treated groups significantly decreased the clinical signs during 7 to 17 dpi. This approach allowed the

validation of kijimicin as inhibitors of *T. gondii* growth. From our results, kijimicin could inhibit *T. gondii* growth both in an *in vitro* and *in vivo*, increase survival rate of infected-mice, and decrease clinical signs compared to PBS-treated group.

In chapter 3, metacytofilin (MCF) identified from the fungi *Metarhizium* sp. TA2759 was evaluated on anti-*Toxoplasma* activity in an *in vitro* and *in vivo*. The IC_{50} of MCF on intracellular and extracellular *T. gondii* was 1.2 μM and 2.4 μM, respectively. Selectivity index of MCF was 141 and 69.5 for intracellular and extracellular *T. gondii*, respectively. Electron microscopy analysis showed that the electron-dense coat structure, the generation of vacuole-like structures and cellular debris which were filled inside the parasite cells were found in the MCF-treated *T. gondii* tachyzoites. Furthermore, treatment of mice with MCF by intraperitoneal route for 7 days after infection with 5,000 tachyzoites of the *T. gondii* PLK strain increased mouse survival (survival rates: 100% in 30 mg/kg-treated, 83.3% in 10 mg/kg-treated, 33.3% in 3 mg/kg-treated, 0% in untreated mice). In this infection model, the survival rate of the infected mice treated with sulfadiazine 400 mg/l through drinking water for 7 days as a standard treatment was 100%. Next, treatment of the mice with MCF by oral route for 7 days after the infection at the same condition was evaluated. The all mice treated with MCF at 30 and 10 mg/kg/day survived while all mice in the PBS-treated group died within 11 days after the infection. MCF treatment by intraperitoneal route and oral route decreased clinical signs of the infected mice compared with those of the control animals. Moreover, anti-*Toxoplasma* effects of MCF were tested in pregnant mice. In the PBS-treated group, one mouse died at 10 days after the infection with 5,000 tachyzoites of PLK strain and the higher clinical scores were seen during 7 to 30 dpi. On the other hand, all mice survived and the clinical symptoms were decreased in the group of MCF treatment at 10 mg/kg/day. The birthrate of the infected-pregnant mice treated with MCF was 85.7% while all mice did not have the offspring in PBS-treated group. Thus, this result indicated anti-*Toxoplasma* activity of MCF in the pregnant mice. Together, MCF exerts excellent activities against *T. gondii* infection.

From the present results, I elucidated that *P. betle* crude extract, kijimicin and MCF had anti-*Toxoplasma* activity in both *in vitro* and *in vivo*. These materials and compounds will be valuable sources for future development of novel anti-*Toxoplasma* drug.