Form No.5

Abstract of Thesis/Dissertation

Applicant

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Title: **Vaccine development based on gene-edited parasite of *Neospora caninum* and identification of drug candidates from wild medical plants for control of protozoan infection**

（遺伝子編集したネオスポラカニナムを基盤としたワクチン開発および原虫感染に対する野生薬用植物からの薬剤候補の同定）

Abstract

Neosporosis is a parasitic disease affecting the health of dogs and cattle worldwide. It is caused by *Neospora caninum*, an obligate intracellular apicomplexan parasite. Dogs are its definitive host, it mostly infects livestock animals, especially cattle that acts as an intermediate host. It is necessary to have well-established models of abortion and vertical transmission in experimental animals, in order to determine basic control measures for the *N. caninum* infection. I evaluated the role of *N. caninum* dense granule antigen 7 (NcGRA7) in the vertical transmission of *N. caninum* using the C57BL/6 pregnant mouse model. I inoculated mice on day 3.5 of pregnancy with parental Nc1 or NcGRA7-deficient parasites (NcGRA7KO). Post-mortem analyses were performed on day 30 after birth and the surviving pups were kept until day 30 postpartum. The number of parasites in brain tissues of offspring from NcGRA7KO-infected dams was significantly lower than that of the Nc1-infected dams under two infection doses (1×10^6 and 1×10^5 tachyzoites/mouse). The vertical transmission rates in the NcGRA7KO-infected gr

oup were significantly lower than those of the Nc1-infected group. To understand the mechanism by which the lack of NcGRA7 decreased the vertical transmission, pregnant mice were sacrificed on day 13.5 of pregnancy (10 days after infection). Although parasite DNA was detected in the placentas, no significant difference was found between the two parasite lines. Histopathological analysis revealed a greater inflammatory response in the placentas from NcGRA7KO-infected dams than in those from the parental strain. This finding correlated with upregulated chemokine mRNA expression for CCL2, CCL8, and CXCL9 in the placentas from the NcGRA7KO-infected mice. In conclusion, these results suggest that loss of NcGRA7 triggers an inflammatory response in the placenta, resulting in decreased vertical transmission of *N. caninum*.

Live vaccination is the most protective method against bovine neosporosis, which is the major cause of bovine abortion globally. Here, parenteral stain Nc1 and less virulent NcGRA7KO line were evaluated as potential live vaccines. Pregnant and non-pregnant BALB/c mice were subcutaneously inoculated with high (1×10^5) or low (1×10^4) doses of tachyzoites. Non-pregnant mice were challenged intraperitoneally with 2×10^6 Nc1-GFP tachyzoites at 45 days post-vaccination (dpv), and postmortem examination was performed at 30 days post-challenge infection (76 dpv). At both doses, NcGRA7KO-vaccinated animals showed higher bodyweight gain and fewer clinical signs than unvaccinated animals. Although there was no significant difference in the survival rate among experimental groups, the parasite burden in the brain of vaccinated animals was lower than that of unvaccinated animals. For pregnancy conditions, mating was started at 3 weeks post-vaccination, and challenge was performed with 1×10^5 Nc1-GFP at day 10.5 of pregnancy. Postmortem examination was performed at 30 days postpartum. For high-dose vaccination, there was no significant difference in the survival rates of offspring between vaccinated (27.8%) and unvaccinated dams (39.1%), whereas for low-dose vaccination, the offspring from vaccinated dams had a significantly higher survival rate (75.0%) than those from unvaccinated dams (6.9%). The amount of challenge (Nc1-GFP) parasite DNA in the brain of offspring from vaccinated dams was significantly lower than that of

offspring from unvaccinated dams. These results suggest that NcGRA7KO parasites have potential for use as a live attenuated vaccine against *N. caninum* infection.

Since there is no available chemotherapy against different protozoan infections as toxoplasmosis, Neosporosis and malaria, treatment with medicinal plants will help to overcome the drug toxicity and parasite resistance compared with the commercially used drugs. Wild medicinal plants from the desert have been traditionally used as antimicrobial agents in Egypt. However, little is known about the antiprotozoal efficacy of these plants. Here, I evaluated the in vitro activity of extracts from certain wild Egyptian desert plants against *Toxoplasma gondii* and *N. caninum*. Of the 12 plant extracts tested, the methanolic extracts from *Artemisia judaica*, *Cleome droserifolia*, *Trichodesma africanum*, and *Vachellia tortilis* demonstrated potent activity against the growth of *T. gondii*, with half-maximal inhibitory concentrations 50 (IC_{50}) of 2.1 μg/ml, 12.5 μg/ml, 21.8 μg/ml, and 24.5 μg/ml, respectively. Their mean selectivity index (SI) values were 150.8, 29.6, 18.9, and 22.6, respectively. These extracts, along with an ethanolic extract of *P. undulata*, were then evaluated against *N. caninum*. *C. droserifolia*, an ethanolic extract of *P. undulata*, *T. africanum*, *A. judaica*, and *V. tortilis* demonstrated potent efficacy against the growth of *N. caninum* in vitro, with mean IC_{50}s of 1.0 μg/ml, 3.0 μg/ml, 3.1 μg/ml, 8.6 μg/ml, and 17.2 μg/ml, respectively. Their mean SI values were 370.9, 18.5, 133.2, 36.8, and 32.2, respectively. These findings indicate that *A. judaica* is most potent against *T. gondii*, while both *C. droserifolia* and *T. africanum* extracts were more effective to *N. caninum*. Our data suggest these extracts could provide an alternative treatment for *T. gondii* and *N. caninum* infections.

Finding a novel treatment for malaria is still challenging, and various extracts from different wild desert plants have been reported to have multiple medicinal uses for human public health, in my study, I had evaluated the antimalarial efficacy of several Egyptian plant extracts. I assessed the cytotoxic potential of 13 plant extracts and their abilities to inhibit the in vitro growth of *Plasmodium falciparum* (3D7), and to treat infection with non-lethal *Plasmodium yoelii* 17XNL in an in vivo malaria model in BALB/c mice. In vitr

o screening identified four promising candidates, *T. africanum*, *A. judaica*, *C. droserifolia*, and *V. tortilis*, with weak-to-moderate activity against P. *falci parum* erythrocytic blood stages with mean IC_{50} of 11.7 μg/ml, 20.0 μg/ml, 32.1 μg/ml, and 40.0 μg/ml, respectively. Their mean SI values were 35.2, 15.8, 11.5, and 13.8, respectively. Morphological alterations, such as cell s hrinkage, and parasite fragmentation were observed following treatment wit h plant extract concentration of 50 μg/ml as well as after treatment with th e positive control drug, chloroquine, in comparison with untreated parasites. Among these four candidates, *T. africanum* crude extract exhibited the hig hest parasite suppression in a murine malaria model against *P. yoelii*. As it causes the significantly reduced level of parasitemia observed from 6-15 days post-infection after oral treatment. My study identified novel natural an timalarial agents of plant origin that have the potential for development into therapeutics for treating malaria.

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