論 文 の 内 容 の 要 旨

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論文題目　**Studies on the effects of sulfated polysaccharides on malaria infection**

（マラリア感染への硫酸化多糖類の効果に関する研究）

Malaria is a global concern. Concerted efforts have been geared to eradicate malaria especially in endemic areas, which are mostly developing countries. Vaccines that can prevent malaria infection are currently under testing while the screening for new antimalarial drugs is also a continuing process. Most of the antimalarial drugs that are currently being used during the blood stage of malaria when the *Plasmodium* parasites have already invaded red blood cells (RBCs) and the clinical symptoms of disease, including fever, are already presented. So there is a current interest in developing vaccines and drugs that can prevent the invasion of RBCs, so that the infection is halted altogether. Heparin is a sulfated glycosaminoglycan that can inhibit the entry of the *Plasmodium* parasites into the RBCs. However, it is not recommended for use in clinical malaria for its anticoagulant effects. Other sulfated polysaccharides like carrageenans from seaweeds were shown to have the same inhibitory effect on *Plasmodium* parasites

in vitro. But their effect *in vivo* are not yet extensively studied. This work explored a novel anticoagulant, gellan sulfate, derived from the microbial polysaccharide,gellan gum, and other derivatives of carrageenans on their effects on the growth and invasion of malaria parasites *in vitro* and *in vivo*.

Gellan sulfate and oversulfated κ-carrageenan were prepared by adding DMF-SO_3 to gellan gum. λ-Carrageenan was modified by acid hydrolysis. The sulfation of gellan and κ-carrageenan were determined by nuclear magnetic resonance spectroscopy and the level of sulfation were measured by elemental analysis.

Gellan sulfate, but not oversulfated κ-carrageenan and hydrolyzed λ-carrageenan, nor the native gellan gum, was shown to inhibit the growth and invasion of RBCs by *P. falciparum* in *in vitro* inhibition assays. Gellan sulfate was also shown to have low cytotoxicity and anticoagulant effects and thus, it was further assessed in *in vivo* studies using rodent malaria models.

In the 4-day suppressive test of growth *in vivo*, BALB/c and C57BL/6 mice were infected with the lethal parasites, *P. yoelii* 17XL and *P. berghei* ANKA, respectively. Gellan sulfate and native gellan gum were tested against artesunate (20mg/kg), an artemisinin derivative which is one of the recommended treatments for severe malaria. With various tested doses, of 20, 25, and 50mg/kg given to mice intraperitoneally, gellan sulfate and native gellan gum were found to be ineffective in inhibiting growth of the parasites *in vivo*. The parasitemias continued to rise after suppressive treatment and the mice eventually succumbed to the disease from 6 to 30 days post-infection. In addition, at 50mg/kg, gellan gum and gellan sulfate treated mice were observed to have weight loss and ruffled hair. This might indicate that at this dose, gellan gum and gellan sulfate were toxic to the mice.

Carrageenans have been shown to inhibit the growth and invasion of RBCs by *P. falciparum in vitro*. However, λ-carrageenan was also shown to increase the permeability of the blood brain barrier when administered to rats. Therefore, the use of carrageenans in malaria may actually cause cerebral malaria (CM). In this work, I examined the effect of carrageenans on the development of CM in the BALB/c mouse, considered as resistant strain, using *P. berghei* ANKA, a standard model for experimental CM. It is found that λ-carrageenan (25mg/kg) can induce symptoms and histopathological lesions related to CM.