論　文　内　容　要　旨

Antimicrobial Photodynamic Therapy with the photosensitizer TONS504 eradicates *Acanthamoeba*

（アカントアメーバに対する光線力学的抗微生物化学療法（PACT）

の効果）

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Acanthamoeba keratitis was first known as a sight threatening corneal infection caused by free living organism Acanthamoeba spp. Acanthamoeba spp. is ubiquitous and has two form, active infected trophozoite form and dormant cyst form. Recently treatment for Acanthamoeba keratitis is consists of debridement of focal lesion and administration of biguanides, diamidines, and antifungal drugs. Moreover, these treatments usually failed to treat AK and also harmful to the cornea. Photodynamic therapy was known as a treatment modality for oncological diseases. Besides the oncological disease, it also could be applied to inactivate microorganism. Photodynamic is consists of combination of photosensitizer and light irradiation. Upon the irradiation the photosensitizers were transfer their energy directly to the substrate or protein in the cells. Currently, we have been developing photodynamic antimicrobial chemotherapy (PACT) for infectious keratitis. Our PACT is consist of photosensitizer, TONS504, a cationic chlorine derivative, it has a molecular weight of 1116.9 and an absorption spectrum about 667 nm. The light source used was LED with wavelength 660 nm. In this study we aimed to investigate the invitro efficacy of TONS504-PACT against Acanthamoeba *castellani* trophozoite and cyst, microorganism caused Acanthamoeba keratitis. Acanthamoeba were grown in agar with lawn of *E. coli*, until reach 96% exponential growth of trophozoite form. Encystment were induced by transferred the trophozoite form to Acanthamoeba saline. Acanthamoeba trophozoite and cysts form were then pelleted and centrifuge in 1000 g for 10 minutes. PACT with TONS504 (1 mg/L and 10 mg/L for trophozoite and 10-20 mg/L for cyst) and irradiation (10 J/cm^2 (single 3-min exposure) and 30 J/cm^2 (three 3-min exposures separated by two 1-min rest periods) for trophozoite and 30 J/cm^2 and 60 J/cm^2 for cyst were applied to the Acanthamoeba in liquid medium. After treatment, acanthamoeba activity was observed by culture and apoptosis/necrosis immunostaining. Our study demonstrated that combination of TONS504-PACT in the highest dose of TONS504 10mg/L and irradiation energy 10-30 J/cm2 inhibited the trophozoites growth about 68% and 77%, respectively. Regarding the inhibition of Acanthamoeba cyst, it was required higher dose of TONS504 and irradiation energy, our TONS504-PACT could eliminate 54% of cyst in dose 20 mg/L of TONS5404 and 60J/cm2 for irradiation energy. Immunofluorescence was also revealed that Acanthamoeba trophozoite and cyst was killed by apoptosis and necrosis process after being exposed by TONS504-PACT. These results suggest that TONS504-PACT can induce death of Acanthamoeba by a mechanism dependent on TONS504 concentration and light energy. Trophozoites were substantially more susceptible to TONS504–aPDT than cysts, likely reflecting the ability of the photosensitizer to bind to or accumulate within the targeted stage of the *Acanthamoeba* life cycle. Further studies are thus warranted to evaluate the efficacy of TONS504–PACT in animal model.