**Background and Purpose**

Acanthamoeba infection in immunocompromised patients may attack the central nervous system and immunocompetent subjects may develop Acanthamoeba keratitis (AK). AK incidences have been reported with increasing frequency worldwide [1-3], particularly in contact lens wearers [4, 5]. AK is a serious, sight-threatening disease, in which patients need long-term treatment. However, up-to-date, no standardized treatment is available. All antiamoebic drugs in clinical use are off-label.

In order to optimize clinical treatment of AK, the purpose of this study was to analyze the concentration dependent effect of biguanides (polyhexamethylene biguanid (PHMB)), hexamidine-diisethionat (HD), propanidin-isoethionate (PD), dichlorodimethyl-dithiocarbamate (DD), miltefosine (MF), povidone iodine (PVP-I), and chlorin e6 photodynamic therapy (PDT) [4-8] on Acanthamoeba castellanii trophozoites and cysts, in vitro.

**Materials and Methods**

Acanthamoeba castellanii 1BU strain was cultured in 712 peptide-yeast extract-glucose (PYG) medium. Thereafter, trophozoites or cysts were cultured in 0.005-0.02% PHMB, CH or 0.25-0.1% HD, PD, DD, or 5% NM or 0.001625-0.00065% MF or 0.25-1% PVP-I containing PYG medium for 4 hours or underwent Chlorin e6-PDT (Table 1).

The percentage of dead trophozoites was determined by CytoTox 96 Non-Radioactive Cytotoxicity assay and trypan blue staining, and those of dead cysts using trypan blue staining. Treated cysts were also inoculated on non-nutrient agar Eshericia coli (CH) or 0.25-0.1% HD, PD, DD, or 5% NM or 0.001625-0.00065% MF. Then, the percentage of encysted trophozoites was determined (Figure 1).

**Results**

All concentrations of different antiamoebic agents had a significant cytotoxic effect on AK trophozoites and cysts (p<0.05), except 0.02% PHMB or CH, for trophozoites, and 0.005% CH or Ce6-PDT for cysts, using trypan blue assay (Figures 1-3).

Observing the agar plates, PHMB, CH, HD, PD, NM and PVP-I led to morphological changes of Acanthamoeba trophozoites, which could not form cysts again within 5 weeks (Figure 4). The strange-shaped structures appeared after 24-72 hours, and could move out from the center to the peripheral area of the plate. Some of them had double wall (like cysts), with discontinuous outer wall (Figure 5). DD and MF treated cysts could excret and later encyst again (Figure 4).

**Conclusions**

In vitro analysis of treatment efficacy of different antiamoebic agents, especially the non-nutrient agar Eschericia coli plate assay may provide us information on specific treatment of different Acanthamoeba strains.

18U Acanthamoeba castellanii was more vulnerable to PHMB, CH, HD, PD, NM and PVP-I treatment than DD and MF. However, none of these agents could completely eradicate trophozoites and cysts.