Abstact

In-vitro investigation of the effectivity of Polyhexamethylene biguanide and Pentamidine isethionate bound to gold nanoparticles on growth inhibition or elimination of trophozoites and cysts of some *Acanthamoeba* genotypes

Introduction: The opportunistic free living amoebas, wide spread in natural resources and usually live in warm and polluted water and humid soils. *Acanthamoeba* species can cause severe disease, including amoebic keratitis (AK), granulomatous encephalitis and cutaneous ulcers. The treatment includes Propamidine Isethionate, Polyhexamethylene Beguanide (PHMB) and Panthamidine Isethionate in single or combination. Despite many studies, definitive treatment for amoebic keratitis has not been reported due to the trophozoite transformation into resistant cysts and consequently, cysts' resistance to the drug.

The most commonly drugs, gold conjugated Polyhexamethylene Beguanide and Pentamidine Isethionate were used as single gold nanoparticles well developed surface chemistry and chemical stability which are suitable and usable tools in this biological study. The gold nanoparticles act as a drug carrier and the drug molecules act as a nanoparticle stabilizer. Binding of gold nanoparticles to the above drug compounds can increase the efficacy of the drug. Infact, one of the most important objectives of the present study is to determine the antiacanthamoebic potency of gold conjugated drugs in *Acanthamoeba* T4 and T11 genotypes; trophozoites and cysts *in vitro*.

Materials and Methods: During 1396-1398, lenses and corneal chip samples were collected from fifty patients with suspected corneal keratitis in Tehran medical centers. Then they were cultured on non-nutrient agar. After microscopic examination, cloning and separation of microorganisms were performed on DNA extracted using PCR method and sequencing of free amoebic genotypes were identified. Two positive samples with T4 and T11 genotypes were selected. In order to synthesize Au-conjugated PHMB nanoparticles, the gold chloride solution (III) was added to PHMB solution in cold condition and reduced by injection of Sodium Borohydride granules. The toxicity of PHMB, Au-PHMB and Pentamidine Isethionate was evaluated by using MTT method. Characterization of gold nanoparticles attached to PHMB was performed using measurement by TEM as an evaluating method in stability studies.

Results: The effect of Pentamidine Isethionate with IC50 470 µM was able to kill 50% of *Acanthamoeba* cysts and with IC50 97.4 µM was able to kill 50% of trophozoites. However, in MTT

assay the IC50 of Pentamidine isethionate on Vero cells was 115.4µM after 24 h. As a result, he drug has a high toxicity which prevented the drug conjugation.

In the present study, the effect of Au-PHMB on the trophozoites of *Acanthamoeba*; T4 and T11 genotypes was more effective than PHMB ($P < 0.05$). Also the effect of Au-PHMB on *Acanthamoeba* T4 genotypes cysts was more effective compared to PHMB. But there was no significant difference ($P > 0.05$). However, the effect of Au-PHMB cysts of the T11genotype was more effective than the the free form of the drug with a significant difference ($P < 0.05$). The effect of Pentamidine isethionate on T4 *Acanthamoeba* cysts and trophozoites in different concentrations had shown significant difference ($P < 0.05$).

Discussion: Our results in evaluation of the pathogenicity of T4 genotype trophozoite with Vero cells by different concentrations of Au-PHMB in comparison to PHMB, show the importance of 12.5 µM concentration, because Au-PHMB was able to kill more parasites compared to PHMB. According to the statistical analysis, ANOVA shows a significant difference between the groups of 25, 50 100 and 200 µM and confirms that in these concentrations, due to gold toxicity not only parasite, but also Vero cells were killed.

In assessing the pathogenicity of T11 genotype trophozoites, Vero cells and certain concentrations of Au-PHMP and PHMB, according to ANOVA statistical analysis, the concentration of 12.5 µM is important, because Au-PHMB kills more parasites than PHMB and more Vero cells survive. These results also indicate that, gold killed Vero cells due to the toxicity as well as the parasites. The results of pathogenicity evaluation of *Acanthamoeba* T4 and T11 genotypes by osmotolerane and thermotolerance tests using Au-PHMB and PHMB, show less resistance in *Acanthamoeba* T11 genotype. The pathogenicity evaluation results of Pentamidine Isethionate on cysts of *Acanthamoeba* T4 genotype, showed high resistance to effective concentrations of the drug.

Conclusion: The increased lethality of the Au-PHMB is due to high reaction of nanoparticles with living cells, as well as the easy and convenient transfer of the conjugated form into the living cell and the effectiveness increase of the drug. However, the exact mechanism of enhancing the lethal effects of amoeba with a gold nanoparticle conjugated drug has not been well understood.

Keywords: Treatment, Drug, *Acanthamoeba*, Polyhexamethylene Beguanide, Pentamidine Isethionate, Gold nanoparticles, Trophozoite, Cyst, *in vitro*