Abstract

Evaluation of inhibitory effects of antisense oligomers on 3D7 *Plasmodium falciparum* Rh5 gene expression to assess its inhibitoryrole against malaria parasite evasion to red blood cells and growthinhibitory effects

Introduction: Malaria is a life-threatening disease. For the past 50 years, *Plasmodium falciparum's* resistance to therapeutic drugs has threatened all major malaria control gains. Since most chemical drugs have many side effects and also some of these drugs are contraindicated in pregnant mothers, so the use of drugs that do not have side effects and also have a high anti-parasitic effect seems necessary. Recently, new techniques have been identified that allow the manipulation of gene expression. Therapeutic antisense technology is one of the new ways to overcome the therapeutic problems in diseases whose treatment is threatened by drug resistance and also there is no effective vaccine to control and treat them.

Materials and Methods: Cultivation of *Plasmodium falciparum* parasite was performed by Tranger & Jensen method on the 3D7 strain. RH5 gene studies were performed by Vector NTI 10 software and mfold and sfold forms, and the best gene position was selected for antisense design of oligonucleotides, and 2-generation antisense (2-O-methyl) was designed in Gamper form. Drug susceptibility testing of IC50 to chloroquine was performed on *Plasmodium falciparum* strain 3D7. Oligonucleotide antisense with concentrations of 1, 0.5, 0.1, 0.05, 0.01µM on *Plasmodium falciparum* with 0.6% parasitism and 5% hematocrit was affected *in vitro*; the microscopic effect of both chloroquine and oligonucleotide antisense was examined at concentrations of 0.2, 0.1 and 0.05µM respectively on *Plasmodium falciparum in vitro*. Then the effect of antisense on Plasmodium and the effect of chloroquine and antisense together were performed by real-time PCR.

Results: The effect of different concentrations of antisense against different regions of RH5 mRNA showed that in antisense against translation region (-7-11) and the region between intron and exon gene (77-58), concentrations higher than 0.01 µM caused more than 85% inhibition on parasite growth in red blood cells. This inhibitory effect was less in the case of antisense against the 261-280 region by 0.01 µM, while in the concentration of more than 0.1 µM, inhibition of about 90% was observed. The results of Real-Time PCR on the mRNA of the target gene at different concentrations of the studied antisense show a decrease of 8-30 times in RH5 expression compared to the 18s-rRNA reference gene in treated and untreated cells. The co-effect of chloroquine (IC50 = 0.2 µM) and antisense (IC50 = 0.05-0.1 µM) indicates the synergistic effect of chloroquine and antisense.

Conclusion: According to the obtained results, RH5 mRNA in *Plasmodium falciparum* is a suitable target in the form of 2´-O-Methyl oligonucleotide and these antisenses can enter red blood cells in the naked form (Gymnosis) without the need for biological carriers and successfully inhibits parasite growth. This inhibitory effect is increased with

chloroquine and can be used in the treatment of patients infected with Plasmodium, especially in patients infected by drug-resistant strains of this parasite.

Keywords: *Plasmodium falciparoum*; Gene RH5; Antisense; *In vitro*