

***In vitro* effect of antiparasitic drugs on the expression of the genes code receptors: AchR and GABA in *Anisakis simplex* L3 larvae**

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Anisakis simplex is a gastrointestinal parasitic nematode with a complex life cycle; the definitive hosts are marine mammals as well as humans. As anisakiasis is a serious condition that can be fatal to humans and may cause allergic reaction in patients sensitive to *A. simplex* allergens. There are still no effective vaccines for controlling these infections, it is necessary to intensify research of depending of the levamisole and γ -aminobutyric acid receptors on the parasite's larvae.

The major group for neurotransmitter-gated ion-channel that includes nicotinic acetylcholine receptor, and γ -aminobutyric acid which has been studied as a potential drug targets for nematodes. The nematode nicotinic acetylcholine receptors (nAChRs) on muscle are the target of the cholinergic anthelmintics levamisole; such compounds cause these ligand-gated ion channels to open, leading to prolonged muscle contraction and spastic paralysis of the parasite. IVM, a macrocyclic lactone, is a broad-spectrum anthelmintic, by binding to nematode GABA-gated chloride channels.

The aim of this study was to investigate the expression of genes encoding receptors: nAChRs (*unc29* KM496571; *unc38* KM496570) and GABA (*gaba1* KP640594; *gaba2* KM496572) in the invasive L3 larvae of *A. simplex* and determine *in vitro* (Iglesias i in., 2001) effect of ivermectin, levamisole (6.25; 12.5; 25; 50 $\mu\text{g/ml}$) on the expression of these mRNA receptors after 3, 6, 12 and 24h. Control wells contained 0.1% DMSO. Isolation total RNA according to the manufacturer's instructions (A&A Biotechnology). RT PCR TranScriba kit (A&A Biotechnology). Quantitative real-time PCR was performed using a SYBRGreen PCR-MIX Taq™ (A&ABiotechnology) on an lightcycler (Applied Biosystem, FAST7500). The data were analyzed and normalized relative to *efl1a1* (KP326558) transcript levels by an AB analysis software (7500v2.0). The mean value \pm SD was used for analysis of relative transcript levels for each time point using the $\Delta\Delta\text{Ct}$ method.

The expression of GABA receptors decreased (10-fold) in ivermectin treated larvae was associated with an decreasing drug concentration (6.25 and 12.5 $\mu\text{g/ml}$) after 6h. The expression of nicotinic acetylcholine receptors (*unc29*; *unc38*) increased at higher concentration of ivermectin (25 ad 50 $\mu\text{g/ml}$) after 12h. GABA and nAChRs receptors could be implicated in the mechanism of action of ivermectin and levamisole in this parasite.