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EVALUATING EXPRESSION OF OXIDATIVE STRESS GENES IN RESPONSE TO TRICHINELLS SPIRALIS INFECTION

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ABSTRACT

Trichinella nematodes are distributed worldwide and have a great variety of hosts, including human. The host cells must fight against infection by this parasite in different situations such as oxidative stress-induced by T. spiralis. There are different enzymes involved in response to oxidative stress in human cells such as super oxide dismutase, peroredoxin and glutathione s-transferase. Here, we have investigated the expression levels of these genes to show their relevance to infection by T. spiralis. We have shown that expression levels of GST, POX and SOD are significantly increased in cells infected by T. spiralis. This suggests that these genes are involved in fighting against parasite infection.

KEYWORDS: Trichinella spiralis, trichinellosis, Oxidative stress, HCT-8 cells

The nematode T. spiralis, the most common cause of human trichinellosis, is a member of a clad that diverged early in the evolution of the Nematoda(Ashour and Elbakary, 2011). It is substantially different in biological and molecular properties from other groups. Trichinellosis is a food-borne zoonotic disease caused by the ingestion of raw or poorly cooked meats infected with parasites of the genus Trichinella(Shimony et al., 2007). Human trichinellosis has been reported in 55 (27.8%) countries worldwide(Cui and Wang, 2011). Trichinella spiralis is a nematode parasite, occurring in rats, pigs and humans. It is also referred to as the "pork worm" because it is commonly found in undercooked pork products(Kang et al., 2013). Trichinella species have unusual life cycle and are the smallest parasites of humans which are one of the most widespread and clinically important parasites in the world(Kocie□cka, 2000). The small adult worms grow and become mature in the intestines of a host such as pig. Each adult female produces several larvae which penetrate the intestinal wall, enter the blood, and are transferred to striated muscle. At this point, they are encysted, or become enveloped in a capsule(Zivojinovic et al., 2013). Using infected meats such as pork, humans will be infected when(Kang et al., 2013).

Once host invasion, T. spiralis must cope with oxidative and nitrosative stress from various sources including varying oxygen within the intestinal lumen and reactive oxygen and nitrogen species (ROS and RNS) produced by the immune system in response to pathogen invasion and tissue destruction(Blum et al., 2013; Dupouy-Camet, 2000). It is postulated that there is a correlation between oxidative and nitrosative stress resistance and pathogenesis for different parasites on the basis that virulent species have higher transcript levels of genes involved in oxidative stress resistance, display a higher resistance to oxygen exposure and oxidative and nitrosative stress(Cheema et al.). A large number of genes are involved in response to oxidative stress and proteomic analysis has shown significant changes in expression of some genes in response to oxidative stress(Rastew et al., 2012). The most significant changes were related to signaling pathways and repair of mis-folded proteins. The list of genes involved in stress response also includes several candidates linked to virulence-related cellular processes such as adherence to host cells and ability to destroy and phagocytosis in bacteria and humans(Raj et al.).However, most of the ROS and RNS responsive genes (especially the genes with the highest induction levels) code for hypothetical proteins of unknown function(Selkirk et al., 1998).

Previous studies have shown different enzymes in parasites involved in stress responses including superoxide dismutase, peroredoxin, glutathione S-transferase(Becker et al., 2004). Here, for the first time, we have investigated expression of these genes against infection by Trichinella spiralis.

MATERIALS AND METHODS

Parasite and cell lines

The virulent strain of T. spiralis was obtained from Tehran University of Medical Sciences. Human

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colonic epithelial cell line HCT-8 (from Pasteur institute, Iran) was cultured in RPMI-1640 containing l-glutamine, nonessential amino acids and 10% fetal bovine serum.

Trichinella sp. co-culture with HCT-8 cells

The HCT-8 cell monolayer was overlaid with the bile-activated ML suspended in RPMI-1640 medium (Gagliardo et al., 2002). Following incubation for 18 h at 37 °C in 5% CO2, the ML were collected, washed four times with PBS, suspended in Tris–EDTA buffer and sonicated. The protein concentration was measured by the BCA method.

RNA isolation and cDNA synthesis

RNA was isolated from HCT-8 cells co-cultured with Trichinells spiralis in presence and absence of oxidants using RNX^{TM}plus solution (Cinnagen, Iran). Total RNAs were treated with DNase I (Sigma, USA) at 37ºC for 30 min. The integrity and concentration of extracted RNAs were examined on agarose gel electrophoresis and by spectrophotometer, respectively. Reverse transcription reaction for first strand cDNA synthesis was performed with 3-5 µg of purified total RNA with the RevertAid™ Reverse Transcriptase (Fermentas, Canada) using oligo $(dT)_{18}$in a total 20 µl reaction mixture.

Real-Time Quantitative PCR analysis

mRNA expression levels of SOD and GST genes were estimated with appropriate primers. The relative expression of each gene was assessed in comparison with the housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) with specific primers. Amplifications were carried out using specific primers (Table 1).Quantitative RT-PCR was performed using 7500ABI system (Applied Bio systems, Foster, CA, USA) in final reaction volumes of 20 µl with 20ng cDNA, 10 µl of SYBR Green I master mix (Takara, Shiga, Japan) and 200 nM of forward and reverse primers, according to the manufacturer's instructions. The PCR reaction was performed as follows: starting with denaturation of templates at 95ºCfor 3 min, followed by 40 cycles of denaturation at 95 ºC for 15 sec and annealing/extension at 60ºC for 30 sec. Specificity of PCR products was examined running on a 2% agarose gel to verify their size and dissociation curve analysis. To obtain a standard curve and amplification efficiency for each primers of gene transcript a serially diluted cDNA was used. For all genes expression analysis, appropriate negative controls containing no template controls were subjected to the same procedure to exclude or detect any possible contamination.

Statistical analysis

Reverse Transcription PCR were analyzed with One way ANOVA followed with T-test was performed using Graphpad Prism 5.0 program and SPSS (SPSS, Chicago, IL, USA). A P-value ≤ 0.05 was considered significant and data were shown as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The effect of oxidative stress on genes expression was analyzed by quantitative Real-time PCR technique. The relative gene expression for each gene was estimated by comparative threshold cycle as described by Livak Briefly, the mean threshold cycle (mCT) was obtained from triplicate amplification during the exponential phase of amplification. Then mCT value of reference gene of internal control gene (GAPDH) was subtracted from mCT value of each Tubb3, and TopIIA genes to obtain ΔCT for each gene. After calculation of ΔΔCT values of each sample the relative expression of each gene was estimated by ratio formula (ratio= $2^{-\Delta\Delta Ct}$).The expression of SOD and GST genes were measured after treatment with OSU-40. Compared with the transcription level of SOD in the untreated control cells, this gene was up-regulated more than 50% in treated cells. Similarly, GST mRNA level was elevated following the treatment. This shows that oxidative response was activated following treatment with an oxidant agent.

Invasive pathogens are frequently exposed to reactive oxygen species as a mechanism of host defense and an organism's ability to survive this aspect of host immunity is critical to establishing infection(Igoillo-Esteve and Cazzulo, 2006). In many systems the transcriptional machinery that regulates gene expression in response to ROS is well characterized(Díaz et al., 2011). Host immune-dependent damage to helminthes parasites is mediated by the generation of oxygen-derived free radicals via a nonspecific defense reaction of the host(Rolo et al., 2012). Due to their participation in the metabolic processes, antioxidants may protect the host against oxidant-mediated damage and the harmful effects of substances produced as a result of the host′s defense response(Mishra and Singh, 2013).

Here, we have investigated the genes involved in oxidative stress response to infection by parasite Tricinella spiralis in HCT-8 cells. As shown in figure 1, GST expression level has increased upon infection by Trichinella compared with control cells in time-dependent manner. Also, it was demonstrated that expression of SOD was increased by time. Another gene involved in oxidative stress response has shown increase compared to control cells (figure3). Results have shown that these genes are significantly increased when infection compared to control cells without infection. Accordingly, this suggests that upon infection in cells by Trichinella spiralis, oxidative stress is stimulated in the infected cells and increasing expression level of the genes involved in stress response could be a possible mechanism to fight against infection by parasites.

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Conflicts of interest: None.

Table 1: The sequence of primers used in this study for gene expression analysis by real-time PCR technique

Genes name(	Primer Sequences	Amplicon size
Sod	Forward: 5'- GCAGGTCCTCACTTTAATCCT -3' Reverse : 5'- AGTCATCTGCTTTTTCATG -3'	200(bp)
Pox	Forward: 5`-CTGATCCCACTGGGGCCTTTG-3` Reverse: 5`-GACGTCGATTCCCAAAGATG-3`	160(bp)
Gst	Forward : 5'- TGAGGTCCTAGCCCCTGGCTGC -3' Reverse : 5'- GGGAGGTTCACGTACTCAGGG -3'	120(bp)
GAPDH	Forward : 5'- GTGAACCATGAGAAGTATGACAA -3' Reverse : 5'- CATGAGTCCTTCCACGATAC -3'	123(bp)

FIGURE LEGENDS

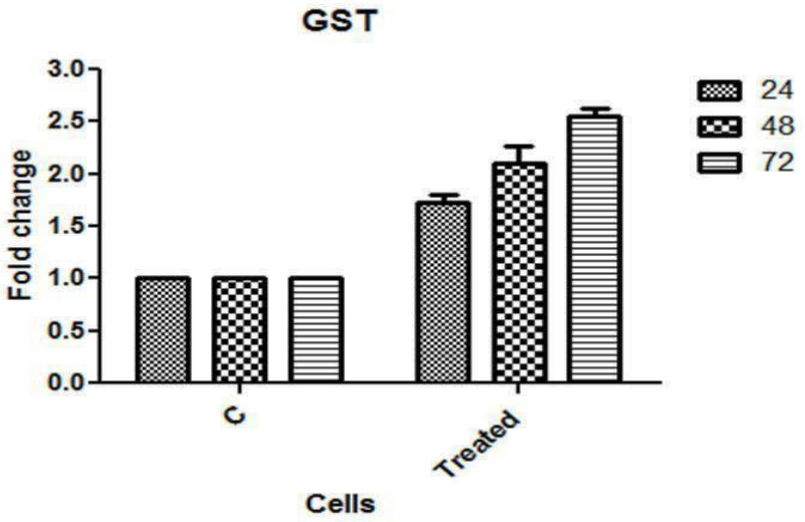

Figure 1: The expression level of GST gene up-regulated HCT-8 cells after infection with T. spiralis. Each real-time PCR examination was carried out at least in triplicate. Data are shown as fold change in relative expression compared with GAPDH on the basis of Comparative Ct ($2^{-\Delta\Delta Ct}$) method. Values are shown as mean ± SD

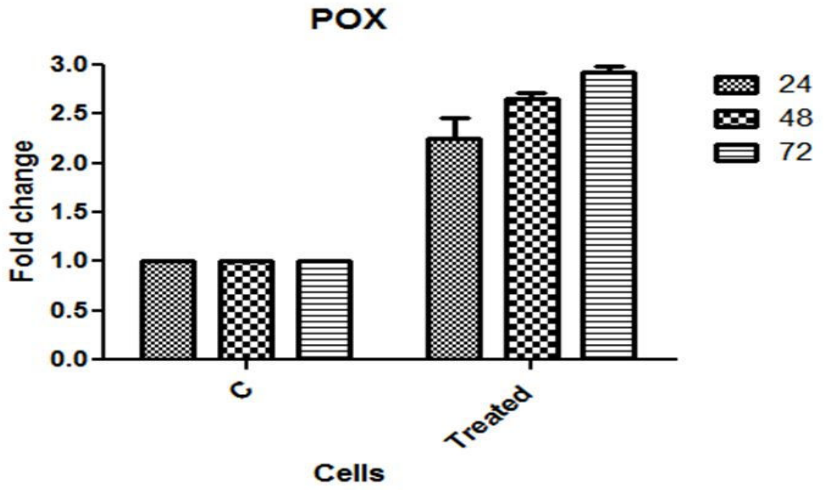

Figure 2: The expression level of POX gene up-regulated HCT-8 cells after infection with T. spiralis. Each real-time PCR examination was carried out at least in triplicate. Data are shown as fold change in relative expression compared with GAPDH on the basis of Comparative Ct ($2^{-\Delta\Delta Ct}$) method. Values are shown as mean ± SD

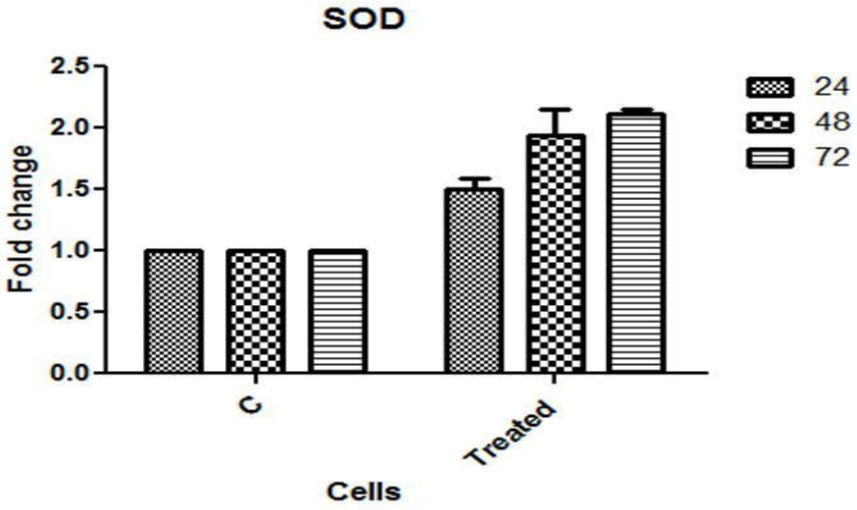

Figure 3: The expression level of SOD gene up-regulated HCT-8 cells after infection with T. spiralis. Each real-time PCR examination was carried out at least in triplicate. Data are shown as fold change in relative expression compared with GAPDH on the basis of Comparative Ct ($2^{-\Delta\Delta Ct}$) method. Values are shown as mean ± SD

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