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Gene Expression Profiling in Practitioners of Sudarshan Kriya

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Introduction :

The human body is constantly under various kinds of stress and attack by reactive oxygen species (ROS) either generated by external agents or during normal metabolic process. ROS can damage cellular functions by damaging lipids, proteins and DNA in the cell [1, 2]. Increasing evidence suggests that chronic psychosocial stress may increase oxidative stress which in turn may contribute to the pathophysiology of atherosclerosis, coronary heart disease, cancer and other chronic diseases [3,4]. Cells can transduce stress into biochemical signals. Numerous cellular functions can be influenced by the presence of stress. Several genes and pathways of stress signalling molecules are found to be activated under conditions of stress. Some of them being heat shock proteins, mitogen-activated protein kinases, stress-activated protein kinases and molecules involved in the oxidative stress pathway [5]. Meditation, SK and other techniques have been known to relieve stress by causing a relaxation response, a complex compensatory mechanism. SK is a breathing technique involving breathing in three different rhythms [6]. We have shown earlier [6] that SK helps lower blood lactate levels and provides better antioxidant defense. However, the biochemical effects and the signal transduction pathways of stress removal have not been studied. In this study an attempt has been made to study the effect on long term practitioners of SK on genes involved in oxidative stress, DNA damage, cell cycle control, aging, apoptosis and several pathways of stress signalling molecules.

METHODS

Study group :

Blood samples were collected from 42 practitioners of Sudarshan Kriya and from 42 age and sex matched healthy normal, not practicing any kind of stress management technique which served as controls. The practitioners included subjects in the age range of 22-64 years, and comprised of 20 males and 22 females. The Sudarshan Kriya practitioners had been regularly doing this technique for more than a year. The controls and practitioners included in the study had the same socio-economic status, had comparable body weights (+10%), were vegetarians and non-smokers. They were not taking any dietary supplements.

Sample Collection :

15 ml of blood was collected by venipuncture, in EDTA containing tubes, from the study subjects as well as controls, after informed consent. Blood was layered onto Ficoll-Hypaque solution and WBCs were separated by centrifugation. The WBCs were pelleted and stored at -70^{0}C until further use for gene expression analysis by RT-PCR.

RNA Isolation and Reverse Transcription:

RNA extraction was done using Tri-reagent. cDNA construction was carried out using Stratascript Reverse Transcriptase (Stratagene, USA) with 2μg of RNA using manufacturer's protocol.

Polymerase chain reaction (PCR):

PCR amplifications were performed with 2μl of cDNA as template in a 25μl volume containing 300μM each of dNTPs, 0.2μM each of forward and reverse primers 10mM of KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.7), 0.1% Triton X-100, 0.1mg/ml of BSA, 1.5mM $MgCl_2$ and 1U of Taq DNA polymerase (Roche Biochemicals, Gemany). PCR products were analyzed by electrophoresis on 1% agarose gels. G3PDH was used as an internal control. The results of densitometry of the above mentioned genes were normalized with that of G3PDH and the results were expressed as Arbitrary Units (AU).

Statistical analysis :

Mean difference between the controls and practitioners was assessed by Mann-Whitney test. Correlation between different parameters was calculated using Pearson's correlation coefficient.

Results :

Effect of SK on some stress related gene expression levels as seen by RT- PCR :

We studied the effect of SK at the transcriptional level of the antioxidant enzymes. There was a significant increase in expression of Glutathione-S-transferase (p=0.034) in practitioners of SK as compared to the normal controls. An increase in the expression of anti-oxidant Cu-Zn SOD, Mn SOD, Glutathione peroxidase and Catalase genes was observed in practitioners but it was not statistically significant. Antiapoptotic Cox-2 (p=0.035) and HSP-70 (p=0.044) showed a significant increase in SK practitioners. Though aging-related hTERT and anti-apoptotic Bcl-2 showed an increasing trend in SK practitioners but it was not statistically significant.

Discussion

There are several pathways of stress signalling molecules, of these the most important examples are heat shock proteins and molecules involved in the oxidative stress pathways. Relaxation is a tendency of a physiological system to return to its original state and is a compensatory mechanism. Techniques that decrease stress and elicit the relaxation response include transcendental meditation, focussed breathing/SK, repetition of a word, phrase or song [6]. This study is the first of its kind to help gather insights into the effects of SK on the gene expression level at RNA level of the genes, which affect several stress related signalling/ metabolic pathways in the human body. This study shows a better antioxidant status at the transcription level in SK practitioners in comparison to matching controls as seen by increased levels of glutathione peroxidase and catalase. These subjects also showed increased GST levels, thereby suggesting better environmental stress regulation.

Cyclooxygenase-2 (Cox-2) is the inducible isoform of cyclooxygenase and is also an inhibitor of apoptosis. Egan et al [7] have shown that upregulation of COX-2 and subsequent production of atheroprotective prostacyclin offers atheroprotection in mice. The observed upregulation of Cox-2 in SK practitioners perhaps suggests a prolonged life span of WBCs by inhibition of apoptosis and also a better protection against cardiovascular diseases. Bcl-2 contributes to transformation by blocking apoptosis rather than increasing proliferation [8]. We observed an increase in Bcl-2 at mRNA level. Upregulation of antiapoptotic Bcl-2 along with Cox-2 gene expression in lymphocytes of SK group also suggests a better immune regulation by prolonging the lifespan of lymphocytes in these subjects. A prominent process in aging is oxidative stress with generation of ROS. ROS affect all the cellular components including telomeric DNA leading to early senescence. hTERT encodes for catalytic component of the telomerase complex [8]. The observed increase in hTERT expression in SK practitioners perhaps suggests delayed replicative senescence of lymphocytes in these subjects thereby providing better immune response.

Another class of molecules known as heat shock proteins (HSPs) provide protection against stress. Although heat is the typical inducer of these proteins, but other stress factors, oxidative stress [9] and proinflammatory cytokines [10] can also induce them. HSP70 is the inducible form of HSPs. It protects cells against a variety of toxic conditions such as oxidative stress [11]. Its overexpression has been shown to be protective against apoptotic death. A significantly higher expression of HSP though in practitioners suggests its cytoprotective role in lymphocytes and prevents them from undergoing apoptosis.

It appears that SK helps in overcoming oxidative stress. It prolongs the life span of lymphocytes by upregulating the anti-apoptotic Cox-2, Bcl-2 and cytoprotective HSP-70 thereby improving the immune response and providing atheroprotection. Increase in hTERT further imparts a longer life to lymphocytes by delaying replicative senescence.

Taken together these results suggest that SK practicing subjects show higher resistance to oxidative stress, and perhaps have a higher level of protection from cancer and cardiovascular diseases.

Acknowledgement

This study was inspired by Sri Sri Ravi Shankar. This work was carried out in collaboration with Ved Vigyan Mahavidyapeeth. We acknowledge them for their support and active participation of Delhi chapter of Art of Living. The financial support provided by Indian Systems of Medicine and Homeopathy, Ministry of Health and Family Welfare, Govt. of India.

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