RESEARCH ARTICLE

ACTA Vol. 16, No. 3, 2018, 243-248
NEUROPSYCHOLOGICA

Received: 20.06.2018
Accepted: 12.10.2018

A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

INCREASED LEVELS OF INTERLEUKIN-10 EXPRESSION COMPARED TO INTERLEUKIN-6 IN THE LEUKOCYTES OF HEALTHY SUBJECTS. COULD THIS BE USEFUL IN THE FUTURE FOR THE DIAGNOSIS OF DEPRESSION?

Agata Grzybkowska[1*(A-F)], Katarzyna Anczykowska[1*(A-F)], Joanna M. Pyczek[2(A-D,F)], Małgorzata Żychowska[3(A-C,F,G)]

[1] Gdansk University of Physical Education and Sport, Faculty of Physical Education, Department of Biochemistry
[2] University of Physical Education in Cracow, Poland, PhD student
[3] Gdansk University of Physical Education and Sport, Faculty of Physical Education, Department of Life Sciences

SUMMARY

Background: It is well known that physical activity promotes mental health. Physically active people relatively rarely suffer from psychosomatic and depressive disorders. It is possible that the differences in the gene activity in peripheral blood leukocytes may be associated with depression, especially those genes participating in an inflammatory response. Thus, the aim of our study was to investigate the levels of *IL6* and *IL10* mRNA and *IL10/IL6* ratio in peripheral blood leukocytes in healthy, physically active individuals.

Material/ Methods: One hundred healthy young men (20-23 years old) participated in this study. All of them declared their regular participation in physical activity. Participants were non-smokers, and consumed alcohol occasionally. To access genes expression, 2 ml of venous blood was collected. RNA isolation was performed and then the relative expression of *IL6* and *IL10* was calculated using the quantitative real-time polymerase chain reaction (Q-RT-PCR). The pro and anti-inflammatory balance was calculated as 2^relative of *IL10*/ 2^relative expression of *IL6*.

Results: A low expression of the tested genes was found in healthy young men. Mean expression for *IL6* was 2^0.051 (n=90) and for *IL10* 2^0.08 (n=98). Mean ratio *IL10*/*IL6* was 2^1.58.

Conclusions: A higher expression of *IL10* compared to *IL6* may be essential not only for physical performance but also for any mental health evaluation. Diverse reports in the subject literature may be associated with choosing control groups that were too diverse, i.e., sedentary or older individuals. It is possible that measuring the expression of *IL6* and *IL10* (especially the ratio *IL10*/*IL6*) in peripheral blood leukocytes may be useful in the assessment of depressive disorders. Thus, a molecular study of active young men can confirm the need for physical activity among people suffering from depression, but further studies are needed, particularly among people with psychosomatic disorders to establish the potential therapeutic effect.

Key words: depressive disorder, gene expression, inflammation, physical activity, peripheral blood mononuclear cells

* Katarzyna Anczykowska and Agata Grzybkowska declare an equivalent contribution.

BACKGROUND

According to WHO, depression is the most common mental disease, one which affects approximately 300 million people of all ages worldwide, and results in as much as 800,000 deaths caused by suicides each year (WHO, 2018). Currently the most common type of treatment is pharmacological management along with psychotherapy (Gautam et al., 2017) but due to the various side effects of medication, safer treatments are being researched. A few dietary interventions have shown promising results, such as introducing various seafood and leafy greens into the diet (LaChance et al., 2018). The promising results are mainly based on the anti-inflammatory and anti-oxidative properties of those foods (Frutos et al., 2018). Physical activity also seems to be an effective treatment strategy for patients with depression, but the underlying mechanisms have yet to be discovered (Agudelo, 2014). This kind of treatment might be less burdensome and more affordable than pharmacotherapy (Moore, 2018). The objective of the present study was to show the changes in gene expression of the genes *IL6* and *IL10* with the emphasis on the *IL10*/*IL6* ratio in healthy, physically active subjects. Regarding the well known fact that physically active people are less likely to develop depressive disorders, it is important to determine the differences between both groups. In our opinion it is important to determine the marker genes and the dysregulation of their expression that will enable professionals to diagnose their patients easier.

MATERIAL AND METHODS

Subjects and study design

One hundred healthy young men (20-23 years old) participated in this study. All of them declared participation in regular physical activity (minimum 3 times a week of 45 minutes of activity). Participants were on a standard, healthy diet, all were non-smokers and consumed alcohol occasionally. No individual declared depression episodes prior to the experiment. All subjects were informed of the purpose of this study and the possible risks involved before giving their written consent.

The study was approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk. The authors of this study were obliged to respect the principles of the Helsinki Declaration.

Experimental Procedure

The methodology used in this experiment was consistent with one described earlier (Kochanowicz et al. 2017; Żychowska et al. 2017). To access gene expression, 2 ml of venous blood samples were taken from the antecubital vein with vacutainers with EDTA as a anticoagulant. The collected blood was then mixed with the RBCL buffer (A&A Biotechnology, Gdynia) and incubated on ice for at least 15 minutes to lyse red blood cells. The samples were then spun at

3000 rcf for 10 minutes at 4°C. The resulting pellet was washed again with the hemolysis buffer and the remaining white blood cells were lysed using Fenozol (A&A Biotechnology, Gdynia).

RNA extraction and reverse transcription

The isolation of the total RNA was carried out using the Chomczynski and Sacchi method (Chomczynski & Sacchi, 1987). The purity and integrity of the RNA were determined by a spectrophotometer (Eppendorf, BioPhotometer Plus, Germany) by absorbance at UV 260/280 and the ratio >1.9 was accepted as a pure RNA suitable for further analysis. RNA was then reverse transcribed to cDNA using oligo(dT) and Transcriptor First Strand cDNA Synthesis Kit as per the manufacturer's instructions (Roche, Poland).

Quantitative Polymerase Chain Reaction assay

Quantitative real-time PCR (qPCR) was carried out on the AriaMx Real-Time PCR System (Agilent Technologies, Poland) using FastStart Universal SYBR® Green Master (ROX) according to the manufacturer's protocol (Roche, Poland) on 96-well PCR plates in triplicate for each sample. The thermal cycling conditions were as follows – activation step: 10 min at 95°C followed by 40 cycles of annealing and extension step: 15 sek at 95°C, 1 min at 60°C. Additionally melt curve analysis was performed for each reaction. The β-tubulin (*TUBB*) was used as a reference gene. The relative expression of *IL10* and *IL6* was calculated using a quantitative real-time polymerase chain reaction (Q-RT-PCR). The primer sequences used in this study are written down below:

TUBB
Forward primer: TCCACGGCCTTGCTCTTGTTT
Reverse primer: GACATCAAGGCGCATGTGAAC
IL6
Forward primer: TCCACGGCCTTGCTCTTGTTT
Reverse primer: GACATCAAGGCGCATGTGAAC
IL10
Forward primer: GAATCCAGATTGGAAGCATCC
Reverse primer: AATTCGGTACATCCTCGACGG

Statistical analysis

To determine relative expression Schmitten and Livak delta delta C_t method (2008) was used (Microsoft Excel, 2017). The mean value and standard deviation was calculated in GraphPad Prism 6.0. The pro and anti-inflammatory balance was calculated as 2^relative of IL10/ 2^relative expression of IL6.

RESULTS

A lower expression of *IL6* mRNA compared to *IL10* mRNA in rest samples was observed in the samples of all participants as well as in the mean value.

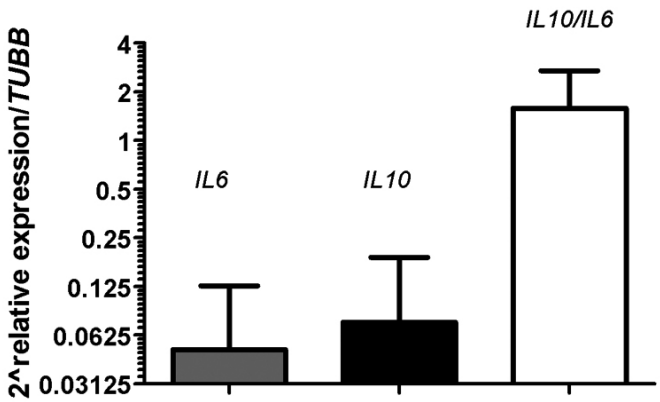

Figure 1. 2^relative expression of IL6 (n=90) and IL10 (n=98) in healthy, physically active young men and the IL10/IL6 ratio.

Relative expression was 2^0.05 for *IL6* and 2^0.075 for *IL10* mRNA. The *IL10/IL6* ratio was 2^1.58. These results indicate that the anti-inflammatory response was higher than the inflammatory in physically active people.

DISCUSSION

Physically active people showed a lower expression of *IL6* compared to *IL10* mRNA, which suggests that the anti-inflammatory response prevails over the inflammatory response. This observation is also visible in the *IL10/IL6* ratio which indicated 3.16 fold more copies of *IL10* (2^1.58 fold responds 3.16 fold in linear value). In our study active people constituted a model group, as no individual had previously suffered from depressive disorders.

Devorak et al. (2015) postulated that the main biological mechanism triggering depression is inflammation. Therefore, in our opinion, physical activity for people affected by depression is needed and could support their therapy. One of the main adaptive changes to exercises on the molecular level depends on the decrease of the *IL6* expression and the increase of the *IL10* expression (Żychowska 2017; Ziemann et al. 2013). That is the reason why active people had a higher expression of *IL10* in the rest state.

There are some reports in the subject literature in which changes in genes encoding interleukins were analyzed. Horowitz et al. (2014) postulated that the high expression of *IL6* could be associated with depression. Furthermore, upregulation of the expression of genes associated with inflammation was postulated by Pace et al. (2006). Trystuła et al. (2017) in their research evaluated genes expression in a 54-old patient with depressive disorder following a transient ischemic attack and found a higher expression of *IL6, IL10* and *CRP* mRNA compared to the control group. Despite the relatively high *IL10* mRNA of this patient, *IL6* mRNA was definitely higher (2^1000-fold compared to 2^400-fold for *IL10*).

Therefore, in our opinion the calculation of the *IL10/IL6* mRNA ratio in the rest samples is crucial.

In recent years, the development of medical diagnostics is closely related to molecular diagnostics, especially with the analysis of genes expression. In our opinion the determination of genetic markers and/or dysregulation in genes expression will be a major advance to facilitate the diagnosis of mental illnesses. Unfortunately, there are still only a few studies in this area. Only some studies in the literature are associated with the expression of genes encoding interleukines. Usually these are studies conducted on stuttering patients (Kahl et al., 2004, Pace et al., 2006, Półrola et al., 2016), sometimes in patients with depression or schizophrenia (Freudenreich et al., 2010, Chase et al., 2015). In our opinion, it is important, for further diagnostics, to carry out more well-planned studies on a larger group of patients. It is also crucial, to perform such studies before introducing pharmacotherapy to the patients, as the medication modulates the immune response.

In conclusion, we suggest that in patients with depressive disorders, the determination of genes expression, especially associated with interleukin, could be useful in difficult diagnostics. Moreover, because of the positive changes caused by physical activity, including appropriate exercising programs in the treatment process, it might be beneficial for the patients.

REFERENCES

Agudelo L. Z., Femenia T., Orhan F., Porsmyr-Palmertz M., Goiny M., Martinez-Redondo V., et al. (2014). Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 159 33–45.

Chase KA, Rosen C, Gin H, Bjorkquist O, et al. (2015). Metabolic and inflammatory genes in schizophrenia. Psychiatry Research 225:208-211.

Chomczynski P., Sacchi N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry. 1987;162(1):156–159.

Devorak J, Torres-Platas SG, Davoli MA, Prud'homme J, et al. (2015). Cellular and molecular inflammatory profile of the choroid plexus in depression and suicide. Frontiers Psychiatry 6:138.

Freudenreich O, Brockman MA, Henderson DC, Evins AE, et al. (2010). Analysis of peripheral immune activation in schizophrenia using quantitative reverse-transcription polymerase chain reaction (RT-PCR). Psychiatry Research 176: 99-102.

Frutos MJ, Valero-Cases E, and Rincon-Frutos L. (2018). Food components with potential to be used in the therapeutic approach of mental diseases, Current Pharmaceutical Biotechnology 19: 1.

Gautam S, Jain A, Gautam M, Vahia VN, Grover S. (2017). Clinical Practice Guidelines for the management of Depression. Indian Journal of Psychiatry. 2017;59(Suppl 1):S34-S50.

Góral-Półrola J, Zychowska M, Ziółkowski A, Pąchalska M (2015). Changes in genes expression associated with cell stress in the patient with chronic perseverative stuttering. Acta Neuropsychologica 13:417-424.

Horowitz MA, Wertz J, Zhu D, Cattaneo A, et al. (2014). Antidepressant compounds can be both pro- and anti-inflammatory in human hippocampal cells. International Journal of Neuropsychopharmacology 18:pyu076.

Kahl KG, Kruse N, Rieckmann P, Schmidt MH (2004). Cytokine mRNA expression patterns in the disease course of female adolescents with anorexia nervosa. Psychoneuroendocrinology 29:13-20.

Kochanowicz A, Sawczyn S, Niespodziński B, Mieszkowski J, Kochanowicz K, Żychowska M (2017). Cellular Stress Response Gene Expression During Upper and Lower Body High Intensity Exercises. PLoS ONE 12(1): e0171247.

LaChance LR, Ramsey D. (2018). Antidepressant foods: An evidence-based nutrient profiling system for depression. World Journal of Psychiatry. 2018;8(3):97-104.

Moore, A.; Beidler, J.; Hong, M.Y. (2018). Resveratrol and Depression in Animal Models: A Systematic Review of the Biological Mechanisms. Molecules 2018, *23*, 2197.

Pace TWW, Mletzko TC, Alagbe O, Musselman DL, et al. (2006). Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. American Journal of Psychiatry 163:1630-1633.

Schmittgen T.D., Livak K.J. (2008) „Analyzing real-time PCR data by the comparative C(T) method." Nature Protocol 3:1101-1108.

Trystuła M, Żychowska M, Wilk-Frańczuk M, Kropotov JD, Pąchalska M. (2017). Dysregulation of gene expression in a patient with depressive disorder after transient ischemic attack confirmed by a neurophysiological neuromarker. Genetic and Molecular Research, 2017:16;1.

WHO Depression Fact Sheet. Available online: http://www.who.int/mediacentre/factsheets/fs369/en/ (accessed on 13 September 2018).

Ziemann E, Zembroñ-Lacny A, Kasperska A et. al. (2013). Exercise training-induced changes in inflammatory mediators and heat shock proteins in young tennis players. Journal of Sports Science and Medicine 2013;12: 282-289.

Żychowska M. (2017). Changes in expression of selected cellular stress response genes after passive body overheating in sauna and moderate exercise in judo athletes and untrained people. Archives of Budo 2017:13.

Żychowska M, Kochanowicz A, Kochanowicz K, Mieszkowski J, Niespodziński B, Sawczyn S. (2017). Effect of Lower and Upper Body High Intensity Training on Genes Associated with Cellular Stress Response. Biomed Research International 2017(2).

Address for correspondence:
Małgorzata Żychowska
Gdańsk University of Physical Education and Sport,
Department of Life Sciences, Gdańsk, Poland
ul. Górskiego 1
80-336 Gdańsk
e-mail: zychowska.m@gmail.com