biopsy and pathohistological assessment). Immediately after rectal examination a sample of urine was collected into sterile tubes in the amount not less than 35 ml. Since mRNA is rather unstable molecule susceptible to degradation, the sample was transported to the Laboratory of human genetics of the Institute of genetic engineering and biotechnology within 12 hours after collection. When it was not possible to observe the procedure, a sample was stored refrigerated at temperature of 4°C until processing. Total RNA was extracted using NucleoSpin® RNA isolation kit, according to the protocol recommended by manufacturer (Macherey - Nagel GmbH & Co., Duren, Germany). Extracted RNA was quantitated by fluorometry using Qubit® FS RNA Assay kit and Qubit® 2.0 fluorometer (Invitrogen, Life Technologies, Oregon, USA). Reverse transcription reaction was done using GeneAmp® Gold RNA PCR Core Kit (Applied Biosystems, USA) on an Eppendorf Mastercycler Gradient PCR unit. After checking successfulness of reverse transcription, the cDNA was used as a matrix in the Real time PCR in the quantitative amplification of KLK3 gene by using the SYBR green chemistry (“ Applied Biosystems”, Ca., USA). All samples were amplified in triplicate with chosen genes. Cycle threshold (Ct), as a basic number parameter of quantitative PCR is used as a measure of amplificability of cDNA template.

**Results.** RNA was isolated from 133 out of 162 collected urine samples which makes 82% success rate for RNA extraction from urine. The average concentration of total RNA was 4.60 ng/μl with highest concentration of 96 ng/μl. Reverse transcription reaction was done for RNA samples with minimum concentration of 1 and maximum concentration of 96 ng/μl.

GAPDH had a minimum Ct value of 15, the average Ct value was 24, and the maximum measured Ct value was 33. Taking into consideration possible instability of RNA molecule which is high even in the samples of blood and tissue samples which are considered to be ideal, possibility of successful extraction of 82% can be considered as high. Urine, as a byproduct of many physiological reaction, is a very demanding material for this type of isolation due to the presence of a large number of various compounds affecting RNA degradation.

Conclusions. Although the extraction of total RNA from the urine obtain relatively low concentration, it is possible by reverse transcription to get quality enough cDNA to contend with different types of analysis. According to our estimates, critical step in extraction of total mRNA from urine is quick transport to the laboratory where extraction will be carried. Also, equally important factor is the stabilization of the urine sample in the laboratory for what we used PBS buffer and centrifugation step prior to extraction. Selected kits for RNA extraction (NucleoSpin® RNA isolation kit, Macherey - Nagel GmbH & Co., Duren, Germany) and reverse transcription kit (GeneAmp® Gold RNA PCR Core Kit, Applied Biosystems, Ca., USA) proved to be good enough to from a relatively small amount of extracted starting material synthesized quality material for further work and analysis.

**Key words:** prostatitis, Prostate Specific Antigen, Urine, RNA, gene expression, Real time PCR.

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MARKERS FOR CELLULAR RESPONSE IN J774A.1 MACROPHAGE CELL CULTURE IN A MODEL OF BACTERIAL LIPOPOLYSACCHARIDES STIMULATION

Miglena Todorova, Oskan Tasinov, Milena Pasheva, Bistra Galunska, Deyana Vankova, Diana Ivanova, Yoana Kiselova

Department of Biochemistry, Molecular Medicine and Nutrigenomics, Medical University, Bulgaria

Cell cultures appear to be useful instrument in studies of molecular mechanisms of variety biological processes, diseases, action of drugs and natural products etc. Treatment with bacterial lipopolysaccharides (LPS) stimulate inflammatory response in variety cell types and this is a widely used model for studies related to immune function. Oxidative stress is also a component of such treatment. For the purpose of such experiments it is important to have specified markers to identify presence of a cellular response and to determine most appropriate LPS concentration where this response is most prominent. The aim of the study was to test the effect of LPS stimulation on J774A.1 macrophages on expression of selected genes, related to inflammation, antioxidant defense and necroptosis.

**Materials and methods.** J774A.1 cell line was cultured in standard conditions in complete DMEM medium with 4.5 g/L glucose, L-glutamine and supplemented with fetal bovine serum to final concentration of 10% and penicillin/streptomycin mixture to final concentration of 100U/mL each. To test the effect of LPS application, cells were seeded in 6 well flasks at density 2x10⁵. After overnight incubation cells were treated with increasing concentrations of LPS (50, 100, 200 and 300 ng/mL final concentration) in complete growth medium for 20h. At the end of the experiment RNA purification with TRI Reagent and DNase treatment were performed according manufacturers’ instructions. For gene expression analysis Real-Time PCR was applied with KAPA SYBR® FAST qPCR Kit. Gene expression levels were calculated using the 2-ΔΔCt method and expressed as relative units compared to the untreated controls where the level of gene expression of interest was considered to be equal to 1. Inflammatory cytokines IL-1β, IL-6 and TNF-α, inflammatory enzyme COX2, glutamate-cysteine ligase (GCL) and necroptosis-related protein RIP3 were analyzed.

**Results.** The most prominent response in regard to GCL, TNF-α and IL-6 expression was observed in the group treated with 100 ng/mL LPS where the levels were 9.1 (p<0.01), 3.4 (p<0.1) and 11.5 (p<0.001) times higher as compared to untreated control. IL-1β was affected at most in cells treated with 100ng/mL LPS and the levels were 146.6 (p<0.001) higher than the control. COX2 expression levels were 2.1 (p<0.001) times elevated. RIP3 expression did not change significantly at any LPS concentration applied.
Prospects for further research. All of the studied inflammatory cytokines increase significantly in this model, as well as the GCL enzyme and appear to be useful markers to observe in order to confirm inflammatory response in J774A.1 macrophages in a model of LPS stimulation. Although necroptosis is a process related to inflammation, RIP3 as a marker does not provide robust confirmation for cellular response. Each laboratory should perform preliminary experiments in order to identify most appropriate LPS concentrations depending on cell density and other parameters of the experiment.

Key words: J744A.1, LPS, markers, inflammation, oxidative stress, necroptosis

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IN VITRO EFFECT OF THE EXTRACT OF WALNUT PREPARATION ON THE PRE-IMMUNE RESISTANCE IN PATIENTS WITH CHRONIC TONSILLITIS

1Danilov L., 2Ghinda Serghei, 1Ababii P., 1Luca V., 1Trofimciuc M.
1Department of Otolaryngology, Nicolae Testemițanu State University of Medicine and Pharmacy, Chisinau, Moldova
2Laboratory of Immunology and Allergology of the Institute of Phthisiopneumology Chiril Draganiuc, Chisinau, Moldova.

The pre-immune resistance is the most ancient type of defense that appeared before the acquired immunity. Studies showed that that there is a unified system of protective factors in the body, including phagocytosis, which plays an important role at the initial stages of body protection from infections [1,2]. Preparations isolated from various herbs and seeds have great importance for the regulation of altered parameters of pre-immune resistance [3]. Study of the effect of some plant polyphenols on E. coli bacteria in vitro showed that quercetin and tannin possessed the greatest antioxidant activity [4]. The preparation EN was prepared from walnut, which contains polyphenols up to 20-22%, free amino acids up to 0.92-1.02% (of which essential ones are up to 0.17-0.20%, immunoactive ones being up to 0.20 - 0.22%), iodine - up to 0.2 mg/l and iron - up to 0.4 mg / l. In addition, the drug had shown antimicrobial activity. In this regard, the aim of the study was to investigate the in vitro effect of the EN preparation on neutrophil activity.

Material and methods. The study included 39 patients with chronic tonsillitis (age 20±1.5) and 116 healthy people (age 22±2.7). A standard in vitro NBT (Nitro-Blue-Tetrazolium) test was performed (phagocytic activity of neutrophils was assessed in Nitro-Blue-Tetrazolium) in all patients. A suspension of leukocytes was used as control and compared to leukocyte suspension mixed with EN preparation diluted 1/128.

Results. The analysis of the effect of the drug EN on NBT-test showed that the EN preparation had stimulating effect on NBT-test in 60% of patients (Table 1). In 25% patients the EN preparation had no modulating action and in 15% - the EN preparation showed suppressive effect on NBT-test. Thus, EN had a stimulating effect in bigger part of cases.

The NBT-test in the group of healthy subjects was 0.12 ± 0.003, which is significantly higher than in the 1 subgroup of patients with the stimulating effect (p<0.001) and significantly less than in the 3 subgroup of patients with a suppressive effect (p< 0.001). The NBT-test in the 1 subgroup, where the stimulating effect was noted, was initially significantly lower than in the 2 and 3 subgroups (p<0.001 in both cases), and under the influence of the EN preparation it increased to the level of healthy ones. The NBT-test in group 3, where the suppressive effect was noted, was initially significantly higher than in the 1 and 2 subgroups (p<0.001 in both cases), and under the influence of the EN preparation it decreased to the level not significantly different from the healthy ones.

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<td>NBT-test (healthy)</td>
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<td>NBT-test (control)</td>
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<tr>
<td>NBT-test (EN stimulation)</td>
<td>0.12±0.002</td>
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■ - reliability between 1-2 subgroups, ○ - reliability between 1-3 subgroups, □ - reliability between 2-3 subgroups

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