Clinical Medicine Research
2018; 7(3): 75-79
http://www.sciencepublishinggroup.com/j/cmr
doi: 10.11648/j.cmr.20180703.14
ISSN: 2326-9049 (Print); ISSN: 2326-9057 (Online)

Research/Technical Note

Expression and relationship of PPARγ and COX-2 in Peripheral Blood Mononuclear Cells of Multiple Organ Dysfunction Syndrome Patients

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To cite this article:
Xu Fei, Qiao Wanhai, Wang Liming. Expression and relationship of PPARγ and COX-2 in Peripheral Blood Mononuclear Cells of Multiple Organ Dysfunction Syndrome Patients. *Clinical Medicine Research*. Vol. 7, No. 3, 2018, pp. 75-79.
doi: 10.11648/j.cmr.20180703.14

Received: June 5, 2018; **Accepted**: July 12, 2018; **Published**: August 8, 2018

Abstract: To study the expression and relationship of cyclooxygenase-2(COX-2) and peroxisome proliferators activated receptor-γ (PPARγ) in peripheral blood monouclear cells (PBMCs) of multiple organ dysfunction syndrome patients. In the emergency department and intensive care unit (ICU) of our hospital, 60 MODS patients were divided into three groups with 20 cases in each group. They were MODS1 group (APACHE II score 0-10 points), MODS 2 groups (APACHE II score 11-20 points), MODS 3 groups (APACHE II score> 20 points) and control groups of 20 healthy subjects. Venous blood were sampled 1d, 3d, 6d after diagnosis of MODS, reverse transcriptase -polymerase chain reaction (RT-PCR) were used to detect the expression of PPARγ, COX-2 in PBMCs. The expression of PPARγ, COX-2 in the same period were compared between the MODS groups and the healthy control group. The study fond that the expression of COX-2 and PPARγ were low in vehicle control group. In MODS1 group the expression of PPARγ and COX-2 was higher than in vehicle control group; during the treatment period, PPARγ was significantly increased ($P < 0.05$), but the expression of COX-2 was significantly lower ($P < 0.05$). PPARγ expression in MODS 2 group and MODS 3group was lower than that in vehicle control group, and its expression was progressively decreased during the treatment ($P < 0.05$);in contrast, Expression of COX-2 in MODS2 and MODS3 group was higher than that in vehicle control group, and its level was progressively increased during the treatment ($P < 0.05$). The study revealed a significant negative correlation between COX-2 and PPARγ in MODS1 group, MODS2 group and MODS3 group. (MODS1 group $r=-0.761$, $P<0.01$; MODS2 group: $r=-0.782$, $P<0.01$; MODS3 group: $r=-0.791$, $P<0.01$). There was no significant correlation in vehicle control group ($r = -0.185$, $P > 0.05$). Thus, PPARγ may protect the MODS patients by repressing the expression of cox-2 therefore it can be used as an important indicator and potential target to determine the condition and treatment of MODS.

Keywords: Multiple Organ Dysfunction Syndrome (MODS), Peroxisome Proliferator-Activated (PPARγ), Receptors-γ, Cyclooxygenase-2 (COX-2)

1. Introduction

Multi-organ dysfunction syndrome (MODS) is the greatest challenge to critically ill medicine, with high morbidity and mortality and no satisfactory treatment. The research progress on pathogenesis of MODS is mainly reflected in the discovery of inflammatory factors, and many studies show that inflammatory factors play a decisive role in the development of MODS. The presence of high levels of inflammatory cytokines suggests a significant increase in MODS. There are many kinds of inflammatory factors involving MODS, and the interaction between them is complex, presenting the

characteristics of "network sex". Therefore, regulating inflammatory factors and blocking the development of inflammation is the hot spot for treating MODS. In recent years, the role of peroxisome proliferation-activated receptor (PPARs) in the pathogenesis of MODS has received more and more attention. The study found that PPAR has a wide range of functions, and plays an important role in lipid metabolism, glucose metabolism, cell proliferation, differentiation, apoptosis, wound healing and inflammation. PPARs [1] includes PPAR alpha, PPAR beta and PPAR gamma 3 subtypes. In the in vivo and in vitro experiments found that PPAR gamma and its ligands in the cell molecular level control inflammation and immune response, the activation of PPAR gamma by blocking cox-2 to implement is immune to all kinds of factors which can adjust the immune function. Epoxy synthase (cyclooxygenase, COX) is the prostaglandin (PGS) synthesis of speed limit of a key enzyme in the process, it can be arachidonic acid metabolism for a variety of prostaglandin product, to participate in the activities of the body's vital physiological. The two isomers of the cox-1 (cyclooxygenase - 1, cox-1) and cox-2 (cox-2, cox-2, cox-2) are mainly composed of cox-1 (cyclooxygenase - 1, cox-1) and cox-2 (cox-2). Cox-1 is expressed in various tissues to stabilize the cell structure and participate in normal physiological activities. Cox-2 is induced enzyme, not express or trace expression in normal tissue, but in the body "severe trauma, infection, shock" stimulating factors, the expression of cox-2 rapid increase [2], inducing the synthesis of a variety of PGs, causes the body's inflammatory response to be amplified and continuous development, cox-2 is activated at the same time after can inhibit the release of TNF alpha and other cytokines, have anti-inflammatory effect. Arachidonic acid metabolites generated by cox-2 - delta - 15 - to oxygen (12, 14) prostaglandin J2 (15 d - PGJ2), is the most important natural endogenous ligand of PPAR gamma, combined with PPAR gamma, by inhibiting the activity of cox-2, thus inhibiting the expression of inflammatory factor, the synthesis of cox-2 regulation; In addition, the 15d-pgj2 can be combined with the PPAR gamma receptor in the nucleus to reduce cox-2 activity through the PPAR gamma-cox-2 negative feedback pathway.

2. Data and Methods

2.1. General Data

Selection of 60 cases of MODS patients in the emergency department and ICU of the hospital in this hospital were the experimental group. According to the patients of acute physiology and chronic health evaluation system Ⅱ (APACHE II) score into MODS1 groups (APACHE Ⅱ score 0-10), MODS group 2 (APACHE Ⅱ scoring 11-20), MODS 3 groups (APACHE Ⅱ score > 20) 3 groups, 20 cases in each group. In the same period, 20 patients with age, gender, and physical fitness were treated as a healthy control group. All 60 MODS patients were eligible for the 2001 international sepsis meeting on MODS diagnostic criteria. Exclusion criteria: (1) not conforming to MODS diagnostic criteria. (2) age <18 years, pregnant or lactating women. (3) taking anti-tumor drugs, receiving radiotherapy, chemotherapy and immunosuppressive therapy, and receiving organ transplant patients with leukemia, lymphoma, AIDS and leukocyte reduction. Sixty cases of MODS, 36 males and 24 females. Average age (56.7 + 13.2 years); In the 20 cases, 10 were male and 10 were female. Average age (51.8 plus or minus 12.48 years old).

2.2. Main Reagent and Instrument

(1) the peripheral blood lymphocyte separation biological technology co., LTD. (tianjin) (2) mRNA detection: total RNA RNA extraction kit, two footwork rt-pcr kit, AMVcDNA first chain synthesis kits, with PCR amplification kit are bought from pioneer technology co., LTD Taq DNA polymerase, PCR Markers (Promega, USA); TDL - 40 b type centrifuge (Shanghai anting scientific instrument factory), photographic microscope (push around - BH - 2) Olympus company (Japan), table (Haerus, west Germany), high-speed centrifuge frozen, ultraviolet spectrophotometer (Gene Quant pro UK) thermal cycler system for cryogenic refrigerator (Sanyo Japan) DNA (Perkin Elmer, USA), gel imaging system (Bio - RAD Laboratories - Segrate Italy)

The research conforms to the medical ethics standard and obtains the approval of the hospital ethics committee.

2.3. Test Indexes and Methods

2.3.1. Specimen Collection

1d, 3d and 6d of the patients were selected for the detection of PPAR gamma and cox-2mrna expression levels in 1d, 3d and 6d after diagnosis. 20 patients in the healthy control group were selected 2ml venous blood test on the day of the physical examination.

2.3.2. Separation of Peripheral Blood Mononuclear Cells (PBMCs)

Ficoll density gradient method is used to isolated peripheral blood mononuclear cell (PBMC), add the mononuclear cells isolated, 1 ml - 70°C cracking fluid cryopreserved for a determination.

2.3.3. Total RNA Extraction

RNA extraction kit was used to extract total RNA and follow the instructions. The absorbance value of total RNA was determined by ultraviolet spectrophotometer (A260 / A280), and the results were both 1.8-2.0, indicating the high purity of RNA extracted.

2.3.4. Expression of PPAR Gamma and COX-2

PPAR gamma, cox-2 expression determination PPAR gamma upstream primer (5 '-3'): tccaccttattattattctg; Downstream primer (5 '-3'): atttgtctgtctttcc. Upstream primers (5 '-3') of the endoginseng beta-actin (beta actin): -atcgtgcgtgtgacattaaggag -A downstream primer (5 '-3'): -aggaaggaaggctggaagg -. Cox-2 upstream primer (5 '-3'): 5 '-tggtctggtgcctggtctgg - 3'; Downstream primer (5 '-3'). (the

primer sequence was synthesized by Beijing orco biotech company). RT-PCR on PCR. General product 77.4 ul: 2 x Taq PCR MasterMix 25ul, ddH2O 50ul, PPAR gamma or beta actin upstream and downstream primer each 1.0 ul, cDNA template 0.4 ul. The PCR reaction conditions: 94°C for 4 min degeneration, 94°C for 30 SEC, 55°C for 30 SEC, 72°C for 30 SEC, 35 cycles. The rt-pcr reaction product 5ul was absorbed, and the sample buffer was fully mixed with the 1ul DNA. The 80 v constant-pressure electrophoresis was obtained in the 80 v of agarose gel, and 0.5mu g/ml bromide was stained for 10 min. After the electrophoresis, observe the results under the uv light and follow the image.

2.3.5. Image Collection and Analysis

Application of BIO - PROFIF line of gray scale scanning image analysis system, snapping the beta actin stripe scanning value as a standard, calculation of PPAR gamma mRNA/beta actin, NF - kBp65mRNA/beta actin absorbance ratio, record PPAR gamma mRNA, cox-2 p65mrna relative expression.

2.4. Statistical Processing

Data statistical analysis USES SPSS17.0 statistical software. Each parameter is represented by mean or minus standard deviation (or plus or minus S). The analysis of the variance of normal and homogeneity of homogeneity in the data of normal and variance is studied. There was a difference between the LSD test and the Dunnett test. There was no significant difference between P and BBB 0 0.05, P <0.05 was significant, and P <0.01 was significant.

3. The Results

3.1. PPAR Gamma Expression in PBMCs

PPAR gamma expression in PBMCs was changed in PBMCs. The expression of PPAR gamma mRNA in normal group PBMCs was less, and compared with normal group, the expression of PPAR gamma mRNA in MODS1 group was higher and higher in time, reaching the peak value of the 6th day (P < 0.05). P< 0.01) and so on. The expression of PPAR gamma mRNA in MODS2 group and MODS3 group was lower and lower during the 6th day. P < 0.01). The expression of PPAR gamma mRNA at the same time was not statistically significant in MODS3 group and MODS2 group (P BBB 0 0.05). The differences between MODS2, MODS3 and MODS1 were statistically significant (P < 0.05).

Table 1. *Expression of PPAR gamma mRNA in PBMCs in different groups (plus or minus S) Category 1 day 3, day 3, day 6($\bar{x}$ ±S).*

group	Day 1	Day 3	Day 6
Normal control group	1.01±0.15	1.01±0.15	1.01±0.15
The MODS1 group	1.10±0.20 *	1.25±0.23 *	1.68±0.24 **
The MODS2 group	0.95±0.15 *^	0.78±0.12 **^	0.59±0.11**^
The MODS3 group	0.98±0.14 *	0.70±0.14 **	0.51±0.13**

Compared with the normal control group; * P < 0.05, P < * * 0.01; Comparison with MODS1 group; ^ P < 0.05.

3.2. COX-2 mRNA Expression in PBMCs

Normal group of peripheral blood mononuclear cells of cox-2 mRNA expression quantity is less, compared with normal group, group MODS1 cox-2 mRNA expression is lower and lower over time, to heaven to minimum 6 (P < 0.05; P< 0.01) and so on, the expression of cox-2mrna in MODS2 and MODS3 was higher and higher over time, reaching a peak value of 6 days (P < 0.05). P < 0.01). There was no statistically significant difference between the MODS3 group and the MODS2 group at the same time (P BBB 0 0.05). The differences between MODS2, MODS3 and MODS1 were statistically significant (P < 0.05)

Table 2 *Expression changes of pbmcscox-2mrna in different groups (plus or minus S) Category 1 day 3, day 3, day 6($\bar{x}$ ±S).*

group	Day 1	Day 3	Day 6
Normal control group	0.11±0.02	0.11±0.02	0.11±0.02
The MODS1 group	2.35±0.21 **	1.76±0.17 **	0.69±0.08 **
The MODS2 group	2.46±0.22 **^	2.49±0.24 **^	2.51±0.25**^
The MODS3 group	2.48±0.21 **	2.51±0.22 **	2.55±0.21**

Compared with the normal control group; * * P < 0.01; Comparison with MODS1 group; ^ P < 0.05.

3.3. PPAR Gamma and COX-2 Correlation Analysis

PPAR gamma and cox-2 expression in PBMCs were negatively correlated with MODS1 group, MODS2, MODS3 group (MODS1 group r= -0.761, P< 0.01; MODS2: r= -0.782, P< 0.01; MODS3 group r= -0.791, P< 0.01). No significant correlation was found in the normal control group (r= 0.183, P BBB 0 0.05).

Multi-organ dysfunction syndrome (MODS) is a clinical syndrome of two or more organs or systemic dysfunction that occur after the patient is severely affected by severe infection and trauma [3]. MODS is a dangerous and complicated mechanism with a high fatality rate. At present, it is believed that the large release of inflammatory mediators and the chain reaction of the "cascade" of uncontrolled inflammation are the main causes of MODS.

In recent years, PPAR gamma has been found to be a key point in the regulation of inflammatory response, and the study on PPAR gamma anti-inflammatory effects found that PPAR gamma inhibited various immunological factors by preventing the activation of nf-kappa B. Studies have shown that PPAR gamma ligands can prevent mononuclear - macrophages from inflammatory reactions, producing anti-inflammatory and/or desensitization phenotypes. PPAR gamma has become a new target of regulating monocyte gene expression [4]. Many studies [5] show that activation of PPAR gamma can have physical effects with NFAT and cox-2 to block the activation of downstream transcription factors.

The present study showed that the expression level of PPAR gamma in MODS1 group was higher than that in the normal control group (P< 0.05), and the expression level of PPAR gamma increased with the improvement of the disease as the treatment time increased. The expression level of PPAR

gamma in MODS2 group and MODS3 group was lower than the normal control group (P< 0.05), and the expression level of PPAR gamma decreased with the severity of the disease, indicating that the expression of PPAR gamma was closely related to the occurrence and development of MODS. The early PPAR gamma expression of MODS was elevated, which was beneficial to the body, the inflammation was suppressed, and the organ function improved. With the aggravation of the disease, the PPAR gamma showed progressive decline, the inflammatory factor was released from the control, and the function of the organ was further aggravated. It is proved that PPAR gamma has the function of blocking inflammatory reaction and protecting the function of viscera in MODS.

4. Discussion

Related studies have found that cyclooxygenase is a kind of important speed limit enzyme, which makes the peanut tetrachlic acid (AA) broken down into prostaglandins, the central part of the inflammatory response. One of the products, 15d-pgj2, is a very important natural ligand of PPAR gamma, which can inhibit the inflammatory reaction with PPAR gamma, and its synthesis is regulated by cox-2 [4]. High expression of cox-2 can increase the 15 d - PGJ2 synthesis, through activation of PPAR gamma, inhibit the nf-kappa B to show anti-inflammatory effect, on the other hand, 15 d - PGJ2 receptors with PPAR gamma phase conjugation, through the PPAR gamma - the nf-kappa B negative feedback circuit, the activity of cox-2 down [4]. So cox-2 has a dual function.

The study confirmed that cox-2 was induced and expressed on a large scale during the occurrence of MODS, resulting in increased inflammatory response [5-6]. Cox-2 is an important speed limit enzyme in the synthesis of ppar-gamma natural ligand 15d- PGJ2, which suggests that ppar-gamma may be the downstream gene involved in the disease of cox-2 [7]. The mechanism of ppar-gamma activation to inhibit inflammatory response is mainly by inhibiting the activation of nf-kappa B, thereby inhibiting the expression of various cytokines and inflammatory factors such as il-1, il-8, TNF alpha, etc. [8]. It can also regulate the expression of cox-2 through the feedback mechanism [9].] in this study, cox-2 content in peripheral blood mononuclear cells in the normal control group were significantly lower than that of patients with MODS and its mRNA expression, and survival in patients with significantly lower than the death of patients, that cox-2 content in cells is closely related with illness weight. The c0x-2 gene contains the binding site of nf-kb, LPS, tnf-a, free radical and other external factors can activate nf-kb, and the combination of nf-kb and COX-2 gene sites leads to the increase of cox-2 expression. NF-KB is the key factor of gene transcription and is involved in early response regulation of body defense function and inflammatory response. Therefore, the study on the activation of nf-kb and its target gene cox-2 has become an important therapeutic means for many diseases, so it is important to see that nf-kb and its target gene cox-2 are important in MODS [10]. Importantly, PPAR expression or ligand activation had major impacts on clonogenicity and/or tumor volume. Thus, PPARγ could be therapeutically targeted for the treatment of squamous cell carcinomas [11].

5. Conclusion

This study showed that the expression level of cox-2 in MODS1 group was higher than that in the control group (P< 0.05), and the expression level of cox-2 decreased with the improvement of the treatment time as the treatment time increased. It was suggested that cox-2 was involved in the development of MODS through its downstream product, 15d-pgj2. 15 d - PGJ2 PPAR gamma is the strongest natural ligand, PPAR gamma expression to rise, thereby blocking the inflammatory cascade effect, inhibit the release of inflammatory cytokines, feedback inhibition of cox-2 activity, organ damage, suggests that cox-2 can lessen the effect of MODS patients with multiple organ dysfunction. The expression levels of cox-2 in MODS2 and MODS3 were significantly higher than that in the control group (P< 0.01). With the progress of the disease, the cox-2 expression was elevated, the inflammatory factors were released and the organ dysfunction was further aggravated.

The anti-inflammatory properties of PPAR gamma are gradually recognized and accepted by people, indicating the broad prospects of PPAR gamma in the prevention and control of MODS, and many scholars have used PPAR gamma as a new target and new method to control MODS. This study based on the research of previous experimental studies, preliminarily PPAR gamma, cox-2 interaction mechanisms at different times of the patients with MODS, but due to various interventions for the treatment of drug in clinical research process and cannot be used agonists, antagonists, and many other conditions, PPAR gamma, cox-2 inflammation factors such as the relationship and effect in the human body there are still many unknown fields is worth our further research. It is believed that with the in-depth study, PPAR gamma and cox-2 will provide a new method for the prevention and treatment of clinical critical diseases such as MODS.

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