Original Article
Expression of COX-2 in different types of arthritis

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Abstract: The specific overexpression of COX-2 is not only affected by the function of normal cartilage cells, but also lead to metabolism imbalance of chondroproteoglycan, which is mediated by inflammatory cytokines, so as to make arthritis irreversible progress and aggravate the disease. Study the mRNA and protein differential expression of COX-2 in the periosteum of rheumatoid arthritis, rheumatic arthritis, bone arthritis, and psoriatic arthritis in order to provide a theoretical basis for the specific treatment of different types of arthritis. From January 2010 to December 2013 in Peking University Hospital 117 cases of patients with arthritis and 30 cases of controls were collected. Suprapatellar bursa fluid was extracted. The COX-2 protein content in articular tissue liquid was measured by ELISA, expression of mRNA in tissue fluid of COX-2 was detected by RT-PCR, quantitative analysis of COX-2 protein was carried out by Western blot. A total of 80 cases were supervised, according to the Ct value of β-actin and COX-2 gene, the expression of mRNA was calculated, and the expression of COX-2 protein was significantly increased in patients with osteoarthritis, rheumatoid arthritis and psoriatic arthritis, while the expression of COX-2 in rheumatic arthritis and control group was not significantly changed, there was not statistically significant. The expression of COX-2 in different types of arthritis had a significant difference, which needed to take different treatment measures.

Keywords: Cyclooxygenase, osteoarthritis, gene expression

Introduction

Arthritis is a kind of disease induced by inflammatory reaction that occur in human joints and the surrounding tissues, which are divided into dozens of types. Widely occurred in the world, China has more than 100000000 arthritis patients, and the number has been increasing. Clinical manifestations show red, swollen, hot, pain, dysfunction and deformity in joint, which seriously influence the life quality of patients [1]. It is one of the most serious diseases.

Arthritis is caused by mental stimulation, cold, damp, wind, strain, postpartum, trauma and other incentives. Early systemic manifestations may be fever, fatigue, muscle soreness, fatigue, anorexia, weight loss and anemia etc. The common local symptoms are that the patients feel only a small number of (1-2) joints pain, but there is no obvious swelling and fever. A few weeks and months later, a few numbers of joints are found swelling and limitation of activity and gradually involving other symmetrical joints. Furthermore, there are obvious stiffness and similar degenerative joint disease joint stiffness [2, 3]. According to the classification of different arthritis, it has different diagnostic methods and different treatment methods.

Different arthritis has different etiology, clinical manifestations, treatment and prognosis, which mainly related to inflammation, autoimmune reaction, infection, metabolic disorders, trauma, and other factors related to the disease. So it is important to study the pathogenic factors of all types of arthritis [4, 5].

There is a correlation between the occurrence of arthritis and the fatigue of the joints. If the joints are too tired combined with dietary imbalance, which will lead to acidic constitution, with the accumulation of acidic substances in the body, the inflammatory reaction occurred. On the other hand, the acidic environment has an effect on the cartilage, which can partially degrade cartilage, and at the same time, cartilage synovial fluid will be reduced. Along with

the two reasons and further influence, caused by spur production, further aggravated the arthritis [5, 6].

In addition, an acidic body will cause trace elements to lose, especially the loss of calcium related arthritis is an important reason for the occurrence of arthritis. Moreover, the imbalance of calcium is not significantly correlated with the type of arthritis, and most types of arthritis are related to calcium imbalance [7, 8].

Cyclooxygenase (COX) is a necessary enzyme for prostaglandin (PGs) synthesis, which is also a key enzyme in the initial step of synthesis, which is called epoxide hydratase. In general, the effective expression of COX1 is able to ensure the normal operation of the body function and the normal physiological function of the tissues and organs. But COX2 is manifested as the physiological function of the opposite, when the high expression of COX2 precisely the inflammation or tumor occur. The expression of COX2 is highly expressed in arthritis, which can increase the expression of PGE2 and result in the accumulation of free radicals in the body. But when inflammation occur, the disease is further aggravated, the clinical manifestation such as edema, pain, etc appear [9-11]. The use of molecular antagonists can relieve symptoms.

Materials and methods

Research object

The experimental groups were the arthritis patients in the departments of orthopedics, general surgery, dermatology and other related departments of Sir Run Run Shaw Hospital of Medical College of Zhejiang University from January 2011 to December 2014. After clinical diagnosis of arthritis, the patients were registered with the clinical data.

Tissue fluid sampling was obtained from lateral margin of the patella by patellar puncture, location was carried out at the junction between upper edge of patella and the vastus lateralis muscle. Press the junction between the depression of the patellar lateral femoral muscle and pierce 0.5-1 cm sticking a snail, appearance of frustration sense would meet requirement. Its characteristics: the distribution of neurons was little, the pain assessment was good, and the feeling was not sensitive, the tissue was thin to operate easily. Near the suprapatellar bursa, the fluid of suprapatellar bursa was down crowded, therefore, the liquid was extracted more thoroughly, and the fluid was directly extracted by the needle moving upward.

Control groups: random patients hospitalized in Sir Run Run Shaw Hospital of Medical College of Zhejiang University during the same period, which were designed according to the ratio of male: female 1:1. The ages of patients were 33-70 years old, whose average age were 58.39±9.41 years old. There was no significant difference in age and sex distribution between the two groups (P>0.05).

The study protocol was approved by the Research Ethics Committee of Sir Run Run Shaw Hospital of Medical College of Zhejiang University, and all patients gave their informed consent before study commencement.

ELISA

The ELISA kit was purchased from IBL (Cat. No. 27186, Japan). 300 μl IBL lysate were added into 500 μl joint tissue liquid, mixing with oscillator mix or with pipette gun, rotating for 30 minutes in 2-8°C, and then centrifuging with 10000 rpl for 10 minutes. 200 μl treating fluid were detected for ELISA, and detecting steps were carried out according to the instructions.

RT-PCR

Total RNA was extracted from the tissue fluid, according to the manual operation, 50 μl free RNAase water was used to wash off. 1% agarose gel electrophoresis was used to determine the total RNA quality; the ratio of A260/A280 was more than 1.8 which was measured by UV spectrophotometry. The purity and the concentration of total RNA meet the requirements.

Reverse transcription reaction of total RNA: Reverse transcription of total RNA was carried out using a one-step reverse transcription kit (Qiagen). According to the kit instruction, after being centrifuged, 11 μL DEPC H_2O and RNA (1 μg) were add into EP tube, the mixtures were put on the ice, when RNA final concentration reached 0.5 g/L, 1 μL oligo (dt) was added, gently mixed evenly, short time of centrifugal. 5× buffer 4 μL, ribonucleotide enzyme inhibitors 1 μL, 10 mm dNTP MIX 2 μL, M-Mulu Reverse transcriptase 1 μL were added in turn, after being mixed, short time of centrifugal. The

Table 1. Primer sequences and related information

Gene Name	Gene primer sequence (5'-3')	Gene product length	Gene product value
COX-2	FW: TCGCTGTGCCTGATGATTCC RV: TCGCTTATGATCTGTCTTCC	190 bp	86°C
β-actin	FW: TGACGTGGACATCCGCAAAG RV: CTGGAAGGTGGACAGCGAGG	205 bp	82.5°C

above reactions were placed on the ice, and the samples were added to the PCR to react.

RT-PCR primer design and synthesis: PCR primer was designed and assessed by PrimerPremier 6.0 and Oligo7 software, respectively, which were synthesized by shenggong Shanghai (**Table 1**).

Real-time PCR detected Ct value of gene amplification, the Ct value was negatively related to DNA initial copy number. ΔCt value of each sample was analyzed according to an internal reference named β-actin by relative quantification. ΔCt = Target gene Ct-internal reference gene Ct, ΔCt was the data of analysis value.

Western-blot

GABAAR in lung tissue was detected by Western blot. 12 μl proteins were dissolved in 2× SDS buffer, after being boiled 5 minutes, the samples were transferred to the (PVDF) membrane by SDS-PAGE, after the membrane being incubated with evaporated skimmed milk for 1 hour, PVDF membrane was incubated with one antibody, after washing 3 times with TBST membrane and HRP labeled two antibody was being incubated for 1 hour. After washing with TBST, display color belt were analyzed by ECL chemiluminescence.

Statistics analysis

Statistical analysis of laboratory test results was carried out by SPSS19.0 software. Comparison among multiple groups, homogeneity test of variance, analysis of variance (ANOVA) and pairwise comparison (q test) were performed in turn. T test and X^2 test were performed between the two groups. Measurement data was showed with x ± s, $P<0.05$ for the difference was significant.

Results

General clinical data

80 cases of arthritis patients were selected from Gansu Second People's Hospital, including 23 males and 57 females, aged from 41 to 78 years, with an average age of 56.4 years. The types of arthritis were shown in **Table 2**. All cases were consistent with the diagnostic criteria of knee joint OA formulation of USA Rheumatism Association [2], and the X-ray classification of OA was 1~3 grade according to Kellg-ren standard [3]. The score table named psoriasis area and severity index (PASI) was used for classification and diagnostic criteria of psoriatic arthritis. All selected cases were not treated with the anti-chemokine drugs, or the follow-up was guaranteed to stop other related drugs for more than 2 weeks. Exclusion of patients with active gastrointestinal ulcers, kidney, liver disease or blood coagulation dysfunction, as well as AIDS and other infectious diseases of blood, the patients were diagnosed inflammatory arthritis, gout or acute knee joint injury by clinical diagnosis.

ELISA detection of Cox-2 in articular tissue fluid

Preparation of standard product: 0.5 ml of distilled water were put into the standard bottle, get 140 ng/ml standard product of human COX-2 after being mixed. 230 μl standard product was mixed in the No. 1 tube, and then 230 μl standard product extracting from No. 1 tube was added to No. 2 tube, followed by continuous dilution in turn and the standard range was 70~1.09 ng/ml. The OD value was read at A450. Before drawing the standard curve, the OD values of all the detection holes (including standard and unknown sample) were subtracted from sample blank hole. In the logarithmic coordinate paper, the corresponding concentration of standard OD based on which a standard curve was made.

mRNA results of COX-2 being detected by fluorescence RT-PCR

Internal reference gene named β-actin and target gene named COX-2 were amplified by fluorescence quantitative PCR, respectively, and the fluorescence amplification curves were obtained, which appeared obvious S-type amplification curve. Dissolve curve and dissolve peak were obtained by making dissolve curve, the results showed that Tm value of beta-actin in each reaction hole was 82.5°C, the Tm values

Table 2. General clinical data analysis of the research object

Groups	Case number (n)	Average age (years)	Male/female ratio	General assessment			Kellgren classification		
				Good	Medium	Bad	1 grade	2 grade	3 grade
Osteoarthritis	30	56.4	0.76	0	7	23	0	11	19
Rheumatic arthritis	30	23.2	1.31	1	12	17	0	11	19
Rheumatoid arthritis	30	43.8	0.63	0	10	20	0	11	19
Psoriatic arthritis	27	45.6	1.43	0	4	26	0	2	28
Control	30	44.7	1.22	27	3	0	28	2	0

Table 3. Difference statistics between the expression of objective gene and target protein

Groups	Content of COX-2 by ELISA test	ΔCt of COX-2 mRNA by (F) RT-PCR test	Protein by Western blot test
Osteoarthritis*	50.32 ng/ml	27.21	1.845
Rheumatic arthritis	2.08 ng/ml	34.12	0.041
Rheumatoid arthritis*	47.63 ng/ml	28.33	1.699
Psoriatic arthritis*	57.89 ng/ml	25.88	2.385
Control	0.07 ng/ml	37.99	0.000
P value	<0.05	<0.05	<0.05

Superscript *represented statistical significance (P<0.05, versus control).

of the COX-2 gene of the target genes of each sample were 86°C. There only one peak in each of the amplification curves, which proved that PCR only produce one product, and which was not affected by the primer dimer.

Western blot

Protein bands were analyzed by gray scale after ECL chemiluminescence. The expression of COX-2 protein was significantly increased in patients with osteoarthritis, rheumatoid arthritis group and psoriatic arthritis group, but there was no obvious change in the expression of rheumatic arthritis and control group (**Table 3**). It was showed that the expression of COX-2 was not associated with the occurrence of rheumatic arthritis.

Discussion

The incidence of arthritis is often associated with mutiple internal causes and external causes including inheritance, bacterial/viral infections, environmental factors. Arthritis may occur at any stage in life. Moreover, incidence rate of arthritis is gradually increasing with ages, especially higher in women aging 40 to 60. The facet joints of hands were generally involved in arthritis progress, such as finger joints and wrist joints, and the bilateral lesions occurred simultaneously, which seriously impacted on the quality of life and work. Patients were accompanied by a morning stiffness of the joints, which lasted more than 1 hour and limited joint activity. When the disease was serious, the whole body organs could be involved. So early diagnosis and treatment were particularly important [11, 12].

In daily life, many people have joint pain problems. Many reasons result in arthritis. Given multiple factors of patients, including age, sex, location of onset and symptoms, common diagnosis were made as soft tissues arthritis, cartilagious arthritis, osteoarthritis or inflammatory arthritis. Arthritis, regardless of its cause, generally could be cured or relieved if timely medical treatment and symptomatic treatment was promptly performed [12, 13].

According to the research analysis, the occurrence of osteoarthritis is closely related to age and obesity. The prevalence of osteoarthritis is only 2% under 45 years old, and the prevalence rate of 65 years old is 68%. Age and obesity are the risk factors of osteoarthritis, which clinical manifestation show all joints are involved, but not limited to the joints of upper hand. It can be found that the patients with negative rheumatoid factor in the blood [13, 14].

Rheumatic arthritis belongs to allergic disease, which is one of the main manifestations of rheumatic fever. With acute onset of fever and joint pain, typical clinical manifestations are mild/moderate fever and migratory multiple joints pain, and most of the involved joints are knee, ankle, shoulder, elbow, wrist and other large joints. Common performance has a joint transfer to another joint, the lesion shows red, swollen, hot, pain, some patients also have the

incidence of several joints at the same time. Subtypical patients have only joints pain but no other joint inflammation, acute inflammation is generally subside after 2-4 weeks, leaving no sequelae, but which frequently repeated attack. If the rheumatic activity affect the heart, myocarditis and even cardiac valvular lesions may occur [14, 15].

The pathogenesis of gout arthritis is due to endocrine causes, which is related with produce increasing and discharge reduction of uric acid. When uric acid is unable to discharge normally, it will precipitate in the key place, especially in small joints, which is characterized by acute onset and rapid relief, which can be caused by excessive eating. The clinical manifestations are red, swollen, heat and pain. Single joint involvement was more common. That is a need for an appropriate drug intervention in a timely manner, but often relapse again [12-14].

The types of arthritis are various and the pathogenesis is complex. Recent experiments in vitro confirm that the special expression of COX-2 is not only affect the function of normal articular cartilage cells, but is mediated by inflammatory cytokines in the metabolic imbalance, so as to make arthritis irreversible progress and aggravate the disease [14, 15]. The study of COX-2 has become a new trend of the treatment of arthritis.

Expressed rarely in normal circumstances, COX-2 is an enzyme which was produced and induces effect with induction of intracellular or intercellular inflammatory cytokines, for example, some immune cells such as mononuclear cells and macrophages can express COX-2. In addition, some of endothelial cells such as synovial cells, fibroblasts and endothelial cells also can express COX-2 [16, 17]. In pathological conditions, the high expression of COX-2 can promote the formation of new blood vessels and help tumor cells escape immune surveillance and other effects to promote the occurrence and development of arthritis by inhibiting apoptosis. Anti-inflammatory effect is positive associated with inhibition level of COX-2 [18-20]. Different types of arthritis have different expressions of COX-2. Our study found that ΔCt value of rheumatoid arthritis inflammatory group and the control group both was over 30, while the ΔCt value of bone arthritis, rheumatoid arthritis inflammatory group and psoriatic arthritis group were between 25-28, due to the ΔCt value and protein expression was negatively correlated, therefore COX-2 expression of bone arthritis and rheumatoid arthritis inflammatory group and psoriatic arthritis inflammation group in mRNA level was much higher than that of rheumatic arthritis group and control group. Western blot showed that the expression of COX-2 was not associated with the occurrence of rheumatoid arthritis. Consequently, COX-2 inhibitor treatment was effective for osteoarthritis, rheumatoid arthritis and psoriatic arthritis, while no efficacy of COX-2 inhibitor was observed for rheumatoid arthritis due to absence of targeting molecule COX-2.

In addition, COX-2 inhibitors have many advantages, such as promising analgesic effect and less gastrointestinal adverse reactions, which have been widely used in clinical treatment [21-23]. Based on our results, the effect of COX-2 inhibitors on the clinical treatment of arthritis warrants further exploration.

Disclosure of conflict of interest

None.

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