Original paper

Activities of antioxidant enzymes and concentrations of selected oxidative stress biomarkers in synovial membranes of patients diagnosed with osteoarthritis

Aktywność enzymów antyoksydacyjnych i stężenie wybranych biomarkerów stresu oksydacyjnego w błonach maziowych pacjentów z chorobą zwyrodnieniową stawów

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Słowa kluczowe: błona maziowa, choroba zwyrodnieniowa stawów, stres oksydacyjny, enzymy antyoksydacyjne, dialdehyd malonowy.

Abstract

Introduction: Osteoarthritis is a chronic degenerative process of multifactor etiology, still largely unexplained. The activity of antioxidant enzymes and concentration of lipid peroxidation products in synovial membrane can be indirectly inferred from its oxidation-reduction status. The role of synovial membrane in the pathogenesis of osteoarthritis is relatively poorly understood, probably due to the difficulties in the acquisition of the material.
Aim of the research: We sought to understand the association between osteoarthritis development and the activities of several antioxidant enzymes and levels of selected oxidative stress biomarkers in synovial membranes of osteoarthritic patients.
Material and methods: The synovial tissue samples were collected from 67 patients. Of these patients, 48 underwent endoprosthetic total hip replacement due to osteoarthritis (the examined group), while 19 underwent surgical repair of a traumatic femoral neck fracture (the control group). Homogenates of synovial membrane were prepared according to our published method. The biochemical analysis included: activity of copper-zinc superoxide dismutase (CuZn-SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), concentrations of protein, malondialdehyde (MDA), thiol groups, and total oxidant status (TOS).
Results: When comparing the examined group with the control group, the activity of CuZn-SOD was significantly elevated and the activity of GPx and the level of thiol groups were significantly decreased, whereas the activities of GR, CAT, the levels of protein, MDA, and TOS remained unaltered.
Conclusions: Osteoarthritis is associated with oxidative stress and altered activities of the antioxidant enzymes in synovial membrane. However, lipid peroxidation seems not to be important for the etiology and development of osteoarthritis.

Streszczenie

Wprowadzenie: Choroba zwyrodnieniowa stawów jest przewlekłym procesem zwyrodnieniowym o wieloczynnikowej etiologii, wciąż niewyjaśnionym. Aktywność enzymów antyoksydacyjnych i stężenie produktów peroksydacji lipidów w błonie maziowej pośrednio wnioskuje o jej stanie oksydacyjno-redukcyjnym. Rola błony maziowej w patogenezie choroby zwyrodnieniowej stawów jest stosunkowo słabo poznana, prawdopodobnie ze względu na trudności w pozyskiwaniu materiału.
Cel pracy: Określenie związku między rozwojem choroby zwyrodnieniowej stawów a aktywnością enzymów antyoksydacyjnych i poziomem wybranych biomarkerów stresu oksydacyjnego w błonach maziowych pacjentów z chorobą zwyrodnieniową stawów.
Materiał i metody: Próbki tkanki maziowej pobrano od 67 pacjentów. U 48 pacjentów wykonano endoprotezoplastykę stawu biodrowego z powodu choroby zwyrodnieniowej stawów (grupa badana), a u 19 pacjentów – chirurgiczną plastykę urazowego złamania szyjki kości udowej (grupa kontrolna). Homogenaty błony maziowej przygotowano zgodnie z autor-

ską metodą. Analiza biochemiczna obejmowała: aktywność miedziowo-cynkowej dysmutazy ponadtlenkowej (CuZn-SOD), peroksydazy glutationowej (GPx), reduktazy glutationowej (GR), katalazy (CAT), stężenia białka, dialdehydu malonowego (MDA), grup tiolowych i całkowitego statusu oksydacyjnego (TOS).
Wyniki: Porównując grupę badaną z grupą kontrolną, aktywność CuZn-SOD była istotnie podwyższona, a aktywność GPx i stężenie grup tiolowych istotnie obniżona, natomiast aktywność GR, CAT, poziom białka, MDA i TOS pozostały niezmienione.
Wnioski: Choroba zwyrodnieniowa stawów związana jest ze stresem oksydacyjnym i zmienioną aktywnością enzymów antyoksydacyjnych w błonie maziowej. Jednak peroksydacja lipidów wydaje się nie mieć istotnego znaczenia dla etiologii i rozwoju choroby zwyrodnieniowej stawów.

Introduction

Osteoarthritis (OA) is the most common chronic joint disorder [1] that affects especially knees, hips, and hands [2]. OA develops progressively over the years and results in pain, swelling, stiffness, deformity, and instability of the joints [3, 4]. Consequently, OA is one of the major causes of disability among elderly people [5].

There are many known risk factors for OA, such as age, gender, injuries, obesity, family history of OA, focal lesions, and metabolic disorders. The central feature of this disease is degradation of the articular cartilage [1]. However, OA also affects subchondral bone, ligaments, periarticular muscles, and synovial membrane [6]. Degeneration of these structures results in cartilage loss, synovitis, and osteophyte formation, bone remodeling, subchondral sclerosis and attrition, which are the basis of radiological diagnosis [2, 7].

The pathophysiology of OA is still not fully understood. However, recent studies indicate that oxidative stress is involved in OA progression [3, 8]. Oxidative stress is defined as an imbalance between reactive oxygen species production (ROS) and scavenging abilities of the antioxidant defense system. Overproduction of ROS promotes cartilage matrix destruction, apoptosis of chondrocytes, and inflammation development [3, 9]. Additionally, ROS cause senescence of chondrocytes through shortening of telomeres as well as decreasing the number and function of mitochondria [10].

OA is usually initiated by joint injury or overuse. Mechanical strain results in increased synthesis of cytokines, such as interleukin-1 (IL-1) or tumor necrosis factor-α (TNF-α) in chondrocytes. This leads to induction of matrix metalloproteinases (MMP) that digest cartilage matrix. Simultaneously there occurs intensified conversion of phospholipids from damaged cell membranes to arachidonic acid. Further enzymatic activity converts arachidonic acid to inflammatory mediators, such as cytokines and eicosanoids. These inflammatory mediators attract white blood cells (WBC) to the joint and induce NADPH oxidase, TNF-α, and IL-1. Activated WBC liberate ROS [11].

Chondrocytes are also able to produce ROS [11]. In normal conditions, chondrocytes display metabolism adapted to anaerobic conditions. Nevertheless, in pathological conditions, an ischemia–reperfusion phenomenon results in fluctuations of oxygen tension in cartilage. As a consequence of oxygen pressure variations, chondrocytes produce abnormal levels of ROS [9].

Products of cartilage degradation and oxidized molecules may induce synovial macrophages and fibroblasts to generate a broad range of inflammatory mediators including cytokines, MMP, and ROS [6]. Additionally, cartilage breakdown products and cytokines are believed to result in mononuclear cell infiltration and neovascularisation in the osteoarthritic synovial membrane [12, 13]. These mechanisms lead to synovial inflammation, which, in turn, accelerates degradation of the nearby cartilage, creating a vicious cycle. Synovitis occurs in early and late phases of OA and manifests histologically as synovial hypertrophy and hyperplasia with an increased number of lining cells and lymphocytes in the subline tissue [2, 10, 13].

Therefore, many authors state that changes caused by OA involve not only the cartilage but also the synovial membrane [2, 6, 10, 14]. Moreover, synovial inflammation corresponds to clinical symptoms of OA, such as joint swelling or inflammatory pain [2].

Research in OA has focused mainly on cartilage and there have only been a few reports on the role of synovial membrane. Due to the fact that oxidative stress plays an important role in the pathogenesis of OA, there are many studies about levels of oxidants and antioxidant enzymes in the blood and the synovial fluid of patients with OA, but there are no studies about the oxidative status of osteoarthritic synovial membrane.

Aim of the research

We sought to understand the associations between OA development and the activities of several antioxidant enzymes and concentrations of thiol groups and lipid peroxidation products in synovial membranes of patients diagnosed with OA.

Material and methods

Study population

The experimental protocol has been approved by the Bioethics Committee of the Medical University of Silesia in Katowice No. NN-013-157/03.

The synovial tissue samples were collected from 67 patients. Of these patients, 48 underwent endoprosthetic total hip replacement due to OA and were classified as the examined group (the OA group),

while 19 patients underwent surgical repair of a traumatic femoral neck fracture and were classified as the control group.

The OA group included male patients with radiologically and clinically diagnosed OA of the hip joint. Patients' mean age was 63.0 ±12.9 years. Patients' mean weight was 82.7 ±13.1 kg. Mean OA duration was 8.2 ±7.3 years. Medical reports of 28 patients revealed past or present history of chronic diseases, such as diabetes, hypertension, myocardial infarction, coronary heart disease, or transient ischemic attack (TIA). Patients with inflammatory rheumatic diseases, or infectious- or endocrine-related arthropathies were excluded. In view of the age, weight, and presence of comorbidity, the OA group was divided into different subgroups (based on the median value).

The control group included male patients with traumatic femoral neck fracture. Patients' mean age was 57.7 ±19.7 years. Patients' mean weight was 82.1 ±14.6 kg. Medical reports of 12 patients revealed past or present history of chronic diseases, such as hypertension, myocardial infarction, or coronary heart disease. Patients with diseases of the joints were excluded from this group.

Laboratory procedures

Homogenates of synovial membrane were prepared according to our method [15]. Briefly, immediately after excision the synovial tissue was placed in ice-cold saline. The obtained samples were dissected to remove the underlying fibrous tissue and fat from the membrane. Then, synovial tissue was cut into small fragments, suspended in saline to give a final tissue concentration of about 10% (w/v), homogenized, and stored at –20°C. After defrosting, the samples were ultrasonically homogenized and centrifuged. In supernatants, the biochemical analysis was performed.

The protein concentrations were indicated by the method of Lowry [16]. All the values of other parameters were expressed per mg of protein or per 100 mg of synovial tissue.

The activity of copper-zinc superoxide dismutase (CuZn-SOD) was measured by the method of Oyanagui [17] and expressed in nitric units. The activity of SOD is equal to 1 nitric unit (NU) when it inhibits nitric ion production by 50%. Catalase (CAT) activity was measured by the Johansson and Borg [18] method. Glutathione peroxidase (GPx) activity was measured by the kinetic method of Paglia and Valentine [19]. The activity of glutathione reductase (GR) was measured according to Richterich [20].

The concentration of malondialdehyde (MDA) was measured fluorometrically, as Ohkawa described [21]. Total antioxidant status (TOS) was measured according to Erel [22]. The level of thiol groups was measured by the method of Koster [23].

Statistical analysis

The statistical analysis was performed using Statistica 10.0 PL software. The statistical methods included the mean and standard deviation. Levene's test was used to verify the homogeneity of variances, while the Shapiro-Wilk test was used to verify normality. The statistical comparisons between the study groups and control group were made using the *t*-test, the *t*-test with separate variance estimates, or the Mann-Whitney *U* test. A value of $p < 0.05$ was considered to be significant.

Results

There were no significant differences in age and weight between the examined and control groups. By contrast, the activity of CuZn-SOD was significantly elevated in the OA group compared to the control group. Nevertheless, the activity of GPx and the level of thiol groups were significantly decreased in the OA group.

The activities of GR and CAT were unaltered in the OA group in comparison to the control group. The levels of protein, MDA, and TOS were also unchanged. Additional analyses were made in the OA group using median body mass index (BMI) (28.4 kg/m^2), age (63 years) and comorbidity to create subgroups (Table 1).

When comparing the subgroup of OA patients with mean BMI = 25.3 ±2.95 kg/m^2 (n = 22) with the subgroup of OA patients with mean BMI = 32.2 ±2.86 kg/m^2 (n = 26) the activity levels of CuZn-SOD and TOS were insignificantly decreased in patients with higher weight, while GPx activity and thiol groups level were significantly elevated in patients with higher weight. There were no significant differences in the other parameters (Table 2).

When comparing the subgroup of younger OA patients with mean age 51.87 ±9.24 years (n = 23) with the subgroup of older OA patients with mean age 73.16 ±4.82 years (n = 25), the activities of GPx and GR were significantly elevated in older patients. There were no significant differences in the other parameters (data not shown).

There were no significant differences in the analyzed parameters between the subgroup of OA patients with comorbidity (n = 28) and the subgroup of patients diagnosed only with OA (n = 20) (data not shown).

Discussion

The development of OA was thought to be related not only to excessive free radical production but also to diminished capacity of antioxidative protection [8, 24]. One of the primary antioxidant enzymes is SOD. CuZn-SOD is a cytosolic form of the enzyme [25]. SOD catalyzes the dismutation of superoxide anions to hydrogen peroxide that is eliminated by CAT or

Table 1. Activity of copper-zinc superoxide dismutase (CuZn-SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and concentration of malondialdehyde (MDA), total antioxidant status (TOS) and level of thiol groups (PSH) in control and OA groups

Parameter	Control group		OA group		*P*-value
	Mean	SD	Mean	SD	
Age [years]	57.7	19.71	63.0	12.94	0.203
Weight [kg]	82.1	14.6	82.7	13.1	0.892
CuZn-SOD [NU/100 mg tissue]	13.54	14.2	23.82	21.5	0.027
CuZn-SOD [NU/mg protein]	8.16	11.4	16.05	16.8	0.017
CAT-Px [IU/100 g tissue]	498.75	250.9	536.18	161.2	0.471
CAT [IU/mg protein]	291.26	166.4	321.40	166.5	0.516
GPx [IU/100 g tissue]	823.04	937.9	446.85	401.5	0.025
GPx [IU/g protein]	490.08	496.0	309.14	425.2	0.146
GR [IU/100 g tissue]	11.00	10.8	9.91	11.2	0.718
GR [IU/g protein]	9.27	12.4	10.11	30.0	0.906
PSH [µmol/100 g tissue]	133.28	155.2	75.83	62.2	0.033
PSH [µmol/g protein]	62.00	52.6	46.97	90.8	0.501
MDA [µmol/100 g tissue]	4.22	3.6	4.03	2.5	0.811
TOS [µmol/100 g tissue]	2.75	2.08	3.03	2.07	0.644

Table 2. Activity of copper-zinc superoxide dismutase (CuZn-SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and concentration of malondialdehyde (MDA), total antioxidant status (TOS) and level of thiol groups (PSH) in lower and higher weight subgroups of OA

Parameter	Lower weight BMI = 25.3 ±2.95 kg/m^2		Higher weight BMI = 32.2 ±2.86 kg/m^2		*P*-value
	Mean	SD	Mean	SD	
Weight [kg]	71.5	8.30	91.7	8.57	< 0.001
CuZn-SOD [NU/100 mg tissue]	27.8	29.0	15.2	16.3	0.068
CuZn-SOD [NU/mg protein]	19.7	22.3	10.2	11.9	0.072
CAT-Px [IU/100 g tissue]	507.6	116.2	561.7	191.2	0.262
CAT [IU/mg protein]	328.2	178.5	320.6	161.0	0.880
GPx [IU/100 g tissue]	289	147	574	498	0.009
GPx [IU/g protein]	164	94.8	431	547	0.032
GR [IU/100 g tissue]	10.0	11.0	10.1	11.7	0.976
GR [IU/g protein]	6.68	7.27	13.08	40.07	0.474
PSH [µmol/100 g tissue]	63.5	51.4	85.7	70.2	0.232
PSH [µmol/g protein]	28.1	14.6	62.8	121.5	0.048
MDA [µmol/100 g tissue]	3.77	2.08	4.21	2.81	0.562
TOS [µmol/100 g tissue]	3.69	2.54	2.47	1.47	0.049

GPx [6]. CAT converts hydroperoxide to water and oxygen, while GPx catalyzes the degradation of hydrogen peroxide and other peroxides by oxidizing the reduced form of glutathione (GSH) [26]. GSH is a common tripeptide and the principal non-protein thiol component of the antioxidant defense system. The oxidized form of glutathione (GSSG) is converted to GSH by GR [27].

The increased activity of CuZn-SOD observed in the present study may be due to an adaptive re-

sponse to increased oxidative stress in the synovial membrane. However, decreased activity of GPx and unchanged activities of CAT and GR, which were observed simultaneously in our study, may cause the accumulation of hydrogen peroxide that is overproduced by CuZn-SOD. Hydrogen peroxide oxidizes primarily high-sensitive thiol groups. That is the possible explanation for decreased levels of thiol groups reported in the present study. Additionally, decreased concentration of thiol groups may be secondary to GSH depletion. Nevertheless, a decrease in GPx activity could be due to the massive production of hydrogen peroxide which overrides enzymatic activity.

Many reports have associated OA and antioxidant status. However, collected data are difficult to compare because activities of the antioxidant enzymes and levels of GSH or thiol groups were measured in different materials, such as erythrocytes, serum, plasma, and synovial fluid. Also, difficulties in comparing the values obtained in a particular study may arise from different epidemiological parameters of the examined groups. Therefore, it is not surprising that the results are inconsistent [28].

The study by Surapaneni and Venkataramana [4] supports our research partially, stating that the activities of SOD and GPx were increased in erythrocytes of OA patients compared with the healthy controls, whereas GSH content in erythrocytes and CAT activity in plasma were decreased. In addition, decreased CAT activity, decreased level of thiol groups, and decreased total antioxidant capacity (TAC) were found in serum by Altindag *et al.* [9].

Sutipornpalangkul *et al.* [5] examined the synovial fluid and did not observe any changes in the activities of SOD and GPx and the content of GSH. By contrast, Ostalowska *et al.* [29] reported increased activities of SOD, CuZn-SOD, GPx, and GR in the synovial fluid of patients with knee OA. Sumii *et al.* [30] also found increased activity of SOD in the synovial fluid, but the activity of SOD in serum was decreased in the same OA patients compared to the healthy controls.

Activities of the antioxidant enzymes change with age. The activity of SOD decreases with age, whereas the activity of GPx was found to be higher in elderly people [25]. These findings are in agreement with the present study. Therefore, the significant increase in the activity of GPx and the insignificant decrease in the activity of CuZn-SOD that were observed in older OA patients may be only a result of obsolescence and may not be related to OA development.

Differences in the activities of the antioxidant enzymes were also observed when comparing the subgroups of OA patients composed according to weight. Obesity is one of the major risk factors for the development of OA. For overweight people, joint damage might be caused not only by the overload effect on joint cartilage, but also by systemic factors such as adipokines or C-reactive protein, IL-6, and plasminogen activator inhibitor [2, 11]. In our study, the alterations in the antioxidant defense system are more significant in patients with lower weight than in those with higher weight. Also, we noted an insignificant tendency for increased values of TOS in patients with lower weight compared to patients with higher weight. Taking into account the fact that weight does not influence the activities of the antioxidant enzymes [31], it is possible to state that patients who developed OA without a major risk factor for OA, such as obesity, have a less sufficient synovial antioxidant defense system than obese individuals with OA. This dysfunction of the antioxidative defenses can be associated with the accumulation of hydrogen peroxide as a result of simultaneous upregulation of the CuZn-SOD activity and exhaustion of GPx and GSH.

Hydrogen peroxide can be transformed into the hydroxyl radical through the Fenton reaction. The hydroxyl radical may, in turn, initiate lipid peroxidation [6]. The major product of the lipid peroxidation is MDA [10]. MDA not only is a marker of oxidative stress, but also contributes to DNA damage and protein modifications [32]. The concentration of MDA in the present study was not significantly changed. Similarly, no significant alterations in TOS were observed when comparing the OA group with the control group. These results led us to conclude that oxidative stress in osteoarthritic synovial membrane does not affect cellular membranes and lipid peroxidation is limited. Consistently with our findings, Ostalowska *et al.* [29] observed no statistically significant alterations of MDA level in synovial fluid of patients with knee OA. Nevertheless, elevated levels of MDA were reported by several authors [4, 12], who examined erythrocytes and plasma of patients with OA. As in the case of antioxidant enzymes, it is difficult to compare MDA levels that were measured in different materials.

Conclusions

In synovial membrane OA is associated with oxidative stress and altered activities of the antioxidant enzymes. Diminished reducing potency of thiol groups in synovial membrane was the only result of oxidative stress that was observed in the present study. In consequence, lipid peroxidation seems not to be crucial for OA etiology and development.

The role of the synovial antioxidant defense system in OA progression may be different in obese and slender patients.

Conflict of interest

The authors declare no conflict of interest.

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