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Superoxide dismutase and xanthine oxidase activities in New Zealand rabbits treated with different types of glycosaminoglycans (GAGs) after osteoarthritis surgery

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Abstract

Chondroitin sulphate is one of the glycosaminoglycans (GAGs) generally acquired from animal tissues. CS has an anti-inflammatory, anti-apoptotic and antioxidant properties. The GAGs have been recently reported to possess the ability to influence oxidative stress known to lead to free radical mediated biological damage. Herein, we report the effect of some GAGs on superoxide dismutase (SOD) and xanthine oxidase (XO) activities in experimental animal models submitted to osteoarthritis surgery. In this respect, SOD and XO activities were investigated in New Zealand rabbits treated with different types of glycosaminoglycans (GAGs); commercial chondroitin sulphate (CCS) and bacterial chondroitin sulphate (BCS) (produced by using E. coli). The results of this study revealed that both CS sources significantly ($P < 0.05$) decreased the levels of SOD and XO activities in the animal models; however, this decrease was more prominent in the animals treated with BCS than in those treated with CCS in terms of SOD activity. Based on these results, it can be concluded that treatment of the animals with the bacterial chondroitin sulphate showed beneficial effects as revealed by oxidative models. This study confirms the antioxidant properties of GAGs, suggesting the hypothesis that chondroitin sulphate could function as a beneficial substance to control oxidative stress.

Keywords: Glycosaminoglycans, superoxide dismutase and xanthine oxidase activities, animal models, commercial and microbial chondroitin sulphate

Introduction

GAGs are important structural elements of the extracellular matrix [1,2]. Depending on the composition of the disaccharide, the type of linkage and the presence of sulphate groups, GAGs are divided into four main groups as hyaluronic acid (HA), chondroitin sulphate (CS), heparan sulphate (HS) and keratan sulfate (KS) [3]. CS is a homopolymeric GAG containing the repeating disaccharide unit (4GlcAβ1-3GalNAcβ1-; GlcA, glucuronic acid; GalNAc, N-acetylgalactosamine) [1].

Current chondroitin sulfate preparations used in many applications including nutritional supplements are extracted from various animal tissues such as cattle, pigs, birds and fish [4–6]. However, due to the risk of mad cow disease and foot-and-mouth disease in cattle, influenza in birds, possible allergic reactions in fish and the possible presence of other diseases (bacterial residues, viruses and prions), the products containing CS produced from these sources reveal safety concerns for consumers [7]. In such products, there is also a risk of contamination of nucleic acids, proteins and other (macro) molecules as well as other polysaccharides. These potential problems have led researchers seek alternative sources. One of these alternative sources is microbial chondroitin sulphate [8,9].

Chondroitin sulfate is useful for supporting bone formation, accelerating bone healing process, blocking angiogenesis and tumor growth, regulating blood lipids, improving atherosclerosis, repairing and regenerating the central nervous system, and joint-related pathologies [10–12]. It is also known to have anti-inflammatory, antithrombotic, anticoagulant and antioxidant effects. Many disorders are affiliated with free oxygen radicals formed in the body. It is suggested that free oxygen radicals have an effect on this event [13]. In this respect, chondroitin sulfate is a prominent material since a great number of recent studies have focused on the biomaterials having bioactive properties belonging especially antioxidant properties. In this respect, glycosaminoglycans were reported to reduce oxidative damage induced by copper (Cu+2), iron (Fe+2) and hydrogen peroxide (H2O2) in human fibroblast cultures and commercial GAGs at different doses were shown to possess beneficial effects in all oxidative models when they were treated with commercial GAGs at different doses [14].

Measuring the activities of xanthine oxidoreductase and superoxide dismutase is among of the efficient tools to revel oxidative stress in animal models. Xanthine oxidoreductase is a member of the

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hydroxylase enzyme family. The enzyme weighs 300 kDa and has a dimeric structure. Each subunit is an enzyme composed of 1333-1358 amino acid residues containing molybdenum-iron in the flavin cofactor binding shade [15]. Xanthine oxidoreductase catalyzes the last two steps of purine breakdown in purine metabolism. It is the enzyme that controls purine catabolism. It causes the formation of free radicals while catalyzing the conversion of hypoxanthine to xanthine and xanthine to uric acid [16]. Cellular superoxide dismutase (SOD) is a group of metalloenzymes bearing various prosthetic groups. It is an essential enzyme for every cell in the organism. While SOD reduces two superoxide radicals to H2O2, catalase and selenium-dependent glutathione peroxidase (GPx) reduce H2O2 to water. The SOD shows its antioxidant effect by preventing the formation of superoxide and hydroxyl radicals [17]. In this context, this study focus on investigation on the effect of different types of GAGs (commercial and bacterial chondroitin sulphate) on superoxide dismutase and xanthine oxidase activities in animal models using New Zealand rabbits submitted to osteoarthritis surgery.

Material and Methods

Materials

Commercial chondroitin sulphate was purchased from Sigma-Aldrich (Germany) with 39455-18-0 CAS number. This product was of animal origin and obtained from bovine trachea. Bacterial chondroitin sulfate was obtained from the researchers (Erenler, Geckil, Karabulut, Akpolat, Sevimli, Ulke, Aliyeva, 2019) as a pure form that was produced using Escherichia coli. Briefly, capsular chondroitin synthesis genes (kfA, kfoC, kfoF) were cloned into a plasmid structure and this plasmid was transferred into a non-pathogenic E. coli strain (C2987). After transformation stage, chondroitin sulphate was synthesized by E. coli and purified under laboratory conditions [18]. All the chemicals and solvents were of analytical grade and purchased from Sigma–Aldrich (USA). Deionized water was used in all the experiments.

Animal experiments

Animal model and practice

The animal experimental protocol was approved by the ethics committee of Medicinal Faculty of Inonu University, Malatya, Turkey with the research protocol number (2015/A-67). Eighteen adult New Zealand rabbits weighing between 3000–4000 g were used in this study. Animals were sourced from the Experimental Animals Application and Research Center of İnönü University (Malatya, Turkey) and individually housed in standard conditions at 22 ± 2 °C with 60% humidity and 12 hours light/dark cycle.

Surgical procedure

Anterior cruciate ligaments were cut to achieve osteoarthritis in the experimental animals. For this purpose, 0.1 ml / kg 2% xylazine hydrochloride and 20 mg/kg ketamine hydrochloride were administered intramuscularly to the animals for anesthesia. The right knee joints were reached with an anterior longitudinal incision. After medial parapatellar arthrotomy, the patella was dislocated laterally and the anterior cruciate ligament was cut. Anterior drawer test was applied to control whether the cruciate ligament was completely cut or not. Experimental animals were left to normal cage activity in the postoperative period.

Feeding procedure of the animals

Water and feed were given ad libitum for eight weeks. Four weeks after osteoarthritis surgery, experimental animals were divided into three treatment groups as below:

a) Control group (n=8): Standard rabbit diet was given for 12 weeks.

b) Commercial chondroitin sulphate (CCS) group (n=5): Along with the standard diet, the animals were treated with CCS daily at 17 mg/kg dose by gavage for 12 weeks.

c) Bacterial chondroitin sulphate (BCS) group (n=5): Along with the standard diet, the animals were treated with BCS daily at 17 mg/kg dose by gavage for 12 weeks.

Sampling of serum samples

After 12 weeks of feeding procedure, all rabbits were sacrificed by intramuscular high dose ketamine administration. Inter cardiac blood samples were taken into biochemistry tubes. Approximately 10 ml of blood samples from each animal was taken into biochemistry tubes and serum was collected by centrifugation for 15 minutes at 3000 rpm at + 4°C. The collected serums were portioned into tubes labeled as “pre-experiment” and stored in the freezer at –80 0C.

Biochemical measurements

Serum superoxide dismutase (SOD) enzyme activity was determined based on the production of H2O2 from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium as previously described [19]. The product was evaluated spectrophotometrically at 560 nm. Results are expressed as U/g protein. Serum xanthine oxidase (XO) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm, according to Prajda and Weber’s method [20]. Results are expressed as U/L.

Statistical analyses

The consistency of continuous variables to normal distribution was examined using the Shapiro Wilk test. Since the variables did not provide the assumption of normality, Kruskal-Wallis test was used to evaluate the differences between treatment groups. When, there was a significant difference between the groups, Mann-Whitney test was used to discriminate each group. The significance levels of pre- and post experiment results were determined by the Wilcoxon Signed Rank test. IBM SPSS Statistics 23.0 (IBM Corp. Released 2014, IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp) was used for statistical analysis and calculations. The differences were determined at the statistical significance levels; $p < 0.05$ and 0.01.

Results

It was extensively evidenced that reactive oxygen species and other free radicals play a considerable role in human disease

states [21,22]. The increased generation of superoxide radicals is mainly due to the oxidative stress in cells and tissues. In this event, superoxide excessively reacts with SOD, which results in the production of large amounts of intracellular hydrogen peroxide. Although the free radicals are not highly toxic, they become dangerous when they are converted hydroxyl radicals like detrimental OH• through a Fenton's reaction or Haber-Weiss reaction in the existence of metal ions [23]. In this respect, GAGs were reported to have beneficial effects in terms of decreasing the oxidative stress. GAGs are linear acid polysaccharides that are comprised of alternating hexuronic acid and hexosamine units. By their interaction with a number of proteins, they can act as cellular organizers [24]. Some GAGs were reported to possess antioxidant activity which could inhibit lipid peroxidation [25–28]; therefore, they could be used as therapeutic agents leading to some positive results [29–31]..

Table 1. Effect of different types of glycosaminoglycans (GAGs) on superoxide dismutase and xanthine oxidase activities in New Zealand rabbits

	SOD (U/g) Med.(min-max)			**XO (U/L) Med.(min-max)**		
Treatment groups	**Treatment periods**			**Treatment periods**		
	Before	**After**	***P***	**Before**	**After**	***p***
Control	21.60 (20.00-26.40)^{a,y}	30.80 (30.00-34.60)^{a,x}	0.01	2.26 (2.23-2.29)^{A,Y}	2.66 (2.65-2.67)^{A,X}	0.01
CCS†	24.80 (20.00-27.20)^{a,x}	16.00 (13.60-16.53)^{b,y}	0.01	2.23 (2.22-2.25)^{A,Y}	2.46 (2.43-2.47)^{B,X}	0.01
BCS‡	24.00 (20.00-25.60)^{a,x}	7.47 (5.80-10.40)^{c,y}	0.01	2.23 (2.21-2.24)^{A,X}	2.24 (2.23-2.25)^{C,X}	0.01
P	0.248	0.01		0.055	0.01	

† CCS: commercial chondroitin sulphate.
‡ BCS: Bacterial (E. coli based) chondroitin sulfate.
$^{a-b}$ In each column, the median SOD values with different superscript lowercase letters indicate significant differences ($p<0.05$; 0.01) between treatment groups.
$^{x-y}$ In each row, the median SOD values with different superscript lowercase letters indicate significant differences ($p<0.05$; 0.01) between treatment periods.
$^{A-C}$ In each column, the median XO values with different superscript uppercase letters indicate significant differences ($p<0.05$; 0.01) between treatment groups.
$^{X-Y}$ In each row, the mean XO values with different superscript uppercase letters indicate significant differences ($p<0.05$; 0.01) between treatment periods.

Discussion

In the present study we investigated different types of glycosaminoglycans (GAGs) in terms of their possible antioxidant activities that were assessed in animal models using New Zealand rabbits by evaluating possible reduction of superoxide dismutase and xanthine oxidase activities. Table 1 shows the effect of different types of GAGs; namely commercial (CCS) and bacterial (BCS) chondroitin sulphate on superoxide dismutase and xanthine oxidase activities in New Zealand rabbits. As can be seen, treating the rabbits with CCS and BCS resulted in a decrease ($P < 0.01$) in the SOD and XO activities; however, this decrease was more prominent in the animals treated with BCS than in those treated with CCS in terms of SOD activity. Regarding XO activity, a similar pattern was observed, but both GAGs were more effective on SOD activity than on XO activity. The treatment periods were shown to have a significant ($P < 0.01$) effect on SOD activity, revealing that these types of GAGs could limit SOD activity more than XO activity.

The effect of GAGs to reduce free radical overproduction could be hypothetically attributed to their ability to bind the transition metal ions as Cu+2 or Fe+2 which are known in turn to be responsible for the initialization of Fenton's reaction [25,26]. Some GAGs possess counterpart secondary structure including carboxylic groups in the same spatial position, leading that some charged react with the transition metals ions like Cu+2or Fe+2 [32,33] which probably limits by a chelation mechanism, the availability of dangerous cations. Accordingly, consistent results were reported by Campo et al. who determined that commercial glycosaminoglycans reduced oxidative damage induced by copper (Cu+2), iron (Fe+2) and hydrogen peroxide (H2O2) in human fibroblast cultures [14]. Based on these results, it can be said that the treatment of the animals with bacterial chondroitin sulphate caused a decrease in oxidative stress in the animals, evaluated by the analysis of superoxide dismutase (SOD) and xanthine oxidase (XO) levels. The results of this study proved the reported antioxidant properties of GAGs, but further revealing the effect of bacterial chondroitin sulphate.

Conclusion

In this study, the effect of some GAGs on superoxide dismutase (SOD) and xanthine oxidase (XO) activities were investigated in experimental animal models. In this respect, New Zealand rabbits submitted to osteoarthritis surgery were treated with different types of GAGs; commercial chondroitin sulphate (CCS) and bacterial chondroitin sulphate (BCS). The results showed that both CS sources remarkably decreased the levels of SOD and XO activities in the animal models in comparison to those in control animal models; however, this decrease was more prominent in the animals treated with BCS than in those treated with CCS in terms of SOD activity. The above results clearly revealed the effectiveness of bacterial chondroitin sulfate on these biochemical parameters especially on SOD activity after treatment, suggesting that treatment of rabbits with this type of GAG could decrease oxidative stress in the animals to some extent, but further investigations should be done to verify such hypothesis.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The financial support no have.

Ethical approval
The animal experimental protocol was approved by the ethics committee of Medicinal Faculty of Inonu University, Malatya, Turkey with the research protocol number (2015/A-67).

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