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REVIEW ARTICLE

Effects of Proteoglycans on Oxidative/Nitrative Stress

Márta Sárközy[a,†], Renáta Gáspár[a,†], Kamilla Gömöri[a], László Dux[a], Csaba Csonka[a,#] and Tamás Csont[a,*,#]

[a]*Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary*

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Abstract: Enhanced production of reactive oxygen and/or nitrogen species in biological tissues leads to oxidative and nitrative stress, a general pathophysiological phenomenon playing a role in the development of various human diseases including cardiovascular and neurological disorders. Reactive oxygen and/or nitrogen species interact with lipids, DNA and proteins *via* oxidative or radical-mediated reactions, potentially leading to cell damage or death. Proteoglycans are among the most important structural and functional macromolecules in most tissues. The chemical structure of these molecules consist of a core protein onto which one or more negatively charged glycosaminoglycan (GAG) chain(s) are covalently attached. Interaction of proteoglycans with oxidative/nitrative stress has been demonstrated in various experimental systems. In this review, we discuss the modulatory effects of proteoglycans on tissue oxidative/nitrative stress and consequent cellular function especially in cardiovascular and neurological disorders. Proteoglycans have been implicated in both deleterious and potentially cytoprotective mechanisms. The protective mechanisms include chelation of positively charged transitional metal ions (*e.g.* iron and copper), scavenging superoxide anions by extracellular superoxide dismutase, building pericellular net and mediation of signal transduction pathways. Although these results may implicate proteoglycans as potential therapeutic targets, more research should be done to better explore proteoglycans as modulators of reactive oxygen/nitrogen species and to determine their possible therapeutic value in disorders accompanied by oxidative/nitrative stress.

Keywords: Proteoglycan, mucopolysaccharide, GAG, ROS, EC-SOD, perineural net, chelation, superoxide, nitric oxide, peroxynitrite.

1. OXIDATIVE/NITRATIVE STRESS

Oxidative and nitrative stress in the living organisms is characterized by elevated levels of reactive oxygen and/or nitrogen species (ROS and RNS, respectively) [1, 2]. The reason for the development of oxidative/nitrative stress could be excess formation and/or insufficient removal of ROS/RNS. ROS are highly reactive small molecules derived from molecular oxygen by redox reactions. Most important ROSs include oxygen radicals [*e.g.* superoxide anion ($O_2^{\cdot-}$); hydroxyl ($^{\cdot}OH$), peroxyl ($RO_2^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals] and non-radicals that are oxidizing agents and/or molecules that can be easily converted into radicals [*e.g.* hydrogen peroxide (H_2O_2); hypochlorous acid (HOCl); ozone (O_3); and singlet oxygen ($^1O_2$)] [3]. Nitrogen-containing reactive molecules including *e.g.* nitric oxide ($^{\cdot}NO$); nitrogen dioxide radical ($^{\cdot}NO_2$); dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$) are called RNS [2, 4].

The sources of increased ROS/RNS levels in biological systems can be both endogenous and exogenous. Exogenous ROS can be produced by ionizing radiation, air pollutants, smoke, toxic molecules, drugs, *etc.* Endogenous enzymatic sources for ROS/RNS formation include the mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, cyclooxygenases, lipoxygenases, nitric oxide synthase (NOS), peroxidases, peroxisomes, thymidine phosphorylase, *etc.* [1, 3, 5]. Moreover, some ROSs can be converted to other ROSs spontaneously, enzymatically, or *via* metal-based catalysis such as the Fenton-reaction or Haber-Weiss reaction. Formation of ROS/RNS may occur in any cellular compartments including the mitochondria, nucleus, cytosol, membranes, and even the extracellular space.

In biological systems, the availability of ROS/RNS is limited by antioxidant mechanism including antioxidant metabolites, antioxidant enzymes, and metal ion sequestration. Antioxidant metabolites (*e.g.* glutathione, bilirubin, uric acid, vitamin C, *etc.*) are small molecules capable of reacting with ROS/RNS, thereby preventing cellular macromolecules (*e.g.* lipids, nucleic acids, proteins) from ROS/RNS–induced chemical alterations and subsequent structural and functional damage. Endogenous antioxidant enzymatic systems decrease the number of ROS/RNS molecules and include superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, and heme oxygenase (HO) [1, 3, 5].

Increased oxidative/nitrative stress induces cellular redox imbalance which has been found to be detrimental to different tissues and cell types in certain diseases [6, 7]. Experimental evidence obtained in various disease models has demonstrated that excess ROS/RNS are generated in parenchymal, endothelial, and inflammatory cells during atherosclerosis, cardiovascular diseases, stroke, circulatory shock, aging, diabetic complications, chronic inflammatory diseases, neurodegenerative disorders, cancer *etc.* [6, 7]. Some compounds contribute to antioxidant defense by chelating transition metals (*e.g.* iron or copper) thereby reducing the production of metal-catalyzed free radicals in cardiovascular tissues as it has been reported in diabetes mellitus [8, 9] or in atherosclerotic lesions [10]. Among ROS/RNS, superoxide, nitric oxide and peroxynitrite seem

*Address correspondence to this author at the Metabolic Diseases and Cell Signaling Research Group, Department of Biochemistry, Faculty of Medicine, University of Szeged, P.O. Box: H-6720, Szeged, Hungary; Tel/Fax: +36-62-545-096, +36-62-545-097; E-mails: csont.tamas@med.u-szeged.hu;
†equally contributed; #equally contributed.

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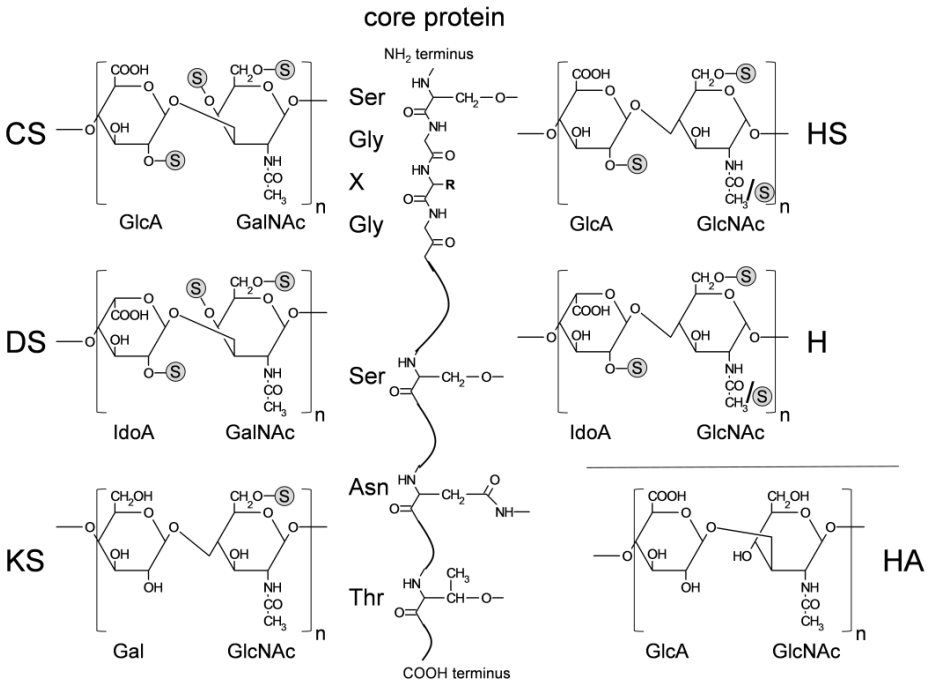

Fig. (1). Structure of proteoglycans Repeating disaccharide units of the glycosaminoglycan chain covalently attached to the central core protein of proteoglycans *via* a glucuronic acid-galactose-galactose-xylulose tetrasaccharid bridge. Different sulfation patterns can be formed by esterification of disaccharide units at various positions (in HA and H even the N-acetyl group can be substituted for sulfate group) as indicated by "S" enclosed by a circle forming:

i. chondroitin sulphate (CS, D-glucuronic acid (β-1, 3) D-N-acetylgalactosamine sulfate linked *via* β-1, 4; left top),
ii. dermatan sulfate (DS, L-iduronic acid (α-1, 3) D-N-acetylgalactosamine sulfate linked *via* β-1, 4; left middle),
iii. keratan sulfate (KS, D-galactose (β-1, 4) D-N-acetylglucosamine sulfate linked *via* β-1, 4; left bottom),
iv. heparan sulfate (HS, D-glucuronic acid sulfate (β-1, 4) D-N-sulphoglucosamine sulfate linked *via* α-1, 4; right top),
v. heparin (H, L-iduronic acid sulfate (α-1, 4) D-N-sulphoglucosamine sulfate linked *via* α-1, 4; right middle) and the non-sulfated
vi. hyaluronic acid (HA, D-glucuronic acid (β-1, 3) D-N-acetylglucosamine linked *via* β-1, 4; right bottom).

The glycosidic OH (on the right side of every disaccharide units) are covalently attached to their respective core proteins *via* hydroxyl groups of serine (O-linked glycosylation, CS, DS and HS), where the serine is found in a general sequence of -Ser-Gly-X-Gly- (X can be any amino acid residue except proline). KS can be covalently attached to its core protein *via* the amide nitrogen of asparagine (N-linked glycosylation) and *via* O-linked glycosylation of serine or threonine.

to play a major role in the pathomechanisms of the aforementioned diseases [6, 7]. Although NO is generally considered to be an antioxidant molecule and a physiological regulator in biological systems, evidences indicated that cytotoxic effects attributed to NO are rather due to peroxynitrite, produced from the diffusion-controlled reaction between NO and superoxide anion [6, 7]. Peroxynitrite and other ROS/RNS interact with lipids, DNA, and proteins *via* direct or indirect oxidative reactions and/or radical-mediated mechanisms. These reactions trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury committing cells to death [6, 7].

2. PROTEOGLYCANS

Proteoglycans (PGs) are biomacromolecules consisting of a so called core protein and one or more covalently attached glycosaminoglycan (GAG) chain(s) (Fig. **1**) [11]. The GAG chains are long, unbranched, linear, and highly negatively charged heteropolysaccharides due to sulfate and/or uronic acid groups (Fig. **1**) [11, 12]. GAG chains contain repeating disaccharides composed mainly of N-acetylated hexosamines (N-acetyl-D-galactosamine or N-acetyl-D–glucosamine) and D/L-hexuronic acid (D-glucuronic acid and L-iduronic acid) (Fig. **1**) [11, 12]. According to the hexosamine component there are six types of GAGs. The galactosaminoglycans consist of chondroitin sulfate (CS) and dermatan sulfate (DS), and the glucosaminoglycans consist of keratin sulfate (KS), heparin, heparan sulfate (HS), and hyaluronic acid (HA) (Fig. **1**) [13-16]. GAGs can be also classified into sulfated and non-sulfated groups as well. Sulfated GAGs include CS, DS, KS, heparin and HS [13-16]. Hyaluronic acid (HA) is the only non-sulfated GAG, however, it could not be classified as a component of proteoglycans because it lacks a core protein [11]. In addition, HA is interestingly the only GAG which is biosynthetised at the cell membrane and not at the Golgi apparatus [12]. KS is another unique GAG because its repetitive disaccharide structure contains D-galactose but no hexuronic acid units (Fig. **1**) [12]. The majority of sulfated GAGs (CS, DS and HS) are covalently attached to their respective core proteins *via* hydroxyl (-OH) groups of serine (Ser) residues (O-linked glycosylation of the core protein) (Fig. **1**) [11, 17]. The Ser residue is generally in the sequence -Ser-Gly-X-Gly- (where X can be any amino acid residue except proline) (Fig. **1**). KS can be covalently attached to its core protein *via* the amide nitrogen N-H of asparagine (Asn) residues (N-linked glycosylation of the core protein) and hydroxyl (-OH) groups of Ser or threonine (Thr) residues (O-linked glycosylation of the core protein) [11, 17] (Fig. **1**). In this case of O-glycosylation, the linkage regions of KS are distinct from all other GAGs [11, 17]. HS and heparin also contain N-sulfate residues [11] Both N-linked and O-linked glycosylation of nascent core proteins takes place in the endoplasmic reticu-

Table 1. Classification of Proteoglycans.

Classification of Proteoglycans			
Location	Classification	Name	Predominant GAG
Intracellular	Secretory granules	Serglycin	Heparin
Cell surface	Transmembrane	Syndecan (1-4)	Heparan sulfate
		NG2	Chondroitin sulfate
		Betaglycan	Chondroitin sulfate/ Heparan sulfate
		Phosphacan	Chondroitin sulfate
	GPI-anchored	Glypican (1-6)	Heparan sulfate
Pericellular	Basement membrane zone	Perlecan	Heparan sulfate
		Collagene (XVIII)	Heparan sulfate
		Agrin	Heparan sulfate
Extracellular	Hyalectan, Lectican	Aggrecan	Chondroitin sulfate/ Keratan sulfate
		Versican (0-3)	Chondroitin sulfate
		Brevican	Chondroitin sulfate
		Neurocan	Chondroitin sulfate
	Small leucine-rich PGs	Biglycan	Chondroitin sulfate
		Decorin	Dermatan sulfate
		Fibromodulin	Keratan sulfate

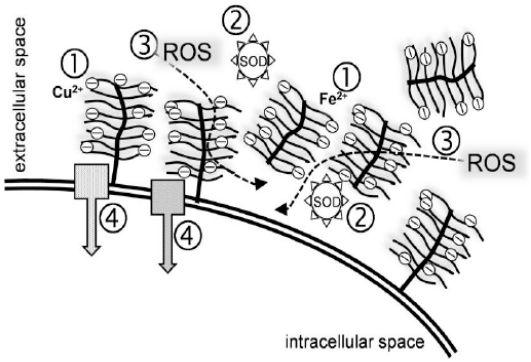

Fig. (2). The possible mechanisms of cytoprotection conferred by PGs (1) Chelating positively charged iron and copper ions, (2) scavenging of superoxide anions by EC-SOD, (3) building pericellular net and interfering with diffusion of potentially harmful molecules and (4) mediating signal transduction pathways.

lum (ER) as well as the Golgi apparatus and entails many different enzymes [14]. Details of the synthesis, transport and sorting of PGs have been reviewed elsewhere [18, 19].

According to their cellular and subcellular localization and the sequence homology of core protein, PGs can be classified into four families: i) intracellular; ii) cell surface; iii) pericellular-basement membrane and iv) extracellular ones, as suggested previously [12, 20] (Table **1**). PGs may interact with a number of growth factors, cytokines, chemokines, cell surface receptors and extracellular matrix components as well as other cells mainly through their GAG chains or *via* their core proteins [12]. Therefore PGs play an important role in physiological and pathophysiological processes including cell signaling, proliferation, migration, differentiation, adhesion and apoptosis [12]. One of the most important roles of PGs is organization of extracellular matrix by the formation of a scaffold and embedding cells within this extracellular matrix scaffold [12]. As we have outlined in the previous section, ROS/RNS may interact with proteins and other biomolecules *via* oxidative or radical-mediated reactions. Nevertheless, articles summarizing the effects of PGs on ROS/RNS are missing. Therefore, here we aim to review currently available data on the effects of PGs on oxidative/nitrative stress in different tissues.

3. EFFECTS OF PROTEOGLYCANS ON OXIDATIVE/NITRATIVE STRESS

To date, the effect of PGs on oxidative/nitrative stress is not entirely clear, and some controversies exist. The role of some proteoglycans have been implicated in the initiation of atherosclerosis, and thus suggested to be deleterious to vascular tissues. On the contrary, ameliorating effects of PGs against oxidative/nitrative stress and subsequent cellular function have been also demonstrated in various experimental systems by different mechanisms. These possible mechanisms include i) the inhibition of lipid (per)oxidation by chelating positively charged iron and copper ions by negatively charged GAG chains of PGs [21], ii) scavenging of superoxide anions by PG-bound extracellular superoxide dismutase (EC-SOD), iii) building pericellular net especially in the central nervous system to interfere with diffusion of potentially harmful molecules, and iv) mediating intracellular signal transduction pathways (Fig. **2**). Moreover, age-induced decrease of GAG content in extracellular matrix was shown to be associated with reduced antioxidant effects [21]. Several diseases may affect the protective effects of PGs on oxidative/nitrative stress. In this review, we summarize the effects of PGs on oxidative/nitrative stress especially in the cardiovascular and central nervous system (Table **2**).

3.1. Cardiovascular System

3.1.1. Subendothelial Lipoprotein Binding and Oxidation

Lipoproteins are special amphipathic particles consisting of lipid droplets surrounded by a single layer of phospholipid molecules bound to proteins termed apo-lipoproteins. This structure allows lipid droplets including triacylglycerol, cholesterol and cholesterol esters to be transported in the hydrophilic blood serum and through cell membranes. It is well known that elevated serum cholesterol level, especially in the forms of increased low density lipoprotein (LDL) and oxidized LDL (oxLDL) levels correlates with the development and progression of atherosclerosis [5]. Atherosclerosis is the main factor leading to the development of stroke and coronary artery diseases including acute myocardial infarction and heart failure, which are the leading cause of mortality in the world [5].

Investigations focusing on the effect of GAGs and PGs on lipoprotein binding and oxidation have reported conflicting results. Lipoproteins cross the endothelial cell membrane mainly by a non-specific vesicular transport [22]. A selective interaction of apoB100-containing lipoproteins including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and LDL particles have been shown with certain proteoglycans of the subendothelial layer resulting in retention and accumulation of lipoproteins in the extracellular matrix [22]. These lipoprotein-PG interactions alter the structural and charge characteristics of LDL particles [22]. Moreover, lipoproteins have been reported to be more susceptible to copper- and iron-catalyzed oxidation after binding to GAGs or PGs possibly due to structural modifications of the particle [21]. Thus, retention of apoB100 containing lipoproteins including LDL in the extracellular matrix increases the chances of oxidative modifications leading to oxLDL and Lipoprotein(a) is an LDL-like particle binding covalently the specific apolipoprotein(a) to the apoB100 apoprotein of the LDL-like particle formation [22]. The complex of lipoprotein receptor (HS/CS PG), lipoprotein particles including LDL, oxLDL, lipoprotein(a), and calcium may be interpreted as an arteriosclerotic plaque formed on the molecular level before any cellular reactivity [23]. These aforementioned altered LDL particles are taken up by macrophages *via* scavenger receptor-mediated endocytosis, leading to cholesteryl ester accumulation and foam cell formation [22].

Furthermore, it was also demonstrated that oxLDL is able to specifically bind to macrophage surface CS PGs, and that the content of CS is increased in macrophages under oxidative stress induced by preincubation with angiotensin II [24]. OxLDL binding and degradation by mutant by mutant Chinese hamster ovary (CHO) 677 epithelial cells lacking cells lacking heparan sulfate but having increased levels of chondroitin sulfate was found to be increased compared to oxLDL binding to the wild-type CHO cells [24]. Therefore, the interaction between macrophage CS and oxLDL might contribute to enhanced uptake of oxLDL with the formation of cholesterol-loaded foam cells, and accelerated atherosclerosis [24].

On the other hand, in another study, HS/CS proteoglycans have been shown to be receptor sites for lipoprotein binding, especially for the anti-atherogenic high density lipoprotein (HDL) particles [23]. Therefore, HS/CS proteoglycans binding HDL particles seem to be a counteracting mechanism against atherogenic apoB100-containing lipoprotein deposition on surface membranes and in subendothelial spaces [23].

3.1.2. EC-SOD Binding by Vascular Endothelial Cells

Endothelial dysfunction results in or contributes to cardiovascular risk factors including hypercholesterolemia, hypertension, diabetes mellitus, chronic smoking as well as heart failure [25]. It thought to be a key event in the development of atherosclerosis and has been shown to develop many years before clinically obvious vascular pathology [25]. It has been shown to be partly dependent on the production of ROS such as superoxide and the subsequent decrease in vascular bioavailability of nitric oxide [25]. EC-SOD is a secretory glycoprotein bound to the surface of vascular endothelial cells with a high affinity for HS PG, and it is a major enzymatic scavenger of superoxide anions in the extracellular matrix [26, 27]. It has been shown that decomposed derivatives of NO donors, sodium nitrite and homocysteine decreased the binding of EC-SOD to vascular endothelial cell surface in human and bovine aortic endothelial cell cultures [27]. These results suggest that excess NO produced under inflammatory circumstances and homocysteine - a well-known risk factor of cardiovascular diseases - result in a loss of protection of the vascular endothelial cell surface from oxidative stress [26, 27]. In contrast, another study has shown that EC-SOD is bound not only to HS PG, but also to other ligands in Drosophila Schneider cells as well as CHO cells stably expressing EC-SOD [28]. This study has demonstrated that fibulin-5 (also known by embryonic vascular endothelial growth factor (VEGF)-like repeat containing protein and developmental arteries and neural crest EGF-like) is required for binding of EC-SOD to vascular tissue and control vascular redox state in the extracellular matrix [28]. Indeed, the decrease in tissue-bound EC-SOD levels in aortas isolated from fibulin-$5^{-/-}$ mice was associated with an increase in vascular superoxide levels [28].

3.1.3. Protection of Cardiomyocytes from Stress

Acute myocardial infarction facilitated by hypercholesterolemia and atherosclerosis as well as other risk factors is the leading cause of mortality and morbidity worldwide [5]. It occurs when myocardial ischemia exceeds a critical threshold and overwhelms myocardial repair mechanisms. Myocardial ischemia means a reduced blood supply to the heart caused by coronary occlusion by a thrombus superimposed on the atherosclerotic plaque. Long lasting ischemia leads to irreversible myocardial cell damage or death. The state of the art treatment of acute myocardial infarction includes the attempt to reopen the occluded coronary artery (termed reperfusion) by coronary intervention procedures or thrombolytic therapies in order to reduce infarct size [5]. Thus the majority of patients with acute myocardial infarction undergo ischemia/reperfusion injury which is accompanied by increased oxidative/nitrative stress [5]. Besides cell death (infarction) and contractile failure, another major life threatening consequence of ischemia/reperfusion injury is the development of cardiac arrhythmias including ventricular arrhythmias [29, 30]. Interestingly, it has been shown that the mechanism of reperfusion induced ventricular fibrillation involves down-regulation of the expression and activity of HO-1, a well-known antioxidant enzyme, in both non-diabetic and diabetic rats [31, 32]. However, literature data is very limited on the effects of PGs on oxidative/nitrative stress in cardiomyocytes under ischemia/reperfusion or other circumstances.

Biglycan, a small leucine-rich PG component of the extracellular matrix, contains 2 GAGs that can be either CS or DS. Although, biglycan has been suspected to contribute to the development of atherosclerosis as discussed above, our group has demonstrated that the overexpression of biglycan in transgenic mice induced expres-

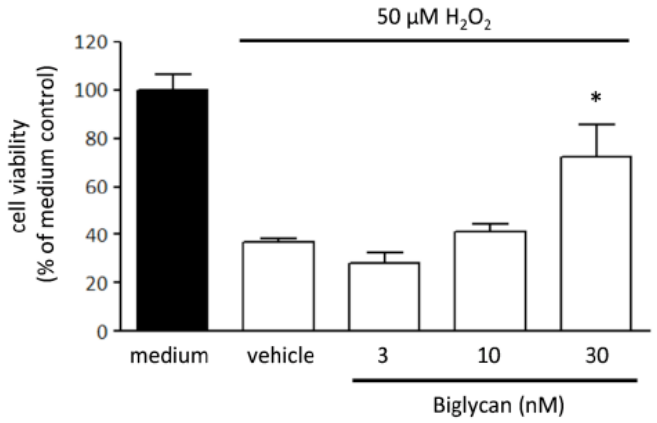

Fig. (3). Biglycan exerts a dose-dependent protective effect against H_2O_2-induced cell death. Primary neonatal cardiomyocytes were pretreated with 0, 3, 10, or 30 nM biglycan (n = 12-12) for 20 h, and then subjected to 50 μM H_2O_2 treatment for 24 h with corresponding biglycan treatment. At the end of the protocol calcein assay was used to assess cell viability. Values are normalized to medium control group and expressed as mean±SEM, *p<0.05 vs. vehicle-treated group, one-way ANOVA.

sion of cardioprotective genes including NOS isoforms in the heart [33]. We have also shown that biglycan treatment leads to cytoprotection against hypoxia/reoxygenation injury in primary cardiomyocytes isolated from neonatal rats [34]. Moreover, this phenomenon was partially mediated by an NO-dependent mechanism [34], as treatment of cardiomyocytes with biglycan resulted in enhanced expression of endothelial NOS and increased production of NO [34]. Moreover, pretreatment of primary neonatal cardiomyocytes with biglycan dose-dependently prevented H_2O_2-induced cell death in our recent experiments (unpublished results) (Fig. **3**). In these experiments, primary cardiomyocyte cultures from 1-3 days old Wistar rats were prepared as described earlier [34]. One day old cultures were treated with 0, 3, 10, or 30 nM biglycan for 20 hours followed by 50 μM H_2O_2 treatment for 24 h during which appropriate biglycan treatments were maintained. At the end of the protocol, cell viability was measured by calcein assay. We found that H_2O_2 resulted in a marked loss of cells, and 30 nM biglycan significantly attenuated H_2O_2-induced cell death (Fig. **3**). These data suggest that biglycan might have a role in attenuation of oxidative stress.

3.2. Central Nervous System (CNS)

The CNS, composed of the brain, spinal cord, and retina, has a limited capacity to spontaneously regenerate after traumatic injury or diseases [35]. Increased oxidative/nitrative stress is a common feature and contributing factor of cell death irrespective whether it is caused by traumatic injury, inflammation, ischemia/reperfusion injury or primary neurodegeneration [36]. Therefore, reducing oxidative/nitrative stress by pharmacological treatment strategies or regenerative medicine strategies may possibly delay the progression of CNS diseases or promote tissue regeneration.

3.2.1. Neurons with Perineural Net

There is a specialized form of extracellular matrix in the CNS, the so-called perineuronal net (PN) surrounding a subtype of neurons [36] including visual cortex, barrel cortex, deep cerebellar nuclei, substantia nigra, hippocampus, and spinal cord, *etc*. [37]. It is now clear that the main role of the PN is the control of synaptic plasticity both during development and in adulthood [37]. The main components of PN are CS PGs including lecticans (aggrecan, brevican, neurocan and versican) and phosphacan. Furthermore, hyaluronan, the large matrix glycoprotein tenascin R (TN-R) and link proteins (cartilage link protein Crtl-1/HAPLN-1 and brain link protein Bral2/HAPLN-4) are the main components of PNs [36, 38]. These components interact with each other and with hyaluronan, which provides the molecular backbone of the PN, thereby creating a stable scaffold around cell bodies and proximal dendrites of neurons [36, 38]. Several studies showed evidence that CS PGs and PN had neuroprotective features against oxidative stress [36]. Suttkus *et al.* has demonstared in different knockout mice strains, each being deficient for a different component of PNs, that aggrecan, link proteins and TN-R were essential for the neuroprotective properties of PN [36]. However, brevican did not seem to play a role in neuroprotective effects of PNs [38]. Neuroprotective effects of PN seem to be directly mediated by the net structure, the high negative charge and the correct interaction of net components [38]. The complex molecular network may limit the diffusion of transitional metals and reactive oxygen and nitrogen species formed in the ECM thereby contributing to the protection of neurons. The polyanionic character of PN PGs suggests that perineuronal nets also potentially contribute to reduce the local oxidative potential in the neuronal microenvironment by scavenging and binding iron, thus providing some neuroprotection against iron-catalyzed oxidative processes to net-associated neurons [39, 40].

3.2.2. Other Cell Types in the CNS

An additional mechanism of antioxidant properties of CS has been demonstrated by Canas *et al*. [41]. In that study, CS reduced the generation of free radicals in human neuroblastoma SH-SY5Y cells by induction of the antioxidant enzyme heme oxygenase (HO-1) [41]. Canas *et al*. showed that CS activated protein kinase C, which phosphorylated Akt and induced the expression of HO-1 by almost 2-fold *via* the Akt/PI3K pathway [41]. Coincubation of SH-SY5Y cells with the protein kinase C inhibitor chelerythrine or the Akt/PI3K inhibitor LY294002 reduced the overexpression of HO-1 to basal levels demonstrating that protein kinase C and Akt/PI3K were responsible for the overexpression of HO-1, induced by CS [41]. In addition, the importance of HO-related mechanisms has been implicated in several diseases including *e.g*. myocardial ischemia/reperfusion injury and cardiomyopathy as well [42].

Moreover, neuroprotective effects of a combination of HS and CS have been shown in hippocampal neurons, in an *in vitro* model of Alzheimer disease [43]. Here, the neuroprotective effect of HS/CS was explained by the binding capacity of sulfated GAGs due to their anionic charge and the ability of GAGs to bind EC-SOD, a well-known radical scavenger [43].

Interestingly, it has been shown that N2 fragment of the beta-cleaved neurodegenerative disease-associated prion protein is able to protect cells from intracellular oxidative stress *via* interaction of its N-terminal polybasic region with cell surface PGs [44]. Another protective mechanism against intracellular oxidative stress has been reported by Maus *et al*. [45]. In that study, NG2 proteoglycan characteristically expressed by oligodendrocyte progenitor cells (OPC) and also by aggressive brain tumours could protect OPCs against oxidative stress *via* an antiapoptotic mechanism [45]. NG2 proteoglycan binds OMI/HtrA2, a mitochondrial serine protease which is released from damaged mitochondria into the cytosol in response to stress. In the cytosol, OMI/HtrA2 initiates apoptosis by proteolytic degradation of anti-apoptotic factors [45]. The proapoptotic protease activity of OMI/HtrA2 in the cytosol could be reduced by the interaction with NG2 [45]. Therefore, binding of NG2 to OMI/HtrA2 might protect cells against oxidative stress-induced cell death [45].

Table 2. The effect of proteoglycans on oxidative/nitrative stress.

	Proteoglycan	Effect on Oxidative Stress	Reference(s)
Cardiovascular system			
lipoproteins	PGs	binding of apo-B containing lipoproteins inducing atherosclerosis	[22]
	chondroitin sulfate PGs	binding of ox-LDL, increased by oxidative stress inducing atherosclerosis	[24]
	syndecan, perlecan	heparan sulfate/chondroitin sulfate PG binds lipoproteins, cations, and polyelectrolytes leading to endothelial dysfunction	[23]
endothelial cells	heparan sulfate PGs	excess NO decreases the binding of EC-SOD to glycosaminoglycan	[26]
		homocysteine decreases the binding of EC-SOD to vascular endothelial cells by degradation of endothelial heparan sulfate PG	[27]
	fibulin-5	essential to EC-SOD binding to vascular tissue	[28]
cardiomyocytes	biglycan	increases NO level by inducing NOS leading to cardioprotective effect	[33, 34]
Central nervous system			
neurons with perineuronal net	aggrecan, tenascin-R	neuroprotection against iron-induced cell death by perineuronal net	[36]
	chondroitin sulfate PGs, hyaluronan, tenascin-R	perineuronal net increases iron-binding capacity	[40]
	chondroitin sulfate PGs, hyaluronan, tenascin	perineuronal net scavenges and binds iron	[39]
neuroblastoma SH-SY5Y	chondroitin sulfate PGs	induces the synthesis of the antioxidant protein heme oxygenase-1 *via* PI3K/Akt pathways	[41]
neurons	heparan sulfate, chondroitin sulfate	neuroprotection by free radical scavenging	[43]
neurons	heparan sulfate PGs	N-terminus of PrPC binds to extracellular PGs decreasing intracellular ROS	[44]
oligodentrocyte progenitor cells	NG2 (chondroitin sulfate PG4)	protects against oxidative stress by sequestering and reducing the protease activity of the apoptosis-inducing serine protease, OMI/HtrA2	[45]
astrocytoma	versican	enhances cell attachment and expression of beta-1 integrin and fibronectin	[46]
Other organs			
podocyte	syndecan	ectodomain shed from syndecan-4 increases generation of ROS	[47]
kidney	FYGL (exogenous PG)	protective role against oxidative damage due to its antioxidant potential	[48]
serum, liver	FYGL (exogenous PG)	FYGL treated db/db mice had lower levels of malondialdehyde, 8-hydroxydeoxyguanosin and protein carbonyl, and significant increase in the antioxidant enzyme activities, including SOD, CAT and GSH-peroxidase, in both serum and liver	[49]

Another study using astrocytoma U87 cells showed that stable expression of an aggregating CS PG, versican or its C-terminal domain significantly decreased H_2O_2-induced cellular apoptosis [46]. This study demonstrated that astrocytoma U87 cells in adherent monolayer were more resistant to H_2O_2-induced apoptosis than cells cultured in suspension [46]. While vigorous trypsinization caused integrin cleavage and rendered the cells more susceptible to H_2O_2-induced damages, expression of versican or its C-terminal domain increased cell attachment and expression of β1 integrin as well as fibronectin. These results suggest that versican plays an important role in reducing oxidant injury *via* an enhancement of cell-matrix interaction [46].

3.3. Other Organs

It has been reported that a membrane glycoprotein, syndecan-4 (Sdc4) could be cleaved to release a soluble ectodomain capable of paracrine and autocrine signaling [47]. A study showed that Sdc4 ectodomain increased surface abundance of TRPC6 channels, generation of ROS, activation of Rac1, nuclear abundance of NFATc1 as well as total β3-integrin and reduced activation of RhoA in podocytes [47]. Therefore, locally generated Sdc4 might play a role in regulating TRPC6 channels, and may contribute to glomerular pathology [47].

Interestingly, FYGL, a novel proteoglycan from Ganoderma lucidum fruiting bodies, has been shown to ameliorate diabetic nephropathy *via* its antioxidant activity in C57BL/6 db/db mice [48]. FYGL dose dependently reduced blood glucose concentration, kidney/body weight ratio, serum creatinine and urea nitrogen as well as albuminuria in diabetic mice *via* increasing the activities of renal superoxide dismutase, glutathione peroxidase and catalase compared to untreated diabetic mice [48]. In the same study, the decrease of renal malondialdehyde content and 8-hydroxy-2'-deoxyguanosine expression were also observed in FYGL-treated diabetic mice compared to the untreated diabetic mice, along with

an amelioration of renal morphologic abnormalities [48]. In another study, FYGL showed antidiabetic, antihyperlipidemic and antioxidant effects in db/db mice [49]. Pan *et al*. showed that FYGL reduced glycated hemoglobin level, and increased insulin as well as C-peptide levels in db/db mice [49]. In that study, FYGL also increaseg glucokinase activities, and simultaneously lower phosphoenol pyruvate carboxykinase activities, accompanied by a reduction in the expression of hepatic glucose transporter protein 2, while the expression of adipose and skeletal glucose transporter protein 4 was increased [49]. Moreover, the antioxidant enzyme activities including SOD, catalase and glutathione peroxidase were also increased in the serum and the liver by FYGL treatment as compared to untreated diabetic mice [49]. In addition, it has been reported that FYGL is a hyperbranched heteropolysaccharide attached to the core protein *via* both serine and threonine residues by O-type glycosidic bonds. Also it was demonstrated that FYGL is able to inhibit protein tyrosine phosphatase 1B activity by a competitive mechanism *in vitro* [50].

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Oxidative and nitrative stress in human tissues has been implicated as a pathophysiological mechanism in different diseases including cardiovascular, neurological and other disorders. In this review we have summarized available literature data on the interactions of PGs with ROS/RNS conferring mostly cytoprotective effects in different organs and diseases (Table **2**). The possible mechanisms of cytoprotection conferred by PGs include chelating positively charged iron and copper ions, scavenging superoxide anions by EC-SOD, building pericellular net especially in the CNS to interfere with diffusion of potentially harmful molecules, and mediation of signal transduction pathways. Although oxidative/nitrative stress occurs frequently in various diseases, the number of publications investigating the effects of PGs on oxidative/nitrative stress and their possible citoprotective mechanisms is very limited. Nevertheless, based on these data, PGs may be implicated as potential pharmacological tools and/or targets to modulate oxidative/nitrative stress. However, further research is needed to explore more completely the role of PGs in the control of tissue ROS/RNS and to determine their possible therapeutic value in disorders accompanied by oxidative/nitrative stress.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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