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Galectin-3 Deficiency Reduces Cardiac and Renal Antioxidant Capacity in Mice

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Original article

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Galectin-3 (Gal-3) has increasingly been recognized as a modulator of inflammation, oxidative/nitrosative stress, fibrogenesis, and tissue remodeling. The objective of the current pilot study was to investigate the influence of Gal-3 on cardiac and renal antioxidant capacity using biochemical and histopathological examinations. Two groups (n=7 each) of male mice were tested: 1. control (CON) group (wild type of C57BL/6 mice) and 2. GAL-3$^{-/-}$ group (galectin-3$^{-/-}$ knockout mice). After overnight fasting, mice were sacrificed by exsanguination in ketamine (100mg/kg intraperitoneally). Then, cardiac and renal tissue samples were taken to determine the parameters of oxidative/nitrosative stress and antioxidant capacity. The levels of malondialdehyde and nitrites+nitrates was not significantly different in the GAL-3$^{-/-}$ group vs. the CON group. The total superoxide dismutase activity in the renal tissue of the GAL-3$^{-/-}$ mice was significantly lower compared to the CON group. Cardiac and renal catalase and glutathione S-transferase activity was significantly reduced in the GAL-3$^{-/-}$ group vs. the CON group, respectively. A significant decrease in glutathione level was also registered in hearts of the GAL-3$^{-/-}$ group vs. the CON group. Our findings indicate that Gal-3 deficiency does not lead to lipid peroxidation and nitrosative stress in cardiac and renal tissue in mice. However, the lack of this beta-galactoside-binding lectin does reduce antioxidant capacity in both of the investigated tissues.

Key words: Galectin-3, heart, kidney, antioxidant capacity, mice.

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Lectins (type C and P, pentraxins and galectins) are evolutionary conserved, ubiquitous, carbohydrate-binding proteins with a specific affinity for oligosaccharides (DRICKAMER and TAYLOR 1993). There are 16 types of galectin found across the eukaryotic domain, including mammals, birds, amphibians, fish, nematodes, fruit flies, sea sponges, and fungi (TIMOSHENKO 2015).

Galectins are classified on the basis of their structure into three groups: (1) prototypical galectins, that contain one carbohydrate-recognition domain (Gal-1, 2, 5, 7, 10, 11, 13, 14, and 16), (2) tandem-repeat galectins, that contain two covalently linked carbohydrate-recognition domains connected by a small peptide domain (Gal-4, 6, 8, 9 and 12) and (3) galectin-3 (Gal-3), a chimeric galectin, which consists of one carbohydrate-recognition domain covalently linked to tandem repeats of proline- and glycine-rich short domains (YANG *et al.* 2008; TIMOSHENKO 2015).

Gal-3 is an ubiquitous and multifunctional β-galactoside-binding lectin, that is primarily expressed in the cytoplasm of cells in numerous tissues (NEWLACZYL & YU 2011; SCIACCHITANO *et al.* 2018).

However, it can also be localized in the nucleus, or on a cell's surface, as well as in the extracellular matrix (NEWLACZYL & YU 2011**)**. Namely, Gal-3 is primarily found in the epithelial and endothelial cells of various tissues and organs, sensorineural cells, and in cells of the immune system (monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils and mastocytes (LEFFLER *et al.* 2004; DUMIC *et al.* 2006; YANG *et al.* 2008; NEWLACZYL & YU 2011; SCIACCHITANO *et al.* 2018**)**. Furthermore, Gal-3 affects various physiological and pathological processes, including apoptosis, angiogenesis, oxidative/nitrosative stress, and inflammatory response (SCIACCHITANO *et al*. 2018). Gal-3 is deeply involved in acute inflammatory response, including the activation and adhesion of neutrophils, monocyte and macrophage hemotaxis, the opsonization of apoptotic neutrophils, and mast cell activation (KARLSSON *et al.* 2009; SCIACCHITANO *et al.* 2018). Moreover, numerous *in vitro* and *in vivo* studies have shown that Gal-3 acts as a powerful proinflammatory sensor in chronic inflammatory states (BERTOCCHI *et al.* 2008; KARLSSON *et al.* 2009; IACOBINI *et al*. 2009; HENDERSON & SETHI 2009; METHI-MATUTE *et al.* 2014; MARTINEZ-MARTINEZ *et al.* 2016; SCIACCHITANO *et al.* 2018). Thus, an increased expression of Gal-3 has been documented in chronic inflammatory diseases, such as liver cirrhosis, chronic pancreatitis, idiopathic pulmonary fibrosis, and experimental glomerulonephritis (SCIACCHITANO *et al.* 2018). In addition, recent studies have found that Gal-3 has a key role in the fibrogenesis of various organs, such as the heart, kidneys, lungs, and liver (BERTOCCHI *et al.* 2008; SAVIC-RADOJEVIC *et al.* 2017; SCIACCHITANO *et al.* 2018). This galectin is also recognized as a predictor of heart and kidney failure, as it is a powerful mitogen in fibroblast cultures. In other words, Gal-3 stimulates both macrophage function and extracellular matrix production, and in this manner leads to the observation of fibrogenesis through the so-called "Gal-3/macrophage/fibroblast axis" (SCIACCHITANO *et al*. 2018). According to this concept, the progressive forms of cardiac and kidney failure are in correlation with a significantly increased expression of Gal-3 in cardiac muscle and kidney tissue (BERTOCCHI *et al.* 2008; SAVIC-RADOJEVIC *et al.* 2017; GEHLKEN *et al.* 2018; SCIACCHITANO *et al*. 2018). Experimental data have also shown that the increased expression of Gal-3 could be an important factor in leptin-induced cardiac fibrosis (MARTINEZ-MARTINEZ *et al.* 2014). On the other hand, published data suggest a possible protective role of Gal-3 in tissue fibrogenesis with prolonged exposure to toxic agents (PUGLIESE *et al.* 2001; NOMOTO *et al.* 2006; IACOBINI *et al.* 2009).

Gal-3 was found to increase the production of reactive oxygen species (ROS) in macrophages (LIU *et al.* 1995). Moreover, Gal-3 acts as a positive hemoattractant for neutrophils (VOLAREVIC & LUKIC 2012), which are an important source of ROS and proinflammatory cytokines [tumor necrosis factor alpha (TNF-α), interleukin-1beta (IL-1ß), and interleukin-6 (IL-6)]. Oxidative stress leads to the upregulation of certain galectins, such as Gal-3, in alveolar macrophages (SUNIL *et al.* 2015), and breast cancer cells (DE OLIVEIRA *et al.* 2015). Additionally, oxidative stress was shown to change the galectin gene expression profiles in HL-60 cells including a significant upregulation of Gal-3 (VINNAI *et al.* 2017). Furthermore, it has been reported that the activity of galectins depends on the microenvironment redox potential (GUARDIA *et al.* 2014). However, the precise mechanisms by which redox potential reactions are regulated in the adjacent microenvironment of galectins have not been fully explained (VINNAI *et al.* 2017). Therefore, having in mind all the previously mentioned facts, the aim of the present pilot study was to investigate the influence of Gal-3 on cardiac and renal antioxidant capacity in mice.

Materials and Methods

Animals

The experiment was performed on male inbred C57BL/6 and galectin-$3^{-/-}$ knockout mice (Gal-$3^{-/-}$), weighting 21-25 g (8 weeks), that were raised in the vivarium of the Medical Military Academy in Belgrade. The animals were kept in individual cages under standard laboratory conditions (ambient temperature 22 ± 2°C, relative humidity 50 ± 10%, 12/12 h dark/light cycle with lights turned on at 9.00 a.m.) with free access to tap water and appropriate food. All experimental procedures were in full compliance with the Directive of the European Parliament and of the Council (2010/63/EU) and approved by the Ethical Committee of the University of Belgrade (Permission N°. 692/2).

Experimental design

Two groups (n=7 for each) of mice were compared: 1. The control group (wild type of C57BL/6 mice) and 2. The Gal-$3^{-/-}$ mice. The mice were fasted overnight and were then sacrificed by exsanguination in ketamine anesthesia (100 g/kg intraperitoneally /i.p./) (LEVIN-ARAMA *et al.* 2016). Cardiac and renal tissue samples were taken in order to determine the parameters of oxidative/nitrosative stress and antioxidant capacity.

Reagents

The following reagents were used in the experimental procedure: bovine serum albumin (Serva, Feinbiochemica,

Heidelberg, New York), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) (Sigma-Aldrich Co., St. Louis, USA), epinephrine (Sigma-Aldrich Co., St. Louis, USA), Griess reagent (Sigma-Aldrich Co., St. Louis, USA), hydrogen peroxide (H_2O_2) (Sigma-Aldrich Co., St. Louis, USA), potassium phosphate buffer (Serva, Feinbiochemica, Heidelberg, New York), sodium carbonate buffer (Serva, Feinbiochemica, Heidelberg, New York), and thiobarbituric acid (Sigma-Aldrich Co., St. Louis, USA).

Sample preparation

Cardiac and renal samples (each of 0.5 g) were washed with cold normal saline and homogenized in 2.5 volumes of ice-cold 0.1 M potassium phosphate buffer (pH 7.4). The resulting homogenate underwent two cycles of centrifugation at 600 g and 10000 g, and the supernatant was collected and stored at -70°C to measure the tissue content of oxidative biomarkers and antioxidant enzymes (ABDEL-DAIM *et al.* 2017). Proteins were determined using the Lowry method with bovine serum albumin as the standard (LOWRY *et al.* 1951**)**.

Biochemical analyses

Determination of oxidative/nitrosative stress parameters

Lipid peroxidation, measured as malondialdehyde (MDA) level, was determined spectrophotometrically in a reaction with thiobarbituric acid as described by GIROTTI *et al.* (1991). The results are expressed as nmol of MDA per milligram of proteins (nmol/mg prot.).

The concentration of nitrites + nitrates (NOx) as a measure of nitric oxide (NO) production was determined by using a Griess reagent. After reduction of the nitrates, the total nitrites were reacted with sulfanilamide and *N*-(1-naphtyl) ethylenediamine to produce an azo dye, which was measured spectrophotometrically at 492 nm (HIBBS *et al.* 1988). The results are expressed as nmol of NO per milligram of proteins (nmol/mg prot.).

Determination of parameters of antioxidative capacity

The activity of total superoxide dismutase (EC 1.15.1.; SOD) in the heart and kidneys was measured spectrophotometrically at 480 nm. After the addition of 10 mM of epinephrine, analysis was performed in the sodium carbonate buffer (50 mM, pH-10.2) containing 0.1 mM of EDTA (SUN & ZIGMAN 1978). Enzyme activity is expressed as units per milligram of protein (U/mg prot.).

Catalase (CAT) activity in cardiac and renal tissue homogenates was determined using the ultraviolet (UV)-kinetic method in the presence of hydrogen peroxide (H_2O_2) (AEBI 1984).

The activity of glutathione S-transferase (GST) was measured spectrophotometrically, as a determination of the conjugates formed upon the action of 1-chloro-2,4-dinitrobenzene and GSH (HABIG & JACOBY 1981).

The content of reduced glutathione (GSH) was determined spectrophotometrically using 5,5-dithio-bis-2-nitrobenzoic acid (DTNB). DTNB reacts with aliphatic thiol compounds at a pH of 8.0 forming yellow p-nitrophenol anion whose absorption is measured spectrophotometrically at 412 nm (ANDERSON 1986). Results are expressed as nmol of GSH per milligram of proteins (nmol/mg prot.).

Histopathological analysis

Cardiac and renal tissues were incubated in 10% formalin solution at room temperature. After fixation, the heart and kidney samples were processed using the standard method (FOX *et al.* 1985). Tissues were incorporated in paraffin, sectioned at 4 μm and then stained with hematoxylin and eosin dye (H&E) (LUNA 1966). They were then prepared for light microscopy analysis. All samples were evaluated by an experienced histopathologist, who was unbiased in terms of the experiment. Preparations were analyzed and photographed by means of a combined photobinocular light microscope Olympus BX51 equipped with Artcore 500 MI Artray, Co. Ltd. Japan Camera.

Statistical analysis

All results are expressed as mean±SEM. The differences in the baseline characteristics of the two groups were evaluated using the *t* test. A value of $p<0.05$ was considered statistically significant. For statistical analysis, the computer software SPSS 15.0 was used.

Results

There was no statistically significant difference in both the cardiac or renal tissue MDA concentrations between the wild type of C57BL/6 mice (CON group) and the GAL-$3^{-/-}$ mice ($p>0.05$) (Table 1). Similarly to MDA, there was no significant difference in both the cardiac or renal tissue NOx levels in CON mice compared to the GAL-$3^{-/-}$ mice ($p>0.05$) (Table 1).

Total SOD activity in the cardiac tissue of the CON group (2.8±0.3 U/mg prot.) was not significantly different compared to the GAL-$3^{-/-}$ mice (2.5±0.4 U/mg prot.) ($p>0.05$) (Fig. 1a). On the other hand, in the renal tissues of the CON group, total SOD activity was significantly higher (14.9±0.3 U/mg prot.) than in the GAL-$3^{-/-}$ mice (13.6±0.9 U/mg prot.) ($p<0.05$) (Fig. 1a).

In contrast to SOD, the CAT activity in the CON group was significantly higher in the cardiac tissues (9.9±1.5 U/mg prot.) compared to the GAL-$3^{-/-}$ mice (7.0±1.0 U/mg prot.) ($p<0.05$) (Fig. 1b). Also, a significant increase in CAT activity was found in the re-

Table 1

Concentration of malondialdehyde (MDA) and nitrites+nitrates (NOx) in kidney and heart tissues of the investigated animals ($\bar{x}$±SEM)

Parameters	Groups	
	CON	GAL $3^{-/-}$
Kidneys		
MDA (nmol/mg prot.)	10.0±0.12	10.7±0.18
NOx (nmol/mg prot.)	1.1±0.04	1.0±0.04
Heart		
MDA (nmol/mg prot.)	9.9±0.14	10.3±0.16
NOx (nmol/mg prot.)	2.2±0.09	2.0±0.12

nal tissues of the CON group (98.3±13.0 U/mg prot.) compared to the GAL-$3^{-/-}$ mice (86.3±10.9 U/mg prot.) ($p<0.05$) (Fig. 1b).

Similarly to CAT activity, GST activity was significantly higher in the cardiac tissues of the CON group (93.9±9.3 U/mg prot.) compared to the GAL-$3^{-/-}$ mice (82.4±6.4 U/mg prot.) ($p<0.05$) (Fig. 1c). A significant increase in GST activity was also registered in the renal tissues of the CON group (55.1±10.3 U/mg prot.) compared to the GAL-$3^{-/-}$ mice (32.4±9.6 U/mg prot.) ($p<0.01$) (Fig. 1c).

Cardiac tissue GSH content in the CON group (41.1±6.2 nmol/mg prot.) was significantly increased in comparison with the GAL-$3^{-/-}$ mice (21.5±4.2 nmol/mg prot.) ($p<0.01$) (Fig. 1d). In contrast to this result, a nonsignificant decrease in renal tissue GSH content was registered in the CON group (21.8±5.1 nmol/mg prot.) when compared to the GAL-$3^{-/-}$ mice (22.3±5.9 nmol/mg prot.) ($p>0.05$) (Fig. 1d).

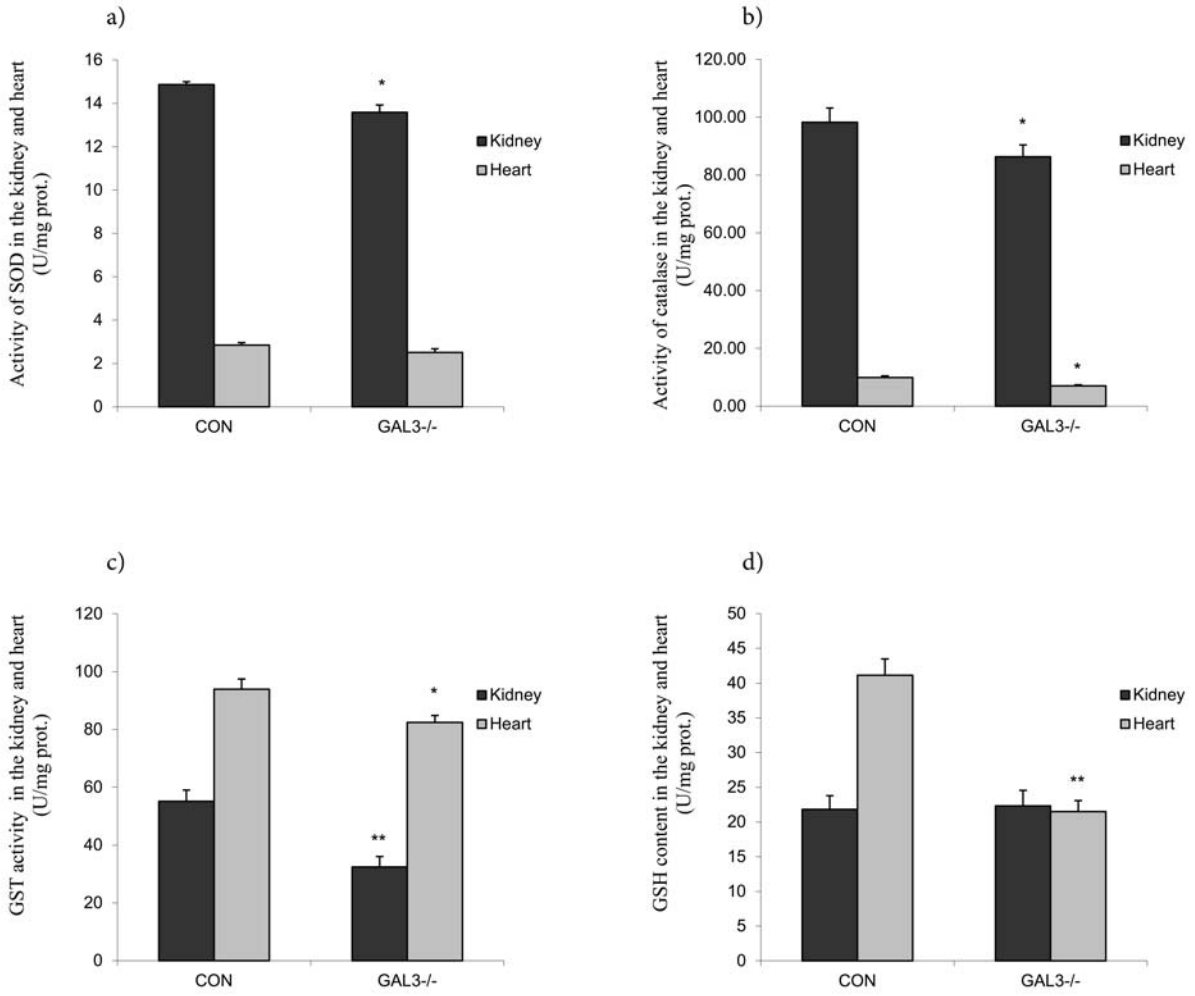

Fig. 1. Values of SOD (a), catalase (b), GST (c), and GSH (d) in the kidney and heart tissues of the investigated animals ($\bar{x}$±SEM; n=7); CON – control group; GAL-$3^{-/-}$ – mice lacking galectin-3 gene; SOD – superoxide dismutase; CAT – catalase; GST – glutathione; S – transferase; GSH – reduced glutathione.
Statistically significant difference estimated by *t* test (*$p<0.05$; **$p<0.01$ compared to control).

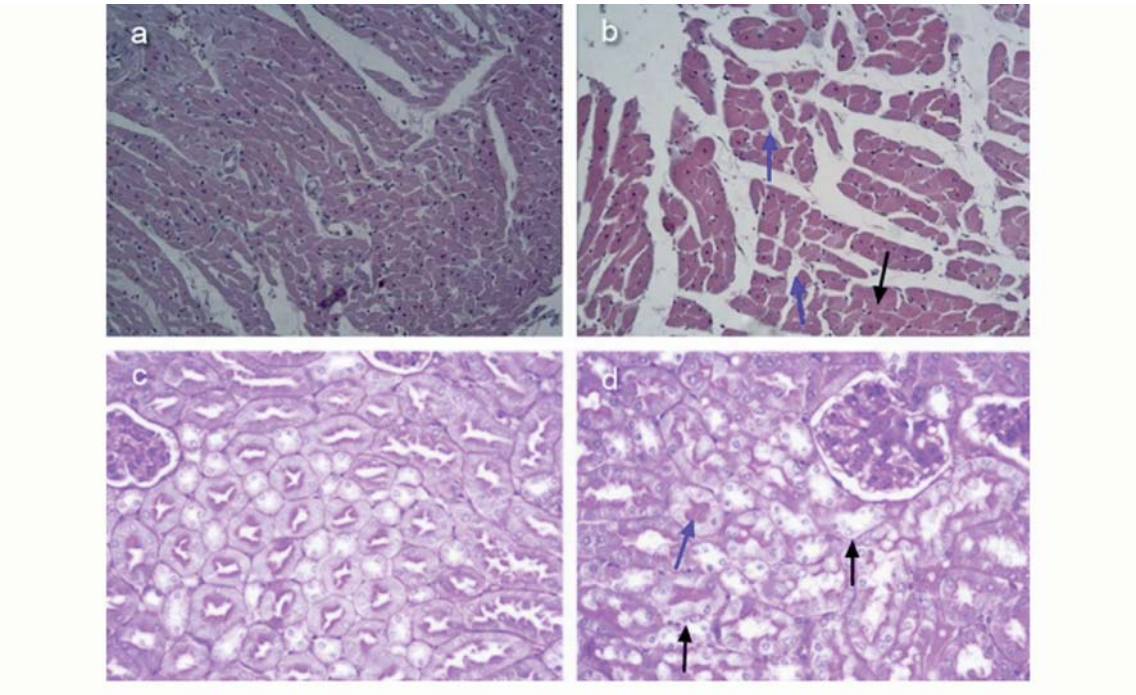

Fig. 2. Histopathological findings of the heart and kidney tissues of the investigated animals (H&E, x20). The hearts of the control mice (a); Increased interstitial space (mild edema) (blue arrow) and distorted intercalated discs (black arrows) (b); The kidneys of the control animals (c); Mild, scattered epithelial desquamation (black arrows) and an intraluminal cast formation in the proximal tubules (blue arrow) of the GAL-3$^{-/-}$ mice (d).

Histopathological findings

The histological appearance of the heart was normal in the CON group (Fig. 2a). On the other hand, an increased interstitial space (mild edema) and a distorted intercalated disc was observed in the cardiac tissues of the GAL-3$^{-/-}$ mice (Fig. 2b).

The histological appearance of the kidneys was normal in the CON group (Fig. 2c). Unlike the control group's histopathological finding, mild scattered epithelial desquamation and intraluminal cast formation was found in the proximal tubules of the GAL-3$^{-/-}$ mice (Fig. 2d).

Discussion

The exact mechanism by which Gal-3 alters equilibrium between oxidants and antioxidants in the heart and kidney tissues is still an insufficiently explored field. Our investigation showed that a lack of Gal-3 is interconnected with the depletion of cardiac and renal antioxidant capacity, and may be related to morphological changes in the heart (Fig. 2b) and kidneys (Fig. 2d).

A recent study showed that the increased expression of Gal-3 in renal tissue is directionally linked with fibrosis, inflammation, and structural damage, while a blockade of Gal-3 via modified citrus pectin (MCP) may prevent these pathological changes (MARTINEZ-MARTINEZ *et al.* 2016). Thus, there is a scientifically-based assumption that Gal-3 deprivation in relation with a decrease of renal antioxidant capacity contributed to the histopathological alteration of the renal tissues of the GAL-3$^{-/-}$ mice in our experiment (Fig. 2d).

A broad spectrum of roles has been attributed to Gal-3, including neutrophil adhesion, the initiation and propagation of oxidative/nitrosative stress, the migration and degranulation of mast cells, as well as the production of proinflammatory cytokines (SCIACCHITANO *et al.* 2018). Published experimental data demonstrate the role of Gal-3 in renal tissue damage triggered by ischemia and reperfusion injury. Namely, in the GAL-3$^{-/-}$ mice, acute tubular necrosis was evident to a lesser extent, while the tubular regeneration was the most significant finding in comparison with the control group. In addition to, proinflammatory cytokine expression (monocyte chemotactic protein-1 /MCP-1/, IL-6, IL-1β, etc.), macrophage infiltration and free radical production were less prominent in the control group compared to the GAL-3$^{-/-}$ mice (BERTOCCHI *et al.* 2008). Taking these facts into consideration, in the current study, Gal-3 may be implicated in the maintenance of redox status and structure in the kidneys of C57BL/6 mice (Fig. 2c).

Gal-3 expression and secretion is altered during fibrosis and inflammation of the cardiac tissues as well (IACOBINI *et al.* 2009; METHI-MATUTE *et al*. 2014; SAVIC-RADOJEVIC *et al*. 2017; GEHLKEN *et al.* 2018). Moreover, Gal-3 can be easily determined and measured as an appropriate biomarker, due to its secretion into the circulatory system (SAVIC-RADOJEVIC *et al.* 2017; GEHLKEN *et al.* 2018). Also, a recent study found that Gal-3 may be an independent prognostic factor for the rehospitalization and mortality of patients with chronic heart failure (GEHLKEN *et al.* 2018). Importantly, Gal-3 is also considered to be a useful prognostic factor in patients with acute heart failure, since its increased expression is recognized as a marker for fibrosis and myocardial remodeling in congestive heart failure (SAVIC-RADOJEVIC *et al.* 2017; GEHLKEN *et al.* 2018). Having these reported data in mind, in the present study it is expected that under physiological conditions, Gal-3 is involved in the maintenance of cardiac redox status and structure in the C57BL/6 mice (Fig. 2a).

MDA is the final product of lipid peroxidation, that affects all lipid structures, including cell membrane lipoproteins (IACOBINI *et al.* 2009). The current study has shown nonsignificant differences in cardiac and renal tissue MDA and NOx concentrations in the GAL-3$^{-/-}$ mice compared to the control values (Table 1). These results are similar to documented data, which report that Gal-3 may exert beneficial effects in cardiac and renal diseases associated with oxidative/nitrosative stress and chronic inflammation (PUGLIESE *et al*. 2001; NOMOTO *et al.* 2006; IACOBINI *et al.* 2009). In relation to this, it is important to note that Gal-3 deficient mice with diabetes mellitus have rapidly occurring glomerulopathy, manifested with mesangium expansion and an increased expression of genes, which are of great importance for the production of extracellular matrix components (PUGLIESE *et al.* 2001). Similarly, Gal-3 deficient mice develop advanced atherosclerosis followed by the accumulation of modified lipoproteins (oxidized, low density lipoproteins /oxLDLs/, etc.) (IACOBINI *et al.* 2009). The results of the present study are also in agreement with the findings published by BERTOCCHI *et al.* (2008). According to this investigation, the lack of Gal-3 expression in experimental animals has a protective role in renal tissues during ischemia-reperfusion injuries (BERTOCCHI *et al.* 2008).

In our study, a significant decrease in total SOD activity in renal tissue was observed in the GAL-3$^{-/-}$ mice (Fig. 1a). On the other hand, the activity of this enzyme in the cardiac tissues of the GAL-3$^{-/-}$ mice was not significantly changed compared to the control group (Fig. 1a). Our findings correspond to the results of other studies indicating that the biological function of galectin can vary from tissue to tissue, depending on the availability of suitable ligands. Namely, it is known that the biological function of galectins in the extracellular matrix, in the presence of oxygen depends on their affinity to bind ligands, resulting in the prevention of free cysteine residue oxidation, as well as the sensitivity of galectins to proteolysis (DRICKAMER & TAYLOR 1993; LEFFLER *et al.* 2004). Furthermore, galectins interact with numerous glycoconjugates on the surface of cells, including transmembrane proteins, triggering signaling events during different processes (cellular proliferation and differentiation, cytokine production, antioxidant defense, tissue organization, etc.) (LEFFLER *et al.* 2004; YANG *et al.* 2008).

In the present study, CAT activity in cardiac and renal tissues was significantly lower in the GAL-3$^{-/-}$ mice compared to the control values (Fig. 1b). This result may be related to published data that highlight the importance of further investigations regarding the precise mechanisms by which galectins in the state of interaction between oxidative stress and the process of cellular differentiation may lead to the initiation of the signaling cascade ROS → altered galectins expression → cellular differentiation (LIU *et al.* 1995; VINNAI *et al.* 2017). Besides, previous clinical cardiomiopathy and nephropathy studies have shown significantly higher oxidative activity in patients with primary chronic heart failure and people with primary renal damage (OZBEK 2012; AHMAD *et al.* 2013). Moreover, the heart, as an organ with higher metabolic requirements than the kidneys, additionally leads to an increase of enzymatic and nonenzymatic antioxidant systems consumption (AHMAD *et al.* 2013). Furthermore, in such circumstances, the inactivation of the Gal-3 gene, or its therapeutic modulation was shown to halt the progression of cardiac remodeling, attenuate myocardial fibrogenesis, reduce the size of atherosclerotic lesions, and preserve ventricular function in rats and mice (NACHTIGAL *et al.* 2008; PAPASPYRIDONOS *et al.* 2008; MACKINNON *et al.* 2013; LALA *et al.* 2015; FORT-GALLIFA *et al.* 2017).

The results of our investigation found that cardiac and kidney GST activity was lower in the GAL-3$^{-/-}$ mice, while GSH level was decreased in the cardiac tissue only, compared to the control values (Fig. 1c, 1d). Consistent with our results, similar changes in antioxidant capacity are observed in a state of acute heart failure and renal tissue injury (OZBEK 2012; AHMAD *et al.* 2013; GEHLKEN *et al.* 2018). On the other hand, it is also established that increased GST activity represents a cellular response to oxidative stress or proinflammatory stimuli, both of which are present in patients suffering from chronic heart failure (SAVIC-RADOJEVIC *et al.* 2017). These antioxidants play a vital role in cellular detoxification, antioxidant defense, and redox status maintenance (AHMAD *et al.* 2013; SAVIC-RADOJEVIC *et al.* 2017). Related to this, it is suggested that Gal-3 could be a predictor of cardiorenal syndrome, since there is a positive correla-

tion between the serum concentration of this lectin and the development of cardiac and renal fibrosis in these patients (MEDVEDEVA *et al.* 2016).

In conclusion, based on our results it can be confirmed that Gal-3 deficiency has an influence on the cardiac and renal antioxidant capacity in mice. Our findings indicate that the lack of Gal-3 does not lead to lipid peroxidation and nitrosative stress, but reduces cardiac and renal antioxidant defense. However, the precise mechanism by which Gal-3 deprivation causes a reduction in antioxidant capacity will require further investigation. Having more data on Gal-3 as a potential agent of oxidative/nitrosative tissue injuries with profibrotic properties versus its involvement in the prevention of oxidative/nitrosative stress and fibrosis, could be relevant for better understanding the etiopathogenesis of fibrotic diseases (fibrosis of cardiac muscle, kidneys, and other organs), which represents a major cause of morbidity and mortality worldwide. In this context, the results of the current pilot study may also be useful for further examination of the role of Gal-3 in cardiac and renal tissues in the course of heart and kidney failure, especially in light of the development of Gal-3 pharmacological inhibitors and antioxidant molecules for the treatment of fibrosis.

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Author Contributions

Research concept and design: D.M., B.J.; Performance of the experiment: D.M., B.J., D.V.; Evaluation of statistical data: M.V.; Date analysis and interpretation: S.B.; Describing experimental methodology: R.J.; Writing the article and carrying out the experiment: D.V., A.R., M.R.; Drafting the manuscript: J.S., B.H.; Final approval of article, project coordinator, and supervision of the research group: T.R.. All authors read and approved the final manuscript.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplementary Materials

Supplementary Materials to this article can be found online at:
http://www.isez.pan.krakow.pl/en/folia-biologica.html

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