RUDN Journal of Agronomy and Animal Industries. ISSN 2312-797X (Print). ISSN 2312-7988 (Online)
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2024;19(3):497—506
http://agrojournal.rudn.ru

Veterinary science
Ветеринария

DOI: 10.22363/2312-797X-2024-19-3-497-506
УДК 619:618.96:569.822.2-086
EDN CIGQPD

Research article / Научная статья

Oxidative stress in dogs with cardiorenal syndrome caused by endocardiosis

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Abstract. Oxidative stress and decreased antioxidant defense were registered in dogs with endocardiosis. Complication of underlying pathology in the form of cardiorenal syndrome leads to aggravation of lipid peroxidation processes and causes further decrease in activity of enzymes of antioxidant defense system of animal organism. The aim of the study was to evaluate the pathophysiological significance of oxidative stress in the processes of formation and progression of cardiorenal syndrome in dogs with endocardiosis. Concentration of malondialdehyde, ceruloplasmin, diene conjugates, activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase were measured in venous blood serum samples from 24 dogs with uncomplicated forms of endocardiosis, 31 dogs with endocardiosis complicated by cardiorenal syndrome, and 22 healthy dogs. Compared with the group of healthy dogs, dogs with endocardiosis were diagnosed with statistically significantly higher median of serum concentrations of malondialdehyde, ceruloplasmin, diene conjugates, as well as statistically significantly lower activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, which indicates activation and progression of lipid peroxidation processes against the background of simultaneous decrease in parameters of antioxidant defense system. Presence of cardiorenal syndrome in dogs with endocardiosis led to

a sharp increase in manifestations of oxidative stress, which should be considered by clinicians when optimizing therapeutic and preventive measures. Lipid peroxidation products, as well as indicators of enzyme systems of antioxidant defense can be used as potential biomarkers of the development of cardiorenal complications in dogs with endocardiosis.

Keywords: cardiorenal syndrome, pathogenesis, biochemistry, pathochemistry, dogs, endocardiosis, heart failure

Author contributions. Vatnikov Y.A. — research concept, work with literature, conducting experiments, writing the paper; Vilkovyskiy I.F. — conducting experiments, collecting data, writing the paper; Shchurov I.V. — conducting experiments, validating methods, working with literature; Yarovenko E.M. — conducting experiments, interpreting data; Rudenko A.A. — administration, work with literature, data processing, analysis and generalization of research results.

Conflict of interests. The authors declared no conflict of interests.

Acknowledgments. The research was supported by the Russian Science Foundation grant No. 24-26-00090, https://rscf.ru/project/24-26-00090/

Article history: Received: 17 July 2024. Accepted: 9 August 2024.

For citation: Vatnikov YA, Rudenko AA, Shchurov IV, Vilkovyskiy IF, Yarovenko EM. Oxidative stress in dogs with cardiorenal syndrome caused by endocardiosis. *RUDN Journal of Agronomy and Animal Industries*. 2024;19(3):497—506. doi: 10.22363/2312-797X-2024-19-3-497-506

Introduction

Hyperproduction of reactive oxygen species can cause a powerful pathological effect on body of sick animals [1]. This pathological condition is associated with lipid peroxidation and is called oxidative stress [2]. Under physiological conditions, the animal body has an effective defense system in the form of antioxidant system [1, 2]. However, an imbalance of prooxidant and antioxidant factors can initiate processes of uncontrolled lipid peroxidation and damage to proteins and nucleic acids in various cellular structures [1]. In this regard, risk of damage to cardiovascular and excretory systems as multimorbid pathology is of particular concern [3—6]. Previously, we studied oxidative stress and its role in hepatocardial syndrome [7]. However, even greater problem in veterinary therapy is cardiorenal syndrome, which occurs against the background of primary cardiopathology and is manifested by pronounced decrease in renal function in the form of azotemia [8—10].

It is known that oxidative stress causes the phenomenon of cytotoxicity, affects neuroendocrine and immune systems, induces production of proinflammatory cytokines and has negative ionotropic effect [10, 11]. General markers of oxidative stress are well known in the literature and include products of lipid peroxidation (e.g., diene conjugates, malondialdehyde, 8-F2a-isoprostane, etc.), oxidized DNA (e.g., 8-hydroxydeoxyguanosine) or protein modifications (e.g., protein carbonyls) [12]. Ceruloplasmin is also considered to play an important role in the development of oxidative stress [4]. Endogenous antioxidant defense system is quite complex, diverse and includes such enzyme systems as catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, free

radical neutralizers (retinol, tocopherol, ascorbic acid) and metal chelators [10, 13—14]. The state of antioxidant system and lipid peroxidation processes in dogs with endocardiosis during the development of complications in form of cardiorenal syndrome are not presented in the literature.

The aim of the study was to evaluate pathophysiological significance of oxidative stress in processes of formation and progression of cardiorenal syndrome in dogs with endocardiosis.

Materials and methods

Dogs with endocardiosis were studied in the research. The diagnosis of endocardiosis in dogs of risk group breeds was established based on the presence of pronounced holosystolic murmur at the apex of heart on the left side of chest, enlarged left atrium (ratio of the left atrium size to aorta size is more than 1.7), a deformed and thickened mitral valve with or without simultaneous damage to tricuspid valve, and signs characteristic of mitral regurgitation according to echocardiography in B-, M-mode and color Doppler mapping [15—19]. All sick animals admitted for initial appointment to veterinary clinics in Moscow and Moscow region had signs of congestive heart failure: hyperemia, pulmonary edema, pleural effusion or ascites. The studies were conducted on 22 physiologically healthy dogs and 55 dogs with endocardiosis. Echocardiographic and Dopplerographic examination methods were performed on *Mindray DP-60* apparatus [14]. Dogs with endocardiosis were divided into 2 groups: I — free from cardiorenal complications (n = 24), II — with cardiorenal syndrome (n = 31). The serum biochemical profile was assessed for each dog to determine compliance with the criteria for participation in the experiment. Dogs included in the control group were classified as physiologically healthy based on physical examination, normal echocardiography data and serum biochemical profile. All dogs with other severe diseases (e.g., oncopathology, liver failure, diabetes, anemia, sepsis, infectious and parasitic diseases) were excluded from the study. There were no differences between the control and experimental groups in body weight, age, sex distribution and breeds. All dog owners signed an informed consent form for voluntary participation in the clinical experiment. Azotemia, manifested by increased concentration of creatinine in the blood serum (≥ 200 μmol/L), was considered a clinically important criterion for the presence of cardiorenal syndrome in animals. Blood samples were collected from dogs on empty stomach from subcutaneous vein of forearm into vacuum tubes with blood coagulation activator in the morning after preliminary fasting regimen of at least 10 hours. Intensity of lipid peroxidation processes and antioxidant system in blood serum of dogs with cardiorenal syndrome was assessed using commercial *RANDOX Laboratories Ltd* kits according to the manufacturer's instructions on *UN2CO-WFT2100* spectrophotometer.

Mann — Whitney and Kruskal — Wallis methods were used to analyze statistically the obtained digital data in STATISTICA 7.0 [17—19]. The experimental data were described by the median Me and the interquartile range IQ.

Results and discussion

The median concentration of malondialdehyde in blood serum of dogs with uncomplicated forms of endocardiosis and in the group of animals with cardiorenal syndrome was significantly higher compared to the control group (table).

Antioxidant status and biomarkers of oxidative stress in dogs with mitral valve endocardiosis complicated by cardiorenal syndrome

Indicator	Group of animals						Kruskal — Wallis test
	Control (*n* = 22)		I (*n* = 24)		II (*n* = 31)		
	Me	IQ	Me	IQ	Me	IQ	
Malondialdehyde, μmol/L	2.75	2.40...2.80	3.80***	3.00...4.10	4.00***	3.70...4.20	*H* = 35.7 *p* < 0.001
Ceruloplasmin, mmol/L	1.30	1.10...1.80	2.15***	1.80...2.60	3.00***###	2.50...3.10	*H* = 46.2 *p* < 0.001
Superoxide dismutase, U/ml	50.0	46.0...55.0	38.0***	35.5...40.0	27.0***###	25.0...30.0	*H* = 57.0 *p* < 0.001
Catalase, U/ml	1.55	1.40...1.90	0.70***	0.50...0.90	0.40***###	0.30...0.50	*H* = 53.5 *p* < 0.001
Glutathione reductase, U/ml	1.45	1.30...1.60	0.70***	0.60...0.90	0.80***	0.60...0.90	*H* = 45.2 *p* < 0.001
Glutathione peroxidase, U/ml	2.95	2.70...3.30	2.35***	1.90...2.65	1.80***###	1.70...2.00	*H* = 44.2 *p* < 0.001
Diene conjugates, units/ml	1.95	1.50...2.30	2.95***	2.50...3.35	3.80***###	3.70...4.10	*H* = 57.2 *p* < 0.001

Note. Me — median; IQ — interquartile range; * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$ — reliability of the difference between the indicators of groups I, II and clinically healthy animals (Mann — Whitney test); # — $p < 0.05$; ## — $p < 0.01$; ### — $p < 0.001$ — reliability of the difference between the indicators of groups I and II (Mann — Whitney test).

However, the median of serum malondialdehyde concentration did not statistically differ in dogs with endocardiosis depending on the presence or absence of cardiorenal complications (Table). At the same time, Kruskal — Wallis analysis showed a high level of reliability, which indicates that the values of this biochemical parameter in animals of different groups do not belong to a single general population.

The median of concentration of ceruloplasmin in serum of dogs with endocardiosis, both in absence and presence of cardiorenal syndrome, was statistically significantly higher compared to the control. In addition, in the group of sick dogs with cardiorenal syndrome, concentration of ceruloplasmin in blood serum was statistically significantly

higher than in the group of sick animals without cardiorenal complications. Kruskal — Wallis analysis of serum concentration of ceruloplasmin in dogs of different experimental groups established the presence of high statistical significance of the results obtained.

The medians of serum superoxide dismutase activity in dogs of different experimental groups do not belong to the same general population according to the Kruskal — Wallis analysis. Activity of this enzyme in blood serum of dogs with endocardiosis was statistically significantly lower both in absence and in presence of cardiorenal syndrome compared to the control group. However, it should be noted that activity of superoxide dismutase in blood serum was lower in the group of dogs with cardiorenal syndrome compared to the intact group of dogs.

With regard to the median of catalase activity in blood serum of dogs of different groups, Kruskal — Wallis analysis established high level of statistical significance. In dogs with endocardiosis, both without cardiorenal complications and with them, the median of serum catalase activity was statistically significantly lower than in the control group. However, it should be noted that activity of this enzyme was significantly lower in the group of dogs with endocardiosis with cardiorenal syndrome than in the group of animals with uncomplicated forms of pathology.

The serum glutathione reductase activity in the group of dogs with endocardiosis and cardiorenal syndrome and in the group of dogs with uncomplicated forms of cardiopathology was lower than in the control group. No statistically significant changes in serum activity of this enzyme were found between the group of dogs with uncomplicated forms of endocardiosis and animals with complications in the form of cardiorenal syndrome. However, Kruskal — Wallis analysis showed statistically significant differences between the different experimental groups of dogs.

Kruskal — Wallis method verified statistically significant differences between serum glutathione peroxidase activity in different experimental groups of animals. At the same time, compared to the control group, activity of this enzyme in the group of dogs with uncomplicated forms of endocardiosis was statistically significantly lower. Presence of cardiorenal syndrome in dogs with cardiorenal syndrome led to even more significant decrease in activity of glutathione peroxidase in blood serum compared to the group of animals with uncomplicated endocardiosis.

Concentration of diene conjugates in blood serum of dogs with endocardiosis both in absence and presence of cardiorenal syndrome was statistically significantly higher compared to the control. In addition, in the group of dogs with cardiorenal syndrome, concentration of diene conjugates in blood serum was statistically significantly higher than in the group of sick animals without cardiorenal complications. Conducting Kruskal — Wallis analysis for serum concentration of diene conjugates in dogs of different experimental groups established the presence of high statistical significance of the obtained results.

Dogs with endocardiosis complicated by cardiorenal syndrome in this study had more significant oxidative stress than those with uncomplicated forms of cardiovascular pathology, as indicated by higher values of concentration of diene conjugates in blood serum. Diene conjugates are direct products of lipid peroxidation. They are synthesized in

the process of double bond rearrangement during free-radical oxidation of polyunsaturated fatty acids.

Malondialdehyde is a less reliable biomarker of oxidative stress [1]. It is synthesized in body of humans and animals during the breakdown of arachidonic acid and other polyunsaturated lipids by active oxygen species [2]. Compared with the control, this metabolite statistically significantly increased in serum of dogs with endocardiosis. However, presence of cardiorenal syndrome in sick dogs did not initiate a further increase in its concentration in blood serum.

Copper-containing protein ceruloplasmin plays a vital enzymatic role in body of animals and humans, namely: it catalyzes oxidation of polyamines and polyphenols in blood serum [4], and is a potential biomarker of oxidative stress. Our study established that during the development of cardiorenal syndrome in dogs with endocardiosis, concentration of ceruplasmin in blood serum was higher than in patients without cardiorenal complications and intact animals.

Superoxide dismutase is an enzyme of antioxidant defense [6]. It catalyzes dismutation of superoxide into oxygen and hydrogen peroxide. In dogs with endocardiosis, activity of superoxide dismutase in blood serum was significantly reduced compared to clinically healthy animals. A particularly noticeable decrease occurred with the development of cardiorenal syndrome.

Catalase is a heme-containing enzyme belonging to oxidoreductases [12]. This enzyme is directly involved in decomposition of hydrogen peroxide into water and oxygen. In dogs with endocardiosis, serum catalase activity was significantly reduced compared to the norm. A particularly pronounced decrease was noted in the group of animals with cardiorenal syndrome.

Glutathione reductase is an enzyme that restores disulfide bond of oxidized glutathione in its sulfhydryl form due to the energy of NADPH formed in pentose cycle [13]. In red blood cells, under conditions of constant high risk of oxidative stress, almost 10% of consumed glucose is used to restore glutathione by glutathione reductase. In dogs with endocardiosis, compared to healthy ones, there is a decrease in serum activity of glutathione reductase. However, presence of cardiorenal syndrome in sick animals did not lead to a more significant decrease in activity of this enzyme.

Glutathione peroxidase is an enzyme that protects body of humans and animals from the effects of oxidative stress [11—13, 20, 21]. Glutathione peroxidase restores fatty acid hydroperoxides to the corresponding alcohols, as well as hydrogen peroxide to water. Our study showed that, compared to healthy dogs, serum activity of this enzyme was significantly reduced in dogs with endocardiosis, and presence of cardiorenal syndrome led to more significant decrease.

It is obvious that increased production of active oxygen species induced by various triggers associated with the severity of chronic heart failure in dogs with endocardiosis contributes to the formation and progression of cardiorenal syndrome. At the same time, decrease in pulmonary capillary blood flow in cardiopathology initiates the production of active oxygen species by mitochondria of various cells in body of sick animals, which requires further study.

Conclusion

Oxidative stress markers (malondialdehyde, ceruloplasmin, and diene conjugates in serum) increase in dogs with mitral valve endocardiosis compared to clinically healthy dogs. The increase in these substrates was significantly higher in the presence of complications such as cardiorenal syndrome. In addition, serum activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase) was found to decrease consistently in dogs with endocardiosis. Depression severity of these enzyme systems was the lowest in dogs with cardiorenal complications. Lipid peroxidation products and antioxidant defense enzyme system indices can be used as potential biomarkers of cardiorenal complications in dogs with endocardiosis.

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Оксидативный стресс при кардиоренальном синдроме у собак, возникшем на фоне эндокардиоза

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Аннотация. Окислительный стресс и снижение антиоксидантной защиты были зарегистрированы у собак с эндокардиозом. Осложнение основной патологии в виде кардиоренального синдрома приводит к усугублению процессов перекисного окисления липидов и обусловливает дальнейшее снижение активности ферментов антиоксидантной системы защиты организма животных. Цель исследования — оценка патофизиологического значения оксидативного стресса в процессах формирования и прогрессирования кардиоренального синдрома у больных эндокардиозом собак. Концентрацию малонового диальдегида, церулоплазмина, диеновых конъюгатов, активность супероксиддисмутазы, каталазы, глутатионредуктазы, глутатионпероксидазы измеряли в образцах сыворотки венозной крови у 24 собак с неосложненными формами эндокардиоза, у 31 собаки, больной эндокардиозом, осложненным кардиоренальным синдромом, а также у 22 здоровых собак. По сравнению с группой здоровых собак, у больных эндокардиозом собак диагностировали статистически значимо более высокую медиану сывороточной концентрации малонового диальдегида, церулоплазмина, диеновых конъюгатов, а также статистически значимо низкую активность супероксиддисмутазы, каталазы, глутатионредуктазы, глутатионпероксидазы, что свидетельствует об активизации и прогрессировании процессов перекисного окисления липидов на фоне одновременного снижения параметров антиоксидантной системы защиты организма. Фактор наличия кардиоренального синдрома у больных эндокардиозом собак приводил к резкому усилению проявлений оксидативного стресса, что нужно учитывать клиницистам при оптимизации лечебно-профилактических мероприятий. Продукты перекисного окисления липидов, а также показатели ферментных систем антиоксидантной защиты можно использовать как потенциальные биомаркёры развития кардиоренальных осложнений у собак, больных эндокардиозом.

Ключевые слова: кардиоренальный синдром, патогенез, биохимия, патохимия, собаки, эндокардиоз, сердечная недостаточность

Вклад авторов: Ватников Ю.А. — концепция исследования, работа с литературой, проведение экспериментов, подготовка текста; Вилковыский И.Ф. — проведение экспериментов, сбор материала, подготовка текста; Щуров И.В. — проведение экспериментов, валидация методов, работа с литературой; Яровенко Е.М. — проведение экспериментов, интерпретация данных; Руденко А.А. — администрирование, работа с литературой, обработка данных, анализ и обобщение результатов исследования.

Заявление о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов.

Финансирование. Исследование выполнено за счет гранта Российского научного фонда № 24-26-00090, https://rscf.ru/project/24-26-00090/

История статьи: поступила в редакцию 17 июля 2024 г., принята к публикации 9 августа 2024 г.

Для цитирования: *Ватников Ю.А., Руденко А.А., Щуров И.В., Вилковыский И.Ф., Яровенко Е.М.* Оксидативный стресс при кардиоренальном синдроме у собак, возникшем на фоне эндокардиоза // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2024. Т. 19. № 3. С. 497—506. doi: 10.22363/2312-797X-2024-19-3-497-506