Theoretical and Applied Veterinary Medicine

ISSN 2663-1156 (print)
ISSN 2663-1164 (online)
Theoretical and Applied Veterinary Medicine, 11(2), 21–28
doi: 10.32819/2023.11009

Original research

Efficacy of metformin treatment for bitches with the mammary gland carcinoma

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Received: 05 June 2023
Revised: 12 June 2023
Accepted: 26 June 2023

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Abstract. The relevance of improvement and clinical approval of modern treatment regimens is determined by the insufficient efficiency of protocols for the treatment of malignant tumors in dogs and the lack of research on the possibility of repurposing certain pharmacological agents for use in veterinary oncology. The manuscript demonstrates the results of a pilot study of using metformin as part of a chemotherapy protocol in female dogs with poorly differentiated tubular mammary carcinoma after mastectomy. Considering the hypoglycemic effect of metformin, it was used as part of an adjuvant protocol in a metronomic mode in female dogs with a normal and high body mass index. Combined oral administration of cyclophosphamide (endoxan) and metformin at a dose of 12.5 mg/m^2 and 10 mg/kg, respectively, for 6 months prolonged the median survival in female dogs with a normal body index (from 135.8 days, 95% CI 40–234 to 178.6 days, 95% CI 22–394, $p < 0.01$) as well as in overweight patients (from 93.4 days, 95% CI 15–174 to 237.8 days, 95% CI 38–373, $p < 0.001$). Herewith, a high body mass index compared to a normal one recognized as the risk of animal death due to the disease progression. The feasibility of using metformin in overweight patients is consistent with a reliably high glucose level before starting treatment. Herewith, blood content of triglycerides and total cholesterol did not depend on the body weight of the dogs and was within the physiological norm in all dogs. Adjuvant therapy with cyclophosphamide (endoxan) and metformin in a metronomic mode for six months was accompanied by a slight decrease (within the lower limit of reference values) of the blood glucose level against the background of the absence of pronounced changes in the concentration of triglycerides and total cholesterol. Improved long-term prognosis and the insignificant risk of side effects allow recommending metronomic therapy with cyclophosphamide (endoxan) and metformin after mastectomy in female dogs with mammary gland carcinoma.

Keywords: dogs; tumors; mammary gland; metformin; body mass index; mastectomy; chemotherapy

Ефективність метформіну за лікування сук із карциномою молочної залози

Анотація. Необхідність досліджень обґрунтована недостатньою ефективністю протоколів лікування злоякісних пухлин у собак та відсутністю напрацювань щодо можливості перепрофілювання окремих фармакологічних засобів для використання у ветеринарній онкології. У рукописі представлено результати пілотного дослідження використання метформіну в складі хіміотерапевтичного протоколу у сук із низькодиференційованою тубулярною карциномою молочної залози на тлі мастектомії. Враховуючи гіпоглікемічний ефект метформіну проведено його застосування у складі ад'ювантного протоколу в метрономному режимі у сук із нормальним та високим індексом маси тіла. Комбіноване пероральне застосування протягом 6 місяців циклофосфаміду (ендоксану) та метформіну у дозі 12,5 мг/м^2 та 10 мг/кг, відповідно, дозволила подовжити медіану виживання у сук із нормальним індексом тіла (з 135,8 днів, 95% CI 40–234 до 178,6 днів, 95% CI 22–394, $p < 0,01$) та у пацієнтів, які мають надлишкову масу тіла (з 93,4 днів, 95% CI 15–174 до 237,8 днів, 95% CI 38–373, $p < 0,001$). При цьому високий індекс маси тіла, порівняно із нормальним, збільшував ризик загибелі тварин внаслідок прогресування захворювання. Доцільність застосування метформіну у пацієнтів, які мали надлишкову масу тіла, узгоджується із достовірно високим рівнем глюкози перед початком лікування. При цьому вміст у крові тригліцеридів і загального холестерину не залежав від маси тіла собак та знаходився у всіх собак у межах фізіологічної норми. Проведення ад'ювантної терапії циклофосфамідом (ендоксаном) та метформіному у метрономному режимі впродовж шести місяців супроводжувалось незначним зниженням (в межах нижнього кордону референтних значень) рівня глюкози у крові на фоні відсутності виражених змін концентрації тригліцеридів та загального холестерину. Покращення довгострокового прогнозу і незначний ризик побічних ефектів дозволяють рекомендувати метрономну терапію циклофосфамідом (ендоксаном) та метформіном після мастектомії у сук із карциномою молочної залози.

Ключові слова: собаки; пухлини; молочна залоза; метформін; індекс маси тіла; мастектомія; хіміотерапія

Cite this article: Bilyi, D. D., & Kovalenko, M. S. (2023). Efficacy of metformin treatment for bitches with the mammary gland carcinoma. *Theoretical and Applied Veterinary Medicine*, 11(2), 21–28. doi: 10.32819/2023.11009

Introduction

The repurposing of pharmacological agents in human oncology consists in the use of drugs for the treatment of cancer that are not registered for various non-oncological diseases. This direction is a new concept in veterinary medicine. Their lower cost compared to antitumor drugs against the background of sufficient effectiveness led to numerous in vivo and in vitro studies, which obtained promising results. Drug repurposing in veterinary oncology is a very new and attractive concept, with only a few studies published so far.

Pilot studies have shown that the use of metformin is associated with a significant reduction in the progression of neoplasia, particularly breast cancer. In vitro and in vivo observations confirm that metformin inhibits the growth of cancer cells. The mechanisms underlying this protective potential have not been fully studied. Currently, two main ways of the protective effect of metformin have been proven: the first one involves the reduction of endogenous hyperinsulinemia, the second one is associated with the activation of adenosine monophosphate-activated protein kinase (AMPK), which inhibits the synthesis and growth of cellular protein. In any case, the antitumor properties of metformin have clinical value and justify the expediency for further study of its properties (Papanas et al., 2010).

A dose-dependent relationship between the duration of metformin pre-treatment and the protective effect against cancer has been reported with evidence of the involvement of anti-tumour mechanisms such as AMPK activation and mammalian target of rapamycin (mTOR) pathway inhibition (Beck & Scheen 2010).

The results demonstrate the effectiveness of metformin in controlling the angiogenesis process in breast tumors according to the expression level of vascular endothelial growth factor (VEGF) and Hypoxia-Inducible Factor-1 (HIF-1), the most important angiogenic markers. In particular, in vivo study evidence in respect to a decrease in tumor size, protein, and gene expression of HIF-1 and VEGF-A was observed against the background of a decrease in CD31 expression; in vitro – after 24 hours, the viability of cancer cells and the expression of HIF-1 and VEGF proteins and genes decrease (Moschetta et al., 2019).

The areas of metformin application in human and veterinary medicine are quite wide, but currently its pharmacological effects have not been fully studied. Obesity and excess weight are the caused factors in the occurrence of mammary tumors in dogs (Pascoli et al., 2017; Varma et al., 2021). Therefore, a potential field of application of metformin is malignant neoplasms of the mammary gland in female dogs which have excess body weight. The incidence of mammary tumors is higher in overweight/obese dogs (Gupta et al., 2012; Bilyi et al., 2021), mastopathy is also more likely to occur in overweight/obese dogs (Bilyi & Khomutenko, 2022). Mammary gland tumors in dogs can be transformed from benign to malignant (Sorenmo et al., 2009).

Overweight and obese female dogs have shorter breast cancer survival than normal weight ones (Tesi et al., 2017; Tesi et al., 2020; Nicchio et al., 2020). In obese dogs, there is a correlation between elevated blood glucose content and insulin release whereas in dogs with normal weight and different blood glucose levels no significant changes in insulin were detected (Gonzlez-Villar et al., 2022). The results of an experimental model have shown that selective increase in visceral fat in dogs and in vivo insulin sensitivity have no changed (Castro et al., 2015).

Metformin-treated hyperadrenocorticism dogs have significant reductions in glycemia, cholesterolemia, and triglyceridemia (Miceli et al., 2018). Metformin causes a significant decrease in postprandial blood glucose levels in dogs (Balogh et al., 2008). The primary acute effect of metformin on glucose metabolism in dogs is the inhibition of hepatic glucose production rather than stimulation of glucose utilization (Chu et al., 2000). The average oral bioavailability of metformin in dogs is 31% (Johnston et al., 2017).

Higher serum levels of the acute phase inflammation proteins including ferritin (Franco-Martinez et al., 2023), myeloperoxidase (Paulo et al., 2023), C-reactive protein and haptoglobin (Vecchiato et al., 2023), number of neutrophils (Paulo et al., 2023) were observed in obese dogs. In humans, metformin specifically inhibits macrophage proinflammatory cytokine secretion without inhibiting their differentiation or activation (Cameron et al., 2016). Metformin reduces protein markers of multidrug resistance in canine lymphoma cells (Arnason et al., 2022).

Female dogs diagnosed with mammary carcinoma have been found to have significantly lower serum adiponectin level than clinically healthy ones, suggesting a link between cancer and hypoadiponectinemia similar to that observed in humans. (Souza et al., 2019). Overweight dogs have significantly lower serum adiponectin levels compared to normal weight dogs. It has been established that metformin enhances the expression of adiponectin in adipose tissue (Park et al., 2014).

Metformin is a potential selective breast cancer drug as an effective (neo-)adjuvant therapy for suppressing cancer stem cells in human and animal mammary carcinomas (Barbieri et al., 2015). Metformin inhibits the epithelial-mesenchymal transition of breast cancer cells (Vazquez-Martin et al., 2010; Leonel et al., 2017) and tumor growth in xenotransplanted metastatic canine mammary tumor cells (Saeki et al., 2015).

Generalization of preclinical data is problematic because questions remain unanswered regarding the relevant mechanisms, bioavailability, and genetic factors that confer metformin sensitivity. Probably the most important unresolved question is the assessment of the anticancer activity of metformin in nondiabetic patients. Further studies of these issues allow to expand the range of use of metformin as an inexpensive, well-tolerated and effective antitumor agent (Quinn et al., 2013).

Taking into the account the relevance of the problem, the goal of the study was evaluation of metformin effectiveness against poorly differentiated mammary gland carcinomas in female canines with normal and excess body weight combined with metronomic cyclophosphamide chemotherapy.

Materials and methods

A randomized controlled trial carried out at "Best" veterinary clinic in Zaporizhia city. The study included a group of 59 female dogs with clinical stage III of poorly differentiated tubular carcinoma of the mammary gland. A neoplastic lesion of one or two "packages" of the mammary gland was clinically registered in patients, and the

Table 1 – Scheme of the experiment

Group	Body mass index	n	Surgical intervention	Pharmacotherapy
Control	normal	17	mastectomy	cyclophosphamide (12.5 mg/m^2) orally once a day for 6 months
	high	12		
Experimental	normal	13		cyclophosphamide (12.5 mg/m^2) + metformin (10 mg/kg) orally once a day for 6 months
	high	17		

Table 2 – Median survival depending on body mass index of dogs

Group		Average indicator, days	95% CI (days)
Control	with a normal body mass index (n=17)	135.8	40–234
	with a high body mass index (n=12)	93.4	15–174
Experimental	with a normal body mass index (n=13)	178.6[a]	22–394
	with a high body mass index (n=17)	237.8[b]	38–373

presence of metastatic foci in regional lymph nodes was confirmed histopathologically. All animals which took the surgical treatment in the scope of regional or unilateral mastectomy. Animals were divided into four separate groups based on body mass index (Table 1). Body mass index was determined on a 9-point scale, dogs with excess body weight and/or obesity had a score higher than 6 out of 9 (Baldwin et al., 2010).

Controls were two groups whose animals underwent mastectomy at the first stage. The first of them included dogs with a normal body mass index (n = 17), the second - bitches that had excess body weight (n = 12). Subsequently, approximately 8 days after the surgery, cyclophosphamide was prescribed orally, once a day for 6 months at a dose of 12.5 mg/m2.

The treatment of the animals who made up the experimental groups included mastectomy followed (on average, 8 days after surgery) by the prescription of cyclophosphamide (12.5 mg/m2 dose) and metformin (10 mg/kg dose) in the following regimen: orally, once per day for 6 months. Two research groups were formed: animals with a normal body mass index (n = 13) were included into the first one, and female dogs with a high body mass index (n = 13) - into the second one.

The contents of glucose, cholesterol, triglycerides and survival time were analyzed. Glucose was determined according to Trinder's method (GLU 500, Erba, Czech Republic) using BS-230 automatic biochemical analyzer (Mindray, China) (Barham, et al. 1972). Cholesterol content was analyzed according to Trinder's method (CHOL 250, Erba, Czech Republic) with using same analyzer (Allain et al. 1974; Roeschlau et al. 1974). Triglycerides were determined according to the GPO-POD method (TG 250, Erba, Czech Republic) with using same analyzer (Cole et al. 1997).

Control of the tumor progresse was carried out every 30 days using chest X-ray and abdominal ultrasound.

Statistical analysis of the results and graph construction were performed using Statistica 10 software (StatSoft inc., USA, 2011). The data were compared using the Tukey test, taking into the account the Bonferroni error for multiple comparisons, the difference was considered significant at $p>0.05$. Median survival for each group and 95% confidence interval were estimated using Kaplan-Meier survival curves.

Results

The average time from the surgery to the start of chemotherapy based on cyclophosphamide and metfomin was 7.5 days (ranging from 5 to 12 days). One animal of experimental group developed toxic phenomena in the form of macrohematuria development, which passed against the background of cyclophosphamide withdrawal for 3 days.

Analysis of the impact of the proposed treatment protocol on the median survival of the dog patients depending on the body mass index showed significant differences between control and experimental groups (Table 2; Fig. 1). The median survival rate of dogs in the experimental groups was significantly higher than that of control animals, respectively. In particular, its average indicator in female canines of the experimental group with a normal body mass index (178.6 days, 95% CI 22–394) was prolonged in 1.3 times ($p < 0.01$) compared to control group patients (135.8 days, 95% CI 40–234). There was an increase in the average median survival in the high body mass dogs of the experimental group (237.8 days, 95% CI 38–373) by 2.5 times ($p < 0.001$) compared to control female dogs (93.4 days, 95% CI 15–174). Median survival was significantly greater ($p < 0.001$) in animals with a normal body mass index both within the control and experimental groups: the excess was 1.5 and 1.3 times, respectively.

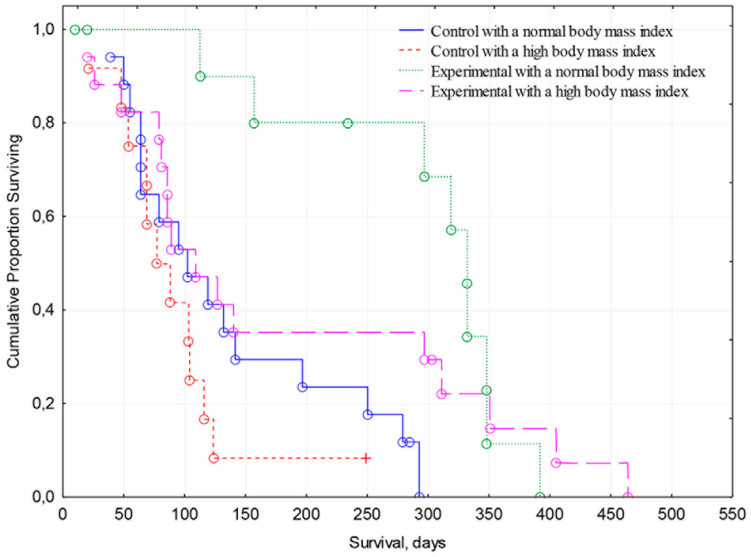

Fig. 1. Median survival and 95% confidence interval in dogs with different treatment regimens

Table 3 – Glucose content and lipid metabolism estimation before treatment

Indicator	Reference values	Group			
		Control		Experimental	
		with a normal body mass index (n=17)	with a high body mass index (n=12)	with a normal body mass index (n=13)	with a high body mass index (n=17)
Glucose, mmol/l	4.4–6.7	4.8 ± 0.76[a]	5.3 ± 0.83[ab]	4.9 ± 0.73[a]	5.8 ± 0.64[ab]
Triglycerides, mmol/l	0,24–0,98	0.88 ± 0.33	0.97 ± 0.47	0.95 ± 0.41	0.88 ± 0.35
Total cholesterol, mmol/l	2,9–6,5	5.07 ± 0.84	5.90 ± 1.59	4.94 ± 0.79	5.09 ± 0.86

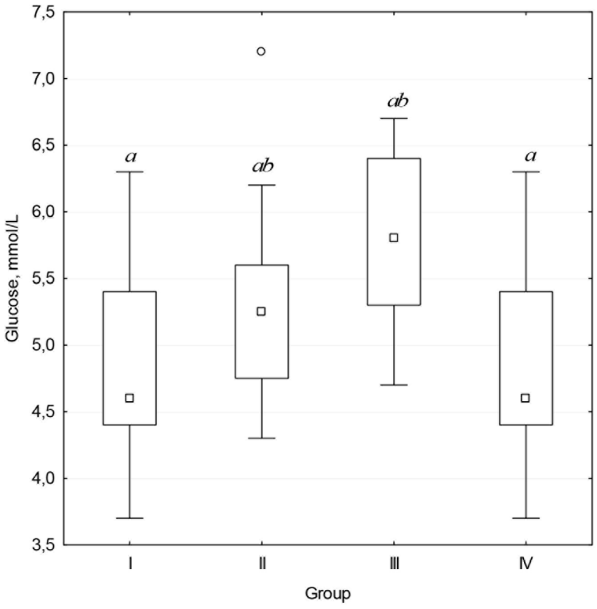

Fig. 2. Glucose level in dogs with malignant neoplasms of the mammary gland

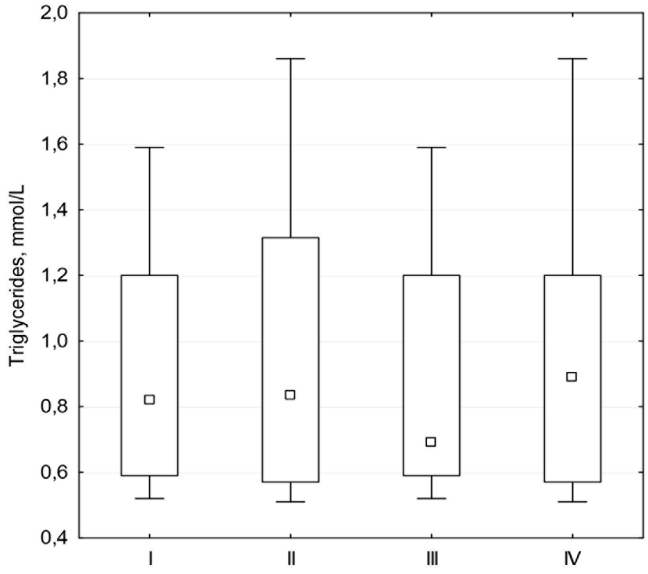

Fig. 4. Concentration of triglycerides in the blood of female dogs with malignant neoplasia of the mammary gland

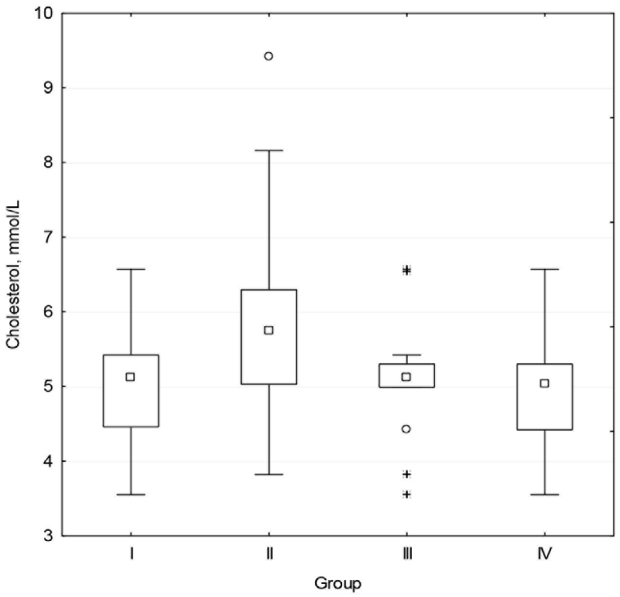

Fig. 3. Total cholesterol content in dogs with malignant mammary tumors

Glucose concentration and those of lipid metabolism (triglycerides and total cholesterol) in dogs with poorly differentiated tubular carcinoma of the mammary gland were equal to the physiological norm. (Table 3, Fig. 2–4). At the same time, according to the Tukey test, a significant difference ($p < 0.005$) in dogs depending on the body mass index was observed only for blood glucose, which was higher in patients with excessive body weight. There was no statistical differences in total cholesterol and triglyceride concentrations.

The results of glucose content measuring, total cholesterol and triglycerides in the blood during treatment (six months) showed dynamic fluctuations in their content within physiological parameters without a statistically significant difference between the experimental and control groups (Table 4–6).

Discussion

Preclinical studies in human medicine have shown that metformin affects on the liver mitochondria state. However, the discovery of a new target on the lysosomal surface may reveal a new mechanism of the treatment with low doses of metformin. Based on data on efficacy and safety in type 2 diabetes, attention is paid to the repurposing of metformin as part of adjunctive therapy for the treatment of cancer, age-related diseases and inflammatory diseases (Foretz et al., 2023).

Table 4 – Dynamics of glucose level (mmol/l) in the blood of bitches with malignant neoplasms of the mammary gland against the background of metformin treatment

Observation term	Group			
	Control		Experimental	
	with a normal body mass index (n = 17)	with a high body mass index (n = 12)	with a normal body mass index (n = 17)	with a high body mass index (n = 13)
Reference values	4.4–6.7			
Before treatment	4.8 ± 0.76[a]	5.3 ± 0.83[ab]	4.9 ± 0.73[a]	5.8 ± 0.64[ab]
Dynamics on the background of treatment				
1 month	5.1 ± 0.86 n = 17	5.2 ± 0.94 n = 11	4.8 ± 0.75 n = 15	5.3 ± 0.53 n = 13
2 month	4.9 ± 0.47 n = 12	5.1 ± 0.63 n = 9	5.2 ± 0.45 n = 14	4.9 ± 0.55 n = 12
3 month	4.8 ± 0.77 n = 10	5.2 ± 0.75 n = 5	4.8 ± 0.89 n = 9	5.7 ± 0.75 n = 11
4 month	5.2 ± 0.66 n = 7	4.7 ± 0.74 n = 3	4.8 ± 0.45 n = 7	5.3 ± 0.62 n = 9
5 month	4.9 ± 0.78 n = 6	5.2 ± 0.00 n = 1	5.0 ± 0.49 n = 6	5.5 ± 0.42 n = 9
6 month	4.9 ± 0.58 n = 6	5.3 ± 0.00 n = 1	5.1 ± 0.32 n = 6	5.4 ± 0.32 n = 8

Table 5 – Blood triglycerides content (mmol/l) in bitches with malignant neoplasia of the mammary gland when using metformin

Observation term	Group			
	Control		Experimental	
	with a normal body mass index (n = 17)	with a high body mass index (n = 12)	with a normal body mass index (n = 17)	with a high body mass index (n = 13)
Reference values	0.24-0.98			
Before treatment	0.88 ± 0.33	0.97 ± 0.47	0.95 ± 0.41	0.88 ± 0.35
Dynamics on the background of treatment				
1 month	0.85 ± 0.32 n = 17	0.93 ± 0.48 n = 11	0.93 ± 0.42 n = 15	0.83 ± 0.33 n = 13
2 month	0.86 ± 0.30 n = 12	1.02 ± 0.54 n = 9	0.99 ± 0.47 n = 14	0.94 ± 0.39 n = 12
3 month	0.90 ± 0.28 n = 10	0.97 ± 0.63 n = 5	1.00 ± 0.44 n = 9	0.89 ± 0.38 n = 11
4 month	0.84 ± 0.24 n = 7	1.06 ± 0.76 n = 3	1.04 ± 0.44 n = 7	0.91 ± 0.36 n = 9
5 month	0.86 ± 0.25 n = 6	1.07 ± 0.00 n = 1	0.91 ± 0.37 n = 6	0.91 ± 0.38 n = 9
6 month	0.81 ± 0.48 n = 6	1.03 ± 0.00 n = 1	0.90 ± 0.68 n = 6	1.07 ± 0.84 n = 8

Table 6 – Dynamic changes in total cholesterol content (mmol/l) in bitches with malignant tumors of the mammary gland using the metronomic treatment protocol

Observation term	Group			
	Control		Experimental	
	with a normal body mass index (n = 17)	with a high body mass index (n = 12)	with a normal body mass index (n = 17)	with a high body mass index (n = 13)
Reference values	2.9-6.5			
Before treatment	5.07 ± 0.84	5.90 ± 1.59	4.94 ± 0.79	5.09 ± 0.86
Dynamics on the background of treatment				
1 month	5.18 ± 0.94 n = 17	5.85 ± 1.59 n = 11	5.09 ± 0.83 n = 15	5.18 ± 0.81 n = 13
2 month	5.09 ± 1.10 n = 12	5.93 ± 1.43 n = 9	5.19 ± 0.92 n = 14	5.43 ± 0.79 n = 12
3 month	5.25 ± 1.03 n = 10	6.54 ± 1.39 n = 5	5.14 ± 0.83 n = 9	5.61 ± 0.77 n = 11
4 month	5.12 ± 1.26 n = 7	5.88 ± 0.4 n = 3	4.99 ± 0.66 n = 7	5.59 ± 0.96 n = 9
5 month	5.12 ± 1.18 n = 6	5.94 ± 0.00 n = 1	4,96 ± 0.76 n = 6	5.49 ± 0.82 n = 9
6 month	5.11 ± 1.19 n = 6	5.96 ± 0.00 n = 1	4.96 ± 0.76 n = 6	5.54 ± 0.86 n = 8

Potential application of metformin includes three areas: improvement of metabolic factors associated with treatment effects, such as insulin resistance and peripheral neuropathy; in combination with the effects of immune checkpoint blockade; endocrine treatment of hormone-dependent cancers. Although the results of metformin as a repurposing agent have been unsatisfactory in some studies, it still has potential for use in some settings of cancer therapy (Wu et al., 2023). The data obtained in our study have showed that the overall disposition pattern and bioavailability of metformin in dogs are similar to those reported in cats and humans. The results of this study showed that a dose of 10 to 15 mg metformin/kg administered orally twice daily to dogs was well tolerated and resulted in steady-state serum concentrations below the upper limit of the human therapeutic range of metformin of 2.5 mg/l.

Standardization of clinical trial protocols, development of dog-specific biological tools, establishment of adequate dog-specific disease biomarkers and identification of targets for the development of new therapies that could improve survival and have less adverse effects than chemotherapy are among the problems that remain unresolved (Vazquez et al., 2023).

The obtained positive results of the use of metformin in the treatment protocols of female canines with malignant mammary tumors are consistent with the data of Fan and coathors that metformin may be a potential chemotherapeutic agent in the treatment of canine mammary tumors due to its ability to inhibit the proliferation of mammary cancer cells through cell cycle arrest, apoptosis induction, adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation and inhibition of AKT/mTOR signaling pathways (Fan et al., 2021). Similar data were obtained by Faria and coathors via detection the complex I of the mitochondrial electron transport chain, AMPK and mammalian target of rapamycin complex 1 (mTORC1) as molecular targets of metformin (Faria et al., 2019).

Antiangiogenesis is currently considered one of the main antitumor strategies due to its protective effect against immediate tumor development and subsequent progression. The demonstrated clinical effectiveness of metformin may be related to its antiangiogenic effect, that is inhibition of HER2-induced tumor angiogenesis through the HIF-1/α-VEGF signaling axis (Wang et al., 2015).

The obtained results regarding the correlation of body weight and the effectiveness of metformin treatment protocols are consistent with published data. Obesity affects the development and progression of canine mammary tumors through higher infiltration of tumor-associated macrophages and enhanced tumor angiogenesis (Lim et al., 2013a), while macrophage infiltration within and around the tumor plays an important role in tumor progression and metastases in dogs (Lim et al., 2022), however, the number of CD204-positive macrophages in mammary tumors does not differ between obese and normal-weight dogs (Seung et al., 2018). Obese dogs have a shorter mean age of mammary tumor onset (Lim et al., 2015b). A positive correlation between body mass index and the histological grade of mammary tumors in female dogs is observed (Tesi et al., 2017; Tesi et al., 2020; Nascimento-Hama et al., 2022), Ki-67 expression is higher in dogs with excessive weight than in dogs of normal weight (Nicchio et al., 2020), which may indicate an important relationship between dyslipidemia and mammary tumor aggressiveness (Costa-Santos et al., 2019).

The determined significant effect of overweight and obesity in dogs on the risk of developing cancer against the background of elevated blood glucose is consistent with the publication of Pecinova et al. (2019), in which the antitumor effect of metformin is substantiated by the modulation of mitochondrial metabolism, which leads to a decrease in blood glucose or activation of antitumor pathways.

A dose-dependent decrease in the level of glycolysis in dogs, regardless of breed, when using metformin was demonstrated by Jimenez et al. (2011). The data of Bashraheel et al. (2023) indicate metformin-mediated suppression of IGF-1R signaling, which is highly activated in malignant breast tumors (primarily HER2-positive). These results may also explain the results we obtained using it in chemotherapy protocols. The results of Queiroz et al.

(2014) showed that metformin has an antiproliferative effect associated with cell cycle arrest and apoptosis, which is mediated by oxidative stress as well as AMPK and FOXO3a activation. Thus, our study is consistent with the results of other researchers and confirms the potential benefit of metformin in the treatment of canine mammary gland cancer and provides new insight into its antiproliferative role in oncogenesis.

Conclusions

In dogs with malignant neoplasia of the mammary gland, the use of a treatment protocol of cyclophosphamide (dose 12.5 mg/m2) plus metformin (dose 10 mg/kg) against the background of regional or unilateral mastectomy provided a statistically significant ($p < 0.01$ and $p < 0.001$) improvement in survival rates regardless of body mass index.

The median survival of dogs with normal body weight exceeded the corresponding values of animals with excessive body weight/obesity by 1.3–1.5 times ($p < 0.01$).

Adjuvant use of metformin in a metronomic regimen does not affect the level of blood glucose, triglycerides and total cholesterol, which fluctuated within the reference values.

The addition of metformin to metronomic therapy protocols may be beneficial for poorly differentiated mammary carcinomas in overweight/obese bitches, but further studies of its effect on carcinogenesis are required.

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