Exp Oncol 2018
40, 3, 205–210

Experimental ONCOLOGY

INFLUENCE OF METFORMIN, SODIUM DICHLOROACETATE AND THEIR COMBINATION ON THE HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF RATS WITH GLIOMAS C6

I.V. Prokhorova, O.N. Pyaskovskaya, D.L. Kolesnik, G.I. Solyanik
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine

***Background*: The efficacy of antimetabolic therapy of malignant neoplasms could not be explained solely by the direct mechanisms of action of such energy metabolism inhibitors as sodium dichloroacetate (DCA) and metformin (MTF). The indirect effects of DCA and MTF on the organs and tissues, which could play significant role in the antitumor activity of these agents, have not been thoroughly explored. *Aim*: To investigate the effect of MTF, DCA and their combination on the survival of rats with C6 glioma and major haematological and biochemical blood parameters. *Materials and Methods*: DCA and MTF were administered orally to inbred female rats for 11 days starting from the second day after tumor cell transplantation at a total dose of 1.1 and 2.6 g/kg, respectively. When combined treatment was used, MTF was administered 3 hours after the administration of DCA. The content of lactate and pyruvate in blood plasma was determined on the ChemWell® 2910 (Combi) automatic analyzer. Blood parameters were determined using the Particle Counter PCE-210 automatic hematology analyzer. *Results:* The administration of DCA did not significantly affect the life span of rats with C6 glioma. Duration of life of rats, which were administered with MTF only, was significantly higher (by 19.1%, $p < 0.01$). Combined administration of DCA + MTF prolonged life span of animals with glioma by 50% ($p < 0.001$). The positive result of antitumor activity of MTF alone and in combination with DCA correlated with a decrease in the mean platelet volume/platelet count (MPV/PLT) ratio by 75.0% ($p < 0.05$) compared with tumor control. In addition, the expressed antitumor effect of combination therapy with DCA and MTF was associated with a decrease ($p < 0.05$) in glucose and lactate levels in blood plasma of rats with C6 glioma by 10% and 41.4%, respectively, compared to tumor control. Analysis of blood parameters showed that the growth of C6 glioma was accompanied by the development of leukopenia, anemia and thrombocytopenia. The introduction of DCA caused the correction of manifestations of anemia and leukopenia, but did not affect the level of platelets in the blood of animals with glioma. MTF alone and in combination with DCA positively influenced the number of white blood cells and caused complete thrombocytopenia correction, increasing platelet count by more than 200% ($p < 0.001$). *Conclusion*: The ability of MTF either used alone or in combination with DCA to influence the development of C6 glioma which is manifested in an increase in the lifespan of rats has been revealed. The most pronounced antitumor effect was recorded against the background of the combined use of these agents, which may be due to their ability to lower the levels of lactate and glucose in the blood of tumor-bearing rats. It is proved that MTF both in monotherapy and in combination with DCA provides correction of anemia and thrombocytopenia, which arise at the background of glioma C6 growth.**

***Key Words*: sodium dichloroacetate, metformin, C6 glioma, hematological indices, lactate, glucose.**

In the last decade significant progress has been achieved in understanding the mechanisms of reprogramming the energy metabolism of malignant cells, and as a result, the efforts of many researchers are now aimed at developing a new direction of antitumor therapy — antimetabolic therapy. Therefore, due to high antitumor activity, such inhibitors of energy metabolism as sodium dichloroacetate (DCA) and metformin (MTF) have been widely studied by oncologists. Both agents have long been used extensively in clinical practice for the treatment of pathologies not related to cancer. For example, the safety and efficacy of DCA has been proven in the case of the correction of lactic acidosis, where DCA therapy effectively reduces the level of lactate in the blood by stimulating the pyruvate oxidation [1]. MTF is a hypoglycemic drug that is used as the first line of therapy for hyperglycemia and insulin resistance for more than 50 years. Recent studies have shown that the ability of MTF to lower blood glucose levels is due to numerous mechanisms, including the activation of 5'-AMP-activated protein kinase, reduction of cyclic AMP production, inhibition of 1st complex of the electron transport chain of mitochondria, and others [2].

The features of the energy metabolism of malignant cells associated with the intensification of glycolysis and the decrease in ATP production due to mitochondrial oxidation have attracted attention of scientists to the possibilities of using DCA and MTF in oncology as potentially simple and effective pharmacological agents for treatment of glycolytic malignant tumors. Numerous studies of these agents have shown their ability to exhibit antitumor properties in many experimental tumor and cell models [3–9]. However, high metabolic plasticity of malignant cells caused significant fluctuations in the effectiveness of antitumor action of these drugs administered in a monotherapy regimen. The most effective approach was the com-

Submitted: September 05, 2018.
*Correspondence: E-mail: oncom@online.ua
Abbreviations used: DCA – sodium dichloroacetate; GR – granulocytes; HCT – hematocrit; Hgb – hemoglobin; LY – lymphocytes; MCH – mean cell hemoglobin; MCHC – mean corpuscular hemoglobin concentration; MCV – mean cell volume; MO – monocytes; MPV – mean platelet volume; MTF – metformin; PCT – plateletcrit; PDW – platelet distribution width; PLT – platelet; RBC – red blood cells; WBC – white blood cells.

bined use of several inhibitors acting on different chains of energy metabolism [10–12].

It should be noted that the analysis of the results of experimental studies suggests that the antitumor efficacy of DCA and MTF in *in vivo* experiments is significantly higher than their cytotoxic/cytostatic effects on malignant cells *in vitro*. This is due to the fact that the reprogramming of the energy metabolism of malignant cells significantly depends on their microenvironment, which is characterized by a metabolic deficiency of the main energy substrates due to inadequate vascularization of malignant tumors [13]. The presence or absence of humoral factors and nutrient substrates in tumor cells microenvironment may also affect the response of these cells to DCA- and MTF-induced reprogramming of energy metabolism. Moreover, DCA and MTF may cause a decrease in the level of such circulating substrates important for tumor cell survival as glucose and lactate, not due to direct action on tumor cells, but indirectly through the influence on normal organs and systems of the body. Unfortunately, the study of the influence of these agents on the hematological parameters of animals with tumors and the possible relation with their antitumor activity were practically not carried out.

That is why the aim of this work was to study the effect of MTF, DCA and their combination on the survival of rats with C6 glioma and basic animal blood parameters.

MATERIALS AND METHODS

The studies were carried out on inbred female rats 2.5–3 months old weighing 100-130 g from the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine (NASU), Kyiv, Ukraine. The research on animals was carried out in accordance with the provisions of the General Ethical Principles of Animal Experiments adopted by the First Congress on Bioethics (Kyiv, 2001) and international requirements in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1986).

As an experimental tumor model, glioma C6 cell line was used, which was obtained from the National Bank of Cell Lines and Tumor Strains of IEPOR NASU. C6 glioma cells were cultured *in vitro* in DMEM with 10% fetal calf serum (Sigma, USA), 2 mM L-glutamine and 40 mg/ml gentamicin at 37 °C in humidified atmosphere with 5% CO_2.

Transfusion of glioma C6 cells was carried out under general anesthesia by intracerebral inoculation of $0.6 \cdot 10^6$ cells in 0.05 ml of physiological saline in the left parietal zone.

After transplantation, the rats were randomized by weight and distributed into 4 groups: rats, which were administered only DCA in a total dose of 1.1 g/kg (n = 12); rats, which were administered with MTF only in a total dose of 2.6 g/kg (n = 12); rats administered with both MTF and DCA (n = 13); rats, which were administered with water only (control, n = 12). As intact controls, healthy animals were used, the age, gender and weight of which fully corresponded to the experimental rats at the start of the research. In combination therapy, the aqueous solution of MTF was administered 3 h after administration of DCA in the total doses of 2.6 and 1.1 g/kg, respectively. The doses of all pharmacologic agents studied corresponded to the range of therapeutic doses for rats and were lower than the maximum tolerated dose [14].

The agents were dissolved in water and administered orally via probe n a volume of 2 ml/animal, once daily, starting from the second day after inoculation of tumor cells. In total 11 administratons were made.

In order to assess the antitumor effect of the agents under study with respect to the C6 glioma, the average life span of animals in each experimental group was assessed.

Research of the main blood indices of rats with C6 glioma after treatment with DCA, MTF and their combination was carried out at the 13th day after inoculation of tumor cells. To determine the content of lactate and glucose, peripheral blood was collected in heparinized tubes, followed by centrifugation at 3000 rpm, and stored at –20 °C until further investigation on a full automatic ChemWell® 2910 (Combi) analyzer. Determination of hemoglobin (Hgb) concentration, hematocrit (HCT) values, erythrocyte, leukocytes, and platelets (PLT) counts, and the calculation of erythrocytic and PLT indices in the whole blood of rats collected in EDTA tubes, was performed using an automatic hematology analyzer Particle Counter PCE-210.

Statistical analysis of the data was performed using descriptive methods, correlation analysis, nonlinear regression analysis, Students t-criterion, Mann — Whitney U-criterion using Microsoft Excel, Statistic and Microcal Origin software. The data are presented as M ± m, where M is the mean value, m is the standard deviation.

RESULTS AND DISCUSSION

The results of the study showed that, after inoculation of glioma C6 cells in, a brain tumor developed in 100% of rats, which (in the absence of therapy) caused the death of all animals on average in 15–16 days. Analysis of blood parameters showed that growth of C6 glioma was accompanied by the development of leukopenia, anemia and thrombocytopenia, whose manifestations can affect the life expectancy of rats. Thus, on the 13th day after transplantation of tumor cells in the blood of rats, there was registered more than twofold ($p < 0.05$) reduction of the total leukocytes (white blood cells — WBC) counts and, respectively, counts of lymphocytes (LY), monocytes (MO) and granulocytes (GR) compared to the corresponding indices in intact animals (Table 1).

Table 1. Leukogram of the rats with glioma C6 after therapy with MTF, DCA and their combination

Blood indices	Intact rats	Tumor control	Type of therapy		
			DCA	MTF	DCA+MTF
WBC ($\cdot 10^3$/μl)	10.6 ± 1.2	4.8 ± 1.3#	7.7 ± 3.0	9.3 ± 0.3*	6.7 ± 1.2#
LY ($\cdot 10^3$/μl)	7.4 ± 0.7	3.4 ± 1.1#	5.5 ± 2.1	6.2 ± 0.2*	4.5 ± 1.0#
MO ($\cdot 10^3$/μl)	1.3 ± 0.1	0.5 ± 0.1#	0.9 ± 0.4	1.2 ± 0.1*	0.8 ± 0.1#
GR ($\cdot 10^3$/μl)	1.9 ± 0.4	0.9 ± 0.2#	1.6 ± 0.6	2.0 ± 0.1*	1.4 ± 0.3

Note: *$p < 0.05$ as compared to tumor control; #$p < 0.05$ as compared to intact control.

The development of anemia in rats with C6 glioma was confirmed by a significant ($p < 0.05$) decrease in the number of red blood cells (RBC) by 15.5%, Hgb levels by 9,6% and HCT by 8,6% compared with those for healthy animals. At the same time, the type of anemia had the character of macrocytic, since mean cell volume (MCV), an index of an average volume of erythrocytes, was significantly higher in the blood of tumor-bearing rats than in intact controls (by 7.8%, $p < 0,05$).

However, this did not affect the RBC indices associated with MCV, i.e. mean cell hemoglobin (MCH; the Hgb content per erythrocyte) and mean corpuscular hemoglobin concentration (MCHC; the Hgb content per volume unit of one erythrocyte), since they were within the normal range (Table 2).

In addition to the registered leukopenia and anemia, the growth of C6 glioma was characterized by the development of severe thrombocytopenia, as shown by a significant decrease ($p < 0.01$) in the counts of PLT by 62,7% and plateletcrit (PCT) by 50,0% (Table 2) as a result of excessive destruction of these cells [15]. Moreover, there is a highly heterogeneous PLT population in the blood of rats with a tumor, indicated by a significant increase in their mean volume (MPV) by 27,0% ($p < 0.05$) and an increase in the distribution width (PDW). This phenomenon occurs in the case of intense production of young PLT and their release in the blood from the bone marrow, which actively compensates their destruction [16].

Influence of MTF, DCA and their combination on survival of rats with C6 glioma and blood indices of animals. It was found that due to the 11-day-long therapy with DCA, MTF and their combination, the positive antitumor effect on C6 glioma was recorded in the groups of rats receiving MTF alone or a combination of MTF and DCA. While DCA therapy did not affect the life span of rats with C6 glioma, the introduction of MTF statistically significantly lengthened the life span of rats with tumors by 19.1% ($p < 0.01$), and the combined administration of the agents gave a significant antigliomic effect and prolongation of life of the animals by 50% ($p < 0.001$). It should be noted that the high effectiveness of antitumor combined therapy with these agents was most likely due to synergistic effects of MTF and DCA on the development of C6 glioma (Fig. 1).

In the absence of antitumor activity of DCA against C6 glioma, the administration of this agent caused a positive effect on some indices of both red and white cell lineages of peripheral blood in rats.

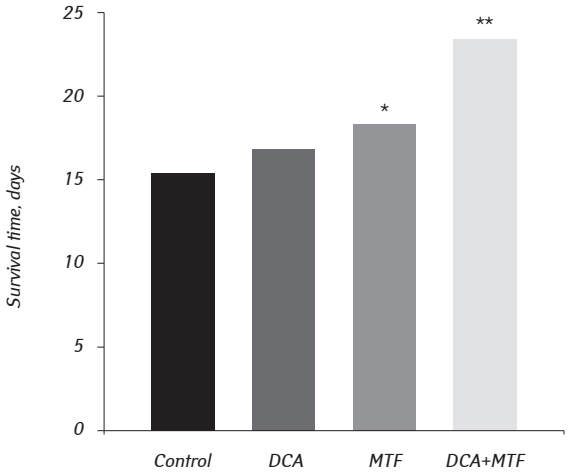

Fig. 1. The average life expectancy of the glioma C6 bearing rats in the groups treated with DCA, MTF or their combination. *$p < 0.01$ as compared to control; **$p < 0.001$ as compared to control

Analysis of hemogram data after DCA therapy indicated that this agent has the ability to correct the manifestations of anemia in rats with C6 glioma. That is, the levels of RBC, Hgb and HCT in the blood of the experimental group of animals were within the normal limits (Table 2), which indicated the normal functional ability of erythrocytes to carry oxygen. A low, however, significant ($p < 0.05$) increase in the MCV index by 4.7% in the blood of tumor-bearing animals after treatment with DCA may be due not only to the glioma development but also to the direct effect of DCA, since intake of this agent may be accompanied by the deficiency of vitamins of group B in the body, which manifests itself in reducing the proportion of RBC against the background of their increased volume.

There was also recorded a marked tendency to increase of the total level of leukocytes in rats after administration of DCA practically to the level of this in intact animals (Table 1). The absence of statistically significant results is due to the large range of deviations characteristic of the studied parameters in the group of animals administered with DCA.

The study of PLT counts and PLT indices showed that DCA did not affect the development of thrombocytopenia in rats with transplanted tumors. Despite the lack of significant changes in HCT, the level of PLT in the

Table 2. Hemogram of the rats with glioma C6 after therapy with MTF, DCA and their combination

Blood indices	Intact rats	Tumor control	Type of therapy		
			DCA	MTF	DCA + MTF
RBC, $\cdot 10^6$/μl	7.1 ± 0.1	6.0 ± 0.1#	6.8 ± 0.3*	6.6 ± 0.4	7.1 ± 0.6*
Hgb, g/dl	12.5 ± 0.2	11.3 ± 0.4#	11.9 ± 0.8	12.0 ± 0.3	12.1 ± 0.8
HCT, %	45.4 ± 0.9	41.5 ± 1.4#	45.3 ± 2.4	46.4 ± 1.4	47.2 ± 2.8*
MCV, fl	63.9 ± 0.7	68.9 ± 1.2	66.9 ± 0.8	70.2 ± 2.1	67.9 ± 1.0
MCH, pg	17.6 ± 0.3	18.8 ± 0.3	17.6 ± 0.5	18.1 ± 0.9	17.1 ± 0.3*
MCHC, g/dl	27.5 ± 0.2	27.2 ± 0.3	26.2 ± 0.5*	25.8 ± 0.4#,*	25.6 ± 0.3*
RDW, %	17.9 ± 0.3	18.2 ± 0.5	18.3 ± 0.7	18.2 ± 0.3	20.5 ± 1.6
PLT, $\cdot 10^3$/μl	311.0 ± 41.0	116.0 ± 54.4#	182.3 ± 49.7#	381.7 ± 12.6**	357.3 ± 57.5*
PCT, %	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0*	0.3 ± 0.0*
MPV, fl	7.4 ± 0.3	9.4 ± 0.9	9.2 ± 0.4#	7.8 ± 0.2	7.8 ± 0.4
PDW, fl	27.1 ± 2.3	27.7 ± 1.4	29.5 ± 0.2	29.1 ± 0.1	28.5 ± 0.9

Note: *$p < 0.05$ as compared to tumor control; **$p < 0.001$ as compared to tumor control; #$p < 0.05$ as compared to intact control.

blood of rats with C6 glioma after action of DCA was almost at the level of tumor control and was lower than that in intact controls by 41.4% ($p < 0.05$). As can be seen from Table 2, the MPV level in the studied animals groups was higher than in intact control by 24.3% ($p < 0.05$), and PDW tended to increase, indicating a predominance of young PLT in the blood of this group of rats at the background of destruction of mature ones.

In contrast to DCA, the introduction of MTF caused a complete normalization of blood leucogram in the animals. As a result of 11 day-long treatment with MTF, the level of WBC and, respectively, LY, MO and GR did not differ from those in intact animals and significantly ($p < 0.05$) exceeded the corresponding tumor control parameters by 93.8; 82.4; 140.0 and 122.2% (Table 1). The obtained data confirm the ability of MTF to normalize the immune system, as previously demonstrated in the study on the role of the immune system in the mechanisms of the therapeutic effect of MTF in patients with type II diabetes mellitus [17].

The most significant positive changes after MTF therapy were recorded in the indicators of the PLT branch of hematopoiesis. As can be seen from Table 2, PLT count significantly increased by 230% ($p < 0.001$) compared with tumor control, reaching the level of this index in the blood of intact rats. At the same time, the MTF had a weak but positive effect on the erythrocytic branch of hematopoiesis: levels of RBC, Hgb, HCT, RDW and MCH did not differ from the corresponding indices of intact rats. An insignificant increase in the volume of erythrocytes in the blood of investigated animals may be due to the fact that MTF has the ability to accumulate in RBC, thereby playing the role of depot of MTF in the body [18–20].

It was shown that due to the combined therapy with inhibitors of energy metabolism, MTF and DCA, the level of WBC in the blood of rats with glioma somewhat increased (by 36.8%, $p < 0.05$) in comparison with the control group but still was significantly lower than the corresponding index in healthy animals, mainly due to the significant ($p < 0.05$) decrease of the LY level by 39.1% (Table 1).

The analysis of the hemogram (Table 2) showed that the combined therapy with MTF and DCA provided the correction of anemia and thrombocytopenia more likely due to the direct effect of MTF on these blood indices. The latter is evidenced by the absence of significant differences between hemograms of rats treated with MTF or combined therapy.

As it is known, under physiological conditions, the PLT count in the blood is in equilibrium, which is supported by a constant ratio of regeneration and elimination. At the same time, the thrombocrit value, reflecting the percentage of PLT volume in the total volume of blood, is directly related to the number of PLT, whereas the MPV index is inversely proportional to these values [21–23]. MPV is considered a good marker for differential diagnosis of thrombocytopenia. An increase in this index is observed in a hyperdestructive form [24–26] and in the case of tumor disease it is supposed to be associated with a negative prognosis. It is worth noting that today the MPV/PLT ratio is considered as one of the markers of the effectiveness of antitumor therapy [27, 28]. At the same time, the decrease of this index to the level typical of healthy animals indicated the effectiveness of therapy. The analysis of the obtained results allowed to calculate the average value and find the range of values of the MPV/PLT ratio for healthy animals, animals with C6 glioma and tumor-bearing rats treated with MTF, DCA and their combination.

It was shown, that the growth of C6 glioma is characterized by the statistically signfcant increase of MPV/PLTs ratio by 300% compared with the corresponding indices of intact control (Table 3). The use of MTF and MTF in combination with DCA significantly reduced this index by 75.0% ($p < 0.05$) compared with tumor control, which correlated with the positive result of antitumor therapy. Despite the tendency to decrease of the MPV/PLT ratio in the animal group after DCA therapy, no significant changes were recorded for this index compared to tumor control, which correlated with the absence of antitumor efficacy of DCA therapy of C6 glioma.

Table 3. Influence of MTF, DCA and their combination on MPV/PLT ratio in the blood of glioma C6 bearing rats

	Intact control	Rats with glioma C6			
		Control	DCA	MTF	DCA+MTF
MPV/PLT	0.02	0.08#	0.05	0.02*	0.02*
Range of (MPV/PLT)	0.02–0.029	0.05–0.167	0.037–0.072	0.019–0.027	0.019–0.021

Note: *$p < 0.05$ as compared to tumor control; #$p < 0.05$ as compared to intact rats.

It is known that brain tumors are characterized by intense glycolysis and are highly dependent on the circulating glucose [29]. Therefore, the influence of DCA and MTF combinaton on the level of lactate and glucose may contribute to their expressed anti-glioma effect. The main antitumor effect of DCA is believed to be realized due to its ability to activate oxidative phosphorylation in tumor cells and depolarize the mitochondrial membrane, which causes increased production of active oxygen species, and, as a consequence, induction of apoptosis [30]. On the other hand, MTF is a hypoglycemic agent widely used in the treatment of type II diabetes mellitus in order to reduce blood glucose levels. In addition, it is an inhibitor of the Ist complex of the electron transport chain of mitochondria, and at the initial respiratory stage, it is able to "shut off" the Krebs cycle.

As can be seen from Fig. 2, the growth of C6 glioma was accompanied by a statistically significant decrease in glucose level in peripheral blood of animals by 22% ($p < 0.05$), which was most likely due to the high need of glycolytic tumor in the level of this energy substrate. Interestingly, after administration of either DCA, or MTF, glucose levels in rat blood increased almost to the level of intact animals.

In the case of DCA, this may be due to the intensification of oxidative phosphorylation and inhibition of glycolysis in the cells of the body by redirecting py-

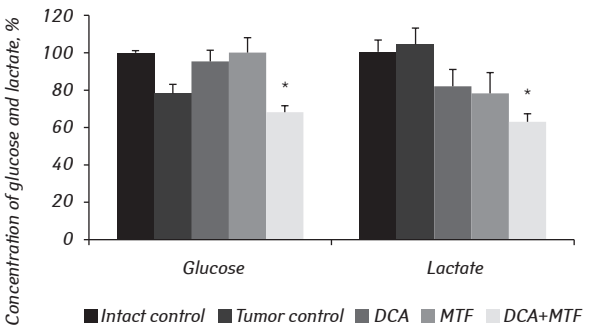

Fig. 2. Influence of MTF, DCA and their combination on glucose and lactate levels in the blood of glioma C6 bearing rats. *$p < 0.05$ as compared to tumor control

ruvate to the Krebs cycle. In favor of this evidenced the decrease in the level of lactate in the peripheral blood of rats after the introduction of DCA. In the case of MTF, an increase in glucose levels in rat blood can occur due to effective anti-glioma activity, and as a consequence of reducing the need of tumor in glucose. The most pronounced changes in both glucose and lactate levels were observed in rats after combined therapy with DCA and MTF. Decrease of glucose level by 10% ($p < 0.05$) in comparison with this index of tumor control can be directly related to the high antitumor effect of combined therapy. However, a significant decrease in the level of lactate by 41.4% ($p < 0.05$) indicated the ability of this combination of agents to significantly reduce the intensity of glycolysis, a factor that can make a significant contribution to the antitumor activity of combination therapy. The question — in which cells (normal or tumor ones) DCA and MTF are capable to significantly inhibit glycolysis, remains open and requires additional research.

CONCLUSION

Thus, this work demonstrated the ability of MTF either used alone or in combination with DCA to influence the development of C6 glioma which is manifested in an increase in the lifespan of rats. The most pronounced antitumor activity was recorded against the background of the combined use of these inhibitors of energy metabolism, which was shown to lengthen the life span of rats with C6 glioma by 50% ($p < 0.001$). In this case, the expressed anti-glioma effect of MTF in combination with DCA may be related to the ability of these agents to reduce the levels of lactate and glucose in the blood of tumor-bearing rats. It is proved that MTF, used as monotherapy or in combination with DCA, provided the correction of anemia and thrombocytopenia developing at the background of C6 glioma growth.

REFERENCES

1. Stacpoole PW, Harman EM, Curry SH, ***et al.*** Treatment of lactic acidosis with dichloroacetate. N Engl J Med 1983; 3**09**: 390–6.

2. Li M, Li X, Zhang H, ***et al.*** Molecular mechanisms of metformin for diabetes and cancer treatment. Front Physiol 2018; **9**: 1039.

3. Seliger C, Luber C, Gerken M, ***et al.*** Use of metformin and survival of patients with high-grade glioma. Int J Cancer 2018. doi: 10.1002/ijc.31783.

4. Sharma P, Kumar S. Metformin inhibits human breast cancer cell growth by promoting apoptosis via a ROS-independent pathway involving mitochondrial dysfunction: pivotal role of superoxide dismutase (SOD). Cell Oncol (Dordr) 2018; doi: 10.1007/s13402-018-0398-0.

5. Fedorchuk AG, Pyaskovskaya ON, Gorbik GV, ***et al.*** Effectiveness of sodium dichloroacetate against glioma C6 depends on administration schedule and dosage. Exp Oncol 2016; **38**: 80–3.

6. Kolesnik DL, Pyaskovskaya ON, Bojchuk IV, ***et al.*** Effect of dichloroacetate on Lewis lung carcinoma growth and metastasis. Exp Oncol 2015; **37**: 126–9.

7. Pereira FV, Melo ACL, Low JS, ***et al***. Metformin exerts antitumor activity via induction of multiple death pathways in tumor cells and activation of a protective immune response. Oncotarget 2018; **9**: 25808–25.

8. Tang D, Xu L, Zhang M, ***et al.*** Metformin facilitates BG45 induced apoptosis via an anti Warburg effect in cholangiocarcinoma cells. Oncol Rep 2018; **39**: 1957–65.

9. Zi F, Zi H, Li Y, ***et al***. Metformin and cancer: An existing drug for cancer prevention and therapy. Oncol Lett 2018; **15**: 683–90.

10. Lu X, Zhou D, Hou B, ***et al.*** Dichloroacetate enhances the antitumor efficacy of chemotherapeutic agents via inhibiting autophagy in non-small-cell lung cancer. Cancer Manag Res 2018; **10**: 1231–41.

11. Zajac J, Kostrhunova H, Novohradsky V, ***et al.*** Potentiation of mitochondrial dysfunction in tumor cells by conjugates of metabolic modulator dichloroacetate with a Pt (IV) derivative of oxaliplatin. J Inorg Biochem 2016; **156**: 89–97.

12. Pyaskovskaya ON, Bojchuk IV, Fedorchuk AG, ***et al.*** Aconitine-containing agent enhances antitumor activity of dichloroacetate against Ehrlich carcinoma. Exp Oncol 2015; **37**: 192–6.

13. Nyberg P, Salo T, Kalluri R. Tumor microenvironment and angiogenesis. Front Biosci 2008; **13**: 6537–53.

14. Quaile MP, Melich DH, Jordan HL, ***et al.*** Toxicity and toxicokinetics of metformin in rats. Toxicol Appl Pharmacol 2010; **243**: 340–7.

15. Zhang S, Cui YL, Diao MY, ***et al.*** Use of platelet indices for determining illness severity and predicting prognosis in critically ill patients. Chin Med J 2015; **128**: 2012–8.

16. Sachdev R, Tiwari AK, Goel S, ***et al.*** Establishing biological reference intervals for novel platelet parameters (immature platelet fraction, high immature platelet fraction, platelet distribution width, platelet large cell ratio, platelet-X, plateletcrit, and platelet distribution width) and their correlations among each other. Indian J Pathol Microbio 2014; **57**: 231–5.

17. Zak KP, Orlenko VL, Popova VV, ***et al.*** The role of the immune system in mechanism of metformin therapeutic effect in patientswith type 2 diabetes. I E J 2017; **13**: 340–6.

18. Tucker GT, Casey C, Phillips PJ, ***et al.*** Metformin kinetics in healthy subjects and in patients with diabetes mellitus. Br J Clin Pharmacol 1981; **12**: 235–46.

19. Lalau JD, Lacroix C. Measurement of metformin concentration in erythrocytes: clinical implications. Diabetes Obes Metab 2003; **5**: 93–8.

20. Wilcock C, Bailey CJ. Accumulation of metformin by tissues of the normal and diabetic mouse. Xenobio 1994; **24**: 49–57.

21. Bessman JD, Williams LJ, Gilmer PR Jr. Mean platelet volume. The inverse relation of platelet size and count in normal subjects, and an artifact of other particles. Am J Clin Pathol 1981; **76**: 289–93.

22. Giles C. The platelet count and mean platelet volume. Br J Haematol 1981; **48**: 31–7.

23. Levin J, Bessman JD. The inverse relation between platelet volume and platelet number. Abnormalities in hematologic disease and evidence that platelet size does not correlate with platelet age. J Lab Clin Med 1983; **101**: 295–307.

24. Bowles KM, Cooke LJ, Richards EM, ***et al.*** Platelet size has diagnostic predictive value in patients with thrombocytopenia. Clin Lab Haematol 2005; **27**: 370–3.

25. Kaito K, Otsubo H, Usui N, ***et al.*** Platelet size deviation width, platelet large cell ratio, and mean platelet volume have sufficient sensitivity and specificity in the diagnosis of immune thrombocytopenia. Br J Haematol 2005; **128**: 698–702.

26. Ntaios G, Papadopoulos A, Chatzinikolaou A, ***et al.*** Increased values of mean platelet volume and platelet size deviation width may provide a safe positive diagnosis of idiopathic thrombocytopenic purpura. Acta Haematol 2008; **119**: 173–7.

27. Xue G, Lixia Z, Daheng Y, ***et al***. Mean platelet volume/platelet count ratio is associated with prognosis in patients with pancreatic cancer. Int J Clin Exp Med 2016; **9**: 16379–86.

28. Inagaki N, Kibata K, Tamaki T, ***et al***. Prognostic impact of the mean platelet volume/platelet count ratio in terms of survival in advanced non-small cell lung cancer. Lung Cancer 2014; **83**: 97–101.

29. Schwartzbaum J, Edlinger M, Zigmont V, ***et al.*** Associations between prediagnostic blood glucose levels, diabetes, and glioma. Sci Rep 2017; **7**: 1436.

30. Stockwin LH, Yu SX, Borgel S, ***et al.*** Sodium dichloroacetate selectively targets cells with defects in the mitochondrial ETC. Int J Cancer 2010; **127**: 2510–9.