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COMPARATIVE STUDY OF SODIUM OXAMATE AND METFORMIN CYTOTOXICITY AGAINST LEWIS LUNG CARCINOMA CELLS UNDER ANCHORAGE-INDEPENDENT GROWTH

Background. The effect of the inhibitors of glycolysis and oxidative phosphorylation on the altered metabolism of neoplasms is considered a promising method of antitumor therapy. However, most studies on the antimetastatic activity of such inhibitors focus on analyzing their effect on the migratory and invasive characteristics of cells. Meanwhile, the survival of circulating metastatic cells and their resistance to anoikis are critically important factors in metastasis. **Aim.** To carry out a comparative study of sodium oxamate (SOX) and metformin (MTF) effects on the survival, proliferative activity, and metabolic plasticity of the low-metastatic variant of Lewis lung carcinoma (LLC/R9) cells under their anchorage-independent growth. **Materials and Methods.** Cell death, apoptosis, cell cycle distribution, reactive oxygen species (ROS) production, glucose and lactate levels, and vimentin expression in LLC/R9 cells under their anchorage-independent growth were assessed following SOX and MTF treatments. **Results.** The cytotoxicity of inhibitors was manifested in a significant decrease in the number of viable LLC/R9 cells and an increase in the number of dead and apoptotic cells, the effects being more pronounced for MTF. In the case of SOX treatment, a correlation was observed between an increase in the percentage of apoptotic cells and ROS level and a decrease in the glucose consumption rate (GCR). MTF increased GCR and the number of apoptotic cells, without changes in ROS levels. Incubation with MTF resulted in a significant twofold increase in the percentage of cells in the S phase due to a decrease in the fraction of cells in the G1/G0 and G2/M phases of the cell cycle. **Conclusions.** Unlike SOX, the cytotoxic effect of MTF on de-adhesive cells was directly related to disrupting energy homeostasis and cell cycle regulation rather than by oxidative stress. Their combined application could potentially reinforce metabolic stress in circulating tumor cells, simultaneously weakening glycolytic and oxidative compensatory pathways, thereby limiting metastatic competence.

Keywords: metastatic cells, anchorage-independent growth, sodium oxamate, metformin, cytotoxicity.

Among many alterations in metabolic pathways observed in tumor cells, the enhanced aerobic glycolysis (known as the Warburg effect) plays a key role [1]. In normal cells, the end-products of glucose metabolism are predominantly carbon dioxide and water formed through oxidative phosphorylation (OXPHOS) by redirection of pyruvate metabolism to the mitochondria. In contrast, in the invasive cancer

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cells, lactate is the predominant end product of glucose metabolism even in the presence of sufficient oxygen. This seemingly inefficient trait of tumor cells provides them with a survival and proliferative advantage, making them resistant to hypoxic conditions. Furthermore, although glycolysis is energetically less efficient than OXPHOS, the energy production rate by glycolysis is approximately two orders of magnitude higher, allowing for rapidly generating energy in the presence of glucose. In addition, lactate is not merely a byproduct of glycolysis; rather, it actively contributes to cell migration, angiogenesis, invasion, and metastasis.

The role of altered metabolism in tumor pathogenesis, progression, and resistance to various treatments has attracted increasing attention in recent years, and its targeting is considered a safe and highly effective cancer treatment option [2]. Over the past decade, numerous studies have investigated the potential antitumor activity of a wide range of glycolysis and OXPHOS inhibitors [3]. Considerable attention has been given to the glycolysis inhibitor sodium oxamate (SOX) and the OXPHOS inhibitor metformin (MTF).

SOX is a non-specific inhibitor of lactate dehydrogenase (LDH), which is considered one of the most promising therapeutic targets for anticancer therapy [4—6]. Numerous studies have shown that most malignant tumors are characterized by an aberrantly high LDH expression, which significantly enhances tumor cells survival and their proliferative potential [7, 8]. SOX (molecular formula $C_2H_2NO_3$) is an isosteric analog of pyruvate, which competes with pyruvate to inhibit LDH by forming an inactive complex with the enzyme. Additionally, SOX can inhibit aspartate aminotransferase (AAT), which, in tandem with malate dehydrogenase, forms a malate–aspartate NADH shuttle. This shuttle is essential for transferring electrons from cytoplasmic NADH, produced during glycolysis, to mitochondrial NADH and creating a proton gradient required for oxidative phosphorylation [9].

Inhibitors of oxidative phosphorylation, such as MTF, are also considered promising anticancer agents. MTF is an anti-diabetic drug used to treat type 2 diabetes and belongs to the biguanidine class [10]. It has drawn considerable attention of cancer researchers due to its ability to inhibit the proliferation of a wide range of malignant cells both in vitro and in vivo, either alone or in combination with traditional anticancer drugs. The main mechanisms of MTF antitumor activity include its effects on energy metabolism, cell growth, proliferation, angiogenesis, and programmed cell death.

It is worth noting that the limited number of studies on the antimetastatic activity of energy metabolism inhibitors has mainly focused on their effects on the migratory and invasive properties of malignant cells [11—13]. However, the survival of the circulating metastatic cells fraction (and their resistance to anoikis) is considered one of the most critical factors in the metastasis. Therefore, the aim of this study was to conduct a comparative analysis of the effects of energy metabolism inhibitors (SOX and MTF) on the survival, proliferative activity, and metabolic plasticity of Lewis lung carcinoma (LLC) cells under anchorage-independent growth conditions (a model of circulating metastatic cells in vitro).

Materials and Methods

Cell line. In the study, we used a low-metastatic variant of Lewis lung carcinoma (LLC/R9) cells. This LLC variant was generated as described earlier [14]. Cells were maintained in vitro at 37 °C in a humidified atmosphere with 5% CO_2 in RPMI-1640 medium containing 10% fetal bovine serum (Biowest, France), 40 μg/mL gentamicin, and 2 mM L-glutamine.

Effects of SOX and MTF on de-adhesive LLC/R9 cells. LLC/R9 cells were seeded into 96-well plates (0.012×10^6 cells per well in 0.1 mL RPMI-1640 medium) or Petri dishes (0.6×10^6 cells for control and 1.2×10^6 cells for experiments) for assessment of the percentage of dead cells, apoptosis level, cell cycle phase distribution, intracellular ROS, glucose and lactate levels, and vimentin and E-cadherin expression. The wells/plates were pretreated with a poly-HEMA solution to model anchorage-independent growth. After seeding, the cells were incubated under standard conditions for 18 h. Then, RPMI-1640 medium with SOX or MTF at various concentrations or only RPMI-1640 medium as control was added to each well, and incubation continued for 48 h. The cell viability was assessed by trypan blue exclusion and direct counting in a hemocytometer. Each concentration of the agents was tested in triplicate.

Cell survival analysis. After 48 h of incubation with SOX or MTF, the number of viable cells (N)

as a percentage of the control was assessed by the crystal violet (Sigma-Aldrich, USA) staining method with measurement of the absorbance on a Synergy HT plate reader (BioTek, USA) at 590 nm. The dose-dependent survival of tumor cells in response to SOX or MTF was analyzed using the exponential model:

$$N = N_R + N_S \times \exp\left(\frac{C - C_0}{\beta}\right), \quad (1)$$

where N_R is the number of cells (expressed as a percentage of the control) resistant to the action of SOX or MTF; N_S is the number of sensitive cells; C_0 is the highest non-cytotoxic concentration of the inhibitor; β is the sensitivity index of the cells to the cytotoxic action of the studied agents. The model parameters were estimated using a nonlinear regression.

Cell cycle distribution and apoptosis. Cell cycle phase distribution and apoptosis were assessed by flow cytometry. Cells were resuspended in hypotonic lysis buffer with propidium iodide and RNase A, and DNA content was analyzed using an FACS Calibur flow cytometer (Becton Dickinson, USA) with a 488 nm argon laser and a 582/42 nm filter. Data were analyzed using ModFit LT 3.0 (BDIS, USA). Apoptosis was determined by the percentage of cells with hypodiploid DNA.

Reactive oxygen species (ROS) levels. Intracellular ROS levels were assessed using 2',7'-dichlorofluorescein diacetate (DCFH-DA) and flow cytometry. Cells were washed twice with phosphate-buffered saline (pH 7.4), incubated in serum-free RPMI 1640 with DCFH-DA at 37 °C for 30 min, washed again, and analyzed using an FACS Calibur with a 488 nm laser and a 530/30 nm filter. 20,000 events per sample were analyzed, with nearly 100% cell staining (99.60%—99.95%). As a measure of the intensity of ROS production per cell, the GeoMean index was used, calculated using the software of the flow cytometer.

Glucose consumption and lactate production rates. Glucose and lactate concentrations in the incubation medium were measured using a Chem-Well 2910 automatic biochemical analyzer with commercial kits according to the manufacturer's instructions. Samples were collected quickly and stored at –20 °C.

Glucose consumption rate (GCR) and lactate production rate (LPR) were calculated as following:

$$GCR(\Delta t_i) = \frac{\left(C_{gl}(t_{i-1}) - C_{gl}(t_i)\right) \times 6 \times 10^{-3}}{N(t_i) \times (t_i - t_{i-1})} \quad (2)$$

$$LPR(\Delta t_i) = \frac{\left(C_l(t_i) - C_l(t_{i-1})\right) \times 6 \times 10^{-3}}{N(t_i) \times (t_i - t_{i-1})} \quad (3)$$

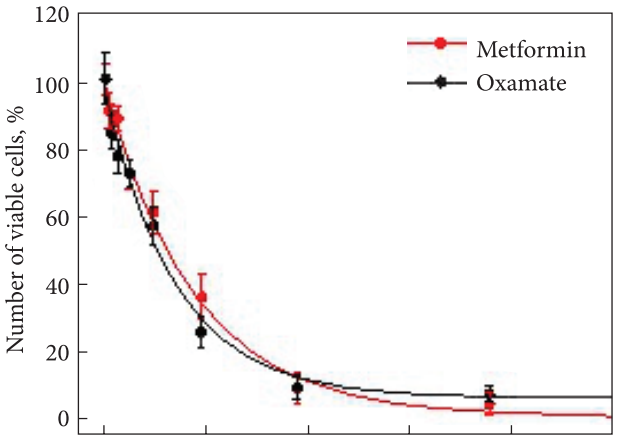
where $GCR(\Delta t_i)$ is the glucose consumption rate and $LPR(\Delta t_i)$ is the lactate production rate at time period from $t_{(i)}$ to $t_{(i-1)}$, μmol/(10^6 cells per day); $C_{gl}(t_i)$, $C_{gl}(t_{i-1})$, $C_l(t_i)$, and $C_l(t_{i-1})$ are concentrations of glucose and lactate (μmol/L) in incubation medium at time points $t_{(i)}$ and $t_{(i-1)}$ respectively, μmol/L; N_{ti} is the number of viable cells at time $t_{(i)}$; $\Delta t = t_{(i)} - t_{(i-1)}$.

Vimentin expression. The vimentin expression level of malignant cells was determined using antibodies to vimentin (ab176512; Abcam, UK). The cells were fixed with paraformaldehyde and then permeabilized in 0.5 mL of 0.1% Triton X-100 in PBS at room temperature for 30 min followed by washing them. After centrifugation at 1,500 rpm for 10 min, 100 μL of a solution of 10% FCS in PBS with antibodies to vimentin at a 1:500 dilution was added to the cell pellet for staining at room temperature for 30 min. After staining and washing with PBS, the cells were analyzed using a Navios EX flow cytometer (Beckman Coulter, USA) in the APC channel (660/20 nm BP light filter). The analysis of the obtained data was performed using the Navios EX tetra program.

Statistical analysis. Statistical analysis included descriptive statistics, Student's *t*-test, Mann — Whitney U-test, and nonlinear regression analysis. The data are presented as M ± SE (mean ± standard error).

Results

Cytotoxic effects of SOX and MTF on de-adhesive LLC/R9 cells. The results of the study on the effects of energy metabolism inhibitors SOX and MTF (in the concentration range from 0 to 150 mM) over 2 days on the viability of LLC/R9 cells under non-adherent growth conditions are presented in Fig. 1, *a*. As shown, increasing concentrations of the inhibitors cause a progressive exponential decrease in the number of viable cells. However, unlike MTF, at high concentrations of SOX, a portion of the cells remains viable, suggesting the presence of a subpopulation that is at least low-sensitive or



a

Model parameters	Oxamate	Metformin
N_R (%)	6.7 ± 2.8	0
N_S (%)	90.3 ± 4.5	97.3 ± 1.9
β (mM)	13.9 ± 2.0	17.6 ± 1.3
IC_{50} (mM)	9.9 ± 3.7	12.1 ± 1.4

b

Fig. 1. Number of viable de-adhesive LLC/R9 cells (in % of control values) as a function of SOX concentration (black symbols and lines) and MTF (red symbols and lines). Symbols — experimental data; (*a*) lines — the best fit of the mathematical model to the experimental data; (*b*) mathematical model parameters of SOX and MTF indexes cytotoxicity against LLC/R9 cells

resistant to the action of this inhibitor. This is confirmed by the results of data analysis on the dependence of LLC/R9 cell viability on SOX concentration using a mathematical model (Fig. 1, *b*) showing the presence of a sensitive cell subpopulation (N_S) and a resistant one (N_R). We have suggested such a heterogeneity of LLC/R9 cells treated with SOX under both adherent and non-adherent growth conditions [15]. However, after two days of incubation, the size of the SOX-resistant subpopulation is small and significantly lower than that observed after 1 day of incubation.

Of note is the similarity in the viability curves of de-adhesive cells in response to SOX and MTF during the 2-day incubation with the inhibitors, which is further supported by the absence of statistically significant differences in IC_{50} values for SOX and MTF (Fig. 1, *b*).

Effects of SOX and MTF on the viability, proliferative activity, and metabolic plasticity parameters of LLC/R9 cells under non-adherent growth conditions. The experiments were conducted after 2 days of incubation of de-adhesive LLC/R9 cells with SOX and MTF at a concentration of 6.5 mM. According to the cytotoxicity parameters of these inhibitors, the concentration of 6.5 mM results in approximately 65% survival of LLC/R9 cells.

The study demonstrated that after 48 h, both SOX and MTF exerted cytostatic/cytotoxic effects on LLC/R9 cells under anchorage-independent growth conditions (Fig. 2, *a*). The number of viable cells decreased by 35% ($p < 0.05$) after SOX treatment and by 48% ($p < 0.01$) after MTF exposure with corresponding increase in the number of dead cells (Fig. 2, *b*). The cytotoxic effect of both inhibitors also included a significant increase in the proportion of apoptotic cells — by 32% ($p < 0.05$) for SOX and 78% ($p < 0.01$) for MTF — compared to the control cells (Fig. 2, *c*). The increase in apoptotic cells following SOX exposure was associated with a significant rise in intracellular ROS levels by 56% ($p < 0.05$) (Fig. 2, *d*). It was accompanied by (or possibly caused by) a significant decrease in GCR and LPR, indicating glycolysis inhibition by SOX (Fig. 3, *c*, *d*).

In contrast to SOX, MTF did not affect the intracellular ROS levels, suggesting that the apoptotic death of de-adhesive LLC/R9 cells under MTF treatment was induced through alternative mechanisms (Fig. 2, *d*).

Noteworthy is the dramatic increase in glucose uptake by tumor cells upon MTF treatment, which leads to glucose depletion in the incubation medium—its concentration dropping to nearly zero after 48 h of MTF exposure (Fig. 3, *a*, *c*) without significant changes in lactate levels (Fig. 3, *b*, *d*).

In addition to their cytotoxic effects, the ability of SOX and MTF to exert cytostatic effects on de-adhesive LLC/R9 cells is also an important factor. Significant differences between the two inhibitors were observed in their influence on the cell cycle phase distribution (Fig. 4). As shown, SOX at the tested concentration did not alter the distribution of cells across the cell cycle phases (Fig. 4, *a*). In contrast, the incubation with MTF led to an almost twofold increase in the proportion of cells in the S phase ($p < 0.005$), due to a significant decrease in the proportions of cells in the G1/G0 ($p < 0.05$) and G2/M ($p < 0.05$) phases (Fig. 4, *b*). This marked that accumulation of cells in the S phase is likely due to cell cycle arrest resulting from DNA damage.

Effects of SOX and MTF on vimentin expression in LLC/R9 cells. Studies have shown that LLC/R9

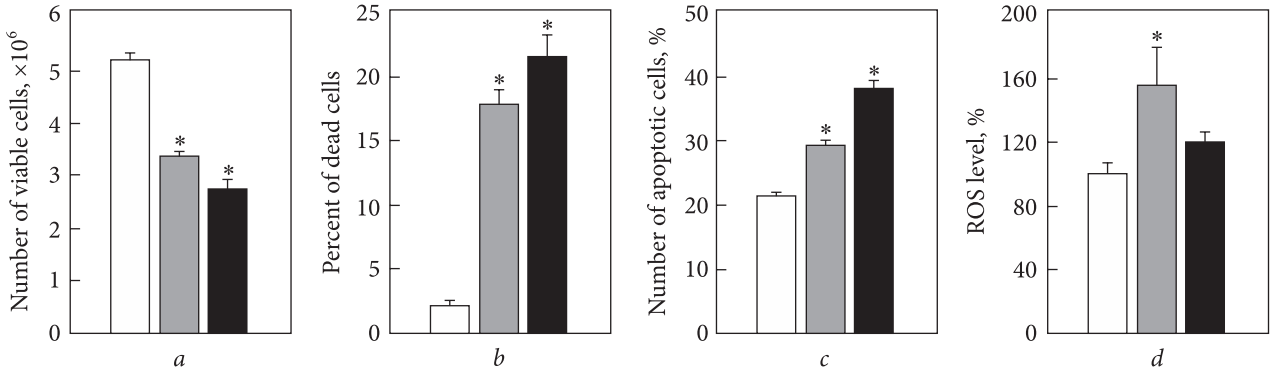


Fig. 2. Effect of SOX (gray bars) and MTF (black bars) on the number of viable de-adhesive LLC/R9 cells (*a*); percent of dead cells (*b*); percent of apoptotic cells (*c*); ROS levels in % of control value (*d*). White bars represent the corresponding values of cells without agents (control value). * the values differ significantly from the corresponding control (in Figs. 2—5)

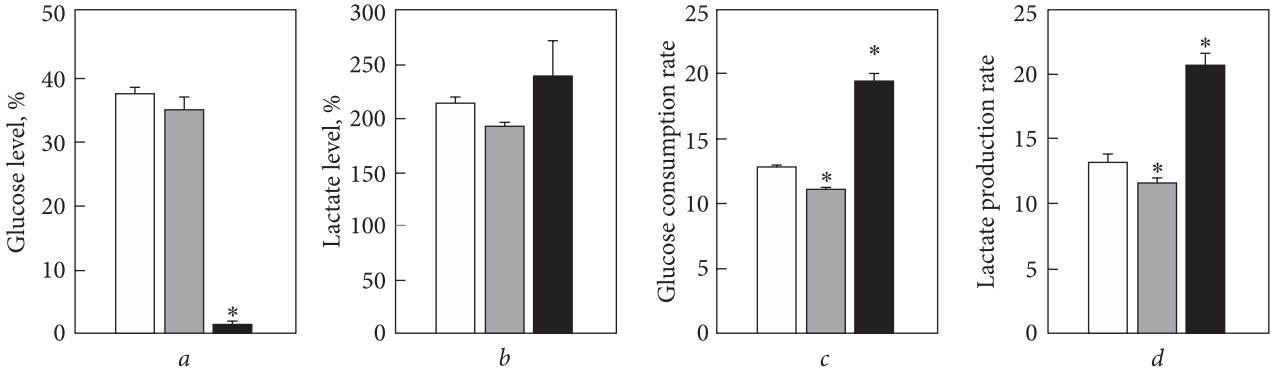


Fig. 3. Effect of SOX (gray bars) and MTF (black bars) on glucose (*a*) and lactate (*b*) level in incubation medium (in % of control value), glucose consumption rate (*c*), and lactate production rate (*d*). White bars represent the corresponding values of control cells

cells do not express E-cadherin, and the effects of SOX and MTF on its expression in these cells were insignificant (data not shown). At the same time, more than 50% of LLC/R9 cells (in the absence of energy metabolism inhibitors) demonstrated a sufficiently high level of vimentin expression.

The incubation with SOX resulted in a significant increase in the number of vimentin-expressing cells by 18%, but did not affect the intensity of expression (Fig. 5). In contrast to SOX, MTF caused a decrease in the vimentin expression level by approximately 55%, without affecting the number of vimentin-expressing cells (Fig. 5).

Discussion

Metabolic reprogramming is one of the most important hallmarks of cancer, and it continuously evolves during tumor progression, particularly in the context of drug resistance development and metastasis [16, 17]. Even though metastasis remains one of the main causes of cancer-related mortality, our current understanding of the mechanisms underlying metastasis and the development of effective antimetastatic therapies lag significantly behind what is known about primary tumor biology and pharmacological approaches for inhibiting its growth. This is largely due to the extremely complex and dynamic nature of the metastatic cascade, which is characterized by constant changes in the cellular microenvironment and requires metastatic cells to possess exceptional metabolic and energetic plasticity.

Virtually all invasive tumors, regardless of their histogenesis, are marked by a specific modification of energy metabolism — namely, the predominance of aerobic glycolysis over OXPHOS (the Warburg effect) [1]. This metabolic shift provides the survival advantages for tumor cells, as they can produce energy and maintain a high level of anabolic activity even under conditions of short-term or prolonged hypoxia [18]. Moreover, aerobic glycolysis ensures a high rate of synthesis of glycolytic intermediates, which serve as essential building blocks for rapidly proliferating cells.

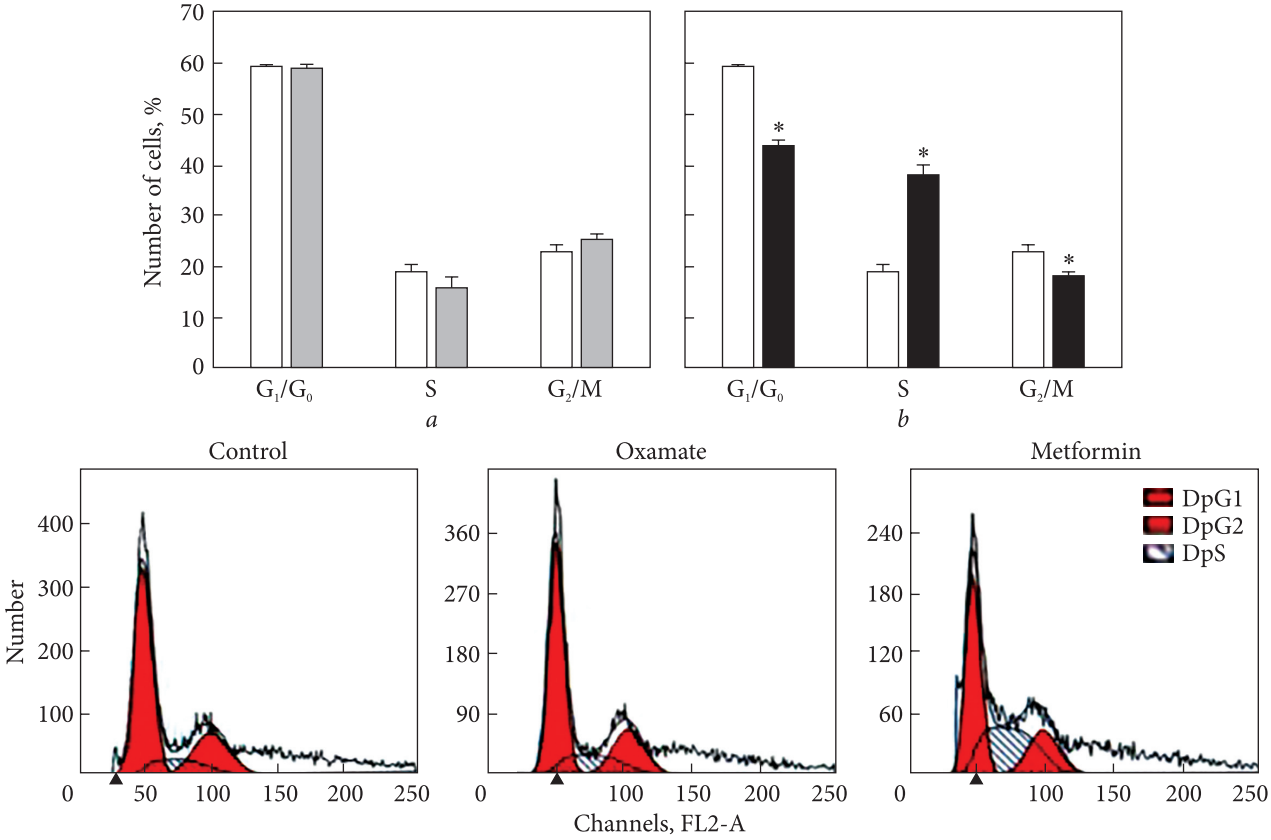


Fig. 4. Effect of SOX (gray bars) (*a*) and MTF (black bars) (*b*) on the cell cycle distribution of de-adhesive LLC/R9 cells. White bars represent the corresponding values of cells without agents (control value)

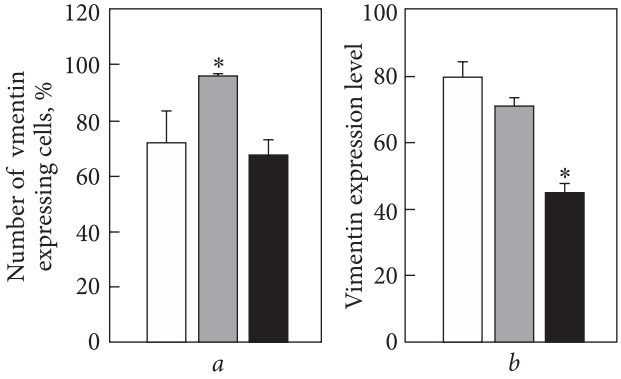


Fig. 5. Effect of SOX (gray bars) and MTF (black bars) on the number of vimentin-expressing cells (*a*) and the level of vimentin expression (*b*) in the de-adhesive LLC/R9 cells. White bars represent the corresponding values of cells without agents (control value)

This unique metabolic profile of tumor cells presents a range of ideal targets for therapeutic intervention aimed at eliminating cancer cells with minimal systemic toxicity [19].

Numerous studies on various glycolysis inhibitors (such as 2-deoxyglucose, SOX) and OXPHOS inhibitors (MTF, DCA, and others) have demonstrated their potential antitumor activity. However, the overwhelming majority of these studies focus on the effects of such inhibitors on the viability and proliferative activity of adherent tumor cells in vitro and on their ability to suppress primary tumor growth in vivo. Investigations into their ability to influence metastasis are typically limited to analyses of tumor cell migratory activity. The effects of inhibitors on the disseminated tumor cells—one of the key components of metastasis—have remained largely overlooked.

This is why our study focused primarily on evaluating the effects of the LDH inhibitor SOX and the OXPHOS inhibitor MTF on the survival and proliferative activity of metastatically active LLC cells under conditions of anchorage-independent growth (considered an experimental model of disseminated tumor cells).

Our results showed that both inhibitors exert a marked cytotoxic effect on de-adhesive LLC cells. However, their incubation with MTF led to a significantly greater reduction in the number of viable cells compared to SOX. The more pronounced inhibitory effect of MTF on the viability of de-adhesive cells correlated with a significantly higher proportion of apop-

totic cells (reflecting MTF cytotoxic action) and a pronounced S-phase arrest of cells, accompanied by a reduction in the proportions of the cells in the G1/G0 and G2/M phases (suggesting the cytostatic action of MTF), compared to the respective effects of SOX.

The difference in the effect of SOX and MTF on vimentin expression is also worth noting. The experimental studies have shown that the metastatic potential of non-small cell lung cancer is directly related to the expression of this mesenchymal marker [20]. In contrast to SOX, which did not affect the level of vimentin expression, MTF caused its significant decrease.

Studies have shown no correlation between intracellular ROS level changes and the proportion of apoptotic cells following MTF treatment. Unlike SOX, which significantly increased both ROS levels and the percentage of apoptotic cells, the increase in the latter under MTF treatment occurred without any change in the intracellular ROS levels.

According to the results of several studies, MTF appears to reduce excessive ROS production and limits early apoptosis—at least in normal cells—through activation of AMPK [21]. Meanwhile, in cancer cell lines, MTF has been shown to induce apoptosis, activate AMPK, and increase ROS levels in tumor cells [22]. It is known that ROS is produced either as a result of NADPH oxidase (NOX) activity or from the mitochondrial respiratory chain. Although MTF influences a broad spectrum of signaling pathways, one of its main mechanisms is believed to be the inhibition of NADH dehydrogenase (complex I of the mitochondrial electron transport chain), an effect associated with a sharp decrease in ATP synthesis and an increase in the mitochondrial reactive oxygen species (mtROS) production.

It should be noted that mitochondrial depolarization induced by MTF can simultaneously trigger apoptosis and activate AMPK via distinct mechanisms [23]. AMP-activated protein kinase (AMPK) is a major energy biosensor and metabolic switch that regulates a wide range of biosynthetic and catabolic pathways in the cell. Its activation supports cellular energy viability in conditions of impaired mitochondrial function by stimulating alternative ATP production mechanisms—primarily glycolysis—through increased glucose uptake via translocation of glucose transporters to the plasma membrane [24].

In our study, we observed an approximately 50% increase in glucose uptake by tumor cells following MTF treatment. This intensification of glycolysis led to complete exhaustion of the incubation medium, with glucose levels approaching zero after 2 days of MTF exposure. At the same time, the increase in lactate production rate (which correlated with increased glucose consumption) did not result in progressive lactate accumulation.

In our experiments, MTF-induced apoptosis in LLC/R9 cells under conditions of unchanged ROS levels may be attributed to drastic glucose deprivation. This is also supported by MTF's effect on cell cycle distribution, which led to a cell cycle arrest in the S-phase without blocking the other cell cycle phases. Numerous studies have demonstrated that glucose deprivation in glycolytic tumor cells (as opposed to normal cells) results in decreased ATP production (even in the presence of elevated glucose uptake), AMPK activation, S-phase cell cycle arrest, and apoptosis via ROS generation [25]. The absence of ROS stimulation by MTF in our study may be related to the measurement of intracellular ROS, which reflects a balance between ROS production (either in the cytoplasm or mitochondria), antioxidant system activity (which is highly dependent on glycolysis), and the degree of ROS binding to cellular structures and molecules, primarily to DNA, which leads to damage.

Thus, our findings indicate that the greater cytotoxicity of MTF against de-adhesive LLC/R9 cells compared to SOX is primarily due to cell cycle arrest at the S phase and induction of apoptosis without significant ROS accumulation. This indicates that MTF exerts cytotoxicity by disrupting energy homeostasis and cell cycle regulation rather than by oxidative stress. These results suggest that the cytotoxic efficacy of MTF as monotherapy against circulating metastatic cells is likely to be low and highlight the potential benefit of combining MTF with glycolysis inhibitors to enhance its antimetastatic activity against disseminated tumor cells.

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ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ЦИТОТОКСИКСИЧНОСТІ ОКСАМАТУ НАТРІЮ ТА МЕТФОРМІНУ ЩОДО КЛІТИН КАРЦИНОМИ ЛЕГЕНІ ЛЬЮЇС В УМОВАХ ДЕАДГЕЗИВНОГО РОСТУ

Стан питання. Вплив інгібіторів гліколізу та окисного фосфорилювання на змінений метаболізм новоутворень вважається перспективним методом протипухлинної терапії. Однак більшість досліджень антиметастатичної активності таких інгібіторів зосереджено на аналізі їхнього впливу на міграційні та інвазивні характеристики клітин. Тим часом виживання циркулюючих метастатичних клітин та їхня стійкість до аноїкісу є критично важливими факторами метастазування. **Мета.** Провести порівняльне дослідження впливу оксамату натрію (SOX) та метформіну (MTF) на виживання, проліферативну активність та метаболічну пластичність клітин карциноми легені Льюїс (LLC) в умовах субстрат-незалежного росту. Загибель клітин, апоптоз, розподіл клітинного циклу, продукцію активних форм кисню (ROS), рівень глюкози та лактату, а також експресію віментину в клітинах LLC за умов деадгезивного росту оцінювали після інкубації з SOX та MTF. Цитотоксичність інгібіторів проявлялася у значному зменшенні кількості життєздатних клітин, збільшенні кількості мертвих та апоптотичних клітин, причому ефекти були більш вираженими для MTF. У випадку застосування SOX спостерігалася кореляція між збільшенням відсотка апоптотичних клітин та рівня активних форм кисню (ROS), а також зниженням швидкості споживання глюкози (GCR). MTF збільшував GCR та відсоток апоптотичних клітин без змін рівня ROS. Інкубація з MTF привела до двократного збільшення відсотка клітин у S-фазі через зменшення частки клітин у фазах G1/G0 та G2/M клітинного циклу. На відміну від SOX, цитотоксична дія MTF на деадгезивні клітини була безпосередньо пов'язана з виснаженням глюкози. Оскільки глюкозодефіцитна ситуація неможлива в крові, де рівень глюкози підтримується на фізіологічному рівні, MTF може виявляти антиметастатичну ефективність щодо циркулюючих пухлинних клітин лише в поєднанні з інгібіторами гліколізу.

Ключові слова: метастатичні клітини, незалежний від закріплення ріст, оксамат натрію, метформін, цитотоксичність.