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The role of PASS and STITCH in the verification of unknown properties of pyruvate and lactate. Literature review and fragments of authors' own research

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ABSTRACT

The aim of the study was to identify the predicted spectrum of biological activity of pyruvate and lactate using modern computer modeling methods and to determine potential protein partners in intermolecular interaction.

Materials and methods. The biological activity spectrum of pyruvate and lactate by the structural formula was determined using the PASS (Prediction of Activity Spectra for Substances) software. Potential protein interaction partners for small molecules were predicted using the Search Tool for Interactions Chemicals (STITCH).

Results. Analyzing the obtained results *in silico* reveals that pyruvate and lactate exhibit diverse biological activities, molecular mechanisms, and pharmacological effects. These include regulation of lipid, protein, and carbohydrate metabolism and effects on enzyme activity and gene expression. The data on the antihypoxic, antiischemic, antitoxic, immunomodulatory, antiinflammatory, antiviral, vasoprotective, and cytoprotective effects are presented. The neuroprotective and antineurotoxic effects of pyruvate and lactate are predicted.

Conclusion. The spectrum of biological activities of lactate and pyruvate were revealed by computer modeling methods, and protein interaction partners were characterized. The small molecules we studied have a coordinating role in the functioning and modulation of mediator, hormonal, receptor, immune, inflammatory, antibacterial, and antiviral responses and gene expression. The use of natural intermediates as therapeutic agents for the treatment of ischemic stroke, acute neurological disorders, and neurodegeneration is discussed, which is underlain by the stimulating effect of metabolites on neuroplasticity. These properties may be manifested through conformational rearrangement of receptors, active binding centers, expression of multiple genes, and changes in the functional manifestations of catalytic and other proteins. The obtained data will obviously expand our understanding of the role of small molecules in intermolecular metabolite – protein interactions.

Keywords: small molecules, pyruvate, lactate, biological activity, computer modeling, PASS, STITCH

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PASS и STITCH в верификации неизвестных свойств пирувата и лактата. Обзор литературы и фрагменты собственных исследований

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РЕЗЮМЕ

Цель исследования заключается в выявлении прогнозируемого спектра биологической активности пирувата и лактата с применением современных методов моделирования, определение потенциальных белковых партнеров для межмолекулярного взаимодействия.

Материалы и методы. Определение спектра биологической активности пирувата и лактата по структурной формуле проводили в программном обеспечении Prediction of Activity Spectra for Substances (PASS). Прогнозирование потенциальных белковых партнеров взаимодействия для малых молекул выполняли в системе Search Tool for Interactions Chemicals (STITCH, инструмент поиска взаимодействующих химических веществ).

Результаты. Анализируя полученные результаты *in silico*, обращает на себя внимание проявление разнообразной биологической активности молекулярных механизмов, оказываемых фармакологических эффектов пирувата и лактата. Среди них регуляция липидного, белкового, углеводного обменов, влияние на активность ферментов, экспрессию генов. Приводятся данные антигипоксического, антиишемического, антитоксического, иммуномодулирующего, противовоспалительного, противовирусного, вазопротекторного и цитопротекторного действий. Спрогнозировано нейропротекторное, антинейротоксическое действие пирувата и лактата.

Заключение. Методами компьютерного моделирования раскрыт спектр биологической активности лактата и пирувата, а также охарактеризованы белки-партнеры по взаимодействию. Изучаемые нами малые молекулы выполняют координационную роль в функционировании и модуляции медиаторного, гормонального, рецепторного ответов, иммунологических, воспалительных, антибактериальных, противовирусных реакций, экспрессии генов. Обсуждается использование естественных интермедиатов в качестве терапевтических средств для лечения ишемического инсульта, острых неврологических расстройств, нейродегенерации, что имеет в своей основе стимулирующее действие метаболитов на процессы пластичности мозга. Проявление этих свойств, вероятно, реализуется через конформационную перестройку рецепторов, активных центров связывания, экспрессии множества генов, изменение функциональных проявлений каталитических и других белков. Полученные знания, очевидно, расширят наше понимание роли малых молекул в межмолекулярных взаимодействиях метаболит–белок.

Ключевые слова: малые молекулы, пируват, лактат, компьютерное моделирование, биологическая активность, PASS, STITCH

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The study of the role of metabolites in intercellular interaction systems is currently relevant. In particular, protein – small molecule interactions can regulate and control a variety of cellular processes, such as transport of substances and transmission of signals, playing a role in maintaining cellular homeostasis [1–3]. For economic and practical reasons, assessing intermolecular interaction of millions of chemical compounds with thousands of ligands in the experiment is difficult, so it is advisable to conduct

a preliminary assessment of the biological activity of specific chemicals *in silico* [4, 5].

Biological activity is the main characteristic of compounds with a known chemical formula, since its presence can be the basis for the use of the substance in medicine or it can limit its use due to the manifestation of undesirable side or toxic effects. The use of computer modeling can reduce the volume of necessary experiments by dozens of times in comparison with a blind search [6].

The focus of our attention is on small molecules pyruvate and lactate. One of the most important intermediate components of metabolism and a source of energy for mitochondria is pyruvate, which participates in the processes of cell matrix remodeling [7, 8]. Lactate is no longer considered as a dead-end product of anaerobic metabolism. Being an energy substrate, it plays an important role in regulating the function of many cells, participating in the processes of tissue proliferation and differentiation and angiogenesis [9, 10].

The article is devoted to *in silico* modeling and elucidation of biological activity and molecular mechanisms underlying the description of molecular structure, taking into account the search for structure – property relationships. Our understanding of cellular signal transduction may contribute to the elucidation of new interactions between lactate and pyruvate with proteins.

The aim of the study was to identify the predicted spectrum of biological activity of pyruvate and lactate using modern computer modeling methods and to determine potential protein partners in intermolecular interaction.

MATERIALS AND METHODS

PASS version 1.917 (Prediction of Activity Spectra for Substances) software is intended for prediction of the biological activity spectrum of a compound by its structural formula based on the analysis of structure – activity relationships using a training sample of compounds. The program has information on structures and known biological activities of over one million molecules. Biological activity for new compounds is predicted using Multilevel Neighborhoods of Atoms (MNA) descriptors, which are necessary to describe the structure of molecules in an organic compound, taking into account the search for structure – property relationships for heterogeneous samples [6].

The spectrum of biological activity predicted by the PASS computer system includes pharmacological effects, specific toxicity, side effects, effects of molecules on metabolism, molecular transport, gene expression, identification of undesirable targets, and molecular mechanisms of action. The prediction result is presented as *Pa* ("to be active") and *Pi (*"to be inactive") probabilities with values from 0 to 1 [11]. We took *Pa* over 0.5 as the optimal value of the probability of activity. The prediction of the biological activity spectrum was presented as an ordered list of *Pa* and *Pi* probability estimates.

Potential protein interaction partners for small molecules were identified using the Search Tool for Interactions of Chemicals (STITCH) version 5.0. The STITCH platform includes over 9,600,000 proteins from 2,031 eukaryotic and prokaryotic genomes and 430,000 chemical compounds. This program combines data on existing protein – small molecules interactions from DrugBank, GPCRligand (GLIDA), Matador, Therapeutic Target Database (TTD), Comparative Toxicogenomics Database (CTD), NCI – Nature Pathway Interactions, Reactome, BioCyc, and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Since there can be overlaps between different manually generated datasets, STITCH considers repetitive interactions only once. Other major sources of intermolecular linkages are experimentally validated interaction data sets, which include data from ChEMBL, PDSP Ki Database, and Protein Data Bank. Sources of protein – chemical interactions are supplemented by automated text analysis and structure-based prediction methods. The text mining pipeline includes collaborative copying and processing of all MEDLINE abstracts and available full-text PubMed Central open access articles; NIH RePORTER grant abstracts were also taken into account [1].

To assess the effect and validity of protein – ligand binding, as well as the variability in affinity of known ligands, it is important to know the binding capacity between the compound and its target. Typically, this binding affinity is quantified as the inhibition constant Ki, and the IC50 (half-maximal inhibitory concentration) value is also taken into account [12].

The standard SMILES entry was used to search for identifiers and common chemical names that are stored in the small molecule information database. The program calculates the parameter *p* – the probability of protein – small molecule interaction. In STITCH, the interaction network can be mapped and tuned using different settings: by degree of evidence, confidence, molecular action or binding affinity. In our work, we

used the binding affinity index. The program predicts intermolecular interactions at a confidence threshold of 0 to 1 (low, medium, high, highest). We used a medium confidence threshold of $p > 0.4$ [1].

RESULTS AND DISCUSSION

PASS program for assessing the biological activity of small molecules. In the PASS computer environment, it is possible to analyze a total of 2,736 types of biological activities. Analyzing the data obtained, we can note the highest number of predicted bioactivities for pyruvate compared to lactate (1,926 and 1,792, respectively). It should be noted that pyruvate has a greater ability to exhibit molecular mechanisms of action and exert pharmacological effects, whereas lactate exhibits more adverse, toxic, and metabolic effects.

The studied predicted manifestations of small molecules and experimentally confirmed data are presented in Table 1. The findings indicate that pyruvate and lactate affect cellular processes: they act as cytoprotectors, stimulators of leuko- and erythropoiesis and platelet aggregation, and inhibitors of thrombopoiesis and platelet adhesion due to their ability to activate the hypoxia-inducible factor-1 alpha (HIF-1α) – erythropoietin (EPO) signaling pathway [13].

Table 1

Predicted effects of pyruvate and lactate in the PASS program

Effect	*Pa* lac	*Pi* lac	*Pa* pyr	*Pi* pyr	Experimentally proven data
Fibrinolytic	0.716	0.024	0.812	0.004	[14]
Lipid metabolism regulator	0.784	0.008	0.812	0.006	[15]
Hypercholesterolemic	0.727	0.019	0.757	0.003	
Leukopoiesis stimulator	0.803	0.003	0.727	0.005	[13]
Platelet aggregation stimulator	0.353	0.009	0.706	0.005	
Erythropoiesis stimulator	0.673	0.007	0.698	0.005	[13, 16]
Cytoprotector	0.554	0.017	0.670	0.009	[17]
Antiviral (rhinovirus)	0.623	0.009	0.663	0.005	[18]
Antihypoxic	0.743	0.004	0.650	0.002	[19]
Neuroprotective	0.646	0.066	0.648	0.05	[16, 20, 37–39, 41]
Antiischemic	0.414	0.154	0.611	0.005	[17, 19, 25, 40]
Restorer	0.480	0.019	0.610	0.011	[21]
Antiviral (picornavirus)	0.588	0.027	0.605	0.018	[18]
Antiinflammatory	0.614	0.026	0.600	0.004	[19, 22]
Platelet adhesion inhibitor	0.592	0.001	0.592	0.014	
Vasoprotector	0.681	0.024	0.564	0.025	[23]
Immunomodulator	0.811	0.023	0.554	0.04	[15]
Antineurotoxic	0.639	0.044	0.545	0.06	[17, 24, 39]
Inhibitor of thrombocytopoiesis	0.854	0.006	0.519	0.004	
Antitoxic	0.599	0.006	0.516	0.012	[21]

Note: *Pyr* – pyruvate, lac – lactate, *Pa* – probability of presence; *Pi* – probability of absence.

Pyruvate and lactate have anti-inflammatory and antiischemic effects, increase myocardial contractility and energy state, protect tissues from ischemic damage and oxidative stress by enhancing sarcoplasmic reticular Ca2+ transport, and increase NADPH production to maintain redox state of glutathione [19].

Pyruvate exerts neuroprotective effects, preventing death of postischemic astrocytes by inhibiting lactate dehydrogenase leakage, reducing redox ratio, and inhibiting activation of apoptotic events, such as cytochrome c release from mitochondria and fragmentation of caspase-3 and poly(ADP-ribose)polymerase. Pyruvate can accelerate its own metabolism by increasing pyruvate dehydrogenase activity and thus restore cellular levels of ATP in postischemic astrocytes [17, 20]. According to V.N. Yartsev, the vasoprotective effect of pyruvate and lactate is probably realized through a direct effect on the neurogenic tone of blood vessels [23]. Due to these predicted and experimentally confirmed properties,

pyruvate has been used as a therapeutic agent for the treatment of stroke and acute neurological disorders in studies by D. Frank [25].

Simulated antiviral effect of pyruvate and lactate against rhinovirus and picornavirus is worth noting. Lactate is highly likely to have an antiviral effect on arboviruses and papillomaviruses. There is an assumption that the virus loses its infectious activity due to conformational rearrangements in the structure and a loss of replication activity [18].

There is evidence of the involvement of these intermediates in various types of metabolism: lipid, protein, and carbohydrate (Table 2). We have identified the effect of pyruvate and lactate on the regulation of lipid metabolism, with a significant hypercholesterolemic effect. It is worth noting that these small molecules inhibit trans-2-enoyl-CoA reductase, which is involved in the biosynthesis of unsaturated fatty acids and elongation of mitochondrial fatty acids. Adipocytes have been experimentally shown to be the main source of lactate in white adipose tissue. Lactate levels range from 0.35–9.67 mM at a fat tissue density of 0.9 g / ml. It has been noted that adipocyte-derived lactate is a signaling metabolite that promotes activation of inflammatory macrophages by direct binding to the prolyl hydroxylase domain protein 2, highlighting the relationship between immunological processes and metabolism in obesity [15].

We revealed that pyruvate and lactate exert an inhibitory effect on enzymes of protein metabolism, such as alanine transaminase, serine-3-dehydrogenase, gamma-glutamyl transferase, and tryptophan transaminase. These intermediates are known to affect a number of carbohydrate metabolism enzymes: glycerol-3-phosphate dehydrogenase, L- and D- forms of lactate dehydrogenase, NAD-dependent malate dehydrogenase, NADP-dependent decarboxylating malate dehydrogenase, malate oxidase, pyruvate dehydrogenase complex [16].

Table 2

Probable molecular mechanisms of pyruvate and lactate action

Molecular mechanism of action	Enzyme code	*Pa* pyr	*Pi* pyr	*Pa* lac	*Pi* lac
Phosphoenolpyruvate phosphotransferase inhibitor	EC 2.7.3.9	0.91	0.001	0.922	0.001
Pyruvate decarboxylase inhibitor	EC 4.1.1.1	0.887	0.002	0.938	0.001
Aspartate – phenylpyruvate transaminase inhibitor	EC 2.6.1.70	0.854	0.003	0.816	0.004
Glutamine – phenylpyruvate transaminase inhibitor	EC 2.6.1.64	0.83	0.004	0.807	0.005
Pyruvate dehydrogenase inhibitor cytochrome	EC 1.2.2.2	0.812	0.002	0.59	0.005
Phenylpyruvate decarboxylase inhibitor	EC 4.1.1.43	0.808	0.002	0.614	0.004
Pyruvate dehydrogenase inhibitor	EC 1.2.4.1.	0.799	0.002	0.856	0.002
L-lactate dehydrogenase inhibitor	EC 1.1.2.3	0.778	0.001	–	–
Phosphoenolpyruvate carboxykinase inhibitor	EC 4.1.1.38	0.769	0.002	0.8	0.002
D-lactate dehydrogenase inhibitor	EC 1.1.2.4	0.765	0.001	0.783	0.002
Oxaloacetate tautomerase inhibitor	EC 5.3.2.2	0.723	0.001	0.881	0.001
Glycerol-3-phosphate oxidase inhibitor	EC 1.1.3.21	0.705	0.002	0.616	0.004
Malate dehydrogenase inhibitor	EC 1.1.1.37	0.674	0.005	0.705	0.004
Oxaloacetate decarboxylase inhibitor	EC 4.1.1.3.	0.674	0.002	0.742	0.003
Glycerol-3-phosphate dehydrogenase inhibitor	EC 1.1.1.8	0.669	0.003	0.698	0.003
Malate dehydrogenase acceptor inhibitor	EC 1.1.1.37	0.62	0.011	0.594	0.018
Malate oxidase inhibitor	EC 1.1.3.3	0.567	0.008	0.522	0.016
D-malate decarboxylating dehydrogenase inhibitor	EC 1.1.1.83	0.557	0.003	0.723	0.002
NADPH-dependent malate dehydrogenase inhibitor	EC 1.1.1.40	0.529	0.003	0.5	0.003
Lactate – malate transhydrogenase inhibitor	EC 1.1.99.7	0.524	0.001	0.808	0.001
Inhibitor of NADP-dependent decarboxylating malate dehydrogenase	EC 1.1.1.82	0.471	0.002	0.522	0.003

The influence of natural intermediates on gene expression is worth noting (Table 3). Pyruvate and lactate upregulate the expression of the *HMOX1* gene, which encodes the stress-induced protein heme oxygenase-1, regulating the viability, proliferation, and differentiation of many cell types. In the studies by R.A. Zager et al. (2014), the use of pyruvate resulted in an increase in cytoprotective messenger

RNA oxygenase-1 and IL-10, a selective decrease in proinflammatory MCP-1 and TNFα, and an increase in ATP levels [26]. Pyruvate attenuates the secretion of amphoterin in cells by inducing heme oxygenase-1 through activation of the phosphatidylinositol-3-kinase pathway, protein kinase, and nuclear factor 2 [27].

It is interesting that pyruvate and lactate increase the expression of the *TP53* gene, a transcription factor that regulates the cell cycle. It is known that the p53 protein acts as a suppressor of malignization, so the *TP53* gene is attributed the properties of an antioncogene [28]. An inhibitory effect of pyruvate on the expression of the gene encoding MMP-9, a protein of the matrix metalloproteinase family, was predicted. An increase in the level of the p53 protein contributes to the inhibition of the growth of non-small cell lung cancer cells, stops their invasion and migration, and induces apoptosis [29]. Pyruvate reduces the expression of the tumor necrosis factor (*TNF*) gene. It is known that this gene encodes a multifunctional proinflammatory cytokine, which is mainly secreted by macrophages and is involved in the regulation of cell proliferation, differentiation, and apoptosis. It has been shown to reduce lung tissue infiltration in radiation-induced lung injury by reducing the level of proinflammatory cytokines IL-1β, IL-6, TNFα, GM-CSF, M-CSF, TGF-β1, and HMGB1 [26, 30].

Table 3

Effect of pyruvate and lactate on gene expression

Effect on gene expression	*Pa* pyr	*Pi* pyr	*Pa* lac	*Pi* lac
BRAF gene expression inhibitor	0.724	0.003	–	–
TP53 gene expression enhancer	0.676	0.031	0.507	0.022
MMP-9 gene expression inhibitor	0.606	0.015	0.15	0.065
Inhibitor of *TNF* gene expression	0.589	0.014	0.163	0.084
HMOX1 gene expression enhancer	0.549	0.027	0.553	0.017
EIF4E gene expression inhibitor	0.544	0.005	0.015	0.005
TERT gene expression inhibitor	–	–	0.621	0.016

The inhibitory effect of pyruvate on the expression of the *EIF4E* gene was predicted by the PASS computer program, and its activation is associated with carcinogenesis. Pyruvate inhibits *BRAF* gene expression. The B-RAF protein, a proto-oncogene, is a part of the RAS / MAPK signaling pathway, which regulates cell growth, proliferation, differentiation, migration, and apoptosis [31]. Lactate has been predicted to inhibit the expression of the telomerase reverse transcriptase (*TERT)* gene, which is the catalytic subunit of the enzyme. The *TERT* gene is mainly active in progenitor and cancer cells, and to a lesser extent – in somatic cells, playing an important role in aging.

STITCH system for identifying intermolecular interaction partners. We searched for intermolecular interaction partners for pyruvate and lactate in the STITCH software. The number of interactions for pyruvate was 109 and for lactate – 384, with a mean significance level $p > 0.4$. Interactions of the studied small molecules with receptors, transfer proteins, hormones, and enzymes were noted, and molecular models were constructed from them. In Table 4, partner proteins typical of pyruvate and lactate are predicted with a high degree of certainty. The interaction of pyruvate and lactate as substrates with lactate dehydrogenase and its various isoforms is confirmed.

The probability of pyruvate and lactate binding to pyruvate kinase, which stimulates POU5F1-mediated transcription activation and plays a common role in the caspase-independent death of tumor cells, is predicted. The ratio between a highly active tetrameric form and an almost inactive dimeric form of pyruvate kinase determines whether glucose carbon will be directed to biosynthesis or used for glycolytic ATP production. The transition between the two forms contributes to the control of glycolysis and is important for the proliferation and survival of tumor cells [32].

Pyruvate and lactate are equally likely to interact with solute carrier family proteins (SLC16, SLC5) and mitochondrial pyruvate carrier (MPC) 1 and 2. Acting through lactate receptors HCAR1 / GPR81 or being transported by monocarboxylate transporters (MCT) across the cell membrane, metabolites influence the functional activity of cells in various tissues (endothelium, adipose tissue cells, neurons), which leads to changes in metabolism, proliferation, and differentiation [33, 34].

The studied natural intermediates pyruvate and lactate are highly likely to act as ligands for vascular endothelial growth factor (VEGF). In particular, the effects of lactate as a proangiogenic factor acting through an increase in VEGF expression are of interest. In some tissues, lactate is taken up and oxidized by macrophages, and lactate-mediated macrophage polarization promotes muscle revascularization and regeneration. The ability of

lactate to alter tumor vascular morphogenesis and perfusion has been established, thereby defining the relationship between metabolism in tumor tissues and angiogenesis [34, 35].

Table 4

Partner proteins in the interaction with pyruvate and lactate

Protein	Description	*p* pyr	*p* lac
PKM	Muscular pyruvate kinase	0.985	0.898
HAO1	Hydroxyacid oxidase	0.973	0.717
LDHA	L-lactate dehydrogenase A	0.969	0.998
LDHC	L-lactate dehydrogenase C	0.968	0.990
LDHB	L-lactate dehydrogenase B	0.968	0.986
LDHAL6A	Lactate dehydrogenase A type 6A	0.960	0.929
LDHAL6B	Lactate dehydrogenase A type 6B	0.957	0.915
LDHD	Lactate dehydrogenase D	0.910	0.911
SLC16A1	Solute carrier family 16, member 1	0.909	0.994
SLC16A3	Solute carrier family 16, member 3	0.909	0.969
SLC16A 7	Solute carrier family 16, member 7	0.900	0.969
SLC5A8	Solute carrier family 5, member 8	0.900	0.954
MPC1	Mitochondrial pyruvate carrier 1	0.900	0.908
MPC2	Mitochondrial pyruvate carrier 2	0.900	0.900
VEGFA	Vascular endothelial growth factor A	0.814	0.879

Note: p – the probability of a small molecule – protein interaction.

Table 4 shows the proteins that indicate interaction of pyruvate with such protein – enzymes as pyruvate carboxylase, mitochondrial phosphoenolpyruvate carboxykinase, dihydrolipoamide dehydrogenase, and dihydrolipoamide transacetylase – the components of pyruvate dehydrogenase complex. The interaction of pyruvate with various malate dehydrogenase isoforms has been predicted: cytosolic NADP-dependent isoform (ME1), mitochondrial NADP-dependent isoform (ME3), and mitochondrial NAD-dependent isoform (ME2). There is evidence that pyruvate is associated with glycerol-3-phosphate dehydrogenase and phospholipase A2, which catalyzes calcium-dependent hydrolysis of two acyl groups in 3-sn-phosphoglycerides. This releases glycerophospholipids and arachidonic acid, which are themselves precursors of signaling molecules.

Pyruvate has been predicted to be a ligand of cystathionine gamma-lyase, which implements methionine sulfidation; it activates sulfurtransferase, which carries out cyanide detoxification and thiosulfate biosynthesis. Both of these proteins generate an endogenous form of hydrogen sulfide, which is a synaptic modulator, a signaling molecule, a neuroprotector, and a regulator of blood pressure [36]. Pyruvate is able to interact with cytochrome b5, which is a membrane-bound hemoprotein that functions as an electron carrier for several membrane-bound oxygenases.

The data on the relationship of lactate with many receptors, including HCAR1, HCAR2, and HCAR3 hydroxycarboxylic acid receptors, which exhibit their effects using the G-protein-mediated pathway, are of interest. In addition, it has been shown that lactate can interact with the alpha-2 adrenergic receptors ADRA2A, ADRA2B, ADRA2C that mediate catecholamine-induced inhibition of adenylate cyclase by G-proteins. To a high degree, the intermediate under study interacts with opioid, dopamine, muscarinic cholinergic, bradykinin, glutamate, lysophosphatidic acid receptor, and G-protein-coupled receptor, which indicates the involvement of lactate in nerve impulse transmission [37]. Neuroprotective effect is probably related to the MTRNR2 protein, which reduces the production of the amyloid beta peptide in Alzheimer's disease [38]. The function of lactate as a ligand for

neurotransmitters, such as glutamate, dopamine, acetylcholine, histamine, and prostaglandin E2, is worth mentioning.

Thus, the metabolic role of lactate in reducing the development of neurodegenerative processes has been predicted and experimentally proven; some scientists consider lactate as a mediator or hormone involved in memory formation and neuroprotection [39]. The use of lactate in clinical studies in the treatment of ischemia and neurodegenerative diseases is discussed, which is determined by the stimulating effect of lactate on neuroplasticity. In particular, the role of lactate as a neuroprotective factor in Alzheimer's disease is of interest, which is determined by its ability to provide metabolic coupling between astrocytes and neurons in active brain regions and participate in the regulation of cerebral angiogenesis [40, 41].

We noted a high probability of interaction of lactate with adrenaline, somatostatin, melatonin, pancreatic polypeptide, and melanin-concentrating hormone. In addition, lactate can interact by a receptor-mediated mechanism with serotonin receptors and galanin and prostaglandin E hormone receptors.

The high ability of lactate and pyruvate to affect immune responses and inflammatory processes, as predicted by the PASS platform, is worth noting. Lactate binds to interleukin (IL)-8 and IL-6 – powerful acute-phase inducers involved in the final differentiation of B cells, lymphocytes, and monocytes; IL-10, which inhibits cytokine synthesis (γ-interferon, IL-2, IL-3, TNF, and GM-CSF); IL-1α, which is involved in the immune response and stimulates the release of prostaglandin and collagenase from synovial cells. Pyruvate, in turn, acts as a ligand for the IL-31 receptor.

These findings have been confirmed in a number of *ex vivo* and *in vivo* experiments. Intracellular pyruvate content has been shown to correlate positively with IL-1β and IL-10 levels in patients with community-acquired pneumonia. Increased levels of pyruvate partially support the ability of hyporeactive monocytes to produce cytokines [42]. Antiinflammatory and potential therapeutic effects of pyruvate in an experimental model of appendicitis in rats were noted [43]. The clinical application and therapeutic effect of pyruvate nasal spray in allergic rhinitis are discussed [44, 45].

The ability of lactate to be an interaction partner for different groups of chemokines has been noted. Thus, the chemokine CCL5 is an attractant for blood monocytes, T helpers, and eosinophils and causes histamine release from basophils. Binding to the chemokines CCR1, CCR3, CCR4, and CCR5, it is one of the major HIV-suppressive factors produced by CD8+ T cells. The chemokine CXCL1 activates neutrophils and may play a role in inflammation by exerting its effect on endothelial cells. D. Zhang et al. (2019) showed that lactylation of lysine residues serves as an epigenetic modification that directly stimulates macrophage chromatin gene transcription. Histone lactylation contributes to the understanding of lactate functions and its role in various pathophysiological manifestations of infectious and noninfectious nature, which may indicate a close functional relationship between the metabolic state and the expression profile of cells [46].

CONCLUSION

The spectrum of biological activities of lactate and pyruvate was revealed by computer modeling methods, and the interaction partner proteins were characterized. The small molecules we studied have a coordinating role in the functioning and modulation of mediator, hormonal, receptor, immune, inflammatory, antibacterial, and antiviral responses and gene expression. The use of natural intermediates as therapeutic agents for the treatment of ischemic stroke, acute neurological disorders, and neurodegeneration is discussed, which is underlain by the stimulating effect of metabolites on neuroplasticity.

These properties may be manifested through conformational rearrangement of receptors, active binding centers, expression of multiple genes, and changes in the functional manifestations of catalytic and other proteins. The obtained data will obviously expand our understanding of the role of small molecules in intermolecular metabolite – protein interactions. Publications in this area appeared only in 2009 and were summarized in a systematic review by X. Li et al. [47]. In 2022, an original viewpoint on the protein – small molecule interaction appeared. The term metabolic entanglement was coined by the authors. The presence of ligand-binding pockets in protein structures for small molecules is to be deciphered, which will allow for integration of metabolomics and protein interactomics [48].

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