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REVIEW

Ji et al: Lactylation in ischemic brain injury

Lactylation in ischemic brain injury—metabolic mechanisms, neuroinflammation, and therapeutic targets: A review

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ABSTRACT

Cerebral ischemic injury, a major cause of mortality and disability, results from reduced or interrupted blood flow to the brain, most commonly in ischemic stroke. Insufficient oxygen and nutrient supply disrupts cellular metabolism, leading to neuronal death, neurological dysfunction, and lasting impairments. Current therapeutic strategies, including thrombolysis, mechanical thrombectomy, and anticoagulation, primarily aim to restore perfusion and provide neuroprotection by preserving the ischemic penumbra. While these interventions can partially rescue viable tissue in the acute phase, their effectiveness is constrained by narrow therapeutic windows, low recanalization rates, and contraindications, leaving significant unmet clinical needs. Consequently, the search for novel, targeted approaches has become a central focus of ischemic stroke research. Recent discoveries have identified lactylation, a newly recognized post-translational modification derived from lactate, as a key regulator of gene expression, protein function, and metabolic reprogramming. Once regarded as a simple glycolytic byproduct, lactate is now known to act as both an alternative energy substrate and a signaling molecule, influencing neuronal metabolism, antioxidant defense, and inflammatory responses. In ischemic brain injury, lactylation modifications of histone and non-histone proteins may either protect neurons—by supporting energy homeostasis, regulating stress-responsive genes, and suppressing apoptosis—or exacerbate injury through neuroinflammation, excitotoxicity, and immune evasion. Evidence indicates that the outcomes of lactylation depend on lactate concentration, timing of accumulation, cell type, and the balance between “writer” and “eraser” enzymes. Therefore, lactylation emerges as a promising yet complex therapeutic target in cerebral ischemia. Modulating lactate metabolism and its downstream modifications offers new opportunities to expand the therapeutic window, attenuate neuronal injury, and improve recovery. This review summarizes the molecular mechanisms linking lactate and lactylation to ischemic injury, highlights current contradictions in experimental findings, and explores the potential of targeting lactylation pathways for innovative treatment strategies.

Keywords: Lactylation modification, cerebral ischemic injury, epigenetic modification, hypoxia-reperfusion, treatment strategies.

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INTRODUCTION

In recent years, most countries have experienced an increase in stroke incidence, mortality, and disability rates, with ischemic stroke accounting for 62.4% of all stroke occurrences [1-3]. This condition arises when cerebral blood vessels become narrowed or obstructed, resulting in a diminished blood supply to the brain and subsequent tissue necrosis. Chronic ischemia in the brain can lead to permanent neuronal damage and neurological impairments (Figure 1), significantly affecting the patient's quality of life and creating a considerable burden on families and society [4-6]. Presently, pharmacological and surgical reperfusion therapies remain the primary effective treatments. The fundamental approach of these neuroprotective therapies is to preserve the ischemic penumbra surrounding the necrotic core, where blood flow is relatively less compromised. Early reperfusion therapy is aimed at salvaging the brain tissue within this ischemic penumbra. However, if ischemia persists for an extended period, the necrotic core can expand, leading to irreversible neuronal damage in the surrounding tissue. The limited therapeutic window, combined with low recanalization rates and numerous contraindications to thrombolysis, reduces the clinical effectiveness of reperfusion therapy [7-9].

Recent studies highlight the significant role of cellular metabolic reprogramming, particularly glycolysis, in the progression of ischemic and hypoxic diseases following ischemia and hypoxia, a process that has drawn considerable attention [10, 11]. Lactic acid, once thought to be a mere byproduct of glycolysis, is now recognized as an essential metabolite. It not only serves as an energy source for various tissues, including skeletal muscle, heart, brain, and cancer cells, but also functions as a signaling molecule involved in immune regulation, fat mobilization, wound repair, and maintaining cellular homeostasis [12-14]. Furthermore, recent research demonstrates that lactic acid contributes to the repair process in ischemic brain injury, with exogenous supplementation of lactic acid helping to reduce ischemic brain damage [11, 15-17]. Lactylation, a post-translational modification of proteins, has become acknowledged as a crucial mechanism in the regulation of cellular metabolism. This modification influences gene expression, protein activity, and processes related to the development of ischemic diseases [18-20]. Lactylation can occur on both histones and non-histones within cells, with enzymes such as histone acetyltransferases (e.g., p300, CBP) and deacetylases (e.g., HDACs) playing vital

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roles in regulating these modifications. Targeting these enzymes could provide novel therapeutic strategies for the treatment of ischemic diseases [21-23].

This review aims to investigate the molecular mechanisms of lactylation modification, its involvement in ischemic brain injury progression, and potential therapeutic strategies targeting lactylation, given its critical role in ischemic disease biology.Since research on lactylation in cerebral ischemic injury (whether in neonatal or adult models) is still in its early stages, the review will also explore future prospects for utilizing lactylation and its regulatory pathways as novel therapeutic approaches.

METHODS

Literature search strategy

To comprehensively review the literature on lactylation modification and ischemic brain injury, a systematic search strategy was implemented. We searched the following databases: PubMed, Google Scholar, and Web of Science, covering the period from January 1, 2000 to June 30, 2025. The search terms used were: "lactylation AND stroke", "lactylation AND ischemic brain injury", "lactate AND cerebral ischemia". The literature search was limited to articles in English, and only peer-reviewed journal articles were selected.The inclusion criteria were: (1) studies that explored the relationship between lactylation modification and ischemic brain injury, (2) both clinical and experimental studies with complete experimental data, (3) high-quality review articles were also included to provide comprehensive background information and references, and (4) peer-reviewed journal articles. Exclusion criteria included: (1) studies that did not directly address the role of lactylation or lactate in ischemic brain injury, (2) conference papers and studies with incomplete data, and (3) studies with poor quality or inadequate experimental design.

Lactic acid and lactylation modifications

Lactic acid, an important metabolic byproduct, is primarily produced in the cytoplasm and plays a key role in glycolysis. Under conditions of hypoxia or elevated metabolic demand, glucose is converted into pyruvate through glycolysis. In cases of limited oxygen availability, particularly in ischemic injuries, pyruvate is unable to enter the mitochondria for oxidative phosphorylation and is instead converted to lactate. This

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conversion is catalyzed by lactate dehydrogenase (LDH), which facilitates the reduction of pyruvate to lactate, while NADH is oxidized to NAD+, ensuring the continuation of glycolysis [24-26].

The production of lactate is closely connected to the energy demands of the cell and is regulated by a range of essential proteins. Key proteins that regulate lactate production include lactate dehydrogenase (LDH), which plays a direct role in lactate generation [27, 28], as well as several enzymes that facilitate glycolysis, including glucose transporter proteins (GLUT), Pyruvate kinase M1/2 (PKM), Hexokinase 2 (HK2), Aldolase A (ALDOA), and Phosphofructokinase platelet type (PFKP), among others [29-32]. For example, the expression and activity of LDH are frequently elevated in tumor cells, promoting the acceleration of lactate production [33-35]. GLUT family members, such as GLUT1 and GLUT3, enhance glucose uptake, ensuring an adequate supply of substrates necessary for glycolysis [36, 37]. Meanwhile, as lactate accumulates, it leads to a decrease in local pH, prompting cells to utilize specific transporters, such as monocarboxylate transporters (MCTs), to export lactate, thus preventing excessive accumulation and helping maintain intracellular pH balance [38-41]. The coordinated actions of these proteins maintain the equilibrium of lactate production, regulating both the cell's metabolic state and its ability to adapt to environmental changes (Figure 2).

Lactylation, like other post-translational modifications, regulates protein function and structure by attaching a lactyl group to the side chain of an amino acid. In 2019, the first identification of lysine lactylation (Kla) occurred, marking a post-translational modification derived from lactate. This modification is evolutionarily conserved and is prevalent across various types of cells [42-44]. Within the cell, lactylation is triggered when lactate—whether produced endogenously or introduced exogenously—reaches a critical concentration threshold [45-47].

The lactylation process begins when lactate serves as the substrate, which is subsequently converted into lactyl-CoA [48, 49]. Lactyl-CoA then acts as a substrate for a specific group of acetyltransferases, which transfer the lactyl group to lysine residues found in both histones and non-histones, leading to changes in protein structure and function. These enzymes, commonly referred to as "writers," include P300/CREB-binding protein (CBP) [50-52] , Methyltransferase-like 3 (METTL3)

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[53-55], Lysine acetyltransferase 2A (KAT2A), and others [56-59]. Meanwhile, "erasers," such as HDACs [60-63], Lysine demethylases (KDMs) [64], and Sirtuins (SIRTs) [65-67], remove the lactyl group from lysine residues on proteins, thus preventing prolonged effects of lactylation. The collaborative actions of both "writers" and "erasers" control the equilibrium of protein lactylation, allowing for stable functional modifications within the body (Figure 2).

Proteins that can undergo lactylation modifications include both histones and non-histones (Figure 2) [68-73]. Initially identified in histones, lactylation modifications have since driven increased research into the regulation of histones, particularly in neurological diseases. For example, it was discovered that histone H3K9 lactylation (H3K9la) promotes temozolomide resistance in glioblastoma by causing MutL homolog-1 (MLH1) intron retention via LUC7-like 2 (LUC7L2). Specifically, temozolomide treatment results in an upregulation of H3K9la levels in glioblastoma cells. This modification occurs within the intronic region of the MLH1 gene, leading to intron retention and interference with normal splicing, thus impairing MLH1 function and facilitating temozolomide resistance. [74]. Moreover, pharmacological inhibition of glycolysis can reduce H3K9 lactylation, consequently increasing the sensitivity of glioblastoma cells to temozolomide.

Although initial lactylation research primarily focused on histones, more recent studies have expanded to examine non-histone proteins [75]. Lactate and lactylation play vital protective roles in ischemic stroke by maintaining neuronal function and promoting cell survival. Lactate provides an alternative energy source for neurons, sustaining cellular energy and preventing neuronal death during ischemia. Lactylation modifications, particularly in histones, regulate gene expression that may help cells adapt to ischemic stress. Recent studies suggest that lactylation in microglia modulates neuroinflammation, contributing to neuroprotection during ischemic events[76]. Additionally, lactylation in ischemic stroke has been shown to regulate genes involved in neuronal survival, enhancing resilience to injury.Excessive lactate accumulation and dysregulated lactylation can exacerbate neuronal injury. High lactate levels lead to acidosis, excitotoxicity, and mitochondrial dysfunction. Dysregulated lactylation further promotes inflammation and neuronal death. Fang et al. showed that LDHA-induced lactylation activates the NLRP3 inflammasome, increasing inflammation and tissue damage in ischemia[77]. Weng et al. demonstrated

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that lactylation of Tufm protein induces mitophagy and neuronal apoptosis, contributing to neuronal injury[78]. Additionally, Yang et al. highlighted how lactylation of Programmed death-ligand 1 (PD-L1) can enhance immune evasion in tumors, showing lactylation's dual, context-dependent effects[76].

The role of lactylation in ischemic brain injury has been a topic of interest

Initial studies have indicated an increase in lactate levels following ischemic brain injury, with these elevated levels potentially serving as biomarkers [79, 80]. Subsequent research has shown that while lactate levels rise after ischemic brain injury, supplementing with exogenous lactate can effectively mitigate damage, suggesting a protective role for lactate in such injuries [81-83]. Table 1. Summary of Conflicting Reports on Lactate and Lactylation Modifications in Ischemic Brain Injury. However, for an extended period, the precise mechanism through which lactate exerts its protective effects remained unclear. Recent findings have shed light on how lactate influences the neuronal GPR81 protein, regulates brain angiogenesis during development, and promotes recovery from hypoxic-ischemic injury [11]. Furthermore, lactate has been found to protect both neurons and astrocytes from ischemic damage by regulating calcium levels [84]. While these studies suggest that lactate treatment enhances protective factors and reduces harmful ones, the mechanisms by which lactate, as a metabolic byproduct, regulates these factors were not previously explored. It was only after the concept of lactylation was introduced that researchers began to understand the intricate relationship between lactate and ischemic brain injury.

Yao Y's team was the first to demonstrate that lactylation modifications are enhanced in ischemic brain injury [85]. They investigated Kla in cortical proteins from the cerebral ischemia-reperfusion injury (CIRI) model in adult rats, noting an increase in lactylation modifications associated with ischemic brain injury. A total of 1003 lactylation sites were identified across 469 proteins. However, their study did not provide any subsequent mechanistic insights, nor did it establish whether lactylation modifications have a protective or detrimental effect on ischemic brain injury.

Sun M et al.'s study provided a comprehensive examination of the mechanisms by which lactylation modifications affect ischemic brain injury [86]. They identified that MeCP2, a key transcriptional regulator, undergoes lactylation, which serves as a

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protective mechanism against neuronal death induced by stroke. From a mechanistic perspective, lactylation at the K210/K249 sites of MeCP2 suppresses the expression of apoptosis-related genes, such as Programmed cell death protein 4 (Pdcd4) and Phospholipase A2 group VI (Pla2g6), thereby diminishing neuronal apoptosis. Moreover, HDAC3 and p300 were found to be crucial enzymes that regulate the lactylation of MeCP2 following a stroke. In conclusion, their findings not only demonstrated that lactate alleviates ischemic brain injury by modulating protein lactylation modifications within neuronal cells but also elucidated the specific sites and regulatory pathways involved.

Numerous studies challenge the previously held view that lactate functions as a protective factor [87-89]. Mitochondrial transfer, which refers to the movement of mitochondria between cells, plays a critical role in protecting against ischemic brain injury by providing energy and enhancing the function of damaged neurons. In their study, Zhou J et al. demonstrated that lactylation modification regulates mitochondrial transfer, which subsequently influences the outcome of ischemic brain injury. Their research specifically identified low-density lipoprotein receptor-related protein-1 (LRP1) as a key surface receptor involved in endocytosis and signal transduction, which regulates essential cellular processes, such as survival, differentiation, and proliferation. In a mouse model of ischemic stroke, inhibiting LRP1 in astrocytes reduced mitochondrial transfer to the injured neurons and worsened ischemia-reperfusion injury. Mechanistically, LRP1 in astrocytes facilitates mitochondrial transfer to neurons by decreasing lactate production and ADP-ribosylation factor 1 (ARF1) lactylation. Additionally, LRP1 suppresses glucose uptake, glycolysis, and lactate production, which ultimately results in a reduction of ARF1 lactylation [90]. Although the study showed that lactate inhibits mitochondrial transfer and aggravates neuronal damage, it did not involve direct lactate application to animals or cells with ischemic brain injury. Moreover, considering the multiple mechanisms at play, mitochondrial transfer is only one protective factor, making it impossible to draw definitive conclusions about whether lactate or lactylation modifications are beneficial or harmful in ischemic brain injury. The only conclusion that can be drawn is that increased lactylation modification in astrocytes may worsen ischemic brain injury. Furthermore, research has shown that lymphocyte cytosolic protein 1 (LCP1) is upregulated in ischemic brain injury models. Silencing LCP1 significantly reduced

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neurological deficits, infarct size, and brain water content in middle cerebral artery occlusion (MCAO) in adult rats, while also decreasing cell apoptosis. In addition, both total lactylation and LCP1 lactylation levels were markedly elevated in cerebral infarction, both in vivo and in vitro. Treatment with 2-Deoxy-D-glucose (2-DG) resulted in a significant reduction in LCP1 lactylation. In conclusion, inhibiting glycolysis lowered LCP1 lactylation and facilitated LCP1 degradation, ultimately reducing the progression of cerebral infarction [91]. Xiong XY et al. conducted a study that demonstrated the effectiveness of inhibiting glycolysis in reducing ischemic brain injury [92]. Their findings were validated through an in vivo model, which also contradicted the conclusions drawn by Sun M [86] et al. Specifically, the research showed that inhibiting LDHA or glycolysis, thus reducing lactate production, resulted in significant brain damage reduction in ischemic stroke mice. However, the supplementation of additional lactate exacerbated the brain injury, possibly due to its association with neuronal death and the activation of A1 astrocytes. Increased lactate levels during ischemia may facilitate the formation of protein Kla, whereas post-reperfusion lactate treatment does not influence the Kla levels of neuroprotective brain proteins. Moreover, pharmacologically inhibiting lactate production or blocking its transport into neurons led to a notable reduction in Kla protein levels in ischemic brains. Further analysis of MCAO results in astrocyte-specific LDHA knockout mice showed that the cKO mice, compared to the control group, exhibited lower Kla protein levels, alongside a decrease in brain infarction volume. In addition, inhibiting the formation of protein Kla using the antagonist A-485, which targets the writer p300, significantly reduced neuronal death and neuroglial activation in brain ischemia. This intervention also lowered Kla protein levels, thereby prolonging the reperfusion window and improving functional recovery in ischemic stroke. These findings suggest that lactate produced by astrocytes contributes to exacerbating ischemic brain injury by promoting the formation of protein Kla. The study highlights two essential points: 1) lactate produced by astrocytes plays a critical role in ischemic brain injury, and 2) inhibiting glycolysis can help alleviate ischemic brain injury. This conclusion directly contradicts the findings of Sun M [86] et al. A closer examination of both studies reveals differences in drug administration timing: one study applied the drug 24 hours before surgery, while the other administered it 40 minutes post-surgery. These discrepancies in experimental conditions cannot fully account for the conflicting conclusions. Additionally, while Sun M [86] et al.'s experiments involving exogenous

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lactate supplementation found it to alleviate ischemic brain injury, Pan XR et al.'s study, under the same experimental conditions, observed that lactate supplementation worsened ischemic brain injury [93]. The numerous contradictory conclusions cannot be explained solely by experimental conditions, suggesting that further research is necessary to clarify the underlying reasons for these discrepancies.

The study conducted by Pan XR and his team is particularly notable, as it highlights the significant reduction of lactate and Kla protein levels in ischemic brain tissue of mice following electroacupuncture (EA) pretreatment, a therapeutic approach combining traditional Chinese medicine techniques. This reduction was also associated with decreased astrocyte activation and less neuronal damage and death. However, the study's interpretation of traditional medicine mechanisms through a Western medical framework introduces unnecessary complexity. The authors did not elaborate on how electroacupuncture regulates the reduction in lactylation levels, nor did they clarify how this reduction contributes to the alleviation of ischemic brain injury. In contrast, certain traditional Chinese herbal formulas have been shown to modulate lactylation modifications and offer protective effects against ischemic brain injury. For instance, Song C et al. found that Buyang Huanwu Decoction (BHD) alleviates ischemic brain injury [94]. However, while earlier studies primarily focused on neuronal cells, their research shifted focus to endothelial cells, demonstrating that BHD inhibits glycolysis and apoptosis by suppressing pan-Kla and H3K18la protein levels, as well as Apoptotic protease activating factor 1 (Apaf-1) transcriptional activity. This action helps prevent the progression of ischemic brain injury. One notable limitation of their study is the uncertainty regarding whether the effects of BHD are solely reliant on lactylation modification regulation in endothelial cells, as it remains unclear whether BHD can directly affect neuronal cells.

Ischemic brain injury and tumor cells both rely on glycolysis for energy production and consequently generate significant amounts of lactate, which represents a shared characteristic in their energy metabolism. However, there are notable discrepancies between ischemic brain injury and tumor cells. For instance, while glycolysis promotes the progression of tumors in cancer cells, ischemic brain injury has resulted in varied and sometimes contradictory conclusions. This contrast is also evident in lactylation modifications, where the effects are inconsistent not only across different cell types but even within the same cell type. Moreover, despite using identical

models and drug interventions, diverse outcomes have been observed, indicating that further, more comprehensive research is necessary to fully understand the role of lactylation modifications in ischemic brain injury.

Lactylation as a regulator of neuroinflammation in brain injury

Recent studies have demonstrated that histone lactylation plays a key role in modulating neuroinflammatory responses, especially in microglial cells. Histone H3 lysine 9 lactylation (H3K9la) has been shown to promote M1-type pro-inflammatory polarization of microglia via activation of the TNF-α signaling pathway, an effect that can be attenuated by inhibiting the histone acetyltransferase P300 or lactate-producing enzyme LDHA [95]. In Alzheimer's disease, a H4K12la–PKM2 positive feedback loop exacerbates microglial activation and promotes neurodegeneration, highlighting the role of lactylation in amplifying immune responses [96]. Similarly, in Parkinson's disease models, H3K9la drives SLC7A11 expression, which promotes glutamate toxicity and microglial dysfunction [97]. These findings reveal a broader role for lactylation beyond energy metabolism, implicating it in chronic neuroinflammatory conditions that are mechanistically and pathologically related to ischemic injury.In addition to classical writers like P300, aminoacyl-tRNA synthetases AARS1 and AARS2 have recently been recognized as enzymes capable of regulating global lactylation levels. Their activity may be influenced by β-alanine administration, which alters lactate-mediated transcriptional programs and inflammatory responses [98].These findings underscore the need to evaluate lactylation dynamics not only in neurons but also in glial subtypes, particularly microglia and astrocytes, as the cell-type specific outcomes of lactylation can be fundamentally different. Moreover, it raises a crucial hypothesis: lactylation may be beneficial when transient, serving as an adaptive response, but harmful when persistent, promoting chronic inflammation and secondary injury. The temporal window of lactylation activity during hypoxia and reperfusion may thus represent a critical therapeutic target.

The therapeutic potential and possible targets of lactylation modifications in ischemic brain injury

Research on lactylation modifications in ischemic brain injury is still at an early stage, with only a limited number of foundational studies conducted. Consequently, no clinical trials have tested drugs specifically targeting lactylation modifications, and no

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drugs have been developed with a focus on these modifications. However, as the body of research on lactylation modifications in ischemic brain injury continues to grow, targeting these modifications shows considerable potential for future therapeutic strategies in brain injury treatment. Therefore, based on the current body of research, we investigate potential targets for lactylation modifications in the treatment of ischemic brain injury, laying the groundwork for future research in this field.

Glycolytic enzyme inhibitors

One approach is to target key enzymes involved in glycolysis, aiming to reduce lactate production and thereby mitigate lactate accumulation in ischemic brain injury [99-101]. For example, experimental studies have shown the potential of drugs that target enzymes such as hexokinase, phosphofructokinase, and lactate dehydrogenase (LDH). Specifically, HK2 inhibitors have been shown to decrease lactate levels, which may help alleviate brain injury and improve neurological function [102-104]. Moreover, targeting pyruvate dehydrogenase kinase, which regulates the conversion of pyruvate into lactate, has demonstrated effectiveness in reducing lactate production and mitigating brain cell damage [105].

MCT inhibitors

Although inhibiting MCTs, particularly MCT1 and MCT4, has shown promise in reducing lactate efflux and mitigating brain injury in preclinical models of ischemic stroke, it is important to note that the complete inhibition of MCTs could lead to potential metabolic disturbances. The efflux of lactate via MCTs is crucial for maintaining cellular pH balance and preventing lactate accumulation. Therefore, while targeting MCTs may provide neuroprotective effects in the context of ischemic brain injury, the potential side effects of complete inhibition, such as the risk of metabolic acidosis or impaired cellular energy metabolism, need to be carefully considered. Further research is necessary to assess the therapeutic window and identify the optimal degree of MCT inhibition that would provide neuroprotection without inducing significant metabolic dysregulation [106-108].

Targeting lactylation or de-lactylation modifications

Targeting lactylation or de-lactylation modifications directly to treat ischemic brain injury seems to be a promising strategy, but before exploring the potential of lactylation modifications, it is essential to first investigate the levels of intracellular

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lactate. Lactate, as a byproduct of glycolysis, plays a crucial role in regulating lactylation, and understanding its intracellular concentrations is key to developing effective therapies. Recent research has revealed that the role of lactylation modifications in ischemic brain injury is highly intricate[87]. Lactylation can affect neuronal function by regulating both histone and non-histone proteins, which can either intensify pathological responses or suppress the expression of neuroprotective genes. Consequently, lactylation modifications can have a dual impact, potentially contributing to brain injury or offering neuroprotection, depending on the molecular interactions involved. Therefore, more in-depth research is needed to better understand the mechanisms through which lactylation modifications influence ischemic brain injury and to explore their therapeutic potential.

DISCUSSION

Ischemic brain injury is a leading cause of severe disability and mortality worldwide. Although treatments such as thrombolytic therapy and mechanical thrombectomy have shown some effectiveness in the acute phase, challenges remain due to the limited treatment window and the brain injury that occurs following reperfusion. Therefore, there has been increasing emphasis on developing innovative therapeutic strategies for ischemic brain injury. In recent years, lactylation modifications have emerged as a novel epigenetic mechanism, garnering significant attention from researchers, particularly for their effects on cellular metabolism, immune regulation, and neuronal function. Lactylation involves the modification of proteins by lactate molecules through acylation, altering their structure and function. As a byproduct of glycolysis, lactate plays a pivotal role in cellular energy metabolism and modulates protein function through lactylation, impacting cell survival, proliferation, and death. Studies have shown that lactylation in ischemic brain injury is a complex mechanism, potentially aggravating damage by enhancing inflammation and cell death or providing protection by improving cellular metabolism and preventing neuronal death. Recent research indicates that the effects of lactylation modifications on ischemic brain injury are closely related to lactate levels, its origins, and the specific sites and molecules targeted by lactylation.

Although some studies suggest that exogenous lactate supplementation may help alleviate ischemic brain injury, the impact of lactate varies significantly depending on

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the experimental conditions. In certain cases, lactate may provide protective benefits by regulating mitochondrial transfer and enhancing the functions of neurons and astrocytes. However, excessive lactate accumulation and increased lactylation modifications, particularly in specific cell types or target proteins, could exacerbate injury. Therefore, future research must focus on balancing lactate production with its modification effects, while also targeting the enzymes and molecules involved in lactylation modifications. Therapeutic strategies targeting key enzymes in the glycolytic pathway, such as hexokinase and lactate dehydrogenase, as well as lactate transporters like monocarboxylate transporters (MCTs), have shown significant promise. These approaches help reduce the production and accumulation of lactate, thereby reducing the incidence of ischemic brain injury. However, therapies targeting lactylation modifications are still in the early stages, with most research focusing on animal models and cell experiments, and lacking sufficient clinical evidence. Moving forward, further investigation is needed to explore the specific mechanisms of action of lactylation modification-based therapies across different forms of brain injury.

The dual role of lactylation modifications in ischemic brain injury offers valuable insights for research. While many aspects remain unclear, further exploration of the mechanisms underlying lactylation modifications and their interactions with other metabolic pathways could pave the way for more precise, targeted therapies for ischemic brain injury in the future. Consequently, the development of drugs aimed at lactylation modifications and associated metabolic pathways could provide new therapeutic options in clinical settings, leading to improved treatment outcomes and prognosis for patients suffering from ischemic brain injury.

However, there are contradictions in the findings of current literature, particularly in terms of experimental design and timing of interventions between studies. For example, Sun M et al. and Xiong XY et al. both explored the role of lactylation in ischemic brain injury, but their conclusions are contradictory due to differences in sample size, timing of interventions, and experimental design. Sun M et al.'s study did not implement randomization, had a small sample size, and lacked control over sample size, which may have limited the statistical significance of their findings. On the other hand, Xiong XY et al. conducted a more complex intervention design, but their study also showed discrepancies in drug administration timing, which could have impacted the outcomes due to inconsistent experimental conditions. These differences

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highlight the lack of standardization in current research and underscore the importance of research quality in interpreting results.Despite the valuable insights into lactylation's role in ischemic brain injury, contradictory results persist in the literature. These discrepancies likely arise due to several underlying factors that should be further explored:（1）Cell-Type Specificity:Different cell types, such as neurons, astrocytes, and microglia, may respond differently to lactylation. For instance, lactylation may play a protective role in neurons but exacerbate inflammation in glial cells. The cellular context in which lactylation occurs can significantly influence its functional outcome, contributing to contradictory results.（2）Timing of Lactate Surge:The timing of lactate accumulation during ischemic brain injury may determine whether it has a protective or damaging effect. Lactate levels rise rapidly during ischemia, but early lactate accumulation may help preserve cellular energy, while later-stage lactate buildup could lead to acidosis and exacerbate neuronal injury. Thus, the timing of lactate surge could be a key factor in reconciling conflicting data.（3）Dose-Dependent Effects:The concentration of lactate plays a critical role in determining its impact on ischemic brain injury. Low lactate concentrations may have protective effects by promoting cellular energy production and survival, whereas excessive lactate accumulation can lead to cellular toxicity, inflammation, and neuronal death. Future studies should aim to define the threshold at which lactate becomes detrimental.（4）Writer/Eraser Imbalance:The balance between lactylation 'writers' (enzymes that add lactate groups) and 'erasers' (enzymes that remove lactate groups) is crucial in determining the effects of lactylation. Dysregulation of this balance may lead to contrasting outcomes. For example, an excess of lactylation or inadequate removal of lactate could promote neuroinflammation and worsen injury, whereas balanced lactylation may be protective.These factors underscore the complexity of lactylation's role in ischemic brain injury and highlight the need for standardized experimental protocols that account for these variables. Understanding how these mechanisms interact will be key to resolving the current discrepancies in the literature.

Future studies should prioritize standardizing experimental designs, using appropriate sample sizes, and incorporating randomization and blinding. Further investigations should also focus on the cell-type specificity of lactylation effects and the temporal

dynamics of lactylation modifications to better understand lactylation's dual role in ischemic brain injury.

CONCLUSION

In conclusion, lactylation modification represents a novel and pivotal epigenetic mechanism in the pathophysiology of ischemic stroke, exhibiting a context-dependent dual role. Its net effect—whether neuroprotective or detrimental—is contingent upon a complex interplay of factors including cell-type specificity , the temporal dynamics and magnitude of the post-ischemic lactate surge, and the precise equilibrium between lactylation writers and erasers. While therapeutic strategies targeting glycolytic enzymes and lactate transporters (MCTs) hold significant promise by modulating lactate flux and subsequent lactylation, current evidence remains largely pre-clinical. Future research must prioritize standardized experimental designs, elucidate the precise spatiotemporal mechanisms of lactylation, and validate these findings in clinical settings to facilitate the development of targeted, lactylation-based neuroprotective therapies for ischemic brain injury.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Summary of conflicting reports on lactate and lactylation modifications in ischemic brain injury

Study	Model	Lactate / lactylation	Observed change	Mechanism / target	Effect on ischemic brain injury
Yao Y et al. [85]	Adult rat CIRI model	Lactylation	↑ Global Kla (1003 sites)	Descriptive proteomics; unclear function	Undetermined
Sun M et al. [86]	MCAO in mice	Lactylation	↑ MeCP2-K210/249 lactylation	↓ Pdcd4 and Pla2g6 → ↓ apoptosis	Protective
Zhou J et al. [90]	MCAO in mice	Lactylation (ARF1)	↑ ARF1 lactylation (↓ LRP1)	Inhibits mitochondrial transfer → ↑ injury	Detrimental
LCP1 study [91]	MCAO in rats	Lactylation (LCP1)	↑ LCP1 lactylation	Promotes infarction, edema, apoptosis	Detrimental
Xiong XY et al. [92]	MCAO + LDHA KO	Lactylation	↓ Kla via LDHA/p300 inhibition	↓ neuronal death, ↓ glial activation	Protective (via inhibition)
Pan XR et al. [93]	MCAO in mice + EA	Lactylation	↓ Lactate and Kla levels	↓ astrocyte activation, ↓ neuronal death	Protective
Song C et al. [94]	OGD-induced HUVECs + BHD	Lactylation	↓ H3K18la and pan-Kla	↓ Apaf-1, ↓ endothelial apoptosis	Protective
Sun M [86] vs.	MCAO models	Lactate supplementati	[86] ↓	Differ by intervention	Contradictory

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Xiong XY [92]	on	injury; [92] ↑ injury	timing

Note: The table summarizes representative studies showing either protective or detrimental effects of lactate and lactylation in ischemic brain injury. Conflicting findings often arise from differences in intervention timing, target proteins, or cell-specific responses. Symbols: → direction of signal transmission; ↑ rising signal; ↓ falling signal. Abbreviations: Kla: Lysine lactylation; MCAO: Middle cerebral artery occlusion; CIRI: Cerebral ischemia-reperfusion injury; BHD: Buyang Huanwu Decoction; LDHA: Lactate dehydrogenase A; KO: Knockout; OGD: Oxygen-glucose deprivation.

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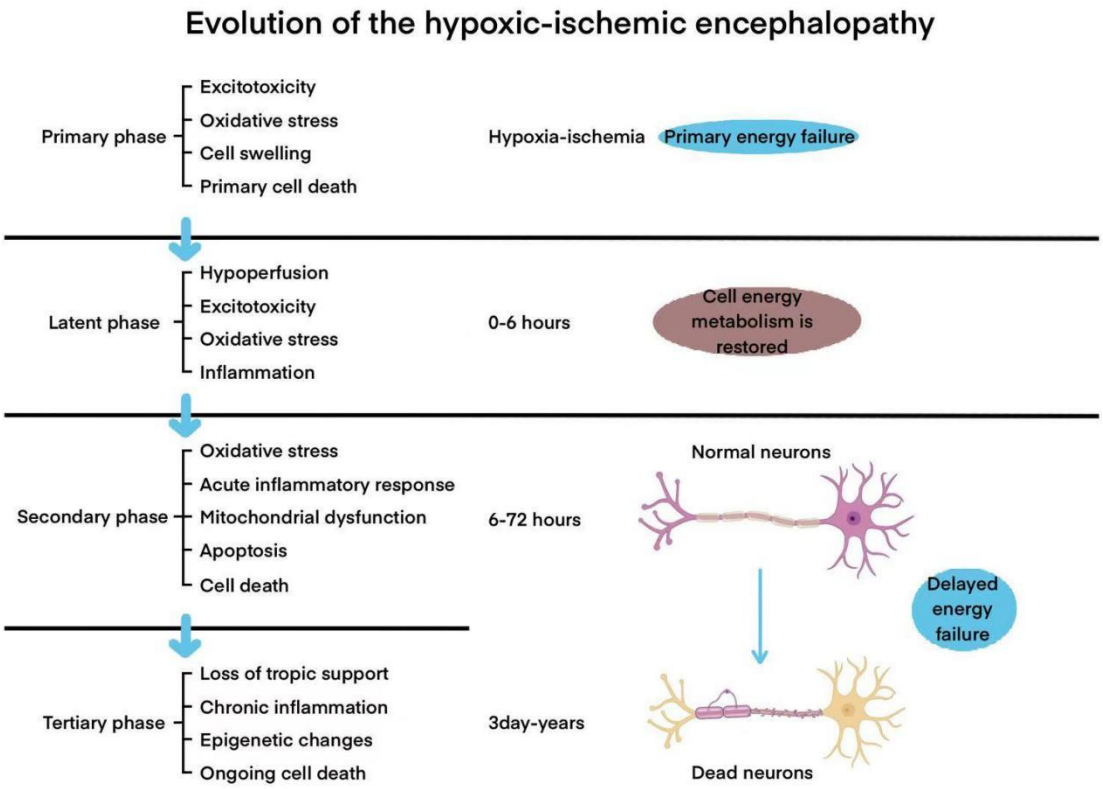

Figure 1. The progression of ischemic encephalopathy. Ischemic brain injury develops in four distinct stages. The first stage is marked by primary energy failure during hypoxic-ischemic events, which triggers detrimental effects, including ATP-dependent pump blockade, lactic acidosis, calcium ion buildup, excitatory amino acid release, toxic edema formation, and necrosis in the brain's most vulnerable regions. This is followed by a latent phase (pre-Stage 2), known as the energy recovery phase during resuscitation. In the subsequent 6-72 hours (Stage 2), the energy consumption process of the brain reoccurs in areas with greater resistance, maintaining excitotoxicity. This stage is characterized by a substantial influx of calcium ions, increased activation of neuronal NOS, oxidative stress, and mitochondrial dysfunction, ultimately leading to secondary energy failure and neuronal death through caspase pathway activation. Mitochondrial deterioration and an acute inflammatory response are key features of this stage, resulting in apoptosis. The third stage is defined by persistent inflammation and epigenetic changes. During this phase, oxidative stress causes direct damage to the central nervous system and activates a cascade of inflammatory responses, thereby accelerating the progression of Stage 3. Prolonged inflammation exacerbates the damage.

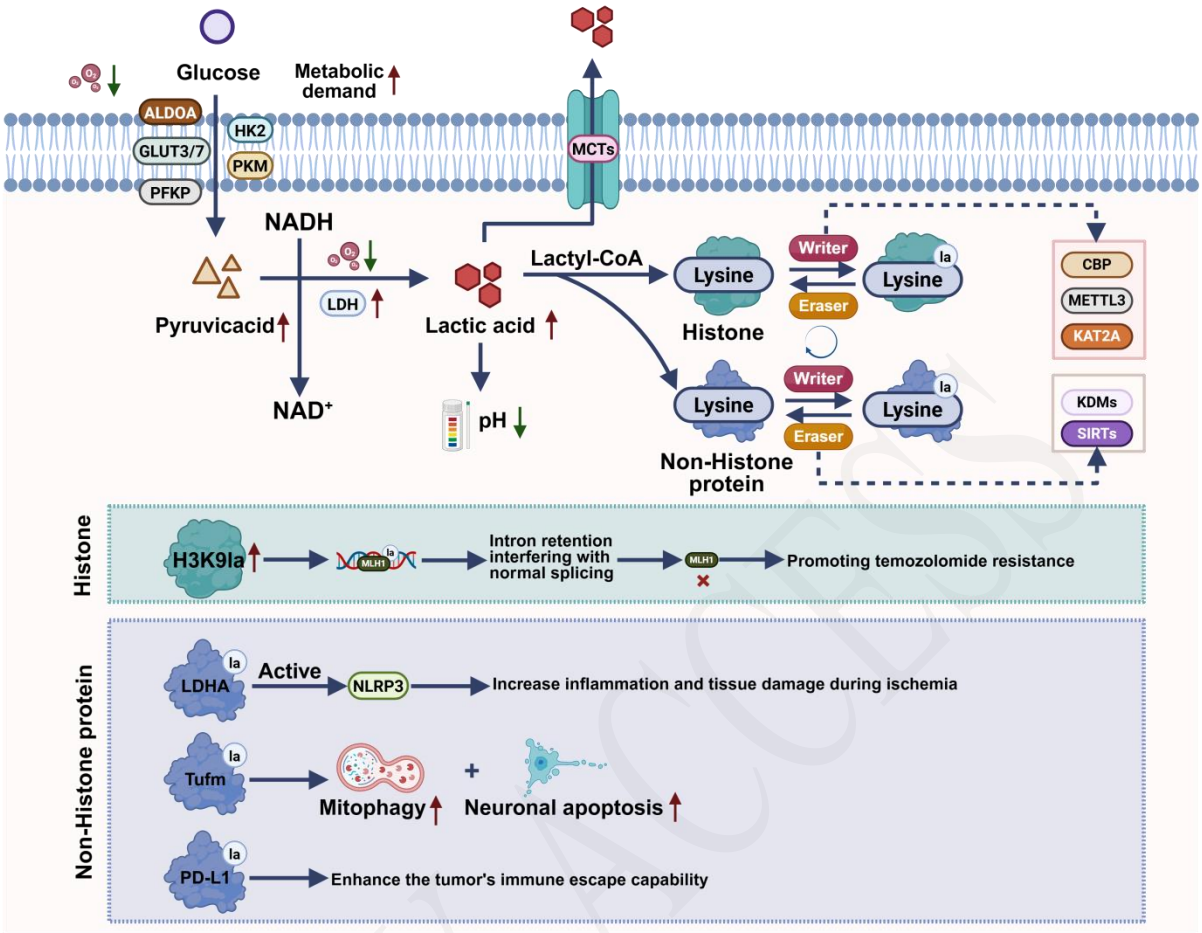

Figure 2. Diagram depicting the processes of glycolysis, lactate production, and lactylation modifications. Glycolysis generates lactate from pyruvate via LDH, with excess lactate exported by MCTs to balance pH. Lactate can form lactyl-CoA, driving lysine lactylation (Kla) on histone and non-histone proteins. Kla is regulated by specific "writers" (CBP, METTL3, KAT2A) and "erasers" (HDACs, KDMs, SIRTs). Histone lactylation (e.g., H3K9la) alters splicing and promotes temozolomide resistance, while non-histone lactylation enhances inflammation (NLRP3), mitophagy and neuronal apoptosis (Tufm), and immune evasion (PD-L1). This highlights lactate–lactylation as a key regulator in cancer and ischemic injury. Abbreviations: ALDOA: Aldolase A; GLUT1/GLUT3: Glucose transporter 1/3; HK2: Hexokinase 2; PKM: Pyruvate kinase M; PFKP: Phosphofructokinase, platelet type; LDH: Lactate dehydrogenase; NAD^+/NADH: Nicotinamide adenine dinucleotide (oxidized/reduced); MCTs: Monocarboxylate transporters; Lactyl-CoA: Lactyl-coenzyme A; H3K9la: Histone H3 lysine 9 lactylation; MLH1: MutL homolog 1; LDHA: Lactate

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dehydrogenase A; NLRP3: NOD-like receptor family pyrin domain-containing 3; Tufm: Tu translation elongation factor, mitochondrial; PD-L1: Programmed death-ligand 1; CBP: CREB-binding protein; METTL3: Methyltransferase-like 3; KAT2A: Lysine acetyltransferase 2A (GCN5); KDMs: Lysine demethylases; SIRTs: Sirtuins.

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