Review Article

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DOI: 10.4103/2394-8108.186256

Pyruvate dehydrogenase complex in cerebral ischemia-reperfusion injury

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Abstract:

Pyruvate dehydrogenase (PDH) complex is a mitochondrial matrix enzyme that serves a critical role in the conversion of anaerobic to aerobic cerebral energy. The regulatory complexity of PDH, coupled with its significant influence in brain metabolism, underscores its susceptibility to, and significance in, ischemia-reperfusion injury. Here, we evaluate proposed mechanisms of PDH-mediated neurodysfunction in stroke, including oxidative stress, altered regulatory enzymatic control, and loss of PDH activity. We also describe the neuroprotective influence of antioxidants, dichloroacetate, acetyl-L-carnitine, and combined therapy with ethanol and normobaric oxygen, explained in relation to PDH modulation. Our review highlights the significance of PDH impairment in stroke injury through an understanding of the mechanisms by which it is modulated, as well as an exploration of neuroprotective strategies available to limit its impairment.

Key words:

Autologous embolus, combination therapy, ischemia/reperfusion injury, oxidative injury, pyruvate dehydrogenase kinase, reactive oxygen species, tissue plasminogen activator

Introduction

In the United States, stroke is the leading cause of adult disability and the fourth leading cause of death.[1] Ischemic stroke, which accounts for approximately 80% of all stroke cases, is caused by occlusion of a major blood vessel in the brain and results in a lack of adequate blood flow to meet metabolic demand.[2] Given the brain's heavy dependence on oxidative metabolic activity and its absence of alternate energy stores, the insufficient delivery of oxygen and glucose contributes to a state of metabolic disorder. The resultant oxidative stress promotes mitochondrial dysfunction, calcium accumulation, and reactive oxygen species (ROS) generation.[3,4] Impairment of mitochondrial enzymes can limit aerobic metabolism and adenosine triphosphate (ATP) generation, ultimately leading to a situation of energy failure and neuronal cell death.[5] Pyruvate dehydrogenase (PDH) complex is a mitochondrial enzyme with a known sensitivity to inactivation during stroke injury.[6,7] The effects of PDH impairment can be particularly devastating due to this enzyme's critical role as the sole link between anaerobic and aerobic cerebral energy metabolism.[8] As such, PDH is an important target of not only ischemia-reperfusion injury but also therapeutic interventions aimed at restoring oxidative energy metabolism.

Pyruvate Dehydrogenase Complex: Structure, Regulation, and Function

Found exclusively in the mitochondrial matrix, PDH catalyzes the conversion of pyruvate to acetyl coenzyme A and, in turn, serves as the metabolic gateway between glycolysis and the tricarboxylic acid cycle. This reaction is critical for generating the reduced form of nicotinamide adenine dinucleotide (NADH), the reducing power of which is utilized by the mitochondrial electron transport chain to drive oxidative phosphorylation.[8] PDH comprises numerous subunits of three enzymes: Pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Activity of PDH is dependent on the presence of five associated coenzymes, namely, nicotinamide adenine dinucleotide (NAD^+), thiamine pyrophosphate, lipoic acid (LA), coenzyme A, and flavin adenine dinucleotide.[9]

PDH's critical role in controlling the flow of metabolites between two major pathways lends it to tight regulation by various

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Submission: 10-04-2016
Revised: 02-06-2016
Accepted: 14-06-2016

How to cite this article: Thibodeau A, Geng X, Previch LE, Ding Y. Pyruvate dehydrogenase complex in cerebral ischemia-reperfusion injury. Brain Circ 2016;2:61-6.

mechanisms. End-product inhibition by either acetyl coenzyme A or NADH is one of the modes of inactivating the complex.[10] Activity of PDH is also influenced by phosphorylation and dephosphorylation events catalyzed by pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP), respectively. Site-specific phosphorylation of the E1α subunit of PDH results in the inhibition of the entire enzyme complex whereas dephosphorylation of E1α activates the complex.[11] Cellular levels of Ca^{2+}, Mg^{2+}, and ATP/adenosine diphosphate (ADP) act as additional modulators of PDH activity.[9]

Pyruvate Dehydrogenase Susceptibility in Ischemia-reperfusion

PDH's inherent complexity, strict cofactor requirements, and tight regulation make it the likely target for damage and subsequent downregulation during stroke injury.[9] Several ischemia-induced effects on cerebral energy metabolism implicate PDH impairment in stroke injury propagation. A loss of PDH activity following ischemic insult may explain why aerobic glucose metabolism decreases while oxidative metabolism of other fuels, including glutamate, γ-aminobutyric acid, and glutamine, increases.[12] The observed hyperoxidation of NAD(H) and electron transport chain components during reperfusion provides additional evidence implicating PDH impairment in ischemia-reperfusion injury.[9,13] The finding that impaired NADH production, as opposed to utilization, is responsible for compromised oxidative phosphorylation suggests that the complicating factor lies upstream of the electron transport chain as is the case for PDH.

Pyruvate Dehydrogenase in Oxidative Injury

The reduction in brain perfusion that occurs during ischemic stroke sets up an anoxic state, impairing oxidative phosphorylation and limiting production of essential cerebral metabolites. Diminished cerebral blood flow also leads to the overproduction of ROS due, in part, to an accumulation of ADP, and the disruption of ion homeostasis, namely, elevated levels of intracellular Ca^{2+} and Na^{+}.[14] Oxidative stress and subsequent ROS generation are known to play an important role in pathogenesis of stroke by disruption of mitochondrial activity.[4] Furthermore, ROS have been shown to impair activity of PDH.[8,15,16] Studies have revealed that elevated ROS production with a corresponding decrease in levels of PDH activity and expression occurs during stroke.[17] Inactivation of PDH, which serves as a key moderator of oxidative phosphorylation, not only limits the normal production of ATP but also further increases the generation of ROS upon reperfusion. Such enhancement of ROS propagates oxidative damage to proteins, lipids, nucleic acids, and other cellular targets.[4] Thus, PDH downregulation potentiates metabolic stress and, in turn, exacerbates neuronal injury.[18] The neuroprotective influence of interventions that facilitate the removal of PDH from ROS inhibition may be understood by their ability to promote metabolic recovery through restoration of mitochondrial oxidative metabolism.

Mechanisms of Neurodysfunction in Relation to Pyruvate Dehydrogenase

Oxidative stress

The decrease in PDH activity observed during ischemic stroke may be attributable to one or several potential mechanisms of enzyme inactivation. Oxidative stress is widely acknowledged as one such mechanism responsible for the loss of PDH activity following reperfusion.[8,19] The mitochondrion is both a major source of ROS and a primary target of oxidative damage, with PDH being one of the principal mitochondrial constituents susceptible to inactivation.[8] Studies indicate that levels of the ROS superoxide ($\cdot O_2^-$), hydroxyl radical (·OH), nitric oxide (·NO), and peroxynitrite (ONO_2^-) are enhanced during reperfusion of ischemic tissues.[20,21] The influence of these free radicals and oxidants on PDH activity was investigated by incubating PDH in the presence of a superoxide radical-generating system (xanthine oxidase/hypoxanthine). This ROS exposure resulted in a concentration-dependent decrease in PDH enzymatic activity. In addition, this inactivation was partially disrupted by the antioxidant superoxide dismutase and almost completely prevented by catalase exposure.[16] Similarly, hydroxyl radical and peroxynitrite were each found to induce an inhibitory effect on purified porcine PDH activity.[8] Peroxynitrite, a reactive nitrogen species (RNS) that is formed by the reaction of superoxide with nitric oxide, is known to oxidatively modify proteins by S-nitrosylation and tyrosine nitration.[22,23] Elevation of 3-nitrotyrosine, an established biomarker of "nitroxidative stress," is associated with a reduction in PDH activity in the hippocampus of animals subjected to a hyperoxic resuscitation protocol.[8] The molecular mechanism by which peroxynitrite and other oxidative species inhibit PDH enzymatic activity has been explored by investigating the exposure of rat brain to 1,3-dinitrobenzene (1,3-DNB), an inducer of oxidative stress. Evidence shows that 1,3-DNB-induced inhibition of PDH is linked to a reduction in LA immunoreactivity, coenzyme modification as a potential mechanism of redox-based PDH dysfunction. Moreover, alpha-ketoglutarate dehydrogenase, which is structurally very similar to PDH, is not as sensitive to 1,3-DNB, further implicating PDH as the molecular target lying upstream of the hyperoxidized electron transport chain during reperfusion.[24] An alternative mechanism of RNS-mediated modulation of PDH activity that has been proposed is the disruption of enzyme complex dephosphorylation, thereby maintaining PDH in an inactive state. However, *in vitro* models demonstrate that peroxynitrite targets purified, dephosphorylated PDH following exposure to reperfusion, suggesting that the enzyme in its active, rather than inactive, state is subjected to modification.[8]

Altered regulatory control by pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase

Altered regulatory control of PDH via the phosphorylating and dephosphorylating enzymes PDK and PDP, respectively, has also been explored as a potential source of PDH impairment during ischemia-reperfusion injury. So far, four isozymes of PDK (PDK1-4) and two isozymes of PDP (PDP1-2) have been identified, with PDK2 and PDP1 found to be the most abundantly expressed in rat brain.[10,25] A change in the relative

rates of these PDH-specific kinases and phosphatases has been examined as a means of reducing the proportion of active complex during reperfusion.

Impairment of PDP is one such mechanism that has been proposed in the reduction of PDH activity.[8] Since PDP is responsible for activating PDH by removal of a phosphate group, it is reasonable to assume that preventing this dephosphorylation from occurring would have an inhibitory effect on enzymatic activity. Preclinical studies evaluating the mechanism of traumatic brain injury (TBI) identified increased expression of PDK2 and decreased expression of PDP1. The results suggest that changes in these regulatory protein levels may maintain PDH in a hyperphosphorylated state, contributing to the impaired oxidative glucose metabolism characteristic of both TBI and stroke.[25,26] In addition, speculation of PDP deficiency as the cause behind certain cases of chronic congenital lactic acidosis has prompted investigation into whether PDP loss may explain the similar accumulation of lactic acid that occurs during the ischemic cascade.[27] To experimentally examine the possibility of altered PDP expression as a mechanism underlying brain injury, samples collected from animals exposed to ischemia followed by hyperoxic reperfusion were prepared in the presence of exogenous PDP plus its required divalent metal ions Mg^{2+} and Ca^{2+}, ensuring complete dephosphorylation and, thus, maximal PDH activation. The lack of significant difference in PDH activity when compared to samples prepared without supplementation suggests that PDP impairment is not a direct cause of the reduced PDH activity observed in reperfusion injury.[8]

Alternatively, upregulation of PDK, which enhances PDH phosphorylation and subsequent inactivation, could account for the reduced PDH activity observed in ischemic injury.[8] The ATP-dependent PDK isozymes are bound to PDH's E2 domain and phosphorylate any of three specific serine residues of the E1α subunit to inactive the enzyme complex. The kinase reaction rate is influenced by relative amounts of a number of mitochondrial metabolites.[10] Elevated ratios of ATP/ADP, NADH/NAD^+, and acetyl CoA/CoA, as well as reduced pyruvate concentration, increase the rate of phosphorylation by PDK.[28] *In vitro* evidence has also identified a specific interaction between PDK2 and the delta isoform of the signal transducer protein kinase C (δPKC), which results in activation of PDK2. Redox-dependent translocation of δPKC to the mitochondria upon reperfusion has been found to be associated with PDK2 activation and, in turn, PDH inhibition. Furthermore, disruption of this process by infusion of the δPKC inhibitor, Tat-$\delta_{V1\text{-}1}$, prevented δPKC translocation and resulted in almost complete regain of PDH activity.[29] Increased expression of hypoxia-inducible factor 1-alpha (HIF1α) may be another mode of PDK2-mediated inhibition of PDH. Using PDH deficient fibroblasts, which are similar to hypoxic cells in that they exhibit increased glycolysis, lactate accumulation, and diminished oxidative phosphorylation, researchers have demonstrated a 1.5-fold enhancement of HIF1α expression.[30] HIF1α is known to induce transcription of an array of genes related to glucose metabolism, including that for PDK.[31] Thus, enhanced HIF1α expression may contribute to the metabolic dysfunction observed in ischemic stroke injury by PDK-mediated modulation of PDH.

Loss of pyruvate dehydrogenase activity

Other studies propose that the reperfusion-induced reduction in PDH activity is attributable to a loss of total PDH activity, rather than the modulation of reversible phosphorylation events.[6,7,32-34] Mitochondria extracted from the postischemic dorsolateral striatum and prepared under fixed phosphorylation conditions demonstrated significant losses in PDH activity when subjected to 3 h (29% decrease), 6 h (36% decrease), and 24 h (57% decrease) of recirculation. In comparison, no significant changes were measured in the activity of two other mitochondrial enzymes, alpha-ketoglutarate dehydrogenase and NADH-cytochrome *c* oxidoreductase.[32] Such PDH-specific loss of total activity further emphasizes the critical role this enzyme complex plays in impaired energy metabolism following reperfusion.

Mechanisms of Neuroprotection in Relation to Pyruvate Dehydrogenase

Antioxidants

Given the critical role of PDH in cerebral energy metabolism and the notion that its reduced activity contributes to ischemic brain injury, interventions that prevent PDH inhibition or that compensate for its impairment have been explored as neuroprotective strategies. Based on data implicating oxidative stress as a cause of PDH inactivation, it is reasonable that antioxidants should provide a beneficial effect. Alpha-LA has been identified as a potent metabolic antioxidant that may serve as an ideal treatment for ischemic injury involving free radical processes.[35-38] The influence of R-(+)-alpha-LA, the naturally occurring enantiomer of LA, on pyruvate metabolism has been documented in primary cultured hepatocytes isolated from 24 h fasted rats. The results showed enhanced pyruvate oxidation and decreased gluconeogenesis. Of note, these changes were associated with significant increases in the activation state of PDH, which may reflect a return of normal metabolic function conferred by antioxidant therapy.[39]

While exogenous antioxidants may improve mitochondrial resistance to oxidative stress, another promising approach utilizes pharmacologic stimulation of endogenous gene expression to protect against metabolic dysfunction.[40] The transcriptional activating factor Nrf2 regulates expression of many genes encoding mitochondrial antioxidant enzymes, as well as targets of oxidative stress.[41,42] Of note, Nrf2 has been found to exert control over key mediators of cellular energy metabolism, including pyruvate dehydrogenase lipoamide β and PDK. Stimulation of the Nrf2 pathway by sulforaphane, a molecule with known antioxidative effects that is obtained from cruciferous vegetables, has proven effective in reducing brain infarct volume and increasing expression of the stress-response protein, heme oxygenase-1, in a rat model of focal ischemic stroke.[43] These results, coupled with additional findings of reduced flux through the PDH pathway in Nrf2 knockdown cells, suggest that PDH or its regulators may be of those proteins under Nrf2 influence.[44] The critical role that PDH plays in energy metabolism and its vulnerability to oxidative stress may explain the protective effect that genetic manipulation by Nrf2 pathway activation has upon cerebral ischemic injury.

Combination therapy with ethanol and normobaric oxygen

Dose-dependent neuroprotection by ethanol (EtOH) has been observed in rat models of middle cerebral artery occlusion.[45,46] EtOH has been found to raise expression levels of PDH and PDP and decrease those of PDK. Other signs of improved oxidative metabolism, including reduced ROS levels, lower ADP/ATP ratios and fewer neurological deficits, accompanied these changes. When these same parameters were assessed in rats treated with EtOH + normobaric oxygen (NBO), it was found that combination therapy conferred a greater therapeutic effect than each agent alone.[17] EtOH's ability to reduce energy demands and to inhibit glucose metabolism more likely accounts for the limited ROS generation detected in EtOH treatment groups.[47,48] Removal of PDH from ROS-mediated inhibition promotes oxidative metabolism and is, therefore, one of the mechanisms that have been proposed in EtOH-induced neuroprotection. Conversely, NBO has been utilized to counteract ischemia-induced hypoxic conditions. Although NBO has been reported to confer neuroprotective effects during ischemic events when administered in clinical settings, its limited time window for efficacy and minor therapeutic effect limit its potential for clinical application. However, when administered concomitantly, NBO enhances the effects of EtOH, evidenced by a greater attenuation of impaired PDH activity and protein expression, which may reflect further facilitation of aerobic metabolism.[49-54] While further studies are needed to characterize PDH modulation by EtOH and NBO at the molecular level, their role in stabilizing cerebral energy metabolism makes these agents promising neuroprotectants in ischemic stroke injury.

Dichloroacetate

Dichloroacetate (DCA), a pharmacologic agent that activates PDH by inhibiting PDK, has also revealed significant neuroprotective potential.[55-57] Administration of DCA has been shown to enhance regional lactate removal and limit the lactic acidosis associated with brain hypoperfusion and metabolic dysregulation.[58-61] In addition, a proton magnetic resonance spectroscopy study revealed that DCA delivered in high dose or within 2 days of ischemic stroke produced similar reductions in lactate levels.[62] Treatment with DCA appears to be most effective during reperfusion by enhancing the postischemic reactivation of PDH.[63-65] This effect on PDH activity has been demonstrated in rat and gerbil models of cerebral ischemia, which exhibited a reduction in lactate levels in addition to a restoration of ATP and phosphocreatine levels later on in reperfusion, but displayed no demonstrable effect during the ischemic phase.[57,66] These findings of reduced lactate production and increased oxidative energy metabolism by DCA administration further implicate PDH impairment in the delayed cerebral energy failure that occurs after ischemic insult. By enhancing activity of the rate-limiting enzyme that links pyruvate production with pyruvate oxidation, DCA promotes oxidative metabolic recovery. Despite early recognition of DCA's selectivity and ease of delivery, clinical studies have raised concern regarding its potential toxicity.[67-69] This includes a randomized, controlled clinical trial evaluating the efficacy of 25 mg/kg/day DCA in patients with mitochondrial encephalopathy with lactic acidosis and stroke-like episodes that resulted in early termination due to associated peripheral nerve toxicity.[70]

Acetyl-L-carnitine

Acetyl-L-carnitine (ALCAR) is an endogenous metabolic intermediate which has been shown to be neuroprotective in cerebral ischemia models when administered at supraphysiologic doses.[71-74] Human and animal studies suggest that ALCAR's neuroprotective effect is derived from its restoration of oxidative energy metabolism. Delivery of ALCAR acetyl groups to the tricarboxylic acid cycle is understood to improve aerobic energy metabolism by providing a fuel supply alternative to pyruvate, allowing for circumvention of the PDH pathway.[75] This hypothesis of metabolically mediated neuroprotection by ALCAR is supported by rat models of global cerebral ischemia. These models exhibit reductions in lactate and inorganic phosphate levels, along with elevations in levels of ATP and creatine-phosphate,[71] consistent with the metabolism of ALCAR acetyl units, and indicative of augmented oxidative cerebral energy production, and diminished anaerobic glycolysis and lactic acidosis. Another proposed mechanism of ALCAR-mediated neuroprotection is by relief of oxidative tissue injury.[76] This effect has been demonstrated in a canine cardiac arrest model by the substance's ability to limit protein carbonyl formation, a marker of oxidative stress, in brain tissue during reperfusion.[77] PDH's critical function in oxidative energy metabolism and its known susceptibility to inactivation by ROS support the role ALCAR may play in attenuating the mitochondrial dysfunction observed during ischemic stroke injury by either preventing PDH inhibition or compensating for its impairment.

Conclusion

The structural and regulatory complexity of PDH, coupled with its important role in aerobic cerebral energy metabolism, makes it a vulnerable and potentially destructive target in ischemia-reperfusion injury. PDH's known reduction in activity upon reperfusion has encouraged exploration of the mechanisms behind its impairment and has led to the identification of oxidative stress and altered regulatory enzymatic control as the likely mediators. As discussed, such investigation has also elucidated the influence of PDH impairment upon metabolic and, consequently, neuronal dysfunction during stroke. This understanding has guided studies focused on evaluating the ability of various agents to protect against PDH impairment by means of PDH upregulation or via the impediment of PDH inhibition. Studies indicate that agents that are able to restore PDH activity are associated with improvements in oxidative energy metabolism and, therefore, may be an efficacious means of conferring neuroprotection.

Financial support and sponsorship

This work was supported in part by the American Heart Association Grant-in-Aid (14GRNT20460246), Merit Review Award (I01RX-001964-01) from the US Department of Veterans Affairs Rehabilitation R and D Service, the National Natural Science Foundation of China (81501141), and the Beijing New-star Plan of Science and Technology (xx 2016061).

Conflicts of interest

There are no conflicts of interest.

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