Renal Cortical Pyruvate as a Potentially Critical Mediator of Acute Kidney Injury

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Key Words

Pyruvate · Acute kidney injury · Injury-induced depletion

Abstract

Pyruvate is a key intermediary in both aerobic and anaerobic energy metabolisms. In addition, a burgeoning body of experimental literature indicates that it can also dramatically impact oxidant, proinflammatory, and cytoprotective pathways. In sum, these actions can confer protection against diverse forms of tissue damage. However, the fate of pyruvate during the evolution of acute kidney injury (AKI) has remained ill defined. Recent experimental studies have indicated that following either ischemic or nephrotoxic renal injury, marked and sustained pyruvate depletion results. While multiple potential mechanisms for this pyruvate loss may be involved, experimental data suggest that a loss of lactate (a dominant pyruvate precursor) and enhanced gluconeogenesis (i.e. pyruvate utilization) are involved. The importance of pyruvate depletion for AKI pathogenesis is underscored by observations that pyruvate therapy can attenuate diverse forms of experimental AKI. This protection may stem from reductions in tissue inflammation, improved anti-inflammatory defenses, and an enhanced cellular energy metabolism. The pieces of information that give rise to these conclusions are discussed in this brief report.

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Introduction

Pyruvic acid, a key glucose metabolite, sits at the crossroads of anaerobic and aerobic energy production. Under aerobic conditions, pyruvate undergoes decarboxylation by pyruvate dehydrogenase, yielding acetyl CoA. Alternatively, it can undergo carboxylation by pyruvate carboxylase, forming oxaloacetate. With acetyl CoA and oxaloacetate entry into, and metabolism within, the Krebs cycle, aerobic ATP production results. Conversely, under conditions of tissue ischemia or hypoxia, lactate dehydrogenase (LDH) converts pyruvate into lactic acid, potentially producing tissue acidosis. This conversion has two potentially important consequences. First, NAD is generated from NADH, which then facilitates anaerobic ATP production via glycolysis. Second, it is now relatively well accepted that tissue acidosis per se can confer dramatic protection against hypoxic/ischemic tissue damage [1]. Indeed, the well-known phenomenon of ‘reperfusion injury’ is likely mediated, at least in part, by the correction of tissue acidosis during reperfusion, hence the loss of the cytoprotective effects of acidosis [1].

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Pyruvate as a Cytoprotective Molecule

Within the past decade, it has become increasingly apparent that, in addition to its central role in energy metabolism, pyruvate can impact a host of potential injury pathways. Seminal studies by Nath and colleagues pointed out that, as an α -ketoacid, pyruvate is capable of scavenging hydrogen peroxide by undergoing decarboxylation and yielding CO_2 and water [2, 3]. The relevance of this pathway is that oxidative stress is a key determinant of most forms of acute as well as chronic kidney injury [4]. Thus, pyruvate stores represent a potentially important antioxidant defense, e.g. analogous to glutathione. Support for the role of pyruvate as an antioxidant is underscored by the demonstration of Nath and colleagues that exogenous pyruvate therapy can attenuate the glycerol model of rhabdomyolysis-induced acute renal failure, which is known to be mediated in part by oxidative stress [2, 3]. More recently, it has become apparent that pyruvate also possesses potent anti-inflammatory effects. In support of this concept are demonstrations that pyruvate can protect against diverse injury pathways in which inflammation plays a critical pathophysiologic role [e.g. the cecal ligation and puncture model of sepsis-induced acute kidney injury (AKI), and tissue inflammation following experimental myocardial infarction and stroke] [5–9]. However, the pathways by which pyruvate exerts its anti-inflammatory effects have remained poorly defined.

AKI Induces a Relative Pyruvate Depletion State

While the literature supports an expanding potential role for pyruvate as a prophylactic agent for protection against experimental and perhaps clinical AKI, the fate of endogenous pyruvate in the aftermath of AKI has until recently remained undefined. We recently questioned whether AKI might deplete endogenous pyruvate stores and thus potentially contribute to ongoing renal damage. A correlate of such an event would be the possibility that pyruvate might have therapeutic utility by replenishing diminished pyruvate stores. To explore this possibility, we recently measured pyruvate concentrations in renal cortical tissues following renal ischemic/reperfusion injury and during the evolution of the glycerol model of rhabdomyolysis-induced acute renal failure [10]. In response to progressive renal ischemia, induced by renal pedicle cross clamping in the mouse, time-dependent reductions in pyruvate levels occurred. Thus, after 60 min of ischemia, >50% pyruvate depletion was observed. As

expected, these pyruvate reductions were quantitatively matched by reciprocal increases in tissue lactate concentrations. However, upon vascular reflow, a surprising result was obtained. Lactate levels fell, but surprisingly they reached subnormal levels (approx. 50% of controls). Furthermore, the pyruvate depletion persisted, indicating that the falling lactate was not simply due to conversion back to the pyruvate pool. To test whether these results were unique to ischemic renal injury, pyruvate and lactate levels were measured in the glycerol AKI model. Again, falling renal cortical pyruvate and lactate levels were observed [10]. Thus, two questions emerged: first, why was lactate not simply converted back to pyruvate, thus replenishing the depleted pyruvate stores? And second, why did pyruvate levels continue to fall, even at a time when lactate levels had stabilized at subnormal values?

Potential Mechanisms Leading to the Depletion of Lactate and Ultimately Pyruvate (see fig. 1)

To address the first issue of falling tissue lactate levels during vascular reperfusion, we hypothesized that lactate was egressing from damaged tubules, thereby leading to a relative lactate depletion. Two pieces of evidence supported this concept [9]. First, following ischemic injury, a 'step up' in plasma lactate concentrations was observed, implying an exodus from injured tubules. Second, when isolated proximal tubules were subjected to *in vitro* injury, lactate readily exited damaged cells even in the absence of lethal cell damage. Second, in response to AKI, progressive LDH reductions were observed. Indeed the degree of renal cortical LDH decrement was strongly correlated with renal injury severity [11]. Thus, we hypothesized that with a reduction in cellular LDH, the ability of cells to convert lactate back to pyruvate is reduced and thus lactate is relatively free to efflux from damaged cells into the systemic circulation.

In addition to a loss of pyruvate substrate (i.e. lactate), there are other potential mechanisms for a failure of pyruvate reconstitution following the induction of AKI. One such possibility would be a failure of glucose conversion to pyruvate via glycolysis. However, the classical view is that proximal tubules are unable to perform glycolysis due to relatively low levels of glycolytic enzymes [12]. Despite the wide acceptance of this principle, we were able to demonstrate, both *in vivo* and *in vitro*, that marked proximal tubule glycolysis can indeed occur. Previous work from Dickman and Mandel [13] and Gullans et al. [14] brilliantly illustrates this point. They observed

that when rabbit proximal tubules were subjected to hypoxia (but not anoxia) or to antimycin-induced mitochondrial blockade, glycolysis was induced. Given these observations, it appears that a failure of glycolysis cannot explain the AKI induction of pyruvate depletion.

A third possibility for postischemic pyruvate depletion that was explored was the converse of diminished glycolysis: i.e. post-AKI-induced enhanced gluconeogenesis. In this scenario, the relative pyruvate depletion would arise via its possible conversion to glucose. Indeed, the experimental data [10] obtained strongly supported this notion based on 3 sets of observations: (i) in the aftermath of ischemia- or glycerol-induced AKI, increased renal cortical glucose levels were observed; (ii) when AKI mice, but not normal mice, were given an exogenous pyruvate load, increased renal cortical glucose formation resulted, and (iii) a correlate of the increase in glucose levels was a concomitant increase in renal cortical glycogen content. Thus, the increases in glucose reflected increased production rather than glycogenolysis.

A fourth explored possibility was that pyruvate could be depleted as a result of injury-induced H_2O_2 production, leading to pyruvate decarboxylation, as discussed above. However, we found that in both the glycerol and the ischemic AKI models the H_2O_2 levels fell rather than rose, and paradoxically pyruvate administration normalized the H_2O_2 content, likely due to improved mitochondrial electron transport with increased superoxide/ H_2O_2 production, as will be discussed. Finally, we questioned whether pyruvate depletion could have resulted from increased pyruvate conversion to acetyl CoA via the action of pyruvate dehydrogenase. However, this pathway seemed implausible given that both ischemic and glycerol-induced AKI significantly lowered rather than raised pyruvate dehydrogenase levels [10]. Thus, based on all of the available evidence, the 3 dominant pathways by which AKI would appear to induce pyruvate depletion are: (1) lactate (pyruvate precursor) loss; (2) relative LDH depletion, causing a failure of lactate capture, and (3) enhanced pyruvate consumption via gluconeogenesis, culminating in post-AKI increments in both glucose and glycogen pools.

New Insights into Pyruvate-Mediated Protection against AKI

Given that both ischemic- and glycerol-induced AKI were associated with reductions in renal cortical H_2O_2 levels, and given that pyruvate therapy paradoxically re-

Fig. 1. Metabolic pathways leading to potential changes in tissue pyruvate levels. Pyruvate is produced from glucose via the glycolytic pathway. Conversely, pyruvate may be consumed via its conversion back to glucose and glycogen via the process of gluconeogenesis. LDH is a bidirectional enzyme, either converting pyruvate to lactate or vice versa. Thus, LDH is a critical determinant of tissue pyruvate levels. LDH, like lactate, can efflux from sublethally damaged cells. The consequence of these changes is presumably the depletion of lactate and secondarily pyruvate. Under the action of pyruvate decarboxylase, an enzyme complex, pyruvate can ultimately be converted to acetyl CoA with subsequent metabolism via the Krebs cycle. Finally, pyruvate can be consumed by serving as an H_2O_2 scavenger.

stored them towards normal, alternative potential interactions between pyruvate therapy and evolving AKI were addressed [10]. As a first step, we were able to confirm that, indeed, pyruvate administration conferred marked protection against both glycerol-mediated AKI and a chemical model of renal ischemia: maleate nephrotoxicity [15]. With this information in hand, new potential insights were sought. To assess the degrees of tissue inflammation in these models, two proinflammatory mediators, i.e. $TNF-\alpha$ and monocyte chemoattractant protein 1 (MCP-1) mRNA levels, were assessed. In both cases, pyruvate therapy led to striking reductions in the mRNAs of these proinflammatory molecules. To examine the potential mechanisms for these reductions, we tested wheth-

er pyruvate therapy led to an increase in two anti-inflammatory and cytoprotective molecules, i.e. interleukin-10 and heme oxygenase-1. In both cases, their mRNA levels rose in response to pyruvate therapy [10]. Lastly, given that pyruvate sits at the crossroads of both anaerobic and aerobic energy metabolisms, tissue ATP concentrations with and without pyruvate therapy were assessed, and indeed pyruvate induced modest ATP increases. That ATP and H_2O_2 levels rose in a concomitant fashion is consistent with increased electron transport down the mitochondrial electron transport chain, leading to both increased ATP and mitochondrial H_2O_2 production. Hence, the rising H_2O_2 levels were, in a sense, a marker of improved energetics rather than simply a potential mediator of oxidative stress. The relative importance of each of these changes for the overall capability of pyruvate to induce renal cytoprotection will require much more in-depth exploration.

Conclusions

Although it is well recognized that pyruvate is a key substrate in energy metabolism, increasing evidence indicates that it can have protean metabolic actions that can directly impact the evolution of AKI. Exogenous pyruvate administration is able to capture at least some of these actions and thus confer protection against diverse forms of AKI. Alternatively, it is important to recognize that a consequence of AKI is rapid and sustained pyruvate depletion which can potentially contribute to ongoing tissue damage. Further studies will be required to determine the potential of pyruvate not just as a prophylactic but also as a therapeutic agent, with the target being reconstitution of injury-induced depletion of pyruvate stores.

Acknowledgments

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