Pyruvate in the Correction of Intracellular Acidosis: A Metabolic Basis as a Novel Superior Buffer

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Key Words

Pyruvate · Lactate · Bicarbonate · Glucose · Ischemia · Intracellular pH · Acidosis

Abstract

The review focuses on biochemical metabolisms of conventional buffers and emphasizes advantages of sodium pyruvate (Pyr) in the correction of intracellular acidosis. Exogenous lactate (Lac) as an alternative of natural buffer, bicarbonate, consumes intracellular protons on an equimolar basis, regenerating bicarbonate anions in plasma while the completion of gluconeogenesis and/or oxidation occurs via tricarboxylic-acid cycle in mitochondria mainly in liver and kidney, or heart. The general assumption that Lac is 'metabolized to bicarbonate' in liver to serve as a buffer has been questioned. Pyr as a novel buffer would be superior to conventional ones in the correction of metabolic acidosis. Several likely biochemical mechanisms of Pyr action are discussed. Experimental evidence, in vivo, strongly suggested that Pyr would be particularly efficient in the correction of severe acidemia: type A lactic acidosis, hypercapnia with cardiac arrest, and diabetic and alcoholic ketoacidosis in animal experiments and clinic settings. Because of its multi-cytoprotec-

tion, Pyrs not only correct acidosis, but also benefit the underlying dysfunction of vital organs. In addition, Pyr is also a potential buffer component of dialysis solutions. However, the instability of Pyr in aqueous solutions restricts its clinical applications as a therapeutic agent. Attempts to create a stable Pyr preparation are needed.

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Since 1970s, pyruvate (Pyr) has become increasingly attractive in the protection of dysfunctional vital organs, particularly in myocardial ischemia and reperfusion injury, pointing to a potential therapeutic value for the dysfunctional myocardium [1–3]. Also, findings strongly indicated that Pyr would be a novel buffer in many clinic settings [4–6]. This review discusses its distinctive effects on intracellular pH (pHi), proposing that Pyr may be superior to lactate (Lac) and bicarbonate in the correction of severe intracellular acidosis.

Lactate Metabolism and Effects on pHi and Lactic Acidosis

Lac has been traditionally accepted as the best replacement for bicarbonate in the treatment of metabolic acidosis since early 1930s [7]; it has since become part of solutions for dialysis and continuous renal replacement therapy for several decades.

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However, the widely propagated theory that Lac is ‘metabolized to bicarbonate’ on an equimolar basis in liver represents a simplification. In fact, Lac is an end product of anaerobic glycolysis with Pyr as its only outlet. As an alkalizer, it must be converted to Pyr by lactic dehydrogenase (LDH) in most mammalian tissues, which in turn can either be completely oxidized to CO₂ and H₂O in the mitochondria mainly in liver, kidney, heart and brain for the titration of a proton or enter gluconeogenesis from two Lac anions in the mitochondria and cytosol of liver and kidney for the titration of two protons. Both Lac oxidation and Lac-based gluconeogenesis are a mitochondrial tricarboxylic-acid cycle (TCA cycle)-dependent pathway. The overall reactions can be described as shown in equations 1 and 2; whichever route of Lac disposal is followed, one proton (H⁺) is consumed per Lac anion metabolized (Lac⁻: H⁺ = 1:1):

The consumption of a proton in cytosol effectively means the regeneration of a bicarbonate anion, thus raising pHi and preserving the blood bicarbonate level stoichiometrically [8, 9].

The uptake of Lac or Pyr by cells and the entry of Pyr into mitochondria depend on the Lac⁻/H⁺ cotransport system, monocarboxylate transporters (MCT, fig. 1) with H⁺ ([H⁺] in fig. 1) flux in symport. The system is located in the cytoplasmic membrane and inner membrane of mitochondria. The oxidation of exogenous Lac to Pyr by LDH is NAD⁺-dependent in cytosol, which releases two hydrogen ions with one (H⁺ in fig. 1) left in the hydrogen pool of cytosol as shown in equation 3. Both MCT-transported and Lac-released protons initially decrease pHi [10, 11], while the other hydrogen ion (negative hydrogen or hydride ion) released from Lac reduces cytosolic NAD⁺ ([NAD⁺]c) to NADH ([NADH]c). Such a decrease of reducing equivalents has been called cellular ‘pseudohypoxia’. In aerobic conditions, [NADH₂]c ([NADH + H⁺]c) may be transferred into mitochondria and oxidized with the generation of 1 H₂O and 2.5 ATP. Thus, the proton released from Lac is eventually consumed. The administration of Lac raises the Lac/Pyr ratio and decreases the [NAD⁺/NADH]c ratio in cytosol, which may critically inhibit glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase (G-3-PD) [12]. It also reduces the glycolytically derived ATP production that is vital

for sustaining cellular functions in anaerobic conditions. Pyr oxidative decarboxylation, then, is catalyzed by the Pyr dehydrogenase complex (PDC) in mitochondria, coupled with the reduction of mitochondrial NAD⁺ ([NAD⁺]m) to [NADH]m, yielding acetyl-CoA. It depends on oxygen as an electron acceptor in mitochondria that one pair of [NADH₂]m ([NADH + H⁺]m) as well as transferred [NADH₂]c is oxidized through the electron transfer chain, consuming the two hydrogen ions of [NADH₂]m with 1 H₂O and 3 ATP generations.

Catalyzed by PDC, one hydrogen (hydride) ion of [NADH + H⁺]m is transferred via [FADH₂] from the reactive thiol group (-SH) of coenzyme A, HS-CoA, which does not affect pHi; the other hydrogen ion (proton) is picked up in the hydrogen pool in the mitochondria matrix [13]. The overall reaction is the oxidation of imported proton via MCT and raises pHi as reported in cardiomyocytes of ischemic reperfused rabbit hearts and failing human myocardium [10, 11]. Thus, for every exogenous Lac anion metabolized in aerobic conditions either by oxidative phosphorylation or with glyconeogenesis, one endogenous proton is consumed, leading to the preservation of blood bicarbonate levels on a 1:1 molar basis.

In the mitochondria of many cell lines, mainly hepatocytes, carbonic anhydrase isoenzyme (CA V) exists, which catalyzes hydration (-HCO₃ or H₂CO₃) of CO₂ and H₂O. The bicarbonate so generated in the mitochondria is vital for the activity of bicarbonate-requiring Pyr carboxylase (PC) that is essential for the TCA cycle and gluconeogenesis [14, 15]. Also, acetazolamide, an inhibitor of most CA isoenzymes including CA V, may decrease Lac-induced blood alkalization and induce lactic acidosis [15]. Although these facts support that CA V plays an important role in the pHi modulation, they do not prove the general assumption that ‘the irreversible oxidation of Lac generates alkali from the conversion of CO₂ to bicarbonate by CA’, which then corrects metabolic acidosis [16, 17]. The traditional concept that Lac as a buffer is ‘metabolized to bicarbonate’ in liver mitochondria catalyzed by CA V to correct acidosis lacks direct evidence. It is well known that the Lac oxidation induces cytotoxic effects in tissue ischemia or hypoxia, such as the depletion of reducing equivalents, inhibition of glycolysis and release of protons in cytosol. Hence, in hypoxic conditions, the administration of sodium Lac may aggravate, rather than attenuate, intracellular acidification if the ability of Pyr oxidation is limited and gluconeogenesis is suppressed in cells such as hepatocytes, cardiomyocytes and macrophages, which have abundant mitochondria.

Fig. 1. Pyruvate metabolic pathways and their relationship with the consumption of protons in intracellular compartments. Pyr and/or Lac enter cytosol via the MCT system with an extracellular $[H^+]$ flux in symport. The oxidation of Lac to Pyr with LDH releases an H^+ coupled with NAD^+ reduction to NADH in cytosol, inhibiting glycolysis at the G-3-PD step. Both MCT-imported $[H^+]$ and Lac-released H^+ initially decrease pH_i , whereas the Pyr reduction to Lac consumes the $[H^+]$, restoring pH_i and glycolysis in anaerobic conditions. In aerobic conditions, the Pyr entry into mitochondria with $[H^+]$ yields $NADH + [H^+]$ by PDC. Oxidative phosphorylation of both $NADH + [H^+]$ ($[NADH + H^+]_m$) and transferred $NADH + H^+$ ($[NADH + H^+]_c$) generates ATP and H_2O , eventually consuming $[H^+]$ and H^+ , respectively. The overall oxidation of exogenous Pyr and/or Lac consumes endogenous $[H^+]$ on an equimolar basis, raising pH_i and pH_e . Excess Pyr simultaneously activates the PDC activity and enhances anaplerotic pathways via PC, accelerating the TCA cycle flux and the $[H^+]$ consumption. Pyr-based gluconeogenesis consumes an additional pair of $[NADH + H^+]_c$ at the step of G-3-PD in cytosol, compared with Lac-based gluconeogenesis. The preservation of a near physiologic pH_i and the acceleration of TCA cycle by excess Pyr enhance the Lac oxidation in lactic acidosis and the oxidation of ketone bodies and fatty acids in ketoacidosis. The shuttle of $[NADH + H^+]_c$ transference from cytosol to mitochondria is not shown. See the text for details. (1) = Gluconeogenesis-coupled reactions in mitochondria; (2) = gluconeogenesis-coupled reactions in cytosol; G-3-PD = glyceraldehyde-3-phosphate dehydrogenase; K_m = Michaelis constant; LDH = lactic dehydrogenase; MCT = monocarboxylate transporters; ME = malic enzyme; NAD^+ = oxidized nicotinamide adenine dinucleotide; NADH = reduced nicotinamide adenine dinucleotide; PC = pyruvate carboxylase; PDC = pyruvate dehydrogenase complex; PFK-1 = phosphofructokinase-1; pK_a = dissociation constant; TCA cycle = tricarboxylic-acid cycle.

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In cells with no or a few mitochondria (e.g., erythrocytes or neutrophils, etc.), exogenous Lac would just deteriorate the acidosis and depress cell function [18] even in normoxymia.

Hyperlactatemia and lactic acidosis could be corrected with dichloroacetate (DCA) or THAM [16, 19], but their values in clinic settings are not clear due to the negative survival rate and/or potential toxicity [19, 20]. The efficacy of Lac therapy in patients with lactic acidosis has not been systemically evaluated. In principle, severe acidemia: Type A lactic acidosis, hypercapnia with cardiac arrest and diabetic ketoacidosis, which account for over 90% of severe acidosis in clinic settings with excess mortality [19], are contraindications of the Lac administration. The Lac infusion should be avoided in cases of elevated serum Lac. Although sodium Lac itself is almost obsolete in the treatment of clinical acidosis, several recent studies have demonstrated that lactated Ringer's solution (28 mM) was beneficial in the resuscitation of massive hemorrhagic shock accompanied with lactic acidosis. It significantly improved acidosis and survival rates [17, 21–23]. In rat working hearts after hemorrhagic shock accompanied with lactic acidosis and impaired PDC activities, Lac at 8.0 mM in the presence of palmitate in perfusates saturated with 95% oxygen improved cardiac efficiency [21]. Most likely the intracellular acidosis was attenuated although pH_i data were not determined. The paradoxical effect of exogenous Lac on experimental lactic acidosis above has not been fully interpreted, but may be associated with several reasons: different species of models, early stage of shock, over 40 mm Hg of mean arterial pressure of shock and anesthesia conditions [17, 21–23]. The above findings indicated that the TCA cycle function in vital organs remained, at least partially, intact for Lac oxidation [24]. Notably, Lac-based gluconeogenesis in liver and kidney may still be preserved in the early stage of shock [25, 26]. In particular, kidney is as important a gluconeogenic organ as liver for Lac disposal. It is estimated that one-half of glucose production within the Lac Cori cycle takes place in the kidney cortex and Lac accounts for approximately 50% of renal gluconeogenesis. The Lac uptake remains relatively stable in hemorrhagic shock dogs until blood loss reaches 40% of total blood volume [26, 27]. While acidosis significantly depresses hepatic uptake of Lac, renal gluconeogenesis is markedly enhanced by ischemic injury or acidosis [26, 28] due to the rapid stimulation of phosphoenolpyruvate carboxykinase [29]. Further, a recent clinical investigation found that in patients with early postoperative cardiogenic shock accompanied with lactic acidosis, the

clearance of exogenous Lac was not significantly altered, compared with controls [30]. In contrast, if shock despite causes in origin are severe [31] or combined with alcoholic intoxication [23], preexisting liver diseases, diabetes or multiple organ injuries, the Lac infusion most likely exacerbates lactic acidosis. Therefore, it should be taken into account in the deduction of Lac effects on acidosis in above-mentioned experimental results if the oxidative phosphorylation of TCA cycle or gluconeogenesis in vital organs was, at least in part, sustained during early shock (hypoxia) or recovered after shock (reoxygenation). On the other hand, because the anoxia (hypoxia) of tissues and the dysfunction of TCA cycle commonly coexist in lactic acidosis, exogenous Lac should be avoided in such clinical settings even in the early stage of shock if the patient is critically ill, preferably in consideration of a new buffer, Pyr, instead.

Pyruvate Improvement of Intracellular Acidosis

Pyr was initially used in mid-1970 to buffer intracellular hydrogen ions and improve redox status in cardiomyocytes. The administration of exogenous Pyr promisingly improved glycolysis that was depressed from ischemia-reperfusion injury, preserved cardiac contractile function and prolonged the survival time of ischemic swine hearts [32]. Also, Pyr (10 mM), as a sole exogenous substrate, significantly attenuated the pH_i decrease in the perfused rat ischemic myocardium. It was first discovered in 1994 that animals treated with the Pyr infusion did not develop an anesthetic agent-induced mild metabolic acidosis presented in controls [33]. The finding confirmed that the intravenous Pyr would induce 'systemic alkalization' that was not emphasized in its conception until 1999 [34]. The comparison of Pyr with Lac on pH_i in human cells was first conducted with neutrophils in 1995 [4]. In an acidic milieu, following a slight pH_i decrease, Pyr (40 mM) maintained neutrophilic pH_i close to a physiological value, whereas an identical Lac level brought about a prompt and substantial pH_i decline. The extracellular pH [pH_e] of mixture solutions with Pyr was significantly higher than that with Lac counterparts as well. A subsequent report confirmed the phenomenon [Perit Dial Int Abstracts, 1997;17(suppl 1):S33]. The impact of Pyr on systemic acidosis, however, at present has not been well appreciated [31], and potential mechanisms whereby Pyr preferably corrected severe acidosis have not been fully elucidated either [6, 34]. There are several likely bio-

(1) By reducing to Lac with LDH, one exogenous Pyr anion consumes one proton from the cytosolic hydrogen pool, raising $[\text{NAD}^+/\text{NADH}]_c$ and pHi , as described in equation 3:

$$\text{CH}_3\text{COCOO}^- + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{CH}_3\text{CHOHCOO}^- + \text{NAD}^+ \quad (3)$$

pyruvate lactate

The $[NADH/NAD^+]_c$ ratio by the law of mass action is the major determinant of reactive direction. For any given cellular level of Pyr, the ratio determines what fraction of Pyr is converted to Lac. When mitochondrial oxidative reactions are impaired, the ratio rises, and the conversion of Pyr to Lac increases. Pyr inhibits glycolysis in aerobic conditions, however, it is of pivot importance that Pyr favors anaerobic glycolysis due to the increase of $[NAD^+/NADH]_c$ ratio and restoration of pH-sensitive phosphofructokinase (PFK-1) activity [5, 32, 37]. This would preserve glycolytic ATP production that is essential for maintaining sarcoplasmic membrane function, modulating intracellular protons [38, 39], and prolonging cell survival of vital organs during hypoxia [2, 40]. In the swine model of hemorrhagic shock manifested lactic acidosis, the intravenous infusion of Pyr further raised blood Lac levels, indicating that the Pyr reduction occurred in cytosol, but lowered the blood Lac/Pyr ratio and efficiently avoided or corrected lactic acidosis [6, 34, 41, 42].

Accordingly, Pyr ‘systemic alkalization’ proposed originally referred to this cytoplasmic reduction and the mitochondrial oxidation of exogenous Pyr [34]. In this respect, Pyr may also consume a proton in neutrophilic cytosol with the reduction to Lac in addition to its weaker buffering capacity, both raising neutrophilic pH_i [4].

(2) By oxidizing into CO_2 and H_2O via the TCA cycle in mitochondria, Pyr anions also consume protons on an equimolar basis ($\text{Pyr}^-:\text{H}^+ = 1:1$) as equation 4 shows:

$$\text{CH}_3\text{COCOO}^- + \text{H}^+ + 2 \frac{1}{2} \text{O}_2 \xrightarrow{\text{TCA cycle}} 3 \text{CO}_2 + 2 \text{H}_2\text{O} (+15 \text{ ATP, oxidation}) \quad (4)$$

pyruvate

As mentioned above, the exogenous Pyr consumes endogenous protons stoichiometrically in both anaerobic glycolysis and aerobic oxidation, raising pH_i, and Pyr does not release a proton as Lac does, but merely consumes it in the oxidation. In addition, Pyr oxidizes with a less oxygen consumption rate, compared with Lac.

(3) Supraphysiologic doses of exogenous Pyr would greatly stimulate the activity of PDC although there has been no data from human beings yet. It has been demonstrated with various animal tissues in rat, rabbit, bovine and swine that Pyr at several millimolars simultaneously stimulated the PDC activity by inhibiting Pyr dehydrogenase kinase in association with the improvement of cardiac performance and cerebral and hepatic functions [6, 41–43].

(4) Although Lac may replenish the TCA cycle by the malate-aspartate shuttle, an anaplerotic pathway that transfers Lac-reduced $[NADH_2]_c$ to mitochondria [21], it is with Pyr, other than Lac or other keto acids, that anaplerosis via carboxylation can be carried out by PC to oxaloacetate or by malic enzyme to malate. In a near physiological condition, Pyr carboxylation may account for, *in vivo*, only 5% of the citrate formation in swine hearts [44]. However, it may increase as many as 7 times in perfused rat hearts [24]. The increased availability of Pyr as a substrate and the PDC stimulation together with enhanced anaplerotic pathways would greatly increase the TCA cycle flux and accelerate the consumption of protons and the ATP generation. In addition, anaplerosis pathways, *per se*, fix CO_2 in mitochondria. Importantly, during acute acidosis Pyr may increase the CO_2 fixation in the mitochondria of liver and kidney [45], which may be of significance in improving local hypercapnia in ischemic tissues.

(5) Gluconeogenesis is an energy-requiring process. It consumes equivalently 3 high-energy phosphate bonds (2 ATP and 1 GTP) for 1 mol of glucose synthesized from 1 mol of Lac. However, the experimental evidence in pH_i within the rat hepatic lobule revealed that gluconeogenesis from Lac is a proton-consuming process [46]. Compared with one proton consumed by every Lac anion in Lac-based gluconeogenesis (equation 1), every Pyr anion uses two protons in Pyr-based gluconeogenesis ($\text{Pyr}^-:\text{H}^+ = 1:2$). In the cytosolic process of gluconeogenesis, a pair of $[\text{NADH} + \text{H}^+]\text{c}$ is required at the step of G-3-PD. It is supplied by the Lac oxidation to Pyr in Lac-based gluconeogenesis, but is additionally consumed in Pyr-based gluconeogenesis. Hence, Pyr-based gluconeogenesis in liver and kidney would be of particular significance in the preservation of a near physiologic arterial pH (pH_a) in shock. In this regard, it was affirmed with rat hepatic intralobular mapping results that protons are consumed stoichiometrically when weak acids are converted into neutral products (glucose, CO₂ and H₂O) [46]. The statement may be the case of Lac, other than the case of Pyr in terms of gluconeogenesis.

As mentioned earlier, the entry of Lac or Pyr into cells is carried by a proton-linked MCT family, which includes seven isoforms in a range of mammalian cells. They exhibit distinct cellular localization and have slightly different properties. Isoforms act as Lac^-/H^+ symport with a 1:1 stoichiometry. In contrast to the MCT in human neutrophils, which has a similar affinity for both Lac and Pyr [5], the isoforms of hepatocytes and cardiomyocytes in rats have much higher Michaelis constant (K_m) values for L-Lac than those for Pyr (4.7 and 2.74 mM vs. 1.3 and 0.2 mM), respectively [47, 48]. Thus, Lac may more readily induce a pH_i decline than Pyr when high extracellular concentrations of both are comparable in human vital organs. On the other hand, Pyr has a weaker buffering capacity due to the dissociation constant (pK_a) of 2.49, compared with Lac pK_a of 3.9 [4]. The weaker buffering capacity usually results in a higher pH value. Owing to these properties, equimolar doses of external Pyr and Lac are not expected to result in equivalent pH_i reductions in human cells as shown in neutrophils [4]. In rat cardiomyocytes, the property of MCT K_m was also considered in the Pyr effect on pH_i [49].

Based on its biochemical and biophysical characteristics, Pyr may be valuable in the treatment of severe metabolic acidosis such as type A lactic acidosis, hypercapnia with cardiac arrest, diabetic ketoacidosis and alcoholic intoxication although no clinic data have been reported yet. In theory, Pyr with insulin might be a more efficient regime in the correction of diabetic ketoacidosis mainly due to their potential synergetic effects on the oxidation of ketone bodies. Pyr in the regime might be also valuable to restore the sensitivity of insulin receptors inhibited by severe acidosis and reduce the insulin dosage to avoid hypoglycemia. Moreover, because of its better consumption of intracellular protons in the absence of the direct production of CO_2 in hypoxia, Pyr might be also beneficial in respiratory acidosis. However, these deductions require experimental and clinical validations.

Pyruvate Superiority in the Correction of Acidosis

Pyr and Lac in the Correction of Metabolic Acidosis

The effect of intravenous Pyr on acid-base balance was compared with equimolar Lac in anesthetized dogs in late 1970s. Lac infusion was associated with a significantly slower rate of bicarbonate level rising in comparison with Pyr, indicating that the additional metabolic steps are required [50]. The oxidation of Lac-reduced $[\text{NADH}_2]\text{c}$

may take place either in cytoplasm-coupled reactions associated with gluconeogenesis, or a mitochondrial route in the presence of oxygen. In addition to the difference of the proton consumption in gluconeogenesis processes between Pyr and Lac, the mitochondrial route is an indirect pathway, which depends on the reducing equivalent of carrier systems. The malate-aspartate shuttle is the major step involved in transporting $[\text{NADH} + \text{H}^+]\text{c}$ into the mitochondria in cells of heart, liver and kidney. The shuttle operation is driven by the electrical potential across mitochondrial membrane. Thus, the oxidation of Lac to Pyr is an energy-dependent step and operates slowly (in minutes) in physiological conditions [51]. In perfused rabbit cardiomyocytes, the pH_i recovery was delayed by 12 min. with Lac-added perfusates compared with Pyr-added counterparts in the presence of DCA [10]. This rate-limited step, which would display while the $[\text{NADH}_2]\text{c}$ load is increased, may be another interpretation of the retardation of pH_i recovery in addition to the elevated proton load, per se, from the Lac oxidation and the $[\text{NADH}]\text{c}$ increment [10]. Although the ratio of $[\text{NAD}^+/\text{NADH}]\text{c}$ determines the balance between Pyr and Lac (equation 3), the reaction is in favor of the direction from Pyr to Lac as the lesser rise in blood Pyr concentration with Lac infusion than in blood Lac level with equal Pyr infusion [50]. Several recent findings evidently supported that Pyr would be superior to Lac in the treatment of clinic metabolic acidemia including lactic acidosis. In the rat peritoneal dialysis model, preliminary results revealed that uremic acidosis was better corrected and the functions of peritoneal cells were more preserved with Pyr-buffered dialysates than with Lac counterparts [J Am Soc Nephrol Abstracts, 1998;9:236A; also Chin J Nephrol 2001;17:365–368]. Also, in the rat model of severe hemorrhagic shock manifested lactic acidosis, commonly seen in a clinical setting, the intravenous infusion of Pyr improved, whereas Lac exacerbated, metabolic acidosis. Pyr Ringer's solution resulted in a higher blood base excess level and a lower blood Lac level, compared with Lac Ringer's counterparts [31]. The study also substantiated that Pyr, in vivo, significantly decreased the expression of apoptosis markers, whereas Lac increased the markers after resuscitation in rat lungs [31] as observed, in vitro, in human neutrophils and endothelial cells [5, 52]. Although mechanisms of Pyr attenuation of apoptosis in shock resuscitation are not fully understood, it is well recognized that acidosis may be a triggering factor [5, 53]. Studies in hemorrhagic shock repeatedly confirmed that Pyr effectively prevented or corrected severe lactic acidosis in swine [6, 34, 41, 42]. A human trial demonstrated that

Pyr-fortified cardioplegia solutions provided cardioprotection superior to Lac-based counterparts during surgical cardiac arrest although pH_i data were absent [54]. These findings strongly suggested that Pyr was preferable in the correction of intracellular acidosis in both anaerobic and aerobic conditions.

In brief, because of the prevalence of LDH in mammalian cells, Pyr as an oxidant improves pH_i and cellular functions, which is opposite to the deleterious effects on pH_i and cytotoxic characteristics of Lac as a reductant in molecular and cellular levels particularly in anaerobic conditions. Meanwhile, due to the Lac oxidation to Pyr with LDH, both of them also exhibit somewhat similar metabolic and biological effects including the modulation of pH_i in aerobic conditions.

Pyr and Bicarbonate in the Treatment of Metabolic Acidosis in Cardiogenic Shock and Dialysis

Bicarbonate as a natural buffer was not questioned in the treatment of metabolic acidosis until two decades ago. The bicarbonate corrects acidosis through chemical neutralization, which depends on a fair respiratory function, whereas Pyr exerts a metabolic titration of intracellular acidosis without directly producing CO₂. Bicarbonate often benefits patients with metabolic acidosis in the absence of tissue hypoxia (e.g., uremic, diarrheic, and renal tubular acidosis). However, patients with cardiac arrest or cardiogenic shock (or septic shock, hepatic failure and diabetic ketoacidosis, etc.) usually have impaired tissue oxygen delivery. In these circumstances, the primary causes of acidosis result from the accumulation of lactic acid, the proton release from ATP hydrolysis and the retardation of local tissue CO₂ ('metabolic' hypercapnia). And limited glycolytic ATP production may lead to a progressive pH_i decline to <6.0 in cardiomyocytes [19, 53]. Bicarbonate administration in hypoxic states does not correct the underlying tissue hypoxia and is generally not successful in improving the acidosis or patients' general conditions. It usually results in severe adverse effects, leading to the exacerbation of hypercapnia and lactic acidosis and further decrease of tissue pH_i in liver, brain, skeletal muscle and red cells [19, 55]. In the presence of high P_{CO_2} and high non-bicarbonate buffers in plasma, bicarbonate, per se, would considerably decrease pH_i although pH_a may increase due to the readily entrance of CO₂ (but not -HCO₃) into intracellular spaces [56]. Nevertheless, severe acidosis (pH_a < 7.1) must be timely corrected at any clinical circumstances although mild acidosis may be beneficial. Without an ideal base in the clinic setting, it is strongly recommended that the bicarbonate

infusion should be cautious in the management of cardiopulmonary arrest in order to maintain pH_a in 7.10–7.20 after the airway is established [57]. Clinical evidence also revealed that bicarbonate therapy provided little improvement of diabetic ketoacidosis and suggested that it should not be used unless pH_a is <7.0 [19, 58]. On the other hand, DCA, which stimulates the PDC activity by directly inhibiting PDC kinase as one of Pyr actions and corrects lactic acidosis as a specific agent, also can significantly improve myocardial performance and acidosis in hypoxia [14, 20]. In comparison, Pyr may be superior to bicarbonate or DCA in the treatment of hypoxic acidosis. Its unique metabolic and chemical characteristics may be ideal for the correction of severe acidosis in cardiogenic shock or hypercapnia with cardiac arrest as it consumes protons and benefits in myocardium performance without risking pH_i deterioration in anaerobic conditions. Further, Pyr protects cellular functions of multiple cell lines and vital organs as a strong antioxidant. It also inhibits inflammation mediators (e.g., TNF- α , NF- κ B and NO), improves Ca²⁺ homeostasis, attenuates apoptosis and preserves carbohydrates metabolic pathways in hyperglycemia and promotes lipid metabolism [2, 3, 5, 6, 52]. Pyr as an intermediary metabolite of glucose is also an energy-yielding substrate without any known cytotoxicity. An intravenous Pyr loading test demonstrated its safety in human subjects [59].

In addition, Pyr might be also a promising buffer to correct uremic acidosis in patients undergoing peritoneal dialysis (PD), which markedly improves the biocompatibility of PD solutions [5, 36]. It may efficiently correct chronic acidotic status in hemodialysis patients as well, which is not satisfactorily treated at present [60]. Pyr as one of the buffer components may decrease bicarbonate or Lac concentrations in dialysis solutions, reduce oxidative stress induced by high bicarbonate levels [61] and Lac toxicity. It can also improve glucose and lipid metabolism in dialysis patients particularly with cardiovascular complications and diabetes. However, the instability of sodium Pyr in aqueous solutions restricts its clinical applications at the present time. It is well known that Pyr in aqueous solutions rapidly undergoes an aldol-like condensation, forming parapyruvate to inhibit mitochondrial functions [62]. Efforts to overcome Pyr instability in solutions creating Pyr therapeutic preparations suitable for intravenous infusion and dialysis are urgently warranted. Several clues point to a possibility.

In conclusion, Pyr as a novel generation of buffers would be superior to conventional ones both in animal experiments and in clinic settings, particularly in the

treatment of severe acidemia, which still lacks effective agents to deal with at present. Intravenous administration of Pyr would not only correct both pH_a and pH_i and acidotic symptoms, but also improve underlying cell dysfunctions to a large extent in critically ill patients with heart, liver, kidney or brain disorders. Further evaluation of Pyr effects on acid-base balance disorders and their underlying mechanisms in both animals and clinical trials is required. Attempts to create stable therapeutic preparations of Pyr solutions are warranted.

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