Review

Bench-to-bedside review: Amelioration of acute renal impairment using ethyl pyruvate

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Abstract

Inflammation and oxidative stress cause renal impairment. Renal failure exacerbates the effect of oxidative stress on many organ systems. Antioxidants can prevent or treat renal failure in various experimental models and clinical situations. Pyruvate is an endogenous antioxidant with beneficial effects in animal models of oxidative stress. Because sodium pyruvate rapidly degrades in solution, a simple derivative of pyruvic acid, namely ethyl pyruvate, has been investigated as a therapeutic agent in preclinical studies. Ethyl pyruvate reduces organ system damage in ischaemia/reperfusion injury and haemorrhagic and endotoxic shock, at least in part through its antioxidant action. In addition, ethyl pyruvate appears to have direct beneficial effects on cytokine expression and proinflammatory gene regulation. The effect is long lasting and, importantly, even when it is administered after the onset of inflammation it can ameliorate organ damage and improve survival. Ethyl pyruvate is a widely used as a food additive and was shown to be safe in phase I clinical trials. We suggest ethyl pyruvate warrants further evaluation in the management of acute renal impairment.

Introduction

Acute renal failure is associated with oxidative stress [1]. This relationship appears to be both cause and effect because dialysis reduces markers of oxidative stress in patients with acute renal failure [2] and various antioxidants are able to ameliorate experimental renal injury [3,4]. Patients with multiple organ failure have reduced antioxidant defence mechanisms, and this effect is substantially accentuated when acute renal failure is also present [5].

Antioxidants have been used successfully to prevent or treat acute renal failure in humans. *N*-acetylcysteine attenuated oxidative stress and nephropathy caused by intravenous radiocontrast, and was more effective than standard intravenous fluid prophylaxis [6]. Infrarenal abdominal aortic aneurysm repair commonly causes ischaemia/reperfusion injury to the kidney, producing a similar oxidative stress to that of radiocontrast. A mixture of antioxidants (including *N*-acetylcysteine, allopurinol, mannitol, and vitamins E and C) given to patients undergoing infrarenal abdominal aortic aneurysm repair produced a significantly better creatinine clearance (compared with that in placebo control individuals) 48 hours postoperatively [7].

Ethyl pyruvate as an antioxidant

Pyruvate is an endogenous antioxidant and free radical scavenger [8,9]. Sodium pyruvate scavenges H_2O_2 via a nonenzymatic reaction (oxidative dephosphorylation) that yields acetate, water and carbon dioxide as products:

$$CH_3COCOO^- + H_2O_2 \rightarrow CH_3COO^- + CO_2 + H_2O$$

In so doing, sodium pyruvate suppressed H_2O_2-induced lipid peroxidation of rat kidney homogenate, and prevented cytosolic ^{51}Cr release (a marker of cellular injury) by renal epithelial cells exposed to H_2O_2 [10]. Sodium pyruvate also attenuated the proteinuria induced by *in vivo* selective renal administration of H_2O_2 in rats [10]. In an *in vivo* model of renal failure caused by intramuscular glycerol, sodium pyruvate reduced the rise in serum creatinine at 24 hours after glycerol injection, prevented structural damage (as assessed by histology) and lessened the fall in glomerular filtration rate after glycerol injection [10]. Additionally, sodium pyruvate has beneficial effects on other organ systems in other animal models of redox stress (for review, see Fink [11]).

Unfortunately, the clinical utility of sodium pyruvate is limited because it rapidly undergoes an aldol-type addition reaction in aqueous solution to form parapyruvate. This latter compound inhibits the oxidative decarboxylation of α-ketoglutarate to form succinyl coenzyme A (a component of the Krebs cycle) [11]. Ethyl pyruvate may be more stable than pyruvate [11] and is considered safe for human consumption

IL = interleukin; iNOS = inducible nitric oxide synthase; LPS = lipopolysaccharide; NF-κB = nuclear factor-κB; TNF = tumour necrosis factor.

as a food additive [12]. Ethyl pyruvate is as effective as pyruvate (on a millimole for millimole basis) as a scavenger of H_2O_2 (Engert JA, Fink MP, unpublished observations), although the rate of this reaction has not been measured. Ethyl pyruvate has been shown to scavenge phenoxy radicals, but the biological significance of this phenomenon is unknown (Englert JA, Kagan VE, Fink MP, unpublished observations). Ethyl pyruvate is more lipophilic than pyruvate anion, and (like most esters) presumably diffuses readily into cells, thereby acting on both intracellular and extracellular radicals. The antioxidant potential of ethyl pyruvate with regard to other radicals and its interactions with other oxidant and antioxidant species in complex biological systems have not yet been characterized. Ethyl pyruvate itself is not a free radical.

Ethyl pyruvate has beneficial effects on a number of organ systems when it is administered to animals subjected to ischaemia/reperfusion injury and haemorrhagic or endotoxic shock. For example, ethyl pyruvate reduced structural and functional damage to intestinal mucosa caused by mesenteric ischaemia and reperfusion [13]. It reduced hepatic necrosis and expression of proinflammatory cytokines after partial warm hepatic ischaemia and reperfusion [14]. In a rat model of myocardial ischaemia/reperfusion [15] it diminished infarct size and improved myocardial function, and in a rat model of severe haemorrhagic shock [16] it improved survival and ameliorated ileal mucosal permeability. In a mouse model of resuscitated haemorrhagic shock [17] ethyl pyruvate improved survival at 24 hours, reduced bacterial translocation to mesenteric lymph nodes, and prevented the development of increased ileal mucosal permeability. It prolonged survival time and was associated with significantly lower circulating concentrations of nitrite/nitrate and IL-6 and higher plasma levels of IL-10 in a rats given a bolus of lipopolysaccharide (LPS) [18]. Finally, in a murine model of acute pancreatitis [19] ethyl pyruvate improved survival, reduced pancreatic tumour necrosis factor (TNF) and IL-6 expression and nuclear factor-κB (NF-κB) binding, and reduced alveolar permeability.

Only one published study to date has failed to find a beneficial effect of ethyl pyruvate in an animal model of critical illnesses [20]. Moderate haemorrhagic shock (involving loss of approximately 35% blood volume) was induced in pigs and maintained for 90 min, after which the animals were resuscitated with Ringer's lactate either with or without ethyl pyruvate over the next 60–90 min. Ethyl pyruvate did not change heart rate, mean arterial pressure, cardiac output, oxygen delivery or uptake, or lactate concentration. At the end of the resuscitation phase, the oxygen saturation in the stomach and the cytoplasmic pH in the hind limb were both significantly lower in animals given ethyl pyruvate, although the physiological significance of this is unclear. It is possible that the dose of ethyl pyruvate used was not adequate (40–60% of that currently being used in a human phase II trial of patients undergoing cardiopulmonary bypass). It may also be that the moderate degree of haemorrhage induced less ischaemia/reperfusion injury than in the other studies, and that the time course over which the drug was applied, and its effect quantified, was insufficient.

Other anti-inflammatory actions of ethyl pyruvate

Ethyl pyruvate appears to be a more effective anti-inflammatory agent than other antioxidants. This was apparent when ethyl pyruvate was compared with sodium pyruvate in lens cell culture [21], and a similar trend was observed in an *in vivo* model of rat mesenteric ischaemia and reperfusion [13]. Treatment with ethyl pyruvate was more effective than sodium pyruvate in preventing the proinflammatory cytokine stimulated hyperpermeability and induction of inducible nitric oxide synthase (iNOS) in Caco-2 human enterocyte-like monolayers *in vitro*, and in protecting against ileal mucosal hyperpermeability, bacterial translocation and hepatocellular injury in mice given LPS *in vivo* [22].

The enhanced anti-inflammatory effect of ethyl pyruvate is probably due to its ability to modulate cytokine expression and proinflammatory gene regulation, in addition to the biochemical antioxidant properties described above. For example, rats given a bolus of LPS had raised plasma levels of IL-6 and lower levels of IL-10, an effect that was reversed by ethyl pyruvate [18]. Also, treatment with ethyl pyruvate initiated 24 hours after caecal puncture in mice significantly increased survival (from 30% to 88%). In animals with established endotoxaemia or sepsis, ethyl pyruvate treatment significantly reduced circulating levels of the newly recognized late-phase proinflammatory cytokine high mobility group 1 (HMGB1). In macrophage cultures, ethyl pyruvate specifically inhibited activation of p38 mitogen-activated protein kinase and NF-κB, two signalling pathways that are critical for cytokine release [23].

Additional effects of ethyl pyruvate have been described. In RAW 264.7 murine macrophage-like cells, stimulation with LPS led to an increase in glutathione concentration [24]. Contrary to what might be expected based on its antioxidant properties, ethyl pyruvate blocks this effect [24]. Extramitochondrial glutathione depletion is known to alter the thiol-disulfide redox state, which in murine hepatocytes inhibited nuclear binding of NF-κB and subsequent expression of iNOS [25]. More recently, ethyl pyruvate was shown to reduce NF-κB DNA binding in RAW 264.7 macrophages, which similarly reduced LPS-induced iNOS expression and luciferase reporter gene function [26]. The mechanism of this effect was further explored. IκB degradation was unaffected. However, ethyl pyruvate directly inhibited binding of the p65 subunit of NF-κB to DNA while having no effect on the p50 subunit.

It is possible that ethyl pyruvate inhibits activation of NF-κB simply by scavenging H_2O_2. However, several observations

suggest that ethyl pyruvate mediated inhibition of NF-κB activation may entail more than an antioxidant effect. First, oxidative stress (1–10 mmol/l H_2O_2) fails to activate NF-κB in Caco-2 [27] or DLD-1 [28] enterocyte-like cells, but ethyl pyruvate blocks activation of NF-κB in cytomix-stimulated Caco-2 cells [29]. Second, other well known scavengers of reactive oxygen species, such as pyrollodine dithiocarbamate and dimethyl sulfoxide, fail to block IL-1β induced NF-κB activation in Caco-2 cells [27], but ethyl pyruvate effectively inhibits cytomix-induced NF-κB activation in the same cell line [29]. Third, *N*-acetylcysteine, an effective reactive oxygen species scavenger, fails to inhibit activation of NF-κB induced by TNF or IL-1β in ECV304 endothelial cells [30]. *N*-acetylcysteine also fails to block LPS-induced NF-κB activation in J774.1 murine macrophage-like cells [31]. In contrast, ethyl pyruvate inhibits activation of NF-κB induced by cytomix (TNF, IL-1β and interferon-γ) in Caco-2 cells [29] or by LPS in RAW 264.7 murine macrophage-like cells [23]. Indeed, when Song and coworkers [24] carried out a head-to-head comparison of ethyl pyruvate and *N*-acetylcysteine in experiments using LPS-stimulated RAW 264.7 cells, ethyl pyruvate was clearly a more potent inhibitor of NF-κB DNA binding when the two compounds were compared on a millimole for millimole basis. These data support the view that ethyl pyruvate has anti-inflammatory effects that are independent of its antioxidant properties.

Timing of administration of ethyl pyruvate

As might be expected following inhibition of NF-κB DNA binding, the effects of ethyl pyruvate appear to persist for some time. Pretreatment of mice with ethyl pyruvate, with the last dose 6 hours before injection of LPS, was almost as effective at preventing hepatic iNOS mRNA expression and circulating nitrite/nitrate as was ethyl pyruvate given both before and after the insult. A similar durability of effect was seen with ethyl pyruvate pretreatment of Caco-2 cells *in vitro*, even when the cells were washed extensively to remove the compound [22]. The durability of the anti-inflammatory effects of ethyl pyruvate in these studies constitutes another piece of evidence in support of the view that this compound exerts anti-inflammatory actions via one or more mechanisms that do not depend on scavenging of H_2O_2 or other reactive oxygen species. In order to react preferentially with reactive oxygen species, a scavenger must be present in cells when these compounds are being formed. However, transient exposure of cells to ethyl pyruvate (followed by washing to remove the compound) confers durable protection against a subsequent proinflammatory stimulus, making it unlikely that scavenging of reactive oxygen species is the underlying mechanism.

Most importantly, from a clinical standpoint ethyl pyruvate appears to retain beneficial effects even when it is given after a proinflammatory stimulus has become well established. In the study conducted by Miyaji and coworkers [32] ethyl pyruvate, even when given at 6 or 12 hours after caecal ligation and puncture, decreased injury in many organs (liver, pancreas, muscle and kidney) when assessed at 24 hours. Similarly in mice, LPS-induced ileal mucosal hyperpermeability, bacterial translocation to mesenteric lymph nodes and hepatocellular injury were reduced to a similar extent by ethyl pyruvate (compared with Ringer's lactate solution), regardless of whether it was given 1 hour or 6 hours after LPS injection. These beneficial effects on organ system function translate to mortality benefits; ethyl pyruvate reduced mortality in mice that underwent caecal ligation and puncture, even when treatment with the compound was started 24 hours after the operation [23].

Ethyl pyruvate and renal failure

Ethyl pyruvate decreases sepsis-induced acute renal failure *in vivo*. Mice subjected to caecal ligation and puncture developed multiple organ failure (as indicated by increased circulating levels of several enzymes, including aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase and amylase) and a biphasic rise in serum TNF concentration, despite fluid resuscitation and treatment with broad spectrum antibiotics. Circulating creatinine concentrations were also reproducibly increased, with histological evidence of tubular damage. A single dose of intraperitoneal ethyl pyruvate immediately after surgery partially inhibited muscle, liver and pancreatic injury. Renal function was also substantially protected, as indicated by lower plasma urea and creatinine levels, as well as reduced histological tubular damage. Furthermore, ethyl pyruvate inhibited the induction of mRNA for TNF, tissue factor, plasminogen activator inhibitor-1 and tissue plasminogen activator, all of which are believed to have deleterious effects in sepsis [32]. In a more recent study from the same laboratory [33] ethyl pyruvate reversed renal dysfunction in aged mice 20 hours after caecal ligation and puncture, as detected by dendrimer-enhanced magnetic resonance imaging.

Possible adverse effects of ethyl pyruvate

Attenuation of inflammation in sepsis may well be a double-edged sword, because inflammation is integral to host defences against infection. In this regard, the enhancement of immune function in a rat model of burn injury is encouraging. Rats given intraperitoneal ethyl pyruvate 6 hours after a 30% full-thickness burn did not exhibit the reduction in splenic lymphocyte proliferation rate at days 1–7 that was observed in control animals [34]. Ethyl pyruvate also enhanced IL-2 production. While reducing nonspecific inflammation, it appears that ethyl pyruvate can also enhance specific cell-mediated immunity by an as yet unexplored mechanism.

Ethyl pyruvate is unlikely to be harmful to humans, given its close similarity to an endogenous metabolite, its safety profile in animals and its common use as a food supplement in humans. Phase I clinical studies carried out by Critical Therapeutics, Inc. (Lexington, MA, USA) confirm the safety of ethyl pyruvate. The results of these studies have not been

reported by the company in the peer-reviewed literature but were submitted to the US Food and Drug Administration. Since the Food and Drug Administration has permitted the company to carry out a phase II randomized controlled trial of ethyl pyruvate in high-risk patients undergoing cardiac surgery and cardiopulmonary bypass [35], major safety concerns apparently were not raised by the agency.

Conclusion

It would seem from these animal and cellular models that ethyl pyruvate reduces oxidative stress and the damage done by oxygen free radicals in a variety of tissues. All of the *in vivo* models in which survival was examined have shown ethyl pyruvate to have a beneficial effect. Two studies specifically demonstrated a beneficial effect in acute renal failure, even when the drug was given at a relatively advanced stage in the disease. The kidneys are particularly sensitive to oxidative stress and the deleterious effects of excessive inflammation, usually being the first organ system after the lungs to exhibit clinical and biochemical signs of impairment in critical illness. Accordingly, we believe that ethyl pyruvate warrants evaluation in a clinical trial for the prevention and/or amelioration of acute renal failure in high-risk intensive care patients.

Competing interests

MPF is a cofounder of and a consultant to Critical Therapeutics, Inc., a biotechnology company that is developing several compounds, including ethyl pyruvate, as therapeutic agents. MPF receives funds from Critical Therapeutics. Critical Therapeutics holds the patient for ethyl pyruvate.

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