Pancreatology 19 (2019) 209–216

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Ethyl pyruvate and analogs as potential treatments for acute pancreatitis: A review of *in vitro* and *in vivo* studies

Linbo Yao [a, 1], Chunru Cheng [b, 1], Xinmin Yang [a], Chenxia Han [a], Dan Du [c], Tingting Liu [a], Michael Chvanov [d], John Windsor [e], Robert Sutton [f], Wei Huang [a, *], Qing Xia [a, **]

[a] Department of Integrated Traditional Chinese and Western Medicine, Sichuan Provincial Pancreatitis Centre and West China-Liverpool Biomedical Research Centre, West China Hospital, Sichuan University, Chengdu 610041, China
[b] School of Chemistry and Pharmaceutical Engineering, Institute of Pharmaceutical Engineering Technology and Application, Key Laboratory of Green Chemistry of Sichuan Institute of Higher Education, Sichuan University of Science and Engineering, Zigong 643000, China
[c] West China-Washington Mitochondria and Metabolism Centre, West China Hospital, Sichuan University, Chengdu 610041, China
[d] Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool L69 3BX, UK
[e] Centre for Surgical and Translational Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland 92019, New Zealand
[f] Liverpool Pancreatitis Study Group, Royal Liverpool University Hospital, Institute of Translational Medicine, University of Liverpool, Liverpool L69 3GE, UK

ARTICLE INFO

Article history:
Received 9 November 2018
Received in revised form
10 December 2018
Accepted 15 December 2018
Available online 25 December 2018

Keywords:
Acute pancreatitis
Pancreatic acinar cells
Ethyl pyruvate
Medicinal chemistry
Fluid therapy

ABSTRACT

Ethyl pyruvate (EP) has been shown to improve outcomes from multiple organ dysfunction syndrome (MODS) in experimental animal models of critical illness. This review aimed to summarise *in vitro* and *in vivo* effects of EP analogs on acute pancreatitis (AP) with the objective of proposing medicinal chemistry modifications of EP for future research. *In vitro* studies showed that both sodium pyruvate and EP significantly reduced pancreatic acinar necrotic cell death pathway activation induced by multiple pancreatic toxins. *In vivo* studies using different murine AP models showed that EP (usually at a dose of 40 mg/kg every 6 h) consistently reduced pain, markers of pancreatic injury, systemic inflammation and MODS. There was also a significant increase in survival rate, even when EP was administered 12 h after disease induction (compared with untreated groups or those treated with Ringer's lactate solution). Experimental studies suggest that EP and analogs are promising drug candidates for treating AP. EP or analogs can undergo medicinal chemistry modifications to improve its stability and deliverability. EP or analogs could be evaluated as a supplement to intravenous fluid therapy in AP.

Introduction

Acute pancreatitis (AP) is a common disease with a rising global incidence [1,2]. A quarter of patients have severe AP [3], defined as developing persistent organ failure (OF) [4] or multiple organ dysfunction syndrome (MODS) [5], that is associated with a mortality of 30–50% [6–8]. The mainstay of early AP treatment is fluid therapy using crystalloid solution [3,9], although we do not know which type of fluid to use, how fast to give it and how best to monitor the response to it [10]. Despite recent advances in understanding the role of reactive oxygen species (ROS) [11–15], mitochondrial injury [16–18], endoplasmic reticulum stress [19–21], inflammasome [22–25], and immune signalling pathways [26] in AP, there is no specific treatment for AP that targets outcome determining pathophysiology, such as MODS [27,28].

Pyruvate (CH_3COCOO^-), the anionic form of 2-oxo-propionic acid, plays a key role in intermediary metabolism being the final product of glycolysis and the starting substrate for the tricarboxylic acid (TCA) cycle [29,30]. It is also an effective scavenger of ROS and hydrogen peroxide [30]. Pharmacological administration of

Abbreviations: AP, acute pancreatitis; APACHE, Acute Physiology, Age and Chronic Health Evaluation; CDE diet, choline-deficient diet with DL-ethionine; CER, caerulein; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C chemokine receptor 4; DAMPs, damage-associated molecular pattern molecules; EP, ethyl pyruvate; HMGB1, high mobility group box-1; IL, interleukin; LPS, lipopolysaccharide; MODS, multiple organ dysfunction syndrome; NaTC-AP, sodium taurocholate-induced AP; NF-kB, nuclear factor-kB; OF, organ failure; RAGE, receptor for advanced glycation end products; RLS, Ringer's lactate solution; ROS, reactive oxygen species; SP, sodium pyruvate; TCA, tricarboxylic acid; TNF-α, tumour necrosis factor-alpha.

* Corresponding author.
** Corresponding author.
E-mail addresses: Dr_Wei_Huang@163.com (W. Huang), xiaqing@medmail.com.cn (Q. Xia).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.pan.2018.12.007
1424-3903/

pyruvate has been shown to improve organ function in animal models of oxidant-mediated cellular injury. However, its use as a therapeutic agent appears limited because of the relatively poor stability in aqueous solution [30]. In an effort to develop a more stable aqueous form of pyruvate, Sims et al. [31] studied the biological effects of a closely related compound, ethyl pyruvate (EP), an ester formed by the condensation of pyruvic acid with the 2-carbon alcohol, ethanol [32]. EP is also a ROS scavenger, but appears to have pharmacological and anti-inflammatory effects that are quite distinct from those of pyruvate anion [29,30]. EP itself can act directly as a metabolic substrate to fuel the TCA cycle or as a cell-permeable source of pyruvate [30]. Treatment with EP has been shown to ameliorate organ dysfunction and/or improve survival in a wide variety of preclinical models of critical illnesses, such as severe sepsis, acute respiratory distress syndrome, AP and stroke [29,30,32,33]. While EP has these documented therapeutic benefits, the biochemical mechanisms responsible are not well understood [30].

Search methods and results

A comprehensive systematic literature search was carried out in PubMed, Web of Science, EMBASE, Science Citation Index Expanded, the Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library and Google Scholar to identify relevant articles. The MeSH terms were “acute pancreatitis”, “pancreatitis”, “pancreatic acinar cells”, “pancreatic acini”, and “AR42J cells” were combined with MeSH terms “pyruvate”, or “ethyl pyruvate” as well as “pyruvate medicinal chemistry”. Studies reporting the effects of EP derivatives on other cell types investigating inflammatory pathways were also scrutinised. Only articles in English were included. Published reviews regarding this topic were manually examined for other relevant studies.

Effects of EP and analogs on AP

In vitro studies – pancreatic acinar cells

Two studies [34,35] investigated the role of sodium pyruvate (SP) or EP in pancreatic toxin-induced acinar cell injury (Table 1). In freshly isolated rat pancreatic acinar cells, Wang et al. [34] investigated the role of EP in trypsinogen activation peptide-induced damage-associated molecular pattern molecules (DAMPs) release (e.g. high mobility group box-l, HMGB1) and acinar cell injury. They found that EP (28 mM) significantly reduced HMGB1 mRNA expression up-regulation induced by trypsinogen activation peptide (3 nM) at all time points (3, 6, 12 and 24 h) tested. These results were confirmed by marked suppression of HMGB1 protein at 12 and 24 h and increased cell viability at 24 h in the EP supplemented group compared with the group treated with trypsinogen

Table 1
Summary of effects of ethyl pyruvate derivatives on different cell types.

Cell type	EP and derivatives	Key findings
Mouse [35] and rat [34] acinar cells	EP or SP	EP reduced trypsinogen activation peptide-induced HMGB1 release and cell death (Trypan blue) SP attenuated pancreatic toxin-induced Ca^{2+} overload, ATP loss and necrosis
Mouse peritoneal macrophages [39], mouse alveolar macrophages [53], mouse BM-derived macrophages [50], RAW 264.7 [43,46,52,55–57,59], PBMC-derived human macrophages [39]	EP, 2-arachidonic acid or diethyl oxaloproprionate	EP had activities that counteracted LPS-induced oxidative (HO-1, iNOS), cell death (caspase-1 and NLRP3 activation, HMGB1 release) and inflammatory mediators (NF-κB, TNF-α, IL-6) The other EP derivatives had similar effects
THP-1 cells [39], PBMC-derived human monocytes [52]	EP, ethyl lactate	EP suppressed inflammatory (TNF-α, IL-1β, IL-6 and IL-8) and cell death (NLRP3) pathways induced by LPS as well had immunoregulation activities (HLA-DR, CD14 and CD91)
Murine myeloid cell line, 32Dcl3 (CRL-11346) [40]	EP	EP inhibited both LPS- and fMLP-induced autophagy in neutrophils
Human platelets [41]	EP	EP inhibited thrombin-induced PI3K/Akt and PKC activation and phosphatidylserine exposure
Lung epithelial cells A549 [42,44,47–49,54], murine respiratory epithelial-like cells (MLE-15 and MLE-12 cells) [53], human vascular endothelial cell line [37], and HUVECs [45,51]	EP, SP	EP reduced glucose deprivation-, LPS- or TNF-α-induced oxidant production, ERS activation, inflammation (NF-kB, IL-8, neutrophil adhesion, KC and MCP-1 release), HMGB1 release, and necrosis SP had much less effects compared with EP
Caco-2 cells [55,58]	EP, 2-arachidonic acid or diethyl oxaloproprionate	EP inhibited cytomix[a]-induced inflammation and (NF-kB, TNF-α) and oxidative stress (NO, iNOS) The other EP derivatives had similar effects
Renal tubular epithelial cells (LLC-PK cells) [60]	SP	SP ameliorated hydrogen peroxide-induced lipid peroxidation
The human hepatocellular carcinoma cell line Huh7[42], human hepatoma cell line (HepG2 cells) and rat hepatocyte cell line (RLC-16 cells) [38]	EP, SP, pyruvate acid, phosphorpyruvic acid	EP reduced N-acetyl-p-benzoquinone imine-, IL-1β- or TNF-α-induced oxidative (peroxynitrite and hydroxyl radicals), inflammatory (IL-6, IL-8 and neutrophil adhesion), apoptotic (annexin-V) and necrotic (propidium iodide) responses SP and pyruvate acid had similar protective effects as EP but not phosphorpyruvic acid

EP, ethyl pyruvate; SP, sodium pyruvate; HMGB1, high mobility group box-1; BM, bone marrow; PBMC, primary blood mononuclear cells; LPS, lipopolysaccharide; HO-1, haeme oxygenase-1; iNOS, inducible nitric oxide synthase; NLRP3 nucleotide-binding domain, leucine-rich-containing family, pyrindomain-containing-3; NF-κB, nuclear factor-κB; TNF-α, tumour necrosis factor-α; HLA-DR, human leukocyte antigen-DR; fMLP, N-Formyl-Met-Leu-Phe; PI3K/Akt, phosphatidylinositol 3-kinase/serine-threonine kinase; PKC, protein kinase C; KC, keratinocyte-derived chemokine; MCP-1, monocyte chemoattractant protein 1.

[a] A cocktail of pro-inflammatory cytokines called cytomix (1000 U/ml IFN-γ plus 10 ng/ml TNF-α plus 1 ng/ml IL-1β).

activation peptide alone. In the other study, Peng at al. [35] showed that SP (10 mM) significantly protected ATP loss and necrosis in mouse pancreatic acinar cells induced by palmitoleic acid (50 μM), bile acid mixture (0.06%) and asparaginase (200 IU/ml), which can cause AP. In our own study (conference abstract) [36], apoptotic cell death pathway activation induced by 10 nM cholecystokinin in pancreatic acinar cells was significantly and equivalently inhibited by 1 mM SP and EP (both $P < 0.001$) 12 h post stimulation. Similarly, necrotic cell death pathway activation induced by 10 nM cholecystokinin was also significantly reduced by 1 mM SP and EP (both $P < 0.001$) 8 h post stimulation and EP showed a greater effect than SP at 12 h.

In vitro studies – immune, epithelial and endothelial cells

Findings from other *in vitro* studies [34,35,37–60] collectively suggest that EP suppressed oxidative stress [37,38,46,50,55–57,60], endoplasmic reticulum stress [45], inflammation [39,42,44,45,47–53,55–59] and cell death [35,40,54] as well as having immunoregulation effects [42,44,52] on a range of immune, epithelial and endothelial cells known to be injured with AP. The main protective mechanisms of EP in AP are described in Fig. 1.

In vivo studies

There were no human studies that investigated the effects of EP and analogs on AP. There were a total of 11 studies using murine models of AP (Table 2). One study investigated SP and 10 investigated EP. The endpoints used in these studies included pancreatic injury, pain, systemic inflammation parameters, MODS (e.g. lung, gut, kidney and liver) and survival rate.

Pancreatic injury

In a mouse model of AP induced by a choline-deficient diet with DL-ethionine supplementation and with caerulein and lipopolysaccharide challenge (CDE diet/CER/LPS-AP), the effects of Ringer's lactate solution (RLS) were compared with RLS supplemented with EP (RLS-EP) on markers of pancreatic injury [61]. RLS-EP significantly attenuated serum amylase, pancreatic wet weight, minimised pancreatic necrosis, neutrophil infiltration and preserved the pancreatic islets. It also decreased pancreatic nuclear factor kB (NF-κB) DNA binding activity and pancreatic tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6.

In a series of studies [62–65] conducted by Luan et al., the effects of EP on pancreatic injury were investigated in sodium taurocholate-induced AP (NaTC-AP) in rats. Histopathologically, EP treatment significantly decreased intralobular oedema, inflammatory cell infiltration, necrosis and haemorrhage of pancreas. Pancreatic malondialdehyde, myeloperoxidase, HMGB1, TNF-α and NF-kB DNA binding activity were also suppressed. Consistent with these findings, a most recent study [66] showed that EP treatment improved pancreatic oedema, neutrophil infiltration and necrosis in NaTC-AP in rats.

In another study, pre-treatment with SP decreased the pancreatic histopathology score (mean 9 vs. 5, $P < 0.05$) in CER-AP in rats, this was associated with decreased pancreatic protein carbonyl concentrations, consistent with the antioxidant activity of pyruvate [67].

Pain

Recently, Irie et al. [68] reported the first study linking DAMPs (i.e. HMGB1) and pain in AP. They found that macrophage-derived HMGB1 mediates pancreatic pain by targeting RAGE (receptor for advanced glycation end products) and CXCL12 (C-X-C motif chemokine ligand 12)/CXCR4 (C-X-C chemokine receptor 4) signalling axis in the early stage of CER-AP in mice. In their experiment, prophylactic use of EP inhibited HMGB1 release from macrophages and prevented the hyperalgesia determined by Von Frey filaments

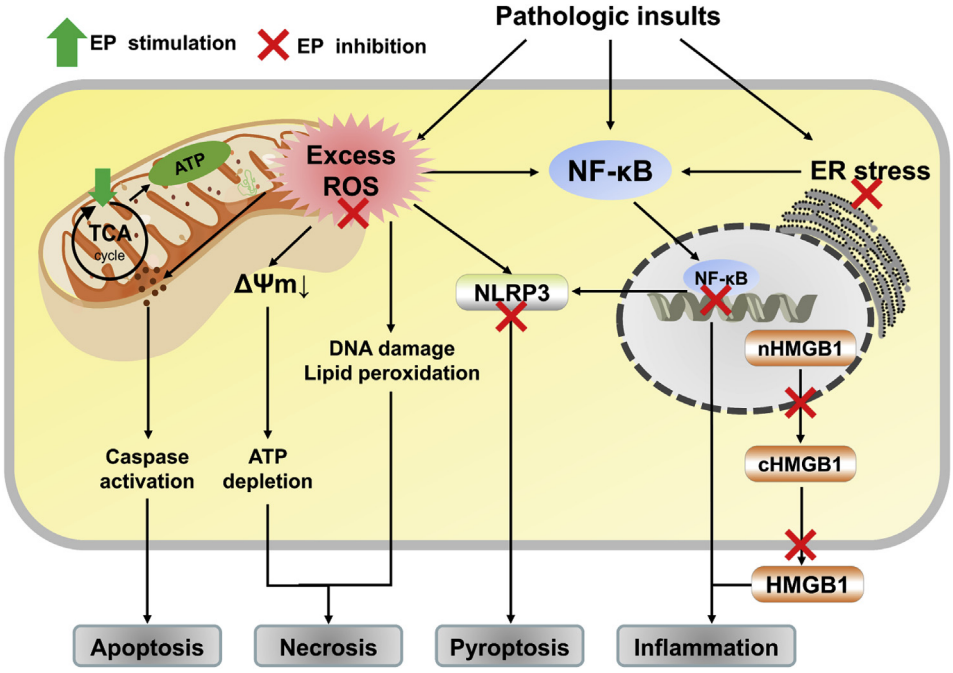

Fig. 1. The proposed ethyl pyruvate protection mechanisms in acute pancreatitis. A range of pathological celluar signalling pathways are activated in pancreatic acinar cells, immune cells, and cells from vital organs during acute pancreatitis. Ethyl pyruvate can act directly or indirectly (hydrolysed to pyruvate and ethanol) as a metabolic substrate to fuel the TCA cycle, promoting ATP production. Ethyl pyruvate can also scavenge ROS and inhibit ER stress, NF-kB, NLRP3 inflammasome as well as prevent HMGB1 release. ΔΨm, mitochondrial membrane potential; EP, ethyl pyruvate; ER, endoplasmic reticulum; cHMGB1, cytosolic HMGB1; HMGB1, high mobility group box-l; NF-kB, nuclear factor-kB; nHMGB1, nuclear HMGB1; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrindomain-containing-3; ROS, reactive oxygen species; TCA, tricarboxylic acid.

Table 2
Effects of ethyl pyruvate and its derivatives on local and systemic injury of experimental acute pancreatitis.

Study	Year	Model	Species	Regimen	Dissolving solution (injection volume)	Sampling	Key findings
Yang et al. [61]	2004	CDE diet (24 h)/CER (7×50 μg/kg/h; i.p.)/ LPS (4 mg/kg; i.p.)	Mice	EP vs. RLS (both 50 mg/kg; i.p.), given at 10 min before and 6 h after first CER	RLS (28 mM EP, 0.4 ml)	7 or 10 h after first CER	EP significantly reduced pancreatic, lung, liver and gut injury compared with RLS group
Cheng et al. [69]	2007	NaTC (3% or 5%, 1 ml/kg; i.d.)	Rats	EP (40 mg/kg; i.v.), given at 12 h	RLS (28 mM EP, 3 ml)	24 h	EP significantly attenuated liver and kidney injury together with reduced serum cytokines, LDH, CRP and HMGB1
Ziolkowski et al. [67]	2008	CER	Rats	SP (250 mg/kg/h for 4 h; i.v.)	NaCl (0.9%)	NA	SP significantly reduced pancreatic injury
Yang et al. [70]	2008	NaTC (5%, 1 ml/kg; i.d.)	Rats	EP (30 mg/kg; i.v.) given at 12, 18 or 30 h	RLS (28 mM EP, 2.3 ml)	24 or 48 h	EP significantly reduced lung, liver, kidney injury, serum cytokines and HMGB1
Yang et al. [71]	2009	CDE diet (24 h)/CER (7×50 μg/kg/h; i.p.)/ LPS (4 mg/kg; i.p.)	Mice	EP vs. RLS (both 40 mg/kg; i.p.), given at 2 h after LPS injection, q6h	RLS (0.6 ml)	48 h	EP significantly reduced liver injury and maintained circulating blood volume compared with the RLS group
Luan et al. [62]	2010	NaTC (5%, 1.5 ml/kg; i.d.)	Rats	EP (40 mg/kg, i.v.), beginning at 6 h, q6h	RLS (28 mM EP, 3 ml)	24 h	EP significantly attenuated pancreatic and gut injury
Luan et al. [63]	2012	NaTC (5%, 1.5 ml/kg; i.d.)	Rats	EP (40 mg/kg, i.v.), beginning at 6 h, q6h		24 h	EP significantly reduced pancreatic and liver injury
Luan et al. [64]	2013	NaTC (5%, 1.5 ml/kg; i.d.)	Rats	EP (40 mg/kg, i.v.), beginning at 0 h, q6h		24 h	EP significantly improved pancreatic injury
Luan et al. [65]	2013	NaTC (5%, 1.5 ml/kg; i.d.)	Rats	EP (40 mg/kg, i.v.) beginning at 0 h, q6h		24 h	EP significantly reduced pancreatic and lung injury
Matone et al. [66]	2013	NaTC (2.5%, 1 ml/kg, i.d.)	Rats	EP vs. RLS (both 50 mg/kg, i.p.), given at 3 and 6 h	RLS (28 mM EP, 5 ml)	8 h	EP significantly attenuated lung injury when compared with RLS group
Irie et al. [68]	2017	CER (6×50 μg/kg/h; i.p.)	Mice	EP (80 mg/kg, i.p.), given at 1 h before first CER	NA	7 h	EP prevented pancreatitis-associated hyperalgesia without significantly improving pancreatic injury

EP, ethyl pyruvate; CDE, choline-deficient ethionine-supplemented; CER, caerulein; i.p., intraperitoneal; LPS, lipopolysaccharide; RLS, Ringer's lactate solution; NaTC, sodium taurocholate; i.d., intra-ductal; i.v., intravenous; LDH, lactate dehydrogenase; CRP, C-reactive protein; NA, not available; HMGB1, high mobility group box-1 protein.

[68]. It was noted that this analgesic effect was not associated with reduced pancreatic tissue weight or plasma amylase activity [68].

General systemic inflammatory markers

Consistent with its protective effects on local and distant organ injury, EP treatment significantly decreased serum TNF-α, IL-1β, IL-6 levels in rats with severe AP [69]. EP treatment also reduced serum HMGB1, C-reactive protein, and lactate dehydrogenase levels [62,69,70], all markers of systemic inflammation. The above results show that EP has pharmacological activity for anti-inflammatory effects and can inhibit the release of various early inflammatory cytokines and late inflammatory mediators such as HMGB1 in experimental AP.

Acute respiratory failure

There were 4 studies [61,65,66,70] investigated the effect of EP on acute respiratory failure secondary to AP. In CDE-diet/CER/LPS-AP in mice, Yang et al. [61] used fluorescein isothiocyanate-albumin to detect the integrity of pulmonary alveolar endothelial/epithelial cells and found that EP significantly ameliorated pulmonary hyperpermeability and histopathologic evidence of pulmonary inflammation compared with RLS alone group. These findings were in accord with significantly decreased lung wet to dry weight ratio, TNF-α, IL-1β and HMGB1 expression, nuclear NF-kB translocation as well as reduced malondialdehyde concentration, myeloperoxidase activity, and lung histopathological scores (congestion/oedema, neutrophil infiltration and intra-alveolar haemorrhage) which resulting in improved lung permeability in NaTC-AP in rats [65,70]. Similarly, Matone et al. [66] found that RLS-EP significantly reduced IL-6 and matrix metalloproteinase-2 in the lungs in NaTC-AP in rats [66].

Acute gut dysfunction or failure

There were only 2 studies [61,62] that investigated the role of EP on intestinal barrier function in experimental AP. In one study [61], RLS-EP almost completely prevented bacterial translocation to isolated mesenteric lymph nodes in CDE-diet/CER/LPS-AP in mice, compared with RLS alone. In the other study [62], EP significantly improved ileal histopathology (villous height and mucosal thickness) and decreased myeloperoxidase activity, plasma diamine oxidase activity and endotoxin levels in NaTC-AP in rats.

Acute renal failure

Two studies [69,70] investigated the effect of EP on the risk of acute renal failure. In NaTC-AP and taurodeoxycholate-induced AP, respectively, Yang et al. [70] and Cheng et al. [69] showed that EP significantly reduced blood urea nitrogen and serum creatinine levels, indicating decreased renal dysfunction.

Acute liver injury

There were 5 studies [61,63,69–71] that investigated acute liver injury with NaTC-AP in rats. Serum levels of alanine and aspartate aminotransferases were significantly elevated in AP group, and EP significantly decreased them by 60% [61,69,70]. EP also dramatically suppressed histopathological changes in the liver including oedema, degeneration of cells, hepatocyte necrosis, and inflammatory cells infiltration [63,71]. Furthermore, EP markedly decreased NF-κB DNA binding activity and decreased malondialdehyde concentration, myeloperoxidase activity, haeme oxygenase-1 and HMGB1 protein in the liver [63,71]. Compared with RLS treatment alone, RLS-EP significantly decreased hepatic mRNA expression of TNF-α, IL-1β, IL-6, inducible nitric oxide synthase and cyclooxygenase-2 through its anti-inflammatory and antioxidant action [63,71]. RLS-EP had no apparent effect on hepatic apoptosis [71].

Survival rate

There are 5 studies [61,64,66,69,70] that examined the effect of EP treatment on survival rate in murine models of severe AP (Table 3). In both CDE diet/CER/LPS-AP (mice) [61] and NaTC-AP (rats) [64,66,69,70], RLS-EP treatment significantly increased survival rate compared with no treatment or RLS alone. In one study [69], the impact of dose and timing of EP treatment was examined. The authors demonstrated the survival rate in rats treated with EP was dose-dependent with 40 mg/kg being more effective than lower doses of 0.4 and 4 mg/kg (63% vs. 13% and 6%, respectively, both $P < 0.05$); There was no difference on the effect of EP on survival when given 2 h before or 12 h after the induction of AP (69% vs. 63%, $P > 0.05$). The survival rate decreased dramatically when EP was given 24 and 48 h compared with given 12 h after the induction of AP (38% and 6% vs. 63%, respectively, both $P < 0.05$).

Medicinal chemistry for EP analogs

Given the treatment efficacy of EP and SP in the studies reported, the question arises as to whether the chemical structure of EP and analogs can be further modified to improve their stability and deliverability.

Previous medicinal research of EP analogs

EP is a analog of pyruvic acid, which is the final product of glycolysis and the starting substrate for the tricarboxylic acid (TCA) cycle. The original design of EP aimed to improve the relatively poor stability of pyruvic acid, which can go through a condensation reaction in aqueous solutions to form 2-hydroxy-2-methyl-4-ketogultarate, a potentially toxic inhibitor in the mitochondrial TCA cycle [72].

Pyruvic acid has a chemical structure of α-ketone acid, which can react rapidly and nonenzymatically with hydrogen peroxide [73] and play an important role in ROS scavenging. In order to investigate the relationship between the chemical structure of pyruvate and its anti-oxidant and anti-inflammation effects, a series of commercial available compounds have been screened and compared [55,74–76]. Besides the core chemical structure of α-ketone acid, the results indicate that the lipophilicity of the molecule is very important in the pharmacological effects of the pyruvate derivatives - the greater the lipophilicity is, the more effective the molecule is [74]. As the α-ketone acid and the ester both exhibited similar anti-inflammatory effects, the presence of an ester linkage is not considered essential for the pharmacological effects [74]. However, the ester form of pyruvate was more effective than SP due to increased membrane permeability and lipophilicity [77]. Recently, a novel series of clovamide derivatives have been synthesised and screened. Among them, S-ethyl 2-oxopropanethioate, a novel EP, significantly suppressed the inflammatory response in LPS-activated BV-2 cells [78].

Tautomerization has also been considered for the α-ketone acid derivatives. The compound with a dicarbonyl group can enolize to form a chemical structure of α,β-unsaturated ketone (or ester, acid), a carbon-carbon double bond conjugated to a carbonyl group, which is chemically stable. In aqueous solution, the keto-enol equilibrium for pyruvate significantly favours the keto form [79]. The rate of enolization is substantial, especially in the presence of a divalent cation, such as ionised calcium or magnesium [80]. Therefore, it appears that the pharmacological effects of EP are partially mediated by the enol tautomer. However, when comparing the anti-inflammatory effects of benzoylformic acid and EP, Cruz et al. [74] pointed out that the ability to form an enol tautomer is not essential for the pharmacological effects of pyruvate analogs. The compounds with the chemical structure of α,β-unsaturate ketone (or ester, acid) can undergo Michael addition reaction with nucleophiles, such as thiol groups, amino groups. Therefore the enol form of the pyruvate analog has a tendency to covalently modify nucleic acids and proteins, which may result in toxicity. EP, which mainly exists as keto form in aqueous solution, has low toxicity and is effective as an hydrogen peroxide scavenger.

Structure optimisation and screening for EP analogs

EP is a potential candidate for the treatment of AP, based on the review of *in vitro* and *in vivo* findings. There is scope for chemical structure optimisation to create new compounds with stronger activity and favourable drug-like properties. For instance, it might be worthwhile to increase the lipophilicity of the molecule to increase the pharmacological effects based on the previous studies. Different lipophilic groups (R, R'), either aliphatic or aromatic, can be used to link the core chemical structure (α-ketone acid). On the other hand, when the core structure of EP covalently linked to a different carrier (see below), it is possible to selective deliver pyruvate to the mitochondria with a 100-fold increase in concentration and this may provide further improvements in pharmacological effects (Fig. 2). These mitochondria-targeted carriers include lipophilic cations (for example triphenyl phosphonium) and Szeto-Schiller peptides. There is scope to synthesise other compounds with different specific structure-activity relationships and these will need to be screened in *in vitro* and *in vivo* AP models.

Another potential strategy is to undertake nanoparticle formulation of EP to enhance cellular uptake, increase the bioactivity *in vitro* and superior bioavailability *in vivo*. Thus the strategy should

Table 3
Effects of EP on survival in experimental acute pancreatitis.

Study	Year	Model	Species	Regimen	Period	Survival rate
Yang et al. [61]	2004	CDE diet (24 h)/CER (7 × 50 μg/kg/h; i.p.)/LPS (4 mg/kg; i.p.)	Mice	EP vs. RLS (both 50 mg/kg; i.p.), given at 10 min before and 6 h after first CER	7 d	Control group vs. RLS group vs. EP group: 100% vs. 10% vs. 60%
Cheng et al. [69]	2007	NaTC (3% or 5%, 1 ml/kg; i.d.)	Rats	EP (0.4, 4 and 40 mg/kg; i.v.), given at 12 h, tid, for 3 d	7 d	0.4 mg/kg vs. 4 mg/kg vs. 40 mg/kg: 6.3% vs. 12.5% vs. 62.5%
				EP (40 mg/kg; i.v.), given at −2, 12, 24 and 36 h	7 d	−2 h vs. 12 h vs. 24 h vs. 36 h: 68.8% vs. 62.5% vs. 37.5% vs. 6.3%
Yang et al. [70]	2008	NaTC (5%, 1 ml/kg; i.d.)	Rats	EP (30 mg/kg, i.v.), given at 12, 18 and 30 h	3 d	Untreated group vs. EP group: 0% vs 60%
Luan et al. [64]	2013	NaTC (5%, 1.5 m/kg; i.d.)	Rats	EP (40 mg/kg, i.v.), given at 0 h, q6h	48 h	Control group vs. Untreated group vs. EP group: 100% vs. 13.3% vs. 40%
Matone et al. [66]	2013	NaTC (2.5%, 1 ml/kg; i.d.)	Rats	EP vs RLS (both 50 mg/kg, i.p.), given at 3 and 6 h	3 d	Untreated group vs. EP group: 0% vs. 90%

EP, ethyl pyruvate; CDE, choline-deficient ethionine-supplemented; CER, cerulein; i.p., intraperitoneal; LPS, lipopolysaccharide; RLS, Ringer's lactate solution; NaTC, sodium taurocholate; i.d., intra-ductal; i.v., intravenous; tid, three times per day.

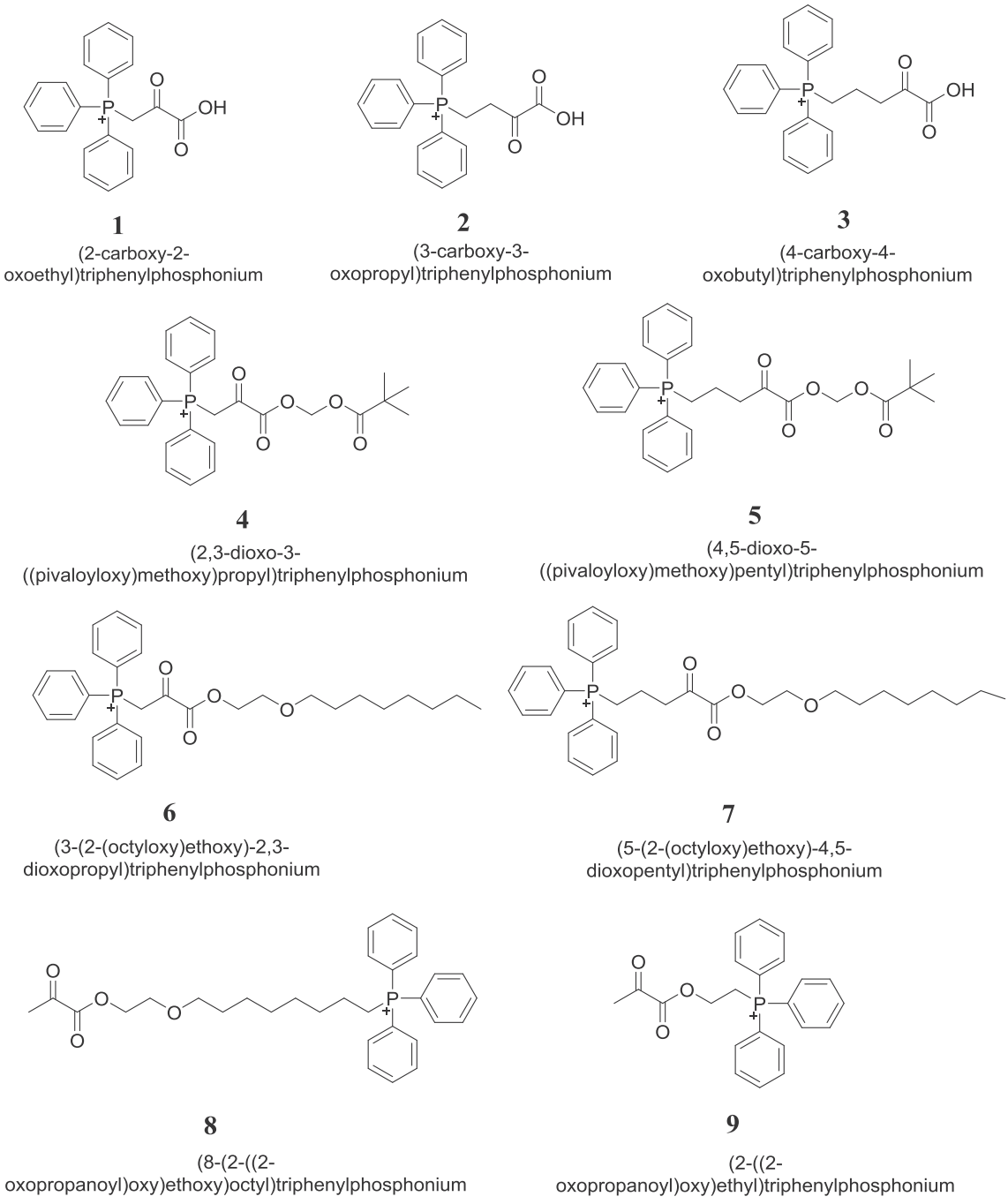

Fig. 2. Chemical structures of newly designed mitochondria-targeted ethyl pyruvate analogs using triphenyl phosphonium.

be to conjugate EP with a number of different carriers including phospholipids, cyclodextrin, phosphatidyl choline, liposomes and nanoparticles. Pharmacokinetics and pharmacodynamics studies will then be required to compare the *in vivo* amount and velocity of EP uptake. This will be necessary before clinical studies are undertaken.

Therapeutic potential of EP in AP: summary and perspectives

In vitro, EP and some of its analogs protect primary acinar cells, immune cells, and cells from vital organs through a number of pathways, including anti-oxidative stress, endoplasmic reticulum stress, inflammation and inflammasome pathways. *In vivo*, limited animal studies with variable methodology show that EP and/or SP significantly reduced pancreatic injury, pain, systemic inflammation and severity of MODS as well as improved survival in murine models of AP. This effect is present even when treatment is given 12 h after the induction of AP.

Fluid resuscitation is a cornerstone for early treatment of AP. RLS has been recommended as the fluid of choice in AP [3,9], although the current American Gastroenterology Association Practice Guidelines have raised concerns because of the risk of lactic acidosis [81]. Lactate alone has been shown to reduce pancreatic and liver injury in Toll-like receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity [23]. The supplementation of RLS with EP may be more effective as

a resuscitation fluid than RLS alone, since EP provides extra fuel for the TCA cycle to provide additional ATP to meet the metabolic demands. It is surprising that there have been no clinical trials of EP or its analogs in human AP. The only existing phase II trial investigated EP and fluid therapy after cardiac surgery[137], while the safety profile of EP was demonstrated, it was not possible to show any improvement in outcomes. As pointed out the efficacy of EP might be enhanced by medicinal chemistry modifications to EP analogs, for example linking pyruvate to a lipophilic mitochondria carrier or liposome. This review has underlined the opportunities that exist to improve the efficacy of fluid resuscitation by using EP (or an analog) as a supplement. Further experimental and clinical studies are required.

Software used for structure drawing

ChemDraw Ultra.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work is supported by NZ-China Strategic Research Alliance 2016 Award (China: 2016YFE0101800, Q.X. and W.H.; NZ: J.A.W.); Key Technology R&D Programme of Sichuan Provincial Department of Science and Technology (2015SZ0229, L.D. and X.Q.); the Introduction Programme of Scientific Researchist of Sichuan University of Science and Engineering (2017RCL61); the Scientific Research Fund of the Sichuan Provincial Education Department (18ZA0360); Students Innovation Fund of Sichuan Province (grant number 201810622007); and National Institute for Health Research Senior Investigator Award (R.S.).

References

[1] Xiao AY, Tan ML, Wu LM, et al. Global incidence and mortality of pancreatic diseases: a systematic review, meta-analysis, and meta-regression of population-based cohort studies. Lancet Gastroenterol Hepatol 2016;1:45–55.
[2] Peery AF, Crockett SD, Barritt AS, et al. Burden of gastrointestinal, liver, and pancreatic diseases in the United States. Gastroenterology 2015;149:1731–41. e3.
[3] Tenner S, Baillie J, DeWitt J, et al. American College of Gastroenterology guideline: management of acute pancreatitis. Am J Gastroenterol 2013;108:1400–15. 1416.
[4] Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013;62:102–11.
[5] Huang W, Windsor JA. Fulminant or early severe acute pancreatitis is overlooked by classifications of severity. Crit Care Med 2017;45:e744–5.
[6] Guo Q, Li A, Xia Q, et al. The role of organ failure and infection in necrotizing pancreatitis: a prospective study. Ann Surg 2014;259:1201–7.
[7] Kadiyala V, Suleiman SL, McNabb-Baltar J, et al. The Atlanta classification, revised Atlanta classification, and determinant-based classification of acute pancreatitis: which is best at stratifying outcomes? Pancreas 2016;45:510–5.
[8] Schepers NJ, Bakker OJ, Besselink MG, et al. Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis. Gut 2018;0:1–8.
[9] Working Group IAPAPAAPG. IAP/APA evidence-based guidelines for the management of acute pancreatitis. Pancreatology 2013;13:e1–15.
[10] Haydock MD, Mittal A, Wilms HR, et al. Fluid therapy in acute pancreatitis: anybody's guess. Ann Surg 2013;257:182–8.
[11] Leung PS, Chan YC. Role of oxidative stress in pancreatic inflammation. Antioxidants Redox Signal 2009;11:135–65.
[12] Booth DM, Mukherjee R, Sutton R, et al. Calcium and reactive oxygen species in acute pancreatitis: friend or foe? Antioxidants Redox Signal 2011;15:2683–98.
[13] Booth DM, Murphy JA, Mukherjee R, et al. Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. Gastroenterology 2011;140:2116–25.
[14] Hackert T, Werner J. Antioxidant therapy in acute pancreatitis: experimental and clinical evidence. Antioxidants Redox Signal 2011;15:2767–77.
[15] Chvanov M, Huang W, Jin T, et al. Novel lipophilic probe for detecting near-membrane reactive oxygen species responses and its application for studies of pancreatic acinar cells: effects of pyocyanin and L-ornithine. Antioxidants Redox Signal 2015;22:451–64.
[16] Shalbueva N, Mareninova OA, Gerloff A, et al. Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis. Gastroenterology 2013;144:437–446 e6.
[17] Mukherjee R, Mareninova OA, Odinokova IV, et al. Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP. Gut 2016;65:1333–46.
[18] Shore ER, Awais M, Kershaw NM, et al. Small molecule inhibitors of cyclophilin D to protect mitochondrial function as a potential treatment for acute pancreatitis. J Med Chem 2016;59:2596–611.
[19] Lugea A, Tischler D, Nguyen J, et al. Adaptive unfolded protein response attenuates alcohol-induced pancreatic damage. Gastroenterology 2011;140:987–97.
[20] Lugea A, Gerloff A, Su HY, et al. The combination of alcohol and cigarette smoke induces endoplasmic reticulum stress and cell death in pancreatic acinar cells. Gastroenterology 2017;153:1674–86.
[21] Biczo G, Vegh ET, Shalbueva N, et al. Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. Gastroenterology 2018;154:689–703.
[22] Hoque R, Sohail M, Malik A, et al. TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. Gastroenterology 2011;141:358–69.
[23] Hoque R, Farooq A, Ghani A, et al. Lactate reduces liver and pancreatic injury in Toll-like receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity. Gastroenterology 2014;146:1763–74.
[24] Hoque R, Mehal WZ. Inflammasomes in pancreatic physiology and disease. Am J Physiol Gastrointest Liver Physiol 2015;308:G643–51.
[25] Kang R, Chen R, Xie M, et al. The receptor for advanced glycation end products activates the AIM2 inflammasome in acute pancreatitis. J Immunol 2016;196:4331–7.
[26] Gukovskaya AS, Gukovsky I, Algul H, et al. Autophagy, inflammation, and immune dysfunction in the pathogenesis of pancreatitis. Gastroenterology 2017;153:1212–26.
[27] Moggia E, Koti R, Belgaumkar AP, et al. Pharmacological interventions for acute pancreatitis. Cochrane Database Syst Rev 2017;4. CD011384.
[28] Afghani E, Pandol SJ, Shimosegawa T, et al. Acute pancreatitis-progress and challenges: a report on an international symposium. Pancreas 2015;44:1195–210.
[29] Fink MP. Ethyl pyruvate. Curr Opin Anaesthesiol 2008;21:160–7.
[30] Kao KK, Fink MP. The biochemical basis for the anti-inflammatory and cytoprotective actions of ethyl pyruvate and related compounds. Biochem Pharmacol 2010;80:151–9.
[31] Sims CA, Wattanasirichaigoon S, Menconi MJ, et al. Ringer's ethyl pyruvate solution ameliorates ischemia/reperfusion-induced intestinal mucosal injury in rats. Crit Care Med 2001;29:1513–8.
[32] Fink MP. Ethyl pyruvate: a novel anti-inflammatory agent. J Intern Med 2007;261:349–62.
[33] Yang R, Zhu S, Tonnessen TI. Ethyl pyruvate is a novel anti-inflammatory agent to treat multiple inflammatory organ injuries. J Inflamm 2016;13:37.
[34] Wang G, Liu Y, Dui D, et al. Trypsinogen activation peptide induces HMGB1 release from rat pancreatic acinar cells. Open Med 2017;12:293–8.
[35] Peng S, Gerasimenko JV, Tsugorka TM, et al. Galactose protects against cell damage in mouse models of acute pancreatitis. J Clin Invest 2018;128:3769–78.
[36] Huang W, Xiong JJ, Cheng CR, et al. Therapeutic potential of pyruvate in acute pancreatitis: in vitro findings and a systematic review. Pancreas 2015;44:1380.
[37] Rossmann C, Nusshold C, Paar M, et al. Ethyl pyruvate inhibits oxidation of LDL in vitro and attenuates oxLDL toxicity in EA.hy926 cells. PLoS One 2018;13, e0191477.
[38] Nagatome M, Kondo Y, Kadowaki D, et al. Ethyl pyruvate attenuates acetaminophen-induced liver injury and prevents cellular injury induced by N-acetyl-p-benzoquinone imine. Heliyon 2018;4, e00521.
[39] Li S, Liang F, Kwan K, et al. Identification of ethyl pyruvate as a NLRP3 inflammasome inhibitor that preserves mitochondrial integrity. Mol Med 2018;24:8.
[40] Zhu Q, Wang H, Wang H, et al. Protective effects of ethyl pyruvate on lipopolysaccharideinduced acute lung injury through inhibition of autophagy in neutrophils. Mol Med Rep 2017;15:1272–8.
[41] Li W, Yang X, Peng M, et al. Inhibitory effects of ethyl pyruvate on platelet aggregation and phosphatidylserine exposure. Biochem Biophys Res Commun 2017;487:560–6.
[42] Relja B, Omid N, Wagner N, et al. Ethanol, ethyl and sodium pyruvate decrease the inflammatory responses of human lung epithelial cells via Akt and NF-kappaB in vitro but have a low impact on hepatocellular cells. Int J Mol Med 2016;37:517–25.
[43] Shin JH, Kim ID, Kim SW, et al. Ethyl pyruvate inhibits HMGB1 phosphorylation and release by chelating calcium. Mol Med 2015;20:649–57.

[44] Relja B, Omid N, Schaible A, et al. Pre- or post-treatment with ethanol and ethyl pyruvate results in distinct anti-inflammatory responses of human lung epithelial cells triggered by interleukin-6. Mol Med Rep 2015;12:2991–8.
[45] Wang G, Liu K, Li Y, et al. Endoplasmic reticulum stress mediates the anti-inflammatory effect of ethyl pyruvate in endothelial cells. PLoS One 2014;9, e113983.
[46] Jang HJ, Kim YM, Tsoyi K, et al. Ethyl pyruvate induces heme oxygenase-1 through p38 mitogen-activated protein kinase activation by depletion of glutathione in RAW 264.7 cells and improves survival in septic animals. Antioxidants Redox Signal 2012;17:878–89.
[47] Mizutani A, Maeda N, Toku S, et al. Interaction of ethyl pyruvate in vitro with NF-kappaB subunits, RelA and p50. Eur J Pharmacol 2011;650:151–6.
[48] Mizutani A, Maeda N, Toku S, et al. Inhibition by ethyl pyruvate of the nuclear translocation of nuclear factor-kappaB in cultured lung epithelial cells. Pulm Pharmacol Therapeut 2010;23:308–15.
[49] Johansson AS, Palmblad J. Ethyl pyruvate modulates adhesive and secretory reactions in human lung epithelial cells. Life Sci 2009;84:805–9.
[50] Dave SH, Tilstra JS, Matsuoka K, et al. Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis. J Leukoc Biol 2009;86:633–43.
[51] Johansson AS, Johansson-Haque K, Okret S, et al. Ethyl pyruvate modulates acute inflammatory reactions in human endothelial cells in relation to the NF-kappaB pathway. Br J Pharmacol 2008;154:1318–26.
[52] Hollenbach M, Hintersdorf A, Huse K, et al. Ethyl pyruvate and ethyl lactate down-regulate the production of pro-inflammatory cytokines and modulate expression of immune receptors. Biochem Pharmacol 2008;76:631–44.
[53] van Zoelen MA, de Vos AF, Larosa GJ, et al. Intrapulmonary delivery of ethyl pyruvate attenuates lipopolysaccharide- and lipoteichoic acid-induced lung inflammation in vivo. Shock 2007;28:570–5.
[54] Lim SC, Choi JE, Kim CH, et al. Ethyl pyruvate induces necrosis-to-apoptosis switch and inhibits high mobility group box protein 1 release in A549 lung adenocarcinoma cells. Int J Mol Med 2007;20:187–92.
[55] Sappington PL, Cruz Jr RJ, Harada T, et al. The ethyl pyruvate analogues, diethyl oxaloproprionate, 2-acetamidoacrylate, and methyl-2-acetamidoacrylate, exhibit anti-inflammatory properties in vivo and/or in vitro. Biochem Pharmacol 2005;70:1579–92.
[56] Han Y, Englert JA, Yang R, et al. Ethyl pyruvate inhibits nuclear factor-kappaB-dependent signaling by directly targeting p65. J Pharmacol Exp Therapeut 2005;312:1097–105.
[57] Song M, Kellum JA, Kaldas H, et al. Evidence that glutathione depletion is a mechanism responsible for the anti-inflammatory effects of ethyl pyruvate in cultured lipopolysaccharide-stimulated RAW 264.7 cells. J Pharmacol Exp Therapeut 2004;308:307–16.
[58] Sappington PL, Han X, Yang R, et al. Ethyl pyruvate ameliorates intestinal epithelial barrier dysfunction in endotoxemic mice and immunostimulated caco-2 enterocytic monolayers. J Pharmacol Exp Therapeut 2003;304:464–76.
[59] Ulloa L, Ochani M, Yang H, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. Proc Natl Acad Sci U S A 2002;99:12351–6.
[60] Salahudeen AK, Clark EC, Nath KA. Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo. J Clin Invest 1991;88:1886–93.
[61] Yang R, Uchiyama T, Alber SM, et al. Ethyl pyruvate ameliorates distant organ injury in a murine model of acute necrotizing pancreatitis. Crit Care Med 2004;32:1453–9.
[62] Luan ZG, Zhang H, Ma XC, et al. Role of high-mobility group box 1 protein in the pathogenesis of intestinal barrier injury in rats with severe acute pancreatitis. Pancreas 2010;39:216–23.
[63] Luan ZG, Zhang H, Ma XC, et al. Therapeutic treatment with ethyl pyruvate attenuates the severity of liver injury in rats with severe acute pancreatitis. Pancreas 2012;41:729–37.
[64] Luan ZG, Ma XC, Zhang H, et al. Protective effect of ethyl pyruvate on pancreas injury in rats with severe acute pancreatitis. J Surg Res 2013;181:76–84.
[65] Luan ZG, Zhang J, Yin XH, et al. Ethyl pyruvate significantly inhibits tumour necrosis factor-alpha, interleukin-1beta and high mobility group box 1 releasing and attenuates sodium taurocholate-induced severe acute pancreatitis associated with acute lung injury. Clin Exp Immunol 2013;172:417–26.
[66] Matone J, Moretti AI, Apodaca-Torrez FR, et al. Ethyl-pyruvate reduces lung injury matrix metalloproteinases and cytokines and improves survival in experimental model of severe acute pancreatitis. Acta Cir Bras 2013;28:559–67.
[67] Ziolkowski W, Wierzba TH, Kaczor JJ, et al. Intravenous sodium pyruvate protects against cerulein-induced acute pancreatitis. Pancreas 2008;37:238–9.
[68] Irie Y, Tsubota M, Ishikura H, et al. Macrophage-derived HMGB1 as a pain mediator in the early stage of acute pancreatitis in mice: targeting RAGE and CXCL12/CXCR4 Axis. J Neuroimmune Pharmacol 2017;12:693–707.
[69] Cheng BQ, Liu CT, Li WJ, et al. Ethyl pyruvate improves survival and ameliorates distant organ injury in rats with severe acute pancreatitis. Pancreas 2007;35:256–61.
[70] Yang ZY, Ling Y, Yin T, et al. Delayed ethyl pyruvate therapy attenuates experimental severe acute pancreatitis via reduced serum high mobility group box 1 levels in rats. World J Gastroenterol 2008;14:4546–50.
[71] Yang R, Shaufl AL, Killeen ME, et al. Ethyl pyruvate ameliorates liver injury secondary to severe acute pancreatitis. J Surg Res 2009;153:302–9.
[72] Montgomery CM, Webb JL. Metabolic studies on heart mitochondria. II. The inhibitory action of parapyruvate on the tricarboxylic acid cycle. J Biol Chem 1956;221:359–68.
[73] Meijer FG, Gruber M, Monfoort CH. Influence of aneurin and aneurin pyrophosphate on anaerobic pyruvate metabolism of pig heart muscle in vitro. Nature 1949;163:914.
[74] Cruz Jr RJ, Harada T, Sasatomi E, et al. Effects of ethyl pyruvate and other alpha-keto carboxylic acid derivatives in a rat model of multivisceral ischemia and reperfusion. J Surg Res 2011;165:151–7.
[75] Leelahavanichkul A, Yasuda H, Doi K, et al. Methyl-2-acetamidoacrylate, an ethyl pyruvate analog, decreases sepsis-induced acute kidney injury in mice. Am J Physiol Renal Physiol 2008;295:F1825–35.
[76] Lee HK, Kim ID, Kim SW, et al. Anti-inflammatory and anti-excitoxic effects of diethyl oxopropanamide, an ethyl pyruvate bioisoster, exert robust neuroprotective effects in the postischemic brain. Sci Rep 2017;7:42891.
[77] Varma SD, Devamanoharan PS, Ali AH. Prevention of intracellular oxidative stress to lens by pyruvate and its ester. Free Radic Res 1998;28:131–5.
[78] Min S, More SV, Park JY, et al. EOP, a newly synthesized ethyl pyruvate derivative, attenuates the production of inflammatory mediators via p38, ERK and NF-kappaB pathways in lipopolysaccharide-activated BV-2 microglial cells. Molecules 2014;19:19361–75.
[79] Chiang Y, Kresge AJ, Pruszynski P. Keto-enol equilibria in the pyruvic acid system: determination of the keto-enol equilibrium constants of pyruvic acid and pyruvate anion and the acidity constant of pyruvate enol in aqueous solution. J Am Chem Soc 1992;114:3103–7.
[80] Wilton DC. The mechanism of adduct formation between NAD+ and pyruvate bound to pig heart lactate dehydrogenase. Biochem J 1979;177:951–7.
[81] Vege SS, DiMagno MJ, Forsmark CE, et al. Initial medical treatment of acute pancreatitis: American gastroenterological association Institute technical review. Gastroenterology 2018;154:1103–39.