

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

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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.

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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 ($\text{CH}_3\text{COCOO}^-$), 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- κ B, nuclear factor- κ B; 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- α .

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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-1, 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 oxalopropionate	EP had activities that counteracted LPS-induced oxidative (HO-1, iNOS), cell death (caspase-1 and NLRP3 activation, HMGB1 release) and inflammatory mediators (NF- κ B, 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-1 β , 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- κ B, 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 oxalopropionate	EP inhibited cytomix ^a -induced inflammation and (NF- κ B, 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-1 β - 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, pyrin domain-containing-3; NF- κ B, nuclear factor- κ B; 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-1 β).

activation peptide alone. In the other study, Peng et al. [35] showed that SP (10 mM) significantly protected ATP loss and necrosis in mouse pancreatic acinar cells induced by palmitoleic acid (50 μ M), 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 κ B (NF- κ B) DNA binding activity and pancreatic tumour necrosis factor- α (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- κ B 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

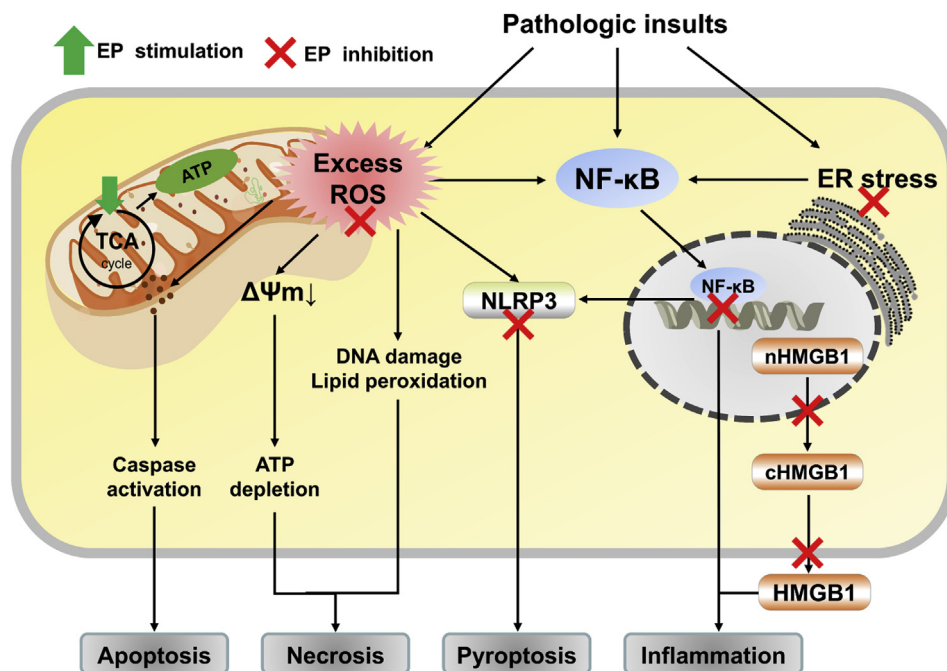


Fig. 1. The proposed ethyl pyruvate protection mechanisms in acute pancreatitis. A range of pathological cellular signalling pathways are activated in pancreatic acinar cells, immune cells, and cells from vital organs during acute pancreatitis. Ethyl pyruvate can act directly or indirectly (hydrolysed 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- κ B, NLRP3 inflammasome as well as prevent HMGB1 release. $\Delta\Psi_m$, mitochondrial membrane potential; EP, ethyl pyruvate; ER, endoplasmic reticulum; cHMGB1, cytosolic HMGB1; HMGB1, high mobility group box-1; NF- κ B, nuclear factor- κ B; nHMGB1, nuclear HMGB1; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-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-ducal; 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-1 β , 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-1 β and HMGB1 expression, nuclear NF- κ B 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- κ B 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-1 β , 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-ketoglutarate, 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 -unsaturated 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 EP (40 mg/kg; i.v.), given at -2, 12, 24 and 36 h	7 d 7 d	0.4 mg/kg vs. 4 mg/kg vs. 40 mg/kg: 6.3% vs. 12.5% vs. 62.5% -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.

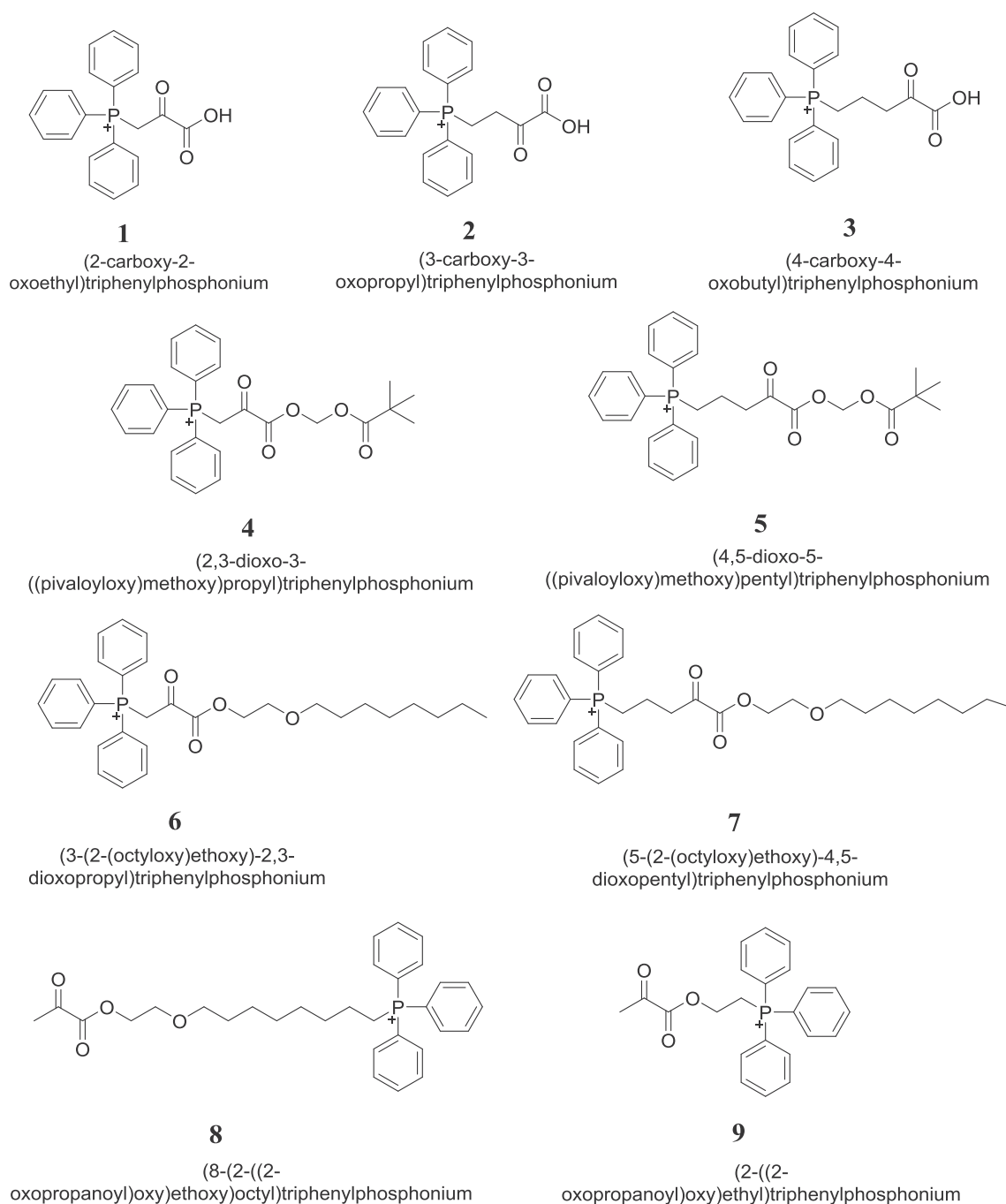


Fig. 2. Chemical structures of newly designed mitochondria-targeted ethyl pyruvate analogs using triphenyl phosphonium.

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 surgery¹³⁷, 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.

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