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RESEARCH PAPER

Therapeutic treatment with poly(ADP-ribose) polymerase inhibitors attenuates the severity of acute pancreatitis and associated liver and lung injury

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Background and purpose: The mortality associated with acute pancreatitis (AP) is largely attributable to abnormalities that occur in distant organs and supportive care remains the only treatment for patients with these complications. Recently, prophylactic pharmacological blockade of poly(ADP-ribose) polymerase (PARP) enzymes has been shown to attenuate the severity of the disease. However, the clinical relevance of PARP inhibitors administered after the onset of AP remains uncertain. The aim of the present study was to investigate the therapeutic effects of PARP inhibitors in established AP.
Experimental approach: Mice were fed a choline/methionine-deficient/ethionine-supplemented (CMDE) diet to induce AP. PARP inhibitors were given at 36 h after the onset of CMDE diet. Severity of pancreatitis was assessed by measurements of serum amylase, lipase, IL-1β and IL-6, and histological grading. Serum hepatic enzymes, myeloperoxidase (MPO) activity and morphological changes were measured as indicators of hepatic insult. Lung injury was evaluated by MPO activity and morphological changes. Survival rates of mice were monitored for 7 days.
Key results: CMDE diet administration resulted in a significant increase in serum amylase, lipase, IL-1β, IL-6, alanine aminotransferase and aspartate aminotranferase levels, indicating AP and associated liver injury. Analysis of the histopathological changes in pancreas, liver and lung revealed extensive tissue damage. Treatment of mice with PARP-inhibitors after the onset of AP was associated with a reduction in the severity of AP and, accordingly, with a reduced mortality rate.
Conclusions and Implications: Our results support the therapeutic application of PARP inhibitors in the treatment of established AP.
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Keywords: acute pancreatitis; poly(ADP-ribose) polymerase; therapeutic administration; lung injury; liver injury; inflammation

Abbreviations: ALT, alanine aminotransferase; AP, acute pancreatitis; AST, aspartate aminotransferase; CMDE, choline/methionine-deficient/ethionine-supplemented; H&E, hematoxylin and eosin; MPO, myeloperoxidase; NADPH, nicotinamide adenine dinucleotide phosphate; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species

Introduction

Acute pancreatitis (AP) is a common disease of variable severity ranging from a mild and self-limiting condition to a severe form, the latter still being associated with a mortality rate of 15–25% (Imrie, 1997; Steer, 2002). AP involves a complex cascade of local and systemic events. A still unknown triggering event within the pancreas converts digestive proenzymes into their active forms, leading to membrane disruption, oedema, interstitial haemorrhage and necrosis accompanied by an inflammatory response with infiltrating leucocytes that contributes to the progression of both, the local pancreatic destruction and the subsequent systemic manifestations. In fact, the morbidity and mortality associated with severe AP are mostly attributable to abnormalities that occur within distant organs, such as liver and lungs (Steinberg and Tenner, 1994; Mentula *et al*., 2005).

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Hepatic injury during severe AP has long been recognized as evidence by the inclusion of serum hepatic enzymes in Ranson's criteria predicting the severity of AP. Hepatocellular insult is also apparent in experimental models of severe necrotizing pancreatitis, with increased serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Yang *et al*., 1998; Gray *et al*., 2006).

Despite advances in the diagnosis and treatment of inflammatory pancreatic disease, most therapies are supportive and target the haemodynamic effects by removal of precipitating factors that include alcohol and biliary obstructing calculi (Banks, 1997; Baron and Morgan, 1999). Recently, it has been shown that genetic or pharmacological blockade of the enzyme poly(ADP-ribose) polymerase-1 (PARP-1) before the onset of AP, attenuated the severity of the disease in mice (Mota *et al*., 2005; Mazzon *et al*., 2006). However, the clinical relevance of treatment with PARP inhibitors after the onset of AP remains to be established.

PARP-1 belongs to a family of enzymes that, using NAD^+ as a substrate, synthesize and transfer ADP-ribose polymers onto glutamic acid residues of various acceptor proteins involved in chromatin structure and DNA metabolism (Schreiber *et al*., 2006). Members of the PARP family share a catalytic domain that contains the PARP signature motif, a highly conserved sequence that forms the active site. PARP-1 specifically detects DNA-strand breaks generated by different genotoxic agents. Moreover, PARP-1 plays a relevant role in cell necrosis and organ failure in various diseases associated with inflammation (Cuzzocrea, 2005). Accordingly, inhibition of PARP-1 might control the severity of AP by preventing both the local and systemic inflammatory response, as well as controlling the mechanisms involved in pancreatic cell necrosis mediated by neutrophils via the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Mota *et al*., 2005). Neutrophil NADPH oxidase initiates the generation of reactive oxygen species (ROS), including hydrogen peroxide and superoxide (Gukovskaya *et al*., 2002). Rapid DNA single-stranded breaks are induced by ROS, leading to over-activation of PARP and depletion of cellular energy stores resulting in mitochondrial-free radical generation and cell necrosis (Szabo and Dawson 1998; Hwang *et al*., 2002).

The aim of this study was to investigate the therapeutic effects of PARP inhibitors in controlling the morbidity and mortality associated with severe AP in an experimental model based on the feeding of young female mice with a choline/methionine-deficient/ethionine-supplemented (CMDE) diet. To achieve this, PARP inhibitors were given after the clinical, biochemical and pathological signs of the disease had already appeared in the animals. Our results support the therapeutic use of PARP inhibitors in the control of severe AP.

Materials and methods

Animals and treatments

Female Swiss–Webster mice (3–4 weeks old) were purchased from The Harlam Iberica Laboratory (Barcelona, Spain). The animals were kept under standardized conditions with a 12-h light/dark cycle. Mice were fasted from solid food for 24 h (but allowed water *ad libitum*) and then fed the choline/methionine-deficient diet (Harland Teklad, Madison, WI, USA) supplemented with 0.5% DL-ethionine (Sigma, St Louis, MO, USA) (CMDE) for 3 days. To ensure equal exposure by all animals, the diet was replaced with clean and fresh CMDE diet every 24 h. Following this period of CMDE-diet administration, animals were given a normal diet to permit estimation of the 7-day mortality rate.

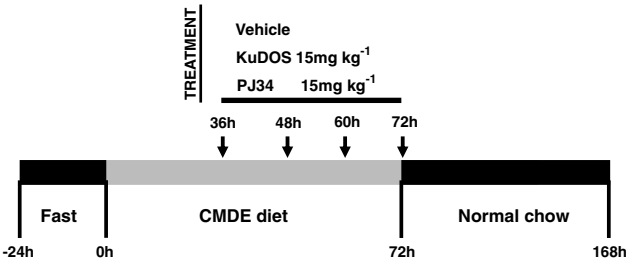

Figure 1 Treatment protocol to evaluate the therapeutic effects of PARP inhibitors in AP. AP, acute pancreatitis; PARP, poly(ADP-ribose) polymerase.

Pharmacological inhibition of PARP activity was carried out by treating mice (intraperitoneally) with either *N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethyacetamide HCl (PJ34) (Alexis, Carlsbad, CA, USA) (15 mg kg^{-1} every 12 h) or KuDOS (KU0058684) (KuDOS Pharmaceutical Ltd., Cambridge, UK) (15 mg kg^{-1} every 12 h), starting 36 h after the CMDE diet was provided, till regular chow replaced the toxic diet (Figure 1). Both, PJ34 ($EC_{50} = 20$ nM) (Garcia-Soriano *et al*., 2001) and KU0058684 ($IC_{50} = 3.2$ nM) (Farmer *et al*., 2005) are competitive inhibitors with respect to the PARP substrate NAD^+. PJ34 and KU0058684 were dissolved in dimethylsulphoxide (DMSO) at 20 mg ml^{-1} and then diluted in saline (0.9% NaCl). Saline containing 5% DMSO was used as vehicle for control mice.

All experimental procedures were performed in accordance with the University of Murcia approved institutional animal care guidelines that are in compliance with regulations in Spain (RD 1201/2005), Europe (86/609) and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Sample collection

At the indicated times, mice were killed by CO_2 inhalation. Blood, pancreas, liver and lungs were removed for further studies. Serum samples were obtained by centrifugation of blood at 1100 *g*, aliquoted and frozen at −70°C until analysed. Organs were either fixed in 4% formaldehyde or rinsed in saline buffer, snap-frozen in liquid nitrogen and stored at −70°C until analysed.

Myeloperoxidase determination

Myeloperoxidase (MPO) activity was measured photometrically. Tissue samples were thawed, homogenized in 1 ml of 20 mM phosphate buffer (pH 7.4) and then centrifuged at 10 500 *g* for 12 min at 4°C. The resulting pellet was resuspended in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (Sigma).

Homogenates were then frozen in liquid nitrogen and thawed on four consecutive occasions before a final 40 s sonication. Samples were then centrifuged at 10 500 *g* for 12 min at 4°C and supernatants were collected for MPO assay. The assay mixture consisted of 10 μl supernatant, 25 μl 3,3′5,5′-tetramethylbenzidine (Sigma) (final concentration, 1.6 mM), 25 μl H_2O_2 (Sigma) (final concentration, 0.3 mM) diluted in 80 mM phosphate buffer (pH 5.4) and 40 μl of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide. This mixture was incubated for 2 min at 37°C. The reaction was stopped with 1N HCl and absorbance was measured at 450 nm. An enzyme unit is defined as the amount of enzyme that produces an increase of 1 absorbance unit min^{-1}.

Histological analysis

For histopathological analysis, formaldehyde-fixed specimens were embedded in paraffin, cut in 4 μm sections and stained with hematoxylin and eosin (H&E). Analysis of the histopathological changes in pancreas, liver and lung was carried out under light microscopy by two expert pathologists who were unaware of the treatments. Histological grading was scored by assigning a subjective value: 0, absent; 1, mild; 2, moderate and 3, severe to certain typical tissue injuries. Necrosis, leucocyte infiltration and vacuolization were analysed for liver sections; necrosis and infiltration of inflammatory cells were scored in pancreas samples and necrosis, leucocyte infiltration, capillary congestion and alveolar membrane thickening were scored in lung samples.

Serum amylase, lipase, ALT, AST and cytokine determination

Serum concentration of lipase, amylase, ALT and AST were measured in a Modular P800 photometric analyzer (Roche, Mannheim, Germany) using commercial kits according to the manufacturer's protocols (Roche). The serum levels of interleukin (IL)-6 and IL-1β were determined by enzyme-linked immunosorbent assay, according to the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Results are presented as mean values ± standard error of mean (s.e.m.). Statistical analyses of variables were performed by using unpaired *t*-test. For analysis of survival rates, the Kaplan and Meier method was used. A *P*-value <0.05 was considered statistically significant.

Results

Effects of PARP inhibitors on established CMDE diet-induced AP

To examine the therapeutic effect of PARP inhibitors in the control of severe AP, we used an experimental necrotizing AP model based on the administration of a CMDE diet to young female mice (Lombardi *et al*., 1975) (Figure 1). Thirty-six hours of CMDE diet administration, resulted in a significant increase in serum amylase and lipase levels compared to the levels in mice fed with normal chow (Figure 2a). Three days of CMDE feeding caused an even higher increase in the levels of amylase and lipase in serum. Treatment of mice at 36 h after the onset of the CMDE diet with PARP inhibitors, either PJ34 or KuDOS, resulted in a significant reduction in the serum levels of both pancreatic enzymes (Figure 2b). Administration of PARP inhibitors at 48 h post-CMDE diet had no effects on amylase levels and only displayed a small beneficial effect on lipase levels (data not shown).

Morphological analysis of pancreas sections from mice after 3 days of CMDE feeding revealed massive dilation of the

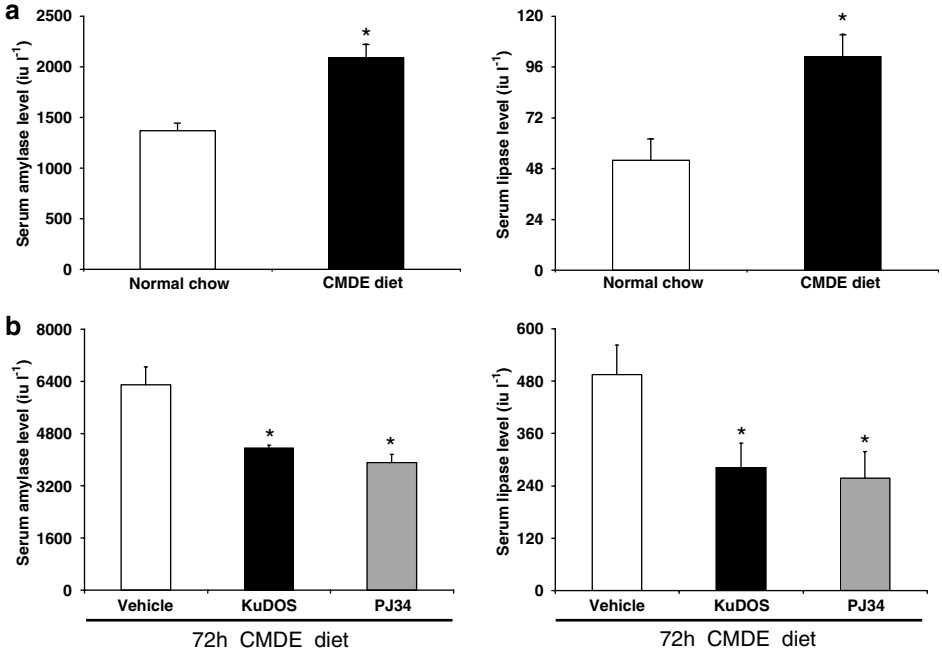

Figure 2 Serum amylase and lipase levels in mice at 36 h (**a**) and at 72 h (**b**) after the onset of the CMDE diet. Mice were treated with either vehicle or PARP inhibitors (PJ34 or KuDOS). Results are expressed as mean ± s.e.m. from 18–20 mice per group. *$P<0.05$. CMDE, choline/methionine-deficient/ethionine-supplemented; PARP, poly(ADP-ribose) polymerase.

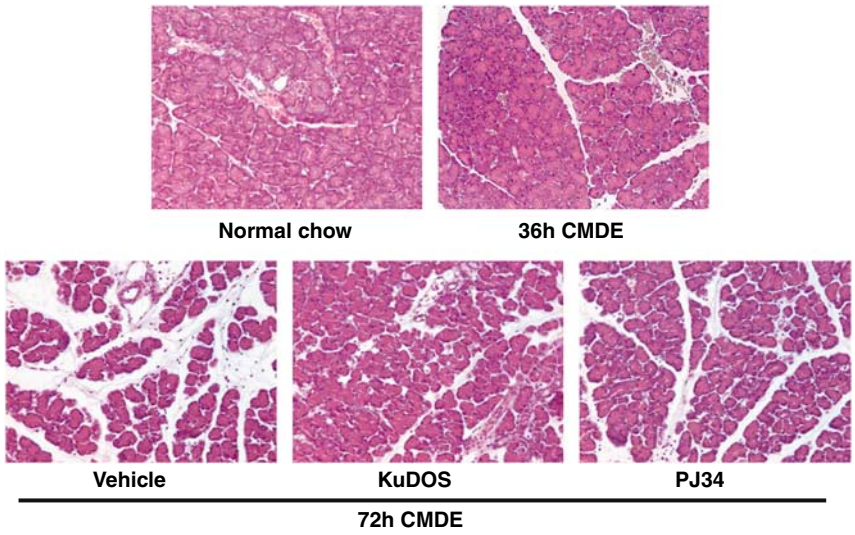

Figure 3 Morphological changes in CMDE diet-induced AP. Representative light micrographs of pancreas sections stained with H&E after feeding mice with a CMDE diet. Mice were treated with either vehicle or PARP inhibitors (PJ34 or KuDOS). Magnifications $\times 20$. AP, acute pancreatitis; CMDE, choline/methionine-deficient/ethionine-supplemented; H&E, hematoxylin and eosin; PARP, poly(ADP-ribose) polymerase.

septa caused by oedema and leucocyte infiltration that disrupted acinar cell morphology, signs representative of AP (Figure 3). These pathological effects were greatly ameliorated by treatment with PARP inhibitors, 36 h after the onset of the CMDE diet. Regular chow fed mice showed no morphological evidence of pancreas injury (Figure 3). To assess the morphological changes in a semi-quantitative fashion, slides were scored by two pathologists, unaware of the treatments. As summarized in Table 1, treatment of mice with either PARP inhibitor markedly reduced the severity of the CMDE diet-induced pancreatitis. Scores for pancreatic inflammation and necrosis and the total pancreatic histological score were all decreased.

Table 1 Histological scoring of pancreatic lesions

	Infiltration	*Necrosis*	*Total score*
Vehicle	2.21 ± 0.15	1.67 ± 0.22	3.88 ± 0.16
KuDOS	1.50 ± 0.22*	0.90 ± 0.14 *	2.40 ± 0.15*
PJ34	1.54 ± 0.23*	0.75 ± 0.3*	2.29 ± 0.19*

*$P < 0.05$.

Hepatic injury associated with a CMDE diet is attenuated in mice treated with PARP inhibitors

Thirty-six hours of the CMDE diet resulted in a significant increase in serum ALT and AST levels, compared to levels in mice fed with normal chow (Figure 4a). Three days of CMDE feeding caused an even higher increase in the levels of ALT and AST in serum. Treatment of mice at 36 h after the onset of the CMDE diet with PARP inhibitors, either PJ34 or KuDOS, resulted in a significant reduction in the serum levels of both hepatic enzymes (Figure 4b). Analysis of the histopathological changes in the liver from mice fed on CMDE diet for 3 days revealed massive infiltration of polymorphonuclear and mononuclear cells and disruption of the whole parenchyma. Most hepatocytes appeared completely dilated, displaying signs of vacuolar degeneration and nuclear apoptosis and/or degeneration. This liver damage was attenuated after treatment with either PJ34 or KuDOS, as shown histologically in Figure 5 and by the scores in Table 2. Regular chow fed mice showed no morphological evidence of hepatic injury.

The infiltration of neutrophils into the liver was also evaluated by hepatic MPO activity 3 days after the start of CMDE diet administration. Pharmacological inhibition of PARP significantly diminished liver MPO activity compared to mice treated with vehicle (Figure 6).

Lung injury associated with CMDE diet-induced AP is attenuated after the therapeutic administration of PARP inhibitors

We assessed the extent of lung injury associated with CMDE diet-induced AP in either vehicle or PARP inhibitors-treated mice 3 days after the onset of CMDE diet administration. Morphological analysis of lung sections from vehicle-treated mice showed thickened alveolar-capillary membranes, hyperaemia, cell infiltration and signs of apoptosis in alveolar type I cells and alveolar macrophages. PARP inhibitors given at 36 h after the onset of the CMDE diet markedly attenuated the CMDE diet-associated insult to lungs of mice under the same experimental conditions (Figure7 and Table 3). Regular chow fed mice showed no morphological evidence of lung injury (Figure 7). MPO activity in lung tissue 72 h after the onset of CMDE diet was significantly higher in vehicle-treated mice than in mice treated with the PARP inhibitors (Figure 8).

Therapeutic administration of PARP inhibitors reduced the systemic inflammatory response associated with CMDE diet-induced AP

To assess the systemic inflammatory response to CMDE diet-induced AP, serum concentrations of IL-6 and IL-1β were

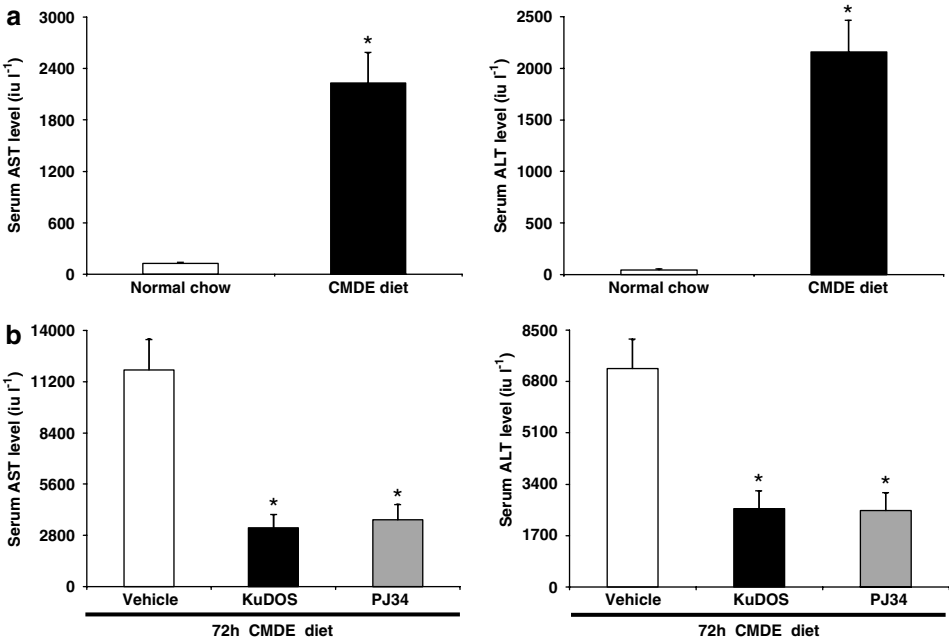

Figure 4 Serum ALT and AST levels in mice at 36 h (**a**) and at 72 h (**b**) after the onset of the CMDE diet. Mice were treated with either vehicle or PARP inhibitors (PJ34 or KuDOS). Results are expressed as mean ± s.e.m. from 18–20 mice per group. *$P<0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMDE, choline/methionine-deficient/ethionine-supplemented; PARP, poly(ADP-ribose) polymerase

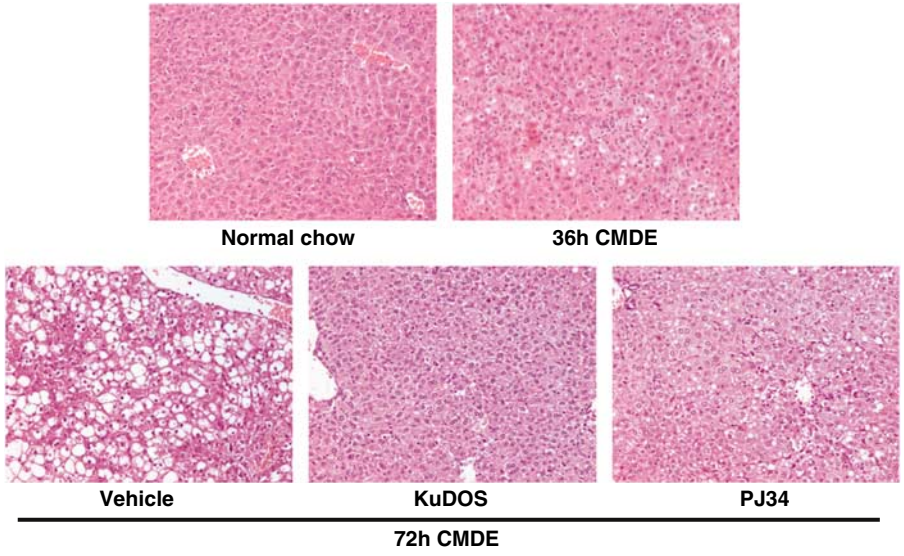

Figure 5 Morphological changes in liver after administration of CMDE diet. Representative light micrographs of liver sections stained with H&E after feeding mice with a CMDE diet. Mice were treated with either vehicle or PARP inhibitors (PJ34 or KuDOS). Magnifications × 20. CMDE, choline/methionine-deficient/ethionine-supplemented; H&E, hematoxylin and eosin; PARP, poly(ADP-ribose) polymerase.

measured. As shown in Figure 9, CMDE diet-induced pancreatitis in mice is associated with an increase in serum levels of IL-6 and IL-1β. However, this increase was significantly attenuated by the therapeutic administration at 36 h after the onset of the CMDE diet of PARP inhibitors (Figure 9).

PARP inhibitors improved the survival rate of mice in established CMDE diet-induced AP

The survival of mice following the administration of a CMDE diet for 3 days was monitored for a total of 7 days (see Figure 1). Treatment with pharmacological inhibitors of PARP (either PJ34 or KuDOS) after the onset of the clinical, biochemical and pathological signs of the disease (36 h after the onset of the CMDE diet) resulted in a significant reduction in the mortality rate (Figure 10).

Discussion

AP can be a severely debilitating if not lethal disease in humans. Recently, using the well-characterized cerulein-

Table 2 Histological scoring of liver lesions

	Necrosis	*Infiltration*	*Vacuolization*	*Total score*
Vehicle	2.60±0.24	2.75±0.25	2.75±0.25	8.10±0.09
KuDOS	1.13±0.5*	1.50±0.23*	1.50±0.29*	4.13±0.14*
PJ34	1.85±0.1*	1.85±0.1*	1.80±0.1*	5.50±0.05*

*$P<0.05$.

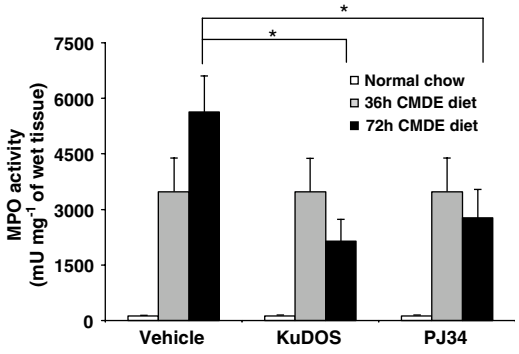

Figure 6 Liver neutrophil infiltration as measured by MPO activity. MPO activity was measured, at 72 h after starting the feeding with a CMDE diet, in the liver of mice treated with vehicle or PARP inhibitors (PJ34 or KuDOS). Data are expressed as mean MPO activities (mU mg^{-1} of wet tissue)±s.e.m. from eight mice per group. *$P<0.05$. CMDE, choline/methionine-deficient/ethionine-supplemented; MPO, myeloperoxidase; PARP, poly(ADP-ribose) polymerase.

induced murine model of AP, our group has demonstrated that PARP inhibitors administered before the onset of AP ameliorated disease severity (Mota *et al*., 2005). This result has also been confirmed by Mazzon *et al*. (2006). However, in the cerulein-induced murine model of AP, it is difficult to test therapeutic effects of PARP inhibitors because of the early appearance of clinical, pathological and biochemical signs of the disease. Indeed, histological examination of pancreas sections revealed tissue damage characterized by inflammatory cell infiltrates and acinar cell necrosis, as early as 6 h after the injection of cerulein (Mazzon *et al*., 2006). Until now, there have been no reports of the use of PARP inhibitors to treat AP after the onset of the disease. Differentiation between the results of prophylactic and therapeutic treatments is of major importance in assessing the potential of a new drug. In fact, therapies such as antiproteases, somatostatin and antiplatelet activating factor given late in the disease course had little effect on the clinical outcome in spite of their utility in prophylaxis (Norman, 1999). Because of our interest in assessing the clinically relevant therapeutic effects of PARP inhibitors, we have used a lethal model of experimental pancreatitis, based on the administration of a CMDE diet to young female mice (Lombardi *et al*., 1975). The relatively lengthy time course of this model (Niederau *et al*., 1992), in contrast to the cerulein-induced model, allowed us to design a protocol to evaluate the real therapeutic effects of PARP inhibitors in controlling the morbidity and mortality associated with severe AP.

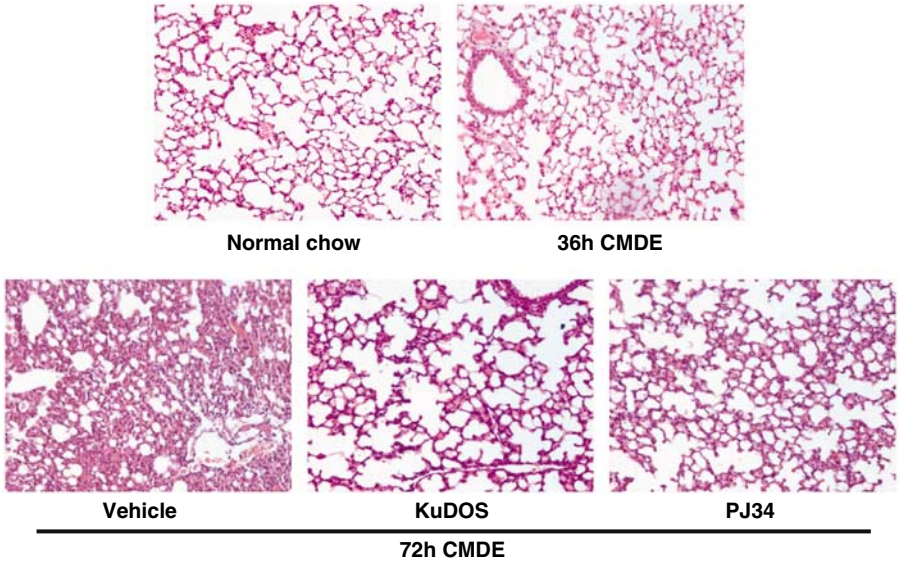

Figure 7 Morphological changes in lung after administration of CMDE diet. Representative light micrographs of lung sections stained with H&E after feeding mice with a CMDE diet. Mice were treated with either vehicle or PARP inhibitors (PJ34 or KuDOS). Magnifications ×20.CMDE, choline/methionine-deficient/ethionine-supplemented; H&E, hematoxylin and eosin; PARP, poly(ADP-ribose) polymerase.

Table 3 Histological scoring of lung lesions

	Necrosis	*Infiltration*	*Capillary congestion*	*Alveolar membrane thickening*	*Total score*
Vehicle	2.31±0.21	2.67±0.29	2.44±0.22	2.29±0.28*	9.46±0.04
KuDOS	1.31±0.16*	1.63±0.16*	1.67±0.19*	1.69±0.17*	6.38±0.1*
PJ34	1.31±0.18*	1.65±0.19*	1.50±0.23*	1.65±0.22*	6.11±0.08*

*$P<0.05$.

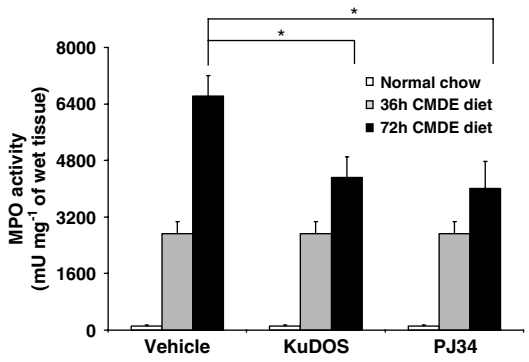

Figure 8 Lung neutrophil infiltration as measured by MPO activity. MPO activity was measured, at 72 h after starting feeding with a CMDE diet, in the lung of mice treated with either vehicle or PARP inhibitors (PJ34 or KuDOS). Data are expressed as mean MPO activities (mU mg^{-1} of wet tissue) $\pm$ s.e.m. from eight mice per group. *$P<0.05$. CMDE, choline/methionine-deficient/ethionine-supplemented; MPO, myeloperoxidase; PARP, poly(ADP-ribose) polymerase.

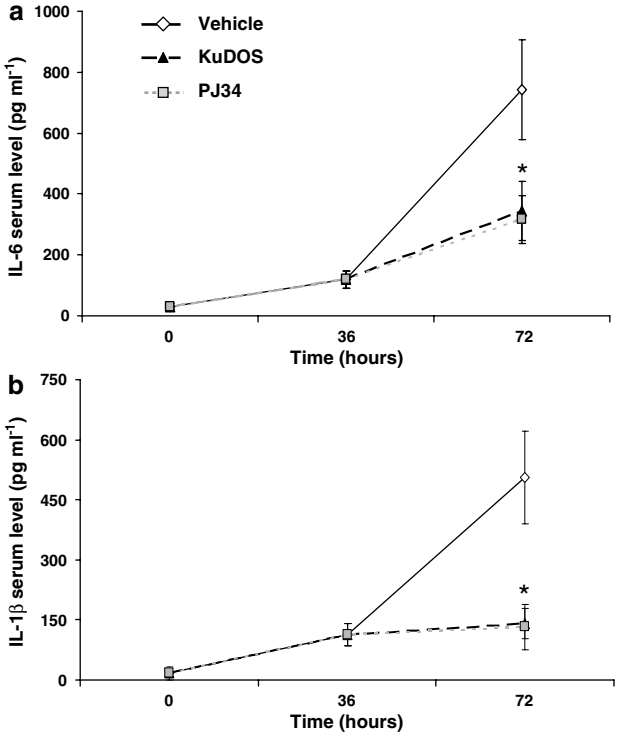

Figure 9 Serum levels of IL-6 and IL-1β in CMDE diet-induced AP. Serum levels of IL-6 (**a**) and IL-1β (**b**) were quantified by ELISA in mice treated with either vehicle or PARP inhibitors (PJ34 or KuDOS), at different times after CMDE diet administration. Results are expressed as mean $\pm$ s.e.m. from 7–10 mice per group. *$P<0.05$. AP, acute pancreatitis; CMDE, choline/methionine-deficient/ethionine-supplemented; ELISA, enzyme-linked immunosorbent assay; IL, interleukin.

Our goal was to initiate therapy with PARP inhibitors after the clinical, biochemical and pathological signs of the disease had appeared in the animals. Thus, the first time point for PARP inhibitors therapy (36 h) was chosen to coincide with an increase in serum amylase and lipase and clear histological

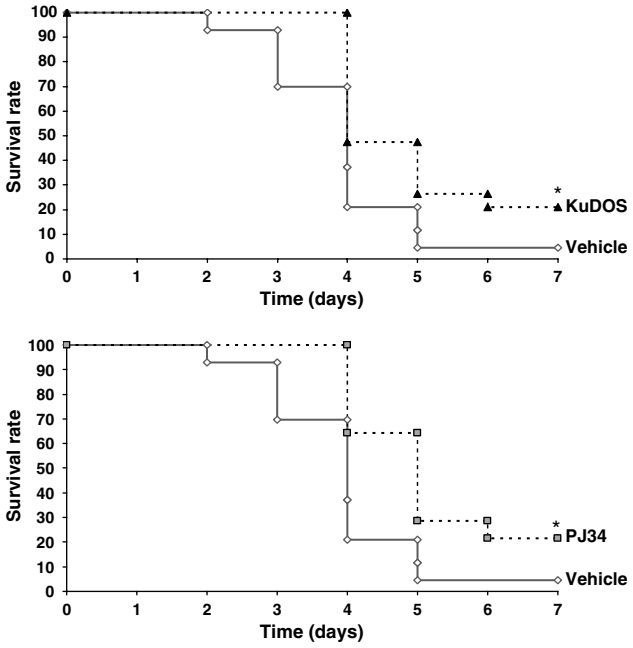

Figure 10 Effects of pharmacological inhibition of PARP on CMDE diet-induced AP mortality. Young female mice were fed with a CMDE diet for 72 h and then regular chow for the following days. Vehicle or PARP inhibitors (PJ34 or KuDOS) were administrated 36 h after the onset of the CMDE diet. Mortality rates were analysed using the Kaplan–Meier method. *$P<0.01$ for PARP inhibitors-treated mice versus vehicle-treated mice. This figure shows data for 20 mice per group. AP, acute pancreatitis; CMDE, choline/methionine-deficient/ethionine-supplemented; PARP, poly(ADP-ribose) polymerase.

evidence of injury to the pancreas. Interestingly, at this time point, we also found a significant increase in the hepatic enzymes, AST and ALT, in serum. Liver injury is a clinical prognostic indicator in AP (Gallagher *et al.*, 2005) and has also been associated with experimental severe diet-induced AP (Gloor *et al.*, 1998, 2000; Jaffray *et al.*, 2000; Gray *et al.*, 2006). However, the early liver injury we observed in the CMDE diet-induced AP model could also be related to the development of a fatty liver as a consequence of ethionine administration (Farber, 1967). In addition, histological examination of liver and lung sections also showed important tissue damage as early as 36 h after CMDE feeding.

Treatment with PARP inhibitors was continued through to the end of the CMDE diet feeding (72 h) and was able to attenuate all of the pathological parameters associated with CMDE diet-induced AP and pancreatitis-associated lung and liver injury. Interestingly, PARP inhibitors were quite effective at attenuating the elevation of serum ALT and AST, in agreement with other experimental hepatic injury models (Gero and Szabo, 2006). In accordance with the attenuation of all of these parameters of morbidity, the mortality rate recorded in the CMDE diet model was significantly lower in mice treated with PARP inhibitors after the onset of the disease than in those treated with vehicle. In the absence of PARP inhibitors, our survival data are in agreement with the survival data reported for the lethal model of AP based on the administration of a CMDE diet, in which the first animals die at about 60 h after the onset of the toxic diet and none of the animals die later than 5 days after start of the

CMDE diet (which is fed for a total period of 72 h and is then replaced by regular chow) (Niederau *et al*., 1992). Niederau *et al*. (1992) also demonstrated that in the surviving mice, resolution of functional alterations occurs after 2–3 weeks and thus earlier than resolution of morphological alterations, which is complete only after 4–6 weeks.

PARP inhibition might control the severity of AP by preventing both the local and systemic inflammatory response and the mechanisms involved in pancreatic cell necrosis mediated by neutrophils via the enzyme NADPH oxidase (Mota *et al*., 2005). Moreover, we did not observe any side effects of PARP inhibitors in the mice. Indeed, PARP inhibitors have now progressed through the preclinical efficacy and safety stages of drugs development and have entered human clinical testing for some pathologies, such as myocardial infarction, thoracoabdominal aortic aneurysm and malignant melanoma (Jagtap and Szabo, 2005). It appears that, as far as the cytoprotective, anti-inflammatory aspects of PARP inhibitors are concerned, acute, life-threatening diseases such as AP may represent some of the major indications for their clinical use. Development of PARP inhibitors for use in chronic pathologies might prove to be more challenging, as a variety of long-term safety issues need to be resolved.

In conclusion, our results show for the first time that treatment with PARP inhibitors in established AP, and after clear signs of liver and lung injury, was associated with a reduction in the severity of AP and, accordingly, with a reduced mortality rate in our experimental model of severe AP. These findings support the therapeutic use of PARP inhibitors in the treatment of patients with severe AP and associated liver and lung injury.

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Conflict of interest

The authors state no conflict of interest.

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