INDUCTION OF APOPTOSIS IN PANCREATIC ACINAR CELLS In-Vitro

Ang A. D.¹ and Bhatia M.²

Department of Pharmacology, Faculty of Medicine, National University of Singapore, 10 Kent Ridge Crescent, 119260 Singapore.

ABSTRACT

In acute pancreatitis (AP), acinar cell death occurs both by necrosis and apoptosis (programmed cell death). The clinical severity of this disease varies widely, from a mild, self-limiting form with interstitial edema to severe, necrotizing pancreatitis with a high mortality rate. Mild pancreatitis is characterized by very little necrosis but a high degree of apoptosis suggesting that the severity of acute pancreatitis is inversely related to the degree of apoptosis. It has been hypothesized and to an extent proven that induction of apoptosis in pancreatic acinar cells reduces the severity of AP. This study attempts to induce apoptosis in murine pancreatic acinar cells in-vitro. The apoptotic inducers used were Caerulein and Menadione. The method of apoptotic quantification was by measuring caspase-3 activity of the cell lysate. It was found that both treatment with Menadione and Caerulein showed increased apoptotic activity. There is potential for the inducers of apoptosis to be further investigated as possible prophylactic or therapeutic approaches for AP.

INTRODUCTION

Acute pancreatitis is a common disorder with potentially devastating consequences. Among the major causes are biliary stones and excess alcohol consumption. The pathophysiological events leading up to acute necrotizing pancreatitis is still unclear. Despite significant improvement of our knowledge in recent years, there is still a great deal of uncertainty and controversial discussion with regard to the role and importance of the various etiological and pathophysiological mechanisms, the relevance of prognostic scores, the clinical assessment of the course of the disease, or recent therapeutic concepts for patients with acute pancreatitis. Deciding what constitutes an adequate therapeutic approach for patients with acute pancreatitis is still a major clinical problem, especially in cases of acute necrotizing pancreatitis. Until now, the current strategy is to treat acute pancreatitis symptomatically.

This study attempts to address acute pancreatitis at the point of acinar cell injury itself. Previous reports attribute acinar cell injury in the form of necrosis and apoptosis as the initiating event of acute pancreatitis (Gukovskaya, 1996). Kaiser (1995) indicates that severe forms of acute pancreatitis primarily involve pancreatic necrosis, whereas mild forms predominantly involve apoptosis and marginal amounts of necrosis. Necrosis and apoptosis are each forms of cell injury that result in cell death, but the former is associated with loss of membrane integrity, rupture of cell walls, and the evolution of inflammatory response, whereas the latter is an active, energy-consuming process that requires gene expression and that finally results in cell shrinkage and

¹ Student
² Lecturer
internucleosomal DNA degradation in most cell types (Kaiser et al. 1995). Bhatia et al. (1998) found that apoptosis might be the a favourable response to acinar cell injury and that interventions which favour induction of apoptotic, as opposed to necrotic acinar cell death might reduce the severity of an attack of pancreatitis.

This research focused in particular on the in-vitro induction of apoptosis of acinar cells. Primary murine acinar cells were used as the model of this study. The apoptotic inducers used in this study were Menadione and Caerulein (CCK). Apoptotic activity was measured from the cell lysate using a fluorogenic Caspase-3 assay. The use of Caspase-3 as the apoptotic indicator was based on current knowledge of the apoptosis pathway. Hengartner’s (2000) review on apoptosis suggests caspase-3 as the downstream protein in the convergence of reported death signaling pathways. The results of this study shows increase of apoptotic activity in Menadione and Caerulein treated cells. This finding is encouraging for further investigation on the effect of apoptotic induction in acute pancreatitis. However, in-vivo studies have to be carried out first to establish parallel results with the in-vitro.

MATERIALS AND METHODS

Materials
In-vitro study was carried out on mice of the BalbC and Swiss strain. The mice were housed in a climate controlled room with an ambient temperature of 24±2 °C. They were fed standard laboratory food and given water ad libitum. The apoptotic inducers used were Menadione and Caerulein. Menadione was purchased from Sigma. Caerulein is a Cholecystokinin (CCK) analogue purchased from Bachem. Quantification of Caspase-3 activity was done using a spectrofluorometer, SpectrafluorPlus by Tecan.

Cell Preparation
Primary single acinar cells or small clusters were prepared by a previous established method of collagenase digestion (Gerasimenko et al. 2001). Briefly, the removed pancreas was perfused with type IV collagenase (200UI) using a 29G syringe, minced with a sharp tip surgical scissors till a fine suspension was achieved, and incubated in collagenase for 10 minutes at 37 °C. The isolated cells were then subjected to a centrifugation wash with buffer. Cell isolation procedure and preparation of collagenase were performed with a standard Na HEPES.

Induction of Apoptosis
The prepared acini were distributed into 1.7ml microfuge tubes containing Na HEPES buffer using a micro pipette. Apoptotic inducers such as Caerulein and Menadione were then added to the mixture. Working concentration of Caerulein is 10⁻⁷ M (Gukovskaya et al. 2002) and Menadione is 20µM (Gerasimenko et al. 2002). The tubes were then left to incubate at 37 °C for varying periods of 1, 3 and 6 hours.

Quantitation of Apoptosis
The extent of apoptosis was determined by assaying for Caspase-3 activity. This was done by obtaining the lysate from the incubated cells. The cell lysate was obtained by centrifuging at maximum speed (13000rpm) for 30 mins. It was then subjected to a fluorometric assay using Ac-
DEVD-AFC as the fluorophore Capsase-3 substrate. The excitation and emission wavelength used for the assay were 394nm and 535nm respectively.

DNA Estimation

DNA estimations were performed to normalize the amount of Caspase-3 activity per amount of DNA present in the sample as a means to correct the error of cell distribution among the samples. The estimation of DNA was performed with a fluorophore DNA binding dye Hoechst H 33258. The excitation and emission wavelength used for the assay were 360nm and 450nm respectively. A standard curve of DNA was then generated to extrapolate the relative amount of DNA in the samples. DNA standards and samples were prepared using a PO4 Buffer at pH of 7.4.

RESULTS

With reference to Figure 1(A), apoptosis of acinar cells were measured in terms of fluorescence unit (Rfu) per DNA content (ug) against time (minutes). From these graphs, a percentage increase of fluorescence per minute (Rfu/min) as compared with the 0 hour controls was generated at a fixed time interval of 25-70mins for all experiments done. These data was then used as the indicator of apoptotic activity. Figure 2 (B) shows the averaged caspase-3 activity of multiple experiments done (n=6). From these figures, it is observed that both Caerulein and Menadione treated samples shows increased caspase-3 activity. Figure 2 (B) shows a 4 fold increase in caspase-3 activity in 1 hour Menadione treated samples as compared with the control. Caerulein treated samples shows a 2 fold increase (Figure 2 B). It was also found that incubation periods also affected the outcome of each treatment. Menadione treatment showed increased activity at 1hour of incubation but did not have the same effect with 3 hours of incubation (not shown in results). Caerulein on the other hand did not show any increase with 1 hour incubation (not shown in results) but showed significant increase with 3 hours of incubation.

Figure 1 : (A)Shows a results of a Caspase-3 assay from one of the experiments done. Values are represented by fluorescent unit per ug DNA at every 5 minute interval. Both Caerulein and Menadione treated cells show increase in Caspase-3 activity with respect to the controls. (B) Shows averaged readings from experiments carried out. Values are represented by percentage increase of fluorescence units per ug DNA a minute over the 0hr controls. Again, both Caerulein and Menadione show a 2.5 fold and 4 fold increases respectively over the relevant controls.
DISCUSSION

Kaiser et al (1995) showed the relationship between severity of acute pancreatitis, necrosis and apoptosis in five experimental models. The report found that in the induction of severe pancreatitis marked necrosis but very little apoptosis was found. In contrast to the findings in severe pancreatitis, mild pancreatitis was characterized by very little necrosis but a high degree of apoptosis. Their finding that the severity of acute pancreatitis is inversely related to the degree of apoptosis suggests that apoptosis may be a teleologically beneficial response to acinar cell injury in general and especially in acute pancreatitis. Similar findings were reported by Gukovskaya et al (1996).

This study and previous ones (Gukovskaya et al 2002; Gerasimenko et al 2002; Bhatia et al 1998) have shown that it is possible to induce apoptosis in pancreatic acinar cells using specific inducers. A subsequent report (Bhatia et al 1998) found that the event of apoptosis reduces acinar cell injury and severity of pancreatitis in-vivo. Therefore it would be possible that Menadione and Caerulein too have the potential of reducing the severity of acute pancreatitis and hence could be developed to form a therapeutic or prophylactic solution towards pancreatitis. However, a crucial finding made by Bhatia et al (1998) is that the reduction of acinar cell injury and severity of pancreatitis in-vivo (by induction of apoptosis) is only true for a small window of opportunity when the cell has committed to apoptosis and before it is deleted. Therefore the events that take place in this window are important in understanding the mechanism of prevention of acinar cell injury (necrosis).

REFERENCES


