Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, acinar cell damage, and systemic inflammation in acute pancreatitis

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Seyhun E, Malo A, Schäfer C, Moskaluk CA, Hoffmann R, Göke B, Kubisch CH. Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, acinar cell damage, and systemic inflammation in acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 301: G773–G782, 2011. First published July 21, 2011; doi:10.1152/ajpgi.00483.2010.—Acute pancreatitis is an injury to acinar cells, but the exact mechanisms are still incompletely understood (54). The disease is characterized by dysregulation of endoplasmin (ER) stress responses, balances the folding capacity and folding demand within the organelle. It is achieved through an upregulation of ER-resident chaperones and selected transcription factors, ER enlargement, downregulation of gene transcription, and increase in ER-associated degradation (ERAD) (6).

The chaperone-protein association provides an optimal protein-folding environment employing several mechanisms to achieve correct protein trafficking (51, 60). The capacity of the ER to fold proteins is presumably limited by chaperone resources and can be exceeded by events such as a high cellular protein demand, protein overexpression, and oxidative imbalance. When such stresses occur, an accumulation of malfolded proteins in the ER takes place, provoking the ER stress response (52).

The unfolded protein response (UPR), as part of the ER stress response, involves the downregulation of pJNK. In controls without pancreatitis, TUDCA showed cytoprotective effects including pPERK signaling and activation of downstream targets. We concluded that ER stress responses activated in acute pancreatitis are grossly attenuated by TUDCA. The chaperone reduced the UPR and inhibited ER stress-associated apoptotic pathways. TUDCA has a cytoprotective potential in the exocrine pancreas. These data hint at new perspectives for an employment of chemical chaperones, such as TUDCA, in prevention of acute pancreatitis.

chemical chaperone

NORMAL EXOCRINE PANCREAS FUNCTIONS are associated with high levels of protein trafficking (9). The zymogens are folded into distinct 3D arrangements, which have to remain stable. Overall, the process of protein folding and maturation is crucial in the transmission of gene expression into specific biological functions. Folding and stabilization take place inside the endoplasmic reticulum (ER). In the exocrine pancreas, after a protein enters the ER, it begins its chaperone-assisted folding and stabilization by posttranslational modifications (4). To support its prominent role in the synthesis of digestive enzymes, the exocrine pancreatic acinar cell has particularly abundant ER (9, 18).

Heavy chain binding protein (BiP, also known as glucose-related protein 78), is a dominant folding-assisting chaperone. It binds folding intermediates of many proteins (36, 41). If the folding is incomplete or incorrect, the export is inhibited by an ER quality control system. The proteins are retained in the ER bound to chaperones until the folding process is complete. If the correct folding cannot be achieved, proteins are targeted for ER-associated degradation (ERAD) (6).

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The unfolded protein response (UPR), as part of the ER stress response, involves the downregulation of pJNK. In controls without pancreatitis, TUDCA showed cytoprotective effects including pPERK signaling and activation of downstream targets. We concluded that ER stress responses activated in acute pancreatitis are grossly attenuated by TUDCA. The chaperone reduced the UPR and inhibited ER stress-associated apoptotic pathways. TUDCA has a cytoprotective potential in the exocrine pancreas. These data hint at new perspectives for an employment of chemical chaperones, such as TUDCA, in prevention of acute pancreatitis.

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of production and secretion of zymogens (particularly the inhibition of pancreatic secretion and premature activation of digestive enzymes), cytoplasmic vacuolization, edema formation, and recruitment of inflammatory cells into the pancreas (32, 50). The disease can spread systematically though an inflammatory response that is driven by increased expression of proinflammatory molecules as well as elevated concentrations of pancreatic enzymes in blood serum (5). Respiratory dysfunction is frequent in AP and is a major component of AP-associated multiple-organ distress syndrome (44). Mediators of lung injury are cells that regulate the migration and pulmonary infiltration of neutrophils, where they cause protease-induced injury and breakdown of the pulmonary parenchyma (40). The neutrophil-induced production of reactive oxygen and nitrogen species can have deleterious effects on pulmonary endothelial and epithelial functions (5, 7).

Solid evidence suggests that ER stress responses are involved in the early stages of AP. Recently, we have shown that tauroursodeoxycholic acid (TUDCA), a bile salt with ER chaperone-like properties, reduced hallmarks of acinar cell overstimulation and ER stress in isolated rat acinar cells in an in vitro system (35).

Here we extend the study of the potential of TUDCA to reduce ER stress and its consequences in an in vivo experimental AP model system.

MATERIALS AND METHODS

Materials. Caerulein was obtained from Sigma-Aldrich (Steinheim, Germany). TUDCA and tauroursodeoxycholic acid (TDCA) were dissolved in aqua bidest from Calbiochem (Darmstadt, Germany). Phadebas amylase test was purchased from Magle Life Sciences International (Lund, Sweden). The protein assay was from Bio-Rad Laboratory (Hercules, California). Caerulein was obtained from Sigma-Aldrich (Steinheim, Germany), TUDCA and tauroursodeoxycholic acid (TDCA) were dissolved in aqua bidest from Calbiochem (Darmstadt, Germany). Phadebas amylase test was purchased from Magle Life Sciences International (Lund, Sweden). The protein assay was from Bio-Rad Laboratory (Hercules, California). Caerulein was obtained from Sigma-Aldrich (Steinheim, Germany), TUDCA and tauroursodeoxycholic acid (TDCA) were dissolved in aqua bidest from Calbiochem (Darmstadt, Germany). Phadebas amylase test was purchased from Magle Life Sciences International (Lund, Sweden). The protein assay was from Bio-Rad Laboratory (Hercules, California).

Animals. Male Wistar rats (150–200 g) (Charles River Laboratories, Sulzfeld, Germany) were used for all experiments. All animals had free access to regular chow and water ad libitum. Rats were kept in a room at 22°C on a 12-h:12-h light/dark cycle and received care in accordance with the national and international guidelines as outlined in the Guide for the Care and Use of Laboratory Animals. All experiments were performed in accordance with protocols approved by the University of Munich Animal Care and Use Committee and the local veterinarian office.

Induction of AP. For caerulein-induced pancreatitis, animals were fasted overnight. Rats received one intraperitoneal (i.p.) injection containing a supramaximally stimulating dose of caerulein (50 μg/kg body wt) in 0.9% sodium chloride into the left lower abdomen. Control rats were administered comparable injections of saline. Half the animals (caerulein and control rats) received 2 hr before one i.p. injection of TUDCA (250 mg/kg body wt) or TDCA (250 mg/kg body wt). The other half (controls) received a comparable injection of saline. Thus a total of six animal groups were used: 1) two injections of saline (named thereafter control-NaCl); 2) one injection of TUDCA and a second injection of saline (control-TUDCA); 3) one injection of saline and a second injection of caerulein (CER); 4) one injection of TUDCA and the second injection of caerulein (TUDCA + CER); 5) one injection of TDCA and a second injection of saline (control-TDCA); and 6) one injection of TDCA and a second injection of caerulein (TDCA + CER). Thirty minutes and 1, 2, and 4 hr after the final injection, animals were killed by decapitation under isoflurane anesthesia.

Quantification of caerulein-induced injuries. Blood and pancreatic tissue were processed as described previously (56). Serum amylase activity was determined with Phadebas amylase test and expressed in percent increase compared with control-NaCl. The extent of pancreatic edema was determined by measuring tissue water content. The difference between the wet and dry tissue weights was calculated and expressed as a percentage of tissue wet weight.

Quantification of pancreatic trypsin activity. Pancreatic samples were homogenized in ice-cold freshly prepared MOPS buffer and processed as described (29). Trypsin activity was measured fluorometrically using Boc-Glu-Ala-Arg-AMC.HCl as substrate. Activity levels were calculated from a slope using a standard curve generated with purified trypsin. Protein content was measured, and trypsin activity was expressed as femtomole per milligram protein (fmol/mg).

Quantification of neutrophil content in pancreas and lung tissue. Tissue neutrophil content served as a marker for the inflammatory reaction to AP, locally in the pancreas, and systemically in lung tissue. It was determined by measuring tissue myeloperoxidase (MPO) activity. Tissue samples were immediately frozen in liquid nitrogen, stored at −80°C until processed as described (19). The absorbance at 460 nm was measured over 4 min, calculated of the slope, and expressed as milliunits per milligram tissue protein (mU/mg) after measurement of the protein content of each sample.

Western blotting of pancreatic acini proteins. Preparation of whole cell lysates and Western blotting were performed as described (29). Antibodies were used in the following concentrations: anti-BiP, 1:2,000; anti-phospho-PERK, 1:250; anti-ATF4, 1:1,000; anti-erbB1, 1:500; anti-caspase 3, 1:500; anti-caspase 12, 1:2,000; anti-CHOP, 1:500; anti-phospho-JNK, 1:2,000; and anti-actin, 1:5,000, as an appropriate IgG HRP-conjugated secondary antibody (1:10,000). Antibody binding was detected by chemiluminescence radiography. Membranes were scanned, recorded digitally, and processed using ImageJ software.

Evaluation of pancreatic morphology. A portion of each pancreas was fixed overnight at 4°C in fresh formaldehyde in phosphate-buffered saline (PBS), pH 7.4. The tissue was embedded in paraffin, cut in 3-μm sections, and processed for hematoxylin and eosin (H&E) staining by standard procedures. Histological images were taken using an Olympus BX 41 microscope with a digital camera and processed using the cellA software (Olympus, Hamburg, Germany). Multiple randomly chosen microscopic fields were analyzed and evaluated for each tissue sample of at least three rats. Three histological features were scored: 1 for interstitial edema, 2 for epithelial injury, and 3 for cell death. Interstitial edema was scored as 1 if lobular units of pancreatic tissue were separated by interstitial edema, as 2 if smaller acinar units within the lobule were also separated by interstitial edema in <50% of the pancreatic tissue, and as 3 if acinar edema involved >50% of pancreatic tissue. Epithelial injury was identified as vascular degeneration of the cytoplasm and was scored as 1 if vascular degeneration was present in less than one-third of pancreatic acinar epithelial cells, as 2 if vascular degeneration was present in greater than one-third but less than two-thirds of acinar cells, and as 3 if vascular degeneration was present in greater than two-thirds. Cell death was identified as morphological evidence of apoptosis (nuclear fragmentation) or necrosis (loss of nuclear outlines and chromatin staining). Cell death was
scored as 1 if identified in less than <5%, as 2 if identified in 5–25%, and as 3 if identified in >25% of pancreatic epithelial cells.

Statistical analysis. Serum amylase, pancreatic water content, MPO activity, histological features, and quantitative Western blot data were analyzed using a Kruskal-Wallis analysis of variance by ranks. Because the Kruskal-Wallis test was highly significant, data were analyzed using the Dunn test for multiple comparisons vs. a control group. The results reported represent means ± SE values obtained from multiple determinations in separate experiments. All statistical perturbations were performed using SigmaStat 3.0 statistical software (SYSTAT Software, Chicago, IL).

RESULTS

Effects of TUDCA and TDCA preincubation on the severity of AP. To demonstrate that the effects of TUDCA are specific and related to ER function and ER stress accomplishment, we used another bile acid TDCA, which does not have ER chaperone properties, as a negative control, in addition to a mock saline treatment. TDCA and TUDCA were given in the same i.p. dose to matched experimental animals before the induction of AP, and key parameters of AP were measured, such as amylase in serum, trypsin activation, and edema formation in the pancreas.

Treatment of rats with caerulein (50 μg/kg body wt) with mock saline pretreatment led to an enhanced serum amylase activity, pancreatic trypsin activity, and edema formation (Fig. 1, A–C). Amylase started to rise in blood serum at 30 min after caerulein administration. It reached its maximum at the 4-h time point (853.44 ± 26.66 U/l vs. control-NaCl 427.54 ± 40.80 U/l). Interestingly, after TUDCA or TDCA pretreatment and caerulein administration, serum amylase showed a similar progression as in the mock treatment experiments (maximum at 4 h after TUDCA and caerulein administration 899.00 ± 10.31 U/l, and after TDCA and caerulein administration 772.86 ± 43.59 U/l). TUDCA or TDCA pretreatment alone (no caerulein) had no effect on amylase serum levels.

Trypsin activity started to rise 30 min after caerulein administration. It peaked at 1 h (2,882.26 ± 459.76 fmol/mg protein vs. control-NaCl 54.91 ± 7.31 fmol/mg protein) with a decrease thereafter. Pretreatment with TUDCA reduced the trypsins activity after AP induction significantly to about two-thirds of the maximum at 1 h after caerulein administration 1,032.22 ± 262.29 fmol/mg protein). TDCA before caerulein was not able to reduce the trypsin activation at any time point. TUDCA and TDCA pretreatment alone showed no effect compared with saline without caerulein administration.

The relative water content indicating inflammatory edema formation in pancreatic tissue started to rise 30 min after caerulein administration. It reached its maximum at 4 h after AP induction (91.53 ± 1.97% vs. control-NaCl 81.59 ± 0.56%). TUDCA pretreatment showed a significant reduction starting from 1 h after AP induction (maximum at 4 h after caerulein administration 86.26 ± 1.96%). Pretreatment with TDCA before caerulein administration was not able to reduce the edema formation. No significant difference can be demonstrated between control-TDCA and CER rats in pancreatic water content.

Effects of TUDCA on MPO activity. To further evaluate the effects of TUDCA, we continued to measure MPO activity locally in the pancreas and as a systemic reaction to AP induction MPO activity in lung tissue (Fig. 2, A and B). MPO in the pancreas started to rise at 30 min after caerulein administration and stayed elevated until 4 h. MPO in lung tissue started to rise at 1 h after caerulein administration to its maximum at 4 h (maximal 16.82 ± 1.16 mU/mg weight). With the TUDCA preincubation before AP induction, MPO was significantly lower in the pancreas at all time points and at the 2- and 4-h time points in lung tissue (CER at 4 h 16.82 ± 1.16 mU/mg weight vs. TUDCA + CER 9.58 ± 1.09 mU/mg weight). Effects of TUDCA on pancreas morphology. In addition to the biochemical hallmarks of AP, we analyzed the effect of TUDCA on histological changes in pancreatic exocrine tissue (Fig. 3). Portions of all pancreas heads were harvested, fixed, and stained with H&E and examined and scored morphologically. Saline-injected rats and TUDCA-injected rats showed little to no histological evidence of characteristics of AP (Fig. 3A, left and middle left). Treatment with caerulein resulted in intracellular vacuolization, increased edema, inflammation, and single cell necrosis starting at the 1-h time point and increasing thereafter (middle right). In contrast, pretreatment with TUDCA resulted in a reduced vacuolization after caerulein and to markedly attenuated inflammatory changes (right).
Caerulein alone caused no significant changes in ATF4 expression at any given time point compared with NaCl-controls. Surprisingly, sole TUDCA without AP induction caused a huge increase in pPERK in the exocrine pancreas starting after 30 min up to 4 h with its maximum at 1 h (362.44 + 90.53% increase of NaCl-control) and a decline thereafter but not back to basal levels. pPERK was only modestly pronounced in TUDCA + CER-treated organs but anyway not significantly different compared with CER alone.

ATF4 is a transcription factor selectively upregulated by pPERK. To investigate the consequences of TUDCA-induced pPERK we analyzed ATF4 protein expression by Western blotting after caerulein administration (Fig. 5A). Caerulein alone caused no significant changes in ATF4 expression at any time points compared with NaCl-controls. Parallel to PERK phosphorylation, TUDCA preincubation alone without AP induction caused a significant increase in ATF4 starting after 30 min up to 4 h with peaking the 4-h time point (572.27 + 206.54% increase of NaCl-control). ATF4 expression in TUDCA + CER compared with CER alone showed no significant difference.

IRE1 is a second ER stress sensor and transducer, bound to BiP under physiological conditions. Upon stress, IRE1 becomes active as an endonuclease. Active IRE1 targets cytoplasmic XBP-1 mRNA and generates a splice variant that converts XBP-1 into sXBP-1, an active transcription factor. Subsequently, sXBP-1 induces the transcription of several ER stress-related genes involved in the biogenesis of the organelle itself. For our experiments, we analyzed the 28-kDa small splice variant sXBP-1 to describe changes in IRE1 activity (Fig. 5B). Caerulein-induced mild AP caused a significant increase in XBP-1 splicing compared with NaCl-control at 1 and 2 h (maximal at 2 h 248.17 + 35.17% increase of NaCl-control). Control-TUDCA and TUDCA + CER showed no changes in sXBP-1 expression compared with controls.

We also analyzed the phosphorylation of JNK. Activation of IRE1 can lead via binding and clustering of TNF receptor-associated factor 2 to pJNK. Several isoforms are known with different molecular weights, JNK1 (46 kDa), JNK2/3 (55 kDa). Using Western blotting with a phospho-specific JNK antibody, we investigated the phosphorylation status of JNK in the exocrine pancreas after AP induction with or without TUDCA pretreatment (Fig. 4B). For the quantification of our data we analyzed the levels of phospho-JNK1 exemplary (Fig. 6A). As described earlier (24) pJNK is a very early response element after AP induction. After caerulein administration the phospho-specific Western blotting signal increased to its maximum after 30 min (1,321.25 + 364.42% increase of NaCl-control). Phospho-JNK1 levels rise up to almost the same level after TUDCA preincubation and AP induction (1,232.81 + 313.83% increase of NaCl-control). There is no statistical difference at 30 min, but, thereafter, phospho-JNK1 signals decrease faster after TUDCA preincubation. This is significant at 2 and 4 h. TUDCA preincubation alone shows no difference compared with control-NaCl.

Another proapoptotic pathway emanating from the ER involves the ER initiator procaspase 12, which is exclusively activated by cleavage under prolonged ER stress conditions and activates the effector caspase 3, leading to apoptosis. Using Western blotting from exocrine pancreas cell lysates, we investigated the expression of the 38-kDa splice variant of active caspase 12 (Fig. 6B). As shown in the Western blotting
analysis, caspase 12 appears very early and significantly 30 min after AP induction. It peaks 2 h after caerulein administration, and TUDCA pretreatment was able to almost abolish activation at all analyzed time points. Control-NaCl and control-TUDCA show no increase in caspase 12 activation at any time point.

Further downstream in the signal cascade of caspase 12 is caspase 3. To show an effect of the ER chaperone on the effector-caspase 3, the 17-kDa splice variant of active caspase 3 was analyzed too (Fig. 7). Our results show an increase in activated caspase 3 after caerulein-induced AP. It peaks at 1 h (615.13 ± 158.78% increase of NaCl-control) with a decrease thereafter. After TUDCA preincubation and caerulein administration, the active caspase 3 increased too, but the protein level in Western blotting is significantly lower compared with caerulein alone (278.16 ± 65.34% increase of NaCl-control).

DISCUSSION

In this present study, TUDCA pretreatment reduced intracellular trypsin activation and edema formation in experimentally induced AP, whereas TDCA, a bile salt without chaper-
one-like properties, does not reduce those key parameters of AP. Furthermore, TUDCA prevented cell damage, the activation of MPO, the caerulein-induced BiP upregulation and reduced XBP-1 splicing, caspase 12 and 3 activation, and accelerated the downregulation of pJNK. TUDCA showed cytoprotective effects via pPERK and its downstream targets in controls without pancreatitis. These findings are of interest because ER stress and its responses are relevant in the physiological function of the exocrine pancreas and in the development of AP. Histological examination was consistent with early morphological changes of the ER during the onset of AP.

In this context, gene-profiling studies show significant alterations in ER stress key regulators, and in a model of necrotizing pancreatitis all ER stress sensors, PERK, IRE1, ATF6, and their downstream signal pathways become activated (1, 22, 25, 29, 33, 37). It has also been observed that stimulation of isolated pancreatic acini by different secretagogues can generate a distinct ER stress response (28).

From those previous studies we conclude that pancreatic stimuli in physiological concentrations stimulate and increase zymogene production and secretion in the exocrine pancreas with the help of an accelerated ER function in protein folding and trafficking to the Golgi apparatus. Supraphysiological stimulation, e.g., cholecystokinin (CCK) overstimulation or induction of AP, may lead to protein accumulation attributable to the ER folding capacity being exceeded. It putatively prompts the activation of the ER stress sensors. This finally

![Fig. 4. Effects of TUDCA preincubation on binding protein (BiP) expression and protein kinase-like ER kinase (PERK) phosphorylation. BiP induction as endoplasmic reticulum (ER) stress-inducible chaperone was analyzed by Western blotting from pancreatic tissue lysates with or without preincubation with 250 μg/ml TUDCA and AP induction. A: representative Western blots of 5 individual experiments. For quantification, Western blot membranes were counterstained with anti-actin (row 5). A quantitative analysis of n = 5 independent experiments is shown below. *P < 0.05 compared with caerulein stimulation alone. B: representative Western blots of pPERK of 5 individual experiments. For quantification, Western blot membranes were counterstained with anti-actin (row 5). Each point represents the mean ± SE. *P < 0.05 compared with caerulein stimulation alone.](http://ajpgi.physiology.org/)

![Fig. 5. Effects of TUDCA preincubation on X-box protein (XBP)-1 splicing and activation transcription factor (ATF4) expression. A: data for ATF4 expression. B: representative Western blot of an experiment repeated 4 times. For quantification, Western blot membranes were counterstained with anti-actin (row 5). Below it shows the quantitative Western blot data from 4 individual experiments. Each point represents the mean ± SEM. *P < 0.05 compared with caerulein stimulation alone. A quantitative analysis of the Western blot data from 5 independent experiments is shown below. Each point represents the mean ± SEM. *P < 0.05 compared with caerulein stimulation alone.](http://ajpgi.physiology.org/)
results in a general stop of transcription and translation, induction of chaperones, and expansion of the ER itself, an attempt to match demands of protein folding and secretion from the acinar cell upon stimulation. Such effects very likely increase the efficiency of the ER function and export and promote a proper trafficking of proteins (11, 45, 48). So far it is known that CCK in supraphysiological doses induces acinar cell damage, activates trypsin, and induces proapoptotic signals and ER stress. On the other hand, in endocrine pancreatic cells TUDCA was a useful tool to reduce ER stress (31). We tested this bile salt derivate with ER chaperone-like characteristics in exocrine acinar cells, thereby reducing ER stress, trypsin activation, and apoptosis (35).

Özcan et al. (43) described TUDCA as a bile salt conjugate with chemical or pharmaceutical chaperone properties. Treatment of hepatoma cells with TUDCA suppressed ER stress-induced pPERK and XBP-1 splicing. In leptin-deficient (ob/ob) mice, as a model of severe obesity and insulin resistance, oral administration of TUDCA exhibited a potent antidiabetic activity. TUDCA enhanced insulin response and impaired glucose tolerance and suppressed PERK, IRE1, and JNK activation. TUDCA improved insulin resistance and reduced the obesity-induced lipid accumulation in liver. De Almeida et al. (13) showed a protective effect of TUDCA on ER stress induced by mutant protein aggregates of the mutated HFE C282Y protein associated with hereditary hemochromatosis.

Furthermore, the expression of mutated proteins can lead to ER stress. DNA point mutations may result in the production of misfolded proteins that are transcribed but are unable to reach their functional designation. Misfolded proteins are retained inside the ER and induce ER stress. Important examples of misfolding diseases are Alzheimer’s disease and cystic fibrosis (15, 64). Thus extraordinary efforts have been made to design therapeutic interventions preventing or even correcting the structural abnormality of misfolded proteins. In this regard, rescue of misfolded “trafficking-defective” proteins or protein congestion of the ER by pharmacological chaperones is emerging as one of the most promising therapeutic strategies for such disorders (11, 45, 53).

TUDCA in the present in vivo study had no effect on serum amylase with contrast to our previous in vitro findings. Still, this is concordant to observations that serum amylase is important in the diagnosis of AP but of no prognostic value, as the levels do not correlate with the severity, course, or outcome of pancreatitis (2, 27, 30).
Interestingly, TUDCA employed in vivo reduced trypsin activation, edema formation, and histological changes in acinar cells. It further had positive influence on the inflammatory reaction to AP by reducing MPO activity in pancreas and lung. This is important because the systemic inflammatory response to AP is a major component of pancreatitis-associated morbidity and mortality (8, 44, 47).

In terms of ER stress and its downstream pathways TUDCA pretreatment was able to reduce BiP expression, as a sensitive parameter of ER stress. TUDCA reduced XBP-1 splicing and inhibited ER-associated proapoptotic pathways emitting from the organelle. This, at least in part, is mediated by a reduction in ER stress-specific caspase 12 activation and its effector caspase 3 and the accelerated downregulation of pJNK. The release of cytochrome c from mitochondria downstream of the caspase activation should be investigated in future studies because it is important in AP (42). Beyond that, TUDCA is able to phosphorylate cyclic adenosine monophosphate-responsive element-binding protein and activates phosphatidylinositol 3-kinase-dependent Bad signaling as demonstrated earlier (10, 63).

Surprisingly, TUDCA by itself, without an induction of AP, induced the phosphorylation of PERK. To further investigate this phenomenon, we determined the consequence of pPERK. One target of pPERK is eukaryotic initiation factor-2α (eIF-2α) (52). As a consequence of pPERK, it becomes phosphorylated, resulting in repression of mRNA translation and protein synthesis (23). At the same time, active eIF-2α specifically increases the translation and expression of ATF4 (20). In our study this pathway became activated just by incubation with TUDCA. As a consequence ATF4 acts as a transcription factor to induce genes important in translation, transport, and metabolism of amino acids, secretion, resistance to oxidative stress, and protein degradation (34). As Lu et al. (46) describe, pPERK and downstream targets of ATF4 can protect cells from lethal effects of oxidants of relevance in AP. Bardag-Gorce et al. describe a unique upregulation of ATF4 in liver tissue induced by the use of proteasome inhibitors (14a). The second target of pPERK Nrf2 was neither regulated by proteasomal inhibitors nor involved in the cytoprotection from oxidative stress (3). Therefore, PERK and eIF-2α phosphorylation may impact on the pancreas at two levels: it could reduce the rate of translation initiation and protein synthesis and prevent the synthesis of proapoptotic proteins. At the same time, it may initiate signaling in cytoprotective gene expression pathways. Activation of those pathways can single-handedly promote a stress-resistant preconditioned state (34). Both effects may contribute to the cytoprotection seen in our AP experiments, and it supports the concept that cells are able to activate only one or two stress sensors or selective targets of ER stress sensors and with this a particular branch of the ER stress response in accordance to the unfavorable circumstance that the cell has to overcome or to accommodate. It is interesting, however, that persistent activation of this UPR branch is tolerated for extended periods in the context of hibernation, a physiologically quiescent stress-resistant state in mammals (16, 17). Therefore, pharmacological agents that promote eIF-2α phosphorylation and ATF4 induction without adding to cell stress may be useful tools in a wide range of clinical situations in anticipation of cellular injury. It may further explain successful treatments of idiopathic and autoimmune pancreatitis by bile salts demonstrated in several case reports and small pilot studies in humans (49, 57, 59). Thus this treatment strategy may become important, even since a recent publication showed a connection between ER stress and chronic hereditary pancreatitis (26). Overall, this TUDCA effect is a major in vivo result of this study but has not been seen in vitro in acinar cells. This difference may be due to the third phase of AP, the extra-acinar cell phase that involves inflammatory responses to acinar cell injury and systemic generation of cytokines, chemokines, and activation of complement factors, as well as systemic complications (55, 63). Furthermore, acinar cells interact in vivo with endocrine pancreatic islet cells andstellate cells, and the exocrine pancreas shows significant disturbances in microcirculation under the condition of AP (62, 67). Though the intracellular crosstalk and organ change cannot be considered in vitro, it may influence experimental results in vivo. Furthermore, TUDCA is able to increase intracellular calcium by inhibiting the sarcoplasmic reticulum Ca2+-ATPase pump (63), and this may be the sublethal stress that most greatly influences PERK phosphorylation because this is the ER stress pathway involved in inflammatory reactions. Although BiP expression and XBP-1 splicing is evident at low levels after TUDCA pretreatment alone at several time points in our experiments, we believe that these changes are not as significant as the pathways involved in protein folding and trafficking.

In summary, our data show that the ER stress response of the exocrine pancreas to caerulein administration can be pharmacologically modulated. Associated with this modulation are diminished morphological and biochemical markers of AP that are normally induced by caerulein. These findings suggest that ER stress is an early molecular mechanism of at least some forms of pancreatitis and a potential therapeutic target for chemoprevention of this disease. Future studies in humans may open new perspectives on the use of chemical chaperones as therapeutics in pancreatitis.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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