L-arginine-induced experimental pancreatitis

Péter Hegyi, Zoltán Rakonczay Jr, Réka Sári, Csaba Gőg, János Lonovics, Tamás Takács, László Czakó

Abstract
Despite medical treatment, the lethality of severe acute pancreatitis is still high (20-30%). Therefore, it is very important to find good animal models to characterise the events of this severe disease. In 1984, Mizunuma et al. developed a new type of experimental necrotizing pancreatitis by intraperitoneal administration of a high dose of L-arginine in rats. This non-invasive model is highly reproducible and produces selective, dose-dependent acinar cell necrosis. Not only is this a good model to study the pathomechanisms of acute necrotizing pancreatitis, but it is also excellent to observe and influence the time course changes of the disease. By writing this review we illuminate some new aspects of cell physiology and pathology of acute necrotizing pancreatitis. Unfortunately, the reviews about acute experimental pancreatitis usually did not discuss this model. Therefore, the aim of this manuscript was to summarise the observations and address some challenges for the future in L-arginine-induced pancreatitis.

Hegyi P, Rakonczay Jr Z, Sári R, Gőg C, Lonovics J, Takács T, Czakó L. L-arginine-induced experimental pancreatitis. World J Gastroenterol 2004; 10(14): 2003-2009 [http://www.wjgnet.com/1007-9327/10/2003.asp]

EFFECT OF EXCESSIVE DOSES OF L-ARGININE ON DIFFERENT TISSUES
Mizunuma et al. were the first who studied the effect of an excessive dose of L-arginine (Arg) on different tissues in rats[1]. After a single intraperitoneal (ip.) injection of 500 mg/100 g body mass (bm) Arg, the liver cells showed slight vacuolar degeneration. The kidney contained eosinophilic compositions in some proximal convoluted tubules, but showed no degenerative changes. Adipose tissues around the pancreas showed fat necrosis. There were no changes in the weight of different organs (liver, kidney, spleen, thymus), except for the pancreas. Due to the effect of pancreatitis the weight of the pancreas nearly doubled by the end of the first 24 h. No evidence of pathophysiological lesions was observed in the lung, heart, intestine, testis, spleen and thymus[1]. After the first observation Kishino et al. examined the pancreas by electron microscopy[2]. They found that degeneration started with disorganization of the rough endoplasmic reticulum. The main changes in acinar cells after 24 h were partial distension of the endoplasmic reticulum. At this time large sequestered areas in the cytoplasm contained disarranged rough endoplasmic reticulum and degraded zymogen granules. Forty-eight hours after Arg-injection, dissociation and necrosis of acinar cells were noted. Subsequently, the necrotic cells were replaced by interstitial tissue composed of leucocytes and fibroblasts. The early morphological changes of the acinar cells may be related to metabolic alterations associated with the endoplasmic reticulum. The final conclusion was that an excessive dose of arginine was toxic to the rat pancreas when injected intraperitoneally[3]. Tani et al. continued this work by observing the effect of Arg on the pancreas[3]. They clearly proved that excessive doses of Arg could cause a severe acute necrotizing pancreatitis. On the other hand, Delaney and Weaver showed that long term administration of Arg caused pancreatic atrophy with insufficiency, therefore high doses of Arg were also suitable for the induction of chronic pancreatitis[3,4].

INDUCTION OF PANCREATITIS
Mizunuma et al. induced acute necrotizing pancreatitis by a high dose of Arg ip. (500 mg/100 g bm), which evoked selective pancreatic acinar cell damages without any morphological change in the Langerhans islets[3]. After this first observation, researchers investigating Arg-induced pancreatitis usually modified the method of pancreatitis induction. Tani et al. tried to use higher doses of Arg, but found that Arg at the dose of more than 500 mg/100 g bm killed most of the treated rats within a few hours[3]. When a single dose of 500 mg/100 g bm of Arg was injected, 70-80% of the pancreatic acinar cells were necrotized within 3 d[3]. When rats were given additional 3 injections of 250 mg/100 g bm over 10 d, there was up to 90% acinar destruction[3]. The longest treatment of Arg was performed by Weaver et al. Daily administration of 350 mg/100 g bm of Arg for 30 d resulted in severe pancreatic necrosis by wk 4, only isolated single acinar cells remained within a fibrous connective tissue matrix[3]. Most of the authors, who studied the pathomechanisms of this pancreatitis used 250 mg/100 g bm of Arg twice at an interval of one hour[6-8]. On the other hand, when the regenerative processes were studied after pancreatitis, a smaller dose of Arg (200 mg/100 g bm of Arg ip. twice at an interval of 1 h) was used[9,10].

All in all, the dose- and time-dependency of the effects of Arg gives an excellent opportunity to study the different phases of pancreatitis. A higher dose of Arg is suggested to study the pathomechanism of acute pancreatitis, while a smaller dose of Arg seems more suitable to characterize the regenerative processes. Long-term administration of Arg is suggested to study chronic pancreatitis (Table 1).

PATHOMECHANISM OF L-ARGININE INDUCED PANCREATITIS
The mechanism by which Arg causes pancreatitis is not fully known. Accumulating evidence suggests that oxygen free radicals[7,8,11-13], nitric oxide (NO)[14], inflammatory mediators[6,12,15,16] all have a key role in the development of the disease.

Changes in cytokine levels
We found that both serum tumour necrosis factor-α (TNF-α) and interleukin (IL)-6 level were already significantly increased...
Table 1  Induction of pancreatitis: differences in methods

| Dose of L-Arginine | Reference |
|--------------------|-----------|
| **Single dose**    |           |
| 500 mg/ 100 g bm ip.| Mizinuma et al. 1994, Kishino et al. 1984, Tani et al. 1990, Shields et al. 2000, Kihara et al. 2001, Tachibana et al. 1997, Tashiro et al. 2001 |
| 450 mg/ 100 g bm ip.| Tashiro et al. 2001 |
| 400 mg/ 100 g bm ip.| Tashiro et al. 2001, Rakonczay et al. 2002 |
| 300 mg/ 100 g bm ip.| Tashiro et al. 2001, Rakonczay et al. 2002 |
| 250 mg/ 100 g bm ip.| Pozsar et al. 1997 |
| 200 mg/ 100 g bm ip.| Tashiro et al. 2001 |
| **Double dose**    |           |
| 2x250 mg/ 100 g bm ip.| Takacs et al. 1996, Varga I. et al. 1997, Toma et al. 2000, Czako et al. 2000, Czako et al. 2000, Takacs et al. 2002, Toma et al. 2002 |
| 2x230 mg/ 100 g bm ip.| Takacs et al. 2002 |
| 2x200 mg/ 100 g bm ip.| Hegyi et al. 1997, Hegyi et al. 1999, Hegyi et al. 2000, Takacs et al. 2001 |
| **Multiple dose**  |           |
| 350 mg/ 100 g bm ip. daily from 1 to 4 wk | Weaver et al. 1994 |
| a single 500 mg/ 100 g bm ip. vs controls | Delaney et al. 1993 |
| (d 4, 7, 10)  |           |

Table 2  Changes of inflammatory mediator levels in Arg-induced acute pancreatitis

| Mediator | Dose of Arg/ 100 g bm | Effect | Reference |
|----------|-----------------------|--------|-----------|
| MDA      | 2x250 mg              | ↑      | Czakó et al., 1998 |
| NSG, MDA | 300 mg                | ↑      | Rakonczay et al., 2003 |
| Protein carboxyl | 300 mg | ↑      | Rakonczay et al., 2003 |
| Mn-, Cu, Zn-SOD | 2x250 mg | ↓      | Czakó et al., 1998 |
| Catalase | 2x250 mg              | ↓      | Czakó et al., 1998 |
| glutathione peroxidase | 2x250 mg | ↓↑ | Czakó et al., 1998 |
| Serum TNF-α, IL-6 | 2x250 mg | ↑      | Czakó et al., 2000 |
| Serum TNF-α, IL-1, IL-6 | 2x230 mg | ↑      | Rakonczay et al., 2002 |
| Pancreatic IL-1β | 300 and 400 mg | ↑      | Rakonczay et al., 2003 |
| Pancreatic TNF-α | 300 and 400 mg | ↑      | Rakonczay et al., 2003 |
| Pancreatic cNOS | 2x250 mg | ↓↑ | Takács et al., 2002 |
| Pancreatic INOS | 2x250 mg | ↑      | Takács et al., 2002 |
| CCK      | 2x250 mg              | ↑      | Czakó et al., 2000 |

↓ : decreased activity, ↑ : increased activity, Arg: L-arginine, MDA: malondialdehyde, NSG: nonprotein sulfhydryl group, SOD: superoxide dismutase, TNF: tumor necrosis factor, IL: interleukin, CCK: cholecystokinin-octapeptide.

48 h after administration of 2×250 mg/100 g bw Arg[10]. Among the endogenous scavengers Mn-superoxide dismutase (SOD) and catalase activities decreased significantly throughout the entire study vs the control. Cu, Zn-SOD activity decreased only at 12 h, while the glutathione peroxidase activity decreased at 6 and 12 h after Arg injection (Table 2). Pretreatment with the xanthine oxidase inhibitor allopurinol (100 and 200 mg/kg) prevented the generation of reactive oxygen metabolites and ameliorated the severity of Arg-induced pancreatitis.

Later on, we showed that the pancreatic IL-1β level significantly decreased at 1 h after ip. administration of 300 or 400 g/100 g bm Arg[12]. The IL-1β levels increased significantly at 12 h after Arg injection, peaked at 24 h and decreased thereafter (Table 2). The pancreatic TNF-α content increased significantly at 6 h, peaked at 18 h, and then remained elevated at a relatively constant level during pancreatitis (Table 2). Pretreatment with antioxidant pyrrolidine dithiocarbamate (PDTC) or methylprednisolone (MP) significantly decreased the pancreatic levels of these proinflammatory cytokines, ameliorated pancreatic oedema and exerted a beneficial effect on pancreatic morphological damage[15]. It can be proposed that these cytokines are involved in the pathogenesis of Arg-induced acute pancreatitis.

Oxidative stress changes

In 1998, we demonstrated that the pancreatic malondialdehyde (MDA) level was significantly elevated at 24 h, and peaked at 48 h after administration of 2×250 mg/100 g bw Arg[10]. Among the endogenous scavengers Mn-superoxide dismutase (SOD) and catalase activities decreased significantly throughout the entire study vs the control. Cu, Zn-SOD activity decreased only at 12 h, while the glutathione peroxidase activity decreased at 6 and 12 h after Arg injection (Table 2). Pretreatment with the xanthine oxidase inhibitor allopurinol (100 and 200 mg/kg) prevented the generation of reactive oxygen metabolites and ameliorated the severity of Arg-induced pancreatitis.

We also showed that 300 mg/100 g bm Arg significantly increased the pancreatic non-protein sulfhydryl group content, malondialdehyde and the protein carbonyl levels vs the control (Table 2)[15]. Pretreatment with PDTC or MP significantly ameliorated these changes and reduced the severity of the disease.

These results suggest that generation of free radicals is an early and perhaps pivotal mechanism in the pathogenesis of Arg-induced acute pancreatitis.

Role of nitric oxide

NO, a highly reactive free radical, is generated from Arg by an enzymatic pathway (NO synthase: NOS) originally demonstrated in vascular endothelial cells[17]. Under physiologic conditions,
constitutive NO synthase (cNOS) results in a low level of NO, while in different inflammatory processes inducible NO synthase (iNOS) produces larger quantities of NO in various cell types. The activity of NOS is specifically inhibited by structural analogues of L-arginine such as NO<sub>2</sub>-nitro-L-arginine methyl ester (L-NAME)[17].

It was demonstrated that the cNOS activity was depleted at 6 h after onset of Arg-induced pancreatitis, it then gradually increased to a level significantly higher than that in controls and decreased thereafter at 48 h[18]. The iNOS activity was significantly increased at 24 and 48 h vs control (Table 2). Treatment with L-NAME significantly reduced the amylase activity, pancreatic oedema, pancreatic vascular permeability and cNOS activity in the pancreas at 24 h after the onset of pancreatitis as compared with those in the control. L-NAME treatment reduced the iNOS activity, but not significantly, and did not exert any beneficial effect on the histological score[19]. It can be concluded that both cNOS and iNOS play an important role in the development of Arg-induced acute pancreatitis in rats.

**Endogenous cholecystokinin**

Endogenous cholecystokinin (CCK) and CCK-A receptors have been suspected to play a role in the development of acute pancreatitis in rats[18,19]. No significant differences in plasma CCK bioactivity were found between the pancreatic animals and the control group during the course of Arg-induced pancreatitis (Table 2). KSG-054, a CCK receptor antagonist did not exert any beneficial effect on the laboratory and morphologic changes observed in Arg-induced pancreatitis[20]. These results suggest that endogenous CCK is not involved in the pathogenesis of Arg-induced pancreatitis.

**Effect of nuclear factor-kappa B activation**

Recent studies have established the critical role played by inflammatory mediators in acute pancreatitis[14,20]. One of the most important transcription factors that control proinflammatory gene expression is nuclear factor kappa B (NF-kB)[21]. Therefore, we set out to investigate NF-kB activation and proinflammatory cytokine synthesis in the pancreas during Arg-induced acute pancreatitis in rats[22]. The dose-response (300 or 400 mg/100 g bm) and time-effect (0.5-96 h) curves related to the action of Arg on the pancreatic NF-kB activation and IL-1β, TNF-α, heat shock protein (HSP) 60 and HSP72 synthesis were evaluated. Also we wanted to establish whether PDTC or MP pretreatment could block the activation of pancreatic NF-kB and their effects on the severity of Arg-induced acute pancreatitis. Our results showed that pancreatic NF-kB and proinflammatory cytokine expressions were activated dose-dependently during Arg-induced acute pancreatitis in rats, although at a later stage as compared with other models. We have established that PDTC and MP pretreatment specifically and dose-dependently can block this NF-kB activation in the pancreas. Furthermore, the inhibition of NF-kB activation and proinflammatory cytokine synthesis has been found to be clearly associated with a protective effect against pancreatic damage[15].

**Apoptosis and gene expression of pancreatitis-associated protein**

Pancreatitis-associated protein (PAP) is an acute phase secretory protein known to be overexpressed in acute pancreatitis[22]. Motoo et al. examined the effects of arginine (1.25, 2.5, 5 or 10 mg/mL) on cellular morphology, PAP expression and apoptosis in rat pancreatic acinar AR4-2J cells[23]. This in vitro experimental design allowed the study of acinar cells without the confounding effects of other cell types involved in acute pancreatitis (e.g. inflammatory cells). The growth of AR4-2J cells was inhibited by Arg in a dose-dependent manner. This inhibition was due to Arg-induced apoptosis of cells which was shown by fluorescence staining, and DNA fragmentation assay. The DNA fragmentation was most prominent at 24 h when the PAP mRNA level was low, whereas it was not seen when the level was high. The expression of PAP mRNA was detected at 2 h and peaked at 6 h, it was highest at a dose of 2.5 mg/mL Arg. Motoo et al. speculated that PAP might inhibit the induction of apoptosis[23].

**Role of transforming growth factor beta 1**

Mild acute pancreatitis is often followed by full recovery of pancreatic tissue structure and function once the primary cause is eliminated[24]. Transforming growth factor beta (TGF-β) and the extracellular matrix are believed to play an important part in this process[25]. TGF-β promotes regeneration in wounded tissues by attracting monocytes and leukocytes and inducing angiogenesis and fibroblast recruitment. Kihara et al. induced acute necrotizing pancreatitis by intraperitoneal injection of 500 mg/100 g bm Arg, and examined the expression of TGF-β1, extracellular matrix proteins and metalloproteinases (degrading a variety of extracellular matrix components). TGF-β, protocollagen types I, III, IV, and fibronectin mRNA expression reached a peak value on d 2.5-3 and gradually decreased thereafter to reach control levels on d 7. Matrix metalloproteinase-2 mRNA levels peaked on d 5, whereas the immunoreactivity was maximal on d 7. TGF-β1 immunoreactivity was detected in disrupted acinar cells on d 3 and 5. Immunoreactivity for fibronectin was detected around disrupted acinar cells and interstitial spaces on d 3 and maximally on d 5. The authors believe that the results suggest an important role of TGF-β in extracellular matrix production during the early phase of acute pancreatitis[26].

**Effect of endotoxaemia in Arg-induced pancreatitis**

Systemic endotoxaemia is a common feature of severe acute pancreatitis[25]. The effect of *E. coli* endotoxin was investigated by Pozsár et al. on the mortality rates and pancreatic histology of Arg-induced (250 mg/100 g bm) acute pancreatitis in rats[27]. The mortality rates of rats treated with 5 and 10 mg/kg endotoxin were 10 % and 30%, respectively (no death was observed in the group with only Arg-induced pancreatitis). The extent of acinar cell necrosis, hemorrhage, oedema and leukocyte infiltration was significantly greater in the endotoxin-treated groups vs the control groups. The authors speculated that systemic endotoxia might exert its effect by stimulating pro-inflammatory cytokine synthesis in granulocytes. The animal experiments were closely related to similar observations made in humans. It was found that high serum endotoxin levels showed a positive correlation with disease severity[28].

**Expression of nerve growth factor**

Nerve growth factor (NGF) is a known mediator of the inflammatory response[27]. It is believed to play an important role in the pathogenesis of pain. NGF expression was investigated after injection of 250 mg/100 g bm Arg twice intraperitoneally[29]. No significant differences in NGF mRNA levels were found between the Arg-injected and control rats before d 3. However, NGF mRNA levels significantly increased On days 3 (10-18 fold) and 5 (3.2-6 fold). NGF protein levels were 2-fold higher than control levels on day 3, but this did not reach statistical significance. On d 5 there was a 4-fold increase in NGF protein levels. The cellular sites of increased NGF production were investigated by immunohistochemistry. In control rats NGF-immunoreactivity was localized to the islets of Langerhans. In Arg-induced pancreatitis, NGF was detected in the cytoplasm of exocrine pancreatic tissues, including acinar
and ductal cells at 2 and 6 h. On d of NGF was predominantly found in ductal cells. It was possible that this change in staining pattern represented a release of stored NGF from the islets to the parenchyma[28].

Effect of Arg-induced pancreatitis on the cytoskeleton
Disruption of the cytoskeleton seems to be a common prominent early feature in acute pancreatitis. Actin cytoskeleton was investigated using rhodamin phalloidin and epifluorescence microscopy combined with Normanski images[29]. In control tissue actin was primarily localised as an intense fluorescent band beneath the luminal membrane. Arg administration (200-500 mg/100 g bm) resulted in changes of the actin cytoskeleton, including reduced actin staining underneath the luminal and basolateral membranes and increased cytoplasmic staining in pancreatic acinar cells. Interestingly, the total actin content of cells was increased twofold at 24 h. The intermediate filaments were investigated by confocal microscopy. A single large dose arginine also induced the disruption of intermediate cytokeratin filaments, which were replaced by a few focal deposits or small aggregates. Sub-basolateral staining appeared with a lower intensity whereas cytoplasmic staining was not presently[29].

Role of heat shock proteins
Heat shock proteins (HSPs) are highly conserved cytoprotective proteins that are present in all species and have essential functions in protein folding, transport, translocation, assembly and degradation. HSP families have been categorized by their molecular mass. HSPs can be induced by a wide variety of conditions. Interestingly, cerulein pancreatitis has been reported to increase[30-32] or decrease[33-35] the synthesis of pancreatic HSP60 and HSP72. Arg-induced pancreatitis was accompanied with large increases in HSP27 and HSP70 levels, peaking at 24 h and localized to acinar cells[36]. Moreover, HSP27 shifted to the phosphorylated forms during pancreatitis. There were smaller increases in HSP60 and HSP90, and no effect on GRP78. Interestingly, a lower dose of Arg induced less pancreatitis, but larger increases in HSP27 and HSP70 expression and phosphorylation of HSP27. The results of Tashiro et al. are in accordance with our findings[36]. The smaller increases in the quantity of HSPs following 400 mg/100 g bm Arg were probably due to tissue necrosis, protein degradation and decreased gene activation. We believe that the increased levels of HSPs most probably act to limit the severity of the disease. In a recent study, Tashiro et al. have shown that hyperthermia and possibly HSPs confer significant protection against Arg-induced (400 mg/100 g bm) pancreatitis[36]. More specifically, the degradation and disorganization of the actin cytoskeleton were prevented. These previous findings are in contrast with ours[36]. We could only demonstrate increases in the serum proinflammatory cytokine (TNF-α, IL-1, IL-6) levels after heatwater (and also cold-water) immersion pretreatment of rats with Arg-induced pancreatitis (2×250 mg/100 g bm), but the biochemical and morphological parameters of the pancreas were not significantly different. The explanation of the discrepancies between the results of the two studies may lie in the different types of pretreatment and the differences in the dose of Arg and the strains of rats used.

Role of vacuole membrane protein 1
In vitro expression of vacuole membrane protein 1 (VMP1) promoted formation of cytoplasmic vacuoles which was followed by cell death[37]. In order to test if VMP1 expression was related to the cytoplasmic vacuolization of acinar cells during acute pancreatitis, Vaccaro et al. studied the in vivo expression of the VMP1 gene during Arg-induced (500 mg/100 g) acute pancreatitis[38]. Northern blot analysis showed that the maximal induction of VMP1 after 24 h remained high after 48 h of arginine administration. Significant increases in the number of apoptotic cells were found during those periods. Twenty-four and 48 h after arginine administration, light micrographs from thin plastic toluidine blue sections revealed numerous vacuoles in the cytoplasm of acinar cells. In situ hybridization studies showed a high expression of VMP1 in acinar cells with cytoplasmic vacuolization. VMP1 mRNA highly and significantly correlated with vacuole formation. The results suggest that VMP1 expression might be involved in the cytoplasmic vacuolization of acinar cells during the early stage of acute pancreatitis[38].

EXTRAPANCREATIC MANIFESTATIONS OF L-ARGININE INDUCED PANCREATITIS
Whereas the mortality of interstitial pancreatitis was close to zero, patients with necrotizing pancreatitis had a considerable mortality. The extrapancreatic manifestations, such as circulatory, pulmonary, renal and hepatic failure (multi-organ failure) contributed significantly to the morbidity and mortality of this disease[39-41]. This model seems to be an appropriate tool to study the extrapancreatic organ damage and its pathomechanisms.

We found that oxidative stress developed not only in the pancreas but also in remote organs during acute pancreatitis induced by 2×250 mg/100 g bm Arg[41]. The MDA concentration was significantly increased at 6 h after Arg treatment in the liver and at 24 h in the kidney. Among the endogenous scavengers, Mn-, and Cu, Zn-SOD and glutathione peroxidase were significantly reduced both in the liver and in the kidney during the course of Arg-induced pancreatitis. The catalase activity was significantly increased in the liver, whereas it was significantly decreased in the kidney. The prophylactic application of 200 mg/kg allopurinol significantly restrained the generation of free radicals in the liver. Histologic examination revealed vacuolar degeneration within hepatic cells and eosinophilic components in proximal convoluted tubules in the kidney.

Hypertonic saline (HTS) could restore the circulating volume in haemorrhagic shock by improving cardiac contractility and peripheral tissue perfusion[42]. The aim of Shields[43,44] was to investigate the effect of HTS resuscitation on the development of end-organ damage in Arg-induced pancreatitis. They demonstrated increased pulmonary endothelial permeability and increased myeloperoxidase activity at 72 h after pancreatitis induction by a single 500 mg/100 g bm Arg injection. Histological examination of the lung revealed marked interstitial congestion and hemorrhage. HTS injections (75 g/L NaCl, 2 mL/kg) were applied at 24 and 48 h after the administration of Arg. Pulmonary oedema, endothelial leak, enhanced neutrophil activity were all attenuated and the pancreatic and pulmonary histological scores were improved in animals treated with HTS.

REGENERATIVE PROCESS AFTER L-ARGININE-INDUCED PANCREATITIS
Time course changes
In the early phase of acute pancreatitis evoked by 200 mg/100 g bm Arg (d 1-3), the laboratory signs of acute pancreatic inflammation predominated in the Arg-treated rats[9]. Pancreatitis revealed an intercellular edema with the infiltration of leukocytes, dilatation of the capillaries, and microfocal necroses in the parenchyma on d 1 after pancreatitis induction. Interestingly, acinar cells surrounding the Langerhans islets remained intact (Figure 1). Following the early acinar cell necrosis, histological examinations revealed a marked adipose tissue deposition at the end of the first week. Accumulation of adipose tissue was a sign of atrophy. The rest of the pancreas (not involved in Arg-induced cell
decreased on d 14. These results proved that there was a regenerative phase on d 7. As far as the pancreatic protein secretion was concerned, in pancreatitis-group the basal secretory protein level was significantly diminished throughout the experiment. The maximal pancreatic protein secretion was significantly decreased in a progressive manner on d 3, 7 and 14. In the pancreatic rats, both the basal and the maximal pancreatic amylase secretion were significantly increased on d 1. The maximal output was significantly decreased only on d 14. Taken together, the pancreatic secretion in the early phase of Arg-induced pancreatitis is characterized by increases in the secretory volume and amylase level, with a simultaneous decrease in protein output. A progressive decrease in pancreatic secretory capacity was then detected confirming the acinar cell damage.

Effect of exogenous CCK on the regenerative process
In the early phase of pancreatitis, administration of low doses of CCK-8 further deteriorated the laboratory and histologic parameters of acute pancreatitis. The mortality rate (15%) also demonstrated the harmful effect of CCK-8 in the early phase of this pancreatitis. Histologic sections demonstrated a more intense mitotic activity due to the effect of CCK-8 on d 7. Moreover, histologic examination revealed that the bulk of hypertrophized pancreatic acinar cells were found surrounding the enlarged Langerhans islets following CCK-8 administration. It appears that the closeness of the Langerhans islets protects acinar cells and accelerates the regenerative process during Arg-evoked pancreatic tissue damage. The reason for this might be the complex interaction of acinar and islet cells. The laboratory changes (such as pancreatic DNA and enzymes content) also proved the positive effect of CCK-8 during the recovery phase.

Secretory changes
Experimental studies have revealed that, suggesting that pancreatic enzyme secretion is reduced after the induction of acute pancreatitis. We characterized the secretory changes in Arg-induced pancreatitis on d 1, 3, 7 and 14 following pancreatitis induction, when the rats were surgically prepared with pancreatic duct and femoral vein cannulae under urethane anesthesia. In pancreatic rats, both the basal and the maximal pancreatic volumes were significantly increased vs controls on d 1 following the induction of pancreatitis. The basal output was still significantly higher vs controls on d 3 and 7, but the maximal output was significantly decreased vs the controls on d 7. No change in basal pancreatic fluid secretion was observed in pancreatic rats, but the maximal output was significantly decreased on d 14. These results proved that there was a conversion from the acute inflammatory phase to the late regenerative phase on d 7. As far as the pancreatic protein secretion was concerned, in pancreatitis-group the basal secretory protein level was significantly diminished throughout the experiment.

Effect of diabetes on Arg-induced pancreatitis
The interesting finding that perinsular acini remained intact during Arg-induced-pancreatitis prompted us to continue studies on the effects of diabetes in the process of pancreatic remodeling. An insulo-acinar correlation was also indicated by the morphological evidence. Acinar cells around the islets could be distinguished from teleinsular acini by their staining characteristics. These perinsular cells were larger, contained larger nuclei and nucleoli, and had more abundant zymogen granules. To achieve our aims, we used streptozotocin (STZ) to evoke diabetes. STZ was specifically toxic to the β-cells of the islets of Langerhans, which was irreversible and dose-dependent. When pancreatitis was induced in diabetic rats, the perinsular acini did not remain intact during Arg-induced-pancreatitis. We also found that in diabetic rats, the pancreatic regenerative processes (mitotic activity of acinar cells) in response to low doses of CCK-8 were markedly diminished. The lack of regenerative effect of CCK-8 may be due to the low insulin level. Furthermore, histologic sections indicated no difference between the peri- and teleinsular acinar cell damage in diabetic rats. This is a morphological confirmation of the pivotal role of insulin in the regulation of the exocrine pancreatic structure. However, other islet cell hormones might also be involved in this process.

When Arg-induced pancreatitis was evoked in STZ-diabetic rats, the significant elevation in serum amylase level was not so obvious as that observed in pancreatic rats. The explanation of this phenomenon is that a diabetic state (in rats without pancreatitis) appeared to shift the normal pancreatic enzyme content (decreased amylase and increased trypsinogen). These data are in accordance with those of Sofrankova et al., who demonstrated a similar pancreatic enzyme pattern in a secretory study.

Early increases were observed in the basal pancreatic fluid and amylase secretion in Arg-STZ-treated rats. However, no CCK-8-stimulated fluid secretory peak following pancreatitis induction was detected in diabetic animals, in contrast with the situation in non-diabetic rats, suggesting that diabetes could moderate the CCK-8-stimulated secretory changes in both the early and late phases of Arg-induced pancreatitis.

In order to clearly prove the effect of β-cells during the process of pancreatitis, we replaced the endogenous insulin with mixed exogenous insulin (2 IU s.c. twice daily, 30% short-acting and 70% intermediate-duration insulin), and found that simultaneous administration of exogenous insulin replaced the hypertrophic effect of low-doses of CCK-8.

Late recovery in normal and diabetic rats
Six months after Arg-induced pancreatitis without diabetes, a major restitution of the pancreatic enzyme content was found. The lipase and trypsinogen contents of the pancreas were recovered, but amylase was significantly decreased vs the controls. In spite of this large-scale restitution of the pancreatic enzyme compositions, marked histologic alterations, periductal fibroses, adipose tissue and tubular complexes, were detected 6 mo following pancreatitis induction. No mitotic activity and centroacinar hyperplasia were observed at this time. When

Figure 1: Histologic alterations in the pancreas following Arg-induced pancreatitis on d 3. The signs of acute inflammation and tubular complexes are visible. Segmental fibroses with blue stained fibroblast are observed. Intact pancreatic acinar cells are presented mainly around the Langerhans islets (Crossmann's trichrome staining x100).
pancreatitis was induced in STZ-diabetic rats, a very considerable recovery of the enzyme content was noted 6 mo following pancreatitis induction. Even at this time, however, the amylase content remained significantly decreased. This may be due to the combined effect of STZ-evoked decrease on amylase biosynthesis and the toxic damage caused by Arg to pancreatic acinar cells[50]. So far as the pancreatic histologic structure was concerned, the morphologic changes were similar to those seen in Arg-induced pancreatic non-diabetic rats.

Effect of fibroblast growth factor-7 and fibroblast growth factor-10, and role of keratinocyte growth factor receptor

Keratinocyte growth factor (KGF) is a member of the rapidly growing fibroblast growth factor (FGF) family of mitogens[57]. FGF-7 and FGF-10 show high structural homology and similar biological characteristics. Both are mainly synthesized by mesenchymal cells and stimulate epithelial cells via KGF receptor (KGRF) which is a splice variant of FGF receptor-2[58].

In normal pancreas, FGF-7 is localized in alpha islet cells, but FGF-10 is not detected. KGRF is also localized in islet cells, ductal cells, and centroacinar cells in the normal pancreas. In the pancreatic tissues of rats with Arg-induced pancreatitis, FGF-7 was localized in alpha cells, whereas FGF-10 was expressed in vascular smooth muscle cells (VSMCs). KGRF was not expressed in centroacinar cells after Arg treatment. These findings suggest that FGF-7 and FGF-10 contribute to the regeneration and differentiation of acinar cells and angiogenesis in acute pancreatitis through KGF[59].

Expression of lumican proteins

Lumican is a member of a small leucine-rich proteoglycan family[59]. It has been reported that lumican mRNA and its protein were ectopically and highly expressed in acinar cells in chronic pancreatitis (CP)-like lesions close to pancreatic cancer cells[60]. CP-like lesions are characterized by acinar and ductal-ductular cell proliferation with expanding fibrosis. Immunohistochemically, the lumican proteins are localized in ductules and a few centroacinar cells in normal pancreas.

After administration of an excessive dose of Arg, an immature fibrosis with fragmented and loose collagen fibers was observed on d 4 after pancreatitis induction. Moreover, lumican immunoreactivity was also detected in the collagen fibers on d 4. Lumican mRNA was barely detected in islet cells in the normal pancreas, but it was strongly expressed in acinar and islet cells on d 1 following the induction of pancreatitis. Lumican mRNA was expressed in many proliferating fibroblasts on d 4. These findings indicate that lumican is transiently synthesized by acinar cells and fibroblasts during Arg-induced acute pancreatitis. Lumican proteins may contribute to immature and transient fibrosis of acute pancreatitis.

CONCLUSIONS AND PERSPECTIVES

We are just starting to understand the pathophysiology of acute necrotizing pancreatitis. By this review we tried to illuminate new aspects of cell physiology and pathology of acute necrotizing pancreatitis. Firstly, we explored the effects of high doses of Arg on different tissues. Then, we concentrated on the pancreas showing that Arg could cause a necrotizing acute pancreatitis. Finally we characterized the early and late phases of this model of acute experimental pancreatitis.

Despite our current knowledge, many hypotheses and questions remain unanswered concerning the effects of Arg. Therefore, it seems to be well worthwhile to continue to explore the pathomechanism of Arg-induced pancreatitis.

REFERENCES

1. Mizunuma T, Kawamura S, Kishino Y. Effects of injecting excess arginine on rat pancreas. J Nutr 1984; 114: 467-471
2. Kishino Y, Kawamura S. Pancreatic damage induced by injecting a large dose of arginine. Virchows Arch B Cell Pathol Incl Mol Pathol 1984; 47: 147-155
3. Tani S, Itoh H, Okabayashi Y, Nakamura T, Fujiji M, Fujisawa T, Koide M, Otsuki M. New model of acute necrotizing pancreatitis induced by excessive doses of arginine in rats. Dig Dis Sci 1990; 35: 367-374
4. Delaney CP, McGeeney KF, Dervan P, Fitzpatrick JM. Pancreatic atrophy: a new model using serial intra-peritoneal injections of L-arginine. Scand J Gastroenterol 1993; 28: 1086-1090
5. Weaver C, Bishop AE, Polak JM. Pancreatic changes elicited by chronic administration of excess L-arginine. Exp Mol Pathol 1994; 60: 71-87
6. Takacs T, Czako L, Jarmay K, Farkas G Jr, Mandi Y, Lonovics J. Cytokine level changes in L-arginine-induced acute pancreatitis in rat. Acta Physiol Hung 1996; 84: 147-156
7. Varga IS, Matkovics B, Hai DO, Kotorman M, Takacs T, Sasvari M. Lipid peroxidation and antioxidant system changes in acute L-arginine pancreatitis in rats. Acta Physiol Hung 1997; 85: 129-138
8. Czako L, Takacs T, Varga IS, Tiszlavicz L, Hai DO, Hegyi P, Matkovics B, Lonovics J. Involvement of oxygen-derived free radicals in L-arginine-induced acute pancreatitis. Dig Dis Sci 1998; 43: 1770-1777
9. Hegyi P, Takacs T, Jarmay K, Nagy I, Czako L, Lonovics J. Spontaneous and cholecystokinin-octapeptide-promoted regeneration of the pancreas following L-arginine-induced pancreatitis in rat. Int J Pancreatol 1997; 22: 193-200
10. Takacs T, Hegyi P, Jarmay K, Czako L, Gog C, Rakonczay Z Jr, Nemeth J, Lonovics J. Cholecystokinin fails to promote pancreatic regeneration in diabetic rats following the induction of experimental pancreatitis. Pharmacol Res 2001; 44: 363-372
11. Czako L, Takacs T, Varga IS, Tiszlavicz L, Hai DO, Hegyi P, Matkovics B, Lonovics J. Oxidative stress in distant organs and the effects of allsporulin during experimental acute pancreatitis. J Pancreatol 2000; 27: 209-215
12. Czako L, Takacs T, Varga IS, Hai DO, Tiszlavicz L, Hegyi P, Mandi Y, Matkovics B, Lonovics J. The pathogenesis of L-arginine-induced acute necrotizing pancreatitis: inflammatory mediators and endogenous cholecystokinin. J Physiol Paris 2000; 94: 43-50
13. Varga IS, Matkovics B, Czako L, Hai DO, Kotorman M, Takacs T, Sasvari M. Oxidative stress changes in L-arginine-induced pancreatitis in rats. Pancreas 1997; 14: 355-359
14. Takacs T, Czako L, Morschel E, Laszlo F, Tiszlavicz L, Rakonczay Z Jr, Lonovics J. The role of nitric oxide in edema formation in L-arginine-induced acute pancreatitis. Pancreas 2002; 25: 277-282
15. Rakonczay Z, Jarmay K, Kaszaki J, Mandi Y, Duda E, Hegyi P, Boros I, Lonovics J, Takacs T. NF-kappaB activation is detrimental in arginine-induced acute pancreatitis. Free Radic Biol Med 2003; 34: 696-709
16. Takacs T, Rakonczay Z Jr, Varga IS, Ivanyi B, Mandi Y, Boros I, Lonovics J. Comparative effects of water immersion pretreatment on three different acute pancreatitis models in rats. Biochem Cell Biol 2002; 80: 241-251
17. Gross SS, Wolin MS. Nitric oxide: pathophysiologic mechanisms. Annu Rev Physiol 1995; 57: 737-769
18. Beglinger C. Potential role of cholecystokinin in the development of acute pancreatitis. Digestion 1999; 60(Suppl 1): 61-63
19. Tachibana I, Shirohara H, Czako L, Akiyama T, Nakano S, Watanabe N, Hirohata Y, Otsuki M. Role of endogenous cholecystokinin and cholecystokinin-A receptor in the development of acute pancreatitis in rats. Pancreas 1997; 14: 113-121
20. Kihara Y, Tashiro M, Nakamura H, Yamaguchi T, Yoshikawa H, Otsuki M. Role of TGF-beta1, extracellular matrix, and matrix metalloproteinase in the healing process of the pancreas after
induction of acute necrotizing pancreatitis using arginine in rats. Pancreas 2001; 23: 288-295

21 Abraham E. NF-kappaB activation. Crit Care Med 2000; 28 (4 Suppl): N100-N104

22 Dussetti NJ, Frigerio JM, Fox MF, Swallow DM, Dagorn JC, Iovanna JL. Molecular cloning, genomic organization, and chromosomal localization of the human pancreatitis-associated protein (PAP) gene. Genomics 1994; 19: 108-114

23 Motoy Y, Taga K, Su SB, Xie MJ, Sawabu N. Arginine induces apoptosis and gene expression of pancreatitis-associated protein (PAP) in rat pancreatic acinar AR4-2J cells. Pancreas 2000; 21: 66-61

24 Takacs T, Czako L, Jarmay K, Hegyi P, Pozsjar J, Marosi E, Pap A, Lonovics J. Time-course changes in pancreatic laboratory and morphologic parameters in two different acute pancreatitis models in rats. Acta Med Hung 1994; 50: 117-130

25 Kivilaakso E, Valtonen VV, Makamaki M, Palkuvaara T, Nikki P, Makela PH, Lempinen M. Endotoxaemia and acute pancreatitis: correlation between the severity of the disease and the anti-enterobacterial common antigen antibody titre. Gut 1984; 25: 1065-1070

26 Pozsjar J, Schwab R, Simon K, Fekete L, Orgovan G, Pap A. Effect of endotoxin administration on the severity of acute pancreatitis in two experimental models. Int J Pancreatol 1997; 22: 31-37

27 Dray A. Inflammatory mediators of pain. Br J Anaesth 1995; 75: 125-131

28 Toma H, Winston J, Micci MA, Shenoys Y, Pasricha PJ. Nerve growth factor expression is up-regulated in the rat model of L-arginine-induced acute pancreatitis. Gastroenterology 2000; 119: 1373-1381

29 Tashiro M, Schafer C, Yao H, Ernst SA, Williams JA. Arginine induced acute pancreatitis alters the actin cytoskeleton and increases heat shock protein expression in rat pancreatic acinar cells. Gut 2001; 49: 241-250

30 Bhagat L, Singh VP, Song AM, van Acker GJ, Agraval S, Steer ML, Saluja AK. Thermal stress-induced HSP70 mediates protection against intrapancreatic trypsinogen activation and acute pancreatitis in rats. Gastroenterology 2002; 122: 156-165

31 Ethridge RT, Ehlers RA, Helmich MA, Rajaraman S, Evers BM. Acute pancreatitis results in induction of heat shock proteins 70 and 27 and heat shock factor-1. Pancreas 2000; 21: 248-256

32 Weber CK, Gress T, Muller-Pfill sach, Lerch MM, Weidenbach H, Adler G. Supramaximal secretagogue stimulation enhances heat shock protein expression in the rat pancreas. Pancreas 1995; 10: 360-367

33 Rakonczay Z Jr, Ivanyi B, Varga I, Boros I, Jednakovits A, Nemeth I, Lonovics J. Takacs T. Nontoxic heat shock protein coinducer BRX-220 protects against acute pancreatitis in rats. Free Radic Biol Med 2001; 31: 1283-1289

34 Strowski MZ, Sparmann G, Weber H, Fiedler F, Printz H, Jonas L, Goke B, Wagner AC. Caerulein-induced pancreatitis increases mRNA but reduces protein levels of rat pancreatic heat shock proteins. Am J Physiol 1997; 273(4 Pt 1): G937-G945

35 Weber H, Wagner AC, Jonas L, Merkord J, Hofken T, Nizze H, Letzmann P, Goke B, Schuff-Werner P. Heat shock response is associated with protection against acute interstitial pancreatitis in rats. Free Radic Biol Med 2002; 32: 1203-1212

36 Tashiro M, Ernst SA, Edwards J, Williams JA. Hyperthermia induces multiple pancreatic heat shock proteins and protects against subsequent arginine-induced acute pancreatitis in rats. Digestion 2002; 65: 118-126

37 Dussetti NJ, Jiayi Y, Vaccaro MI, Tomasinis R, Azizi Samir A, Calvo EL, Ropolo A, Fiedler F, Mallo GV, Dagorn JC. Iovanna JL. Cloning and expression of the rat vacuole membrane protein 1 (VMP1), a new gene activated in pancreas with acute pancreatitis, which promotes vacuole formation. Biochem Biophys Res Commun 2002; 290: 641-649

38 Vaccaro MI, Grasso D, Ropolo A, Iovanna JL, Cerquetti MC. VMP1 expression correlates with acinar cell cytoplasmic vacuolization in arginine-induced acute pancreatitis. Pancreatology 2003; 3: 69-74

39 Steinberg W, Tenner S. Acute pancreatitis. N Engl J Med 1994; 330: 1198-1220

40 Renner IG, Savage WT, Pantoga JL, Renner VJ. Death due to acute pancreatitis. A retrospective analysis of 405 autopsy cases. Dig Dis Sci 1985; 10: 1005-1008

41 Karomagni I, Porter KA, Langevin RE, Banks PA. Prognostic factors in sterile pancreatic necrosis. Gastroenterology 1992; 103: 256-263

42 Kramer GC, Perron PR, Lindsey DC, Ho HS, Gunther RA, Boyle WA, Holcroft JW. Small-volume resuscitation with hypertonestic dextran solution. Surgery 1986; 100: 239-247

43 Shields C, Sookhai S, Winter DC, Dowdall JF, Kingston G, Parfrey N, Wang JH, Kirwan WO, Redmond HP. Attenuation of pancreatic-induced pulmonary injury by aerosolized hypertonestic dextrose. Surg Infect 2001; 2: 215-224

44 Shields C, Winter DC, Sookhai S, Ryan L, Kirwan WO, Redmond HP. Hypertonestic saline attenuates end-organ damage in an experimental model of acute pancreatitis. Br J Surg 2000; 87: 1336-1340

45 Saluja A, Saito l, Saluja M, Houllihan MJ, Powers RE, Mäddolesi J, Steer ML. In vivo rat pancreatic acinar cell function during supramaximal stimulation with cerulein. Am J Physiol 1985; 249: G702-G710

46 Manso MA, San Roman JJ, Dios I, Garcia LJ, Lopez MA. Cerulein-induced acute pancreatitis in the rat. Study of pancreatic secretion and plasma VIP and secretin levels. Dig Dis Sci 1992; 37: 364-368

47 Niederau C, Niederau M, Luthen R, Strohmeyer G, Ferrell LD, Grendel HQ. Pancreatic exocrine secretion in acute experimental pancreatitis. Gastroenterology 1990; 99: 1120-1127

48 Hegyi P, Czako L, Takacs T, Szilvassy Z, Lonovics J. Pancreatic secretory responses in L-arginine-induced pancreatitis: comparison of diabetic and non diabetic rats. Pancreas 1999; 19: 167-174

49 Hellman B, Wallgren A, Petersson B. Cytological characteristics of the exocrine pancreatic cells with regard to their position in relation to the islets of Langerhans. Acta endocrinol 1962; 93: 465-473

50 Kramer MF, Tan HT. The peri-insulin acinar of the pancreas of the rat. Z Zellforsch 1968; 86: 163-170

51 Rakieten N, Rakieten LM, Nadkarni MV. Studies on the diabetogenic action of streptozotocin. Cancer Chemother 1963; 29: 91

52 Junod A, Lambert AE, Orli L, Pictet R, Gonet AE, Renold AE. Studies on the diabetogenic action of streptozotocin. Proc Soc Exp Biol Med 1967; 126: 201

53 Sofrankova A, Dockray GJ. Cholecystokinin- and secretin-induced pancreatic secretion in normal and diabetic rats. Am J Physiol 1983; 244: G370-G374

54 Hegyi P, Rakonczay Z Jr, Sari R, Farkas N, Góg C, Nemeth J, Lonovics J, Takacs T. Insulin is necessary for the hypotherpetic effect of CCK-8 following acute necrotizing experimental pancreatitis. World J Gastroenterol 2004; in press

55 Hegyi P, Takacs T, Tiszlavicz L, Czako L, Lonovics J. Recovery of exocrine pancreas six months following pancreatectis induction with L-arginine in streptozotocin-diabetic rats. J Physiol Paris 2000; 94: 51-55

56 Williams JA. Goldfinch I. The insulin-acinar relationship. In: The Exocrine Pancreas: Biology, Pathobiology and Diseases. Edited by Go VLW et al. Raven Press, New York 1993; pp: 789-802

57 Werner S. Keratinocyte growth factor: a unique player in epiderelial repair processes. Cytokine Growth Factor Rev 1998; 9: 153-165

58 Ishiwata T, Naito Z, Lu YP, Kawahara K, Fujii T, Kawamoto Y, Teduka K, Sugisaki Y. Differential distribution of fibroblast growth factor (FGF-7) and FGF-10 in L-arginine-induced acute pancreatitis. Exp Mol Pathol 2002; 73: 181-190

59 Greiling H. Structure and biological functions of keratan sulfate proteoglycans. Review ES 1994; 70: 101-122

60 Naito Z. Ishiwata T, Lu YP, Teduka K, Fujii T, Kawahara K, Sugisaki Y. Transient and ectopic expression of lumican by acinar cells in L-arginine-induced acute pancreatitis. Exp Mol Pathol 2003; 74: 33-39

Edited by Zhu LH and Wang XL. Proofread by Xu FM