PATHOGENESIS OF BASIC AMINO ACID-INDUCED EXPERIMENTAL ACUTE PANCREATITIS: THE ROLE OF UREA CYCLE METABOLITES AND POLYAMINES

Ph.D. Thesis

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LIST OF FULL PAPERS CITED IN THE THESIS

I. Rakonczay Jr Z, Hegyi P, Dósa S, Iványi B, Jármay K, **Biczó G**, Hracskó Z, Varga IS, Karg E, Kaszaki J, Varró A, Lonovics J, Boros I, Gukovsky I, Gukovskaya AS, Pandol SJ, Takács T. A new severe acute necrotizing pancreatitis model induced by L-ornithine in rats. *Crit Care Med* 2008;36:2117-2127.
IF_{2008}: 6.594

II. **Biczó G,** Hegyi P, Berczi S, Dósa S, Hracskó Z, Varga IS, Iványi B, Venglovecz V, Wittmann T, Takács T, Rakonczay Jr Z. Inhibition of arginase activity ameliorates L-arginine-induced acute pancreatitis in rats. *Pancreas* 2010 (in press).
IF_{2009}: 2.733

III. **Biczó G,** Hegyi P, Sinervirta R, Berczi S, Dósa S, Siska A, Iványi B, Venglovecz V, Takács T, Alhonen L, Rakonczay Jr Z. Characterization of polyamine homeostasis in L-ornithine-induced acute pancreatitis in rats. *Pancreas* 2010 (Epub).
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LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS

IV. Szabolcs A, **Biczó G**, Rakonczay Jr Z, Tiszlavicz L, Halm G, Wittmann T, Takács T. Simultaneous proteosome inhibition and heat shock protein induction by bortezomib is beneficial in experimental pancreatitis. *Eur J Pharmacol* 2009;616:270-274.
IF_{2009}: 2.585

V. Takács T, Szabolcs A, **Biczó G**, Hegyi P, Rakonczay Jr Z. [The clinical relevance of experimental acute pancreatitis models]. *Orv Hetil* 2008;149:1981-1986. [Article in Hungarian]
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INTRODUCTION

Acute pancreatitis is a sudden inflammatory disorder of the pancreas, the pathomechanism of which is not well understood. Although the overall mortality of patients with acute pancreatitis is approximately 5%, a great proportion of deaths is the result of the necrotizing form of the disease. Due to the rapid course of the disease and relative inaccessibility of human pancreatic tissue, a number of animal models have been developed to study the pathomechanism and to test possible treatment options.

One noninvasive, reproducible model of necrotizing pancreatitis is the L-arginine-induced model. Its exact pathomechanism is unknown. L-arginine can be metabolized via a number of different pathways. Two key reactions that are involved in the catabolism of L-arginine are part of the urea cycle. Nitric oxide (NO) synthase catalyzes the conversion of L-arginine to NO and L-citrulline, whereas, arginase hydrolyzes L-arginine to L-ornithine and urea. Polyamines (putrescine, spermidine, and spermine) are direct downstream metabolites of L-ornithine. Polyamine biosynthesis is regulated by the activities of ornithine and S-adenosylmethionine decarboxylases (ODC and SAMDC, respectively), whereas polyamine catabolism is controlled by the rate-limiting spermidine/spermine N^1-acetyltransferase (SSAT). Overexpression of SSAT in transgenic rodents resulted in an immense induction of pancreatic SSAT activity, a profound depletion of spermidine, and spermine pools and acute pancreatitis. It has been shown that pancreatic SSAT activation and subsequent polyamine catabolism are characteristic features of experimental acute pancreatitis models and also of human pancreatic tissue samples from patients with acute pancreatitis. Furthermore, methylated polyamine analogues, which are supposed to fulfill the putative cellular functions of spermidine but are resistant to SSAT-dependent catabolism, prevented acute pancreatitis when administered before the induction of the SSAT

overexpressing transgene. Polyamine analogue administration was also beneficial in other pancreatitis models including L-arginine-induced pancreatitis. The importance of polyamines in the maintainance of pancreatic integrity is further highlighted by the fact that the pancreas contains the highest concentration of spermidine in the mammalian body. However, the exact physiological function of spermidine in the pancreas is unknown. Consequently, metabolites of the urea cycle and polyamine biosynthesis may play a crucial role in the maintenance of pancreatic integrity.

AIMS

The main aims of this work were to determine whether equimolar doses of the L-arginine metabolites L-ornithine or L-citrulline and/or the NO donor sodium nitroprusside cause acute pancreatitis in rats. Large doses of intraperitoneally (IP) injected L-ornithine induced severe acute necrotizing pancreatitis, therefore, we characterized the dose–response and time course changes of L-ornithine administration. Based on these results, we speculated that L-arginine produces a toxic effect on the pancreas, at least in part, via L-ornithine. Therefore, we tested the effects of the irreversible arginase inhibitor (+)-S-2-amino-6-iodoacetamidohexanoic acid (AIHA) on L-arginine-induced acute pancreatitis. As L-ornithine is a direct precursor of polyamines, the question arises how the biosynthesis and catabolism of polyamines change in L-ornithine-induced acute pancreatitis. Furthermore, as we observed pancreatic spermidine catabolism in rats with L-ornithine-induced pancreatitis, we also tested the effects of the metabolically stable polyamine analogue 1-methylspermidine (MeSpd) administration on the disease as treatment to compensate for the loss of natural spermidine.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180 to 250 g were used.

Experimental Protocols

Pilot Study with the Main Direct Downstream Metabolites of L-arginine

Rats (n = 3–5) were injected IP with equimolar (11.7 mL/kg 1.424 M/L) L-arginine-HCl, L-citrulline and/or sodium nitroprusside, L-ornithine-HCl, or D-ornithine-HCl (all chemicals dissolved in physiological saline (PS) and pH set to 7.4). The animals were killed 24 hours after the IP injection. To determine the serum concentrations of arginine, citrulline, and ornithine after injection of L-arginine, rats were killed at 2, 4, 6, and 12 hours.

Dose–response and Time Course Changes of L-ornithine Injection

To study the dose-response (n = 6) of L-ornithine administration, rats were injected IP with 1 to 6 g/kg body weight of L-ornithine-HCl (300 mg/mL, pH = 7.4) and were killed after 24 hours. For the time course studies (n = 4–10), rats were injected with 3 g/kg L-ornithine and were killed 2 to 72 hours, 1 week, or 1 month after the injection. The control animals received PS IP and were killed 24 hours after the injection.

Effect of Irreversible Arginase Inhibition on L-arginine-induced Pancreatitis

In each experimental group, five to eight rats were used. Rats were pretreated with 15 mg/kg AIHA or its vehicle IP 1 hour before injection with PS or 3.5 g/kg L-arginine-HCl (350 mg/mL, pH: 7.4) IP. Rats were sacrificed 24 hours

after the L-arginine or PS injection. Pancreas, liver, kidney, and lung tissue were frozen from control animals for determination of arginase activity.

Characterization of Polyamine Homeostasis in L-ornithine-induced Acute Pancreatitis

Pancreatitis was induced by IP injection with 3 g/kg L-ornithine-HCl. Polyamine homeostasis was studied at 6, 24, 72, and 168 hours after the induction of pancreatitis (n = 5-6). The control animals received PS IP and were killed 24 hours after the injection (n = 5).

Effect of 1-methylspermidine on L-ornithine-induced Acute Pancreatitis

The polyamine analogue MeSpd was synthesized from 3-aminobutanol and was dissolved in PS (25 mg/mL, pH 7.4). Rats were divided randomly into 6 groups. In the O24 group (n = 8), rats were injected with 3 g/kg L-ornithine-HCl IP and received PS IP 4 hours before (n = 4) or 4 hours after (n = 4) the L-ornithine treatment. In the MO24 group (n = 6), rats were pretreated with 50 mg/kg MeSpd IP 4 hours before injection with 3 g/kg L-ornithine IP. In the OM24 (n = 6) group, rats received 50 mg/kg MeSpd IP 4 hours after the L-ornithine (3 g/kg) injection. In the O48 (n = 5) group, rats were injected with 3 g/kg L-ornithine IP and received PS IP 4 and 24 hours thereafter. In the OM48 group (n = 5), rats were treated with 50 mg/kg MeSpd IP 4 and 24 hours after the L-ornithine (3 g/kg) treatment. In the control group (n = 5), rats received PS IP instead of L-ornithine and MeSpd. The „24” and „48” labels in the group names indicate the time points in hours at which rats were sacrificed after the injection of L-ornithine.

In all experimental protocols, rats were killed by exsanguination through the abdominal aorta after anaesthetization with 44 mg/kg pentobarbital IP.

Assays

The pancreatic weight/body weight ratio (p.w./b.w.) was used to evaluate the degree of pancreatic edema. Serum, pancreatic and ascitic amylase activity, serum lipase activity, serum aspartate aminotransferase (ASAT) activity, and concentrations of glucose, calcium, triglyceride, urea, creatinine as markers of acute pancreatitis were measured. Changes in the serum levels of arginine, ornithine, and citrulline in response to IP administration of L-arginine were also determined. Pancreatic trypsin, arginase, myeloperoxidase, ODC and SSAT activities were measured by enzymatic assays. The levels of the natural polyamines (spermidine, spermine, and putrescine) and the polyamine analogue MeSpd were determined by high-performance liquid chromatography. Oxidative stress in the pancreas was assessed by measuring pancreatic nonprotein sulfhydryl group (NSG) content and the activities of glutathione peroxidase (GSH-Px) and superoxide dismutases (SOD). Western blot analysis of pancreatic heat shock protein 27 (HSP27) and HSP72, as well as IκB-α and IκB-β expression was performed. The proinflammatory interleukin-1β (IL-1β) concentrations were measured in the pancreatic cytosolic fractions with an ELISA kit. Apoptosis in the pancreas was analyzed by agarose gel electrophoresis, and quantitated by TdT-mediated dUTP nick end-labeling (TUNEL) assay. Histopathological investigation of the pancreas was also performed in all cases.

Statistical Analysis

Experiments were evaluated by using the analysis of variance followed by Dunnett's multiple comparison *post hoc* test. Values of $p < 0.05$ were accepted as significant.

RESULTS

1. *In Vivo* Effects of Large Doses of the Main Direct Metabolites of L-arginine

Intraperitoneal injection of 2.8 g/kg L-ornithine caused a more severe pancreatitis compared with the L-arginine group. Similar effect was seen with the 3 g/kg L-ornithine dose, so this was used throughout the rest of the study. In contrast, IP administration of 3 g/kg D-ornithine did not result in pancreatic injury. A dose of 2.9 g/kg L-citrulline did not cause an alteration in any of the measured parameters and the pancreas seemed normal in histology. The animals that received sodium nitroprusside (4.95 g/kg) alone or in combination with L-citrulline became lethargic soon after the injection and died by the next morning. Autopsy did not show pancreatitis in these animals.

2. Time Course Changes of Serum Arginine, Citrulline, and Ornithine Concentrations in Rats Injected Intraperitoneally with 3.5 g/kg L-arginine

Serum arginine, citrulline, and ornithine concentrations were all significantly increased after the injection of L-arginine. Importantly, there were much greater increases in serum ornithine versus citrulline levels after L-arginine injection.

3. Dose–response of Intraperitoneal Injection of L-ornithine

The rats injected with 1 or 2 g/kg L-ornithine did not develop any pancreatic lesions. However, 3 g/kg of this basic amino acid caused a severe acute pancreatitis as described subsequently. A dose of 4 to 6 g/kg killed the animals within a couple of hours after the injection after developing lethargy, neurologic and neuromuscular symptoms.

4. Time Course Studies After Intraperitoneal Injection of Rats with 3 g/kg L-ornithine

Macroscopic Observations

The pancreas appeared edematous from 18 to 36 hours, its peak being at 24 hours. Ascites and adhesions of organs were seen from 4 to 6 hours (peaking at 24 hours). Dilated small and large bowels suggesting functional ileus was apparent at 72 hours to 1 week after L-ornithine injection.

Histologic Examination of the Pancreas

The pancreas appeared normal 2 hours after L-ornithine (3 g/kg) injection. At 4 hours, mild interstitial edema and foamy vacuolization of the acini and vascular congestion were observed. At 6 hours, the number of apoptotic bodies was greatly increased and we could also observe focal necrosis (<10%) of acini. At 9 hours, there was interstitial edema, neutrophilic and monocytic adherence, and focal inflammatory infiltration. There were great numbers of apoptotic bodies. The extent of acinar cell necrosis was 15% to 25%. At 12 hours, there was diffuse moderate infiltrate of monocytes and neutrophils, and the necrosis of acinar cells was 26% to 50%. The most severe interstitial edema was observed at 24 hours. Large numbers of neutrophils and monocytes could be observed in the interstitial space. At 48 hours, there was diffuse severe infiltrates of macrophages/monocytes, fibroblasts, and neutrophils. At 72 hours, there was no pancreatic edema, but there was a diffuse severe infiltrate of fibroblasts, macrophages/monocytes, eosinophils, and neutrophils. At 1 week, diffuse moderate infiltrates of fibroblasts and macrophages and relatively smaller number of eosinophils and neutrophils were observed. One month after injection, the pancreas appeared normal, except that part of the parenchyma was replaced by fat Overall, there were no major

pathologic alterations of the pancreatic duct cells, islets of Langerhans, and the liver in the hematoxylin and eosin sections.

Confirmation of Pancreatic Apoptosis Observed on Histologic Examination

Nine hours after L-ornithine injection we could detect a ladder pattern on agarose gel electrophoresis. On the other hand, DNA showed unspecific degradation (smear) 24 h after the administration of L-ornithine indicating severe necrosis. According to the results of the TUNEL technique, the peak of apoptosis was around 6-9 h after the injection.

Activities of Serum, Pancreatic, and Ascitic Amylase

The serum amylase activity significantly increased from 9 to 24 hours, but thereafter (at 48 hours) fell below control values. Pancreatic amylase activity was significantly decreased from 24 hours to 1 month after L-ornithine injection and was just about detectable at 72 and 168 hours. The ascites recovered from rats 24 hours after L-ornithine administration had a huge amylase activity.

Pancreatic Trypsin Activity

Pancreatic trypsin activity was significantly increased 9 to 48 hours after IP injection of 3 g/kg L-ornithine.

Pancreatic Myeloperoxidase Activity

Interestingly, inflammatory infiltration had two phases; the first one coincided with the peak of amylase activity (9–36 hours) and the second one occurred much later (at 72 hours).

Induction of Pancreatic HSP72 Synthesis

Four hours after the injection of 3 g/kg L-ornithine, the levels of HSP72 were significantly increased, peaked at 18 hours, and remained elevated until 1 month.

Degradation of Pancreatic IκB-α and IκB-β and Induction of Interleukin-1β Synthesis

Pancreatic IκB levels in response to L-ornithine injection were significantly decreased from 9 hours. IκB-α levels returned to normal by 36 hours; however, IκB-β level was significantly lower for up to 168 hours after injection. Corresponding to IκB degradation, and consequently to activation of nuclear factor-κB, pancreatic interleukin-1 β synthesis significantly increased from 9 hours.

Pancreatic Nonprotein Sulfhydryl Group Content and the Activities of Glutathione Peroxidase and Superoxide Dismutases

Nonprotein sulfhydryl group content was significantly increased at 6 hours and decreased thereafter in the ornithine-treated group compared with the control group. The activities of GSH-Px and Cu/Zn-SOD significantly increased from 24 hours. In contrast, Mn-SOD activity was significantly decreased at 24 hours and significantly increased at 48 hours versus the control. Taken together these findings suggest the presence of oxidative stress in the pancreas of rats in response to L-ornithine treatment.

Body Weight and Pancreatic Weight/Body Weight Ratio

The body weight of the rats was significantly decreased from 1 day to 1 month after the administration of 3 g/kg L-ornithine versus the PS-treated

control. Pancreatic weight/body weight ratio was significantly elevated at 18 to 48 hours and significantly decreased at 168 hours to 1 month after L-ornithine injection.

Serum Aspartate Aminotransferase Activity and Concentrations of Glucose, Calcium, Triglyceride, Urea, and Creatinine

Serum ASAT activity was significantly increased by approximately five-fold at 24 hours and three-fold at 48 hours after L-ornithine injection. Serum concentrations of glucose were significantly decreased from 24 to 72 hours and returned to normal by 1 week. Serum levels of triglyceride were only significantly effected at 72 hours. Calcium, urea, and creatinine concentrations were not significantly different versus the control.

5. Arginase Activity in Different Tissues and *In Vitro* Effect of AIHA on Arginase Activity

Arginase Activity in Different Tissues

Arginase activity was by far the highest in the liver. Nevertheless, we could also detect arginase activity in the pancreas, lung, and kidney.

In Vitro Effect of AIHA on Arginase Activity

(+)-S-2-amino-6-iodoacetamido-hexanoic acid dose-dependently inhibited liver arginase activity of rat liver homogenate and purified bovine arginase *in vitro*. Sixty μM AIHA (equimolar to an *in vivo* dose of 15 mg/kg) significantly inhibited liver arginase activity by about 25%.

6. Effect of AIHA on L-arginine-Induced Acute Pancreatitis in Rats

Pancreatic Weight/Body Weight Ratio, Serum and Pancreatic Amylase Activity, and Pancreatic Trypsin and Myeloperoxidase Activities

Pancreatic weight/body weight ratio was significantly increased in response to IP administration of 3.5 g/kg L-arginine. Pretreatment with AIHA significantly ameliorated this increase of p.w./b.w. ratio. Serum amylase activity was not significantly altered in any of the groups. Pancreatic contents of amylase were significantly decreased in the L-arginine-treated groups. Pretreatment with AIHA did not influence pancreatic amylase activity in rats injected with L-arginine. Pancreatic trypsin activity was significantly increased by L-arginine administration. Pretreatment with AIHA significantly ameliorated this increased pancreatic trypsin activity. Myeloperoxidase activity was significantly increased at 24 hours after L-arginine injection. Pretreatment with AIHA significantly decreased MPO activity in the L-arginine-induced pancreatitis group.

Pancreatic Nonprotein Sulfhydryl Group Content and the Activities of Glutathione Peroxidase and Superoxide Dismutase

Nonprotein sulfhydryl group content and GSH-Px activity were significantly increased 24 hours after the injection with L-arginine. Pretreatment with AIHA did not influence NSG content, but significantly reduced GSH-Px activity. Activities of Cu/Zn and Mn SOD were unaltered by AIHA pretreatment in the L-arginine-pancreatitis group.

Pancreatic Heat Shock Protein Expression

(+)-S-2-amino-6-iodoacetamidohexanoic acid and/or L-arginine administration resulted in up-regulation of pancreatic HSP27 and HSP72 synthesis versus the PS treated control group. No significant difference was found at

24 hours between the AIHA-treated and untreated L-arginine-induced pancreatitis groups.

Histological Examination

The administration of 3.5 g/kg L-arginine caused severe necrotizing pancreatitis. Injection of AIHA in itself resulted in pancreatic hyperemia and mild inflammatory cell infiltration. However, AIHA pretreatment significantly reduced pancreatic damage in L-arginine-induced pancreatitis.

7. Polyamine Homeostasis in L-ornithine-induced Acute Pancreatitis

Pancreas, Liver, and Lung

Pancreatic spermidine content significantly decreased 24 to 168 hours after the IP injection of 3 g/kg L-ornithine, whereas spermine content significantly increased (by 1.6-fold) only at 72 hours. Pancreatic ODC activity significantly increased at 24 hours; SSAT activity significantly increased from 24 hours and peaked at 72 hours with an almost 10-fold maximum elevation and remained significantly higher than the activity in the control group until 168 hours. Surprisingly, putrescine was not detectable in the pancreas, although putrescine accumulation should be an evident consequence of simultaneously increased activities of SSAT and ODC (if synthesis would not override catabolism).

Hepatic putrescine level showed a 26-fold elevation at 6 hours after L-ornithine injection, but thereafter fell back to control values. Hepatic spermidine level significantly increased from 6 to 72 hours, and spermine content also showed significant elevation from 24 to 72 hours.

Similarly to that observed in the liver, lung putrescine levels showed a significant peak at 6 hours after L-ornithine injection. In contrast to the changes observed in the hepatic polyamine pools, lung spermidine content showed

significantly decreased levels at 6, 72, and 168 hours. Lung spermine content was not significantly altered at the investigated time points.

8. Effect of the Synthetic Polyamine Analogue 1-methylspermidine on L-ornithine-induced Acute Pancreatitis

Pancreatic SSAT Activity, Putrescine, Spermidine and Spermine Content

1-methylspermidine accumulated in the pancreas as a result of both pretreatment and treatment. Pancreatic spermidine content significantly decreased in the L-ornithine-treated groups, whereas spermine contents did not show any alteration. Pancreatic SSAT activity significantly increased in response to L-ornithine injection by more than 4-fold at 24 hours and more than 7-fold at 48 hours. Putrescine was not present in detectable amounts in any of the groups. 1-methylspermidine administration did not affect any of these parameters.

Histological Examination

Interstitial edema, vascular congestion, leukocyte adherence and infiltration and necrosis of acinar cells greatly increased at 24 and 48 hours in response to L-ornithine injection. Apoptosis of acinar cells was detected only at 24 hours. 1-methylspermidine administration did not ameliorate any of the investigated histological parameters.

Serum and Pancreatic Amylase Activities, Serum Lipase Activity, and Pancreatic Weight/Body Weight Ratio

Serum amylase activities did not increase significantly in response to L-ornithine injection. However, MeSpd treatment in the OM24 group significantly increased serum amylase activity, and it was also significant versus the O24 group. Pancreatic contents of amylase significantly decreased in the L-ornithine-treated

groups versus the control group except in the OM24 group. Serum lipase activity significantly increased in the L-ornithine-treated groups at 24 hours versus the control group. Serum lipase activity significantly increased in the OM24 group versus the O24 group. Pancreatic weight/body weight ratio significantly increased at 24 hours in response to 3 g/kg L-ornithine. 1-methylspermidine administration did not influence p.w./b.w., either at 24 hours or at 48 hours.

Pancreatic HSP72 and IκB-α Expression

Injection of 3 g/kg L-ornithine induced pancreatic HSP72 synthesis at 24 hours. Pancreatic IκB-α levels decreased significantly 24 and 48 hours after the L-ornithine injection. 1-methylspermidine administration did not affect HSP72 and IκB-α levels.

Pancreatic Myeloperoxidase Activity, Pancreatic Interleukin-1β Levels and Serum Concentrations of Creatinine and Aspartate Aminotransferase Activity

Pancreatic MPO activity significantly increased in the L-ornithine-treated groups versus the control group. Administration of MeSpd did not influence MPO activity in any of the groups. Corresponding to IκB degradation, and consequently to activation of NF-κB, pancreatic IL-1β synthesis showed significantly elevated levels at 24 and 48 hours after the L-ornithine injection. 1-methylspermidine treatments had no significant effects on proinflammatory cytokine levels, although the analogue treatment seemed to partially prevent the elevation of IL-1β level. Significant elevations of serum creatinine concentrations were detected in the O24 and OM24 groups versus the control group. Serum ASAT activities significantly increased 24 and 48 hours after L-ornithine injection. 1-methylspermidine administration did not affect serum ASAT activity in any of the groups.

SUMMARY

This thesis characterizes a novel model of severe acute necrotizing pancreatitis induced by IP injection of 3 g/kg L-ornithine in rats showing typical laboratory and morphologic signs of that observed in the human disease. This novel pancreatitis model is noninvasive, more diffuse, and reproducible compared with those induced by retrograde ductal injection of bile acids, the closed duodenal loop method, or choline-deficient ethionine diet. L-ornithine-induced pancreatitis is superior to the L-arginine-induced model in that it produces a much severe disease with massive edema and without the confounding effects of possible excessive NO synthesis (at least in the initial phase of pancreatitis induction). Intraperitoneal administration of 4 to 6 g/kg L-ornithine killed the rats within hours (before pancreatitis could develop). The death of these animals may be the result of effects on the central nervous system.

In response to 3 g/kg L-ornithine the pancreas showed massive interstitial edema, apoptosis, and necrosis of acinar cells and infiltration of neutrophil granulocytes and monocytes. The necrotic process lacked morphologic signs indicative of lytic and ischemic damage to the cells and was evidently the result of toxic injury to the acini. The acini eventually regenerated. Acini that did not regenerate were replaced by fat tissue. The rate of pancreatic apoptosis strongly increased at 16 to 24 hours after the administration of 2.5 g/kg or 4 g/kg L-arginine.

Large doses of L-arginine may also produce a toxic effect on the pancreas, at least in part, through L-ornithine, because L-ornithine concentration in the blood was increased 54-fold after IP administration of L-arginine. Arginase is a key enzyme of the hepatic urea cycle, so it was not surprising that the highest enzyme activity was found in the liver of rats. In accordance with values reported elsewhere, arginase activity was several-order magnitudes higher in the liver

compared with other tissues (pancreas, lung, and kidney). Therefore, most likely, large doses of L-arginine are metabolized mainly by the liver.

(+)-S-2-amino-6-iodoacetamidohexanoic acid is an irreversible inhibitor of ODC and arginase and has been reported to have antifertility and antitumor effects. Pretreatment with AIHA reduced the severity of L-arginine-induced pancreatitis most likely by inhibiting arginase activity. It decreased p.w./b.w., pancreatic GSH-Px and MPO activities, and histological damage.

The involvement of polyamines in the pathogenesis of acute pancreatitis was first found in transgenic rats overexpressing SSAT. L-ornithine can serve as substrate for ODC, the initial and rate-limiting enzyme in the polyamine biosynthetic pathway. Therefore, a large dose of L-ornithine/L-arginine will inevitably influence polyamine levels. Injection of large doses of L-ornithine paradoxically induced pancreatic spermidine catabolism, possibly via activation of SSAT, after appearance of the first histological signs of acute pancreatitis. Polyamine levels generally increased in the lung and liver with the exception of lung spermidine levels, which decreased.

Synthetic α-methylated polyamine analogues, such as MeSpd, are metabolically more stable than natural polyamines as they are not substrates for SSAT and are poor substrates for spermine synthase. Nevertheless, they are supposed to fulfill most of the putative cellular functions of natural spermidine and spermine. 1-methylspermidine administration did not influence pancreatic polyamine levels and SSAT activity and failed to ameliorate the severity of L-ornithine-induced pancreatitis. Overall, these data indicate that spermidine catabolism does not take part in the initiation of L-ornithine-induced pancreatitis.

Further studies are needed to determine how basic basic amino acids induce pancreatic injury and to reveal the exact role of polyamine homeostatic processes in the maintenance of pancreatic integrity.

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