Development of a new mouse model of acute pancreatitis induced by administration of L-arginine

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Dawra R, Sharif R, Phillips P, Dudeja V, Dhaulakhandi D, Saluja AK. Development of a new mouse model of acute pancreatitis induced by administration of L-arginine. Am J Physiol Gastrointest Liver Physiol 292: G1009–G1018, 2007. First published December 14, 2006; doi:10.1152/ajpgi.00167.2006.—The pathogenesis of acute pancreatitis is not fully understood. Experimental animal models that mimic human disease are essential to better understand the pathophysiology of the disease and to evaluate potential therapeutic agents. Given that the mouse genome is known completely and that a large number of strains with various genetic deletions are available, it is advantageous to have multiple reliable mouse models of acute pancreatitis. Presently, there is only one predominant model of acute pancreatitis in mice, in which hyperstimulatory doses of cholecystokinin or its analog caerulein are administered. Therefore, the aim of this study was to develop another mouse model of acute pancreatitis. In this study, C57BL/6 mice were injected intraperitoneally with L-arginine in two doses of 4 g/kg each, 1 h apart. Serum amylase, myeloperoxidase, and histopathology were examined at varying time points after injection to assess injury to the pancreas and lung. We found that injection of L-arginine was followed by significant increases in plasma amylase and pancreatic myeloperoxidase accompanied by marked histopathological changes. The injury to the pancreas was slow to develop and peaked at 72 h. Subsequent to peak injury, the damaged areas contained collagen fibers as assessed by increased Sirius red staining. In contrast, L-arginine or other amino acids did not cause injury to the pancreas. In addition, acute inflammation in the pancreas was associated with lung injury. Our results indicate that administration of L-arginine to mice results in severe acute pancreatitis. This model should help in elucidating the pathophysiology of pancreatitis.

ACUTE PANCREATITIS IS AN INFLAMMATORY disease of the pancreas resulting in significant morbidity and mortality (13). Various causes, including gallstones, alcohol, trauma, infections, and genetic alterations, have been implicated in the causation of this disease (1, 23). Although our understanding of the cell biology of the exocrine pancreas and epidemiology of pancreatitis has increased greatly in recent years, our knowledge of its pathophysiology and the ability to prevent or treat pancreatitis remain limited (23). This can partially be attributed to the paucity of clinical material from the early stages of the disease available for research. To overcome this and to study the effect of new therapeutic agents, different experimental animal models of pancreatitis have been developed. Our present limited understanding about the events associated with the development of the disease is based on the use of these experimental models (21). Among the animals used for developing experimental models, the mouse is ideal because of the availability of several genetic manipulations, accessibility of its complete genome, and ease of maintenance. It appears that the future of experimental pancreatitis is inextricably linked to the mouse. Administration of hyperstimulatory doses of cholecystokinin or its analog caerulein results in the development of pancreatitis in mice. This model has been extensively used for studying pancreatitis (7, 18). However, in this model, a relatively mild form of the disease develops, which resolves quickly and is not accompanied by any mortality. The other model that has been used is the choline-deficient, ethionine-supplemented (CDE) diet-induced mouse model, which results in hemorrhagic pancreatitis resembling the human disease; however, this model has several limitations (use of only young female mice, variable response, and high mortality) (19). In fact, this model has been used in relatively few studies in recent years. Clearly, therefore, the need exists to develop another mouse model of acute pancreatitis to supplement the information gained from the caerulein-induced model.

Previously Mizunuma et al. (20) established that intraperitoneal administration of a high dose of L-arginine to rats results in acute necrotizing pancreatitis. However, for reasons stated above, a mouse model of acute pancreatitis is far more advantageous. There are two previous reports in which L-arginine was used for induction of pancreatitis in mice (4, 31). In these studies, the dose and protocol followed were similar to those reported for use in rats. However, despite our repeated attempts to reproduce these protocols with the same dose of L-arginine, we were unable to induce pancreatitis in either Balb/c or C57BL/6 mice. Other groups have also tried to induce pancreatitis in mice by administration of L-arginine using this protocol but did not succeed (personal communication). Therefore, the aim of this study was to develop a reproducible model of L-arginine-induced pancreatitis in mice. We have successfully developed a protocol for induction of severe acute pancreatitis in mice by intraperitoneal injection of L-arginine and report here the dose required, the method followed, and its effect on the different markers of pancreatic and associated lung injury.

MATERIALS AND METHODS

Male C57BL/6 (25–30 g) and Balb/c (18–22 g) mice were purchased from Charles River Laboratories (Wilmington, MA). All animals were housed in standard shoebox cages in a climate-controlled room with an ambient temperature of 23 ± 2°C and 12:12-h light-dark cycle. Animals were fed standard laboratory chow, given

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water ad libitum, and randomly assigned to control or experimental groups. All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School. L-Arginine hydrochloride, L-lysine hydrochloride, L-alanine, glycine, D-arginine hydrochloride, tetramethylbenzidine, and hydrogen peroxide were purchased from Sigma Aldrich (St. Louis, MO). The trypsin substrate Boc-Glu-Ala-Arg-4-methylcoumaryl-7-amide was from Peptide International (Louisville, KY). The auto-analyzer amylase assay kit was from DCL (Oxford, CT), and the microprotein assay kit was from Thermo Electron (Louisville, CO). Propidium iodide was obtained from Molecular Probes (Eugene, OR), and protease inhibitor cocktail tablets were from Roche Diagnostics (Mannheim, Germany).

**Induction of pancreatitis in mice.** A sterile solution of L-arginine hydrochloride (8%) was prepared in normal saline, and the pH was adjusted to 7.0. The sterile solution was administered intraperitoneally to nonfasted mice at a dose of 4 g/kg. Animals were returned to the cages and allowed free access to food and water. After 1 h, animals were administered a second dose of L-arginine (4 g/kg) in saline. Controls received a sham injection of saline alone. Other amino acid solutions were also prepared in normal saline, with pH adjusted to 7.0, and administered intraperitoneally in the same way as for L-arginine. Animals were killed at varying time points by CO2 asphyxia, and a blood sample and tissue were collected. Tissue samples were collected, snap frozen in liquid nitrogen, and stored at −80°C for analysis of myeloperoxidase (MPO) activity. Tissue samples for histology were fixed in 10% phosphate-buffered formalin. For induction of pancreatitis in Balb/c mice, the procedure described by Cui and Bai (4) was followed. Briefly, an L-arginine solution (20 g/l) was administered intraperitoneally at a dose of 2 g/kg twice at an interval of 1 h.

**Measurement of plasma L-arginine levels.** After L-arginine injections, animals were euthanized at different time points; blood was collected in heparinized syringes and centrifuged at 2,000 g for 5 min at 4°C. Plasma (80 μl) was then mixed (by vortexing) with 240 μl of 80% ethanol and centrifuged at 2,000 g for 20 min at 4°C. The supernatant obtained was used for amino acid analysis by reverse-phase HPLC as described by Baran et al. (3).

**Measurement of intrapancreatic trypsin activity.** After L-arginine injection, the pancreas and lung was quantitated by measuring tissue MPO activity. For these measurements, tissue samples were harvested at the time of death and were stored at −80°C. The samples were thawed and homogenized in 10 vol of 50 mM phosphate buffer (pH 7.4) containing protease inhibitors and centrifuged at 16,000 g for 15 min at 4°C. The supernatant was discarded, and the resulting pellet was washed by resuspension in phosphate buffer followed by centrifugation and removal of supernatant. The pellet was suspended in 100 mM phosphate buffer (pH 5.4) containing 0.5% hexadecylmethylammonium bromide and 10 mM EDTA. The suspension was disrupted three times by sonication and freeze-thaw cycles. The suspension was then centrifuged at 16,000 g for 10 min at 4°C, and the supernatant obtained was used for the MPO assay, using a spectrophotometric method where 3,3′,5,5′-tetramethylbenzidine was used as the substrate (24). The protein concentration in the supernatant was measured by the pyrogallol red-molybdate protein dye-binding assay (6) with propidium iodide as the substrate (24). The protein concentration in the supernatant was measured by the pyrogallol red-molybdate protein dye-binding assay (6) with propidium iodide as the substrate (24).

**Assessment of serum amylase activity.** Serum amylase activity was measured by 4,6-ethylidene(G1)-p-nitrophenyl(G2)-α-D-maltohepatoside as a substrate as described by Kruse-Jarres et al. (15). After mice had died, blood was collected in heparinized syringes and centrifuged at 3,000 g for 10 min at 4°C. After centrifugation, the plasma was aspirated and used for measurement of amylase.

**Measurement of intrapancreatic trypsin activity.** For measurement of intrapancreatic trypsin activity, pancreas samples were homogenized in cold (4°C) MOPS buffer (pH 6.5, 250 mM sucrose, 5 mM MOPS, and 1 mM MgSO4) using a motorized glass-Teflon homogenizer. The resulting homogenate was centrifuged (50 g for 5 min), and the supernatant was taken for the enzyme assay. Trypsin activity was measured by a fluorometric method using Boc-Glu-Ala-Arg-methylcoumaryl-7-amide as the substrate according to method of Kawabata et al. (11). DNA was measured in the same supernatant using propidium iodide (17). Trypsin activity was expressed per microgram of DNA.

**Histological examination.** Hematoxylin and eosin-stained sections of pancreas and lungs were evaluated microscopically by a person unaware of the treatment. The extent of injury was calculated by morphometry as described earlier (22). Pancreatic sections were stained for collagen with Sirius red as described by Junqueira et al. (10).

**Statistical analysis.** Results are expressed as means ± SE. Statistical analysis was done by applying unpaired two-tailed Student’s t-test with significance being assigned to P values <0.05.

**RESULTS**

**Administration of L-arginine.** In our initial experiments using the same strain (Balb/c), weight, conditions, and dose of L-arginine (2 g/kg × 2 ip injections) as described by Cui and Bai (4), we did not observe any change in serum amylase or histopathological changes in the pancreas (Fig. 1). After these observations, our studies involved the administration of different doses of L-arginine to mice. Two injections of each dose (1, 2, 3, or 4 g/kg) were given via intraperitoneal injection 1 h apart. The results demonstrate that L-arginine had no effect on serum amylase (Fig. 2A), pancreatic (Fig. 2B) and lung MPO (Fig. 2D), and pancreatic histology (Fig. 2C) when administered at the lower doses (1, 2, or 3 g/kg × 2). In contrast, the higher dose (4 g/kg × 2) caused significant pancreatic injury. Therefore, all future experiments were performed with 4 g/kg of L-arginine administered twice, 1 h apart.

After administration of the first dose of L-arginine (4 g/kg) in saline, mice were sluggish and their lethargy increased with administration of the second dose; also, they had an ungroomed look. This phase lasted for 1–4 h after the second injection, after which they recovered and gradually became active again. With regard to the condition of the mice, we observed that, 72 h after administration of L-arginine, the mice were relatively less active compared with control mice. These changes were not observed in the control group of mice, which were injected with comparable doses of saline alone. However, this effect was not L-arginine specific and was observed with other amino acids as well. With glycine, the effect was more pronounced after the first dose; therefore, the second dose was not administered.

To assess the pharmacokinetics of L-arginine using the injection protocol described (i.e., two injections of 4 g/kg, 1 h apart), plasma concentrations of L-arginine were determined at different time points (Table 1). Our data show that, after a single injection, the blood concentration of L-arginine started to decrease after 60 min, suggesting that the half-life of L-arginine is between 1 and 2 h in mice. However, after administration of a second dose of L-arginine after 1 h, the blood levels 1) increased over and above those obtained with a single injection and 2) remained elevated until 3 h compared with a single injection of L-arginine.

**Plasma amylase in response to L-arginine.** Elevated plasma amylase is an important marker of pancreatic acinar cell injury. Administration of L-arginine resulted in significantly increased plasma amylase levels both after 48 and 72 h compared with results shown in controls (Fig. 3A). Plasma amylase levels returned to normal at 96 and 120 h. To test whether this effect
was specifically because of L-arginine, we also examined plasma amylase levels in response to administration of D-arginine, L-lysine, L-alanine, and glycine (Fig. 3B). It was seen that, 72 h after administration (the time point at which L-arginine resulted in maximal serum amylase), these amino acids had no effect on plasma amylase.

*MPO activity in response to L-arginine.* Development of acute pancreatitis is accompanied by sequestration of neutrophils in the pancreas. Measurement of MPO activity in tissue has been used as a biochemical marker of neutrophil infiltration. Mice administered L-arginine had significantly increased pancreatic MPO activity after 48 h (Fig. 4) compared with the control group (saline alone). MPO activity was maximal at 72 h after administration of L-arginine, consistent with the time point at which serum amylase peaked.

*Histopathological changes in response to L-arginine.* Histopathological evaluation of the hematoxylin and eosin-stained sections of pancreas from the L-arginine administered group revealed significant accumulation of fluid, disruption of histoarchitecture, acinar cell vacuolization, extensive acinar cells necrosis, and neutrophil infiltration (Fig. 5, A and B). In contrast, no histological changes were observed in the control group (administered saline alone).

Histopathological changes were detectable in pancreatic acinar cells as early as 6 h after administration of L-arginine. Early changes included the appearance of vacuoles in acinar cells, which increased in number; with passage of time, the number of cells affected gradually increased. This was followed by the disappearance of zymogen granules from some areas after 24 h of L-arginine injection (Fig. 5C). These histopathological changes observed in pancreatic acinar cells concur with the time point at which both plasma amylase and pancreatic MPO activities started to increase. Seventy-two hours after L-arginine administration, there was accumulation of interstitial fluid, appearance of inflammatory cells, and necrosis of acinar cells. At 96 and 120 h (Fig. 5A), only small areas of intact acinar cells were visible, and remaining areas were replaced by inflammatory and fibrotic cells. Pancreatic islet cells remained unaffected by L-arginine treatment. In addition, pancreatic acinar cells were not affected in response to treatment with D-arginine, L-lysine, L-alanine, or glycine (Fig. 6).

*Activation of trypsin after L-arginine administration.* We studied the activation of trypsin in the pancreas after administration of L-arginine. A significant increase in trypsin was observed at 24 h, after which trypsin activity continued to increase until 120 h (Fig. 7).

*Increased collagen staining in response to L-arginine.* In pancreatic tissue, we studied the appearance of collagen fibers in response to L-arginine administration at different time points using Sirius red staining. At 96 and 120 h, the damaged areas became positive for collagen (Fig. 8). Before 96 h, Sirius red positivity was limited to duct or vessel walls.

*Effect of L-arginine administration on lung injury.* Acute pancreatitis is also accompanied by lung injury. Therefore, to study whether the acute pancreatitis in response to L-arginine administration in mice is associated with secondary lung injury, we studied their lungs for histopathology and MPO activity. There was significantly increased MPO activity in lung tissue from animals in the arginine administered group (Fig. 9). Histopathological observations on lung tissue revealed alveolar thickening and inflammatory cell infiltration (predominantly neutrophils). There were hemorrhages in some areas (Fig. 10).

**DISCUSSION**

This study developed and characterized a new and highly reproducible mouse model of acute pancreatitis that uses a high
The administration of L-arginine (4 g/kg × 2) resulted in significantly increased plasma amylase, pancreatic MPO activity, trypsin activation, and histological changes (including accumulation of fluid, disruption of histoarchitecture, acinar cell vacuolization, extensive acinar cell necrosis, and neutrophilic infiltration) resembling acute pancreatitis in humans.

In contrast to the earlier reports (4, 31) with L-arginine (2 g/kg × 2) for induction of pancreatitis in Balb/c mice, our results demonstrate that administration of L-arginine at lower doses (1–3 g/kg × 2) does not cause pancreatic injury. Our data clearly indicate that a higher dose of L-arginine (4 g/kg × 2) is required for induction of pancreatitis and that lower doses are ineffective. We are unable to provide a clear explanation as
to the reason why our laboratory and others have not been able to reproduce the model described by Cui and Bai (4). It is noteworthy that we attempted to mimic the previously described model as closely as possible and used the same strain, the same age of mice, and the same supplier of L-arginine. In our experience and based on the dose requirement of L-arginine for induction of pancreatitis in mice, it is extremely important that both injections of L-arginine are administered accurately for delivery of the precise dose. Thus the right dose per kilogram of animal is critical for the development of pancreatitis in this model, and an inaccuracy at any level may lead to no pancreatitis.

Hyperstimulation with caerulein in mice induces relatively mild edematous pancreatitis (7, 18). This model is quite reproducible and has been used in many laboratories for understanding the pathophysiology associated with pancreatitis. Although many of the pathological changes observed during caerulein-induced pancreatitis are similar to those observed clinically, this model differs from human disease in that it does not develop into the severe form of the disease (7). In contrast, the CDE diet-induced model of pancreatitis results in a severe hemorrhagic pancreatitis that has some resemblance to human disease but has several limitations restricting its usefulness (19). Only young, female mice can be used for the CDE diet-induced pancreatitis, and most of the mice die within a few days, most likely because of injury to organs other than the pancreas. It is difficult to control the amount of CDE diet consumed by individual mice, and this results in large differences in the extent of injury and mortality in CDE diet-fed mice. Because of these limitations, the CDE diet-induced model of pancreatitis is used very rarely; there have been very few studies in the past 5 years on this model, and it is falling into disuse. Therefore, it can be appreciated that a genuine need exists for another mouse model of pancreatitis that is not strain or gender specific so that we may gain a greater understanding of the pathophysiology of pancreatitis and study the effect of therapeutic agents. Multiple models in the same species can complement the information generated by one model and also help in eliminating the model-specific effects when studying the effect of potential therapeutic agents. As already discussed, the mouse is the desired species for this purpose owing to the availability of transgenic and gene knockout animals.

Administration of high doses of L-arginine is known to cause acute pancreatitis in rats (26), and this has been used by a limited number of laboratories as a model for studying pancreatitis. It has not been fully characterized, and the mechanism

<table>
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<th>Time, min</th>
<th>Plasma L-Arginine, µmol/ml After First Injection of L-arginine (4 g/kg)</th>
<th>Plasma L-Arginine, µmol/ml After Second Injection of L-arginine (4 g/kg)</th>
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<tr>
<td>0</td>
<td>0.27±0.02</td>
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<tr>
<td>60</td>
<td>2.98±0.39</td>
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<td>120</td>
<td>1.48±0.28</td>
<td>4.28±1.34*</td>
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<tr>
<td>180</td>
<td>0.65±0.07</td>
<td>5.52±1.29*</td>
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Values are means ± SE; n ≥ 4. *P < 0.05.
Fig. 5. A: histopathological changes in response to L-arginine administration on mouse pancreas at different time points. Representative H&E-stained micrographs (×20 objective) from control (Con) and L-arginine-treated animals for each time point are presented. B: pancreatic necrosis was significantly increased at all time points in L-arginine-induced pancreatitis (n ≥ 6 for each time point; *P < 0.05). C: percent degranulation (measured as areas without zymogen granules) was significantly increased at early time points after administration of L-arginine (n ≥ 6 for each time point; *P < 0.05).
of L-arginine-induced pancreatitis is still not clear. Therefore, in the present study, we examined whether intraperitoneal administration of L-arginine in mice induces pancreatitis. Administration of the doses used in rat (300–500 mg/100 g) did not cause pancreatic injury. Increasing the dose further caused high mortality in mice. To overcome this problem, we modified the protocol as described in MATERIALS AND METHODS, which enabled us to increase the dose of L-arginine for inducing pancreatitis without causing significant mortality. Administration of two doses of L-arginine (4 g/kg) as 8% solution in normal saline (pH adjusted to 7.0) 1 h apart induced pancreatitis. The changes related to pancreatitis started appearing early, developed gradually, and became pronounced at ~72 h. Therefore, 72 h was chosen as the time point for studying changes in the tissues.

We have shown that L-arginine administration significantly increased plasma amylase compared with the control group, which is indicative of injury to pancreatic acinar cells. Serum amylase and lipase have been used as markers of pancreatitis because their raised levels in patients are highly suggestive of development of pancreatitis. Our observations with amylase were further supported by significantly increased MPO activity in pancreatic tissue from the L-arginine group and histopathological changes. These consisted of accumulation of fluid around acini, vacuolization, disruption of histoarchitecture, and marked necrosis of acinar cells. The extent of necrosis was significantly greater in the L-arginine model than that observed in caerulein-induced pancreatitis. In addition, L-arginine administration resulted in significant infiltration of inflammatory cells, with the majority being neutrophils. Increased MPO activity in the tissue correlates with this observation.

Given that we observed significant pancreatic injury in mice administered L-arginine, we next evaluated its effect on pancreatitis-associated lung injury. We observed significantly increased MPO activity in lung tissue from these animals, which is indicative of infiltration of neutrophils. Lung histopathology showed significant thickening of alveoli, hemorrhages, and infiltration of inflammatory cells. L-Arginine is a potential precursor in the formation of nitric oxide (NO), and this raises the question of whether the injury observed in the lung is due to pancreatitis or results from a direct effect of NO on the lungs. From the dose-response studies, it appears that the dose that causes pancreatitis also causes lung injury; however, from
the data, the possibility of a direct involvement of NO in lung injury cannot be ruled out entirely.

We performed a time course study of pancreatic injury after L-arginine administration by measuring plasma amylase. We observed slightly increased plasma amylase at 24 h, which was followed by significantly increased activity at 48 h and a continued increase up to 72 h. Plasma amylase returned to normal at 96 and 120 h. The decrease in plasma amylase at later time points could be due to significant loss of acinar cells. These results indicate that injury to the pancreas starts quite early after administration of L-arginine but develops gradually and becomes highly pronounced at ~72 h. There was also significantly increased trypsin activity in the pancreas at 24 h, suggesting that high doses of L-arginine cause injury within the acinar cells. In our study, trypsin activity continued increasing up until 120 h. This observation is in contrast to the transient pattern of trypsinogen activation in the caerulein-induced model of pancreatitis. The prolonged increase in trypsin activity observed in this L-arginine-induced model might contribute to the severity of the injury observed in this model.

To rule out the possibility that the pancreatic injury that we observed was specific to L-arginine and was not just a general phenomenon common to other amino acids, we tested the effects of glycine, L-lysine, and L-alanine. Our results clearly demonstrate that these other amino acids do not induce any pancreatic injury, suggesting that the pancreatic injury reported here is specific to L-arginine administration.

An imbalance of amino acids has been suggested as one of the mechanisms by which administration of L-arginine can cause pancreatitis (29). However, our results indicating that administration of a similar concentration of other amino acids, which presumably causes a similar level of amino acid imbalance but fails to induce any pancreatic injury, suggest that the pancreatic injury reported here is specific to L-arginine administration.

An imbalance of amino acids has been suggested as one of the mechanisms by which administration of L-arginine can cause pancreatitis (29). However, our results indicating that administration of a similar concentration of other amino acids, which presumably causes a similar level of amino acid imbalance but fails to induce pancreatitis, refute this possibility. The fact that administration of D-arginine (which does not get metabolized) also did not result in the development of pancreatitis suggests that one of the specific metabolites of L-arginine contributes to causation of the injury observed during development of pancreatitis.

The mechanism by which L-arginine induces pancreatitis in rodents is unclear. However, in the rat model of L-arginine-

Fig. 8. Effect of L-arginine administration on collagen staining in mouse pancreas. Sirius red was used to stain collagen. Representative micrographs stained with Sirius red for collagen (×20 objective) are shown. Note that, at the earlier time points, only the duct walls are positive; however, at 96 and 120 h, Sirius red-positive areas have appeared in parenchyma, indicating the presence of collagen fibers.

Fig. 9. Effect of L-arginine administration on lung injury as assessed by MPO activity at 72 h. There was significantly increased MPO activity in lung tissue from animals in the L-arginine-treated group (n ≥ 6 for each time point; *P < 0.05).
induced pancreatitis, cytoskeletal changes (27), the involvement of oxygen free radicals, NO, and inflammatory mediators (9) have all been suggested to play roles in the induction of pancreatitis. These potential mediators of L-arginine-induced pancreatitis have only been studied at later time points, thereby making it difficult to link these mediators with the initial events. In view of this, it is important to identify the initial early events, which are responsible for triggering the progression of pancreatitis. More recently, endoplasmic reticulum stress (16) has been suggested to be a mediator of L-arginine-induced pancreatitis in rats. Kubisch et al. (16) observed that endoplasmic reticulum stress occurs quite early (4 h), but how administration of high doses of L-arginine causes this stress is not clear.

Although, as mentioned above, the exact mechanism by which L-arginine induces pancreatitis in rodents remains to be fully elucidated, below we discuss a possibility that needs to be addressed in future studies. Given that our data suggest that it is the metabolite of L-arginine that induces pancreatitis in rodents, we believe that one of the enzymes that metabolizes L-arginine might play a role in the initiation of pancreatitis. One such enzyme is nitric oxide synthase (NOS), which is present in acinar cells (5). Induction of inducible NOS has been reported during the late phase of pancreatitis in rats using L-arginine (25). NOS is also capable of producing superoxide radicals in high amounts under certain conditions (2, 28). Interaction of NO and superoxide radicals can generate peroxynitrite radicals, which are capable of modifying proteins that can directly lead to cell injury. In support of the above, there is experimental evidence to indicate that, in endothelial cells in the presence of increased extracellular concentrations of L-arginine, NOS results in increased production of NO (30).

Furthermore, neuronal and vascular endothelial cells (12, 14), which are present in close proximity to pancreatic acinar cells, can also contribute to the increased levels of reactive oxygen and nitrogen species. Further support for our hypothesis that nitrosative stress is a possible mediator for L-arginine-induced pancreatitis comes from our data indicating that similar concentrations of D-arginine (stereoisomer for L-arginine) and other amino acids, which are not substrates for NOS, do not cause pancreatic damage. Moreover, recent studies show that administration of antioxidants to rats given L-arginine decreases pancreatic injury (8).

Mizunuma et al. (20) studied the effect of intraperitoneal administration of a high dose of L-arginine (500 mg/100 g) in rats. They observed slight vacuolar degeneration in liver cells but no changes in the weight of the liver, kidney, spleen, and thymus. Arginine does not appear to affect any organs other than the pancreas (29). This could be due to the relatively high turnover of proteins in pancreatic acinar cells, which causes them to import large quantities of amino acids. This might result in accumulation of a high concentration of L-arginine. We have not directly assessed injury to other organs, but organs other than pancreas and lungs appeared normal macroscopically.

Damage to the pancreas in L-arginine-injected (5 g/kg body wt ip) rats was confined to the exocrine pancreas, with no effect on the islets of Langerhans (20). Our histopathological studies similarly showed intact islet cells, with pancreatic injury restricted to the exocrine pancreas. Another interesting observation in this study was the appearance of collagen fibers in the damaged areas of pancreatic tissue. Whether these changes are permanent or part of the repair process requires further investigation.
To conclude, high doses of l-arginine, using the protocol described, induced acute pancreatitis in mice, and this was associated with injury to the lungs. Injury to the pancreas developed gradually. The success of events associated with the development of pancreatitis appears to be spread over a longer time span in this model, which might be highly advantageous in dissecting out the events related to the pathophysiology of pancreatitis. This is a relatively easy model of pancreatitis. When fully characterized, this model holds the potential of generating information to afford a complete understanding of the molecular mechanisms responsible for the development and progression of acute pancreatitis.

GRANTS

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REFERENCES


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