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Continuous calcium channel blocker infusion in experimentally induced acute pancreatitis: effects on pancreas and liver function.

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Abstract

In this study we examined the effects of continuous calcium channel blocker (CCB) infusion on pancreatic duct-ligated acute pancreatitis (AP) in rabbits. Thirty rabbits were used for this study. Animals in group 1 (n = 10), which served as a control group, underwent dummy operations and received 0.5 microliter/h normal saline via the internal jugular vein. Animals in group 2 (n = 10) with artificially-induced pancreatitis received the same dosage of saline in the same manner. Animals in group 3 (n = 10) with artificially-induced pancreatitis received 180 micrograms/kg/h CCB (Verapamil) via the jugular vein starting from just before pancreatic duct ligation. AP histology score, plasma amylase levels and liver function tests were measured after 48 h. Verapamil infusion did not prevent the rise in plasma amylase levels, nor did it prevent pancreatic inflammation and damage. Serum levels of serum glutamate pyruvate transaminase, serum glutamate oxalacetate transaminase and alkaline phosphatase were significantly elevated in group 2 and significant reductions were seen in the Verapamil treated animals (group 3). The findings in this study imply that a continuous 180 micrograms/kg/h dose Verapamil infusion does not ameliorate the pathogenesis of pancreatitis induced by ligation of pancreatic duct but do not rule out a dose-dependent protective effect. Meanwhile, the lowering of liver function test scores should be considered the beneficial effect of CCBs, and this should be investigated in further studies.

KEYWORDS: acute pancreatitis, duct ligation, calcium channel blocker, liver function test

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Continuous Calcium Channel Blocker Infusion in Experimentally Induced Acute Pancreatitis: Effects on Pancreas and Liver Function

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In this study we examined the effects of continuous calcium channel blocker (CCB) infusion on pancreatic duct-ligated acute pancreatitis (AP) in rabbits. Thirty rabbits were used for this study. Animals in group 1 (n = 10), which served as a control group, underwent dummy operations and received 0.5 μl/h normal saline via the internal jugular vein. Animals in group 2 (n = 10) with artificially-induced pancreatitis received the same dosage of saline in the same manner. Animals in group 3 (n = 10) with artificially-induced pancreatitis received 180 μg/kg/h CCB (Verapamil) via the jugular vein starting from just before pancreatic duct ligation. AP histology score, plasma amylase levels and liver function tests were measured after 48 h. Verapamil infusion did not prevent the rise in plasma amylase levels, nor did it prevent pancreatic inflammation and damage. Serum levels of serum glutamate pyruvate transaminase, serum glutamate oxalacetate transaminase and alkaline phosphatase were significantly elevated in group 2 and significant reductions were seen in the Verapamil treated animals (group 3). The findings in this study imply that a continuous 180 μg/kg/h dose Verapamil infusion does not ameliorate the pathogenesis of pancreatitis induced by ligation of pancreatic duct but do not rule out a dose-dependent protective effect. Meanwhile, the lowering of liver function test scores should be considered the beneficial effect of CCBs, and this should be investigated in further studies.

Key words: acute pancreatitis, duct ligation, calcium channel blocker, liver function test

Although acute pancreatitis (AP) is a severe disease responsible for more than 1% of all hospital admissions (1, 2) resulting in 10-20% of overall mortality, no therapeutic modality has been proven effective in its treatment. Unfortunately, despite the numerous clinical and experimental studies, clinical therapy has not changed and the current therapy is supportive and nonspecific (2, 3). Recently, intracellular calcium influx and the subsequent mediator release are considered the key factors in the development of AP (1, 2, 4-6). Based on this hypothesis, calcium channel blockage has been considered the best means of treating and protecting against AP.

In previous experimental studies, authors chose to induce AP by diet, or intraductal administration of sodium taurodeoxycholate, and treated the animals by calcium channel blockers (CCBs). We have not seen any studies which evaluated the effects of a continuous CCB (Verapamil) infusion on AP artificially induced by pancreatic duct ligation.

Materials and Methods

Thirty adult New Zealand rabbits, weighing between 1,500-2,500 g were used. Animals were anesthetized by an injection of 20 mg/kg thiopenthone sodium via the ear vein. The rabbits were operated on under aseptic conditions. A small incision was made in the neck and the internal jugular vein was freed from the surrounding tissue and canullated with a polyethylene catheter. The catheter was tunneled posteriorly and connected to a miniosmotic pump (ALZET miniosmatic pump, ALZA corporation, Palo Alto, CA, USA) which was placed subcutaneously. The pumps used have a flow moderator for continuous infusion at a rate of 0.5 μl/h. After making an abdominal

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Table I Acute pancreatitis histology score[a]

Microscopic parameters	Highest score
Edema	1
Acinar destruction	2
Acinar vacuolization	2
Neutrophil infiltration	4

[a]: Reference (7).

incision, the pancreatic duct was found and ligated with two silk sutures to induce AP, after which the abdomen was closed in two layers. The animals were allowed water and standard food when they awoke.

Animals were divided into three groups as follows: Animals in group 1 (n = 10), which served as a control group, underwent dummy operations in which laparotomy was performed and their pancreatic ducts were palpated but not ligated. Prior to surgery, these animals also underwent placement of miniosmotic pumps primed to infuse saline intravenously (iv) at 0.5 μl/h before the dummy operations. Animals in group 2 (n = 10) were operated on the same manner except that their pancreatic ducts were actually sutured to induce AP. They also underwent placement of miniosmotic pumps prior to surgery to deliver saline iv at 0.5 μl/h. Animals in group 3 (n = 10) were operated on in the same manner as in group 2 and underwent placement of miniosmotic pumps which delivered a continuous flow of CCB (Verapamil) at a rate of 180 μg/kg/h as described previously (1). Verapamil was a gift from Knoll Pharmaceutical Inc, Istanbul, Turkey.

Animals surviving at 48 h after the initial operation were sacrificed with an air embolozation via ear veins. Blood samples were obtained 48 h after pancreatitis induction. All blood samples were centrifuged at 3,000 rpm for 10 min immediately after collection.

After the animals were killed, their pancreases were harvested and fixed in 10 % formaldehyde, processed in paraffin, and stained with hematoxylin-eosin. One pathologist in a blind fashion performed the microscopic examinations. The severity of pancreatitis was graded as described previously (7) (Table 1).

Results are expressed as mean ± standard error of mean. SPSS 7.5 for Windows was used in the statistical analysis and comparisons between the groups were made using the analysis of variance (ANOVA) test after determination of homogeneity variances. Significance was accepted when $P < 0.05$.

Results

No animals died during the study. Group 1 animals which underwent dummy operations showed no signs of AP. Animals in groups 2 and 3, which underwent ligation of the pancreatic ducts showed symptoms of AP with significantly elevated serum levels of amylase, and elevated pancreatic histology scores ($P < 0.05$; Table 2). Elevated serum glutamate oxalacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), serum levels of bilirubin and lactate dehydrogenase (LDH) were also observed in group 2. Continuous infusion of Verapamil did not prevent the advancement of acute induced pancreatitis although histology scores examined 48 h after pancreatitis induction were slightly reduced, averaging 5.4 ± 0.7 ($P > 0.05$). Continuous infusion of Verapamil did not result in any significant changes in serum amylase and calcium levels in animals suffering from AP ($P > 0.05$). Serum levels of SGOT, SGPT and ALP were significantly reduced in animals which received a continuous of Verapamil (group 3), as compared with pancreatitis animals which received only saline (group 2) ($P < 0.05$; Table 2). Animals treated with Verapamil showed reduced serum levels of bilirubin and LDH, but this was not statistically significant in comparison to group 2.

Discussion

Although other authors have chosen to induce AP by such means as chronic ethanol administration (8), supramaximal stimulation with cerulein (3, 9), changes in diet (2), ligation the bile duct at its entrance the duodenum (10), and intraductal administration of sodium taurodeoxycholate (3, 11), we decided upon the model of AP induced by ligation of the pancreatic duct. This model allowed us to evaluate the effects of CCBs on diffuse, homogeneous, and severe, but not lethal, AP. Other experimental models, such as intraductal injection, it causes rapid severe pancreatitis with high mortality rate (12), and pancreatic injury, in this model, is not homogeneous of the gland (12-14). In contrast, AP induced by a diet rich in ethionine and deficient in choline produces severe lethal pancreatitis (15). In the present study, ligation of the pancreatic duct produced significantly elevated serum amylase levels and pancreatic histology scores in both groups 2 and 3, and there was no mortality.

Table 2 Biochemical and histological assessment in induced acute pancreatitis

	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)
Plasma amylase (U/L)	379 ± 47	1760 ± 340*	1644 ± 408**
Histology score	1.0 ± 0	6.5 ± 0.9*	5.4 ± 0.7**
Calcium (mmol/L)	3.3 ± 0.1	3.2 ± 0.2	3.1 ± 0.3
SGOT (U/L)	55.7 ± 12.7	166.0 ± 24.1*	68.2 ± 15.2***
SGPT (U/L)	69.1 ± 11.6	224.0 ± 75.0*	62.5 ± 15.2***
ALP (U/L)	35.5 ± 1.2	101.3 ± 14.5*	53.6 ± 26.0***
Direct bilirubin (μmol/L)	9.9 ± 3.5	11.6 ± 4.0	6.8 ± 1.2
Total bilirubin (μmol/L)	18.2 ± 5.7	17.8 ± 4.6	12.5 ± 2.2
LDH (IU/L)	331.1 ± 65.2	490.5 ± 126.9	329.2 ± 55.6
Albumin (g/L)	35.8 ± 1.2	36.0 ± 1.1	36.6 ± 0.8
Globulin (g/L)	32.1 ± 4.3	29.1 ± 3.0	22.2 ± 2.1

SGOT: Serum glutamate oxalacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase.
Group 1: Controls, received normal saline, 0.5 μl/h.
Group 2: Acute pancreatitis animals received normal saline, 0.5 μl/h.
Group 3: Acute pancreatitis animals received continuous Verapamil 180 μg/kg/h.
*$P < 0.05$ group 1 *vs* group 2; **$P < 0.05$ group 1 *vs* group 3; ***$P < 0.05$ group 2 *vs* group 3.

The protective effect of Verapamil in experimentally induced AP has been described in other animal models. It has been emphasized that Verapamil administration prevents the increases of TxB2 and 6-keto-PGF1α (3, 4), reduces pancreatic activity of trypsin and chymotrypsin (2), increases pancreatic blood flow and tissue perfusion (11), and inhibits TNFα (1). Additionally, it has been suggested that Verapamil might lessen the loss of zymogen granule membrane and electron density of the pancreatic tissue (2). In these studies, authors noted the protective effects of CCBs, some even reported better survival rates and times. However, in the present study serum amylase levels did not decrease significantly in the Verapamil treated animals. Some authors have suggested that serum amylase levels should not be considered a prognostic sign and do not correlate with the severity of pancreatic injury (16). Despite certain limitations, serum amylase level remains central to the diagnosis of AP (17). It is clear that any therapy to treat or cure pancreatic injury must have the effect of lessening or eliminating increases in serum amylase levels.

Liver function tests are commonly used parameters for the prognosis of AP (16). In the present study, Verapamil treatment significantly reduced serum levels of SGOT, SGPT and ALP. In addition, there were non-statistically significant reductions in serum levels of bilirubin and LDH. The pathogenesis of liver injury in induced-AP remains obscure. It may be that systemic circulatory failure and hypovolemia result in the release of free radicals of oxygen following an ischemic compromise caused by increased capillary volume and congestion and stasis of microcirculation (11). The liberation of calcium ions from the intracellular micro-organelles to cytosol following ischemic compromise, and free calcium ions which activate intracellular enzymes such as phospholipases, are also important causes of cellular injury (18, 19). Verapamil maintains intracellular calcium homeostasis and has been shown to attenuate ischemia and reperfusion injury of liver (20, 21).

It has been speculated that calcium influx plays a critical role in induced pancreatitis and that intracellular calcium is the initial signaling molecule responsible for the onset and development of AP. In our study, the histological results indicated that the microscopic signs of pancreatic damage such as edema, acinar vacuolization, acinar destruction and neutrophil infiltration did not statistically change with continuous verapamil infusion in animals with induced-AP. Therefore, if calcium influx plays a critical role in induced pancreatitis, CCBs should have prevented the development of pancreatic inflammation and damage.

In conclusion, the findings of this study indicate that a continuous flow of CCBs (Verapamil) at a rate of 180 μg/kg/h does not ameliorate the pathogenesis of pancreatitis induced by ligation of pancreatic duct. However, this does not rule out the possibility that different dosages of CCBs may have a beneficial effect. The improvement of liver function test scores may be the beneficial effect of

CCBs in pancreatitis-induced liver injuries, and this needs to be examined in further studies.

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