

Dopamine in models of alcoholic acute pancreatitis

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Abstract

Acute oedematous pancreatitis and acute haemorrhagic pancreatitis were studied using the low pressure duct perfusion models of alcoholic pancreatitis in cats. After creating either form over 24 hours, each pancreas was histologically graded and assigned an inflammatory score (0-16; absent-severe). Urinary trypsinogen activation peptide concentrations were also used as a measure of severity. Using the model of acute haemorrhagic pancreatitis, it was previously shown that low dose dopamine (5 µg/kg.m) reduced the inflammatory score at 24 hours and that this effect was mediated by a reduction in pancreatic microvascular permeability acting via dopaminergic and beta adrenergic receptors. Further studies were conducted and are reported here. In experiment 1 different doses of dopamine in established alcoholic acute haemorrhagic pancreatitis were studied. In group 1 control cats (no dopamine), the inflammatory score was 10.5 (interquartile range (IQR) 4). In groups 2, 3, and 4, haemorrhagic pancreatitis was induced. Twelve hours later dopamine was infused for six hours, in the doses of 2 µg/kg.min, 5 µg/kg.min, and 50 µg/kg.min respectively. The inflammatory score in group 2 was 7 (IQR 0.5, $p < 0.05$ v group 1), in group 3 it was 7 (IQR 2, $p < 0.05$ v group 1), and in group 4 it was 7 (IQR 4, $p < 0.05$ v group 1). This was matched by significantly lower levels of urinary trypsinogen activation peptide at 24 hours. In experiment 2 (group 5) we tried to reduce microvascular permeability further by combining dopamine with antihistamines, but there was no improvement in the inflammatory score. As oedematous pancreatitis is the commoner and milder form of acute pancreatitis in clinical practice, in experiment 3 we looked at the effect of dopamine in this model. In group 6 control cats (no treatment), the inflammatory score was 7 (IQR 3, $p < 0.05$ v group 1). In group 7 cats given dopamine (5 µg/kg.min for six hours) from 12 hours after the onset of acute oedematous pancreatitis, the inflammatory score was reduced to 4 (IQR 2, $p < 0.05$ v group 6). This was matched by a significant reduction in the 24 hour urinary trypsin activation peptide concentration.

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In the low pressure duct perfusion model of acute pancreatitis in cats,¹⁻⁴ intragastric alcohol renders the main pancreatic duct permeable to pancreatic enzymes. Perfusion of a permeable duct with activated pancreatic juice causes acute oedematous pancreatitis (AOP). If 16-16 dimethyl prostaglandin E₂ is also infused intravenously, AOP becomes acute haemorrhagic pancreatitis (AHP). We previously showed that

dopamine reduced the severity of AHP when administered within 12 hours of the induction of this disorder.⁵ This effect was due to a reduction in microvascular permeability mediated by the stimulation of either dopaminergic or beta-adrenergic receptors.^{6,7}

Here, in a further progression of these experiments, we analysed three more issues. In experiment 1 we examined the effect of varying the dose of dopamine in the original model of alcoholic AHP. In experiment 2 we investigated whether the addition of the H₁/H₂ blockers, diphenhydramine and cimetidine, might have a synergistic effect if combined with dopamine as these latter agents would be expected to reduce microvascular permeability by an action through different receptors. In experiment 3 we investigated the effect of dopamine in the model of alcoholic AOP because in humans alcohol often leads to the milder oedematous form of acute pancreatitis.

Methods

THE MODELS

The models have been described in detail previously¹⁻⁴ and are considered briefly here.

Alcoholic AOP

Fasted cats were anaesthetised and after drawing samples for estimating basal serum lipase and urinary trypsinogen activation peptide (TAP), a laparotomy was performed. The body of the stomach was cannulated with the tip of the cannula sited in the antrum. The main pancreatic duct was cannulated in the tail of the pancreas with plastic tubing that had previously been filled with a physiological salt solution (standard per-

TABLE 1 The inflammatory scoring system for acute pancreatitis

Architecture
0 - Normal.
1 - Periductal parenchymal destruction.
2 - Focal parenchymal necrosis away from ducts.
3 - Diffuse parenchymal loss in less than 50% of lobules.
4 - Diffuse parenchymal loss in greater than 50% of lobules.
Inflammatory cell infiltrate
0 - None.
1 - In ducts/around ductal margins/scattered polymorphs.
2 - In the parenchyma in less than 50% of lobules.
3 - In the parenchyma in more than 50% of lobules.
4 - Massive collections/abscesses.
Haemorrhage
0 - None.
1 - Blood in the parenchyma in less than 25% of lobules.
2 - Blood in the parenchyma in 25%-50% of lobules.
3 - Blood in the parenchyma in 50%-75% of lobules.
4 - Blood in the lobules.
Oedema
0 - Normal.
1 - Focally increased between lobules.
2 - Diffusely increased between lobules.
3 - Widely separated lobules/tense acini.
4 - Gross lobular separation/cobblestoning.

fusate) and connected to a constant rate perfusion pump. Twenty ml of 40% ethanol were slowly instilled through the gastric cannula over 10 minutes, and the main pancreatic duct was perfused for one hour with standard perfusate at 0.5 ml/h. At this rate of perfusion, the pressure within the duct never exceeded 10 mm Hg (the maximum secretory pressure of the cat pancreas is less than 20 mm Hg⁸). Then the main pancreatic duct was perfused with activated pancreatic juice for one hour. This juice had previously been collected from donor cats, standardised, and activated just before administration. After two hours when these perfusions were over, the cannula was cut, then ligated, and the abdomen was closed. Anaesthesia was maintained for 24 hours when serum lipase and urinary trypsinogen activation peptide were remeasured. The pancreas was then examined, sections taken for histological analysis, and the cat was killed.

Alcoholic AHP

Alcoholic AHP was induced as follows. Mongrel cats were prepared as above, but in addition, but in addition, at the main pancreatic duct perfusion was started with standard perfusate, an intravenous infusion of 16–16 dimethyl prostaglandin E₂ (PGE₂) in saline (2 µg/kg.hr; 2 ml/h for two hours) was also started to convert AOP to AHP. This infusion was complete at the end of the two main pancreatic duct perfusions. Anaesthesia was maintained for 24 hours, after which serum lipase and urinary trypsinogen activation peptide were measured, the pancreas was examined, and the cat killed.

ASSESSMENT OF PANCREATIC DAMAGE

The effects of treatment with dopamine were assessed in two ways. Firstly, pancreatic inflammation was graded by strict histological criteria and these were then used to make comparisons.^{2,4} Secondly, urinary trypsinogen activation peptide concentrations were measured as this has been shown to be an indicator of the severity of acute pancreatitis.^{9,10}

Inflammatory scoring system

An inflammatory score was assigned to each cat, after examining histological sections in a blinded manner. The inflammatory score (0–16; absent–severe) was the sum of subscores (0–4) based on four criteria: architectural damage (parenchymal necrosis), polymorphonuclear cell infiltrate, intraparenchymal haemorrhage and oedema (Table I).

Assays

Serum lipase was assayed using a standard turbidimetric method.¹¹ Urine for trypsinogen activation peptide was collected by aspirating 2 ml from the bladder of each cat. Twenty µl of 0.5M ethylene-diamine-tetra-acetic acid (final concentration 5 mM EDTA) were added to each sample to prevent degradation of the trypsinogen activation peptide analyte by aminopeptidases

(inhibited by chelating active site zinc). All samples were frozen to minus 70°C until analysed using an enzyme linked immunosorbent assay (ELISA), the non-isotopic equivalent of the original radioimmunoassay (RIA).^{9,12}

EXPERIMENT 1: THE EFFECT OF DIFFERENT DOSES OF DOPAMINE ON THE TREATMENT OF ESTABLISHED ALCOHOLIC AHP

Randomised groups of mongrel cats with AHP were treated with either saline or different doses of dopamine from 12 hours after the main pancreatic duct perfusions used to induce AHP had ended. All infusions were given at 2 ml/h for six hours.

Group 1 (AHP controls): these cats received normal saline intravenously.

Group 2: these cats received an intravenous infusion of 2 µg/kg.min of dopamine.

Group 3: this group received an intravenous infusion of 5 µg/kg.min of dopamine.

Group 4: these cats received an intravenous infusion of 50 µg/kg.min of dopamine.

EXPERIMENT 2: THE EFFECT OF COMBINED DOPAMINE AND HISTAMINE RECEPTOR ANTAGONISTS IN THE TREATMENT OF ESTABLISHED AHP

Group 5 cats received an intravenous infusion of 5 µg/kg.min of dopamine combined with the H₁ receptor blocker diphenhydramine (2 mg/kg) and the H₂ receptor blocker cimetidine (10 mg/kg), starting 12 hours after the end of the main pancreatic duct perfusions used to induce AHP. This infusion was also given at 2 ml/h for six hours.

EXPERIMENT 3: THE EFFECT OF DOPAMINE ON ESTABLISHED ALCOHOLIC AOP

Mongrel cats that were to develop alcoholic AOP in this study, were prepared as described above and were randomised into 2 groups: controls and those receiving dopamine for established disease. The infusions were started 12 hours after the main pancreatic duct perfusions used to induce AHP had finished and were given at the rate of 2 ml/h for six hours.

Group 6 (AOP controls): these cats received normal saline intravenously.

Group 7: these cats received an intravenous infusion of 5 µg/kg.min of dopamine.

STATISTICAL METHODS

The data were expressed as median values (Interquartile range; IQR). Ordinal data were compared by the Mann-Whitney U test when looking at differences between groups and the Wilcoxon test for pairs when looking at changes within groups. Nominal data were analysed using Fisher's exact test. Statistical significance was assumed when $p < 0.05$.

Results

EXPERIMENT 1

The results are shown in Table II. In this

TABLE II Experiments 1 and 2, alcohol haemorrhagic pancreatitis. The effect of different doses of dopamine and the effect of adding antihistamines

Protocol 1	Group 1 Saline (controls) n=8	Group 2 Dopamine (2 µg/kg.min) n=6	Group 3 Dopamine (5 µg/kg.min) n=8	Group 4 Dopamine (50 µg/kg.min) n=6	Group 5 Dopamine 5 µg+ antihistamine n=6
Inflammatory score:	10.5 (4)	7 (0.5)*	7 (2.5)*	7 (4)*	7 (1.5)*
Architectural damage	3 (1)	2	2 (0.5)*	2 (1)*	1.5 (1)*
PMN infiltrate	3 (0.5)	1.5 (1)*	2 (0.5)*	1.5 (1)*	2 (1)*
Haemorrhage	2.5 (2)	2	2 (1)	2 (1)*	2 (2)
Oedema	2 (1)	2	1*	1.5 (1)	1.5 (1)*
Serum lipase (U/l):					
Basal	25 (14)	25 (20)	21 (9)	20 (18)	12 (12)
At 24 hours	145 (75)‡	60 (69)*‡	22 (19)*	22 (25)*	50 (78)†
Urine TAP nmol/l:					
Basal	5 (9)	11 (23)	12 (12)	—	—
At 24 hours	144 (220)‡	110 (20)*‡	108 (89)†‡	—	—

*=p<0.05, versus group 1 controls, Mann-Whitney U test. In addition †=p<0.07, ‡=p<0.05, versus basal values, Wilcoxon test for pairs.

PMN=polymorphonuclear cells; TAP=trypsinogen activating peptide.

experiment, group 1 control cats all developed AHP with a high inflammatory score of 10.5 (IQR 4) and high subscores for haemorrhage 2.5 (IQR 2) and polymorphonuclear cell infiltration 3 (0.5). This was matched by high serum lipase and urinary trypsinogen activating peptide values at 24 hours.

In groups 2, 3, and 4, in which established AHP was treated with different doses of dopamine, the inflammatory score was 7 in all three groups, and this was significantly better than that seen in group 1. A significant reduction in the subscore for pancreatic polymorphonuclear cell infiltration was seen in all three treated groups compared with the control group 1. Serum lipase and urinary trypsinogen activating peptide values at 24 hours were also lower than in group 1.

There was no significant difference in the results between groups 2, 3, and 4 with regard to reduction in the inflammatory score compared with group 1 controls.

EXPERIMENT 2

In group 5 cats in which established AHP was treated with 5 µg/kg.min dopamine combined with H₁ and H₂ receptor blockers, while the inflammatory score was significantly better than that in the control group 1, it was the same as that seen in groups 2, 3, and 4. The subscores for architectural damage, polymorphonuclear cell infiltrate, and oedema were significantly better than in group 1 controls. The serum lipase

TABLE III Experiment 3, alcoholic oedematous pancreatitis. The effect of dopamine 5 µg/kg.min in a model of alcoholic oedematous pancreatitis

Protocol 2	Group 6 Saline controls n=7	Group 7 Dopamine (5 µg/kg.min) n=7
Inflammatory score:	7 (3)	4 (2)*
Architectural damage	2	1 (1.5)
PMN infiltrate	2 (1)	1 (1)
Haemorrhage	2 (2)	1
Oedema	2	1*
Serum lipase (U/l):		
Basal	22 (10)	19 (13)
At 24 hours	41 (62)	41 (30)
Urinary TAP nmol/l:		
Basal	17 (30)	11 (13)
At 24 hours	117 (78)†	59 (17)*†

*=p<0.05 versus group 1 controls, Mann-Whitney U test.

†=p<0.05 versus basal values, Wilcoxon test for pairs.

PMN=polymorphonuclear cells; TAP=trypsinogen activating peptide.

activities at 24 hours were low and significantly lower than in group 1 controls.

EXPERIMENT 3

Table III shows that the inflammatory score was 7 (IQR 3) in group 6 controls. This was significantly better than that seen in group 1 controls who developed AHP. Group 7 cats, who received delayed treatment with dopamine, had a significantly better inflammatory score 4 (IQR 2), largely due to a significant reduction in the subscores for architectural damage and oedema.

Serum lipase activities at 24 hours were not significantly increased from basal values in both groups 6 and 7. Although 24 hour urinary trypsinogen activating peptide values were significantly raised in both groups 6 and 7, group 7 cats had significantly lower values than group 6 control cats.

THE CARDIOVASCULAR EFFECTS OF DOPAMINE

The blood pressure was monitored for the duration of all procedures and remained stable in most animals until the onset of dopamine. Thereafter the pressure became labile and the pulse pressure widened, although the mean arterial pressure remained constant.

Discussion

Most patients who develop acute pancreatitis have the milder oedematous form and recover without sequelae. Between 5% and 20%, however, develop haemorrhagic and necrotising forms of acute pancreatitis which are more severe and have a high mortality.^{13,14} Despite extensive research, no agents have been found that influence the severity of pancreatic inflammation and treatment remains supportive unless complications supervene. Dopamine is a vasoactive drug that is known to improve renal perfusion and has a positive inotropic and chronotropic effect.^{15,16} For these reasons it is frequently used to support critically ill patients, including those with fulminating acute pancreatitis.

Extrapolation from animal models to the human situation is fraught with difficulty. In the low pressure duct perfusion model in cats, intragastric alcohol, intraductal bile salts, and systemic hypercalcaemia all lead to an increase in

ductal permeability. While studies are still awaited to see whether the ingestion of ethanol in humans also leads to an increase in pancreatic ductal permeability, the model used in these experiments is interesting, particularly as ductal perfusion with activated juice leads to acute pancreatitis only in cats with permeable ducts. The models used in these studies are complex but they may have some bearing on the human disease.

Recent experiments in different animal models have shown that when a dopamine infusion is started within 12 hours of the onset of acute pancreatitis it reduces the severity of the pancreatic inflammation.^{5,17,18} In our experiments dopamine was started 12 hours after the induction of acute pancreatitis so as to reproduce the clinical situation in which there is an inevitable delay in initiation of treatment for most patients. Experiment 1 has shown that this anti-inflammatory effect of dopamine is sustained at both low (2 and 5 $\mu\text{g}/\text{kg}\cdot\text{min}$) and high doses (50 $\mu\text{g}/\text{kg}\cdot\text{min}$).

Dopamine stimulates three different receptor systems.^{15,16} In the dose range 1–4 $\mu\text{g}/\text{kg}\cdot\text{min}$, the effect is predominantly on dopaminergic receptors, leading to improved renal and mesenteric blood flow with minimal cardiac effect. In the range 4–10 $\mu\text{g}/\text{kg}\cdot\text{min}$, beta adrenergic receptors are increasingly stimulated leading to a positive inotropic effect on the myocardium. Above this range alpha adrenergic receptors are additionally activated.¹⁵ We have previously shown that the anti-inflammatory effect of dopamine, when infused at 5 $\mu\text{g}/\text{kg}\cdot\text{min}$ is completely blocked by dopaminergic and beta-adrenergic receptor antagonists.⁶ In experiment 1, identical anti-inflammatory effects were seen for the 5 and 50 $\mu\text{g}/\text{kg}\cdot\text{min}$ doses suggesting that there is no alpha-adrenergic receptor contribution to the effects seen in this study. This supports earlier observations from experiments on receptor blockade.⁶

Inflammation of the pancreas is accompanied by the release of vasoactive agents such as histamine, bradykinin, prostaglandins, etc.,^{19,20} both into ascitic fluid and into the systemic circulation. These agents lead to endothelial cell contraction with the formation of gaps in the vascular wall.^{21,22} The concomitant increase in microvascular permeability may be responsible for the genesis of pancreatic parenchymal haemorrhage. We have previously shown that the anti-inflammatory effect of dopamine is the result of a reduction in pancreatic microvascular permeability^{6,7} mediated through the stimulation of dopaminergic and beta adrenergic receptors.⁶ In experiment 2 (group 5) we tried to reduce the local inflammatory response further by inhibiting histaminergic receptors too. We have shown no significant improvement in either the inflammatory score or its subscores in comparison with groups 2, 3, and 4, suggesting that the maximal reversal of microvascular permeability has already been achieved with dopamine alone.

A criticism of the results from the studies in experiment 1 might be that dopamine merely reversed the effect of the exogenously administered 16–16 PGE₂ used to create AHP. It may be argued, however, that the prostaglandin was

administered 12 hours before dopamine was given and that the temporal relationship of these agents would therefore be against such an explanation. Furthermore, the results from experiment 3 showed that low dose dopamine was extremely effective in reducing the degree of inflammation in established alcohol related AOP where exogenous PGE₂ was not administered.

We performed experiment 3 to look at the effect of dopamine in a model of oedematous pancreatitis, because in humans alcohol often leads to mild oedematous pancreatitis which is self-limiting. The effect of dopamine in down-modulating the degree of inflammation in this model could mean an improved speed of resolution in humans. Thus, a case could be made for treating all forms of alcohol pancreatitis with dopamine.

In summary, our experiments in models of alcohol related AOP and AHP have shown that dopamine is effective in reducing the degree of inflammation in established pancreatitis. This is largely independent of dose and the addition of inflammation in established pancreatitis. This is largely independent of dose and the addition of histamine receptor blockers has no potentiating effect. We suggest that either dopamine or its analogue dexamamine should be tried clinically in all patients presenting with acute pancreatitis; a renal dose may be optimal.

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