Significance of prostaglandin E₂ in acute necrotising pancreatitis in rats

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SUMMARY Acute necrotising pancreatitis in rats was induced by injecting 5% sodium taurocholate into the pancreatic duct. Prostaglandin E₂ (100 µg/kg subcutaneously twice) decreased the mortality rate from 100% to 60% (NS). When treatment with prostaglandin E₂ was combined with simultaneous administration of either dazmegrel (UK 38 485, 50 mg/kg bodyweight) or Sibelium (Flunarizine R 14 950, 0.2 mg/kg body weight) a significant decrease in the mortality rate ($p < 0.05$) was recorded. Dazmegrel is a selective thromboxane A₂ synthetase inhibitor and prevents the formation of thromboxane A₂. Flunarizine (a calcium entry blocker) decreases thromboxane A₂ formation and also inhibits the effects of raised thromboxane A₂ concentrations. As plasma thromboxane B₂ (the stable metabolite of thromboxane A₂) concentrations increase and the plasma prostaglandin E₂ concentrations decrease in acute necrotising pancreatitis in rats, the results of the present study indicate that these prostaglandins play a role in the pathophysiology of the disease. It is suggested that restoration of the balance in prostanoid concentrations will have a beneficial effect on the course of acute necrotising pancreatitis.

Several reports have shown that prostaglandins protect visceral organs against a variety of noxious agents.¹ The effect of prostaglandins on the pancreas in acute necrotising pancreatitis (ANP), however, remains controversial.²⁻⁷ Ischaemia and impairment of the pancreatic microcirculation may play an important role in the onset and course of the disease.⁸ Thromboxane A₂ (TXA₂) is a strong vasoconstrictor and as such may be a mediator of ischaemia. We have shown that thromboxane B₂ concentrations raised in ANP,^{9,10} though inhibition of TXA₂ alone does not dramatically alter survival.⁹ We have also shown that plasma concentrations of prostaglandin E₂ (PGE₂) decrease in ANP.¹⁰ PGE₂ has vasodilatory and cytoprotective properties that may protect visceral organs, including the pancreas, against ischaemic damage.¹¹ The administration of exogenous PGE₂, however, alone did not affect survival significantly.^{5,7}

Our previous experiments led us to test the effects of PGE₂ administration on survival when TXA₂ synthesis was inhibited simultaneously with either dazmegrel (UK 38 485) or Sibelium (Flunarizine R 14 950). Dazmegrel is a selective TXA₂ synthetase inhibitor and prevents the formation of the vasoconstrictor TXA₂.¹² Flunarizine (a calcium entry blocker) is an inhibitor of vasoconstriction,¹³ decreases TXA₂ formation⁹ and inhibits the vasoconstrictory effects of raised TXA₂ concentrations.¹³

Methods

ANIMALS

Male Wag/Rij rats, weighing 200-250 g, were used. Acute necrotising pancreatitis was induced by retrograde injection of 5% sodium taurocholate (0.1 ml/100 g body weight) into the biliopancreatic duct, as previously described.⁹

Prostaglandin E₂ (PGE₂) (lot 54F - 0110; Sigma) was stored at -30°C in absolute ethanol; final dilutions in physiological saline (50 µg/ml) were pre-

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pared on the day of injection. The PGE₂ injections (100 µg/kg) were given subcutaneously at the time of the induction of ANP and six hours later. Control animals received 0.5 ml 0.9% sodium chloride instead of each dose PGE₂.

Dilutions of dazmegrel (UK 38485, Pfizer Central Research Laboratories, Sandwich, England) in 0.1 N NaOH (12.5 mg/ml) were prepared on the day of administration. Test animals received a dose of 50 mg/kg body weight dazmegrel through an intragastric tube one hour before the induction of ANP and 12 hours later.

Flunarizine (R 14 950) was obtained as a gift from Janssens Pharmaceuticals, Goirle, the Netherlands. Dilutions in physiological saline (0.1 mg/ml) were prepared on the day of injection. Test animals received one single intravenous injection (0.2 mg/kg body weight) at the time of the induction of ANP.

The rats were randomly assigned to one of four groups: group 1 (eight rats): control group (saline); group 2 (10 rats): test group (PGE₂); group 3 (10 rats): test group (PGE₂+dazmegrel); group 4 (10 rats): test group (PGE₂+flunarizine).

Survival time was recorded and survivors were killed after 72 hours. The amount of ascitic fluid was measured at the time of autopsy by weighing cotton rolls saturated with the fluid. The pancreas was removed, fixed in 4% formalin and embedded in paraffin. Sections were cut and stained with haematoxylin and eosin. The preparations were assessed by light microscopy. The amounts of ascitic fluid for the different groups were compared using the Mann Whitney U test, the survival data using Fischer's test for 2×2 tables.

Results

The effect of treatment with PGE₂ on mortality for rats with acute necrotising pancreatitis is shown in Table 1. Control animals (group 1) exhibited a 100% mortality. Treatment with two doses of 100 µg/kg PGE₂ (group 2) reduced mortality to 60%, an effect that was enhanced by adjuvant treatment with either dazmegrel (group 3) or flunarizine (group 4). According to Fischer's test, a significant difference for groups 3 and 4 with the control group was obtained in the first 36 hours, when the mortality rate was 40% ($P<0.05$).

Ascites developed in all animals that died within 72 hours. The largest amounts of ascitic fluid were found in rats that died in the first 36 hours. The volume of ascitic fluid was not decreased by treatment with PGE₂, but after additional pretreatment with dazmegrel the decrease in fluid was statistically significant ($P<0.05$) (Table 2).

Light microscopy studies of the pancreas showed

Table 1 Mortality rate for acute necrotising pancreatitis in rats

Group	Time period (h)		
	0-36	36-72	0-72
1 AP	100	0	100
2 AP+PGE ₂	60	0	60
3 AP+PGE ₂ +D	40*†	10	50†
4 AP+PGE ₂ +F	40*†	0	40*†

The mortality rate is expressed as the percentage animals that died in each time period. AP=acute pancreatitis; D=dazmegrel; F=flunarizine. * $P<0.05$ statistically significant with respect to group 1; †no statistically significant with respect to group 2 (Fischer's test for 2×2 tables).

Table 2 Amount of ascitic fluid produced in acute necrotising pancreatitis

Group	Time period (h)	
	0-36	36-72
1 AP	7.6 (1.4) [8]	—
2 AP+PGE ₂	8.9 (2.2) [4]	0.6 (0.4) [4]
3 AP+PGE ₂ +F	5.8 (2.9) [3]	1.3 (0.6) [6]†
4 AP+PGE ₂ +D	3.3 (1.0) [4]*†	0.8 (0.9) [6]†

The values, given in grams of ascitic fluid, are expressed as means (SD). The number of animals is indicated in square parentheses. AP=acute pancreatitis; D=dazmegrel; F=flunarizine. * $P<0.05$ significant with respect to group 1; †no statistically significant differences with respect to group 2 (Mann Whitney U test).

Table 3 Amount of necrosis in different groups with acute necrotising pancreatitis

Group	Amount of necrosis (%)*							
	0-36 (h)				36-72 (h)			
%	<10	10-25	25-50	>50	<10	10-25	25-50	>50
AP			4	4				
AP+PGE ₂	3		1	1				3
AP+PGE ₂ +F	2	1						5
AP+PGE ₂ +D		3		1	1	1	3	1

*The amount of necrosis was expressed as a percentage of the whole gland on the basis of a double blind microscopic evaluation of 34 slides. The number of slides is indicated. AP=acute pancreatitis; D=dazmegrel; F=flunarizine.

inflammatory infiltration with large areas of acinar necrosis. The inflammatory infiltrate was not pronounced in the first 24 hours but became severe at 72 hours. Apparently, necrosis continued because at 72 hours sometimes more than 80% of the pancreas was necrotic. Acinar necrosis was less pronounced in the dazmegrel/PGE₂ group, because usually less than 50% necrosis was observed (Table 3). Fat necrosis

was pronounced 24 hours after induction and tended to increase with time too. At 72 hours severe fat necrosis throughout the abdominal cavity was observed in all animals.

Discussion

The present study shows the effects of the administration of exogenous prostaglandin E_2 to rats with acute necrotising pancreatitis. Previously, we reported decreased plasma concentrations of PGE_2 in ANP.¹⁰ In this study we show that supplementation of PGE_2 with exogenous PGE_2 lead to a decrease in the mortality rate though the difference is not significant. When combined with thromboxane A_2 inhibition a significant decrease in mortality rate is achieved.

Prostaglandin E 's are vasodilators which act directly on vascular smooth muscle.¹ These vasodilatory properties may protect visceral organs from ischaemic damage.¹¹ Among the factors associated with vasoconstriction are thromboxane A_2 and other vasoactive mediators. TXA_2 stimulates platelet aggregation leading to the formation of microthrombi, which are detrimental to the microcirculation.¹⁹ The effects of PGE_2 may be antagonised by raised TXA_2 concentrations.

The role of the PGE series in pancreatic physiology and disease has so far been controversial.¹ A protective effect of PGE in acute pancreatitis has been reported by Manabe,² Standfield,³ Coelle,⁴ and Reber,⁶ but a lack of effect was observed by Lankisch,⁵ Martin,⁷ and Crocket,¹⁴ while a deleterious effect was shown in a study by Wedgwood.¹⁵ These studies were not comparable, however, as far as the (animal) model, the PGE analogue and the dosage of PGE are concerned. For example, Manabe,² Standfield,³ and Martin⁷ chose a diet model in mice but administered different dosages of PGE_2 . Crocket¹⁴ used the closed duodenal loop model to induce acute pancreatitis in rats and administered PGE_1 . Using the same model of ANP as described in the present study, Lankisch⁵ could not show any beneficial effect, while Reber⁶ initially found a protective effect which he could not confirm in later studies with cats. Differences in experimental design might, at least partially, explain the discrepancies between results. It also is not known which dose of PGE_2 will reach the inflamed pancreas in a sufficiently high concentration when given subcutaneously.

In the present study treatment with inhibitors of thromboxane A_2 (dazmegrel, Flunarizine) together with the administration of PGE_2 led to a significant improvement in survival rate ($P < 0.05$). The same results were found in another study concerning TXA_2 and prostaglandin I_2 (van Ooijen *et al*, unpublished

results). The use of thromboxane A_2 synthetase inhibitors and Flunarizine alone does not alter the survival rate significantly.⁹ Although it is possible that the significance of the results has been influenced by the small numbers in the several groups our results point to the fact that prostanoids are interrelated and that an imbalance in their concentrations probably need complete correction to have significant effects. In this respect other prostanoid mediators may play a more or less significant role as well.

The mode of action of prostaglandin E_2 and thromboxane A_2 in ANP is not clear. Their balance may protect the pancreas from ischaemic damage. A favourable effect on the pancreatic microcirculation might be the cause for the relatively small percentage necrosis in dazmegrel/ PGE_2 -pretreated animals (Table 3). Prostaglandin E_2 may, by cytoprotection, prevent local and systemic release of activated pancreatic digestive enzymes.³

It is also possible that PGE_2 acts at sites other than the pancreas. Autopsy results seem to indicate that the condition of the pancreas itself may not be of exclusive importance for rat survival. Most animals died within 24 hours at which time the inflammatory reaction in the pancreas and necrosis were not pronounced. At 72 hours, however, necrosis and the inflammatory reaction were severe in animals that survived. Shock and fluid loss probably played a role. The accumulation of ascitic fluid was severe in the first 36 hours. Therapy with PGE_2 did not influence the amount of ascitic fluid formed. Simultaneous administration of dazmegrel, however, significantly lowered the amount of ascitic fluid.

In summary, the results of this study indicate that PGE_2 plays – at least in part – a role in acute necrotising pancreatitis in rats. Administration of PGE_2 significantly increases the survival rate when TXA_2 synthesis is inhibited.

References

- 1 Robert A. Prostaglandins and digestive diseases. *Adv Prostaglandin Thromboxane Res* 1980; **8**: 1533–41.
- 2 Manabe T, Steer ML. Protective effects of PGE_2 on diet induced acute pancreatitis in mice. *Gastroenterology* 1980; **78**: 777–81.
- 3 Standfield NJ, Kakkar VV. Prostaglandins and acute pancreatitis – experimental and clinical studies. *Br J Surg* 1983; **70**: 573–6.
- 4 Coelle EF, Adham N, Elashoff J, Lewin K, Taylor IL. Effects of prostaglandin and indomethacin on diet-induced acute pancreatitis in mice. *Gastroenterology* 1983; **85**: 1307–12.
- 5 Lankisch PG, Goke B, Kunze H, Otto J, Winckler K. Does PGE_2 have a beneficial effect on acute experimental pancreatitis in the rat? *Hepatogastroenterology* 1983; **36**: 148–50.

- 6 Reber HA, Tweedie JH, Mosely JG. The cytoprotective effect of 16,16-dimethyl prostaglandin E2 on bile salt induced damage to the pancreas. *Gastroenterology* 1980; **78**: 1241.
- 7 Martin DM, Someren AO, Nasrallah SM. The value of prostaglandin E2 in ethionine induced pancreatitis. *Gastroenterology* 1981; **80**: 1224.
- 8 Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. *Ann Surg* 1978; **188**: 197–201.
- 9 Van Ooijen B, Ouwendijk RJT, Kort WJ, *et al*. Raised plasma thromboxane B2 levels in experimental acute necrotizing pancreatitis in rats. The effects of Flunarizine, dazoxiben, and indomethacin. *Scand J Gastroenterol* 1988; **23**: 188–92.
- 10 Van Ooijen B, Kort WJ, Zijlstra FJ, Vincent JE, Wilson JHP, Westbroek DL. Prostanoid imbalance in experimental acute necrotizing pancreatitis in rats. *Scand J Gastroenterol* 1988; **23**: 192–8.
- 11 Dawiskiba J, Isaksson B, Jeppson B, Hagerstrand I, Bengmark S. Cytoprotective effect of PGE2 on ischemic splanchnic injuries in the rat. *Eur Surg Res* 1984; **16**: 77–83.
- 12 Fischer S, Struppler M, Bohlig B, Bernutz C, Wober W, Weber PC. The influence of selective thromboxane synthetase inhibition with a novel imidazole derivative, UK-38485, on prostanoid formation in man. *Circulation* 1983; **68**: 821–6.
- 13 De Clerck F, Van Nueten JM. Platelet-mediated vascular contraction. Inhibition by Flunarizine, a calcium entry blocker. *Biochem Pharmacol* 1983; **32**: 765–71.
- 14 Crocket KV, Brackett K, Crocket A, Jacobson ED, Rao SS, Joffe SN. Prostaglandin E1 treatment in experimental acute pancreatitis in the rat. *Eur Surg Res* 1984; **16**: 265–73.
- 15 Wedgwood KR, Farmer RC, Reber HA. A model of hemorrhagic pancreatitis in cats – role of 16,16-dimethyl prostaglandin E2. *Gastroenterology* 1986; **90**: 32–9.