Significance of prostaglandin E₂ in acute necrotising pancreatitis in rats

B VAN OOIJEN, W J KORT, C J TINGA, J H P WILSON, AND D L WESTBROEK

From the Departments of Experimental Surgery and Internal Medicine, Faculty of Medicine, Erasmus University, Rotterdam and Department of Pathology, Bronovo Hospital, The Hague, The Netherlands

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.¹² Ischaemia and impairment of the pancreatic microcirculation may play an important role in the onset and course of the disease.³ 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,⁴⁵ though inhibition of TXA₂ alone does not dramatically alter survival.⁶ We have also shown that plasma concentrations of prostaglandin E₂ (PGE₂) decrease in ANP.⁷ PGE₂ has vasodilatory and cytoprotective properties that may protect visceral organs, including the pancreas, against ischaemic damage.⁸¹¹ The administration of exogenous PGE₂, however, alone did not affect survival significantly.⁹⁻¹⁰

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 formation¹⁵ and inhibits the vasoconstrictory effects of raised TXA₂ concentrations.¹⁶

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.¹⁷

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-
pared on the day of injection. The PGE$_2$ 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 PGE$_2$.

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, Gorihe, 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 (PGE$_2$); group 3 (10 rats): test group (PGE$_2$+dazmegrel); group 4 (10 rats): test group (PGE$_2$+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 PGE$_2$ 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 PGE$_2$ (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 PGE$_2$, 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 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/PGE$_2$ group, because usually less than 50% necrosis was observed (Table 3).
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 E2 to rats with acute necrotising pancreatitis. Previously, we reported decreased plasma concentrations of PGE2 in ANP.10 In this study we show that supplementation of PGE2 with exogenous PGE2 lead to a decrease in the mortality rate though the difference is not significant. When combined with thromboxane A2 inhibition a significant decrease in mortality rate is achieved.

Prostaglandin E’s are vasodilators which act directly on vascular smooth muscle.1 These vaso- dilatory properties may protect visceral organs from ischaemic damage.11 Among the factors associated with vasoconstriction are thromboxane A2 and other vasoactive mediators. TXA2 stimulates platelet aggregation leading to the formation of microthrombi, which are detrimental to the microcirculation.12 The effects of PGE2 may be antagonised by raised TXA2 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,1 Coelle,1 and Reber,11 but a lack of effect was observed by Lankisch,1 Martin,7 and Crocket.13 While a deleterious effect was shown in a study by Wedgwood.14 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,1 and Martin1 chose a diet model in mice but administered different dosages of PGE2. Crocket14 used the closed duodenal loop model to induce acute pancreatitis in rats and administered PGE1. Using the same model of ANP as described in the present study, Lankisch1 could not show any beneficial effect, while Reber11 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 PGE2 will reach the inflamed pancreas in a sufficiently high concentration when given subcutaneously.

In the present study treatment with inhibitors of thromboxane A2 (dazmegrel, Flunarizine) together with the administration of PGE2 led to a significant improvement in survival rate (P<0.05). The same results were found in another study concerning TXA2 and prostaglandin I2 (van Ooijen et al, unpublished results). The use of thromboxane A2 synthetase inhibitors and Flunarizine alone does not alter the survival rate significantly.10 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 E2 and thromboxane A2 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/PGE2-pretreated animals (Table 3). Prostaglandin E2 may, by cytoprotection, prevent local and systemic release of activated pancreatic digestive enzymes.15

It is also possible that PGE2 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 PGE2 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 PGE2 plays a role in acute necrotising pancreatitis in rats. Administration of PGE2 significantly increases the survival rate when TXA2 synthesis is inhibited.

References


Significance of prostaglandin E2 in acute necrotising pancreatitis in rats.

B van Ooijen, W J Kort, C J Tinga, J H Wilson and D L Westbroek

Gut 1989 30: 671-674
doi: 10.1136/gut.30.5.671

Updated information and services can be found at:
http://gut.bmj.com/content/30/5/671

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Pancreas and biliary tract (1949)
Pancreatitis (531)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/