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R. TOMASZEWSKA, A. DEMBIŃSKI, Z. WARZECHA, M. BANAŚ, S. J. KONTUREK, J. STACHURA

# PLATELET ACTIVATING FACTOR (PAF) INHIBITOR (TCV-309) REDUCES CAERULEIN- AND PAF-INDUCED PANCREATITIS. A MORPHOLOGIC AND FUNCTIONAL STUDY IN THE RAT

Department of Clinical and Experimental Pathomorphology and Institute of Physiology, University Medical School, Kraków, Poland

Caerulein-induced acute pancreatitis was studied in rats. Consistent with this type of acute pancreatitis morphological (edema, leukocytic infiltration and acinar cell vaculization) and biochemical (increase in pancreatic protein content, PAF release and serum amylase) changes developed 5 hours after caerulein administration. In addition increase in pancreatic weight and decrease in pancreatic blood flow were noticed. PAF administration caused pancreatic damage similar in some parameters to caerulein-induced pancreatitis, along with reduction of pancreatic blood flow, increase in pancreatic protein content, and serum amylase. TCV-309, a selective PAF antagonist, administered prior to caerulein and/or PAF, reduced caerulein-induced pancreatitis and prevented PAF-induced pancreatitis. Results of our present studies indicate the crucial role of PAF in pathogenesis of experimental acute pancreatitis.

Key words: *pancreatitis, caerulein, PAF, PAF-antagonist.*

## INTRODUCTION

Acute pancreatitis is a severe disease with significant morbidity and mortality. The mechanism of the pancreatic damage has not been fully explained. Caerulein-induced pancreatitis (1, 2) represents a reproducible experimental model to study pathogenesis and treatment of pancreatitis. In the caerulein-induced pancreatitis excessive pancreatic stimulation causes in addition to acinar cells vacuolization, the discharge of secretory proteins into the interstitial space, edema and accumulation of leukocytes (3). Recently it has been reported (4) that pancreatic acinar cells stimulated by caerulein release substantial amounts of platelet activating factor (PAF), a phospholipid which increases vascular permeability, tissue edema and neutrofilic infiltration

leading to haemorrhagic damage of the pancreas (5). Locally administered PAF induces in the rabbit pancreas changes characteristic for acute pancreatitis (6). These findings indicate that PAF may be involved in the progression of acute pancreatitis.

## MATERIAL AND METHODS

Male Wistar rats weighing 250—300 g. were used in this study to examine the pancreatic secretion during induction of pancreatitis and to examine the involvement of PAF in the pathogenesis of acute pancreatitis. Control experiments were first performed in which rats received infusion of saline (control) or infusion of caerulein. After determination that infusion of caerulein resulted in acute pancreatitis five separate groups of rats were given: 1. subcutaneous infusion of caerulein alone 2. intraperitoneal injection of PAF (50 $\mu$k/kg) Bachem Bioscience INC Bubendorf Switzerland, 3. TCV-309 (50 $\mu$k/kg) (Takeda Chemical Industries Ltd., Osaka, Japan), intraperitoneally and 30 mins later subcutaneous infusion of caerulein 4. TCV-309 intraperitoneally and 30 mins later PAF 5. TCV-309 alone. Substances were dissolved in 0.9% NaCl. Pacreatitis was induced by caerulein that was diluted in saline and infused subcutaneously at a dose of 10 $\mu$k/kg-h. About 5h after the beginning of caerulein infusion the animals were anesthetized with ether, the abdominal cavity was opened, and the pancreas exposed for measurement of the blood flow by the laser Doppler flowmeter as described previously (10). Immediately after measurement of pancreatic blood flow the blood was drawn from the *vena cava* into a plastic syringe containing heparin for the measurement of plasma amylase with the method described by Bernfeld (7). The pancreas was then quickly dissected out from its attachement to the stomach, the duodenum and the spleen, rinsed with saline and weighed. Some pieces of pancreatic tissue were excised from the body portion, fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin. The slides were examined histologically by two pathologists unaware of the treatment. Histologic grading of pancreatitis: 1. pancreatic edema 2. inflammatory leukocytic infiltration 3. vacuolization of acinar cells. The histologic grading of edema was made using a scale ranging from 0 to 3: 0- no edema, 1 - interlobular edema, 2- interlobular edema and moderate intralobular edema, 3- interlobular edema and severe intralobular edema. Inflammatory leucocytic infiltration was graded 0 - 3: 0- absent, 1- scarce perivascular leukocytic infiltration, 2- moderate perivascular and scarce diffuse leukocytic infiltration, 3-abundant diffuse leukocytic infiltration. Grading of vacuolization of acinar cells was based on the appropriate percentage of cells involved: 0- absent, 1- less than 25%, 2- 25—50%, 3- more than 50%. A portion of the pancreatic tissue was homogenized in 0,15M NaCl and protein content was determined (8). The content of PAF in pancreatic tissue was determined using bioassay technique as described previously (9). Data analysis. Comparison of the difference between the mean values of the various groups of experiments was made by analysis of variance or the Wilcoxon rank-sum test. A difference with P value of less than 0.05 was considered statistically significant. Results are expressed as means$\pm$SEM.

## RESULTS

Subcutaneous infusion of caerulein for 5 hours consistently produced acute pancreatitis in all tested rats. The pancreas appeared grossly swollen and enlarged with visible collections of edematous fluid. Peritoneal fluid (ascites)

was present in all animals receiving caerulein. In all animals pancreas showed marked interlobular edema and moderate or marked intralobular edema. Proteinaceous fluid infiltrated widened connective tissue septa which underlined acinar structure of the pancreas. Pancreatic acini were separated by edema but showed preserved acinar structure. High acinar cells filled entire acinus, then acinar lumen was only seldom visible. In cases with more than 50% of acinar cells vacuolated acinar architecture was less clear. Cytoplasmic vacuoles varied from minute ones to large occupying most of the cytoplasm-these heavily vacuolated cells resembled signet-ring cells. Giant vacuoles (possibly fused vacuoles from more than one cell) were also observed. Blood vessels, especially venules were filled with blood. Margination of leukocytes and diapedesis into perivascular connective tissue were commonly observed. In addition, diffuse leukocytic infiltration was observed in all but one animals receiving caerulein *(Tab. 1)*. Nuclear pycnosis or acinar cell necrosis were not

*Table 1.* Histological changes such as edema, leukocytic infiltration and acinar cell vacuolization, induced by s. c. infusion of caerulein alone i. p. administration of PAF alone i. p. administration of TCV-309 followed by infusion of caerulein or PAF and by TCV-309 alone. Means ± SEM of 6—8 rats. Asterisk indicates significant decrease below the value obtained with caerulein or PAF.

| | HISTOLOGY | | |
|---|---|---|---|
| | Edema (0—3) | Infiltration (0—3) | Vacuolization (0—3) |
| CAERULEIN ALONE | 2.21 ± 0.11 | 1.66 ± 0.13 | 2.10 ± 0.18 |
| TCV-309 + CAERULEIN | 1.82 ± 0.12* | 1.26 ± 0.16* | 1.85 ± 0.24 |
| PAF ALONE | 1.75 ± 0.22 | 1.52 ± 0.20 | 0.75 ± 0.14 |
| TCV-309 ± PAF | 0.51 ± 0.10* | 0* | 0* |
| TCV-309 ALONE | 0 | 0 | 0 |

observed in this animal group. Langerhans islets were normal by histology *(Figs 1, 2, 3, 4)*. PAF administration resulted in pancreatic swelling. By histology, edema and leukocytic infiltration were observed but less extensive compared to caerulein group. Cytoplasmic vacuoles were observed in less than 25% of acinar cells. Pretreatment with TCV—309 prior to caerulein infusion significantly reduced pancreatic edema. Grossly pancreas was slightly swollen but ascites was minimal. Leukocytic infiltration was observed mostly as perivascular margination and only occasionally was diffuse in the entire pancreas. Acinar cells vacuolization was less extensive compared to rats receiving caerulein alone *(Tab. 1.)*. Pretreatment with TCV-309 prior to the administration of PAF almost completely prevented the histological alterations caused by PAF. The pancreas appeared grossly normal and by histology only slight edema was seen *(Tab. 1)*. Biochemical alterations

*Fig. 1.* Normal pancreas. HE 230×.

*Fig. 2.* Caerulein-induced pancreatitis. Marked intralobular edema, leukocytic infiltration and vacuolization of acinar cells. HE 190×.

*Fig. 3.* Caerulein-induced pancreatitis. Severe, predominantly perivascular leukocytic infiltration. Vacuolization of single acinar cells. HE 230 ×.

*Fig. 4.* Caerulein-induced pancreatitis. Vacuolization of pancreatic acinar cells. HE 300 ×.

*Table 2.* Biochemical and blood flow changes in the pancreas after s. c. infusion of caerulein alone (10 μg/kg-h for 5 h) and treatment with PAF i. p. (50 μg/kg), TCV 309 i. p. (50 μg/kg) infusion or combination of above. Means ± SEM of 8 rats per group. Asterisks indicate significant change ($p < 0.05$) as compared to caerulein alone. Cross indicates significant change as compared to PAF alone.

| Parameter | control | caerulein | PAF | TCV−309 | TCV−309 + caerulein | TCV − 309 + PAF |
|---|---|---|---|---|---|---|
| Pancreatic | 937 | 1664* | 1428* | 966 | $1218^{+}$ | $1103^{+}$ |
| weight (mg) | ±39 | ±98 | ±75 | ±42 | ±70 | ±63 |
| Protein/ | 209 | 262* | 251* | 218 | $222^{+}$ | $209^{+}$ |
| pancreas (mg) | ±13 | ±12 | ±8 | ±10 | ±18 | ±18 |
| Blood flow | 100 | 49* | 58* | 99* | $80^{+}$ | $115^{+}$ |
| (%of control) | ±3,6 | ±4,4 | ±3,1 | ±5,0 | ±2,3 | ±3,3 |
| Amylase | 3333 | 9280* | 7040* | 4160* | $7410^{+}$ | $5278^{+}$ |
| (IU/L) | ±370 | ±740 | ±950 | ±648 | ±600 | ±648 |
| PAF | 1.89 | 11.91* | 12.61* | 2.21 | $4.55^{+}$ | N.T. |
| pg/100 mg of tissue | ±0.9 | ±1.1 | ±3.1 | ±1.0 | ±1.6 | |

caused by caerulein and PAF and TCV-309 pretreatment are presented in table 2. Caerulein infusion and PAF injection increased significantly the weight of the pancreas, protein content and plasma amylase concentration as well as content in pancreatic tissue. Pancreatic blood flow was reduced by about 50% in rats infused with caerulein and/or with PAF. Pretreatment with TCV-309 prior to caerulein or PAF administration significantly reduced the increase in pancreatic weight, protein content and plasma concentration of amylase. Tissue level of PAF was also significantly reduced as compared to that observed after the administration of caerulein of PAF alone. The fall in the pancreatic blood flow after caerulein and PAF was totally reveresed by the pretreatment with TCV-309.

## DISCUSSION

Microcirculatory and secretory pancreatic disturbances caused by caerulein administration may be crucial for pathogenesis of acute pancreatitis in experimental animals. Immediately after caerulein administration increase in protein secretion is observed, which is followed by a rapid decrease to the level slightly above basal values, what was described by us previously, (10). Caerulein-induced morphologic alterations were in other experiments observed as early as 3 hours after the challenge (3). Cytoplasmic vacuoles in injured acinar cells contain active lysosomal enzymes which can activate a spectrum of pancreatic proteolytic enzymes leading to acinar cell destruction and enzymatic leakage to stroma (induction of inflammation) and to the blood (increased

serum amylase) (11). Caerulein decreases pancreatic blood flow leading to ischemia and decreased cellular secretory functions. Previous experiments documented that caerulein induces similar acute pancreatitis in spite of the route of drug administration. Caerulein-induced acute pancreatitis is consistently characterized by edema, leukocytic infiltration and acinar cells vacuolization (1, 2, 3). Six hours after caerulein administration acinar cell necrosis is not observed but is seen 12 hours after caerulein. Pycnotic nuclei (a marker of necrotic acinar cell injury) are also observed only 12 hours after caerulein. This explains, why in our experiments, 5 hours after caerulein pancreatic cell necrosis was not observed, while all markers of acute pancreatitis were consistently present. Our experiments showed that caerulein increases PAF release and decreases pancreatic blood flow. As it was shown by us earlier (10), PAF by itself causes mild acute pancreatitis with pancreatic edema, leukocytic infiltrations and minor cellular vacuolization. Serum amylase is also increased. In addition, PAF alone causes decrease in pancreatic blood flow comparable with that after caerulein. TCV-309, a PAF antagonist reduces pancreatic hypersecretory stimulation, prevents PAF-induced decrease in pancreatic blood flow and prevents PAF-induced morphologic lesions. TCV-309 reduces also all morphological, biochemical and microcirculatory markers of acute caerulein-induced pancreatitis. Beneficial effect of PAF antagonist against caerulein-induced pancreatitis depends on the prevention of inflammatory cells activation and subsequent generation of oxygen radicals within pancreatic tissue (12). Our previous experiments (10) documented that caerulein-induced acute pancreatitis is connected with increased PAF release. Our present experiment shows that PAF alone causes similar to caerulein-induced but milder pancreatitis. This, together with protective effects of PAF antagonist-TCV-309 indicates the crucial role of PAF in progression of caerulein-induced acute pancreatitis.

## CONCLUSIONS

1. Our experiments show that PAF is a mediator contributing to the pathogenesis of caerulein-induced acute pancreatitis. 2. PAF antagonist TCV-309 pretreatment reduces both caerulein- and PAF-induced acute pancreatitis.

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Author's address: Prof. J. Stachura, Department of Clinical and Experimental Pathomorphology, University Medical School, 31—531 Kraków, Grzegórzecka 16, Poland.