INTRODUCTION

Inflammation and coagulation are closely linked processes. Inflammation in response to severe infection or trauma results in systemic activation of coagulation. Inflammation promotes clot formation, reduces the activity of natural anticoagulants and inhibits fibrinolysis (1, 2). Pro-inflammatory cytokines play a main role in activation of coagulation by increasing expression of tissue factor on monocytes and endothelium, leading to thrombin formation (3). Severe infection and inflammation lead to hemostatic abnormalities, ranging from a significant increase in laboratory sensitive tests for coagulation activation to gross activation of coagulation that may lead to disseminated intravascular coagulation (4).

Coagulative disorders are known to occur in acute pancreatitis and are related to the severity of this disease. Various experimental and clinical studies have shown protective and therapeutic effect of heparin in acute pancreatitis. Aim of the present study was to determine the influence of acenocoumarol, a vitamin K antagonist, on the development of acute pancreatitis. Studies were performed on male Wistar rats weighing 250 – 270 g. Acoenocoumarol at the dose of 50, 100 or 150 µg/kg/dose or vehicle were administered once a day for 7 days before induction of acute pancreatitis. Acute pancreatitis was induced in rats by pancreatic ischemia followed by reperfusion. The severity of acute pancreatitis was assessed after 5-h reperfusion. Pretreatment with acoenocoumarol given at the dose of 50 or 100 µg/kg/dose reduced morphological signs of acute pancreatitis. These effects were accompanied with a decrease in the pancreatitis-evoked increase in serum activity of lipase and serum concentration of pro-inflammatory interleukin-1β. Moreover, the pancreatitis-evoked reductions in pancreatic DNA synthesis and pancreatic blood flow were partially reversed by pretreatment with acoenocoumarol given at the dose of 50 and 100 µg/kg/dose. Administration of acoenocoumarol at the dose of 150 µg/kg/dose did not exhibit any protective effect against ischemia/reperfusion-induced pancreatitis. We concluded that pretreatment with low doses of acoenocoumarol reduces the severity of ischemia/reperfusion-induced acute pancreatitis.

**Key words:** vitamin K antagonist, ischemia/reperfusion-induced pancreatitis, acoenocoumarol, interleukin-1beta, lipase activity, pancreatic blood flow, DNA synthesis, D-Dimer
Also there are clinical studies indicating that pretreatment with heparin reduces frequency of post-ERCP pancreatitis (20, 21). Administration of heparin given together with insulin is recommended as a standard treatment in hyperlipidemia-induced pancreatitis (22-24). Moreover, low molecular weight heparin has been shown to be effective in the prevention of encephalopathy in patients with severe acute pancreatitis (25).

The present study was designed to determine the influence of pretreatment with acenocoumarol, a vitamin K antagonist, on the development of ischemia/reperfusion-induced acute pancreatitis.

**MATERIALS AND METHODS**

**Animals and treatment**

Studies were performed on male Wistar rats weighing 250 – 270 g and were conducted following the experimental protocol approved by the First Local Commission of Ethics for the Care and Use of Laboratory Animals in Cracow. Animals were housed in cages with wire mesh bottoms, with normal room temperature and a 12-hour light-dark cycle. Rats were fasted with free access to water for 16 h before induction of acute pancreatitis, earlier food and tap water were available *ad libitum*. Experiments were carried out in two separate series.

The first series of studies were performed to determine appropriate dose of acenocoumarol causing an increase of international normalized ratio (INR) to a range between 2.5 to 3.5. This value of INR is recommended in the most clinical conditions related to coagulation disorders (26).

Thirty six animals were randomly divided into six equal experimental groups: [1] control rats treated with saline (aqueous solution of 0.9% NaCl); [2] – [6] rats treated with acenocoumarol (Acenocumarol WZF, Warszawskie Zaklady Farmaceutyczne Polfa S.A., Warsaw, Poland) given at the dose of 50, 100, 150, 300 or 600 µg/kg/dose. Saline or acenocoumarol were administered intragastrically once a day for 7 days.

The second series of studies were performed to assess the influence of pretreatment with acenocoumarol on the development of ischemia/reperfusion-induced acute pancreatitis. Eighty animals were randomly divided in eight equal experimental groups: [1] control rats treated with saline before sham-operation; [2] rats treated with saline before induction of acute pancreatitis; [3 – 5] rats treated with acenocoumarol given at the dose of 50, 100 or 150 µg/kg/dose before sham-operation; [6 – 8] rats treated with acenocoumarol given at the dose of 50, 100, 150 before induction of acute pancreatitis.

Saline or acenocoumarol were administered intragastrically once a day for 7 day before induction of acute pancreatitis or sham-operation. Acenocoumarol at the dose of 50, 100 or 150 µg/kg/dose was given intragastrically once a day for 7 days before induction of acute pancreatitis.

Before induction of acute pancreatitis rats were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Vetoquinol Biowet, Gorzow Wielkopolski, Poland). Acute pancreatitis was induced by severe pancreatic ischemia followed by reperfusion as described previously in details (27). Briefly, the splenic inferior artery was occluded for 30 min and after that microvascular clips were removed to obtain pancreatic reperfusion. The abdominal cavity for time of reperfusion was closed by suture. In sham-operated control rats, longitudinal laparotomy and mobilization of the celiac artery without clamping was performed.

**Determination of pancreatic DNA synthesis**

In accordance with experimental group, rats were anesthetized with ketamine again after 5-h pancreatic reperfusion or 5 hours after sham-operation. The abdominal cavity was opened and pancreatic blood flow was measured by a laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Jarfalla, Sweden), as described previously (28, 29). Data were presented as percentage of pancreatic blood flow obtained in sham-operated saline-treated rats without induction of acute pancreatitis.

**Biochemical analysis**

After the measurement of pancreatic blood flow, arterial blood was taken from the abdominal aorta. INR was determined in fresh blood, using Alere INRatio® PT/INR Monitoring Systems and Alere INRatio® PT/INR Monitoring System Test Strips (Alere San Diego, Inc, San Diego, California, USA).

Plasma D-Dimer concentration was determined using an immunoturbidimetric assay (Innovance D-Dimer Assay, Siemens Healthcare GmbH, Marburg, Germany) on automatic coagulation analyzer BCS XP System (Siemens Healthcare Diagnostics, Erlangen, Germany).

Serum lipase and amylase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA) using Lipa and Amyl DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Inc., Rochester, NY, USA).

Serum concentration of interleukin-1β (IL-1β) was measured using the Rat IL-1β Platinum Elisa (Bender MedSystem GmbH, Vienna, Austria).

**Determination of pancreatic DNA synthesis**

After the blood withdrawal, the pancreas was carefully dissected out from its attachment to the stomach, duodenum, and spleen. Fat and peripancreatic tissue were trimmed away. Samples of pancreatic tissue were taken for study of DNA synthesis and morphological examination. The rate of DNA synthesis was measured by incubation of minced pancreatic tissue at 37°C for 45 min in 2 ml of medium containing 8 µCi/ml of [3H]thymidine ([6-3H]-thymidine, 20 – 30 Ci/mmol, Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic), as described previously (30, 31). The incorporation of labeled thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as [3H]thymidine disintegrations per minute per microgram DNA (dpm/µg DNA).

**Histological examination of pancreatic damage**

Morphological examination of pancreatic tissue was performed in hematoxin and eosin stained slides as describe previously in detail (32). The histological grading of edema was made using a scale ranging from 0 to 3 (0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = interlobular edema and severe intralobular edema). Leukocytic infiltration was also graded from 0 to 3 (0 = absent, 1 = scarce perivascular infiltration, 2 = moderate perivascular and scarce diffuse infiltration, 3 = abundant diffuse infiltration). Grading of vacuolization was based on the appropriate percentage of acinar cells involved: 0 = absent, 1 = less than 25%, 2 = 25 – 50% and 3 = more than 50% of acinar cells. Hemorrhagia was graded: 0 = no hemorrhagia, 1 = 1 – 2 hemorrhagic foci per slide, 2 = 3 – 5 hemorrhagic foci per slide, 3 = more than 5 hemorrhagic foci per slide. Necrosis was graded: 0 = no necrosis, 1 = less than 15% of pancreatic cells involved, 2 = 15 – 35 % of cells involved, 3 = more than 35 % of cells involved. Results of histological examination have been expressed as a predominant histological grading in each experimental group of animals.
Statistical analysis

Results, except histological data, have been expressed as means ± S.E.M. In the first series of studies, we used six rats in each experimental group; whereas in the second series of studies each experimental group was composed of ten animals. Statistical analysis was made by analysis of variance followed by Tukey’s multiple comparison test. A difference with a P value of less than 0.05 was considered significant.

RESULTS

The first series of studies

Intragastric administration of acenocoumarol once a day for 7 days caused a dose-dependent increase in INR in intact rats (Fig. 1). Acenocoumarol given at the dose of 50, 100 or 150 µg/kg/dose caused around two-, three- and five-fold increase in INR, respectively. INR was non-detectable in rats treated with acenocoumarol given at the dose of 300 or 600 µg/kg/dose because obtained values were out of the Alere INRatio 2PT/INR Monitoring System detection range. Results obtained in the first series of study prompted us to use acenocoumarol at the dose of 50, 100 and 150 µg/kg/dose at the second series of study.

The second series of studies

In control saline-treated sham-operated rats without induction of acute pancreatitis, INR reached a value of 1.11 ± 0.09 (Fig. 2). Morphological features showed that the pancreas in this group of animals exhibits regular histology without damage (Table 1; Fig. 9A). In rats without induction of acute pancreatitis, administration of acenocoumarol given for 7 days at the dose of 50, 100 or 150 µg/kg/dose increased INR to similar values as in the first series of studies (Fig. 2). In those rats, acenocoumarol given at the dose 50 or 100 µg/kg/dose was without significant effect on serum lipase activity; whereas acenocoumarol given at the dose of 150 µg/kg/dose, significantly increased serum lipase activity by around 50% (Fig. 3). In rats without induction of acute pancreatitis, administration of acenocoumarol at the doses used did not significantly affect serum activity of amylase (Fig. 4). Administration of acenocoumarol given alone at the dose of 50 or 100 µg/kg/dose tended to increase serum concentration of pro-inflammatory interleukin-1β (IL-1β), but this effect was statistically insignificant (Fig. 5). In the case of acenocoumarol given alone at the dose of 150 µg/kg/dose, this effect reached statistical significance. In rats without induction of acute pancreatitis, administration of acenocoumarol at the doses used did not significantly affect pancreatic blood flow (Fig. 6), pancreatic DNA synthesis (Fig. 7) or plasma D-Dimer.

Table 1. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment with acenocoumarol given at the dose of 50, 100 or 150 µg/kg/day, applied alone or in their combination (acenocoumarol plus IR) on morphological signs of pancreatic damage

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Numbers represent the predominant histological grading in each group.

Fig. 1. Influence of treatment with acenocoumarol (ACN) for 7 days at the dose of 50, 100, 150, 300 and 600 µg/kg/day on international normalized ratio (INR). Mean ± S.E.M., N = 6 in each group of rats. *P < 0.05 compared to control (C); **P < 0.05 compared to ACN 50; ***P < 0.05 compared to ACN 100. ND = non-detectable.
Fig. 2. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment withacenocoumarol (ACN) given at the dose of 50, 100 or 150 µg/kg/day applied alone or in their combination (acenocoumarol plus IR) on international normalized ratio (INR). Mean ± S.E.M., N = 10 in each group of rats. *P < 0.05 compared to control (C).

Fig. 3. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment with acenocoumarol (ACN) given at the dose of 50, 100 or 150 µg/kg/day, applied alone or in their combination (acenocoumarol plus IR) on serum lipase activity. Mean ± S.E.M., N = 10 in each group of rats. *P < 0.05 compared to control (C); †P < 0.05 compared to IR alone.

Fig. 4. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment with acenocoumarol (ACN) given at the dose of 50, 100 or 150 µg/kg/day, applied alone or in their combination (acenocoumarol plus IR) on serum amylase activity. Mean ± S.E.M., N = 10 in each group of rats. *P < 0.05 compared to control (C); †P < 0.05 compared to IR alone; ‡P < 0.05 compared to ACN 50 plus IR.
Fig. 5. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment with acenocoumarol (ACN) given at the dose of 50, 100 or 150 µg/kg/day, applied alone or in their combination (acenocoumarol plus IR) on serum interleukin-1β concentration. Mean ± S.E.M., N = 10 in each group of rats. 

\[ aP < 0.05 \] compared to control (C);  

\[ bP < 0.05 \] compared to IR alone.

Fig. 6. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment with acenocoumarol (ACN) given at the dose of 50, 100 or 150 µg/kg/day, applied alone or in their combination (acenocoumarol plus IR) on pancreatic blood flow. Mean ± S.E.M. N = 10 in each group of rats. 

\[ aP < 0.05 \] compared to control (C);  

\[ bP < 0.05 \] compared to IR alone.

Fig. 7. Influence of ischemia/reperfusion-induced pancreatitis (IR) and pretreatment with acenocoumarol (ACN) given at the dose of 50, 100 or 150 µg/kg/day, applied alone or in their combination (acenocoumarol plus IR) on pancreatic DNA synthesis. Mean ± S.E.M. N = 10 in each group of rats. 

\[ aP < 0.05 \] compared to control (C);  

\[ bP < 0.05 \] compared to IR alone.
Pancreatic ischemia followed by 5-h reperfusion induced acute hemorrhagic pancreatitis in all tested rats (Table 1; Fig. 9C). In morphological examination, moderate inter- and intralobular edema was accompanied with scare or moderate perivascular and scare diffuse leukocytic infiltration. Vacuolization was observed in 0 to less than 25% of acinar cells. Necrosis was observed in all cases of acute pancreatitis but involved less than 15% of pancreatic cells. Hemorrhage was limited to 1 – 5 foci per slide. Histological findings were associated with biochemical signs of acute pancreatitis. Ischemia/reperfusion-induced pancreatitis caused more than a 10-fold increase in serum activity of lipase (Fig. 3) and more than a 13-fold increase in serum activity of amylase (Fig. 4). Serum concentration of pro-inflammatory IL-1β reached around 340% of a value observed in control rats (Fig. 5); whereas pancreatic blood flow and pancreatic DNA syntheses were reduced by around 68 and 52%, respectively (Figs. 6 and 7). Moreover, ischemia/reperfusion-induced pancreatitis caused a significant increase in INR (Fig. 2) and plasma D-Dimer concentration (Fig. 8) by 52 and 3340%, respectively.

In rats with ischemia/reperfusion-induced pancreatitis, pretreatment with acenocoumarol given at the dose of 50 or 100 µg/kg/dose attenuated the development of acute pancreatitis. In histological examination, this effect was found as a reduction in pancreatic edema, inflammatory infiltration, vacuolization of acinar cells, necrosis and hemorrhages (Table 1; Fig. 9D). Also, in those rats, acenocoumarol given at the dose of 50 or 100 µg/kg/dose significantly decreased serum activity of lipase (Fig. 3) and amylase (Fig. 4), and reduced serum concentration of pro-inflammatory IL-1β (Fig. 5). Moreover, those doses of acenocoumarol partly reversed the pancreatitis-evoked decrease in pancreatic blood flow (Fig. 6) and pancreatic DNA synthesis (Fig. 7). Pretreatment with acenocoumarol at the dose of 100 µg/kg/dose trended to improve beneficial effects of acenocoumarol given at the doses of 50 µg/kg/dose; however difference between those effect was not statistically significant, apart from the effect on serum activity of amylase. In contrast to effects of low doses of acenocoumarol, acenocoumarol given at the dose of 150 µg/kg/dose was without beneficial effect on the pancreatitis-evoked changes of serum pancreatic enzymes activity (Figs. 3 and 4), serum concentration of IL-1β (Fig. 5), pancreatic blood flow (Fig. 6) or pancreatic DNA synthesis (Fig. 7). Morphological features showed that pretreatment with acenocoumarol given at the dose of 150 µg/kg/dose, was without effect on the pancreatitis-evoked pancreatic inflammatory infiltration, vacuolization of acinar cells or pancreatic necrosis (Table 1). Moreover, pretreatment with acenocoumarol given at the dose of 150 µg/kg/dose increased the pancreatitis-evoked pancreatic edema and number of hemorrhages.

Pretreatment with acenocoumarol given at the dose of 50, 100 or 150 µg/kg/dose before induction of acute pancreatitis caused the similar changes in INR as in animals without induction of pancreatitis (Fig. 2). Administration of acenocoumarol in all doses used caused similar and statistically significant reduction in the pancreatitis-evoked increase in plasma D-Dimer concentration (Fig. 8).

**DISCUSSION**

Our present study has shown that pretreatment with low doses of acenocoumarol, leading to inhibition of coagulation, reduces the development of ischemia/reperfusion-induced acute pancreatitis. Aacenocoumarol is a vitamin K antagonist. Vitamin K is required to the synthesis of prothrombin and three other plasma-clotting factors, factor VII, IX and X. These clotting factors are synthesized from biologically inactive precursor proteins (PIVKA - protein induced by vitamin K absence) by γ-glutamyl-carboxylase in liver microsomes (33). Aacenocoumarol, as others vitamin K antagonists, reduces plasma levels of vitamin K-dependent clotting factors and thereby reduces the coagulability of the blood (34).

Previous studies have shown a close interaction between inflammation and coagulation (1, 4, 6). Inflammation results in activation of coagulation, due to tissue factor-mediated thrombin generation, a decrease in the activity of natural anticoagulant mechanisms and inhibition of fibrinolysis. Similarly, activation of coagulation increases the inflammatory responses by presence of active clotting factors (especially thrombin, factor Xa and...
tissue factor-factor VIIa complex), mediators released from platelets and promotion of cell-cell interaction (1, 4, 6). Moreover, there are studies showing that coagulation abnormalities occurring in acute pancreatitis are related to the severity of this disease (13).

Our present study is in agreement with these data and has shown that inhibition of coagulation may affect the development of acute pancreatitis. We have found that pretreatment with low doses of acenocoumarol, inhibits the development of ischemia/reperfusion-induced acute pancreatitis. Protective effect of acenocoumarol on the pancreas was manifested by a reduction in histological and biochemical signs of pancreatic damage. Morphological features of pancreatic tissue has shown that pretreatment with acenocoumarol reduces the pancreatitis-evoked pancreatic edema, necrosis, hemorrhage, leukocyte infiltration and vacuolization of pancreatic acinar cells.

In acute pancreatitis, activation of leukocytes and release of pro-inflammatory cytokines are responsible for local pancreatic damage and development of systemic disorders such as systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) (35). Previous studies concerning the role of cytokines in acute pancreatitis have shown that pro-inflammatory cytokines such as IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) are primary produced within pancreas and subsequently within distant organs, developing organ dysfunction in severe cases of this disease (36). The severity of acute pancreatitis is well-correlated with production of pro-inflammatory cytokine (36). In early phase of inflammation, the release of IL-1β and TNF-α precedes production of IL-6, and this sequence of events has been also found in acute pancreatitis (36). IL-1β plays the essential role in the release other members of pro-inflammatory cytokine cascade and the induction of systemic acute phase response (37). The role of leukocyte activation and IL-1β release in the course of acute pancreatitis has been additionally evidenced by finding that administration of interleukin-1 receptor antagonist prevents a serum rise in IL-6 and TNF-α, and decreases severity of acute pancreatitis (38).

Our present study indicates that protective effect acenocoumarol in the pancreas is associated with a reduction in leukocyte inflammatory infiltration of pancreatic tissue and a decrease in the pancreatitis-induced increase in serum IL-1β concentration. These changes seem to be, at least in part, a result of influence of acenocoumarol on thrombin formation. Induction of acute pancreatitis activates coagulation (13). Acenocoumarol, as other vitamin K antagonists, decreases the liver production of prothrombin and reduces formation of thrombin after induction of coagulation. Thrombin promotes blood coagulation, but it also serves as a signaling molecule by binding to protease-activated receptors (PARs) (6, 39). PARs expression have been found on platelets, endothelial cells and many immune cells including macrophages, monocytes, dendritic cells, lymphocytes and mast cells (6, 39, 40). In platelets, pro-inflammatory effect of thrombin is related to alteration of platelets shape to active phenotype and release of platelet factors, such as serotonin, thromboxane and variety of chemokines and growth factors. Furthermore, thrombin liberates the fibrinogen receptor GPIIb-IIIa integrin complex and P-selectin, as well as mobilizes the CD40 ligand to the platelet surface. CD40 ligand induces

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Fig. 9. Representative morphological features of the pancreas observed in sham-operated saline-treated rats (panel A), rats treated with acenocoumarol at the doses of 100 µg/kg/dose without induction of acute pancreatitis (panel B), rats with ischemia/reperfusion-induced acute pancreatitis (panel C), rats pretreated with acenocoumarol at the doses of 100 µg/kg/dose before induction of acute pancreatitis (panel D). Arrows with E mean edema, with II - inflammatory infiltration, with V - vacuolization, with N - necrosis, with H - hemorrhages. Hematoxylin-eosin counterstain, original magnification ×200.
endothelial cells to secrete chemokines and to express adhesion molecules, leading to generation of signals for recruitment and extravasation of leukocytes (6). Acting directly on PARs on endothelial cells, thrombin and other proteases of the coagulation-fibrinolysis system change shape of these cells into a pro-inflammatory phenotype, increase vascular permeability, mobilize adhesive molecules and stimulate the production of cytokines leading to the local accumulation of platelets and leukocytes (40). Moreover, thrombin is chemotactic for monocytes and promotes the production, and release of pro-inflammatory cytokines in immune cells (6).

The increase in serum activity of lipase and amylase is a well-established index of acute pancreatitis severity with high sensitivity and specificity (41). In our present study, pretreatment with acenocoumarol given at the dose of 50 or 100 µg/kg/day, has reduced the pancreatitis-evoked increase in serum activity of lipase and amylase. This effect seems to be a result of protective properties of acenocoumarol in the pancreas, as well as, a reduction in serum activity of pancreatic enzymes is also one of mechanism reducing the development of tissue damage. Study performed by Keck et al. (42) has shown that presence of active pancreatic digestive enzymes in the circulation up-regulates the expression of adhesion molecules on leukocytes and endothelial cells, leading to increase in leukocyte-endothelial interaction and disturbance pancreatic microcirculation.

Numerous clinical and experimental studies have shown that pancreatic ischemia plays an important role in the development of acute pancreatitis and to the progression of this disease to severe necrotizing pancreatitis (43-46). On the other hand, the improvement of pancreatic blood flow inhibits the development of acute pancreatitis and accelerates the recovery in this disease (47-49). In acute pancreatitis evoked by pancreatic ischemia followed by reperfusion, disturbance of pancreatic blood flow is a primary cause of this disease. Our present study has shown that pretreatment with acenocoumarol, given at the low dose of 50 or 100 µg/kg/day, improves pancreatic blood flow in rats with induction of acute pancreatitis and this effect has been associated with reduction of severity of pancreatic damage. This observation indicates that protective effect of acenocoumarol in the pancreas reducing the development of ischemia/reperfusion-induced acute pancreatitis involves improvement of pancreatic microcirculation. However, this effect seems to be secondary one and related to anticoagulant activity of acenocoumarol.

In our present study, induction of acute pancreatitis by severe ischemia followed by reperfusion has significantly increased INR by 52% and plasma D-Dimer concentration by more than 3000%. These findings indicate that development of ischemia/reperfusion-induced acute pancreatitis is associated with activation of coagulation and formation of thrombi within pancreatic and systemic circulation, and this process is followed by fibrinolysis. This conclusion is based on previous studies showing that experimental animals (8, 13) and patients (10, 13, 50) with acute pancreatitis develop the consumptive coagulopathy. Also, severe acute pancreatitis may result in the development of disseminated intravascular coagulation (12, 51). D-Dimer is a product of plasmin-induced degradation of stabilized fibrin (52, 53) and for this reason it is recognized as a marker of fibrinolysis activation (11, 13, 54).

Our study has also shown that pretreatment with acenocoumarol dose-dependently increased INR and this effect reached a similar rate in animals with or without subsequent induction of acute pancreatitis. On the other hand, pretreatment with acenocoumarol significantly reduced the pancreatitis-evoked increase in plasma D-Dimer concentration. These findings indicate that pretreatment with acenocoumarol reduces activation of coagulation during induction of acute pancreatitis and for this reason inhibits formation of thrombi and reduces creation of fibrinolysis products.

DNA synthesis is an index of tissue cell vitality and cell proliferation and a reduction in pancreatic DNA synthesis is well-correlated with pancreatic damage in acute pancreatitis (55-57). In our present study, pretreatment with acenocoumarol given alone was without significant effect on pancreatic DNA synhesis. This observation indicates that acenocoumarol given alone in doses used does not affect pancreatic cell vitality and proliferation. On the other hand, our present study has shown that pretreatment with low doses of acenocoumarol before induction of acute pancreatitis, attenuates the pancreatitis-evoked fall in pancreatic DNA synthesis. This finding is the additional evidence of the acenocoumarol-evoked protection of the pancreas against the development of ischemia/reperfusion-induced pancreatitis.

In contrast to protective effects of low doses of acenocoumarol, our present study has shown that administration of this vitamin K antagonist at the dose of 150 µg/kg/day given alone, leads to some pancreatic damage. Moreover, pretreatment with acenocoumarol given at this dose has not shown any protective effect against the development of ischemia/reperfusion-induced acute pancreatitis. These findings are most likely a result of excessive reduction in blood coagulation leading to excavation of blood from blood vessels and disturbance of general and organ circulation. This thesis is supported by our present observation that pretreatment with acenocoumarol given at the dose of 150 µg/kg/day causes a five-fold increase in INR. Lack of protective effect of pretreatment with acenocoumarol given at the dose of 150 µg/kg/day is also in harmony with previous observation that pretreatment with high doses of acenocoumarol, such as 1 mg/kg/day or more, increases the severity of experimental acute pancreatitis (58). Aacenocoumarol given at the dose of 300 or 600 µg/kg/dose caused an increase INR above detection range of the Alere INRatio 2PT/INR Monitoring System. On the other hand, in rats with pancreatitis and pretreated with acenocoumarol given at the dose of 150µg/kg/dose D-Dimer concentration reached a similar level as in animals pretreated with lower doses of acenocoumarol. D-Dimer concentration reflects an activity of coagulation and subsequent fibrinolysis. As was shown in our present study, excessive reduction in coagulation by high dose of acenocoumarol aggravates the severity of ischemia/reperfusion-induced pancreatitis. However, acenocoumarol reduces a clot formation and for this reason deleterious effect of acenocoumarol given at the dose 150 µg/kg/dose was not associated with increase in plasma D-Dimer concentration.

Previous animal studies have indicated that pancreatic ischemia may be a causal factor in the pathogenesis of acute pancreatitis development (45, 46, 59, 60). Vascular mechanism plays an essential role in the development of acute pancreatitis in various clinical setting, such as cardiac (44) and aortic (43, 61) surgery, hypovolemic shock (62), hypothermia (63) and transplantation of the pancreas (64, 65). These data and our present observation that pretreatment with low doses of acenocoumarol can inhibit the development of ischemia/reperfusion-induced acute pancreatitis, suggest that pretreatment acenocoumarol could be useful in the prevention of acute pancreatitis development in patients with diseases associated with pancreatic circulation disorders.

Finally, we can conclude that pretreatment with low doses of acenocoumarol reduces the severity of ischemia/reperfusion-induced acute pancreatitis.

Conflict of interests: None declared.
REFERENCES


