The role of adenosine receptors for pancreatic blood flow in caerulein-induced acute pancreatitis

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Abstract

Purpose: The aim of our study was to evaluate the possible influence of adenosine receptors agonists and antagonists on pancreatic blood flow (PBF) and the development of acute pancreatitis (AP) in rats.

Material and methods: Ninety male Wistar rats were subdivided into ten equal groups, nine rats in each. The study was carried out in two stages. In the first one the first group (control) received i.v. saline infusion for 12 hours. The groups 2-5 (the first stage) received i.v. caerulein infusion as in the first group, but with pretreatment with: in the second group – DPCPX (A_1 receptor antagonist), in the third group – CGS 21680 (A_2 receptor agonist), in the fourth group – ZM 241385 (A_2 receptor antagonist), in the fifth group – IB-MECA (A_3 receptor agonist). In the second stage the first group received i.v. caerulein infusion at the dose of 5 μg/kg/h for 12 hours. The groups 2-5 (the second stage) received i.v. caerulein infusion as in the first group, but with pretreatment with: in the second group – DPCPX (A_1 receptor antagonist), in the third group – CGS 21660 (A_2 receptor agonist), in the fourth group – ZM 241385 (A_2 receptor antagonist), in the fifth group – IB-MECA (A_3 receptor agonist). Pancreatic blood flow was measured by laser Doppler flowmetry. Pancreatic inflammation was evaluated by serum α-amylase activity, pancreatic weight and histological changes in the pancreatic tissue.

Results: We observed a significant attenuation of serum α-amylase activity increase (19.1 ± 2.8 kIU/L vs 30.12 ± 2.64 kIU/L), pancreatic weight (expressed as percentage of rat's body weight – 0.85 ± 0.16% vs 1.25 ± 0.14%), and improvement of PBF (79.8 ± 6.1% vs 60.1 ± 3.6%), a reduced degree of pancreatic tissue damage (oedema, leukocyte infiltration, vacuolisation of acinar cells) in the third group (CGS 21680 + caerulein) compared with the first group in the second stage (only caerulein infusion). Neither agonists nor antagonists exerterd any appreciable effects on measured parameters in healthy rats.

Conclusions: Pretreatment with A_2 receptors agonist seems to be protective against the damage to the pancreas during the course of caerulein-induced acute pancreatitis in rats. This effect could be due to improvement of pancreatic blood flow. This finding could have some therapeutic implications.

Key words: adenosine receptors, pancreatic blood flow, acute pancreatitis, caerulein, rats.

Introduction

Adenosine is a potent, endogenous proinflammatory factor released by cells under metabolically unfavorable conditions (hypoxia, ischaemia) [1]. Reperfusion of ischaemic and hypoxaemic tissues results in locally increased permeability of vessels and organ oedema associated with neutrophil accumulation in microcirculation. The interaction of neutrophils and endothelial cells, on the other hand, leads to microcirculation impairment. Adenosine is involved in the inflammatory reactions by its action on neutrophils and A_2 receptors of the endothelium inhibiting chemotaxia, phagocytosis and the adhesion of activated leukocytes to the endothelium, decreasing the oxygen free radical generation and thrombocyte aggregation and activity [2].

Acute pancreatitis leads to hypoxia – the secondary phenomenon to decreased blood supply of this organ. This, in turn, results in the dysfunction of intracellular organelles, which may activate lysosomal and digestive enzymes to initiate the autodigestion and tissue damage within the pancreas. Leukocytes become activated and the expression of adhesive molecules is higher, which enables the margination and adhesion of

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activated leucocytes to the endothelium resulting in their diapedesis into the inflammatory focus. Activated leukocytes are the source of proinflammatory cytokines and oxygen free radicals, which intensify the inflammatory process. Li et al. [3] reported that adenosine and its analogues were likely to partially prevent the activation of the above-mentioned processes during ischaemia in acute pancreatitis.

Adenosine receptors have been found in blood vessels (the smooth muscles and endothelium) and in membranes of cells involved in the cascade of inflammatory processes, i.e. in the neutrophils, basophils, lymphocytes, mastocytes, macrophages and thrombocytes [1]. They mediate the influence of adenosine and its analogues on vasodilatation and modulation of the inflammatory process in acute pancreatitis.

The aim of the study was to determine the influence of substances acting on the adenosine receptors in the development of experimental caerulein-induced acute pancreatitis. The severity of the inflammatory process was assessed on the basis of the serum α-amylase activity, pancreatic weight and intensity of histopathological changes in the pancreatic tissue.

Material and methods

The experiments were carried out on 90 male Wistar rats, weighting 250-300 g, subdivided into 10 equal groups, 9 rats in each. The animals were kept in individual cages; they were fed with standard laboratory chow diet and fasted overnight before the experiment with free access to water. The care was provided and experiments were performed according to current guidelines for the use of experimental animals. The study was carried out in two stages. The first stage was to evaluate the effects of substances stimulating or blocking adenosine receptors on normal pancreas; during the second one we assessed the action of these substances in the course of caerulein-induced acute pancreatitis.

In the first stage, the rats were randomly divided into 5 groups: group 1 – controls receiving only intravenous (i.v.) infusion of 0.15 mmol/L NaCl during 12 hours and groups 2-5, in which the animals were injected intraperitoneally with the substances examined in the following doses: group 2 – DPCPX (A_1 receptor antagonist) 1 mg/kg; group 3 – CGS 21680 (A_2 receptor agonist) 1 mg/kg; group 4 – ZM 241385 (A_2 receptor antagonist) 3 mg/kg; and group 5 – IB MECA (A_3 receptor agonist) 0.5 mg/kg.

In the second stage, the animals were also divided into 5 groups: group 1, in which acute pancreatitis was induced according to the method of Lampel and Kern [4] by a 12 h i.v. infusion of caerulein at the supramaximal dose of 5 μg/kg/h, and groups 2-5 in which identical caerulein infusion was proceeded by intraperitoneal (i.p.) injections of the substances studied in the following doses: group 2 – DPCPX (A_1 receptor antagonist) 1mg/kg; group 3 – CGS 21680 (A_2 receptor agonist) 1mg/kg; group 4 – ZM 241385 (A_2 receptor antagonist) 3 mg/kg; and group 5 – IB MECA (A_3 receptor agonist) 0.5 mg/kg; all substances and the doses as in the first stage.

After the 12 h infusion with saline or caerulein, the rats were anaesthetized with ethyl ether and weighted. Then, the peritoneal cavity was opened and blood samples collected to determine serum α-amylase activities. The pancreas was excised, weighted, the tissue samples were fixed in formaline solution. The ratios of pancreas/body weight were calculated. The α-amylase activity was expressed in IU/L [5]. After the 12 h infusion with saline or caerulein in part of animals the pancreatic blood flow was measured by laser Doppler flowmetry [6] and expressed as percent of pancreatic blood flow of the control group.

Pancreatic inflammation activity was evaluated by serum α-amylase levels, pancreatic weight and histological changes in the pancreatic tissue. The histopathological parameters were evaluated in the descriptive manner. The extension of oedema, degree of leucocyte infiltration and pancreatic cell vacuolization were assessed.

Statistical analysis. The results were presented as mean values ± standard deviation (SD) and the differences were compared using the Student's t-test for unpaired data and Cochran-Cox tests. The differences were considered statistically significant when $p<0.05$.

Results

The rats which received the substances examined before the i.v. infusion of 0.15 mmol/L NaCl, showed no statistically significant changes in the pancreatic weight and serum α-amylase activity as compared to the controls (*Tab. 1*). Moreover, the histological features of the pancreases from the above groups and controls were normal.

The 12 h i.v. caerulein infusion induced acute pancreatitis. The pancreas was enlarged, oedematous and about a twofold increase in its weight was observed. There was almost an 8-fold increase in serum α-amylase activity (*Tab. 1*). Histopathological examination showed interlobular and marked intralobular oedema in all of the animals. Perivascular leucocyte infiltration was observed and in some animals the features of diffuse infiltration were present. The majority of acinar cells showed marked vacuolisation of their cytoplasm (*Tab. 1*).

The i.p. administration of the A_2 receptor agonist (CGS 21680, group 3) before inducing acute pancreatitis, reduced the organ injury. It decreased pancreatic ischaemia caused by caerulein administration, which was observed during laser Doppler flowmetry measurements (*Tab. 1*). The A_2 receptor agonist statistically significantly decreased the pancreatic weight gain, as compared to the control groups. Similar observations concerned the serum α-amylase activity; which was statistically significantly lower after the CGS 21680 administration (*Tab. 1*). The histopathological picture revealed markedly decreased oedema, limited to the interlobular. The leukocyte infiltration of the pancreatic tissue was almost completely reduced. Vacuolization of cytoplasm involved a significantly lower number of acinar cells and as compared to histopathological picture in caerulein-infused animals, its intensity was substantially reduced.

The i.p. administration of the A_2 receptor antagonist (ZM 241385, group 4) before induction of acute pancreatitis enhanced the pancreas damage. The pancreatic oedema was massive and pancreatic weight gain was higher. An increase in the serum α-amylase activity was statistically significant (*Tab. 1*).

Table 1. The role of adenosine receptors agonists and antagonists administration on serum α-amylase activity, pancreatic blood flow and pancreatic weight.

Group	Serum α-amylase (kIU/L)	Pancreatic blood flow** [%]	Pancreatic weight* [%]
I. THE FIRST STAGE			
1. Control	4.15 ± 0.70	100.0 ± 2.2	0.45 ± 0.07
2. DPCPX	1.95 ± 0.50	99.3 ± 4.6	0.46 ± 0.06
3. CGS 21680	2.02 ± 0.58	100.0 ± 5.1	0.45 ± 0.08
4. ZM 241385	1.89 ± 0.65	98.7 ± 4.1	0.43 ± 0.07
5. IB-MECA	2.05 ± 0.65	100.0 ± 4.7	0.43 ± 0.07
II. THE SECOND STAGE			
1. CAERULEIN	30.12 ± 2.64^{A}	60.1 ± 3.6	1.25 ± 0.14^{A}
2. DPCPX+CAERULEIN	30.83 ± 2.55	60.4 ± 5.5	1.28 ± 0.20
3. CGS 21680+CAERULEIN	19.10 ± 2.80^{AB}	79.8 ± 6.1	0.85 ± 0.16^{AB}
4. ZM 241385+CAERULEIN	40.12 ± 2.25^{AB}	55.3 ± 5.2	1.46 ± 0.15^{AB}
5. IB-MECA+CAERULEIN	30.22 ± 2.91	64.5 ± 4.3	1.25 ± 0.21

* pancreatic weight is expressed as percentage of rat's body weight
** pancreatic blood flow as percentage of pancreatic blood flow in control group
^{A} $p<0.05$ compared with control group
^{B} $p<0.05$ compared to the value from the group where caerulein was given alone

The histopathological picture showed marked enhancement of inter- and intralobular oedema, intensive perivascular leucocyte infiltration with the features of diffuse infiltration in the majority of rats in this group. Vacuolization involved all acinar cells and showed high intensity in comparison to the histopathological findings in the caerulein-infused animals.

The A_1 receptor blockade (DPCPX, group 2) as well as A_3 receptor stimulation (IB MECA, group 5) in the animals with induced acute pancreatitis did not affect the pancreatic weight, serum α-amylase activity (*Tab. 1*) or histopathological picture of the pancreas.

Discussion

The majority of the studies, carried out recently, concern the relation between the cascade of events in acute pancreatitis and factors which may modulate it. One of such factors is adenosine, an important physiological modulator of the inflammatory process in its various stages which affects adenosine receptors located on many biological targets [1]. The aim of the present study was to determine the effects of adenosine receptor agonists and antagonists on the development of caerulein-induced acute pancreatitis in rats.

Our study revealed, that the adenosine A_2 receptor stimulation before the induction of acute pancreatitis reduced pancreas damage, while the A_2 receptor blockade enhanced the caerulein-induced injury. The protective action of adenosine in acute pancreatitis has not been fully explained. It seems that it induces the vasodilatation, prevents activation of the transcription factor – NF-κB, responsible for the synthesis of proinflammatory interleukins and adhesive molecules, stimulates the synthesis of antiinflammatory cytokins, inhibits chemotaxis and adhesion of leukocytes to the endothelium, decreases aggregation or activation of thrombocytes and inhibits the production of reactive exygen metabolites by the activated leukocytes.

Reduced damage to the pancreas and decreased serum α-amylase activity observed after the A_2 receptor agonist administration may result from its vasodilating effects preventing a decrease in the pancreatic blood flow in acute pancreatitis. In caerulein-induced acute pancreatitis, a decrease in local blood flow was observed, even by 50% of the baseline value [7].

Adenosine and its analogues may be important mediators of vasodilatation, especially in conditions of impaired oxygen supply. The A_2 receptor located in the smooth muscle cells of blood vessel walls participates in this action. Its participation in the vascular tone regulation was also confirmed by the studies concerning the A_2 receptor antagonist. It has been shown, that ZM 241385 eliminated vasodilatation responses induced by adenosine and caused increased activation of stimulated neutrophils [8,9]. Many authors observed improved organ microcirculation after the administration of adenosine or its analogues.

Vasodilatation and improved blood flow in the pancreas caused by adenosine or its analogues partly prevents the development of pancreatic inflammatory changes. Fenton et al. [10] found, that adenosine acts as a potent vasodilator not only by its effects on smooth muscles. Their findings indicate that it also stimulated the NO production in the arterial endothelium. The protective effects of NO in acute pancreatitis result from its properties.

It is generally accepted that NO is a cellular transmitter. It may diffuse through membranes and affect adjacent cells. This mechanism is thought to be the main mechanism of NO actions in the tissues. NO is a biological signaling molecule, which may also affect main proteins or signal pathways in apoptosis [11]. Its deficiency favours neutrophil accumulation, accompanied by an increase in the number of dead acinar cells in the pancreas. On the other hand, Tsukahara et al. [12] showed the cytotoxic effects of NO, generated in pulmonary macrophages in acute pancreatitis. There are some findings, which do not confirm direct toxic effects of NO (per se), but suggest that NO

plays a permissive role for toxicity induced by glutamic acid. It is increasingly accepted that in ischaemia, NO is likely to be both protective and cytotoxic in action in a dose-dependent manner.

After supramaximal caerulein stimulation, the pancreas shows increased activation of the transcription factor, NF-κB. This factor regulates transcription of proinflammatory cytokines: IL-1, IL-6, IL-8, TNFα, adhesive molecules: ICAM-1, VCAM, E-selectine and P-selectine. These observations indicate that this factor may play an important role in initiating and spreading of the inflammatory process. The studies performed by Li et al. [3] show that adenosine and its analogues prevent the activation of NF-κB transcription in ischaemia.

In acute pancreatitis, numerous proinflammatory cytokines are released (TNFα, IL-1, IL-6, IL-8) whose concentration is related to the severity of the disease and multiorgan complications [13,14]. The antiinflammatory effects of adenosine also exerts an influence on cytokin release. Le Moine et al. [15] showed that adenosine increased secretion of interleukin-10 (IL-10) by monocytes and thus participated in inhibiting the TNFα release, and determined that adenosine inhibited TNFα and IL-6 expression in macrophages. Schmid et al. [16] showed that the IL-1-therapy decreased caerulein-induced pancreatitis. The administration of IL-10 after inducing acute pancreatitis led to a significantly decreased level of mRNA for TFNα and TFNα protein in the pancreas. The mechanism of action of adenosine described above is particularly relevant in the severe phase of acute pancreatitis when the balance between cytokines and their antagonists is disturbed and the disease becomes destructive for remote organs. Some authors suggest that anticytokine treatment is effective in experimental models of acute pancreatitis. However, it should be stressed that the protective effects can be observed only when cytokines are blocked before the failure of remote organs and peak cytokin concentrations have occurred.

In the pathogenesis of acute pancreatitis, the role of platelet activating factor (PAF) has also been confirmed. PAF, similarly to IL-1 and TNFα, was shown to be responsible for multi-organ damage. PAF receptors have been found in the endothelial cells of the pancreatic vessels, pancreatic follicular cells, macrophages, monocytes and neutrophils [17]. Exogenous adenosine or its analogues decreased arachidonic acid release and synthesis of leucotrien B_4 (LTB4), occurring after neutrophil stimulation by PAF. This effect was achieved through A_2 receptors. Adenosine and its analogues reduced the production of reactive oxygen metabolites in the PAF activated neutrophils and the adhesion of those cells to the endothelium.

The phagocyte stimulation of granulocytes observed in acute pancreatitis results in increased production of oxygen reactive forms. The impairment of blood flow leads to a decrease in endogenous oxygen free radical utilization and this reduces tissue antioxidative capacity, which, in turn, results in oxidative stress. Oxygen free radicals through peroxidation of structural lipids in the cell membranes damage the walls of capillaries and follicular cells increasing their permeability. This intensifies the enzyme release and the inflammatory process advances. Bullough [2] showed that through A_2 receptors adenosine inhibited the oxygen free radical formation by stimulated leucocytes, decreased their phagocytosis and adhesion to the endothelial cells. These findings indicate that adenosine may have an important function in protecting the organism against oxidative stress.

Conclusions

The administration of adenosine A_2 receptor agonists reduces the severity of pancreatic damage in caerulein-induced acute pancreatitis in rats. This action is likely to result from the modulating effects of the above-described substances on various stages of the cascade of inflammatory responses and pancreatic ischaemia. This study, however, did not show any effects of the A_1 receptor agonist and A_3 receptor antagonist on the inflammatory process in the presented experimental model of acute pancreatitis.

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