The role of adenosine A2a receptors in experimental acute pancreatitis

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Abstract

Purpose: The role of adenosine and its receptors in acute pancreatitis remains unelucidated. The aim was to evaluate the effects of the adenosine A2a receptor agonist and antagonist in the severe, taurocholate-induced experimental acute pancreatitis (EAP).

Material and methods: The experiments were performed on 80 male Wistar rats, subdivided into 4 groups: C – the control rats, I – the EAP group, IIA – EAP group treated with the A2a adenosine receptor agonist CGS 21680, IIB – EAP group treated with the A2a adenosine receptor antagonist ZM 241385. The blood for α-amylase and lipase and tissues samples for the morphological examinations and immunohistochemistry for A2a receptors were collected in 2, 6, 24 hours of the experiment.

Results: The serum α-amylase tended to decrease in the group IIA as compared to EAP untreated after 6 and 24 h. No significant effect of both treatments on serum lipase was noted. The administration of CGS 21680 resulted in favorable decrease of the inflammatory cell infiltration, hemorrhagic changes, necrosis and vacuolization of acinar cells, without an evident effect on the edema of the interstitial tissue. The administration of ZM 241385 did not affect the scores of necro-hemorrhagic changes and inflammatory infiltration, whereas it decreased the scores of vacuolization and edema. In all groups the expression of A2a receptors was similar.

Conclusions: Our findings suggest, that A2a adenosine receptors are involved in the course of sodium taurocholate EAP. It is probable that the modulation of some subgroups of adenosine receptors could alleviate the course of severe experimental AP.

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Introduction

The essence of AP is the damage to the exocrine cells of the pancreas, with severe consequences for whole organism. This process is caused by the activation of trypsinogen. The local defensive mechanisms do not protect the organ against the effects of successively activated enzymes destroying the gland. The direct factor triggering AP and further pathogenic stages of the disease remains unknown. The studies by Satoh et al. [1] show that, via A2a receptors adenosine is likely to play an important role in the progression of edema formation in edematous, caerulein-induced EAP in rats.

Adenosine is an endogenous nucleoside that participates in a multitude of biochemical and physiological processes throughout the body. Adenosine acts through specific membranous receptors called adenosine receptors-P1 that are present in the brain, heart, kidney, blood vessels, adipose tissue and platelets [2-5]. According to their molecular and biochemical properties the receptors are divided into 4 subtypes: A1, A2a, A2b, A3. The A2a receptors are involved in of many signaling reactions in the organism. The link of nucleoside with A2a receptor on the external surface of the cell membrane results in the activation of adenylcyclase and binding with proteins Gq and Gs [6]. This mechanism is the best-known way of transmitting signals through A2a receptors.

In the alimentary tract the presence of A2a receptors was found in the longitudinal muscular layer of the intestines, parietal cells of the stomach, in the liver, pancreas and spleen [5]. In the pancreas adenosine receptors modulate both endocrine and exocrine functions [5]. Iwatsuki et al. [4,7] suggest that, through A2a receptors, adenosine modulates the secretory response of the pancreatic ductular cells.

The aim of present study was to evaluate and compare the

effects of adenosine A2a receptor agonist and antagonist in sodium taurocholate-induced EAP.

Material and methods

The experiments were performed on 80 white male Wistar rats weighing 250-300 g that were maintained for 24 h without food but were allowed free access to water. The animals were subdivided into 4 experimental groups, 20 animals in each group.

Group C – the control, healthy rats used to determine biochemical norms and standard histological images.

Group I – the rats in which EAP was induced by injecting 5% sodium taurocholate solution (Sigma Chemical Co.) at a dose of 0.08 ml/100 g b.w. into the biliary-pancreatic duct according to Aho and Henckel [8].

Group II – the rats with EAP in which the following compounds were administered (intraperitoneally) 48, 24, 12 and 1 hour before and 1 hour after injection of 5% sodium taurocholate solution into the biliary-pancreatic duet.

Group IIA – CGS 21680 A2a receptor agonist (Tocris Cookson Ltd.) each at a dose of 3 mg/kg b.w.

Group IIB – ZM 241385 A2a receptor (Tocris Cookson Ltd.) antagonist at a dose of 3 mg/kg b.w.

After 2, 6 and 24 hours the animals were anaesthetized with diazepam (0.15 mg/kg b.w.) and ketamine (5 mg/kg b.w.). The blood samples were collected from the left ventricle for biochemical tests. Samples of pancreas were obtained for histopathological and immunohistochemical examinations.

Biochemical assays

The activities of α-amylase and lipase in serum were determined by standard laboratory methods.

Histological examination

For histological examinations under light microscope, the pancreatic sections were fixed in 10% buffered formalin solution, pH 7.4. The sections were embedded in paraffin and cut into 2 μm-thick slices using a microtome. The specimens were stained with hematoxylin and eosin (H+E). Histological features of pancreatitis: the necrotic lesions in the parenchyma and adipose tissue of the pancreas, inflammatory infiltrations, erythrocyte extravasations and hemorrhages, interstitial tissue edema were assessed. The scale according to Satoh et al. [1] was used to evaluate morphological lesions in the individual groups. Histological changes of the pancreas were graded blindly (range 0-4), based on the approximate percentage of acinar cells showing vacuolization and necrosis, interstitial edema, and the approximate areas showing inflammatory cell infiltration and hemorrhage: 0=absent, 1=<5%, 2=5-25%, 3=25-50%, 4=>50%.

Immunohistochemical examination

The reactions were performed in paraffin sections with goat polyclonal antibody anti-adenosine A2A R (R-18): SC 7504 (Santa Cruz Biotechnology, Inc.). The antibodies were visualized with the Cell and Tissue Staining Kit HRP-DAB anti goat (R&D Systems, No. CTS008).

Statistical analysis

The values of biochemical parameters were statistically analyzed. The results were expressed as mean ± standard error of the mean (mean ±SEM). The data were compared using an analysis of variance (ANOVA). A 5% risk of inference error was accepted; $p<0.05$ was considered statistically significant.

Results

Biochemical assays

The results of statistical analysis of the serum α-amylase and lipase activities are compiled in *Tab. 1*. The increase in enzyme activities after the administration of 5% sodium taurocholate in group I, IIA and IIB were found to be statistically significant in comparison to control group (with the exception of the serum lipase in group I). There was some decreasing trend of serum α-amylase (n.s.) after 6 and 24 hours of EAP using the adenosine A2a receptor agonist and some increasing trend (n.s.) after 24 hours of AP using the adenosine receptor antagonist in comparison to EAP untreated. However, the decrease of α-amylase activity after 24 hours of EAP treated with the agonist in comparison to EAP treated with the antagonist was statistically significant ($p<0.05$). The changes of the serum lipase activity after both treatmens in comparison to untreated EAP were non significant.

Histological changes

The typical picture of acute pancreatitis developed during in group I during 24 hours of the experiment. Histological examination revealed that the morphologic changes of the pancreatitis were characterized by the extensive necrosis of the exocrine parenchyma, intensive interstitial edema and focal inflammatory infiltrations of various intensity consisting of neutrophils and single lymphocytes. The pathologic process spread to the peripancreatic adipose tissue in the form of enzymatic necrosis (*Fig. 1*). Damaged walls of intrapancreatic ducts with necrosis of epithelium of the ducts and damaged walls of blood vessels were found, what led to extensive erythrocyte extravasations and hemorrhages to the pancreatic parenchyma. In the cells which were not necrotic, the vacuolar degeneration of the cytoplasm was seen.

In the group IIA, treated with the A2a receptor agonist – CGS 21680, the intensity and extent of inflammatory-necrotic changes of the pancreatic parenchyma, hemorrhages and vacuolization of acinar cells were markedly decreased, while substantial interstitial edema was maintained at the same level (*Fig. 2*, *Tab. 2*)

In the group IIB, A2a receptor antagonist – ZM 241385 treated rats, neither the inflammatory-necrotic changes of pancreatic parenchyma nor hemorrhagic changes were affected by the treatment. However, the interstitial edema and vacuolization of acinar cells were evidently decreased.

The summary of the effects of adenosine A2a receptor agonist and antagonist on the histopathologic findings of EAP is reported in *Tab. 2*.

***Table 1*. The effects of adenosine A2a receptor agonist and antagonist on the serum α-amylase and lipase activities in experimental acute taurocholate pancreatitis in rats (EAP)**

		Mean	SEM	Me	Δ Me	ANOVA	p
Control (C)	Serum amylase IU/L						
		569	61.7	584			
	Serum lipase IU/L						
		24.0	7.48	22.0			
Sodium Taurocholate (EAP)	Serum amylase IU/L						
	2 h	2835	588	2679	2095		
	6 h	2069	558	1962	- 717	16.2	<0.05
	24 h	2603	248	2530	568		
	Serum lipase IU/L						
	2 h	34.4	8.20	36.0	14.0		
	6 h	34.8	11.8	31.5	- 4.50	5.0	n.s.
	24 h	41.3	17.5	34.5	3.0		
EAP + agonist A2a CGS 21680	Serum amylase IU/L						
	2 h	2230	414.7	2403	1 819		
	6 h	1041	156.2	993	-1 411	13.0	<0.01
	24 h	1883*	511.4	1842	849		
	Serum lipase IU/L						
	2 h	39.1	13.6	41.5	19.5		
	6 h	46.0	12.1	44.0	3.5	6.0	<0.05
	24 h	29.4	13.4	27.0	-17.0		
EAP + antagonist A2a ZM 241385	Serum amylase IU/L						
	2 h	1979	419	1936	1 352		
	6 h	1968	655	1848	-88.5	12.0	<0.01
	24 h	3218	286	3274	1400		
	Serum lipase IU/L						
	2 h	36.0	7.29	34.0	12.0		
	6 h	34.0	12.7	32.5	-1.50	12.0	<0.01
	24 h	27.2	9.12	30.2	26.9		

n.s. – non significant; p – statistical significance of differences in comparison to control group (C); * p<0.05 (agonist vs antagonist)

Table 2*. Histological findings of the pancreas in CGS 21680, ZM 241385 – treated rats with EAP

Time	Inflammation	Necrosis	Hemorrhage	Vacuolization of acinar cells	Edema
Sodium taurocholate EAP (untreated)					
2 h	1	1	1-2	1	1
6 h	2	2-3	2-3	2-3	2-3
24 h	3-4	4	4	3-4	3-4
EAP + agonist A2a receptor (CGS 21680)					
2 h	1	0	0	0	1
6 h	0-1	1	1	1	2
24 h	0-1	1-2	2	2	3
EAP + antagonist A2a receptor (ZM 241385)					
2 h	0-1	1	1	0-1	1
6 h	2	2	2	0	1-2
24 h	3-4	4	3	1	1

*) Histological changes of the pancreas were graded (range 0-4) based on the approximate percentage of cell for degree of inflammation, necrosis, hemorrhage, vacuolization and edema as follows: 0, absent; 1 <5%; 2, 5%-25%; 3, 25%-50%; 4, >50% [1]

Figure 1. **Group I (EAP untreated). Necrosis of parenchymal cells and adipose tissue of pancreas, hemorrhage, interstitial edema and inflammatory infiltrates. Focally, the vacuolar degeneration of acinar cells is seen. H+E (x200)**

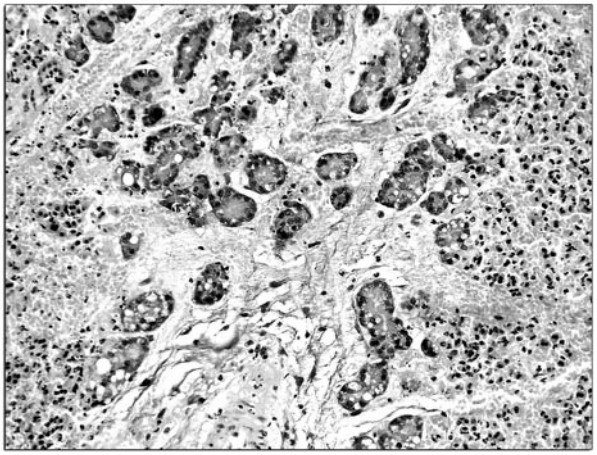
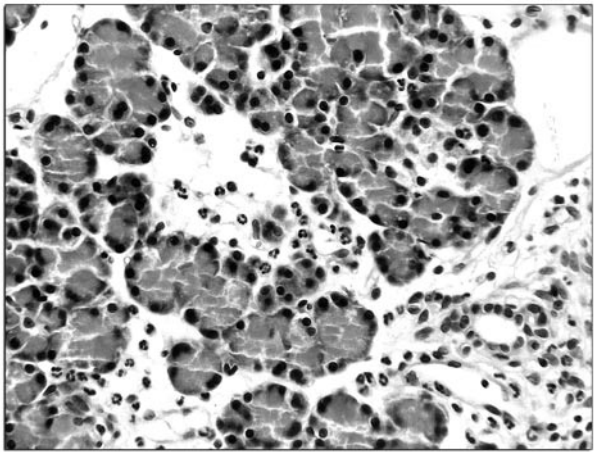


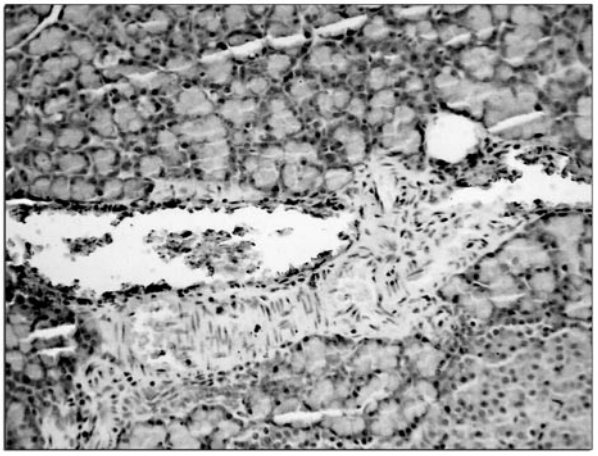
Figure 2. **Group IIA (EAP + CGS 21680). Interstitial edema and sparse inflammatory infiltrates consisting of neutrophils and lymphocytes within the parenchyma of pancreas. H+E (x400)**



Immunohistochemical examinations

The expression of A2a receptors was evaluated in the pancreas of the group C, I, IIA and IIB. In all groups the distribution of A2a receptors was similar. Their expression was observed in the exocrine part of the pancreas within the vascular endothelium (*Fig. 3*) and perivascular nerve fiber bundles. Focally, poor membranous reaction within the epithelial cells of pancreatic ducts was found. The antigen expression was also noted in the endocrine cells of islets of Langerhans located mainly on their periphery. In groups I, IIA and IIB, the expression of A2a receptors was also observed in the inflammatory cells.

Figure 3. **Immunohistochemical reaction of A2a receptors. (Group IIA). Expression of A2a receptors in endothelial cells of blood vessels (x 200)**



Discussion

The studies on etiopathogenesis and causes of unfavorable AP in some patients with necrotic-hemorrhagic form of the disease have been carried out for many years. Adenosine is a purine nucleoside, which modulates numerous physiological functions of the organism and is involved in many pathologic processes: inflammation, damage to tissue continuity, ischaemia and shock [7,9].

Recently it has been demonstrated that adenosine modulates the secretory function of the pancreas and takes part in the pathology of some pancreatic diseases, e.g. mucoviscidosis and AP [1,10]. The role of adenosine in the course of AP is poorly known. The results of the experimental studies reveal that A2a receptor-mediated adenosine may be an important modulator of the individual stages of the inflammatory process [1,11].

In this study, we investigated whether an exogenously added adenosine A2a receptor agonist and antagonist could affect the pathologic outcome of taurocholate – induced rat pancreatitis. The severity of inflammatory process was evaluated on the basis of changes in amylase and lipase activities in serum and the histological examination.

Pretreatment of EAP with the adenosine A2a receptor agonist CGS 21680, tended to decrease the serum α-amylase values after 6 and 24 hours, without statistically significant lipase changes as compared to the untreated group with EAP. In the group pretreated with the adenosine A2a receptor antagonist ZM 241385, no significant changes of α-amylase and lipase were noted.

The administration of CGS 21680 (group IIA) generally alleviated the histological scores of inflammatory processes in the pancreas. The use of highly selective A2a receptor antagonist – ZM 241385 (group IIB) did not intensify the morphological indices of this process.

In the group treated with CGS 21680, the inflammatory-necrotic changes involved only 25% of the adipose tissue and the pancreatic parenchyma after 24 h. Such picture suggests the inhibition of the inflammatory reaction in the pancreas of rats with EAP injected with the A2a receptor agonist. However, the A2a receptor agonist – CGS 21680 resulted in favorable decrease the inflammatory infiltration and necrotic lesions without such effect on the edema formation.

Similar results were reported by Satoh et al. [1] The caerulein model of mild EAP was used in their experiment. The animals were administered CGS 21680 – the selective agonist and DMPX – the antagonist of the A2a receptor. The administration of CGS 21680 also substantially decreased the inflammatory cell infiltration in the pancreas. However, it significantly increased pancreatic edema and vacuolization of the acinar cells. No statistically significant changes in amylase and lipase activities were observed. The reason of such different effects of CGS 21680 still remains unclear. In the immunohistochemical reactions of the rat pancreatic specimens with sodium taurocholate induced EAP (group I), the A2a receptors were found to be distributed on various cells. They were present in the endothelial cells, ducts epithelium, perivascular nerve plexi, pancreatic islet cells (particularly on the periphery) and cells of inflammatory infiltration. Such a varied location of A2a receptors might be responsible for both favorable and unfavorable (edema intensification) effects of CGS 21680 in the course of caerulein EAP. In contrast to their results, in our study with the severe, taurocholate EAP, the same antagonist attenuated not only inflammatory cell infiltration but also necro-hemorrhagic changes and it did not intensify the edema formation or vacuolization of acinar cells. Therefore the general effect of the adenosine A2a receptor agonist in this form of EAP seems to be beneficial.

According to Pearson [12], the main sources of adenosine in the blood are the endothelial cells. Recently, the physiological importance of the endothelium was stressed as a regulator of vasoactive purines due to the system of ecto-nucleopeptidases and the system of membranous transport for adenosine [2]. Thus, the endothelium may regulate the blood concentration of adenosine depending on its amount produced in the tissues [2,12]. Substantial interstitial edema maintained throughout our experiment, is likely to be related to the action of CGS 21680 at the level of endothelial cells.

Inoue et al. [13] suggest that the immunoregulatory action of A2a receptors takes place through their effects on the activity of neutrophils. The activation of these mechanisms of adenosine action through the A2a receptor results in decreased chemotaxis, phagocytosis and the generation of reactive oxygen metabolites by neutrophils. The next stage is the adhesion of inflammatory cells to the endothelium; further monocytes and macrophages are stimulated to secrete pro-inflammatory cytokines. According to Satoh et al. [1], in the caerulein-induced EAP, neutrophil infiltration became smaller due to the CGS 21680 treatment. In our experiment, the group treated with CGS 21680 (group IIA) also showed reduced intensity of inflammatory infiltration in the pancreas compared to the group I untreated animals. The activation of A2a receptors may have, at least partially, the protective effect in the course of AP by decreasing both the intensity of inflammatory infiltration and the release of tissue damaging factors by inflammatory cells.

The pancreatic edema in the caerulein-induced EAP was more intensive after the administration of CGS 21680 and got rapidly smaller under the influence of DMPX [1]. In our rats with taurocholate EAP infused with ZM 241385, the A2a receptor antagonist, the intensification of edema was not observed, particulary 24 hours after the induction of inflammation when the edema was markedly smaller, although the inflammatory-necrotic changes persisted and involved 50-80% of the pancreatic parenchyma. The mechanism of this selective action against edema formation is not clear.

In our experiment, no expression of A2a receptors was observed in the immunohistochemical examinations of the exocrine cells of the pancreas. Therefore, it would be reasonable to assume, that the effects exerted by the agonist or antagonist of adenosine A2a receptors are mediated by such receptors localized on non-parenchymal components of exocrine components and/or in endocrine part of the gland.

Gross et al. [14] showed that adenosine modulates the blood flow of the pancreas. The disorders of the pancreatic microcirculation are one of more important causes of AP progression [14,15]. Some authors suggest that adenosine may intensify vasospasm through the A2a receptors located on the smooth muscle cells of the capillary wall [14,15]. Other studies [1,9] reveal that A2a receptor antagonists slow down the blood flow in the capillaries dilating their lumen. An early sequel of this phenomenon may be the adherence of erythrocytes to the walls of interlobular veins preceded by a marked increase in capillary perfusion.

Thus, a decreased blood flow with simultaneous damage to the vascular wall, could increase the possibility of erythrocytes extravasation and intrapancreatic hemorrhages in EAP, which were observed in our rats with taurocholate EAP. Both, agonist and antagonist of adenosine A2a receptors, used in our study, did not aggravate the hemorrhagic changes, and even some improvement of these changes after the application of agonist was seen. The role of adenosine A2a receptors in the microcirculatory aspects of severe EAP requires further examinations.

Our findings suggest that adenosine A2a receptors are involved in the course of sodium taurocholate-induced EAP. More than one subtype of adenosine A2a receptors may be expressed on the surface of various cells. Thus blockage and stimulation of one type of A2a receptors in our experiment did not provide any clear answer to the question about the role of adenosine receptors in EAP. It is probable that the simultaneous modulation of action of several subgroups of adenosine receptors could slow down the progression of EAP, yet this requires further experimental studies.

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