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Effects of adenosine receptor agonists and antagonists on cytochrome P450 extinction in the liver of rats in the course of acute pancreatitis

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Summary

In the course of acute pancreatitis the liver is an organ that is especially exposed to damage. The presence of adenosine receptors was observed in the whole digestive system. The aim of the experiment was to define the correlation between the extinction of cytochrome P450 in the liver of rats and adenosine receptor agonists and antagonists in the course of necrotizing acute pancreatitis. The experiments were carried out on Wistar male rats weighing 250 g. Acute pancreatitis was induced injecting 5% sodium taurocholate to the biliary-pancreatic duct. Prior to the induction of acute pancreatitis the animals were injected intraperitoneally with selective agonists and antagonists: CGS 21680 (selective A2 agonist), 3 mg/kg, ZM 241385 (selective A2a antagonist), 3 mg/kg, DPCPX (A1 antagonist), 1 mg/kg, 1.3-Dipropyl-8-phenylxantine (selective A1 antagonist), 3 mg/kg, IB-MECA (A3 agonist), 0.75 mg/kg. The determinations were performed in hepatic microsomes obtained according to Guegenrich's method Cytochrome P450 extinction was determined by Matsubar's technique.

The results obtained reveal statistically significantly decreased cytochrome P450 extinction after sodium taurocholate administration. Decreased levels of extinction were also observed after combined administration of sodium taurocholate + Phenylxantine and sodium taurocholate + ZM. The level of IB-MECA remained unchanged in comparison to the controls. However DPCPX and CGS administration increased the extinction of cytochrome P450. The diverse influence of adenosine receptor agonists and antagonists used in the experiment on cytochrom P450 extinction seems to modify the course of the inflammatory process after using 5% sodium taurocholate.

Keywords: acute pancreatitis, cytochrome P450

In the course of acute pancreatitis (AP) resulting from alcohol abuse and gallstones the liver is an organ especially exposed to damage. This is due to many anatomical structures common for the liver and pancreas, like common pancreatic and bile duct, celiac trunk blood supply, venous blood drainage from the pancreas via portal veins to the liver and common lymphatic drainage via the pancreatic duct, which favor the liver damage (4). Other contributors are cytokines and inflammatory mediators produced in the course of AP. In addition to that, mitochondrial function is impaired and liver lisosomal membranes permeability is altered depending on the duration time of the damage and severity of the symptoms (11).

There are two experimental models of AP: ceruleine induced AP (resulting in interstitial pancreatitis) and 5% sodium taurochalate induced AP, which leads to necrotizing form of AP. In the latter model necrotic changes develop faster in comparison to the human but histological pictures are very close. In the early stage damaged lobular cells are exposed to bile salts and long term changes suggest enzymatic self-digestion of the pancreas (2, 3).

Experimental and clinical researches have proved significant involvement of the activated and released inflammatory endogenic mediators in developing AP, which is manifested by intensified granulocyte infiltrates. They provide the source of active proteolitic enzy-

me – elastase. Elastase enhances NADPH-oxidase activity, which stimulates the production of hydrogene peroxides and peroxidative anionoradicals. Another significant source of free radicals is xanthine oxidase, an enzyme present in hepatocytes and endothelium of the small intestine (19). It oxidizes hypoxanthine to uric acid forming anionoradicals, which are transformed into the strongest radical forms i.e. hydroxilic radicals that do not possess inactivating system.

The consequence of alcohol abuse is the induction of cytochrome P450 isoenzyme CYP2E1, which increases the production of oxidative radicals and lipid peroxidation signaling hepatocyte apoptosis. Cytochrome P450 is an important link in xenobiotic metabolism, hydrophobic especially. It takes part in the transformation of fatty acids and eicozanoids. It also catalyzes epoxidation, N- and S-oxidation and dehalogenation. It participates (aside alcohol dehydrogenase) in alcohol detoxication. Its activity is also observed in the course of many inflammatory processes. It seems to be induced by cytokines, inflammatory mediators and free radicals produced in the course of such diseases (13).

Activated metabolism of arachidonic acid leads to the production of thromboxan and prostaglandines, thus altering micro- and macrocirculation. Lipid peroxides that are produced are radicals themselves, which creates the mechanism of „vicious circle" generating the formation of other radicals.

The effects on proteins, both structural and enzymatic take the form of desintegration via the oxidation of thiole group. Hydroxylic radicals cause hialuronic acid depolymerization, collagen degradation and deoxyribonucleic acid (DNA) damage (19).

The search for therapeutic agents to cure AP has turned to substances that stimulate and inhibit adenosine receptors. According to Van Calker's classification (21) there is a class of adenosine receptors, whose main ligand is adenosine, referred to as P1 and a class of purine receptors P2 recognized by adenosine triphosphate (ATP) and adenosine diphosphate (ADP). The class of receptors P1 is further subdivided according to molecular and biochemical properties into 4 subtypes: A1, A2a, A2b and A3. They have been found along the alimentary tract, among others in the liver, pancreas and spleen. A1 mediates adenylocyclase inhibition and affects phospholipase C activity. There are suggestions that they are involved in developing ischemia in the tissues. In the alimentary tract of rats receptor A1 activation brings about the inhibition of the small bowel peristalsis, duodenal muscle relaxation and stomach muscular membrane shrinkage (18).

Adenosine is involved in inflammatory processes via affecting neutrophil and endothelial A2 receptors. A3 receptor increases IP3 level and intracellular Ca^{+2} and inhibits adenylocyclase activity. In humans the highest A3 receptor messenger ribonucleic acid (mRNA) levels are detected in the lungs and liver (17).

Although adenosine receptors have different functions they occur in the same area in the tissues. The expression of more than one adenosine receptor on the same cell may cause many adenosine agonist modulated signal pathways. Adenylocyclase is a typical effecter bound negatively with A1 and A3 and positively with A2, which favors mutual control. Despite intensive research there is no information on adenosine used in the therapy of gastrological illnesses.

The aim of the experiment was to define relation between the extinction of cytochrome P450 in the liver of rats and adenosine receptor agonists and antagonists in the course of experimental necrotizing AP.

Material and methods

Male Wistar rats, weighing 250-300 g were randomly divided according to the principle of timing into study and control groups. The animals were kept in cages for 7-10 days, food and fresh water were provided *ad libitum*; 48 hours prior to the experiment the animals received water alone. The experiments were carried out in constant conditions of the environment (temperature, light, noise). The animals were divided into 4 groups, 10 rats in each group: I – animals shame operated on, 0.9% NaCl 0.3 ml/100/mg body weight (bw) injected into the common bile duct; II – animals operated on, with AP induced by 5% sodium taurocholate injected into the common bile-pancreatic duct, dose 0.3 ml/100 g bw; III – divided into 5 subgroups, 48 hs, 24 hs, 12 hs and 1 h before shame operation the animals received intraperitoneal injection of the following substances: CGS 21680 (selective A2 agonist), 3 mg/kg bw; ZM 241385 (selective A2a antagonist), 3 mg/kg bw; DPCPX (A1 antagonist), 1 mg/kg bw; Phenylxantine (selective A1 antagonist), 3 mg/kg bw; IB-MECA (A3 agonist), 0.75 mg/kg bw; IV – divided into 5 subgroups, operated on and treated, with AP induced by 5% sodium taurocholate injected into the common biliary-pancreatic duct; prior to the induction of inflammation, i.e. 48 hs, 24 hs, 12 hs and 1 h before the experiment the animals received intraperitoneal injection of the following substances: CGS 21680 (selective A2 agonist), 3 mg/kg bw; ZM 241385 (selective A2a antagonist), 3 mg/kg bw; DPCPX (A1 antagonist), 1 mg/kg bw; Phenylxantine (selective A1 antagonist), 3 mg/kg bw; IB-MECA (A3 agonist), 0.75 mg/kg bw. The animals were anaesthetized by intraperitoneall injection of Calypsol (5 mg/kg bw) and Relanium (0.15 mg/kg bw) after 24 hours since the latest dose of substance. Then was made the laparothomy and the liver to the further investigation was taken.

Substances used in the experiment were: sodium taurocholate (Sigma), Relanium (Polfa – Warszawa), Calypsol (Gedeon Richter – Hungary), 0.9% NaCl (Polfarma S.A.), DPCPX, 1.3-Dipropyl-8-phenylxantine, ZM 241385, CGS 21680, IB-MECA (Tocris Cookson Ltd. Great Britain).

The determinations were performed in hepatic microsomes obtained according to Guegenrich's method (8). The livers were homogenized in 4 volumes of 0.1 M Tris-acetate buffer, pH 7.4, containing 0.1 M KCl, 1.0 mM EDTA, and 2.3×10^{-5} M butylated hydroxytoluene in a Waring Blendor for two 40-s intervals, and the mixture was centrifuged at 10 000 × g for 30 min. The pellet was homogenized in a small volume of the buffer mixture, and the supernatant fraction obtained upon centrifugation was combined with the original supernatant fraction and centrifuged at 105 000 × g for 90 min. The resulting microsomal pellet was suspended in a volume of 0.1 M potas-

sium pyrophosphate buffer, pH 7.4, containing 1.0 mM EDTA and 2.3 × 10^{-5} M butylated hydroxytoluene, equal to that of the original homogenate and stored at –70°.

Cytochrome P450 extinction was determined by Matsubar's technique at the protein concentration measured by Lowry's formula (14).

The protein concentration was detected by method of Lowry et al. using bovine serum albumin as a standard (14).

The level of cytochrome P450 was detected by difference spectrophotomotery using a Hitachi two-wavelength double beam spectrophotometer, Model U2000 according to method of Matsubara et al. (14). Preparations were added to 0.1 M potassium buffer (pH = 7.4) and equilibrated with carbon monoxide (CO) for 1 minute. The suspension was divided into two cuvettes (10 mm path length) and sodium dithionite was added to one of the sample to obtain a reduced-CO versus oxidized-CO difference spectrum; the extinction coefficient ($\Delta E_{450-490}$) is 104 mM^{-1} cm^{-1} (8, 14).

The differences between extinction levels of cytochrome P450 for taurocholate sodium and other samples were determined by one side test for arithmetic means by using Statistica software 5.0 version. Significance of statistical difference was determined on $p \leq 0.05$.

Tab. 1. The relation between the extinction of cytochrome P450 in the liver of rats and adenosine receptor agonists and antagonists

The groups	Extinction levels for cytochrome P-450
Taurocholate sodium (TS)	0,014* ± 0,0190
Control	0,036* ± 0,0017
DPCPX	0,036* ± 0,0016
DPCPX + Ts	0,033* ± 0,0140
CGS	0,035* ± 0,0017
CGS + Ts	0,038* ± 0,0180
IB-MECA	0,037* ± 0,0019
IB-MECA + Ts	0,036* ± 0,0230
ZM	0,036* ± 0,0016
ZM + Ts	0,028* ± 0,0320
Phenylxanthine	0,036* ± 0,0017
Phenylxanthine + Ts	0,035* ± 0,0260

Explanation: * $p \leq 0,05$

Results and discussion

The results obtained reveal statistically significantly decreased cytochrome P450 extinction after sodium taurocholate administration. The levels of extinction in groups of all agonists and antagonists of adenosine receptors remained unchanged in comparison to the control. Decreased level of extinction was also observed after combined administration of ZM + Ts, but was not statistically significant. In groups with DPCPX + Ts and CGS + Ts were observed increase levels of extinction of cytochrome P450. These results were statistically significant. However increased levels of extinction after combined administration of Ts + Phenylxantine and IB-MECA + Ts were no statistically significant due to high differences in standard deviations (tab. 1).

The liver is an organ that is on one hand among the first damaged in the course of AP. On the other, it is capable of counteracting the destruction caused by free radicals if defense mechanisms are effective (antioxidants: superoxide dismutase, catalase, glutathione). That is due to, among others, cytochrome P450. Since inosine was found to be endogenic nucleoside of anti-inflammatory potential due to inhibiting proinflammatory cytokines, its receptors have been searched for. Although no inosine specific receptors were found, adenosine A3 receptors were observed to bind inosine, which resulted in decreased inflammatory response (7). There are few reports on liver adenosine receptors. The investigations of ischemic processes and reperfusion of the liver in mice found significant defense reaction of CGS 21680 – selective A2 receptor agonist, on the other hand antagonist ZM 241385 blocked those beneficial effects (6). Acheson et al. (1) observed cytochrome P450 enzymatic induction manifested by faster clearance of antipirine and theophiline in the patients with different liver diseases. A half-life of each substance was decisively lower and its clearance faster in the patients with acute pancreatitis, chronic pancreatitis and in patients with pancreatic cancer. Prokop'eva et al. (16) obtained different results. They investigated the activity of cytochrome P450 1A1, 1A2, 2C6 and 2B1 in the course of experimental AP in rats. In each case the activity was lower in comparison to the controls. The experiments, however, did not include isoform P450 (CYP2E1), which is most likely induced first in the course of AP. Other enzymatic systems are recruited subsequently and hence come divertive results.

In mild 1990 the role of adenosine in metabolism of the liver received much attention. Endogenic adenosine was found to modulate ethanol effects on cyclic adenosine monophosphate (cAMP) in hepatocyte cultures (15). Atamaca and Fry (5) investigated glutatione synthesis in hepatocyte cultures. They found adenosine induced (cAMP) synthesis inhibition and suggested that the processes are not mediated by adenosine receptor agonists or antagonists but via direct influence on ATP level or redox balance. Although adenosine involvement in the course of AP has not been documented yet, the results of experimental studies suggest it can be an important modulator of particular stages of developing inflammation (10).

Adenosine activity mediated by purinergic receptors (A1, A2a, A2b and A3) can occur instantly (egzocytosis, altered concentrations of secondary transmitters) or requires a longer period of time (altered gene expression and cellular proliferation rate) (7). Satoh et al. (17) investigated the effects of A2a receptor stimulation or inhibition on the course of ceruleine induced AP in rats. They used CGS 21680, a selective A2 agonist or DMPX, A2a receptor antagonist. CGS 21680 significantly increased pancreas oedema and vacuolization of the acinar cells but it markedly decreased the infiltration by inflammatory cells. Inoue et al. (9) suggested that A2a receptor immunomodulating activity occurs via its effects on the leukocytes. A2a receptor activated adenosine mechanisms decreases hemotaxis, phagocytosis and causes free radicals release by the neutrophiles. At

the next phase, neutrophile granulocytes adhere to the endothelial cells. Subsequently, monocytes and macrophages are activated and proinflammatory cytokines secreted. According to Satoh et al. (17) ceruleine induced AP and leukocyte infiltration was decreased as a result of CGS 21680 treatment.

Literature data reveal that adenosine present in the pancreas affects endocrine and egzocrine secretion. Iwatsuki et al. (10) tried to assess and compare CGS 21680 – A2a receptor selective agonist with secretine and cholecystokinin 8 (CCK-8), a typical agent stimulating the secretion of the pancreatic juice in dogs. They found that CGS 21680 administered intraarterially increased the secretion of pancreatic juice in a dose dependent way. The response to CGS 21680 activity was abolished by DMPX, A2a receptor antagonist. Their results suggest that adenosine A2a receptors are present in egzocrine cells in dog's pancreas. CGS 21680 induces pancreatic egzocrine secretion, just like secretine does.

The studies by Zhao et al. (22) suggest that double blockage of adenosine A1 and A2 receptors decreases the production of reactive oxygen forms by neutrophile granulocytes stimulated by the platelet activating factor (PAF). Satoh et al. (17) compared cellular adenosine content in healthy animals and in the group of animals with experimental AP (ceruleine and taurocholan induced models). The rats with AP had decisively higher cellular adenosine level in comparison to the controls. In the early phase of AP overproduction of adenosine in the cells damaged occurred due to A1 receptor activation.

Also Smith et al. (20) investigated the influence of IB-MECA – A3 receptor agonist and CGS 21680 – A2a receptor agonist on experimental endotoximia in rats. Both substances demonstrated anti-inflammatory activities and decreased interleukin 10 (IL-10) and tumor necrosis factor (TNF-α) production. According to Luthen et al. (12) cellular ATP content decreased to 38% after 1 h following ceruleine injection.

In this studies was observed decreased cytochrome P450 extinction following sodium taurocholate induced acute pancreatitis. The inflammatory process developing in the pancreas seems to reduce cytochrome P450 enzymes. Increased extinction following the administration of CGS 21680 – selective A2 receptor agonist and DPCPX- A1 antagonist can prove its advantageous influence on cytochrome P450. It remains unclear though if it occurs by direct effects on the liver adenosine receptors or if it results from the activity of the pancreatic adenosine receptors, which decrease inflammatory response in this way.

Conclusions

1. Sodium taurocholate was damaged cytochrom P450 of the liver, what confirm the relationship between pancreas and liver;

2. Increased extinction following the administration of CGS 21680 – selective A2 receptor agonist and DPCPX- A1 antagonist can prove its advantageous influence on cytochrome P450;

3. The diverse influence of adenosine receptor agonists and antagonists used in the experiment on cytochrom P450 extinction seems to modify the course of the inflammatory process after using 5% sodium taurocholate.

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