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Effects of the endogenous adenosine level on biological parameters in the experimental model of acute pancreatitis

Increasingly higher prevalence of acute pancreatitis (AP) resulting from alcohol abuse (especially in men) and cholelithiasis (mainly in women) is a relevant clinical and economic problem. Among the experimental models of AP studied, two are worth mentioning – caerulcin- and sodium taurocholate-induced ones (5). The administration of caerulein results in acute interstitial pancreatitis, while the injection of 5% sodium taurocholate used in the present study leads to the necrotic-haemorrhagic form of pancreatitis (2, 5). In young mice this form may also be induced using choline-free and ethionine-enriched diet. It is believed that the main factor initiating the cell damage in necrotic-haemorrhagic acute pancreatitis is the detergent action of bile leading to destruction of the cell and nuclear membranes and intraplasmic network. The taurocholate model is used in disorders of pancreatic microcirculation, in examinations of arachidonic acid metabolites and in septic-infective complications (6).

Recently, many experimental gastrologic studies are focused on adenosine, which is thought to be a physiological modulator of intercellular communication but mainly is implicated in the individual stages of the inflammatory process, in which it is likely to be a potential mediator through the A2a receptor (1).

The aim of our study is to define the effects of Dipirydamol and Dilazep – the compounds increasing the level of endogenous adenosine, on the basis of lipase and amylase determinations in rats with acute pancreatitis.

MATERIAL AND METHODS

Experimental model. Experimental pancreatitis was induced according to the Aho and Henckel method by administering 5% sodium taurocholate to the biliary-pancreatic duct in the dose of 0.3 ml/body weight (7). The experiments were conducted in Wistar male rats (200–250 g). The randomly selected animals received standard feed and water *ad libitum*. They were divided into 4 experimental groups, 8 rats in each: I – healthy animals to determine internal biochemical standards, II – animals after surgical treatment which were injected 0.9% NaCl to the biliary-pancreatic duct, III – animals in which AP was induced by injecting 5% sodium taurocholate to the biliary -pancreatic duct (3 ml/100 g body weight), IV – animals which prior to the AP induction were administered intraperitoneally the following substances affecting adenosine receptors: IV 1. Dipirydamol – 3 mg/kg – the adenosine transport inhibitor, IV 2. Dilazep – 2 mg/kg – the adenosine uptake inhibitor.

Biochemical examinations. The animals were put to sleep after 2, 6, 24 hours (Ketamine, 5 mg/kg) and the blood samples from the left ventricle (biochemical examinations) and pancreas (mass evaluation, histological and ultrastructural analysis) were collected. The activities of lipase and amylase were determined colometrically according to the Kinetic Colour Test and Immanurea method, respectively.

Statistical analysis. The results were statistically analysed using U Mann-Whitney and Anova-Friedman tests.

RESULTS

The experimental condition of rat pancreases was evaluated by determining the activities of lipase and amylase in blood serum. In group II receiving only NaCl statistically significant differences in the activity of amylase were observed at the individual intervals. In group III, in which acute pancreatitis was induced by injecting 5% sodium taurocholate, the highest values of amylase were found 2 and 24 hours after the induction of AP (Table 1, Fig. 1).

		lg%	P
Control		2.00	
II	2 h	2.18	< 0.05
	6 h	2.23	< 0.05
	24 h	2.23	< 0.05
III	2 h	2.17	< 0.05
	6 h	2.16	Ns
	24 h	2.24	< 0.05
IV 1	2 h	2.13	< 0.05
	6 h	2.22	< 0.05
	24 h	2.35	< 0.05
IV 2	2 h	2.21	< 0.05
	6 h	2.35	< 0.05
	24 h	2.54	< 0.01

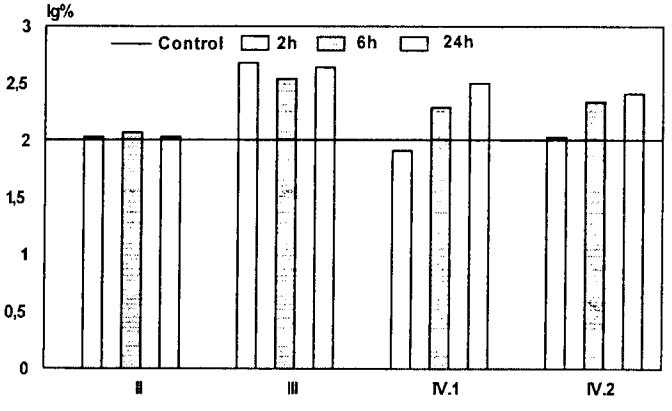

Table 1, Fig. 1. The relative activity of amylase expressed as Ig% of the control value

The levels of lipase in this group were not statistically significantly different (Table 2, Fig.2).

		lg%	P
Control		2.00	
II	2 h	2.18	< 0.05
	6 h	2.23	< 0.05
	24 h	2.23	< 0.05
III	2 h	2.17	< 0.05
	6 h	2.16	Ns
	24 h	2.24	< 0.05
IV 1	2 h	2.13	< 0.05
	6 h	2.22	< 0.05
	24 h	2.35	< 0.05
IV 2	2 h	2.21	< 0.05
	6 h	2.35	< 0.05
	24 h	2.54	< 0.01

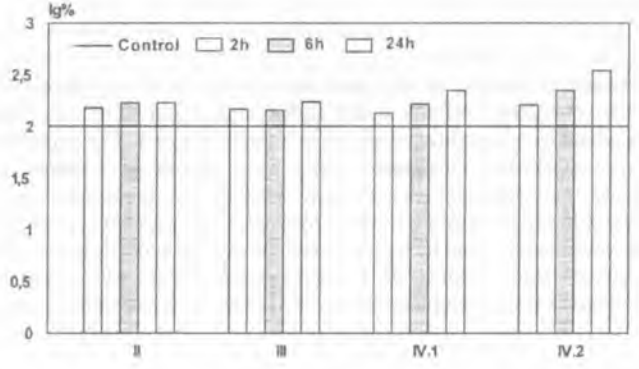

Table 2, Fig. 2. The relative activity of lipase expressed as lg% of the control value

In group IV 1 with induced AP which received Dipirydamol significant differences in amylase and lipase activities were observed at the individual intervals. Compared to group III, a decrease in amylase activity was noted, however the activity of lipase was found unchanged. In group IV 2, in which after the induction of AP the animals received Dilazep, the enzymatic values were similar to the levels found in group IV 1.

DISCUSSION

Acute pancreatitis is not a fully known disease and, therefore, numerous researchers use the experimental models trying to answer the questions concerning its pathogenesis, diagnostic procedures, morphologic changes and therapy. In our experiment the rat model of taurocholate-induced AP was used. The course of AP induced in such a way is rapid; the necrotic changes in the rat glands develop more quickly than in the human organism but histological pictures are very similar. An increase in the inflammatory process was evaluated on the basis of changes in amylase and lipase activities in serum and morphological pictures. The experiments demonstrated significantly reduced histological activities of the inflammatory process in AP induced by sodium taurocholate after administering the adenosine transport inhibitor – Dipirydamol and the adenosine

uptake inhibitor – Dilazep. On the other hand, an increase in serum amylase and lipase activities was found to be statistically insignificant (Table 1, 2. Fig. 1, 2). These findings are consistent with the results observed in caerulein-induced AP in which the adenosine transport inhibitors also caused the regression of the inflammatory process in its histological picture (8).

According to Fishman, the anti-inflammatory action of adenosine may occur via the A3 receptor present in neutrophils, eosinophils and macrophages by the A1 receptor (9). The best known inhibitor of adenosine membraneous transport is Dipirydamol which inhibiting adenosine membraneous transport in the early phase of AP modulates the disease course counteracting further adenosine release. This may lead to oedema intensification and permanent cell damage, which is reflected in the enzymatic picture.

Increasingly the recent reports discuss the role of oxidative stress in AP, the process in which increased generation of oxidants and loss of antioxidants are observed, which results in intensification of the inflammatory process. Iuliano et al. suggest that Dipirydamol is a potent antioxidant, stronger than ascorbic acid and alfa-tocopherol (10). Dilazep, the second compound used in our experiments, which increases the adenosine level in plasma, does not cause such a visible improvement compared to Dipirydamol group. Both compounds inhibit the process of lipolysis, however mostly dilate the vessels and improve the blood flow in many organs, including the pancreas in AP.

To date despite numerous studies concerning experimental acute pancreatitis, no valid conclusions have been reached and the issue remains open. The blockage and stimulation of one receptor type, e.g. A2A in AP does not explicitly explain the role of adenosine receptors as more than one adenosine receptor subtypes may show the expression on the same cell. Since various receptor subtypes have different affinity to the endogenous agonist, the local adenosile level in physiological and pathological conditions may be extremely relevant. The expression of more than one type of adenosine receptor may enable the typical agonist to modulate many signalling pathways. More and more data demonstrate that simultaneous blockage of several adenosine receptor subgroups is likely to stop the development of AP (4). Despite numerous experimental studies on AP no valid conclusions have been reached to date and the issue remains open. The contradictory results of some studies are likely to result from various, difficult to compare experimental models used and different criteria applied to evaluate their effectiveness.

CONCLUSIONS

The experiments reveal that in the groups receiving Dilazep or Dipirydamol, i.e. the substances increasing the levels of endogenous adenosine, the histological features of inflammation decreased and the enzymatic activity slightly increased. These results correlate with the findings reported by Nojie et al. and demonstrate that endogenous adenosine is involved in the inflammatory-necrotic process of AP.

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SUMMARY

The aim of the present study is to assess the role of compounds increasing the level of endogenous adenosine in experimental acute pancreatitis (AP) induced by sodium taurocholate. The experiments were carried out in Wistar white rats. The first group of animals was used to determine the biochemical standards and standard histopathological pictures. In the second group of animals AP was induced by injecting 5% sodium taurochoplate to the biliary-pancreatic duct. Moreover, the rats were administered intraperitoneally the following substances: Dipirydamol, the adenosine transport inhibitor (3 mg/kg body weight) or Dilazep, the adenosine uptake inhibitor (2 mg/kg body weight). The substances were injected 48, 24 and 1 hour before and 1 hour after the induction of pancreatitis. The serum levels of lipase and amylase were determined. The experiments reveal that the adenosine transport inhibitors may decrease the inflammatory process in AP.

Wpływ poziomu endogennej adenozyny na parametry biochemiczne w doświadczalnym modelu ostrego zapalenia trzustki

Celem pracy jest ocena roli związków zwiększających poziom endogennej adenozyny w przebiegu doświadczalnego ostrego zapalenia trzustki (OZT) wywołanego taurocholanem sodu. Badania przeprowadzono na białych szczurach rasy Wistar. Pierwsza grupa zwierząt służyła do wyznaczania norm biochemicznych i wzorcowych obrazów histopatologicznych. W drugiej grupie zwierząt wywoływano OZT przez iniekcję 5% taurocholanu sodu do przewodu żółciowo-trzustkowego. Ponadto szczurom podawano dootrzewnowo: Dipirydamol, inhibitor transportu adenozyny (3 mg/kg m.c.) lub Dilazep, inhibitor wychwytu zwrotnego adenozyny (2 mg/kg m.c.). Substancje te podawano zwierzętom w 48 godzin, 24, jedną godzinę przed i jedną godzinę po wywołaniu zapalenia. W surowicy krwi wykonywano oznaczenia lipazy i amylazy. Z przeprowadzonych badań wynika, że zastosowanie inhibitorów transportu zwrotnego adenozyny może zmniejszyć nasilenie procesu zapalnego w przebiegu OZT.