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Metabolic Function of the Liver in Caerulein Induced Acute Pancreatitis

Czynność metaboliczna wątroby w ostrym zapaleniu trzustki wywołanym ceruleiną

INTRODUCTION

The association of the liver and the pancreas pathology is one of the most important problems in modern gastroenterology. Due to its great sensitivity to the damaging factors and the anatomical proximity of the pancreas the liver is particularly subjected to the functional and morphological disorders in acute pancreatitis.

The collection of the numerous clinical and experimental data did not sufficiently explain the character of the morphology and the functional liver changes in acute pancreatitis (2, 3, 7, 8, 17).

The metabolic function is one of the most significant properties of the liver both in the physiological and the pathological conditions. The impairment of this function takes place in the pathological conditions of the liver itself as well as of some other organs, including the pancreas (3, 6, 7, 12).

One of the methods evaluating the liver metabolic function is the aminophenazone test (11, 12, 20, 25).

The model of the isolated perfused rat liver was chosen as the one excluding the following factors: adsorption, distribution, or extrahepatic elimination (15, 21).

The model of AP included with the excessive caerulein stimulation was selected because of the reversibility of the changes in pancreas after the caerulein withdrawal (19).

The aim of the present study was to answer the following questions:

1. Do any disorders of the liver metabolic function occur in the course of AP?
2. Do such changes depend on the duration and the severity of AP?
3. Does the inhibitor of platelet activating factor (PAF) exert any positive effect on the liver and the pancreas injuries?

MATERIALS

The study was performed in the laboratories of the Department of Pharmacology of the Medical Academy (head of the Department Prof. Zdzisław Kleinrok).

The experiments were performed on 80 white Wistar rats weighing about 250 g. The studied material was divided into 8 groups, 10 rats in each one.

Group I — controls. The liver perfusion was performed for 12 hrs after the infusion of physiological saline through the jugular vein.

Group II — the rats with AP induced by the continuous intravenous caerulein (CR) infusion for 3 hrs in the dose of 5×10^{-6} g/kg/h. The liver perfusion was carried out immediately after CR infusion withdrawal.

Group III — the rats with Ap induced by the continuous intravenous caerulein infusion for 6 hrs in the dose of 5×10^{-6} g/kg/h. The liver perfusion was carried out immediately after CR infusion withdrawal.

Group IV — the rats with Yp induced by the continuous intravenous caerulein infusion for 12 hrs in the dose of 5×10^{-6} g/kg/h. The liver perfusion was carried out immediately after CR infusion withdrawal.

Group V — the rats with Ap induced by the continuous intravenous caerulein infusion for 12 hrs like in group IV but prior to CR infusion the inhibitor of platelet activating factor (BN 52021) was administered in bolus and in the dose of 5×10^{-3} g/kg/h. (Prof. Braquet — France).

Group VI — The liver perfusion performed 24 hrs after CR infusion withdrawal.

Group VII — the rats with AC induced by the continuous intravenous CR infusion in the dose of 7.5×10^{-6} g/kg/h for 12 hrs. The liver perfusion was done immediately after CR infusion withdrawal.

Group VIII — the perfusion of the healthy rat liver with the fluid containing caerulein and aminophenazone.

METHODS

The acute experimental pancreatitis was induced according to the Lampel and Kern's method (19). Caerulein ("Takus", Farmitalia) was given in the doses of 5×10^{-6} g/kg/h, at a rate of 1.2 ml/h using the infusion pump (Unipam type 340A, Poland). The infusion of the isolated rat liver was performed according to the Miller's method with Haft's modification (21).

During the 2 hrs perfusion the fluid was gassed with a mixture of 95% O_2 and 5% CO_2. Prior to the perfusion amidopyrine (aminophenazonum, "Polfa", Pabianice) was added to the fluid, the final concentration being 30μg/ml. After 15 minutes of adaptation the perfusion was performed for 2 hrs. The amidopyrine concentrations in the perfusate were determinated according to the Renicke method after the first and the second hour of perfusion. The amidopyrine concentration was known drug concentrations.

The data were statistically analysed by Student's test assuming 5% error risk and $p < 0.05$ as statistically significant.

RESULTS

The mean aminophenazone (A) concentrations in μg/ml in the perfusate in all the experimental groups are presented in Table 1. The initial aminophenazone concentration in the perfusate before perfusion in all the groups was the same — 30 μg/ml. In group I the mean (A) concentration after 1 h perfusion was 10.5 ±0.36 μg/ml, after 2 hrs perfusion 9.9 ±0.65 μg/ml. The differences in the aminophenazone elimination in groups IV, V, VI were statistically significant in comparison with the control group, which was visible in the increase of (A) concentration in the perfusate both after 1 and 2 hrs' perfusion ($p < 0.001$).

Table 1. The mean aminophenazone concentrations (in μg/ml) in the fluid perfusing the rat livers in the individual experimental groups

Experimental group	The mean aminophenazone concentrations in the perfusate $\bar{x} \pm SD$		The statistically significant difference compared with the control group	
	after 1-h--perfusion	after 2-h--perfusion	after 1-h--perfusion	after 2-h--perfusion
I	10.05 ±0.36	9.90 ±0.56		
II	10.22 ±0.54	9.32 ±0.74	NS	NS
III	10.32 ±0.73	9.29 ±0.80	NS	NS
IV	14.32 ±0.72	13.03 ±1.07	$p<0.001$	$p<0.001$
V	10.92 ±1.07	9.34 ±0.60	NS	NS
VI	12.81 ±0.58	11.37 ±0.44	$p<0.001$	$p<0.001$
VII	16.08 ±1.07	12.20 ±1.09	$p<0.001$	$p<0.001$
VIII	10.52 ±0.69	9.67 ±0.74	NS	NS

The highest mean (A) concentration was found in group VII, in which AP was most severe and induced by the caerulein administration in the dose of 7.5×10^{-6} g/kg/h for 12 hrs. The (A) concentration increase in the perfusate proves the reduced (A) elimination which means the disorder of the metabolic liver function. In group V where the inhibitor platelet activating factor (BN 52021) was given prior to infusion, the (A) elimination was proper, which conforms the correct metabolic liver function. There were no statistically significant differences in the average A nonconcentrations in groups II, III, VIII in comparison with the control group. Group VIII was created to exclude the possibility that intravenous saline infusion itself, given for 12 hrs, may cause the disorders of A elimination. The obtained results in this group were not statistically different compared with the control one.

DISCUSSION

The subject of the present paper was the evaluation of the metabolic liver function in the acute experimental pancreatitis induced by the excessive caerulein stimulation. The morphological liver studies in AP show the inflammatory-

-degenerative changes of the perivenous zones in hepatic lobules, the intensification of which correlates with degrees of the pancreas damage (2, 3, 7—9, 18). In the proper conditions the hepatic cells of these zones show the greatest activity of the aminophenazone metabolizing enzymes (12). The aminophenazone elimination reflects the activity of the enzymatic system dependent on cytochrome P-450, thus allowing the evaluation of the hepatocyte endoplastic reticulum efficiency (4, 26). Cytochrome P-450 contained in liver microsome is an enzyme consisting of 6 polypeptides. Its main purpose is to inactivate the toxins and accelerate their elimination. The changes in the liver metabolic activity of oxidases dependent on cytochrome P-450 may relevantly affect the liver oxidation-detoxication function (11, 12).

One should believe that in the case of liver damage in the course of acute pancreatitis the disorder of the liver aminophenazone elimination occurs. In our studies the diminished aminophenazone elimination was observed in the groups with most severe and long lasting AP. The available literature presents rather small number of papers in which the similar model in acute experimental pancreatitis was used.

The reduced liver aminophenazone elimination in post-alkoholic AP in people was observed by Łukaszyk (20). In biliary AP the aminophenazone elimination was accelerated. However, Acheson et al. (1) described the accelerated aminophenazone metabolism in AP stressing that the biliary acids are the factors stimulating the activity of the oxydases conditioned by cytochrome P-450. Hartlieb et al. (12) showed the diminished aminophenazone liver metabolism in patients with AP which was visible in the prolonged aminophenazone halftime.

Similar results were obtained by Długosz et al. (7, 8) and Andrzejewska et al. (2) who observed the disorder of the oxidation function of liver mitochondria in AP and some other structures of subcellular hepatocytes dependent on the duration and severity of AP in the similar experimental models. The patomechanism of liver damage in the course of AP is not fully explained.

Rinderknecht hypothesis (23), which stresses the great role of the excessive leucocyte and macrophage stimulation leading to the multiorgan failure in the course of AP, seems to be very interesting. Rinderknecht believes that the big initial pancreas injury or the prolonged acute action of the damaging factors results in the accumulation of the numerous cell residues, the dead cells and the toxic factors, which leads to the excessive stimulation of leucocytes and macrophages. When their phagocytic abilities are exceeded the outflow of the digestive granule contents occurs. This leads to the release of the lysosome enzymes and the active oxygenic metabolites from the extracellular spaces, thus resulting in the existing inflammatory changes.

Moreover, neutrophils release the followng enzymes: elastase, collagenase, cathepsin g. The released proteases may be involved in the activation of kinins, complement, clotting sytem and fibrinolysis. The great number of the biologically active substances are produced, among others leucotriens, which may cause the pathological changes in the distant organs, mostly in the liver.

The exceeded phagocytic abilities of macrophages and thus the excessive secretion of cathepsin, the platelet activating factor, and of some other harmful factors leads to the multiorgan insufficiency, especially the liver failure (10, 10, 16). Taking into consideration the reports concerning the positive effect of the platelet activating inhibitor (BN 52021) on acute experimental pancreatitis, in group V the inhibitor was given prior to the 12 hrs' caerulein infusion (5, 24). There are some papers proving the beneficial influence of this inhibitor on the inflammatory and the allergic processes (13, 22, 24). The results obtained by us confirm the good effect of this inhibitor on the liver damage in AP, which was manifested in the normal aminophenazone elimination. This effect may result from the decrease of the pancreas inflammatory infiltration caused by the inhibitor of the platelet activating factor (5, 24).

Analysing the results obtained we answered the previously mentioned questions:

1. In acute experimental pancreatitis induced by the excessive caerulein stimulation the diminished aminophenazone elimination was observed, which proves the impaired metabolic function of the liver.

2. The disorders of the aminophenazone elimination depend on the duration and the severity of AP.

3. The administration of the platelet activating factor inhibitor (BN 52021) significantly improved the liver metabolic efficiency manifesting itself in the proper aminophenazone elimination.

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STRESZCZENIE

Celem podjętych badań było sprawdzenie, czy w ostrym zapaleniu trzustki (ODZT) dochodzi do uszkodzeń wątroby i bliższe poznanie patomechanizmów ich powstawania. Do oceny funkcji metabolicznej wątroby zastosowano perfuzję izolowanej wątroby według metody Millera w modyfikacji Hafta i test z aminofenazonem jako wskaźnik sprawności metabolicznej izolowanej perfundowanej wątroby szczura.

Materiał doświadczalny stanowiło 80 białych szczurów szczepu Wistar, które zostały podzielone na 8 grup, po 10 szczurów w każdej grupie. ODZT wywoływano przez ciągły dożylny wlew ceruleiny przez 3, 6 i 12 godz. w dawce 5×10^{-6} i $7{,}5 \times 10^{-6}$ g/kg/godz.

Uzyskano statystycznie istotne różnice w eliminacji amidopiryny w poszczególnych grupach badanych w porównaniu z grupą kontrolną. W grupach, w których wywoływano ostre zapalenie trzustki o najdłuższym i najcięższym przebiegu, stwierdzono upośledzoną eliminację amidopiryny w porównaniu z grupą kontrolną ($p < 0{,}001$), świadczącą o zaburzonej funkcji metabolicznej wątroby. Natomiast podanie inhibitora czynnika aktywującego płytki (BN 52021) przed infuzją ceruleiny powodowało normalizację eliminacji amidopiryny.