ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 93 SECTIO D 2006

Department of Gastroenterology, Department of Obstetrics and Perinatology
Medical University of Lublin

KATARZYNA LASKOWSKA, BARBARA SKRZYDŁO-RADOMAŃSKA, MARZENA LASKOWSKA, PIOTR RADWAN, MARIA SŁOMKA

The comparison of histamine receptors H_2 antagonist and proton pump inhibitor influence on the course of experimental caerulein-induced acute pancreatitis in rats

Despite the recent improvement in diagnosis and therapy acute pancreatitis still remains a serious illness with high risk of death. Alcohol abuse and gallstones are the main causes of the disease in about 75% of cases. The other reasons are: hyperlipidaemia, hypercalcaemia, drugs, infections, injuries and abdominal surgery. Sometimes, however, the cause still remains unknown (7).

Premature activation of pancreatic enzymes is the central step in the pathogenesis of acute pancreatitis. Pancreatic exocrine secretion is controlled by numerous hormones, mainly cholecystokinine (CCK) and nervous system, especially vagus nerve (n. X) (7, 12). Activation of intrapancreatic cholinergic neurons stimulates pancreatic exocrine secretion directly due to acetylocholine (Ach) and via CCK and other neuropeptides, like vasoactive intestinal peptide (VIP) (7, 11). Cholinergic system is stimulated by acetylocholine (Ach), which when releasing is inhibited by atropine, somatostatin and H_3 presynaptic receptor stimulation (11, 12). CCK acts by CCK-A receptor, placed on afferent endings of n. X and CCK-B receptor placed on pancreatic acini (7). Histamine plays an important role in exocrine pancreatic function not only in physiological conditions, but also during acute pancreatitis. In general, histamine stimulates pancreatic secretion, but less than Ach (12). Histamine activity, as a neurotransmitter, is observed in the central and peripheral nervous system but this biogenic amine is present also in peripheral cells like basophiles, leukocytes, mastocytes, thrombocytes, enterochromaffin-like cells and the histaminergic nervous system. Histamine exerts its influence on digestive tract and blood vessels (4). Histamine, acting via its receptors – H_{1-4}, stimulates not only pancreatic but also gastric and intestinal secretion and inhibits duodenal secretion and somatostatin secretion by duodenal D cells (9).

H_2 receptor is widely spread in the nervous system and peripheral tissues (14) and plays an important role in allergic reactions and inflammatory processes, and increases gastric and intestinal secretion (6). On the other hand, some studies reported that H_2 receptor stimulation, using dimaprit as the receptor agonist, exerts antiinflammatory influence due to inhibition of phagocytosis, chemotaxis, superoxides anions production and synthesis of TNF α (8). Besides, dimaprit decreases synthesis of nitric oxide (NO), which is a known protective factor in inflammatory processes, so dimaprit may increase the severity of acute pancreatitis (10). The exact role of H_2 receptor in pancreatic exocrine function is not clear, and studies results are inconsistent. Some authors reported that H_2 receptor stimulation inhibits pancreatic secretion and H_2 receptor blocking stimulates pancreatic secretion (9). Other investigators postulate quite different effect of H_2 receptor agonists and antagonists (1). Besides, the effects of various H_2 antagonists were different and connected with various influence on the cholinergic system. Ranitidine exerts cholinomimetic action, so it potentiates the effect of caerulein, while cimetidine has cholinolytic activity, and thereby decreases pancreatic secretion (1).

It is generally agreed that the trigger mechanism in the initiation of acute pancreatitis is intracellular activation of pancreatic enzymes and autodigestive processes in the pancreas (2). The further mechanism of development and dissemination of pancreatitis is activation of inflammatory mediators such as tromboxan-TXA_2, leukotrien LTB_4, platelet activating factor (PAF) (15). Among the inflammatory mediators cytokines play an important role, especially interleukins- IL-1β, 4, 6, 8 and TNFα and NF-κB responsible for synthesis and releasing following cytokines and chemokines (13). One of the sources of inflammatory mediators are mastocytes. Disturbances of blood supply, connected with NO synthesis, vasoconstriction and increased vascular permeability are the other pathophysiological mechanisms of acute pancreatitis development (5). pH of gastric fluid entering to duodenum plays an important role in pancreatic exocrine secretion. Acid duodenal content stimulates pancreatic secretion, especially bicarbonates and water (7). An exact role of inhibition of gastric acid secretion on the course of acute pancreatitis is not clear and requires further studies, although some investigators ignore the role of H_2 receptors antagonists and proton pomp inhibitors (7). Also gastric acid secretion may be inhibited by administration of H_3 receptors agonist.

MATERIAL AND METHODS

The study was performed on male Wistar rats. The animals were divided into 6 groups, containing 10 rats in each group. Acute pancreatitis was initiated by 4 subcutaneous caerulein injections in the dose 20 μg/kg in 1-hour intervals. The blood and pancreatic samples were taken 2 h after last caerulein injection. The histamine receptors ligands and proton pump inhibitor were administered for 5 consecutive days prior to initiation of acute pancreatitis. The intensity of pancreatitis was evaluated by serum lipase and amylase activity measured in international units (IU), using a colorimetric method. Inflammatory process in pancreas was also assesed as morphological changes in the light an electron microscopy (interstitial and perivascular oedema, vasodilatation, blood stasis, neutrophils' and lymphocytes' infiltration, vacuolisation, tight junction and cytoskeleton destruction, extension of the endoplasmic reticulum ducts, decreased number of zymogen granules and nuclear chromatine condensation).

I group (N) – treated only with 0.9% NaCl injection, II group (C) – control – treated only with 4 caerulein injections, III group (R1) – treated with the lower dose (2 mg/kg) of ranitidine – H_2 receptor antagonist, IV group (R2) – treated with the higher dose (4 mg/kg) of ranitidine – H_2 receptor antagonist, V group (P1) – treated with the lower dose (1 mg/kg) of pantoprazol – proton pump inhibitor, VI group (P2) – treated with the higher dose (2 mg/kg) of pantoprazol – proton pump inhibitor.

RESULTS

Results of biochemical studies – serum lipase and amylase activity are described in Tables 1 and 2. Results of histological studies were consistent with biochemical results. It is worth to emphasize that no destructive features were observed in pancreatic islets in the studied groups. In group I (N) – treated with 0.9 % NaCl the histological picture showed normal acinar cells with normal nucleus, cytoplazm and zymogen granules. In group II (C) – treated only with caerulein – typical picture of oedematous acute pancreatitis: interstitial and perivascular oedema, vasodilatation and blood stasis, inflammatory infiltrate consisting of neutrophils and lymphocytes, and vacuoles in the cytoplasm and decreased number of zymogen granules in acinar cells were observed. The emphatic intensification of histological inflammatory features appeared in group IV (R2). Intense perivascular and interstitial oedema, intense vacuolisation of cytoplasm and blood stasis in comparison with group II (C) were observed in the light microscopy picture. Besides, intensification of extension of reticulum ducts, mitochondrial oedema and decreased number of zymogen granules and nuclear chromatin condensation were present in the electron microscopy picture. The emphatic amelioration of histological inflammtory features appeared in groups:

V (P1), and VI (P2). Decreased number of vacuoles, neutrophils and lymphocytes and perivascular and interstitial oedema in comparison with group II (C) were observed in the light microscopy picture. Minimal interstitial oedema, single vacuoles and minimal changes in cell's nuleus in comparison with group II (C) were present in the electron microscopy picture.

Table 1. The serum amylase activity in the studied groups

Group	Amylase activity	Statistical significance in comparison with group n	Statistical significance in comparison with group c
I- N	379.4 +/– SD 73.87		*
II-C	6654.4 +/– SD 2071.48	*	
III-R1	6536.6 +/– SD 2131.3	*	
IV-R2	11876.89 +/–SD 2995.74	*	*
V-P1	818.11 +/– SD 218.73	*	*
VI-P2	1012.89 +/– SD 545.17	*	*

* Statistical significance – $p < 0.05$, not statistical significance – $p > 0.05$

Table 2. The serum lipase activity in the studied groups

Group	Amylase activity	Statistical significance in comparison with group N	Statistical significance in comparison with group C
I- N	29.6 +/– SD 7.54		*
II-C	1783.8 +/– SD 938.23	*	
III-R1	1980.8 +/– SD 1313.62	*	
IV-R2	2483.11 +/–SD 497.99	*	
V-P1	43.78 +/– SD 23.9		*
VI-P2	54.33 +/– SD 40.37		*

* Statistical significance – $p < 0.05$, not statistical significance – $p > 0.05$

DISCUSSSION

The aim of this study was to compare the influence of H_2 receptors antagonist with the effect of proton pomp inhibitor – pantoprazol on the course of this disease. The cerulein-induced acute pancreatitis is a common experimental model used in many studies (2). This is a mild, oedematous type of pancreatitis without parenchymal necrosis and haemorrhage. The subcutaneous cerulein administration induces typical elevation of amylase and lipase activities in comparison with the control group (6654 IU versus 379 IU and 1783 IU versus 29 IU respectively). These results were

confirmed by histological changes in both light and electron microscopy. In the light microscopy the interstitial and perivascular oedema, vasodilatation, blood stasis, neutrophil's and lymphocyte's infiltration and vacuolisation were observed (2). In the electron microscopy the tight junction and cytoskeleton destruction, vacuolisation, extension of the endoplasmic reticulum ducts, decreased number of zymogen granules and nuclear chromatin condensation were observed (3).

The exocrine pancreatic function is controlled by the cholinergic system with participation of Ach, and by CCK and secretin (1, 7, 11, 12). This activity is connected with membrane protein G and second messenger system (1,4,5-triphosphate inositole and diacyloglyceride – IP_3 and DAG) and changes of the intracellular calcium level. The acinar cell stimulation causes the increased intracellular calcium level and exocytosis of zymogen granules into the pancreatic ducts (7, 11).

Histamine, acting via its receptors, especially H_2 and H_3 receptors, plays an important role in basal and stimulated pancreatic secretion. This biogenic amine exerts its effect by receptors, membrane G protein and changes in the intracellular calcium level in pancreatic acinar cells (4, 7, 15). The selective histamine receptors ligands were used in our study, however these ligands were not super selective and exerted their different activities on all type receptors. Histamine stimulates pancreatic secretion mainly through the H_1 receptor (9, 12). Histamine is produced by mastocytes, basophiles, neurons and endocrine cells (9, 12), which are activated in inflammatory processes. Mastocytes and some inflammatory infiltration cells produce not only histamine, but also leukotriens (LT), prostaglandines (PG), tromboxanes (TX) etc. Histamine causes increased vascular permeability, smooth muscle contraction, chemokinesis and in this way aggravates inflammation.

In the presented study administration of the H_2 receptor antagonist – ranitidine, especially in the higher dose – aggravated AP, but some authors reported that H_2 receptor antagonist stimulated pancreatic secretion (9) and some investigators noted that ranitidine inhibited pancreatic secretion in basal circumstances (1). H_2 receptors blocking by ranitidine directs histamine to other histamine receptors, especially H_1, and in this way increases not only pancreatic secretion, but also synthesis of prostacyclines, PAF and NO. H_1 receptor stimulation aggravates the course of inflammation and increases exocrine pancreatic secretion, especially water and bicarbonates production (9). Ranitidine – H_2 receptor antagonist – has also cholinomimetic activity and in this way can additionally stimulate pancreatic secretion and increases unprofitable caerulein activity. The other H_2 receptor antagonists, for example cimetidine, have rather anticholinergic activity (1). It must be emphasized that this activity of H_2 antagonists is observed in doses higher than those administered in therapy of ulcer disease (1). So we can say that higher doses of H_2 antagonist are not beneficial in the course of AP, although H_2 receptor stimulation aggravates inflammatory processes. The proton pump inhibitor (PPI) – pantoprazol administration ameliorated the course of AP in the presented study. The decreased lipase and amylase activity and reduction of inflammatory features in histological examination was observed. The gastric acid secretion inhibition was much more effective after PPI than after H_2 receptor antagonist administration. This phenomen is probably connected with an additional effect of H_2 receptor antagonist. Ranitidine, however, exerts its biological activity not only by influence on gastric acid secretion but also by influence on H_2 receptors presented in stomach and pancreas (1). PPI is deprived of cholinomimetic activity of ranitidine. Some investigators reported much more effective gastric acid secretion inhibition after PPI administration in comparison with ranitidine or cimetidine. Long-term PPI therapy may be connected with increased gastrin releasing. Hipergastrinaemia, however, leads to ECL cells hyperplasia, growth processes or atrophic gastritis.

Some authors reported other factors, besides PPI and H_2 antagonists, inhibiting gastric acid secretion, for example antagonists of gastrin and gastrin-related peptide (GRP), H_3 receptor ligands and antagonist of CCK-2 receptors and NO through their influence on gastrin and somatostatin releasing.

CONCLUSIONS

1. Administration of H_2 receptor antagonists, especially in the higher dose, is not beneficial in therapy of acute pancreatitis, although H_2 receptor stimulation aggravates inflammatory processes.
2. Administration of proton pump inhibitor – pantoprazol – is beneficial in therapy of acute pancreatitis.

Acknowledgements. Very special thanks to Prof. Elżbieta Korobowicz and Dr Agnieszka Korolczuk for performing the histological part of the study in the Department of Clinical Pathomorphology, Medical University of Lublin, Poland.

REFERENCES

1. Bertaccini G., Coruzzi G.: Histamine H_2 receptor agonists and antagonists on pancreatic exocrine secretion of the dog. Agents and Actions, vol.16, ¾, 35, 1985.
2. Białek R. et al.: Evidence of intracellular activation of serine proteases in acute cerulein-induced pancreatitis in rats. Scand. J. Gastroenterol., 12, 190, 1991.
3. Fallon M.: Effect of caerulein hyperstimulation on the paracellular barrier of rat exocrine pancreas. Gastroenterology, 108, 1863, 1995.
4. Hill S.J.: Distribution, properties and functional characteristics of three classes of histamine receptor. Pharmacol. Rev., vol.42, No 1, 45, 1990.
5. Jaworek J.: Leptin protects the pancreas from damage induced by caerulein overstimulation by modulating cytokine production. Pancreatology, 292, 89, 2002.
6. Kohno S.: Dimaprite, a histamine H_2 agonist, inhibits anaphylactic histamine release from mast cells and the decreased release is restored by thioperamid (H_3 antagonist) but not by cimetidine (H_2 antagonist). Japanese J. Pharmacol., 62, 1, 75, 1993.
7. Konturek S.J.: Gastroenterologia i hepatologia kliniczna. PZWL, 4th ed. 4, 2001.
8. Nakamura T.: Efficacy of the selective histamine H_2 receptor agonist, dimaprite, in experimental model of endotoxin shock and hepatitis in mice. Eur. J. Pharmacol., 322, 1, 83, 1997.
9. Nguyen T.: Histamine stimulate ion transport by dog pancreatic dog epithelial cells through H_1 receptors. Am. J. Physiol., 275, 76, 1998.
10. Paquay J.P.G.: Nitric oxide synthase inhibition by dimaprite and dimaprite analogues. Br. J. Pharmacol., 127, 331, 1999.
11. Park S.H.: Significant cholinergic role in secretin-stimulated exocrine secretion in isolated rat pancreas. Am. J. Physiol., 274, G413, 1998.
12. Rosado J.A.: Histamine-evoked potassium release in the mouse and guinea pig pancreas. Pancreas, vol.12, No 4, 396, 1996.
13. Slogoff M.I.: COX-2 inhibition results in alteration in nuclear factor NF-κB activation but not cytokine production in acute pancreatitis. J. Gastrointest. Surg., 8, 4, 511, 2004.
14. Van der Goot H., Timmerman H.: Selective ligand as tool to study histamine receptors. Eur. J. Med. Chem., 35, 1, 5, 2000.
15. Zhou W et al.: Evidence for altered cellular calcium in the pathogenetic mechanism of acute pancreatitis in rats. J. Surgical Research, 60, 147, 1996.

SUMMARY

There are many studies investigating pathomechanism leading to the development of acute pancreatitis, however, the issue still remains unclear. The central step in pathogenesis of this disease is premature activation of pancreatic enzymes. Pancreatic exocrine secretion is controlled by hormones and the nervous system, especially vagus nerve and histamine, acting via its receptors. Also pH of gastric fluid, entering to the duodenum, plays an important role in pancreatic

exocrine function. Acid duodenal content stimulates pancreatic secretion, but an exact role of gastric acid secretion inhibition on the course of acute pancreatitis is not clear. The aim of our study was to compare the influence of H_2 receptor antagonist – ranitidine with proton pump inhibitor – pantoprazol on the course of experimental caerulein – induced acute pancreatitis. The study was performed on male Wistar bread rats. Oedematous type of acute pancreatitis was initiated by four subcutaneous caerulein injections. The histamine receptors ligands and proton pump inhibitor were administered for five consecutive days prior to initiation of acute pancreatitis. The intensity of pancreatitis was evaluated by serum lipase and amylase activity and also assesed as a morphological changes in the light and electron microscopy. Biochemical and morphological results were consistent. The emphatic intensification was observed in the group treated with the higher dose of H_2 receptor antagonist. The emphatic amelioration was observed in the group treated with proton pump inhibitor in both administered doses. 1. Administration of H_2 receptor antagonist – ranitidine, especially in the higher dose aggravates inflammation processes in the course of acute pancreatitis. 2. Administration of the proton pump inhibitor – pantoprazol is beneficial in therapy of acute pancreatitis.

Porównanie wpływu antagonisty receptorów H_2 i inhibitora pompy protonowej na przebieg ostrego indukowanego ceruleiną zapalenia trzustki u szczura

Przeprowadzono wiele badań dotyczacych patomechanizmu rozwoju ostrego zapalenia trzustki, ale istota schorzenia nadal pozostaje nie w pełni jasna. Wiadomo, że głównym mechanizmem patogenetycznym jest przedwczesna, wewnątrztrzustkowa aktywacja enzymów trzustkowych. Czynność zewnątrzwydzielnicza trzustki pozostaje pod kontrolą licznych enzymów, układu nerwowego, zwłaszcza nerwu błędnego, oraz histaminy, działającej za pośrednictwem swoistych receptorów. Znaczenie ma także pH soku żołądkowego, dostającego się do dwunastnicy, bowiem kwaśna treść żołądkowa stymuluje wydzielanie trzustkowe, choć dokładna rola hamowania wydzielania kwasu żołądkowego w przebiegu ostrego zapalenia trzustki nie jest zbadana. Celem niniejszej pracy było porównanie wpływu antagonisty receptora H_2- ranitydyny z inhibitorem pompy protonowej – pantoprazolem na przebieg ostrego, eksperymentalnego, indukowanego ceruleiną zapalenia trzustki. Badania przeprowadzono na samcach szczurów rasy Wistar. Obrzękowa postać ostrego zapalenia trzustki była inicjowana przez cztery podskórne iniekcje ceruleiny. Ligandy receptora histaminy oraz inhibitor pompy protonowej były podawane przez pięć kolejnych dni poprzedzajacych ostre zapalenie trzustki. Nasilenie procesu zapalnego trzustki oceniano na podstawie zwyżki aktywności amylazy i lipazy w surowicy krwi oraz na podstawie zmian morfologicznych trzustki ocenianych w mikroskopie świetlnym i elektronowym. Wyniki badań biochemicznych i morfologicznych były zgodne. Nasilenie zapalenia stwierdzono po podawaniu wyższych dawek antagonistów receptora H_2, zaś zmniejszenie procesu zapalnego obserwowano po podawaniu inhibitorów pompy protonowej niezależnie od dawki. Stosowanie antagonisty receptora H_2 – ranitydyny, zwłaszcza w dawce większej, nasila odczyn zapalny w przebiegu ostrego, eksperymentalnego, indukowanego ceruleiną zapalenia trzustki. Podawanie inhibitora pompy protonowej – pantoprazolu, niezależnie od dawki, wywiera korzystny wpływ na przebieg tego schorzenia.