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TEMPORARY ELEVATION OF PANCREATIC LYSOSOMAL ENZYMES, AS A RESULT OF THE OMEPRAZOLE-INDUCED PERIPANCREATIC INFLAMMATION IN MALE WISTAR RATS

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Omeprazole is one of the substituted benzimidazoles, which is not free of side effects. The aim of the study was to evaluate the influence of omeprazole therapy on pancreas. Omeprazole was administered intraperitoneally, twice a day, for 3 days to the male rats in 0.571 mg/kg b.w. and 5.71 mg/kg b.w. doses. Half of animals were sacrificed in the 4th day of the experiment. The remaining rats were raised for another 6 weeks, without any xenobiotics, and sacrificed on the 47th day. The activity of acid phosphatase, beta-galactosidase, cathepsin B, and L, lipase, N-acetyl-glucosaminidase, and sulphatase was evaluated. The slides of the pancreas were examined in light microcopy in hematoxylin-eosin, asan, periodic acid-Schiff (paS) stains. Statistical increase in total activities of acid phosphatase, beta-galactosidase, lipase, N-acetyl-glucosaminidase, sulphatase, and acute inflammatory infiltration in peripancreatic fat tissue without histological pancreas impairment, were observed after the higher dose on the 4th day of experiment. Histological picture and enzymatic profiles were normalized during the next 6 weeks. We concluded that intraperitoneal administration of omeprazole causes tissue inflammation in the peripancreatic lipid tissue and reactive elevation of some pancreatic lysosomal enzymes.

Key words: *omeprazole, pancreas, acid phosphatase, beta-galactosidase, cathepsin B, cathepsin L, lipase, N-acetyl-glucosaminidase, sulphatase, rat*

INTRODUCTION

Chronic peptic ulcer disease, reflux esophagitis and some other ailments dependent on hydrochloric acid exertion, especially those resistant to H2 blokers therapy, are the main indications for omeprazole administration (1, 2). The mechanism of its action is the drugs ability to bind with cysteine's — SH radicals in positions 813 and 892 in segments 5/6, 7/8, and loops connecting H^+/K^+ of ATP-ase (3—5). It results in inhibition of proton pump activity

responsible for H^+ ions excretion to the gastric lumen. This activity is strong enough, to evoke a long-term omeprazole induced hypochlorhydia leading to malignant transformation in gastric mucous membranes, as proven in experimental models (1, 6).

The proton pump of the same or alike mechanism is also present in other organs, e.g. distal part of colon, bile ducts epithelium, and corneal epithelium. Omeprazole, like other substituted benzimidazoles, is activated in organism in a low pH environment. Such pH exists not only in the oxynthic cells but also in lysosomes. It was also observed in other compartments of strong acidity in macrophages, neutrophils, lymphocytes, tissues and organelles of some organs having proton pump. Some of these are: the smooth muscles of blood vessels, bile ducts epithelium, liver and heart muscle's mitochondria. Omeprazole induced thiol groups blockade in these tissues and organelles may result in the temporary inhibition of their physiological function, or may lead to their damage. This mechanism is even more probable due to a fact, that as a result of acid environmental transformation of pro-drug a weak cation is created, for which cellular membranes are relatively impermeable. Therefore, the internal concentration of the active form is much higher than the impermeable form in blood serum (7, 8, 9).

Omeprazole, as a non-selective drug, may block the transformation in all organs were proton pomp is located, which may result in various advarse effects (7, 8). The most common are as follows: diarrhea, nausea, pain, vertigo, gastric complaints, exanthema, flatulence, pruritus and vomiting (1, 3).

This experiment is a part of a large toxicology project, which is designed to investigate acute and late effects of proton pump inhibitors on morphology and activity of lysosomal enzymes in different internal organs as a result of intraperitoneal administration. Intraperitoneal multiinjection in rats is a relevant model of drug administration to the human intravenous injection (10). Lysosomal enzymes were chosen due to their feature of being sensitive indicators of a current organ function. However, their increase can be observed in various ailments, not always directly impairing physiology of the examined organ (11—14). The immediate, as well as the six-weeks post-administration influence of three-days omeprazole administration on pancreas and peripancreatic tissue is determined in this study.

MATERIALS AND METHODS

The whole experiment was based on animal experimental model, and designed according to the guidelines of Bioethical Committee University School of Medicine of Lublin, Poland.

Experiment was conducted on male rats of Wistar breed with initial body weight of 180 ± 15 g. The animals were housed in standard laboratory cages (max. 5 per cage) at a room temperature of

20±3°C in a daylight cycle. Standard laboratory chow and tap water were provided ad libitum. Food and water consumption were monitored daily.

After a two weeks acclimation period, animals were gathered in experimental groups of minimum 10 in a group. Animals were weighed on the 1st, 4th, 11th, 18th, 25th, 39th, and 47th day of experiment.

Tested substance — omeprazole (ASTRA, Sweden) was administered intraperitoneally (right hypogastric region) twice a day (7 a.m., 7 p.m.) for three days. The initial dose — O_1, corresponding to the human therapeutic dose, was 0.571 mg/kg of body weight (1). Due to faster rat's metabolism the second dose — O_2, was increased 10-times and fixed at 5.71 mg/kg of body weight. The drug was dissolved in a volume of 4 ml/kg. In control group — CON (n = 20) animals received the adequate volume of physiological saline (Polfa-Tarchomin, Poland).

Animals in groups O_1I, O_2I, and half of the control group — CON-I, were decapitated in 12 hours after the last injection. The remaining animals, in groups O_1II, O_2II and CON-II were raised for another 6 weeks without any xenobiotics, and sacrificed on the 47th day of the experiment.

Animals were dissected immediately after decapitation. Internal organs were investigated during autopsy. The whole pancreas with surrounding fat tissue and peritoneum was excised. Fragments of organ assigned for histological examination were fixed in 10% buffered formaldehyde solution, and routinely transformed into paraffin sections. Histological preparations were evaluated in light microscope (Axiscop, Zaiss), after hematoxylin-eosin, azan, and histochemical paS (periodic acid-Schiff) stains. The remaining parts of pancreas were frozen in a liquid nitrogen and stored in a temperature −20°C. Pancreases, after being defrosted in the temperature of melting ice, were dissected from surrounding fat tissue and then homogenized. The total activity of lysosomal enzymes such as acid phosphatase, beta-galactosidase, cathepsin B, and L, lipase, N-acetyl-glucosaminidase, and sulphatase in obtained homogenate was evaluated spectrophotometrically, according to the methods described by us (11, 12).

For statistical analysis the ANOVA test was used to compare results between the groups. All data are expressed as Mean±SEM. An associated probability (p-value) of less than 5% was considered significant (10, 15).

RESULTS

None of the animals died in the course of experiment, nor any behavior changes were observed. Throughout the duration of experiment, the omeprazole-treated animals consumed as much food and water as the controls and gained comparable weight ($p > 0.05$).

Histological evaluation revealed the presence of large inflammatory infiltrations in peripancreatic fat tissue in 4 preparations of O_1I group (*Fig. 1b*). Less intense pathological changes of this kind were also observed in two others, and were absent in the remaining three. Single granulocytes in peripancreatic tissue were observed in one animal of O_1I group. These alterations were not seen in the other animals of O_1I group, control, and the ones that were killed on day 47 (*Fig. 1a*).

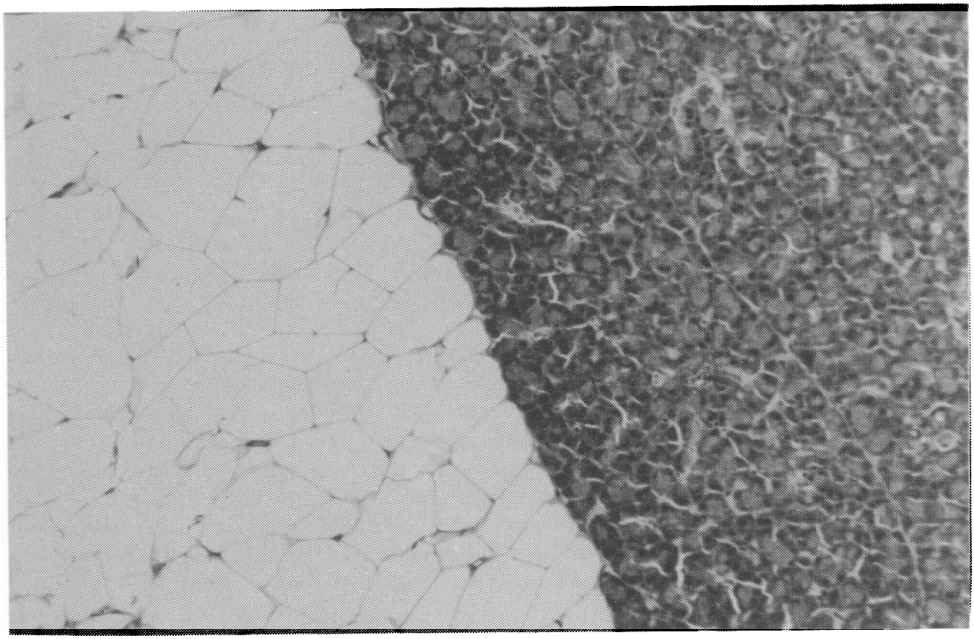

Fig. 1a. Normal (control) pancreas and peripancreatic fat tissue (H+E, ×320)

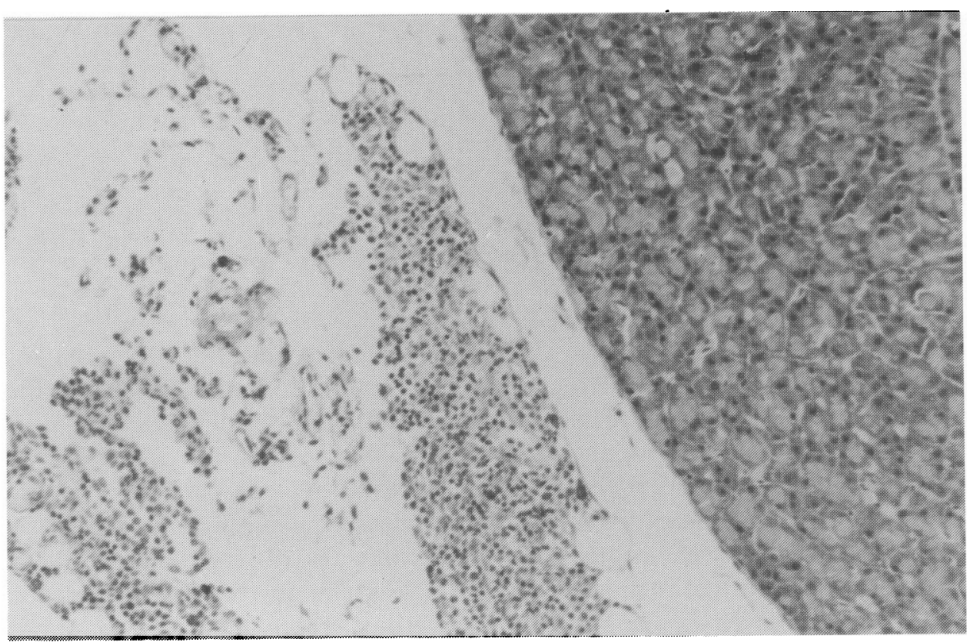

Fig. 1b. Large inflammatory infiltration in peripancreatic fat tissue in omeprazole-treated rat — O_2I group (H+E, ×320)

Numerous single granulocytes were observed in the pancreatic parenchyma, next to the pancreatic ducts. They were sporadically noted in all experimental groups, including the control ($p > 0.05$). No disorders in the appearance of Langerhans' islets, and connective tissue stromal transformation were disclosed.

Mean ± SEM values of activity of particular lysosomal enzymes are shown in *Table 1.*

Table 1. Total activity (mean ± SEM) of pancreatic lysosolomal enzymes in control and omeprazole-treated rats.

Enzymes	CI	CII	O_1I	O_1II	O_2I	O_2II
Acid phosphatase (pmol/1 mg of protein)	8.57 ± 0.34 ***	8.42 ± 0.51 ***	9.54 ± 0.25 **	9.38 ± 0.35	11.94 ± 0.32	9.71 ± 0.30
Beta--galastosidase (pmol/1 mg of protein)	1.41 ± 0.10 **	1.13 ± 0.12 ***	1.27 ± 0.12 ***	1.52 ± 0.09 **	2.23 ± 0.12	1.48 ± 0.07 **
Cathepsin B (nmol/1 mg of protein)	5.48 ± 0.49	6.32 ± 0.37	6.60 ± 0.70	5.88 ± 0.46	6.38 ± 0.27	6.37 ± 0.44
Cathepsin L (nmol/1 mg of protein)	2.50 ± 0.19	2.46 ± 0.15	2.54 ± 0.11	2.23 ± 0.24	2.63 ± 0.16	2.42 ± 0.21
Lipase (pmol/1 mg of protein)	7.81 ± 0.91 *	9.40 ± 0.25	7.33 ± 0.12	8.80 ± 0.86	10.60 ± 0.27	9.39 ± 0.35
N-acetyl--galactosidase (pmol/1 mg of protein)	3.30 ± 0.30 ***	3.80 ± 0.29 **	3.78 ± 0.40 *	4.06 ± 0.30 *	5.63 ± 0.17	4.44 ± 0.25
Sulphatase (pmol/1 mg of protein)	52.07 ± 5.09 **	52.34 ± 6.11 **	43.19 ± 5.66 ***	51.43 ± 9.17 **	91.71 ± 2.64	52.98 ± 5.06 **

* $p < 0.05$ as compared to the value with O_2I, ** $p < 0.01$ as compared to value with O_2I, *** $p < 0.001$ as compared to value with O_2I.

Statistical analysis revealed significant acid phosphatase, beta-galactosidase, lipase, N-acetyl-glucosaminidase, and sulphatase activities increase in animals receiving omeprazole in the higher dose in O_2I Group in

comparison with control group. Other enzyme activities did not demonstrate statistically significant differences.

DISCUSSION

The results show that a short-term omeprazole administration in intraperitoneal injections in dose 5.71 mg/kg of body weight initiates acute inflammation in the peripancreatic fat tissue. These changes were accompanied by the increase of lipase, N-actylgal-galactosidase and sulphatase activities in pancreatic parenchyma. Histological features of organ impairment were absent. Histological images, as well as enzymatic profiles, were normalized during the next 6 weeks of experiment. Concluding, observed changes seem to be of temporary character, and may be interpreted as the organism reaction towards omeprazole.

Acid reaction of the preparation ($pH < 4.5$), that might have evoked granulocytes chemotaxis due to chemical irritation, may be responsible for the initiation of inflammation (9). Lysosomal enzymes activity, observed in O_2I group, seems to be secondary to the inflammatory process in the neighborhood.

Intraperitoneal omeprazole administration introduced in our test, is purely experimental. In clinical practice, it is used as intravenous injections and as pills administered orally (1, 2). Therefore, all the changes observed in our study seem to be of experimental character. However, it seems that similar mechanism may be responsible for other adverse reactions appearing during proton pump inhibitors therapies.

In previous study of omeprazole being administered in single intraduodenal injection to male Wistar rats non-significant elevation of pancreatic lipase and phospholipase activities were observed (16). However, the activities of pancreatic trypsinogen and serum amylase were statistically higher in drug-treated group than in control one.

It is has already been proven that gastric acid plays an important role in pancreatic enzyme synthesis and secretion. They are mediated by gastrin and duodenal cholecystokinin (2, 17—23). Gastric acid inhibition, due to the administration of proton pump inhibitors e.g. omeprazole, evokes dose-dependent trophic changes such as increased stomach weight, thickness and mass of the gastric oxyntic mucosa, which are related to high serum gastrin level. Those were observed in rat, hamster, guinea pig, and chicken (17—19). Elevated plasma gastrin level and histidine decarboxylase activity returned to the control level after omeprazol — free recovery period. The ECL density was the only value that still remained higher than in the age-control group (18). Those temporary, omeprazole-dependent effects are similar to our results. However, authors did not observe any pancreatic changes, probably because of

the different administration technique. The lack of effect of omeprazole oral administration on rat pancreas was also reported by Niederau *et al.* (22). It was also proven by Chen *et al.* (23), who observed insignificant differences in weight and DNA content of the pancreas between experimental and control groups.

CONCLUSIONS

1. Omeprazole peritoneal administration initiates inflammatory changes in peripancreatic lipid tissue and activates pancreatic parenchymal lysosomal enzymes.
2. Observed changes were of temporary character and regressed after drug withdrawal.

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