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Kierownik: prof. dr hab. Zbigniew Wójtowicz

FRANCISZEK BURDAN, BARBARA BURAK, KRZYSZTOF BURSKI,
PIOTR RUCIŃSKI, MAŁGORZATA PLISZCZYŃSKA

Activity of cathepsins and morphological changes in the rat's liver after ranitidine and famotidine administration

Wpływ famotydyny i ranitydyny na aktywność katepsyn
i morfologię wątroby szczura

The new histamine H2–receptor antagonists represented in our study by ranitidine and famotidine are commonly used group of drugs, not only in gastroenterology, but also in other medical specialties. They replace slowly the first introduced drug of this group – cimetidine due to its generally known toxicity. In most of the Western world countries, they belong to OTC (over–the–counter) group of medications, which makes them one of the most spread out and advertised active substances available in the market. However, as well as other xenobiotics, they are not free of various side effects (1, 9).

The aim of this study was to evaluate the influence of famotidine and ranitidine long–term time administration on the activity of liver cathepsins and morphological structure of the liver.

MATERIAL AND METHODS

Our study was conducted on 60 white male rats of Wistar breed. The animals were obtained from Commercial Laboratory Animals Breed Station in Warsaw, Poland. After two weeks of acclimatization, the rats were divided into one control and four experimental groups (n = 12). The examined substances were administered every twelve hours intraperitoneally in the right hypogastric region, in two doses: F1 – 0.28 mg/kg of the body weight, F2 – 2.8 mg/kg of the body weight (famotidine – Gedeon Richter, Hungary); R1 – 0.71 mg/kg b.w., R2 – 7.1 mg/kg b.w. (ranitidine – GlaxoWellcome, UK). In the control group, the animals received 0.9% NaCl. After six weeks' administration of medicaments, the animals were decapitated. Liver samples were taken for biochemical and histological examinations. The activity of cathepsins B, D, and L were determined according to the method described in detail in currently available publication (5).

Fragments of the right lobe of the liver were fixed in 10% buffered formaldehyde solution and processed routinely. Light microscopic examination was performed after hematoxyline and eosin stain (H + E).

The study was fully approved by the Bioethical Commission of Medical University School of Lublin.

Statistical verification of obtained data was elicited using ANOVA test and personal computer (10). An $\alpha = 0.05$ ($p < 0.05$) was considered significant.

RESULTS

At the time of experiment, no changes in the behavioral activity were observed, the mortality rate was zero. Changes in body and liver weight were not statistically significant ($p > 0.05$), data not shown.

Changes in activity of free and bound B, D, L cathepsins' fractions in famotidine and ranitidine groups were not statistically significant in comparison with control and other experimental groups (Tab. 1 – 3).

Eosinophilic degeneration of hepatocytes appeared occasionally in all groups, both control and experimental. Focal cholestasis was found in one out of eight examined livers in R2 group. Local necrosis of hepatocytes with accompanying inflammatory infiltration was noted in two samples of R1, four of R2, three of F1, whilst only one in F2 group. Inflammatory infiltration without any signs of necrosis was observed around central hepatic vein, in one sample of R2, and one of F2 group.

DISCUSSION

The results of our study show that ranitidine and famotidine administered in OTC doses did not cause any significant changes in liver cathepsins activity and only minimal adaptive and degenerative changes in liver tissue.

The above results are fully confirmed by current available literature, and support the thesis that modern H2 blockers are relatively safe drugs that do not impair liver structure, and the function of this organ in both animal and human models (1, 4, 6, 12).

Lack of essential differences in the studied cathepsins activity can be explained by the fact that females were more sensitive to the drug than males in the case of oral administration of ranitidine, as proven in rats, mice, and rabbits by Tamura and co–workers (11). However, the vast range between the obtained data in the particular groups may suggest individual sensitivity for the examined substances.

The activity of cathepsins may be used as a sensitive indicator of various organs impairment, including liver (2, 3). In the erlier studies it was suggested that the increased lysosomal activity after H2 blockers administration could be involved in reducing the quantity of membrane after the membrane flow which occurs on gastric acid inhibition (8). Contrary, the more recent studies showed that the stabilization of lysosomal membranes and thus the prevention of lysosomal leakage may be one of the favourable additional mechanisms of antiulcer activity of H2 antagonists (7).

In normal human volunteers alanine aminotransferase values were increased to at least twice the pretreatment levels in half of the subjects receiving 100–mg q.i.d intravenously for 7 days, and in 4 of 24 subjects receiving 50–mg q.i.d intravenously for 5 days. In spite of general good tolerance of H2

Table 1. Activity of free and bound fraction of the cathepsin B in liver homogenate in experimental and control groups

	Group	Dose (mg/kg)	Activity of cathepsin B (nmol/1 mg of protein)	
			Free fraction Mean ± SD	Bound fraction Mean ± SD
0.9% NaCl	Control		18.816 ± 2.73	19.554 ± 2.36
Famotidine	F1	0.28	34.026 ± 10.40	35.354 ± 10.48
	F2	2.8	29.492 ± 3.42	19.546 ± 4.50
Ranitidine	R1	0.71	27.395 ± 3.07	37.105 ± 9.58
	R2	7.1	34.021 ± 7.97	37.870 ± 12.30

Table 2. Activity of free and bound fraction of the cathepsin D in liver homogenate in experimental and control groups

	Group	Dose (mg/kg)	Activity of cathepsin D (nmol/1 mg of protein)	
			Free fraction Mean ± SD	Bound fraction Mean ± SD
0.9% NaCl	Control		78.608 ± 13.52	62.787 ± 34.56
Famotidine	F1	0.28	89.564 ± 11.23	123.95 ± 61.21
	F2	2.8	80.784 ± 13.06	68.698 ± 13.36
Ranitidine	R1	0.71	70.276 ± 27.67	165.79 ± 75.18
	R2	7.1	101.39 ± 11.76	173.30 ± 57.52

Table 3. Activity of free and bound fraction of the cathepsin L in liver homogenate in experimental and control groups

	Group	Dose (mg/kg)	Activity of cathepsin L (nmol/1 mg of protein)	
			Free fraction Mean ± SD	Bound fraction Mean ± SD
0.9% NaCl	Control		31.508 ± 4.71	59.290 ± 15.8
Famotidine	F1	0.28	37.484 ± 8.02	39.015 ± 5.14
	F2	2.8	25.964 ± 3.61	42.324 ± 17.4
Ranitidine	R1	0.71	39.936 ± 6.09	39.294 ± 4.36
	R2	7.1	34.702 ± 7.52	37.528 ± 25.4

blockers, there have been occasional reports of hepatocellular, hepatocanalicular or mixed hepatitis with or without jaundice. These events were usually reversible, but in exceedingly rare circumstances death has occurred (1).

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STRESZCZENIE

H2 blokery są powszechnie używane nie tylko w gastroenterologii, lecz także w innych dziedzinach medycyny. Tak jak i inne ksenobiotyki leki te nie są wolne od działań ubocznych. Celem pracy była ocena wpływu ranitydyny i famotydyny na morfologię wątroby i aktywność frakcji wolnej i związanej katepsyn wątrobowych. Badania przeprowadzono na szczurach białych szczepu Wistar, którym podawano ranitydynę i famotydynę przez 6 tygodni dootrzewnowo. W homogenatach wątrobowych oznaczano aktywność frakcji wolnej i związanej katepsyn B, D i L. Ocenę histologiczną prowadzono po uprzednim wybarwieniu preparatów metodą hematoksylinowo–eozynową. W pracy obserwowano brak istotnych statystycznie różnic w aktywności badanych katepsyn w grupach doświadczalnych w porównaniu z grupą kontrolną i pomiędzy obu badanymi lekami. Pojedyncze zmiany histologiczne, jakie obserwowano, miały charakter zmian adaptacyjnych i minimalnych zmian degeneracyjnych.