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Activity of cathepsin B, D and L in rat cerebrum after cimetidine and famotidine administration

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Cathepsins are lysosomal enzymes that are used as sensitive markers in various toxicological investigations. The purpose of this study was to evaluate and compare the influence of cimetidine and famotidine on the cerebral cortex, particularly on the activity of cortical cathepsin B, D and L in the frontal lobe of rat brain. The drugs were administered intraperitoneally, twice a day, for six weeks to male Wistar rats in two doses. The initial dose was 2.85 mg/kg for cimetidine and 0.285 mg/kg for famotidine. The second dose was 10 times higher. Control animals were injected with 0.9% NaCl. Half of the animals from each of the drug-treated and control groups were sacrificed on the 42nd day of the experiment. The remaining animals were raised for another 6 weeks without any xenobiotics, and sacrificed on the 84th day. The frontal lobe of the right cerebral hemisphere was taken for biochemical investigation. The activities of free and bound fractions of cathepsin B, D and L were evaluated spectrophotometrically in cortical homogenates. The activity of bound fraction of cathepsin D and L decreased significantly in animals exposed to the higher dose of cimetidine and sacrificed on the 42nd day. Also significant elevation of the free fraction of cathepsin L was noted in the same group of rats. Cathepsin activities were normalized during the next six weeks. No behavioural changes were noted among the observed animals. Unlike cimetidine, famotidine did not change profiles of the cerebral cathepsins.

Keywords: famotidine, cimetidine, H_2-receptor antagonist, drug-toxicity, neurotoxicity, cathepsin B, cathepsin D, cathepsin L, brain, rat

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Famotidine (N'-(aminosulfonyl)-3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl] thio]-propanimidamide) and cimetidine (N"-cyano-N-methyl-N'-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]-ethyl]guanidine) are competitive and reversible inhibitors of the action of histamine at the histamine H_2-receptors, located in the basal membrane of the gastric parietal cells and in other organs and cells, e.g. brain, granulocytes and blood platelets. The drugs are widely used in treatment of duodenal and gastric ulcers, gastroesophageal reflux disease, Zollinger-Ellison syndrome, multiple endocrine adenomas and other pathological hypersecretory conditions (1, 4).

Generally both drugs are well tolerated; hence in some countries they belong to the OTC (over-the-counter) group. Similar to other xenobiotics however, they are not free of side-effects. Headache, dizziness, constipation and diarrhoea are the most common reported adverse reactions that complicate therapy with H_2-receptor antagonists. These reactions mostly occur in patients, who take the medicine orally as a tablet or suspension. Other side-effects such as bronchospasm, gynecomastia, central nervous system adverse reactions (confusion, disorientation, hallucinations, delirium, grand mal seizure, anxiety, paresthesia, decreased libido, lethargy and somnolence) appear at similar frequency without regard to the method of administration (4, 18, 21, 23, 26). The CNS (central nervous system) side-effects induced by H_2 blockers may be due to the actual blockade of H_2 receptor binding of histamine or to some other mechanism (1, 4, 12, 13, 27). Their estimated incidence is low (0.2% or less) in outpatients but dramatically increases in hospitalized patients to 1.6%–80% (12). The adverse reactions are seen more often in elderly people and patients with impaired renal function or hepatic failure since the metabolism of the studied compounds takes place in the liver and kidneys (4, 18, 21, 26).

The purpose of this study was to evaluate and compare the influence of cimetidine and famotidine on the cerebral cortex, particularly the activity of cortical cathepsin B (E.C. 3.4.22.1), D (E.C. 3.4.23.5) and L (E.C. 3.4.22.17). The cathepsins were chosen since they are sensitive, albeit non-selective markers, used in various investigations. They belong to the cysteine lysosomal proteases that are involved in a variety of physiological processes such as proenzyme activation, enzyme inactivation, antigen presentation, hormone maturation, and tissue remodelling (10, 11, 23).

This paper is a continuation of the earlier published data regarding the influence of antisecretory drugs on morphology and function of various organs, and is taken from a large toxicology project, to evaluate and compare tolerability of such drugs.

Materials and Methods

The study was an animal experimental model conducted in accordance with the Practice Regulations for Nonclinical Laboratory Studies (20, 29), with the Guide for the Care and Use of Laboratory Animals (14, 20) and under the guidelines (#0038/2000) of the Bioethical Committee University School of Medicine of Lublin, Lublin, Poland.

The experiment was conducted in outbred male Wistar rats with initial body weight of 180±15 g. The animals were housed in standard laboratory plastic cages (maximum 5 rats per cage) at a room temperature of 20±3 °C in a daylight cycle. Standard laboratory pellet chow LSM (Wytwornia Pasz w Motyczu, Poland) and filtrated tap water were provided *ad libitum* throughout the study. Food and water consumption were monitored daily.

The rats were individually identified by ear clipping done on the 5th day of quarantine. After a two-week acclimation period, the animals were divided into experimental groups, a minimum of 20 in a group. Animals were weighed on days 1, 4, 11, 18, 25, 39 and 47 of the experiment.

The test substances were administered intraperitoneally to the rats, twice daily with a 12-hour interval period, for six weeks in two doses. The initial dose, selected on the basis of the human therapeutic one, was 2.85 mg/kg (C_1) for cimetidine and 0.285 mg/kg (F_1) for famotidine. The second dose (C_2, F_2) was increased 10 times due to the faster rat metabolism (Table I). Control animals (CON) were injected with 0.9% NaCl. The animals were carefully observed for about 1 hour after each injection.

Half of the animals from each of the drug-treated and control groups were sacrificed on the 42nd day of the experiment (I). The remaining animals were raised for another 6 weeks without any xenobiotics, and sacrificed on the 84th day (II).

Immediately after decapitation, the skull was opened, brain removed, separated from meninges and rinsed in physiological saline. The frontal lobe of the right cerebral hemisphere was separated, frozen in liquid nitrogen and stored at temperature –20 °C. After being defrosted at the temperature of melting ice each sample was subjected to biochemical investigation. Each was placed into a 0.3 M sucrose solution (Sigma Chemical Co., St. Louis, MO., USA) at 4 °C and homogenised. The homogenate was centrifuged for 10 minutes at 2,200 g at 4 °C. The supernatant was decanted and centrifuged for 20 minutes at 35,000 g. The obtained sample containing the free fraction of enzyme was assigned as supernatant 1. The precipitate was placed into 5.0 ml of 0.3 M sucrose containing 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO, USA), and stored for 24 hours at 4 °C. Triton was used to dissolve the lysosomal membrane. The precipitate was then centrifuged for 20 minutes at 35,000 g. The supernatant, containing the fraction of bound enzyme, was decanted and assigned as supernatant 2.

Activity of cathepsin B, D and L, and protein level were assayed spectrophotometrically using substrates (Sigma Chemical Co., St. Louis, MO, USA) which form coloured complexes when they react with the proteases. The method is described in detail elsewhere (10, 19).

The homogeneity of variances was examined using Kolmogoroff-Smirnoff test. The inhomogeneous distribution data was analysed by Mann-Whitney U test. An $\alpha=0.05$ ($p<0.05$) was considered significant. Data was analysed using STATISTICA 5.0 on a PC.

Table I

Free and bound fraction activities (Mean±SD) of cathepsins (nmol/mg of protein/hour) in control (CON), cimetidine (C) and famotidine (F) treated groups and their characterisation

	Group	N	Dose mg/kg	Day of sacrification	Cathepsin B		Cathepsin D		Cathepsin L	
					free	bound	free	bound	free	bound
Control	CON-I	10	–	42	19.02±4.83	17.06±3.98	52.14±7.13	57.43±6.38	17.47±2.78	19.98±4.67
	CON-II	10	–	84	18.29±4.53	17.90±3.67	53.23±6.67	58.07±6.99	17.02±3.49	20.89±4.03
Cimetidine	C_1I	10	2.85	42	18.82±4.12	18.02±4.09	54.87±7.89	57.09±7.01	16.88±3.75	18.89±4.23
	C_1II	10	2.85	84	18.98±4.11	18.01±3.76	51.99±8.24	56.09±7.09	17.37±3.02	19.04±4.45
	C_2I	10	28.5	42	19.78±4.00	18.67±4.87	54.98±6.09	53.09±6.28*	19.77±2.24*	15.73±4.15*
	C_2II	10	28.5	84	18.92±3.98	16.99±3.78	54.08±7.00	56.98±6.87	17.73±3.11	18.89±4.15
Famotidine	F_1I	10	0.285	42	17.82±4.00	17.38±4.87	54.87±6.98	58.08±6.09	17.96±2.78	20.43±4.87
	F_1II	10	0.285	84	18.82±3.98	17.94±3.04	52.78±7.10	57.45±6.34	17.09±3.11	20.07±3.77
	F_2I	10	2.85	42	17.99±4.32	18.63±4.00	55.09±7.23	58.09±6.08	16.95±2.99	18.79±4.70
	F_2II	10	2.85	84	18.02±4.11	17.98±4.52	53.13±7.78	58.09±6.87	17.78±3.68	19.79±4.03

* – $p<0.05$ as compared to corresponding control (CON)-

Results

No animal deaths or behavioural changes were observed in the course of the study. Throughout the experiment, the drug-treated animals consumed as much food and water as the control animals and gained comparable weight ($p>0.05$) – data not shown.

The activities of examined cathepsins are presented in Table I.

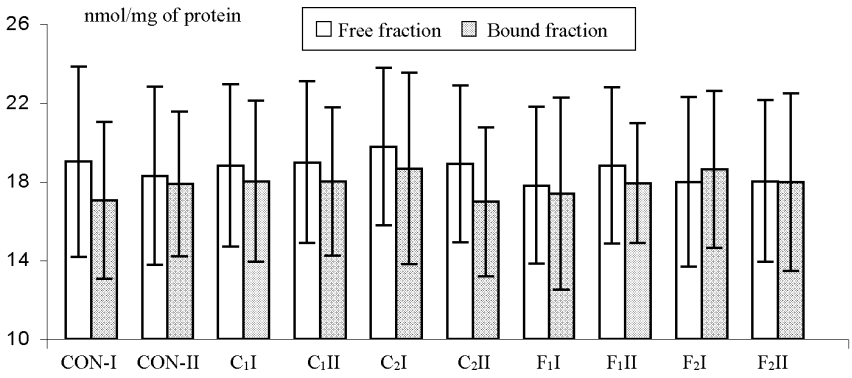

Fig. 1. Activity (Mean ± SD) of free and bound fraction of cerebral cathepsin B in control (CON), cimetidine (C) and famotidine (F) treated groups

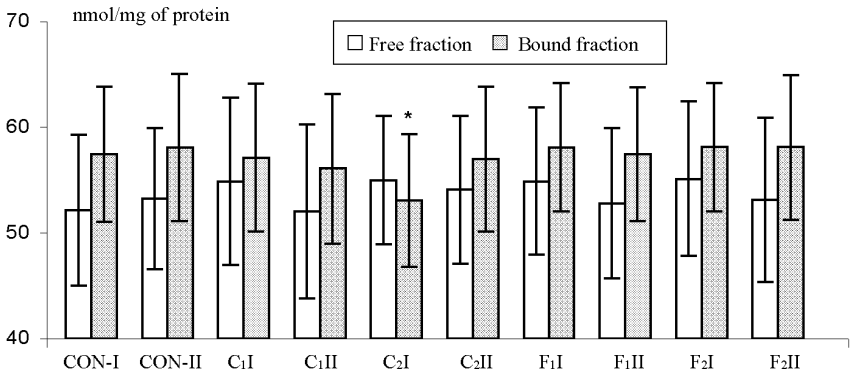

Fig. 2. Activity (Mean ± SD) of free and bound fraction of cerebral cathepsin D in control (CON), cimetidine (C) and famotidine (F) treated groups. *: $p<0.05$ vs control

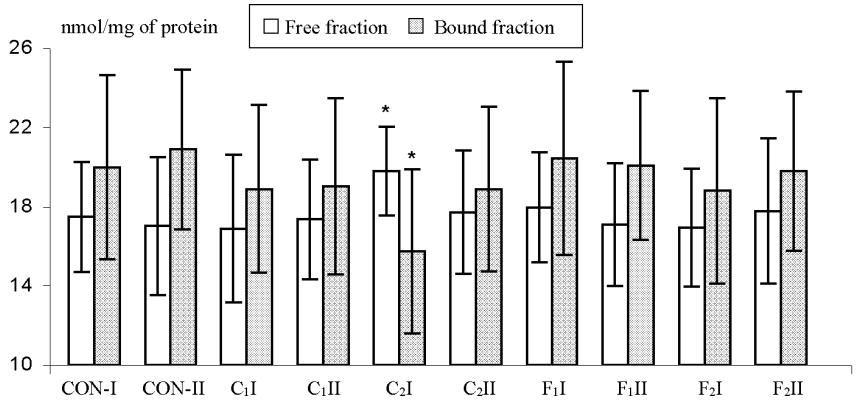

Fig. 3. Activity (Mean ± SD) of free and bound fraction of cerebral cathepsin L in control (CON), cimetidine (C) and famotidine (F) treated groups. *: $p<0.05$ vs control

No changes in activity of cathepsin B was observed in the group of rats examined (Fig. 1). The activity of bound fractions of cathepsin D and L decreased significantly in animals exposed to the higher dose of cimetidine and sacrificed on the 42nd day of the experiment (Figs 2 and 3). Significant elevation of the free fraction of cathepsin L was also noted in that group of rats (Fig. 3). Cathepsin activities were normalized during the next six weeks. Unlike cimetidine, famotidine did not change cerebral cathepsin profiles.

Discussion

Observed changes in the activity of cathepsin D and L seem to be temporary and dose-dependent since they were found only in animals exposed to the higher dose of cimetidine and sacrificed 12 hours after the last injection. No similar alterations in the activity of lysosomal enzymes were seen after a six-week untreated interval, and in groups of rats treated with famotidine. No behavioural changes were observed in drug-treated and control groups.

Similar, but insignificant, changes in free and bound cathepsins fractions activity were observed in livers obtained from rats intraperitoneally exposed to famotidine and ranitidine for six weeks (8). In these cimetidine- and famotidine treated animals, as well as animals exposed to ranitidine, the total lipid peroxidation process – measured as a blood plasma malondialdehyde (MDA) level, was unchanged (6). Such results were also found in animals exposed to the proton pump inhibitor – omeprazole, which is used in similar clinical situations as the discussed H_2 blockers (8–10). However, additional peripancreatic inflammation and lack of change in liver and pancreatic cathepsins activity were seen in rats treated with omeprazole (9, 10).

High activity of brain cathepsins, especially cathepsin B and D was seen in different, clinical demyelinization diseases such as sclerosis multiplex, allergic perinephritis or in their experimental models (5, 22, 25, 28). It was also shown that some non-selective cyclooxygenase inhibitors, e.g. flufenamic and indomethacin could inhibit transformation of the non-active pro-cathepsin form to proteolitical active form of cathepsin (24). Bednarski et al. (2, 3) suggested that inactivation of cathepsins B and L results in a proliferation of lysosomes and that meganeurite generation provides a means of storing residual catabolic organelles. Pinching off the meganeurite could eliminate the accumulated material but this action would result in axotomy in some cases. Reduced cathepsin L activity, increased numbers of lysosomes, and the formation of meganeurites are all reported to occur during brain ageing (3). The activity of lysosomal enzymes, as well as correct proportion between cathepsin D and L are both important for cytoskeleton physiology (2).

In a previous paper Shimokawa et al. (27) reported that high doses of intracerebrally administered H_2 receptor antagonists could induce clonic and/or tonic convulsion in mice and in demyelinization diseases could elevate the activity of cathepsins (2, 22). It was also shown that tonic convulsion can be suppressed by GABA-ergic or glutamatergic anticonvulsants, while clonic convulsion may be associated with the blockade of H_2 receptor in the brain and not be directly associated with the GABA and glutamate-mediated neurotransmission (27). All these results show that the lack of behavioural effect observed in our study was probably secondary to the low doses of the tested drugs and intraperitoneal route of administration, which decreased the drug concentration in blood since their main metabolic pathway occurs in liver (1). However, the significant increase of some of the cathepsin could be interpreted as a sign of the temporary, and reversible neurotoxic effect of cimetidine.

Our results are also similar to those presented in a comprehensive analysis by Cantu and Korek (12) who showed that cimetidine is most frequently associated with CNS adverse reactions among all the H_2 blockers. Human data also suggest that such effects occur during the first 2 weeks of therapy and resolve within 3 days of drug withdrawal, strongly explaining the temporary character of changes observed in this paper.

Poor tolerance of cimetidine and famotidine, as well as other H_2 antagonists, are rarely reported (12, 13, 18, 21, 26). However, a few case reports about serious side effects, especially hypersensitivity exist (15, 16, 31). Orr et al. (23) showed that famotidine significantly reduced sleep latency. In the same study cimetidine revealed a small increase in subjective estimates of sleepiness. However, no effects on sleep-related respiratory parameters were noted. The only risk factor for ranitidine found in a large widespread American study, was for patients who did not have their dosage corrected for renal function. For cimetidine the higher risk of adverse drug reactions occur in patients taking the drug with another medication known to cause a drug interaction and in older patients (4). Such risks were proved in other studies (13, 18, 21, 26).

On the other hand Kedziora-Kornatowska et al. (17) showed significant increase in supraoxide anion generation in patients treated with cimetidine vs those treated with famotidine and ranitidine. All three drugs stimulated superoxide dismutase activity and decreased malondialdehyde concentration. Such results showed beneficial effect on antioxidant processes especially for famotidine, while cimetidine was the least beneficial. However, the authors suggest that cimetidine has a possible role in activated oxygen species generation indicating the necessity of careful administration of this drug.

Intraperitoneal administration presented in our study is purely experimental. In clinical practice, histamine H_2 receptor antagonists are used in pills or intravenous injections (1, 13). Due to this fact and a number of differences between human and laboratory animals, further experimental and epidemiological studies are needed to prove good tolerability of cimetidine and famotidine or to evaluate their adverse reactions, especially the neurotoxic effect.

In conclusion, our investigation shows that six-week intraperitoneal cimetidine administration, unlike famotidine, temporary changes cerebral profiles of cathepsin D and L, and has no effect on cathepsin B in rats.

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