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Activity of Lysosomal Exoglycosidases of Rat Alimentary Tract in Chronic Exposure to N-Nitrosodimethylamine

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Abstract

Increasing pollution of the environment by nitrosocompounds has been observed. Acute intoxication of animals by nitrosocompounds leads to necrosis and chronic exposure to neoplasm. We have little information on the influence of nitrosocompounds on the metabolism of glycoconjugates (glycoproteins, glycolipids and proteoglycans) in animals. The aim of our work is to evaluate the influence of exposure of rats for 10 and 90 days to N-nitrosodimethylamine (NDMA), on activity of lysosomal exoglycosidases (enzymes hydrolysing terminal sugars in oligosaccharides) in the alimentary tract. It was found that intoxication of rats with NDMA increased specific activity of N-acetyl-P-hexosaminidase, β-galactosidase and α-mannosidase in the majority of tissues of rat alimentary tract. Damage to the cells of the alimentary tract by NDMA or its metabolites P, increases activity of exoglycosidases, because damaged tissue elements must be removed by catabolic enzymes before restoration processes can start.

Keywords: N-nitrosodimethylamine, rat, exoglycosidases, alimentary tract.

Introduction

Quality of tissues and the ability to regenerate depends on the quantity and quality of constituting substances, among them glycoconjugates. Glycoconjugates (glycoproteins, glycolipids, proteoglycans) are a group of chemical compounds possessing sugar chains covalently combined to proteins or lipids. Lysosomal exoglycosidases (N-acetyl-p-hexosaminidase, oc-mannosidase, β-galactosidase, sialidase) take part in hydrolyzing terminal sugars of oligosaccharide chains [1-5].

It was recently reported that biosynthesis and catabolism of glycoconjugates, including activity of exoglycosidases, can be influenced by environment, way of life, diet and disease. Toxic N-nitrosoamines appear in the environment: in water, soil and food, at a concentration of from 0.01 to 100 μg/kg or dm^3 [6-8]. Concentration of nitrosocompounds at a range of 100 μg/kg in food create the most important danger to humans and animals.

Nitrosocompounds reach organisms through polluted food, air and water or can be synthesized in organism with nitrites and secondary or tertiary amines. N-nitrosocompounds may change the metabolism of cells, leading to necrosis in acute intoxication, to modification of some metabolic pathways in subacute intoxication, and to neoplasms in chronic exposure [9-12].

It was reported that nitrosocompounds can be formed in stomach and intestines from its precursors with the participation of bacterial flora [8, 13-15], and both endogenic and exogenic xenobiotics are absorbed in the alimentary tract which is first exposed to their action.

The aim of our investigation is to evaluate the influence of chronic exposure to N-nitrosodimethylamine on the activity of lysosomal exoglycosidases in rat alimentary tract.

Materials and Methods

Male Wistar rats (approx. 190 g body weight) fed a standard diet were divided into three groups of 8 animals each: control animals exposed for 10 days to NDMA (at

a dose of 20 μg/dm^3 per day), and animals exposed for 90 days to NDMA at the same dosage.

The rats were killed under ether anaesthesia. Tissues were rinsed with tap water, then with 0.15 M solution of NaCl and homogenized in ice-cold 0.15 M KC1 containing 0.2% Triton X-100. The homogenates were centrifuged at 10,000 x *g* for 30 min. at 4°C. The activity of N-ace-tyl-P-hexosaminidase, β-galactosidase and α-mannosidase in the supernatant were determined by the method of Cha-teriee *et al.* [16] in the modification of Zwierz *et al.* [17]. Protein was determined according to Lowry *et al.* [18] using crystalline bovine serum albumin as a standard.

The results are presented as means SD of 8 identically treated animals. The statistical analysis was performed using Student's Mest and the Wilcoxon test ($p < 0.05$).

Results

Exposure of rats to 20 μg NDMA in 1 dm^3 of drinking water per day corresponds to exposure of the general human population [7, 8].

Intoxication of rats with NDMA for 10 or 90 days increased the specific activity of N-acetyl-β-hexosaminidase in all tested tissues of the alimentary tract, in comparison to control group (Fig. la). A significant increase in N-ace-tyl-β-hexosaminidase activity after 10 days of exposure, in comparison to control group, was noted in duodenum, caecum and large intestine and after 90 days of exposure in all alimentary tissues with the exception of the stomach. Specific activity of β-galactosidase in the alimentary tract increased significantly (in comparison to control) after 10 days of exposure to NDMA in duodenum, jejunum, caecum and large intestine, insignificantly increased in stomach, and slightly decreased in caecum (Fig. lb). After 90 days of exposure to NDMA all tissues of the alimentary tract revealed a significant increase in β-galactosidase activity above control group.

A specific increase in α-mannosidase activity, above control group, after 10 days of exposure to NDMA was noted in jejunum; an insignificant increase in stomach and duodenum (Fig. lc). An insignificant decrease of α-mannosidase activity (in comparison to control) was observed in ileum and a significant decrease in caecum and large intestine. After 90 days of exposure to NDMA a significant decrease was noted in stomach and a significant increase in remaining tissues.

Discussion

The doses of N-nitrosodimethylamine (NDMA) applied in our experiment are related to chronic exposure of the general population to polluted food entering the alimentary tract [7, 8].

In our experiments we noted a significant increase in the activity of exoglycosidases in all tested segments of the alimentary tract (with the exception of the stomach) after 90 days intoxication with NDMA (20 μg/dm^3) in drinking water. Exposition of rats for 10 days on NDMA caused a significant increase of exoglycosidase activity in the majority tissues of alimentary tract, with the exception of α-mannosidase where significant decrease of activity was noted in caecum and colon (Fig. lc).

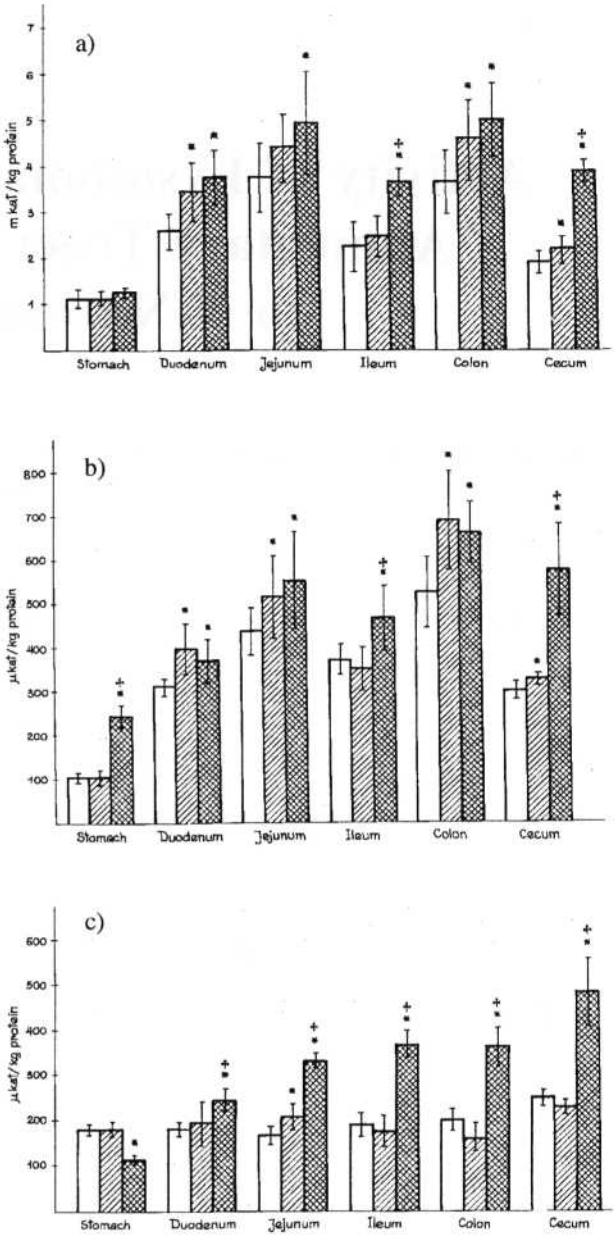

Fig 1. Activity of lysosomal exoglycosidases: (a) N-acetyl-p-hexosaminidase, (b) β-galactosidase and (c) α-mannosidase, of rat alimentary tract after 10 or 90 days exposure to N-nitrosodimethylamine.
*) Statistical significance in comparison with control group at $p < 0.05$ *or* $p < 0.001$.
†) Statistical significance in comparison with group treated with N-nitrosodimethylamine for 10 days *at* $p < 0.05$.

It was reported that 97% of alimentary N-nitrosodimethylamines were absorbed to blood from the small intestine [19], according to concentration gradient, but absorption may be facilitated by specific transporters in intestinal mucosa [20, 21], increased peristaltics and mixing the contents of the alimentary tract [22].

Intensive absorption of NDMA in the alimentary tract can lead to local concentrations of nitrocompounds, which can cause local damage. It was reported that N-nitrosamine penetrating the cell membrane can damage cellular orga-

nelles (mitochondria, lysosomes) and cause necrotical changes in place of contact, with intensity related to xenobiotic concentration and time of exposure [23]. Injured tissues stimulate catabolic processes, which precede repair processes. Cells injured by NDMA can be a chemotactic factor for macrophages, which accumulate in injured tissues. Macrophages have numerous lysosomes which contain catabolic enzymes, among them glycosidases [24]. Therefore glycosidases of macrophages can contribute to the increase in total activity of glycosidases of the alimentary tract.

When one evaluates the influence of NDMA on tissues of the alimentary tract, we must remember that *in vivo* in stomach and intestines are synthesized endogenic nitrosocompounds [6, 8, 15, 25] from their precursors, with participation of bacteria [8, 15, 25]. Endogenic nitrosocompounds may increase the concentration of xenobiotics, and cause potentiation of the toxic effect and increase in catabolic processes with the participation of glycosidases.

It was reported that intestines have a very intensive metabolism of xenobiotics similar to liver activity [26]. Xenobiotics in intestines are metabolized by enzymes which are able to performe first (oxidation, reduction, hydrolysis) and second phase of transformation (conjugation with glucuronic, sulfuric, and aminoacids) [27]. Therefore, an increase in exoglycosidase activities can be caused by the action of biotransformation products of NDMA, produced by the microsomal system in intestine [28, 29]. It was reported that alkylating agents (eg. methyl cation in the case of NDMA) can react with accessible heteroatoms of nitrogen and oxygen constituting nucleophilic centres of DNA and RNA [9, 30]. We cannot exclude the participation of alkylated nucleic acids in increasing the intensity of catabolic processes. It is possible that genes coding exoglycosidases could be included in adaptative response of organism [24] to chronic exposition on NDMA, similarly as gen *ada* coding protein Ada [31], responsible for the activity of methyl transferase O^6-methylguanidine-DNA [24, 26], or gen *aid B* coding dehydrogenase isovaleryl-Co A [21].

Exposure of the tissues of the alimentary tract on NDMA could stimulate cytochrome P-450 to produce excessive amounts of free radicals [32, 33], which can cause damage to cells [13] by peroxidation of cell membranes lipids [11]. Peroxidation of lipids in cell membranes increases permeability of membranes to lactate, sodium, potasium, calcium, and magnesium, which accumulate in rough endoplasmic reticulum, cause swelling and loss of rybosoms [31]. Free radicals may damage nucleic acids, break double strand of DNA and damage nitrogen bases [13]. Action of free radicals could disturb glycoproteins synthesis and catabolism. Defected glycoproteins may be catabolized by glycosidases, and it can cause an increase in exoglycosidases activity.

Increased activity of lysosomal enzymes in the alimentary tract, after exposure to NDMA, reflects an increase in catabolism of glycoconjugates, probably caused by the multiple factors disscussed above. The question of which factors are most important awaits further study.

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