Proteolytic processes in organism of different age rats exposed to xenoestrogens

Journal of Physics: Conference Series **2288** (2022) 012013 doi:10.1088/1742-6596/2288/1/012013

Proteolytic processes in organism of different age rats exposed to xenoestrogens

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Abstract. Endocrine disrupting chemicals (EDCs) are a group of compounds that affect the endocrine system, frequently found in everyday products and epidemiologically associated with several diseases. The human population is now ubiquitously exposed to EDCs in daily life. The main way of getting xenoestrogens to the body is the contaminated food. The effects of xenoestrogens on the proteolytic processes of different age rats were determination. The experiments were conducted on Wistar rats exposed to exogenous estrogen for 45 days. At the beginning of the experiment 3-month-old pubertal animals and 6-month-old sexually mature rats were involved. The research materials were organ tissue and blood serum of the rats. The objects were indexes of activity of trypsin and its obligatory inhibitors α1 - antitrypsin (α1-AT) and α2-macroglobulin (α2-MG), cysteine cathepsins B and L, the molecules of middle mass (MMM) level. In summary, the eating food contaminated by exoestrogens led to changes in the proteolytic system and the development of endogenous intoxication, which are also organ-specific and dependent on the age of the animals: a higher level of activity of the inhibitory link and the content of MMM was observed in rats in the puberty period, which leads to a decrease in the potential of the protective mechanisms of the organism and can become a trigger dysfunctional systems of natural detoxification and biotransformation. Inhibition of apoptosis is the main consequence found in the body of experimental rats. This phenomenon can lead to processes that inhibit one of the main mechanisms that reject damaged cells from the population. Females who were in puberty were more susceptible to dietary synthetic estrogens. In contrast to adult animals of the same sex, whose indicators indicate the importance of age characteristics of the body for the ability to perceive the effects of xenoestrogens. Rats became less sensitive to the effects of these substances with age. The difference in experimental animals was due to changes in the rate of detoxification pathway reactions, and not in the metabolism of estrogens entering the body, in particular, with food.

1. Introduction

The destruction of the endocrine system by chemicals, which are a group of compounds that negatively affect the endocrine system, is an urgent problem up day. Such substances are often found in everyday products and are epidemiologically associated with several diseases. Currently, the endocrine system by chemicals (EDCs) data bank contains 1,000 molecules, including pesticides, natural and industrial products, cosmetics, medicines and food additives, and other

low-molecular-weight xenobiotics [1]. These environmental estrogens can be categorized into the groups: (1) naturally occurring non-steroidal plant estrogens or phytoestrogens; (2) the steroid estrogens – 17β estradiol and estrone from animal and human sources; (3) the mycotoxins, zearalenone and zearalenol; (4) synthetic compounds with phenolic groups; (5) metalloestrogens, such as, arsenic, cadmium, and manganese [2–4].

The human population is now ubiquitously exposed to countless environmental and stochastic factors such chemicals in daily life, in indoor as well as outdoor environments, through their use in pesticides/herbicides, predatory insects, industrial and household products, plastics, detergents, flame retardants and as ingredients of personal care products, oral contraceptives, hormonal therapy [3, 5, 6]. Intake to the human body may be oral, inhalation or dermal absorption [7, 8]. Estrogens are also used in animal husbandry to increase growth. Both farm and urban sewage effluent contained substantial amounts of steroidal estrogen pollutanted water sources such as surface and groundwater [3, 6, 9–12].

After a while, these pharmaceutical hormones mimic estrogen. Also, there were natural links between endocrine disruptors (EEDs) in the environment and changes in microbial ecology, as well as an increase in the level of resistance of pathogenic organisms to antibiotics, toxicity of the aquatic environment and microorganisms, and a decrease in the resistance of human health [6, 13–19].

The Endocrine Society declares what endocrine disrupting chemicals (EDC) are an exogenous chemical, or mixture of chemicals in the Statement of Principles. They interferes with any aspect of action of hormone" and as an exogenous agent. Ones can be connect with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body. EDC are responsible for the maintenance of homeostasis and the regulation of developmental processes [2, 13, 20–22].

Metabolic disorders, changes in lactation, breast density, immune function of the body, and other adverse consequences are the result of deterioration of the endocrine system (for example, obesity, changes in the timing of puberty and menopause [5, 23–25].

Some authors have identified signs of multifactorial hormonal activity in several EDCs. For confirmation, we can consider the pesticide DDT, which is an agonist of the endogenous active substance estrogen, and one of its metabolites is antiandrogenic. The research have shown that the estrogenic activity of bisphenol A (BPA) is an antagonist of thyroid hormones. If we take into account the affinity of estrogen (ER) to nuclear receptors , we can determine that xenoestrogens are usually less effective. The effects that occur at low doses are explained by the fact that they act additively with endogenous estrogens. Chemical bonds of xenoestrogens with plasma carrier proteins have significantly lower affinity. In natural estrogens, this affinity is much better, so they are more easily accessible to target organs [2, 26–32].

Some environmental chemicals may be able to interfere in the endocrine regulation of energy metabolism and adipose tissue structure. This includes compounds to which the human population is exposed in daily life through their use in pesticides/herbicides, industrial and household products, plastics, detergents, flame retardants and as ingredients in personal care products. This has the potential for a vicious spiral not only of increasing obesity but also increasing the retention of other lipophilic pollutant chemicals with an even broader range of adverse actions [10, 26, 33, 34].

The metabolomics analysis identified various metabolites that are affected by various estrogen treatments. There are the increased risk of thrombosis, marked changes in the distal tubules and collecting ducts in the kidneys of rats exposed to phthalate, and hypertrophy in the hepatocytes of the centrilobular zone of the liver [2, 17, 35]. Combinations of gene chains directly regulated by ER, such as lipid metabolism, and other gene networks may be responsible for such changes. Gene networks are activated in response to disruption of physiological liver processes, such as pathways associated with oxidative stress [2, 24, 36].

Exposure to estrogens is associated with increased risk of breast and other types of human cancer [30, 37, 38]. Environmental EDS ligands represent an emerging threat to human bone health [39]. Brain cells and neural circuits are likely to be influenced by estrogenic endocrine disruptors (EEDs) because they strongly dependent on estrogens [1, 8, 40, 41].

Age and/or estrogenic surroundings affected the difference in effects [42]. A process known as developmental reprogramming can permanently reprogram normal physiological responses under the influence of changing environmental conditions. Such changes in physiological responses increase the body's susceptibility to diseases later in life [1, 43, 44].

It is very difficult to develop clear clinical guidelines to address the potential health effects of toxicants that are commonly seen at all levels of the organization among the general population. The lack of a complete understanding of the main mechanisms of exposure to toxicants entering the environment and the level of their impact on a living organism significantly complicates scientific research on this issue. [2, 23, 45].

Proteolysis is an important component of the homeostasis of the body, which serves as a trigger mechanism for many biological processes, maintains a dynamic equilibrium in hemostasis and affects the function of membrane cells. Proteases are involved in the processes of programmed cell death due to selective release of lysosomes in response to various effects [46,47].

The aim of the study was determination of the effect of xenoestrogens on the proteolytic processes of different age rats.

2. Materials and methods

The experiments were conducted on Wistar rats exposed to exogenous estrogen for 45 days. At the beginning of the experiment 3-month-old pubertal animals (group II) and 6-month-old sexually mature rats (group IV) were involved. The control group consisted of intact appropriate age animals (groups I and III). For modeling exogenous estrogen impact rat' meal is treated with the drug "Synestrol" as stilbene derivative differing from steroid hormones estrogen on chemical structure, but by biological and medicinal properties similar to them in the rate of 2 mg per kg. The research materials were organ tissue and blood serum of the rats. The objects were indexes of activity of trypsin and its obligatory inhibitors $\alpha 1$ - antitrypsin ($\alpha 1$-AT) and $\alpha 2$-macroglobulin ($\alpha 2$-MG) [47], cysteine cathepsins B and L [48], integrated indicators of endogenous intoxication syndrome (EI), namely, the molecules of middle mass (MMM) level [49]. The data were treated with standard methods of variation series estimation. The difference between the comparative values was considered probable at $p < 0.05$.

3. Results and discussion

Cysteine cathepsins are important regulators and signaling molecules of many biological processes. Cathepsins B and L are expressed in all tissues of the body and play an important role in the physiological intracellular degradation of proteins. The enzymes are involved in the development of a number of pathological conditions [50]. Cathepsins B and L carry out regulatory action, post-synthetic modification of precursors of peptide hormones and neurotransmitters [50]. Trypsin is secreted by the pancreas in the form of an inactive precursor – trypsinogen. Transformation of zymogen into trypsin occurs more intensively under the influence of cathepsin B, while cathepsin L inactivates trypsin [51].

After conducting research on the determination of the effect of exoestrogen on the proteolytic processes of pubertal and sexually mature females rats the following results were obtained.

For example, the role of genotoxic carcinogens with estrogenic activity formed during meat roasting, on the induction of colon, prostate and mammary gland, is assumed to be influenced by expression in nanomolar concentrations on the expression and activity of matrix proteinases, and in particular, of cathepsins, which mechanically supports tissue-specific carcinogenicity of the like substances caused invasion of tumor cells through the basement membrane [52, 53].

The activity of cysteine cathepsin L was reduced by 15 % in the liver of pubertal females when compared with control and 10 % at the sexually mature individuals of group IV. Cathepsin B was activated by 30 %, respectively (table 1).

Trypsin has been shown to be a nonspecific carrier of steroid hormones, as well as proteolytic cleavage of estrogen receptors [53]. The increase of trypsin index in the experimental group II of rats (by 12 %) was observed in the liver, while in the mature female the enzyme activity was increased by 7,5 % in the IV group (table 1).

Table 1. Proteolytic indices of different age female rats exposed to alimentary estrogens.

Index	I group	II group	III group	IV group
		Liver		
Trypsin, nmol/sek/g protein	0,65+0,03	0,73+0,04*	0,67+0,033	0,72+0,036*
α1-AT, µmol/ sek/g protein	0,46+0,02	0,54+0,03*	0,48+0,024	0,53+0,027*
α2-MG, µmol/ sek/g protein	0,24+0,02	0,22+0,02	0,24 +0,01	0,25+0,01
Cathepsin L, units./ g protein	21,19+1,06	18,01+0,91	20,78+1,04	18,65+0,93
Cathepsin B, units/ g protein	20,88+1,04	27,06+1,05	21,07+1,05	25,34+1,05
MMM, units	8,78+ 0,44	9,83+0,52*	7,68+0,38	8,22+ 0,44
		Brain		
Trypsin, nmol/sek/g protein	0,061+0,004	0,073+0,003*	0,067+0,003	0,072+0,003*
α1-AT, µmol/ sek/g protein	0,25+0,01	0,29+0,01	0,24+0,01	0,26+0,01
α2-MG, µmol/ sek/g protein	0,14+0,01	0,22+0,01*	0,15 +0,02	0,17+0,02
Cathepsin L, units./ g protein	12,74+0,61	13,66+0,68	11,05+0,55	12,48+0,72*
Cathepsin B, units/ g protein	14,07+0,73	15,47+0,77	14,25+0,70	13,11+0,66
MMM, units	4,67+0,23	5,42+0,19*	4,15+0,22	4,73+0,23*
		Kidneys		
Trypsin, nmol/sek/g protein	0,59+0,04	0,67+0,03*	0,62+0,03	0,67+0,05*
α1-AT, µmol/ sek/g protein	0,32+0,02	0,345+0,02	0,34+0,02	0,35+0,02
α2-MG, µmol/ sek/g protein	0,18 +0,01	0,21+0,01*	0,19+0,01	0,20+0,01
Cathepsin L, units./ g protein	15,47+0,77	15,62+0,78	16,03+0,8	16,42+0,72
Cathepsin B,units/ g protein	10,55+0,67	16,47+0,53*	12,17+0,69	15,76+0,66*
MMM, units	8,37+1,42	11,09+1,74*	8,41+1,42	9,34 +1,58*
		Blood serum		
Trypsin, nmol/sek/g protein	1,20+0,09	1,73+0,12*	1,25+0,12	1,54+0,16*
α1-AT, µmol/ sek/g protein	0,15+0,01	0,17+0,02*	0,17+0,01	0,19+0,12
α2-MG, µmol/ sek/g protein	0,014+0,001	0,015+0,001	0,012+0,001	0,014+0,002
Cathepsin L, units./ g protein	1,21+0,14	1,39+0,09*	1,16+0,11	1,24+0,10*
Cathepsin B,units/ g protein	1,17+0,13	1,69+0,11*	1,11+0,17	1,42+0,11*
MMM, units	6,23+0,26	7,38+0,92*	6,74+0,38	7,68+0,62*

Note: * - difference between the index of experimental group to intact appropriate age rats index is considered probable at $p<0.05$

Proteolytic inhibitors perform important physiological functions: delay the premature activation of proteolytic enzymes, protect proteolytic tissue from microbial enzymes, regulate the state of the coagulation system and fibrinolysis, affect arterial pressure and vascular permeability, apoptosis processes. The ratio of systems with mutually opposite action is in a strictly dynamic equilibrium, where each of them has a significant role in the regulation of the vital activity of the organism [54]. For the excess of trypsin, the distribution of the enzyme between the two

major inhibitors α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG) occurs according to their molar content. The index of alpha-1-antitrypsin increases with inflammatory processes, such as acute, subacute and chronic infectious diseases, acute hepatitis and cirrhosis of the liver in active form, necrotic processes, post-operative conditions, Alpha-2 macroglobulin is a glycoprotein, an inhibitor of plasma proteinases, and is used as a marker for cell membrane permeability. α2-MG level increase can be observed in various pathological conditions, such as nephrotic syndrome, hormonal dysfunctions or disorders associated with the development of the child's body. α2-MG suppresses the activity of leukocyte and synovial collagenases, cathepsin B, calicreatin plasma [4].

The level of α1-AT was higher in group II by 17 % when compared with intact rats and dominated in group IV (by 11 %). Due to the positive effect of estrogens on the synthesis of α2-MG, its concentration in women is approximately 20 % higher than that of men. α2-MG has the ability to bind hormones and cytokines (IL, IFN, TNF-a, growth factors) [55]. The activity of α2-MG tended to decrease in the experimental rats of pubertal age and weakened in the experimental sexually mature individuals (table 1).

Exposure of the drug Synestrol in the brain of the rat in the pubertal period has been shown to activate cathepsin B by 10 % compared with the control group of the same age. In the sexually mature female, the activity of the enzyme in the experimental group is reduced by 8%.

Nerve cells contain large amounts of cathepsin L [56]. A cathepsin L function in secretory vesicles is defined as a key protease for the proteolytic processing of proneuropeptides and prohormones in active neuropeptides, which are mediators for synapses in intercellular communications in the nervous system. During the exposure of the Synestrol drug in the rat brain, both subjects underwent cathepsin L activation: 7 % (group II) and 13 % (group IV).

In determining of trypsin activity it was found that in the experimental group I the enzyme activation was dominant over the indicator of the corresponding control group by 20 $^0/$ in the brain. In the experimental group of sexually mature individuals, trypsin activity was 7,5 $^0/$ higher than in female experimental group. There was a tendency to increase of α1-AT enzymatic activity in the experimental groups of pubertal and mature females by 16 % and 8 %, respectively. In the study of α2-MG activity, the deterministic activation was 57 % in the experimental group II, while the deviation of the index between experimental groups of adult-raised animals was 13 % (table 1).

Widespread expose of estrogens has led to the need for studies of biochemical changes in the kidneys. According to the results of studies of shifts in the proteolytic system, it has been found that trypsin activity increased by almost 14 % in the kidneys of puberty female and 8 % in adult individuals. No significant differences were found for the α1-AT index between groups III and IV, for group II, growth was 8 %. A similar trend of change is characteristic for α2-MG: the activation was 5 % and 17 %, respectively (table 1).

According to the references, the assessment of the long-term effects of estrogen on lysosomal enzymes such as cathepsins B and L has shown changes in the activity of enzymes that were more significant at low doses of estrogens: there was no correlation between doses and the activity of lysosomal enzymes [12].

For females of the younger group, the kidney activity of cathepsin B exceeded the control values by 57 %, in older rats - by 29,5 %. There were no differences in the activity of cathepsin L between the experimental and the corresponding control groups. It has been established that the alimentary exposition of estrogens leads to the activation of the proteolytic chain in the study of serum. Thus, for females under the age of 4,5 months, trypsin activation was 44 %, cathepsin B – 44 %, cathepsin L – 15 %. For females aged 7,5 months, activation was 23 $^0/$, 23 % and 7 %, respectively. The reaction of the serum inhibitor in the group II females was 13 % (α1-AT) and 7 % (α2-MG), group IV – 12 % and 17 %, respectively (table 1).

Endogenous intoxication (EI) is a clinical syndrome that arises at various etiology pathological

conditions due to the accumulation in tissues and biological fluids of the body of the metabolic, destructive cell and tissue structures, destroyed protein molecules, accompanied by functional and morphological lesions of organs and body systems. There is a direct correlation between the level of proteolytic activity of the blood with integrated indicators of endogenous intoxication syndrome (EI), namely, the molecules of middle mass (MMM) that can inhibit transformation of lymphocytes, phagocytic activity of leukocytes, cause neurotoxic action and disjunctive effect on processes of tissue respiration and oxidative phosphorylation, inhibit protein synthesis in the non-cellular systems, promote hemolysis of erythrocytes, influence erythropoiesis, sharply disturb the permeability of membranes and cause cellular aggregation. The accumulation of toxic metabolites also contributes to limited proteolysis reactions catalyzed by lysosomal enzymes in the intercellular space, which leads to damage both at the cellular and organ levels [57].

The imbalance in the proteolytic system leads to excessive formation of the peptides (medium molecules) with toxic properties. It has been shown their accumulations in the organs of rats of both experimental groups. Thus, the increase in the content of MMM in female pubertal females consumed Synestrol was 12 % in the liver, 32,5 % in the kidneys, 18,5 % in the blood serum, and 16 % in the cerebrum. In mature females, the increase was 7 %, 11 %, 14 % and 14 %, respectively.

In summary, the eating food contaminated by exoestrogens led to changes in the proteolytic system and the development of endogenous intoxication. A higher level of the inhibitory link activity and the content of MMM were observed in rats in the puberty period, which leads to a decrease in the potential of the protective mechanisms of the organism and can become a trigger of dysfunction systems of natural detoxification and biotransformation. Females were more exposed to dietary synthetic estrogens during puberty than adult animals. Such data prove that age is also a factor in the influence of xenoestrogens on processes in the body. Animals became less sensitive to the effects of these substances with age. This reaction is explained by changes in the rate of detoxification processes, and is not associated with the metabolism of estrogens entering the body, in particular, with food.

Considering the state of processes of proteolysis in the organs of females using xenoestrogens, it is possible to admit of reconstructing of the mediator functioning possibility and enzyme systems, additional enhancement of pathological symptoms. It's assume the above effects can initiate endocrine disruptions, simulate responses depended on steroid hormones receptors, as well as receptor-independent processes in the pathological, namely, promote increased proliferative activity, inhibition of apoptosis, stimulation of neoangiogenesis, cause changes in the epithelium of the organs that affect on the metabolic process, and to be trigger mechanisms for the development of carcinogenesis in hormone-dependent organs, in particular, the mammary gland, formation of general pathological state in the organism. Explaining the effective mechanisms of proteolytic processes will help clarify the importance of risks in environmental changes for an organism with different age indicators. Also, a deeper analysis will provide evidence to reduce the impact of negative factors and, ultimately, reduce the burden of age-related diseases.

4. Conclusions

Thus, the alimentary exposition of rats with exoestrogens leads to changes in the proteolytic system and the development of endogenous intoxication, which are organ-specific and age-dependent animals. The control difference of signs in an organism provides various influence on the following types of interaction, namely a cell - a cell, a cell - an extracellular matrix. In addition, soluble factors can be a trigger for disruption of information transmission by signaling pathways. We believe that the identified phenomena may lead to the suppression of one of the main mechanisms of removal of damaged cells from the population, namely apoptosis.

The observed effects were more expressed in females during puberty than in mature rats. The

obtained results prove high sensitivity of living organisms to exogenous estrogen-like compounds at specific age-related physiological conditions.

Our conclusions prove the need for a comprehensive study to determine the trigger role of xenoestrogens contained in food in the development of pathological changes in the body. The information obtained can be a paradigm for risk assessment and prevention of diseases, the etiology of which is the alimentary intake of xenoestrogens.

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