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Estrogen-dependent activity of kidney lysosomal proteolytic enzymes

Hormonally active steroids, like estrogens are capable of exerting wide-spread effects on metabolic activities throughout the body. During the recent years there increased interest in benefits and risks of hormonal replacement therapy consisting in the improvement of the quality of life of women and prevention of serious illnesses like cardio-vascular diseases, as well as Alzheimer's disease and osteoporosis. Disadvantages of estrogens appear; these are: hypertension, hepatic vein thrombosis. and portal vein thrombosis (8, 4). The risk of adenoma and carcinoma of the liver and kidney as well as of endometrium and breast caused by oral contraceptives has been clarified after relatively long-term experience (2, 3, 5). Most investigations have reported that the use of estrogens has had little effects on the risk of toxical process in parenchymal tissues. Generally speaking, synthetic sex hormones should not be used in acute or chronic kidney diseases. It has been clear at the same time that the lysosomal enzymes could be important indicators of toxicity. Furthermore, it has been well documented that the lysosomal enzymes are the agents that catalyse all biological reactions (7).

The aim of this study was to assess the activity of lysosomal proteolytic enzymes like cathepsins B, D and L (free and bound fraction) in the whole homogenate of the kidney.

MATERIAL AND METHODS

The whole experiment was based on the animal experiment model. The experiment was carried out with the approval of the Ethics Committee of the Medical University of Lublin, Poland. The study was conducted on female rats of Wistar strain with the initial body weight of 180-300g. The animals were subjected to reverse light cycling for 2 to 3 weeks before use. The rats were housed in standard laboratory cages (max 6 pieces per cage). After acclimation period, the animals were gathered in 8 experimental groups of minimum 10 in a group. Oestradiolum benzoicum (Jelfa, Jelenia Góra, Poland) was used for the purpose of this study. Oestradiolum benzoicum was given i. m. for 8 weeks in six different doses: E1 – 0.00075g/kg of the body weight (n=15, number of rats) one time per week; E1.1 – 0.00075g/kg b. w. (n=15), every three days. E2 – 0.0015g/kg b. w. (n=15), one time per week; E2.1 – 0.0015g/kg b. w. (n=15), every three days; E3 – 0.003g/kg b. w. (n=15), one time per week; E3.1 – 0.003g/kg b. w. (n=15), every three days. Two control groups were designed: K0 – the untreated animals (n=15), K1 – the animals received the adequate quantity of *oleum pro injectione* (n=15).

All the animals were killed by decapitation after 9 weeks of experiment. The fragments of kidney after taking parts of organ for histological examination, were frozen in the temperature of liquid nitrogen and stored in the temperature -20°C. After being defrosted the kidney tissues were dissected and then homogenized. Total activity of proteolitic lysosomal enzymes such as cathepsin B, D and L were evaluated in the obtained homogenate, according to the methods described in other publications (1). For statistical analysis the ANOVA test was used to compare results between the groups. All data were expressed as Mean ± SEM. In all analysis and associated probability (p-value) of less than 5% was considered significant.

RESULTS

The results of this study were listed in three tables and figures.

In control groups the activity of free fraction of cathepsin B was similar as well as the activity of bound fraction, but in group K1 these values were higher. In both these groups the activity of total fraction, was higher than the activity of free and bound fractions. Furthermore, the total activity of cathepsin B is not the sum of activity accesses due to different methodology of determination. During the experiment we did not observe the simple relationship between the lysosomal activity and the dose of injected estrogens. Thus, the smallest activity of bound fraction of cathepsin B was noted in group E3 (the higher dose of injected estrogens) and the highest activity of this fraction was noted in group E1 (the smallest dose of injected estrogens). The value of free fraction of cathepsin B observed in group E2 was significantly higher than this activity in control groups and in other experimental groups. The total activity of cathepsin B was also the highest in group E2 (Tab. 1, Fig. 1)

The activity of free fraction of cathepsin D in group K0 was smaller than in the group of animals treated with *oleum pro injectione*. The activity of bound fraction of this enzyme was compared in both control groups. The total activity was higher in group K1. The smallest activity of free fraction of cathepsin D was observed in group E1 and the highest activity of this fraction was in group E3. The activity of bound fraction of cathepsin D was smaller than the observed activity of free fraction in all the assessed groups. The highest value of this fraction – in group E3.1 and the smallest was in group E1 as referred to the control groups. In all the groups, the total value of the observed activity was higher than separately assessed activity of free and bound fractions. Furthermore, the total activity was not the sum of both free and bound activity (Tab. 2, Fig. 2).

The activity of free fraction of cathepsin L was similar in both control groups as well as of bound fractions, but the value of bound fraction was higher than the activity of free fraction of this enzyme in control groups. The total activity was higher than activities of free and bound fractions. The total activity was the highest in group E1.1 and the smallest in group E3. The activity of free fraction of cathepsin L was the smallest in group E1 and the highest in group E1.1 In control groups (K0. K1) the activity of bound fraction of cathepsin L was higher than in other groups. Thus, we did not observe a simple relationship between the activity of enzyme and the dose of injected estrogens similar to the earlier described groups (Tab. 3, Fig. 3).

Table 1. The activity of cathepsin B in the studied groups

GROUP	N	MEAN	SD	MEDIAN	MIN	MAX	VARIANCE
K 0	8	0.086	0.022	0.085	0.050	0.115	0.001
K 1	7	0.096	0.024	0.089	0.069	0.127	0.001
E 1	8	0.189	0.222	0.085	0.049	1.627	0.049
E 1.1	9	0.145	0.058	0.128	0.083	1.266	0.003
E 2	15	0.115	0.035	0.106	0.072	0.192	0.001
E 2.1	7	0.090	0.015	0.090	0.071	0.118	0.001
E 3	9	0.068	0.025	0.072	0.012	0.102	0.001
E 3.1	11	0.120	0.085	0.870	0.062	0.338	0.007

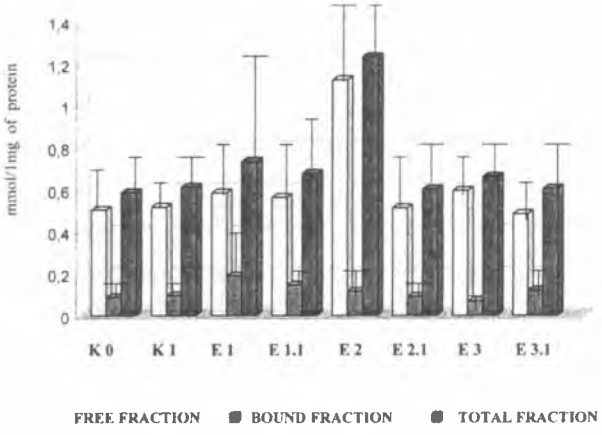

Fig. 1. The activity of cathepsin B in the studied groups

Table 2. The activity of cathepsin D in the studied groups

GROUP	N	MEAN	SD	MEDIAN	MIN	MAX	VARIANCE
K 0	9	0.254	0.053	0.257	0.175	0.339	0.003
K 1	7	0.237	0.066	0.244	0.128	0.339	0.004
E 1	8	0.186	0.077	0.175	0.120	0.362	0.006
E 1.1	9	0.340	0.231	0.271	0.165	0.872	0.053
E 2	15	0.275	0.085	0.270	0.146	0.467	0.007
E 2.1	7	0.242	0.081	0.209	0.131	0.347	0.006
E 3	9	0.201	0.017	0.207	0.174	0.228	0.001
E 3.1	10	0.240	0.073	0.230	0.146	0.363	0.005

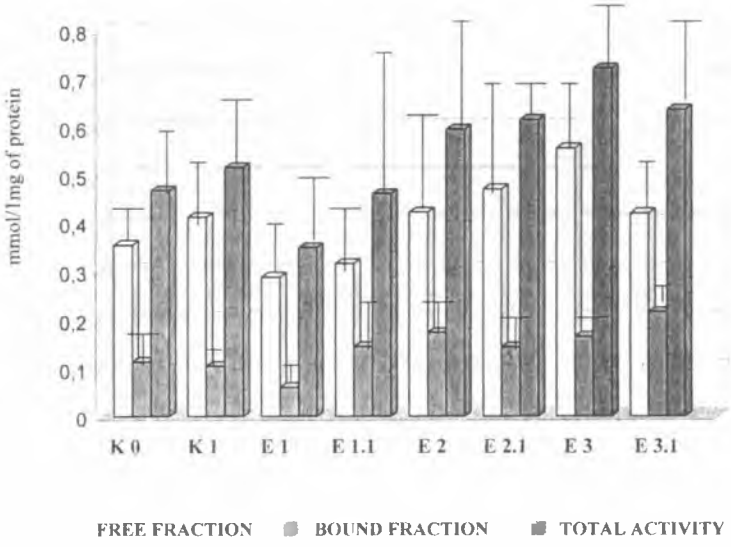

Fig. 2. The activity of cathepsin D in the studied groups

Table 3. The activity of cathepsin L in the studied groups

GROUP	N	MEAN	SD	MEDIAN	MIN	MAX	VARIANCE
K 0	9	0.330	0.025	0.321	0.298	0.382	0.001
K 1	7	0.319	0.028	0.313	0.271	0.351	0.001
E 1	8	0.289	0.030	0.287	0.234	0.321	0.001
E 1.1	9	0.285	0.054	0.272	0.213	0.371	0.003
E 2	15	0.285	0.022	0.278	0.265	0.353	0.001
E 2.1	7	0.282	0.013	0.280	0.264	0.299	0.001
E 3	9	0.200	0.008	0.203	0.183	0.210	0.001
E 3.1	11	0.208	0.110	0.219	0.073	0.484	0.012

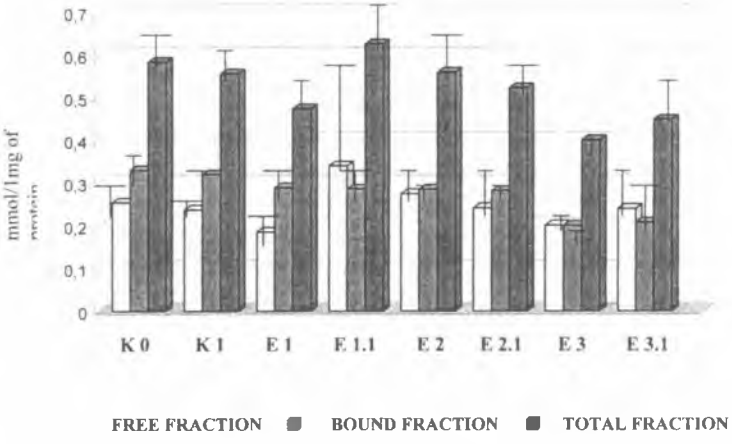

Fig. 3. The activity of cathepsin L in the studied groups

DISCUSSION

The results show that long-term estrogen therapy initiates the changes in the activity of lysosomal proteolitic enzymes. Our results are uncharacteristic. On the base of the experiment there is no obvious evidence at present to suggest that the activity of all fractions of cathepsin B, D and L changes during the treatment with higher doses of estrogens. This results suggest that during estrogen intoxication the lysosomal membranes are impaired.

The enzyme activity in plasma and in homogenate sometimes is completely different. This statement has found confirmation in the Skrzydlewska's study on the activity of lysosomal proteases in the liver and in the plasma from rats intoxicated with methanol. S k r z y d l e w s k a observed the increased level of these enzymes in plasma and also the decreased level of cathepsin B, C, D and L in the liver homogenate (9). Other authors confirmed the cytotoxic effects initiated with the lysosomal proteases on the different tissues of the body (6). Lysosomal enzymes activity, observed in all the experimental groups, so uncharacteristic seems to be secondary to the inflammatory process in the neighbourhood. It was confirmed during the parallelly performed histological study of kidney tissues. Lysosomal proteases are sensitive indicators of a current function of an organ. Their changes can be observed in various ailments like renal cortex, liver, pancreas and heart (10).

Women receiving contraceptive steroids should be alert regarding their potential hazards and the physician must be informed of such risks and observe these patients with special control care. In some cases the control comprises the pathomorphological study. Actually, the knowledge about the relationship between the histological picture of the parenchymal human organs and the activity of lysosomal enzymes is not complete. The lysosomal enzymes could be indicators of toxic damage of parenchymal tissues. This statement needs detailed studies.

CONCLUSIONS

1. The activity of cathepsin B, D and L is uncharacteristic and seems to be secondary to the inflammatory process in kidneys.

2. The activity of proteolytic enzymes changes during estrogen therapy, but the above results do not show the relationship between their activity and the dose of injected estrogens.

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SUMMARY

Drug-induced kidney injury is a potential complication of a number of medicaments. The wide-spread use of estrogens in the form of oral contraceptives and in the form of hormonal replacement therapy, has necessitated extensive studies on the biochemical alterations. The following effects of estrogens on kidney are discussed in greater detail with special regard to enzyme induction.

The aim of the study was to evaluate the influence of long-term activity estrogens on kidney lysosomal enzymes like cathepsin B, D and L.

Female rats were divided randomly into eight experimental groups. Oestradiolum benzoicum was used for the purpose of this study. Estrogens were given i. m. one time per week for 8 weeks in different doses, respectively: E1 – 0.00075g/kg b. w., one time per week; E1.1 – 0.00075g/kg b. w. every three days; E2 – 0.0015g/kg b. w.. one time per week. E2.1 – 0.0015g/kg b. w.. every three days; E3 – 0.03g/kg b. w.. one time per week; E3.1 – 0.003g/kg b. w.. every three days; KO – untreated animals; K1 – treated control, rats received oleum pro injectione at the dose of 1.2ml/100g b.w. The activity of free and bound fractions of lysosomal enzymes, such as cathepsin B, D and L were assayed in kidney homogenates using spectrophotometric methods. Differences between various experimental groups were tested with ANOVA test.

The activity of cathepsin B, D and L fractions showed significant changes compared to control groups. The observed changes were not characteristic in all the studied groups. The most important changes referred to the activity of cathepsin B and D. Differences were noted between the enzymes activity in animals treated with the smallest dose of estrogen and that in control groups. It was smaller in group E1 than in groups K0 and K1. The activity of cathepsin B was higher in group E1 than in control groups. There was no correlation between the dose of injected estrogens and the observed lysosomal activity changes. The changes in lysosomal activity were uncharacteristic.

Wpływ estrogenów na aktywność proteolitycznych enzymów lizosomalnych w nerce

Zastosowanie naturalnych i syntetycznych hormonów, szczególnie w postaci środków antykoncepcyjnych oraz jako składników hormonalnej terapii zastępczej, wzbudziło zainteresowanie wpływem tych związków na zmiany biochemiczne w organizmie człowieka. Około 50% związanych estrogenów jest wydalanych bezpośrednio przez nerki z pominięciem wtórnych przemian metabolicznych w wątrobie. Nerki są odpowiedzialne za eliminację estrogenów.

Celem pracy była ocena aktywności frakcji wolnej i związanej katepsyny B, D i L w nerkach w trakcie długotrwałej terapii estrogenowej. Badania przeprowadzono na albinotycznych szczurach szczepu Wistar. Zwierzęta podzielono na 8 grup doświadczalnych: K0 – zwierzęta, którym nie podawano żadnych ksenobiotyków; K1 – zwierzęta, którym podawano preparat oleisty (oleum pro injectione) w dawce 1,2 ml/100g masy ciała; E1 – 0,00075 g/kg m. c. raz w tygodniu; E1.1 – 0,00075g/kg m. c., co 3 dni; E2 – 0,0015g/kg m. c. raz w tygodniu; E2.1 – 0,0015g/kg m. c. co trzy dni; E3 – 0,003g/kg m. c. raz w tygodniu; E3.1 – 0,003g/kg m. c. co trzy dni. Injekcje domięśniowe wykonywano przez 8 tygodni. W 56 dniu doświadczenia samice dekapitowano. Pobrane w trakcie autopsji nerki homogenizowano. Aktywność frakcji wolnej i związanej katepsyn B, D i L oznaczono metodą spektrofotometryczną.

Aktywność obu frakcji badanych enzymów wykazywała istotne statystycznie zmiany w porównaniu z aktywnością grup kontrolnych. Zależność ta jednak nie była charakterystyczna. Największe zmiany aktywności dotyczyły frakcji (wolnej. związanej i całkowitej) katepsyn D i B. Występowały istotne różnice między aktywnością enzymów w grupach kontrolnych i w grupach zwierząt leczonych najniższą dawką estrogenów. Wartości te były niższe w grupie E1 niż w grupach K0. K1 w odniesieniu do katepsyny D i L. Aktywność obu frakcji katepsyny B była wyższa w grupie E1 niż w grupach kontrolnych. Podsumowując, należy stwierdzić, że aktywność frakcji wolnej i związanej katepsyn B, D i L zmienia się w trakcie terapii estrogenowej, ale nie ma związku z dawką stosowanych estrogenów.