VARIATIONS OF VALUES OF LIVER DETOXIFICATION IN EXPERIMENTAL HYPOTHYROIDISM, DEPENDING ON ACETYLATION PHENOTYPE

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Owing to advances in diagnosis of thyroid disease and accumulation of new information about various aspects of its diagnosis, clinical course and management, the question of interconnection between thyroid and non-thyroid pathologies does not lose its relevance. [8] A wide prevalence of thyroid disease only increases the value of research carried out in this direction. In particular, in hypothyroidism, which is one of the most commonly diagnosed thyroid diseases, biochemical parameters characterizing functional activity of liver may serve as valuable diagnostic parameters [6]. As is well known, according to various studies, liver and intestines, which are the central organs of biotransformation of overwhelming majority of substances that enter the body, are directly involved in the metabolism of thyroid hormones [4, 5, 7]. Moreover, studies dedicated to the dependence of rate and completeness of metabolism of substances on the phenotype of acetylation, without which it is impossible to carry out a full-fledged modern study of thyroid pathology, are gaining popularity [1, 2].

Keywords: liver, hypothyroidism, phenotype

The purpose of current work was to identify differences and patterns in dynamics of blood biochemical parameters, characterizing the activity of the detoxifying function of liver and the intensity of catabolism in experimental hypothyroidism, in accordance with the phenotype of acetylation.

Materials and methods of research

The experiments were carried out on 60 rats with body mass of 180-220g, which were divided into two equal by the number of animals main groups, depending on the type of acetylation: α- slow and β-fast metabolizers (acetylators). An experimental hypothyroidism was simulated in both experimental groups by introducing mercazolil at a dose of 5 mg, given over 14 days [3]. Each group in turn was divided into 5 equal subgroups, depending on the periods of study. In this way, the group of α- "slow" metabolizers (acetylators) was divided into the following subgroups: I_{α} – intact, II_{α} – 4th hour of study, III_{α} – 1st week of study, IV_{α} – 2nd week of study, V_{α} – 3rd week of study. Accordingly, the group β – "fast" metabolizers (acetylators) consisted of: I_{β} – intact, II_{β} – 4th hour of study, III_{β} – 1st week of study, IV_{β} – 2nd week of study, V_{β} – 3rd week of study. The dynamics of values of thyroid hormones, reflecting the development of pathological process, were evaluated as within the given subgroups separately, considering the rate and type of acetylation, as well as in associate $I_{\alpha\beta}$, $II_{\alpha\beta}$, $III_{\alpha\beta}$, $IV_{\alpha\beta}$ and $IV_{\alpha\beta}$ groups regardless of acetylation phenotype. The results of this comparison are shown in Table 2. Likewise, the data of two analogous subgroups, with corresponding terms but different rate of acetylation, were combined and their statistically processed results were compared to those of other combined groups with different periods of study. The comparisons are presented in Table 1, which reflect the changes of the level of thyroid hormones in groups Iαβ $I_{\alpha\beta}$, $II_{\alpha\beta}$, $III_{\alpha\beta}$, $IV_{\alpha\beta}$ and $IV_{\alpha\beta}$.

The changes in the concentration of urea, creatinine, total protein and albumin were examined in the above terms and the parameters were studied as in combined groups, consisting of α and β subgroups (with phenotype of "slow" and "fast" acetylation of metabolic system of body, respectively), as well as within given subgroups separately and the reliability of differences between them were examined.

Acetylation phenotype was identified by the conventional method [1]. Biochemical parameters of concentration of substances in serum were determined by semiautomatic biochemical analyzer "Mindray BA88" (China), with test system "Human" (Germany) [5].

Statistical data processing was performed using Student's criterion using Excel and Biostat. The criterion for statistical significance was the importance of $P < 0,05$.

Results of research and their discussion

Changes of the blood biochemical indicators, such as urea and creatinine, which characterize intensity of catabolic processes, were observed at various stages of the modelled experimental hypothyroidism.

In this way, the level of urea in the 1st week of study was significantly higher than that indicator of animals from intact group, which was higher by 48,2 %, and with respect to the in the 4 hour experimental group of modelled thyroid pathology by 43,4% (Table 1). The level of this indicator in the 2nd week with respect to indicators of animals from intact group and animals from 4 hour group was higher by 48,7% and 43,9%, respectively; the analogous difference in the 3rd week was 64,1% and 58,8%.

Significantly high levels of creatinine were observed only in the 3rd week of experimental hypothyroidism, relative to the indices of animals of intact group, 4th hour, 1st and 2nd weeks, were respectively, 23,6%, 33,9%, 22,1 and 23,8%. It is clear from the provided data that there is a more pronounced difference in the 3rd week of experiment, with respect to the parameters of 4th hour, 1st, 2nd weeks of study, indicating lower serum creatinine values at an early period of experimental hypothyroidism.

As a result of the analysis of the results obtained in groups, separated according to the acetylation phenotype, statistically significant differences were found in the studied parameters (Table 2).

In this way, the level of urea in animals who were β – fast metabolizers (acetylators) at 4 hour of the study was higher by 47,4% relative to the parameters of intact animals with a similar phenotype, and on the 1st, 2nd and 3rd weeks, this difference was 52,3%, 48,9% and 76,0%. In the subgroup with α – "slow" metabolism phenotype (acetylation), a statistically significant increase in the concentration of urea compared with intact animals was observed only on the 3rd week of the study and was 40,0%.

Table 1

Changes in individual blood biochemical parameters of rats in modelled hypothyroidism (M ± m)

Studied groups	Urea (mmol/L)	Creatinine (mcmol/L)
$I_{αβ}$ groups of intact animals	3,90 ± 0,75	57,20 ± 2,6
$II_{αβ}$ groups (4th hour)	4,03 ± 0,87	52,78 ± 2,5
$III_{αβ}$ groups (1st week)	5,78 ± 0,19*^	57,90 ± 1,08
$IV_{αβ}$ groups (2nd week)	5,80 ± 0,12*^	57,08 ± 0,90
$V_{αβ}$ groups (3rd week)	6,40 ± 0,30*^	70,68 ± 1,80*^#&

Notes: * – significant difference (P < 0,05) when comparing results with those in the group of intact animals; ^ – same in comparing results with those in group 1; # – same in comparing results with those in group 2; & – same when comparing results with those in group 3; $ – same in comparing results with data in the same group to a subgroup of slow acetylators.

Table 2

Changes in individual blood biochemical parameters rat modeling of the experimental hypothyroidism subgroup with α- and β-acetylating phenotype (M ± m)

Groups, (term of study)	Subgroups	Urea (mmol/L)	Creatinine (mcmol/L)
$I_{αβ}$, (intact)	$I_α$	3,8 ± 0,8	56,4 ± 1,8
	$I_β$	4,05 ± 0,65	58,1 ± 2,1
$II_{αβ}$, (4th hour)	$II_α$	2,10 ± 0,07	35,07 ± 2,47*
	$II_β$	5,97 ± 0,05*$	70,50 ± 0,09*$
$III_{αβ}$, (1st week)	$III_α$	5,40 ± 0,12^	50,97 ± 0,14*^
	$III_β$	6,17 ± 0,09*$	64,83 ± 0,94*^$
$IV_{αβ}$, (2nd week)	$IV_α$	5,57 ± 0,02^	51,33 ± 0,16*^
	$IV_β$	6,03 ± 0,10*$	62,83 ± 0,70^$
$V_{αβ}$, (3rd week)	$V_α$	5,67 ± 0,05*^	56,33 ± 1,04^#&
	$V_β$	7,13 ± 0,06*^#&$	85,03 ± 0,48*^#&$

Notes: * – significant difference (P < 0,05) when comparing results with those in the group of intact animals; ^ – same when comparing results with those in group 1; # – same when comparing results with those in group 2; & – same when comparing the results with those in group 3; $ – same when comparing results with the data in the same group with α-subgroup of unstable metabolizers.

Compared to the 4th hour studies, in subgroups with β – "fast" phenotype of (acetylation) metabolism, a statistically significant increase in the level of urea was observed in the 3rd week of experiment and comprised 19,4%. In $III_α$, $IV_α$, $V_α$ subgroups, relatively to $II_α$ subgroup, the urea concentration was higher by 2,6, 2,7 and 2,7 times, respectively. In the subgroup with β-acetylation phenotype, higher rates were observed at ultimate terms (3rd week) of experiment compared to the 1st and 2nd weeks, which were 15.6% and 18.2% higher.

Statistically significant differences in the levels of urea among subgroups with different phenotypes of acetylation, except intact animals, were observed in almost all terms – on the 4th hour, 1st, 2nd and 3rd weeks of study and their values were higher by 184,3%, 14,3%, 8,3% and 25,7% respectively, in subgroups with phenotype of β-acetylation compared to phenotype of α-acetylation. Surveying the results of the study, it should be noted that there were insignificant of differences between subgroups with different rates of metabolism in the development of thyroid pathology and only a slight increase in these differences at ultimate term.

The obtained results demonstrate that the concentration of urea at 4th hour term of study

decreased insignificantly in subgroups with α-acetylation phenotype, whereas a statistically significant increase in this parameter was present in subgroups of animals with β-phenotype.

In all subsequent terms of studies, there were higher levels of urea relatively to the 4th hour with subgroups of α-phenotype. At the same time, there were higher levels of urea relatively to intact animals in subgroup with β- phenotype, compared to the concentration of it in α-subgroups. However, the indicator was not noted to change in dynamics, except for the ultimate term, where differences relatively to earlier terms were more pronounced compared to the differences in the values of urea in subgroups with α-acetylation phenotype. In general, dynamics of the urea level may suggest about more active changes of this indicator in the subgroup of β- "fast" metabolizers, compared to metabolizers with α- "slow" phenotype.

As is known, the basic way of neutralization of highly-toxic ammonia in the liver, which results from degradation of amino acids, is the formation of urea that is excreted with urine as a final product of protein metabolism in the body. The observed changes in the urea level may imply the extent of influence of experimental hypothyroidism, as on the intensity of catabolic processes as well as on the efficiency of the detoxifying ability of liver, associated with phenotype of acetylation.

As concerning the level of creatinine, subgroup with α-acetylation phenotype had lower by 60,8 % values at 4 hour term, and in the subgroup with β- "fast" phenotype of metabolism was by 21,3 % higher, compared to indicators of intact animals. In comparison with indicators of intact animals in subgroups with α- and β-phenotype creatinine concentration at the 1st week of study was by 10,7 % lower and 11,6 % higher, respectively. On the 2nd week of experiment, with respect to indicators of intact animals, statistically significant differences occurred in the subgroup with α-phenotype, where serum creatinine was 9,9 % lower and at 3rd week in animals with β-phenotype where this parameter was greater by 46,4 %. Comparing indicators within both "fast" and "slow" subgroups the results of creatinine concentration at 4-hour period were 45,3 % higher and 8,7 % lower, while in the 2nd and 3rd weeks of experimental pathology, relative to the same period of comparison, 46,4 % higher and 12,2 % lower, respectively, and by 60,6 % and 82,3 % higher. Compared with the 1st week results, there were no significant changes observed in IV_{α} and IV_{β} subgroups, and in V_{α} and V_{β} subgroups creatinine level was higher by 10,5 % and 31,2 %, respectively. On the 3rd week, the levels of creatinine relatively to those in the 2nd week of experiment were by 9,7 % higher in the subgroups with α-acetylation phenotype, and by 35,3 % higher in the subgroup with β- "fast" metabolism.

Analizing concentration of creatinine in subgroups with β-phenotype with respect to indicators of α- "slow" metabolizers, significant changes were observed at 4 hour term, where their concentration was twofold higher. On the 1st, 2nd and 3rd weeks this difference comprised 27,2 %, 22,4 % and 50,9 %. As can be noted from the above given results, the biggest difference between the two groups with different metabolic rates was observed in the early and late terms of experimental hypothyroidism.

In the study of changes of creatinine concentration at the earliest term of experiment (4 hour), in comparing the level of these parameters with intact animals, it should be noted decrease of their values was observed in the subgroups with α- "slow" phenotype, and, this trend preserved almost at all study periods, except for the ultimate, where statistically significant differences were not detected. By contrast, creatinine concentration in groups with β- acetylation phenotype increased at all stages of experimental hypothyroidism. The results of changing concentrations of creatinine indicate that phenotype of acetylation has a significant impact on its level in the blood serum in experimental hypothyroidism.

As is known, a significant portion of nitrogen, produced by the process of degradation of amino acids and formed from creatine and creatine phosphate, is excreted from body in the form of creatinine. Thus, creatinine, along with urea, is an important indicator of protein degradation. [9] Significant changes in the direction of creatinine concentration between subgroups with different acetylation phenotype suggest about differences in the mechanisms of utilization of products of protein degradation, which animals have depending on the type and rate of their metabolism in conditions of experimental hypothyroidism. It is known that normally, from total amount of nitrogen excreted with urine fraction of that in urea accounts for about 85 %, in creatinine – about 5 %, the remaining percentage is distributed among ammonium salts, uric acid and other compounds [4]. The obtained results suggest not only changes in the concentrations of urea and creatinine in general, but also their relationship to each other.

In support of what was said, ratios of urea to creatinine in subgroups with α-acetylation phenotype in intact animals, at 4-hour, 1-week, 2-week and 3-week terms of experimental hypothyroidism were 67,4, 58,9, 105,9, 108,5 and 100,7 %, respectively. An analogous correlation between urea and creatinine levels in the groups with β-acetylation phenotype at the same terms of study gave following ratios: 69,7,

84,7, 95,2, 96,0 and 100,7%, respectively. As seen from the above values, if in α-subgroups this ratio drops sharply at early terms, then by the 1st week it sharply increases, growing to 2nd week and slightly decreasing at ultimate term. In groups with β-phenotype, by contrast, even through all stages increase of values of urea to creatinine was noted. In general, comparable in intact with different phenotype animals values of the ratio of urea to creatinine become significantly different at 4 hour term, which with the development of experimental hypothyroidism slightly level out, disappearing toward the ultimate term of experiment.

The results obtained in studying individual parameters, which characterize the activity of the processes of protein degradation and liver detoxification in experimental hypothyroidism, demonstrate a statistically significant difference in the dynamics of changes in the values of these parameters between the subgroups with α- and β-acetylation phenotypes. The most pronounced differences of values among subgroups are shown to be in the group with β- "fast" phenotype of metabolism, unlike those found in a group with α-phenotype of metabolic activity. It should also be noted that the most significant differences of the studied parameters as comparing various periods of study, as well as between subgroups of same terms, appear in the later stages of the simulated experimental hypothyroidism.

Conclusion

1. Experimental hypothyroidism in dynamics, in general characterized by increased concentrations of parameters, which characterize the intensity of protein degradation and detoxification function of liver.

2. From the studied in experimental hypothyroidism parameters the most pronounced differences observed among subgroups with α- and β-acetylation phenotypes were in the levels of creatinine.

3. It is characteristic for animals with β-acetylation phenotype to have more marked changes in the values of creatinine and urea at ultimate terms of studied experimental hypothyroidism, than for animals with α-acetylation phenotype.

References

1. Goldenkova I.V. Comparative analysis of phenotyping and genotyping of N-acetylation polymorphism in humans. Genetics. 2006; 42: 1143–1150. 8.

2. Evgenyev M.I., Harmonov S.Y., Gismyatov R.G., Shitova N.S., Zykov I.E. Non-invasive methods of determination of acetylation phenotype. Issues of Biololgy, Medicine and pharmacist chemistry. 2003; 3: 34–39.

3. Manyuk E.S., Izatullin V.G., Vasilieva L.S. Correction of experimental hypothyroidism with herbal preparation "Bayakon" Bulletin of AUSC RAMS. 2008; 59: 54–57. 1

4. Nikitina L.P., Solovyov N.V., Tsidendambaev P.B. Biochemistry of the liver in health and disease: a learning aid for teachers and medical students, physicians, interns, clinical residents. Chita CHGMA, 2004; 52.

5. Nikolaev A.Y. Biological Chemistry / AY Nikolaev. – 4th ed., Rev. and ext. M.: Medical news agency. 2004; 556.

6. Fadeev V.V. Modern concepts of the diagnosis and treatment of hypothyroidism in adults. Issues of Endocrinol. 2004; 2: 47–53.

7. Tkachuk A. Clinical Biochemistry. ed. pers. corr. Russian Academy of Sciences, Academician of RAMS VA Tkachuk. – 2nd ed., Revised and edited. – M.: GEOTAR-MED, 2004; 512.

8. Lindeman R.D., Romero L.J., Schade D.S. et al. Impact of subclinical hypothyroidism on serum total homocysteine concentrations, the prevalence of coronary heart disease (CHD), and CHD risk factors in the New Mexico Elder Health Survey. Thyroid. , 2003; 13: 595–600. 6.