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Instances of folliculogenesis in rat thyroid: The norm vs. the experiment

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Abstract:
Objective: identification and characterization of the stages of new follicle formation, along with measuring the frequency of folliculogenesis in various parts of the thyroid in normal and experimental conditions.
Materials and Methods. The experiment was performed on 36 mature white rats distributed among three groups: the control (Group I), 60-day exposure to sodium benzoate at a dose of 1,000 mg/kg (Group II), and administration of Mexidol at a dose of 50 mg/kg against the background of a 60-day exposure to sodium benzoate (Group III). The stages of folliculogenesis were studied using light microscopy followed by statistical analysis of its frequency.
Results. The formation of a new follicle begins with the proliferation of thyrocytes on the cell wall of the mature follicle, after which a cavity is formed between the cells into which they secrete colloid. As the follicle grows, the height of the thyrocytes declines, and the size of their cavity and the amount of colloid increase. In Group II vs. Group I, on days 3 and 24 of the experiment, signs of folliculogenesis were detected 1.7 and 1.4 timesless frequently in the thyroid center, and 2 and 1.2 times less often in the thyroid periphery; while in Group III vs. Group II, these were observed1.3 and 1.6 timesmore often in the center of the organ, and 1.3 and 1.3 times more frequently in the thyroid periphery.
Conclusion. During folliculogenesis in the thyroid, focal proliferation of thyrocytes develops in the cell wall of one of the follicles with further formation of the cavity of the newly formed follicle and its growth. Normally, the formationfrequency of new follicles is higher in the organcenter vs. the periphery and lower in both thyroid zones in the group of rats exposed to sodium benzoate. However, in the group of rats where sodium benzoate administration waspharmaceuticallycorrected by Mexidol, the frequency of the young follicleformation increased, albeitit did not reach the control values.

Keywords: thyroid, thyrocytes, follicle, sodium benzoate, Mexidol.

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Introduction

One of the unique properties of living organisms is theircapability to regeneration of damaged cells. The regenerative potential of different cells varies, which is associated with different sources of their origin, structure and functions [1]. Follicular cells of the thyroid (epithelial in nature) have pronounced secretory activity and sensitivity to various exogenous and endogenous influences. The habitualbodyresponse to cell death is to restore the integrity of the tissue via hypertrophy of surrounding cells or their proliferation. However, we could not find published information about the morphological features of the thyrocyte regeneration process and the stages of new follicleformation, as well as its frequency of occurrence in different parts of the organ.

Objective – To identify and describe the stages of new follicleformation, along withmeasuring the frequency of folliculogenesis in various parts of the thyroid in normal and experimental conditions.

Materials and Methods

The experiment was carried out on 36 mature white male rats weighing 200-210 g distributed among three groups: Group I (C) represented the control; Group II (SB2) included rats were exposed to 1,000 mg/kg of sodium benzoate (Eastman Chemical B.V., the Netherlands) for 60 days; Group III (SB2+M) comprised animals exposed, besides sodium benzoate, to 50 mg/kg of Mexidol (Ellara Medical Center LLC). Animals were removed from the experiment by decapitation under ether anesthesia on days 3 and 24 after completion of 60-day exposure to sodium benzoate or combined exposure to sodium benzoate and Mexidol. Histological processing of thyroid tissue was performed according to standard methods, and sections were stained with hematoxylin and eosin. Then we analyzed 10 fragmentsin each section of the thyroid sampled from each animal at a high magnification (600×) (center and periphery of the organ) in the control and experimental groups in order to identify signs of the formation of early follicles from the cell wall of a mature follicle. Considering that each glass slide contained six sections of the organ, we analyzed 60 fragments

of the center and periphery of the thyroid in each animal. Statistical data processing was completed using STATISTICA 12.0 software (StatSoft, Inc., USA) and Microsoft Office Excel 2017. To evaluate the type of data distribution, the Shapiro–Wilk test was employed. Values are presented as M±SE, where M is the sample mean and SE is the standard error of the mean. To compare samples, weusednonparametric tests: the Mann–Whitney U test for independent samples and the Wilcoxon W test for dependent samples. Differences were considered statistically significant at $p<0.05$, where p is the probability of a type I error when testing the null hypothesis. In all instances, two-tailedtests were performed. When comparing several groups with each other, we used the Bonferroni correction for multiple comparisons [2].

Results

In the course of analyzing histological preparationsof the thyroid in all groups, wedetectedfollicles with a specific structure on days 3 and 24 of the experiment. For instance, in some follicles, part of theircell wall was thickened and contained several layers of thyrocytes; while in other follicles, such cells had a cavity of varying sizes. It is worth noting that the cavity was filled with a substance similar to the colloidin its color intensity and was separated by one layer of thyrocytes from the main cavity of the follicle. Taking this into account, we assumed that the process of a young follicle formation from the cell wall of a mature follicle was observed, which morphologically can be divided into three following stages.

I. Proliferation stage (a group of cubic or prismatic thyrocytes of typical morphology located in several layers on the wall of a medium or large follicle).

II. Stage of cavity formation (a spherical or oval-shaped cavity filled with contents similar in color intensity to the colloidappears between the thyrocytes).

III. Stage of a young follicle growth (enlargement of the cavity with colloid and transformation of bordering thyrocytes limiting into low cubic or flat cells) (*Figure*).

In animals of the control group, the frequency of new follicleformation in the central part of the thyroidincreased from 7.5±0.6 to 7.8±0.7 (p=0.943), whereas in the peripheral part, it decreased from 4.5±0.6 to 4.3± 0.3 (p=0.952).

In Group II where animals received sodium benzoate at a dosage of 1,000 mg/kg of their body weight, we detectedsigns of folliculogenesison days 3 and 24of the experiment 2 and 1.2 times less often, respectively,in the center of the thyroid gland, and 1.7 and 1.4 times less often, correspondingly, in the periphery, compared with the control group.

In the Group III in which rats were administered the pharmaceutical corrector Mexidol against the background of 60-day exposure to sodium benzoate at a dose of 1,000 mg/kg of their body weighton days 3 and 24 of the experiment, we observed young follicles forming on the wall of a mature follicle 1.3 and 1.6 times more often, respectively, in the thyroid center, and 1.3 and 1.3 times more often, correspondingly, in the thyroid periphery, compared with the group with isolated administration of sodium benzoate (*Table*).

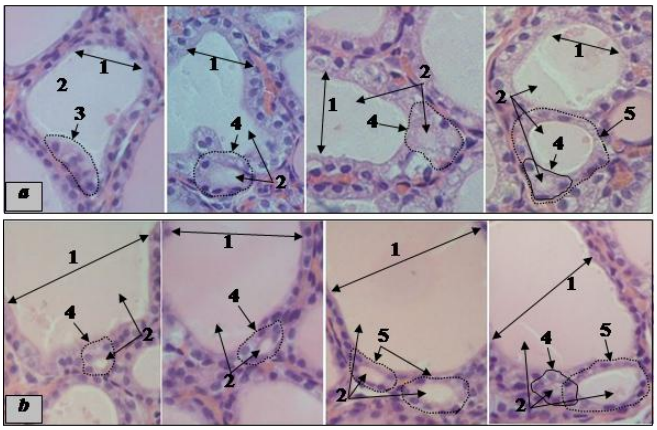

Figure. Histological structure of the central (a) and peripheral (b) sections of the thyroid in rats of the SB2+M group on day 24 of theexperiment: 1 – follicle; 2 – colloid; 3 – proliferation stage; 4 – stage of the cavity formation in a young follicle; 5 – young follicle growthstage. Hematoxylin and eosin staining. Magnification 600×

Table. Formation frequencies of young thyroidfollicles, M±SE

Group, *n*	Days of the experiment				Statistical significance of differences	
	Day 3		Day 24			
	Thyroid				p_{1-3}	p_{2-4}
	Center	Periphery	Center	Periphery		
C (*n*=10)	7.5±0.6	4.5±0.6	7.8±0.7	4.3±0.3	0.943	0.952
SB2 (*n*=10)	4.4±0.5 $p_{к-2}$=**0.003**	2.2±0.3 $p_{к-2}$=**0.014**	5.4±0.6 $p_{к-2}$=**0.047**	3.6±0.3 $p_{к-2}$=0.253	0.423	**0.012**
SB2+M (*n*=10)	5.6±0.6 $p_{к-3}$=0.298	3.6±0.4 $p_{к-3}$=**0.032**	6.8±0.7 $p_{к-3}$=0.306	4.7±0.5 $p_{к-3}$=0.173	0.411	0.227

pk–2, statistical significance of differences between SB2 andC groups; pк–3,statistical significance of differences between SB2 and SB2+M groups;p1–3,statistical significance of differences between day 3 and day 24values in the central zone; p2–4,statistical significance of differences between day 3 and day 24values in the peripheral zone

Discussion

We encounteredpublished evidence that the presence of several rows of thyrocytes occurs as a result of their mitotic division, which is directly related to the level of thyroid-stimulating hormone [3, 4]. It is known that the thyroid is capable of regeneration, but this process is not well studied [5]. What was described earlier suggested that the identified parietal cell clusters were the result of thyrocyte mitosis. The emergence of a cavity between thyrocytes and its filling with secretion may have indicated that the latter was a product of their activity. Consequently, the morphological similarity with typical follicles allowed considering this structure as a young follicle formed from the wall of a mature follicle. Bearing in mind that the activity of thyrocytes in the thyroid periphery was weaker than in its center [6], it seemed feasible to explain the general (for all groups) trend of more frequent formation of young follicles in the center of the organ than in its periphery. In the SB2 group, the general trend of a reduction in the formation frequency of young follicles in different parts of the organ was associated with the mechanism of action of sodium benzoate (which is known to reduce the level of thyroid-stimulating hormone and disrupt the DNA structure both directly and via the initiation of oxidative stress) [7, 8]. The use of Mexidol (which has an antioxidant effect) [9] leveled off the effect of sodium benzoate and increased the frequency of the appearance of young follicles in the thyroid, compared with the data of SB2 group on days 3 and 24 of the experiment.

Conclusion

During the process of folliculogenesis in the thyroid gland, focal proliferation of thyrocytes develops in the cell wall of one of the follicles with further formation of the cavity in the newly formed follicle and its growth. Normally, the formationfrequency of new follicles is higher in the center of the organ than in its periphery, and lower in both thyroid zones in the group of rats exposed to sodium benzoate. However, in the group of rats where the administration of sodium benzoate was corrected by the exposure toMexidol, the frequency of the young follicleformation increased, albeit it did not reach the control values.

Author contributions: all authors contributed equally to the preparation of the manuscript.

Conflict of interest: the authors declare no conflicts of interest

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