Morphological Parameters of the Myocardium in Spontaneously Hypertensive Rats upon Administration of Propylthiouracil

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ABSTRACT

The morphological changes in myocardial tissue in spontaneously hypertensive rat (SHR) occurring after administration of propylthiouracil (PTU) were assessed in the project. PTU at a concentration of 0.1% was given to the animals with drinking water for 47 days. After the end of the experiment, the morphological parameters of cardiomyocytes, the density of cardiomyocytes and fibroblasts in the myocardium, and the average area of the lumen of veins were examined. The progressive development of dystrophic processes in the myocardium was established against the background of PTU intake, which was characterized by a decrease in the cardiomyocyte density and an increase in the volume of connective tissue, venous congestion, and expansion of the lumen of veins.

KEYWORDS: Propylthiouracil; Spontaneously Hypertensive Rat; Myocardium; Cardiomyocytes.

1. INTRODUCTION

Hyperthyroidism is accompanied by damage to the cardiovascular system, which may result in the occurrence of arterial hypertension [1]. Propylthiouracil (PTU) is a "drug of choice" that is used for hyperthyroidism in the first trimester of pregnancy [2]. However, there are facts indicating that PTU causes vasculitis and venous hyperemia of some organs [3-5]. The effect of PTU on the state of the myocardium is still insufficiently studied, especially if it is realized against the background of already existing pathological changes in the cardiovascular system due to hypertension.

Spontaneously hypertensive rats (SHR) can be used as an experimental model for this kind of research, because their pathophysiological and pathomorphological signs of hypertension are similar to those observed in the human body [6].

The aim of this work was to assess morphological changes in myocardial tissue in SHR that occur after long-term introduction of PTU.

2. METHOD(S)

In the experiments, SHR females of 6 months were used. All animal experiments were conducted in accordance with the institutional guidelines and have been approved by the institutional review boards. PTU ("Sigma," USA) at a concentration of 0.1% was introduced with drinking water according to the method of Polychronakos *et al*. [7], and the animals were kept with free access to balanced dry food ("Gora," Ukraine). The rats were sacrificed on the 17th, 25th, 31st, 39th, and 47th days of the experiment (7 animals for each period). Fragments of the heart were fixed in 10% neutral formalin, after which they were subjected to histological processing. Histological specimens were stained with hematoxylin and eosin according to the standard technique. An AmScope XYL-403 microscope with a digital camera was used for microscopic analysis.

In histological preparations, the following were measured: the average area of the cytoplasm of the cardiomyocyte (S_{cc}), the average area of the nucleus of the cardiomyocyte (S_{nc}), the cardiomyocyte nuclear-cytoplasmic ratio (N/C ratio), the density of cardiomyocytes as their number per 0.05 mm^2 (D_c), the density of fibroblasts as their number per 0.05 mm^2 (D_f), and the average area of the lumen of the veins (S_v). Counting was performed on 10 sections obtained from each sample. For measurements, the AxioVision Rel 4.8 program ("CarlZeiss," Germany) was used.

The experimental results are presented as Me (Q1; Q3). The statistical significance of differences between groups was assessed using the Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

The microscopic structure of the myocardium of intact SHR showed the presence of characteristic compensatory changes [8]. Hypertrophy and polymorphism of cardiomyocytes were revealed. We observed both cardiomyocytes containing one centrally located basophilic nucleus and oxyphilic cytoplasm as well as binuclear cardiomyocytes. Single cardiomyocytes with a pyknotic nucleus were visualized. Loose connective tissue with numerous capillaries, oxyphilic thin collagen fibers, and fibroblasts with flat and basophilic nuclei were well identified between cardiomyocytes. Bundles of collagen fibers were concentrated around large- and medium-sized vessels. Areas with thinned, unevenly hypertrophied, tortuous muscle fibers and focal myocytolysis were found.

Application of PTU caused marked changes in the structure of the myocardium in SHR. Especially from the 25th day after PTU intake, degenerative-dystrophic changes were visually observed in the myocardium of the animals, displayed by a decrease in the packing density of cardiomyocytes, a change in the shape of cardiomyocytes and pycnosis of their nuclei. Areas with focal myocytolysis and infiltration were often found. Around the thinned muscle fibers, zones of fibrosis were identified in the form of wide layers of connective tissue with bundles of oxyphilic collagen fibers and numerous fibroblasts.

On days 39 to 47 after PTU intake, necrotic areas were visualized in the myocardium, characterized by the presence of fragmented, destructed, nuclear-free cardiomyocytes with no cross-striation, while the boundaries between cells were poorly visualized. In the interstitial connective tissue around the necrotic areas of the myocardium, the number of fibroblasts decreased, and the capillaries were expanded and filled with blood cells. All vessels were characterized by plethora, their walls were thickened, and connective tissue with collagen fibers and numerous fibroblasts were found around them.

Analysis of quantitative morphometric parameters of the myocardium of SHR after the first 25 days of PTU administration revealed a statistically significant decrease in the area of the cytoplasm of cardiomyocytes S_{cc} (Table 1). At the same time, the area of the nuclei of cardiomyocytes S_{nc} did not change compared to day 0, and a statistically significant increase in their nuclear-cytoplasmic ratio was observed due to a decrease in cytoplasm. From day 31 of PTU intake to the end of observation (day 47), there was a progressive decrease not only in the average area of the S_{cc}, but also in the S_{nc}. In this regard, the nuclear-cytoplasmic ratio decreased almost to the initial values, but this was a sign of nuclear pycnosis and degenerative changes in cardiomyocytes.

Within 47 days of PTU administration, the density of cardiomyocytes D_c decreased 4 times, and the density of fibroblasts D_f increased 2 times. The average area of the veins S_v approximately increased 3 times.

Table 1: Morphological parameters of the myocardium of SHR with PTU intake.

Indicator	Days of PTU intake					
	0	17	25	31	39	47
S_{cc}, mm^2	350 (280; 392)	179 (140; 294) *	180 (148; 246) *	124 (105; 142) *	64 (59; 70) *	77 (65; 87) *
S_{nc}, mm^2	29 (27; 31)	23 (19; 28)	28 (26; 32)	18 (16; 22) *	9 (8; 10) *	8 (7; 9) *
N/C ratio	0.08 (0.07; 0.10)	0.12 (0.08; 0.16) *	0.15 (0.11; 0.19) *	0.14 (0.13; 0.19) *	0.14 (0.11; 0.16) *	0.10 (0.08; 0.10)
D_c	20 (17; 22)	16 (14; 20) *	14 (12; 16) *	9 (7; 12) *	5 (4; 7) *	5 (3; 7) *
D_f	11 (9; 13)	15 (12; 19)*	20 (15; 25)*	20 (17; 22)*	21 (17; 25)*	22 (19; 27)*
S_v, mm^2	1665 (1430; 1900)	2078 (1154; 2245)	3959 (2376; 4118)	2855 (2638; 3282)*	3425 (3095; 3723)*	5161 (4787; 5517)*

*The indicator is statistically significantly different from day 0 ($p < 0.05$).

4. DISCUSSION

As it was determined earlier, the characteristic signs of age-related remodeling of the myocardium in SHR are an increase in the area of cardiomyocytes and their nuclei, as well as a decrease in the density of cardiomyocytes in comparison with normotensive animals [8, 9]. Myocardial hypertrophy in rats of this strain, reaching a 30% increase in heart weight, occurs as a compensatory response to a constant increase in systemic arterial pressure [10, 11]. In addition, as a rule, in rats of this strain, changes in the normal architectonics of the myocardium are found in the form of zones of replacing fibrosis at the site of the lost cardiomyocytes [12].

The use of PTU has a multidirectional effect on the histological characteristics of the myocardium in SHR. At the initial stages of PTU intake, dystrophic processes developed in the myocardium of rats, which were manifested by a decrease in the area of the cytoplasm of cardiomyocytes. However, at the PTU intake for no more than a month, these changes did not go beyond

the cardiomyocytes in normotensive rats [9]. A longer intake of PTU was manifested in a further decrease in the area of the cardiomyocyte cytoplasm and pycnosis of the nuclei. The increase in dystrophic changes in the myocardium was accompanied by an increase in the volume of connective tissue with the achievement of the maximum pronounced value on the 47th day of PTU intake. This indicated that the loss of cardiomyocytes due to PTU intake stimulated the proliferation of fibroblasts, leading to the appearance of extensive zones of myocardial fibrosis.

The histological evaluation showed that the use of PTU led to plethora of blood vessels and capillaries, venous congestion, and expansion of the lumen of veins and capillaries. Since hypertension is accompanied by an increase in vascular permeability [13], such PTU-induced circulatory disorders against this background carry the risk of perivascular edema, the development of heart failure due to ischemia, and dystrophic changes in myocardium.

5. CONCLUSION

Intake of PTU for 47 days in SHR leads to impaired coronary circulation and the development of degenerative processes in the myocardium, which are manifested by a decrease in the area of the cytoplasm of cardiomyocytes, pycnosis of their nuclei, loss of cardiomyocyte, and fibrotic changes.

AUTHOR CONTRIBUTIONS

Study conception and design: K.O. Pobelensky, E.I. Legach. Data collection: K.O. Pobelensky, N.V. Kolot. Analysis and interpretation of results: K.O. Pobelensky, E.S. Protsenko, G.A. Bozhok. Draft manuscript preparation: K.O. Pobelensky, G.A. Bozhok. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest.

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