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The Effect of Changes in Thyroid Hormone Levels on Learning and Memory in Middle Aged Rats

Orta Yaşlı Sıçanlarda Tiroid Hormon Seviyesindeki Değişikliklerin Öğrenme ve Bellek Üzerine Etkisi

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ABSTRACT

Aim: The increase in the frequency of thyroid diseases in old age makes it necessary to reveal the relationship between aging and irregularities in thyroid hormone levels. In the present study, the effects of experimental hypothyroidism and thyrotoxicosis induced in middle aged rats on spatial learning and memory performance were investigated.

Materials and Methods: In this study, 45 Wistar albino 12-month-old rats weighing 400-450 g were used. The rats were divided into three groups according to thyroid hormone levels; euthyroid (n=16), hyperthyroid (n=16) and hypothyroid (n=13). Thyrotoxicosis was induced by intraperitoneal (i.p.) administration of L-thyroxine at a dose of 0.2 mg/kg/day. Hypothyroid state was induced by daily administration of 0.05% 6-n-propyl-thiouracil (PTU) in the drinking water of rats. Spatial memory and learning performance of rats were evaluated by Morris water maze test. Thyroid hormone levels were determined with a commercial ELISA kit.

Results: One-way ANOVA test revealed that the mean distance to the platform ($p<0.05$), escape time ($p<0.01$), swimming speed ($p<0.001$) and distance traveled ($p<0.05$) values of the rats showed significant differences between the groups. According to these results; learning performance of the hypothyroidism group decreased compared to the control group, while learning and memory performance of the thyrotoxicosis group did not differ from the control group.

Conclusion: Hypothyroidism during aging has a negative effect on learning and memory performance compared to thyrotoxicosis. Revealing the changes in thyroid hormone metabolism in middle aged and determining the daily requirement will provide a different perspective on aging-related dementia-type diseases.

Keywords: Aging, hypothyroidism, learning and memory, thyrotoxicosis

ÖZ

Amaç: Yaşlılık döneminde tiroid hastalıklarının sıklığındaki artış, yaşlanma ve tiroid hormon (TH) seviyesindeki düzensizliklerin ilişkisinin ortaya konulmasını gerekli kılmaktadır. Bu çalışmada, orta yaşlı sıçanlarda indüklenen deneysel hipotiroidi ve tirotoksikozun uzamsal öğrenme ve bellek performansı üzerine etkisi araştırılmıştır.

Gereç ve Yöntem: Bu çalışmada 45 adet Wistar albino cinsi 400-450 gr ağırlığında 12 aylık sıçanlar kullanıldı. Sıçanlar TH seviyesi dikkate alınarak üç gruba ayrıldı; ötiroidi (n=16), tirotoksikoz (n=16) ve hipotiroidi (n=13). Tirotoksikoz durumu, her gün 0,2 mg/kg/gün dozda L-tiroksin intraperitoneal (i.p.) olarak uygulaması ile oluşturuldu. Hipotiroidi durumu günlük %0,05'lik 6-n-propyl-tiyourasilin (PTU) sıçanların içme suyuna karıştırılması ile indüklendi. Sıçanların uzaysal hafıza ve öğrenme performansları Morris su tankı testi ile değerlendirildi. TH testleri ticari ELISA kiti ile çalışıldı.

Bulgular: Tek yönlü ANOVA testi, sıçanların platforma olan ortalama uzaklık ($p<0,05$), kaçış süresi ($p<0,01$), yüzme hızı ($p<0,001$) ve kat edilen mesafe ($p<0,05$) değerlerinin gruplar arasında anlamlı farklılık gösterdiğini ortaya koydu. Bu sonuçlara göre; hipotiroidi grubu öğrenme performansı kontrol grubuna göre azalırken tirotoksikoz grubunun öğrenme ve bellek performansı kontrol grubundan farklılık göstermedi.

Sonuç: Yaşlanma sürecinde ortaya çıkan hipotiroidi durumu tirotoksikoz durumuna kıyasla öğrenme ve bellek performansı üzerine olumsuz etki göstermektedir. Orta yaşlılık döneminde TH metabolizmasındaki değişimin açığa çıkarılması ve günlük ihtiyacın belirlenmesi, yaşlanmaya bağlı görülen demans tipi hastalıklara farklı bir bakış açısı sağlayacaktır.

Anahtar Kelimeler: Yaşlanma, hipotiroidi, öğrenme ve bellek, tirotoksikoz

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INTRODUCTION

Biologically, aging is a term used to express the physiological changes that occur in the organism with the advancement of chronological age. Developments in the field of health and social welfare have resulted in an increase in life expectancy and therefore an increase in the proportion of the elderly population in the population. The ratio of the elderly population to the total population is increasing rapidly in our country, as in the rest of the world. An increase of 201% in the elderly population in Turkey between 2008 and 2040 has been reported[1]. While this phenomenon highlights the importance of healthy aging, it also creates the need to focus on the diagnosis and treatment of dementia-type diseases, the incidence of which increases in old age. Many studies on the aging brain have revealed pathological changes such as neuron loss, synaptic dystrophy and decrease in brain volume[2,3].

In various animal species, the aging process is associated with learning and memory disorders that correlate with the decrease in synaptic transmission and plasticity occurring in different regions of the brain[4]. Research on memory has revealed that the hippocampus is the structure that plays a fundamental role in the formation, storage and recall of memories[5]. Revealing the complex circuits between the hippocampus and the entorhinal cortex and other structures, damage to memory-related functions in hippocampal damage, and histological demonstration of place cells have provided further evidence on the role of the hippocampus in memory[6,7]. It is thought that the weakening of cognitive functions in older people will be due to the decrease in synaptic plasticity, which is defined as the ability of the individual to form and eliminate neural connections throughout his life[8]. Numerous data have revealed that granule cells in the hippocampus decrease radically with aging[9]. At the same time, the decrease in these cells with neurogenesis ability with aging emerges as one of the factors suggested for the deterioration of the cognitive process during aging[10].

Changes in thyroid hormone (TH) levels have dramatic effects on cortical functions and neuronal functions both in the intra-uterine and postnatal periods. While studies on the effects of THs on the central nervous system in the early period are widely available in the literature, studies on the irregularities in TH levels seen in adulthood are limited. In line with the roles of THs in growth and development, TH levels are closely monitored during pregnancy or in newborns. However, the frequency of thyroid diseases that begin in childhood and adulthood is equally high. The prevalence of hypothyroidism in children under the age of 10 in Turkey is reported to be approximately 21%[11]. In the general population, subclinical hypothyroidism is seen at a rate of 14-18%[12]. The prevalence of hypothyroidism in people over the age of 74 was found to be 21% in women and 16% in men[13]. Therefore, considering the increase in the frequency of dementia-type diseases characterized by a decrease in cognitive performance and functional weakening with age, there is a need to reveal the relationship between TH and cognitive functions in old age. It has been reported that hypothyroidism and hyperthyroidism in adulthood increase the deterioration in electrophysiological and behavioral indicators that evaluate synaptic plasticity[14]. In this study, the effects of experimental hypothyroidism and thyrotoxicosis induced in old rats on spatial learning and memory performance were investigated. The increase in the incidence and prevalence of thyroid diseases in old age makes it necessary to reveal the relationship between old age and TH irregularities.

MATERIALS AND METHODS

Experimental Animals and Grouping

All experiments were performed on Wistar Albino rats. Forty-five 12-month-old rats weighing 400-450 g were obtained from Erciyes University Hakan Çetinsaya Experimental and Clinical Research Center and were housed in accordance with the National Health Institutes guide for humane animal care and use. The rats were subjected to a 14-hour light-10-hour dark cycle and were fed without water or feed restrictions. Ethics committee approval was received with the decision of Erciyes University Animal Experiments Ethics Committee dated 09.12.2015 and numbered 15/150. Rats; they were divided into three groups as thyrotoxicosis group (n=16), hypothyroid group (n=13), euthyroid (control) group (n=16). Thyrotoxicosis group: L-thyroxine was injected intraperitoneally (i.p.) at 0.2 mg/kg daily for 60 days to induce thyrotoxicosis in 16 12-month-old rats[15]. This induction method and drug dose was preferred because chronic L-thyroxine administration caused a high increase in serum free T3 and free T4 levels[16,17]. Hypothyroidism group: To create hypothyroidism in 13 12-month-old rats, 0.1 ml of 0.5% 6-n-propyl thiouracil (PTU) was added to their drinking water every day for 60 days.

Old age for Wistar albino rats; early old age (equivalent to 25-40 years in humans, 150-300 days for rats), middle old age (equivalent to 40-65 years in humans, 300-600 days for rats), old old age (equivalent to 65-75 years in humans, 600-600 days for rats) and late senility (its equivalent in humans is over 75 years of age, for rats it is 730 days and above)[18].

Measurement of Thyroid Hormone Levels

Rats were anesthetized by i.p. administration of a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg) and blood was collected into heparinized tubes. The obtained plasma was stored at -80 °C for measurement of T4 levels. Measurements were made using the ELISA method with the Multiskan™ FC

Microplate Photometer device at 450 nm, in accordance with the manufacturer's instructions.

Morris Water Tank Test Application

Morris Water Tank (MST) experiments were carried out with the video monitoring and recording system of the water tank (130 cm in diameter and 45 cm in height) located in Erciyes University Physiology Behavior Laboratory (n=16). An escape platform (diameter: 10 cm, height 22 cm) on which the rats could stand was placed in one of the four quadrants of the water tank area, and it was filled with water to a level of 1 cm above this platform. The water was dyed with a blue, non-toxic dye to prevent the rats from seeing the location of the hidden platform. The location of the platform was kept constant during all learning trials and care was taken to keep the water temperature between 20-22 °C. During each learning trial, the rat was placed in the water so that it could see the large clues around it from a quadrant other than the quadrant where the platform was located. The behavior to be learned in these trials is for the rat to find the platform and get out of the water by climbing on it within 2 minutes of swimming. Rats that could not find the platform during this period were helped to find the platform and were left on the platform for 15 seconds to observe the clues.

Learning trials were performed on each rat four times a day (half an hour apart) for 4 days. Twenty-four hours after the last learning trial (16th trial), the platform in the tank was removed and the same test was applied without the platform (Probe trial). While performing statistical analysis, the change in learning performance according to days and the change in learning performance according to trials were recorded, and it was investigated whether learning took place and whether there was a significant difference between the groups. In each trial, the behavior of the rats was recorded by the Noldus Ethovision video-monitoring and analysis system, and the time it took the rats to find the platform, swimming distance, time spent in the platform area, swimming speed and time in each quadrant area were recorded. Trials were carried out every day and at the same time of the day (10:00-14:00).

Statistical Analysis

Statistical analysis of behavioral experiments was performed with Statistical Package for the Social Sciences version 16 package program. Learning performances changing throughout the days were analyzed via factorial ANOVA with repeated measurements. Normality and sphericity evaluations of the data were made with Shapiro-Wilk and Mauchly tests. Since the data had equal variance, comparisons between groups were made with one-way ANOVA test. The probability level for statistical significance was accepted as $p<0.05$. Values are expressed as mean±standard error.

RESULTS

Thyroid Hormone Values

At the end of the behavioral experiments, after the rats were anesthetized with the ketamine+xylazine combination, plasma free T3 (fT3) and free T4 (fT4) levels were measured in the blood taken intracardially. Plasma fT3 (control: 11.48±5.09 pg/mL, hypothyroid: 6.10±1.16 pg/mL, thyrotoxicosis: 29.09±2.92 pg/mL) and fT4 (control: 34.44±6.55 ng/dL, hypothyroid: 23.68±3.67 ng/dL, thyrotoxicosis: 51.09±2.92 ng/dL) values were measured lower in the hypothyroid group than in the control group and the thyrotoxicosis group ($p<0.05$, n=6).

Morris Water Tank Test Results

The distance traveled decreased significantly throughout Day ($F=41.076$, $p<0.001$), Trial ($F=48.26$, $p<0.001$) and Day*Trial ($F=2.491$, $p=0.009$), showing that learning took place (Figure 1A). TH level status variable has shown a statistically significant effect on Day*Group ($F=2.512$, $p=0.025$), Trial*Group ($F=2.673$, $p=0.18$) and Day*Trial*Group ($F=2.654$, $p<0.001$). In the 1st and 2nd trial of the 2nd day, the 3rd trial of the 3rd day, and the 1st and 2nd trial of the 4th day, the hypothyroidism group covered more distance than the control and thyrotoxicosis groups, creating a statistically significant difference.

Escape time values are given in Figure 1B. The significance of Day ($F=48.313$, $p<0.001$), Trial ($F=14.232$, $p<0.001$) and Day*Trial ($F=4.603$, $p<0.001$) during the escape period showed that learning occurred in all groups. While the TH level variable did not show a statistically significant effect on Day*Group and Trial*Group, the Day*Trial*Group effect ($F=2.266$, $p=0.002$) was found to be significant. In the 1st trial and 4th trial of the 1st day, in the 1st trial and 2nd trial of the 2nd day, in the 2nd trial and 4th trial of the 4th day, the hypothyroidism group found the hidden platform in a longer time than the control and thyrotoxicosis groups, creating a statistically significant difference from them.

When the average distance values of euthyroid and experimental group rats to the learning platform were examined, a significant Day ($F=42.913$, $p<0.001$) and Trial ($F=14.543$, $p<0.001$) effects were observed (Figure 1C). Day*Group ($F=2.315$, $p<0.05$) and Day*Group*Trial ($F=1.740$, $p<0.05$) interactions were found to be statistically significant. The hypothyroid group performed worse than the control group in the 1st and 2nd trials of the 2nd day, the 4th trial of the 3rd day, and the 1st and 2nd trials of the 4th day.

Swimming speed values of the groups are given in Figure 1D. Day*Group ($F=4.749$, $p<0.001$), Trial*Group ($F=6.970$, $p<0.001$) and Day*Trial*Group ($F=6.345$, $p<0.001$) interactions showed a statistically significant difference. The groups showed different swimming speed performances on different days and trials.

The hypothyroid group had a lower swimming speed than the control group in the 1st, 3rd and 4th trial of the 1st day, the 3rd trial of the 2nd day, the 1st trial of the 3rd day, and the 2nd and 3rd trial of the 4th day. The thyrotoxicosis group had a higher swimming speed than the control group in all trials of the 3rd day, and in the 2nd and 4th trials of the 4th day.

The percentage values of time spent in the target area in the last trial in which reference memory was evaluated are shown in Figure 2. The time values spent in the target quadrant showed statistically significant differences between the groups. The percentage values of time spent in the target quadrant of the hypothyroidism group created a statistically significant difference compared to the control group. The percentage values of time spent in the target quadrant in the hypothyroid group decreased (p=0.002). This deterioration was not observed in the thyrotoxicosis group (p=0.328).

DISCUSSION

Spatial navigation requires memorizing places and routes so that the organism does not get lost by encoding environmental cues. Many living creatures need to leave their habitats and return safely to their homes in order to find food and water, to mate, and for other needs[19]. MST, one of the tests developed in this context, was developed to evaluate spatial learning and memory in rodents. MST is one of the most frequently used measures of spatial memory formation and retention related to hippocampal functions[20]. This test is based on the fact that rats quickly learn the location of the escape platform with their innate swimming ability and fear instinct. In this study, we evaluated learning performance based on escape time, average distance to the platform, distance traveled to find the platform, and time spent in the target quadrant in the probe trial. Swimming speed averages were also included in the evaluation as a factor affecting other parameters. In our study, the distance traveled by the middle-old euthyroid, middle-old hypothyroid and middle-old thyrotoxicosis groups to find the platform in the tank differed between groups. The distance traveled by hypothyroid rats to find the platform is greater than euthyroid animals. These results show the negative effect of hypothyroidism on spatial learning performance. When

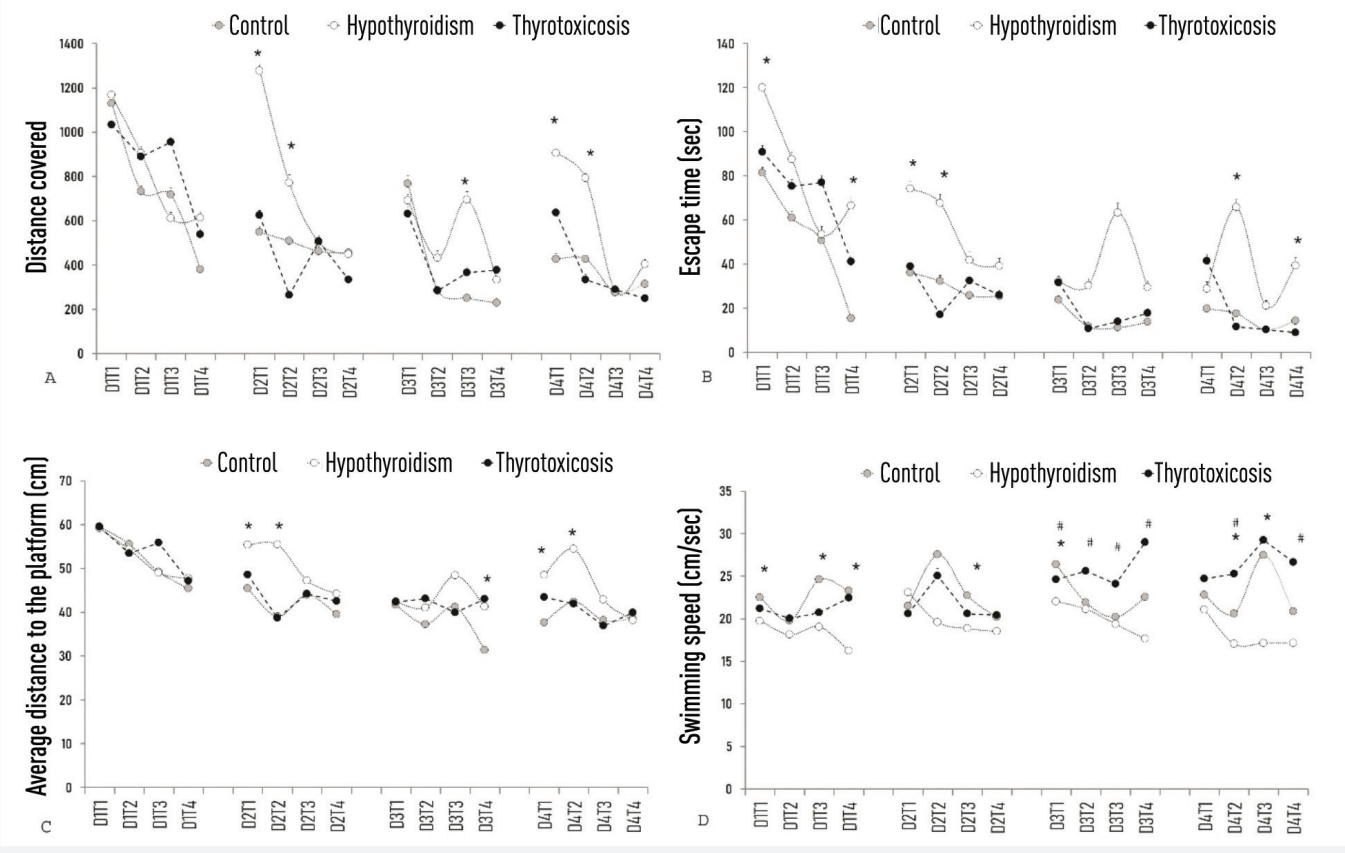

Figure 1. Change in distance covered (A), escape time (B), average distance to the platform (C) and swimming speed (D) during the learning period for the groups. Values are given as mean±standard error (D1: 1st day, T1: 1st trial)

**: Indicates a significant difference between the control and hypothyroid groups. #: Indicates a significant difference between the control and thyrotoxicosis groups*

the effect of thyrotoxicosis on learning was examined, it was observed that it did not differ from the control group. Analysis of escape time values revealed similar results as the distance traveled parameter. While learning performance was impaired in the hypothyroidism group, learning performance was not affected in the thyrotoxicosis group.

Swimming speed evaluation showed that the groups had different swimming speeds on different days and trials. The hypothyroid group exhibited lower swimming speed values than the control group. This finding is consistent with Hosseini et al.'s[21] finding of low swimming speeds observed in rats with methimazole-induced hypothyroidism. These results reflect the necessity of TH for skeletal muscle. The fact that the enzymes that regulate energy production and glycogen accumulation, especially in type 1 skeletal muscle fibers, are affected by hypothyroidism seems to be the underlying reason for these results[22]. Thyrotoxicosis had the opposite effect, causing a higher rate than the control group. Hyperthyroidism above physiological doses and for a long time causes proteolysis in skeletal muscle, causing muscle weakness[23]. However, considering the duration of the experimental groups and the doses in this study, the high swimming speed values may be explained by the fact that the experiment was carried out during the period when catabolism in the muscles was not yet dominant and the positive effects of THs on skeletal muscle contraction were observed[24].

Average distance to the platform values provide more reliable information as it is a parameter that is not affected by swimming speed, unlike the distance traveled and escape time. In this study, rats in the hypothyroid group swam farther from the platform than the other groups. At the same time, probe trials used to evaluate memory performance showed that the percentage values of time spent in the target quadrant of hypothyroid rats were lower than the control group. These results show that in addition to the impairment in learning performance in hypothyroidism, there is also impairment in memory consolidation processes. In the literature, the effect of hypothyroidism on learning has been mostly investigated on young rats, and studies on middle-aged hypothyroidism are limited. There are many studies in the literature showing that learning and memory performance is impaired in young hypothyroid rats[25,26].

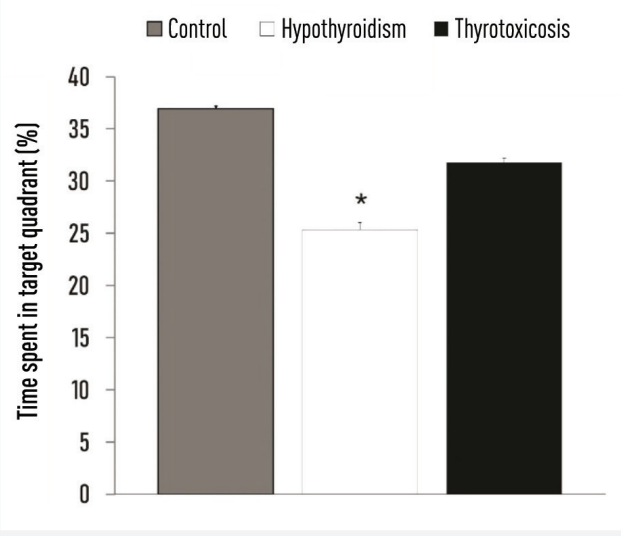

Figure 2. The ratio of the time spent by the groups in the quadrant where the platform was located in the last day's trial where memory was tested, to the total time (values are given as mean±standard error, significance level $p<0.05$ indicates statistical significance)

**: Indicates a significant difference compared to the control group*

Epidemiological studies reveal a striking increase in the incidence and prevalence of thyroid diseases in old age[27]. This relationship between the aging process and thyroid diseases requires further investigation of the hypothyroidism situation in the elderly. In terms of thyrotoxicosis, no effect of thyrotoxicosis on learning and memory was observed in middle-aged rats in this study. In addition to the studies in the literature showing that induced thyrotoxicosis in young rats causes learning impairment[15], there are also studies suggesting that it causes an increase in learning performance in mice administered postnatal L-thyroxine[28]. These differences may result from differences in the induction of thyrotoxicosis, the type of experimental animals selected, and the procedures for behavioral testing.

Study Limitations

In this study, hypothyroidism and thyrotoxicosis status were confirmed using the ELISA method at plasma free T3 and T4 levels. Plasma thyroid-stimulating hormone levels could not be measured. Another limitation of the study is that protein measurements were not made to show the molecular mechanisms of impaired learning and memory performance in hypothyroidism.

CONCLUSION

During the aging process, cognitive functions are more sensitive to the decrease in THs than to the increase in TH levels. Hypothyroidism in middle age has negative effects on learning and memory performance. It should be taken into consideration that diseases affecting TH metabolism may be involved in the etiology of dementia-type diseases seen in middle and late old age and should be included among the diagnostic tests. Conducting detailed studies at the molecular level about the effects of dysthyroidism on the central nervous system during the aging process will provide information about the functions of THs in the aging brain. In addition, the findings may make a significant contribution to determining

the factors that play a role in the development of Alzheimer's disease or other dementia-type diseases.

Ethics

Ethics Committee Approval: Ethics committee approval was received with the decision of Erciyes University Animal Experiments Ethics Committee dated 09.12.2015 and numbered 15/150.

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.K.T., B.T., Concept: R.K.T., N.D., Design: R.K.T., N.D., Data Collection or Processing: N.D., B.T., C.S., Analysis or Interpretation: E.B., B.T., C.S., Literature Search: E.B., C.S., Writing: E.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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