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ANTIELASTASIC ACTIVITY OF ANTISTRESSANTS IN BONE TISSUE OF RATS AFTER STRESS

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Abstract

Background. Stress causes the development of post-stress pathological reactions, which culminate in dysmetabolic disorders, against the background of which most diseases of humans and animals develop. Bone tissue has been found to be one of the most sensitive organs and tissues to the effects of dysmetabolic syndrome.

Aim. Study of the effect of stress on the activity of the proteolytic enzyme elastase in bone tissue and determination of the possibility of preventing elastase activation using anti-stress agents.

Methods. Stress was induced in rats by holding the animals at −20 °C for 30 minutes and elastase activity was investigated on the 4th day after stress. Antioxidant preparations EkSoVit, Quercetin and ascorbic acid were used as anti-stressors, which were administered into the oral cavity 3 days before stress and 3 days after stress in the form of an oral gel in doses (mg/kg): EkSoVit 0.43 and 1.07, Quercetin 0.128 and 0.257 and ascorbic acid 0.257.

Results. In rats without stress, the elastase activity in the femur was (μk-kat/kg) 6.66; in rats after stress 9.61; in rats with stress that received EkSoVit, 8.16 and 6.90; in rats with

stress that received quercetin, 7.20 and 5.60; in rats with stress that received ascorbic acid, 7.20. The antielastase activity in vivo was: EkSoVit – 49.15 and 126.05; quercetin – 112.10 and 135.93 and ascorbic acid – 81.63. The antielastase efficacy of antistressants was after conversion of antielastase activity to a dose of 1 g/kg: for EkSoVit 114.3 and 117.8; for quercetin 875.8 and 528.9; for ascorbic acid 317.8.

Conclusion. After stress, the activity of the proteolytic enzyme elastase increases in bone tissue. The use of anti-stress agents with antioxidant action significantly prevents the activation of elastase. The most effective anti-elastase drug was quercetin (vitamin P).

Keywords: elastase; bone tissue; stress; anti-stress agents.

Introduction

Stress is one of the main causes of human and animal diseases [1]. Pathological processes that occur after stress are caused by the sequential activation of the sympathetic, parasympathetic and neuroendocrine systems [2]. As a result, oxidative stress [3], dysbiotic syndrome [4] and dysmetabolic syndrome [5] develop.

Dysbiotic syndrome is a consequence of impaired intestinal mucosal barrier function, leading to translocation of bacteria and their toxins into the blood [6]. The intestinal endotoxin lipopolysaccharide (LPS) is the most potent pro-inflammatory factor [7].

To prevent the development of pathological complications of stress, antioxidants [8, 9], inhibitors of proteolytic enzymes [10, 11], as well as organoprotectors, in particular hepatoprotectors [12], are used.

Bone tissue was found to be extremely sensitive to the pathological effects of post-stress reactions [13]. Already 3 days after stress, a significant decrease in mineralizing activity was observed in the bone tissue of rats, which could be prevented by the administration of anti-stress drugs.

The aim of this study was to determine the effect of stress on the activity of the proteolytic enzyme elastase, which is an important factor in the development of the inflammatory process [14].

Materials and research methods

1. The structure of experimental studies

The experiments were conducted on white Wistar rats, 5-month-old females, with a live weight of 270-280 g, divided into 7 groups of 5 rats each.

Group I – intact rats;

Group II – rats in which cold stress was reproduced and which received daily, 3 days before stress and 3 days after stress, oral application of a base gel without anti-stressants in an amount of 0.5 ml per rat. The base gel contained 1% sodium benzoate and 3% carboxymethylcellulose Na-salt;

Group III – rats with stress that received the anti-stress agent EkSoVit in the form of a gel with a content of 2% of the drug 3 days before stress and three days after stress;

Group IV of rats received EkSoVit (gel with a content of 5 % of the drug) under similar conditions;

Group V of rats received the anti-stressor quercetin as part of an oral gel containing 0.6 % of the drug;

Group VI of rats received a gel with quercetin containing 1.2 % of the drug;

Group VII of rats received an oral gel containing 1.2 % ascorbic acid.

2. Anti-stressors

EkSoVit is a dietary supplement (TC U 10.8-37420386-010:2025), contains 750 mg of soy extract in 1 g, including 60 mg of protease inhibitors (Kunitz and Bauman-Birk). Gels with a content of 2 % and 5 % EkSoVit were prepared on a base gel (composition: 1 % sodium benzoate, 3 % carboxymethylcellulose sodium salt).

Anti-stressor – Vitamin P (quercetin) – dietary supplement (TC U 10.8-37420386-011:2025), contains 20 mg of quercetin (vitamin P) in 1 ml of fructose syrup. Gels with a content of 0.6 % and 1.2 % quercetin on a base gel were prepared.

Ascorbic acid. A gel containing 1.2 % ascorbic acid was prepared on a base gel.

All gels with anti-stress agents were administered orally to rats in an amount of 0.5 ml per rat for three days before stress and three days after stress.

3. Stress model [13]

Cold stress was reproduced by exposing rats to a temperature of −20 °C for 30 minutes. Animals were euthanized under thiopental anesthesia (20 mg/kg) by total bleeding from the heart on the 4th day after stress.

4. Determination of elastase activity [15]

After euthanasia, femurs were isolated from rats, cleaned of soft tissues, and homogenates were prepared at the rate of 75 mg of bone tissue per 1 ml of 0.1M citrate buffer pH 6.1. Homogenates were obtained by grinding a portion of tissue in the presence of crushed glass. After centrifugation (2500 rpm for 15 minutes), the supernatant was used to determine elastase activity.

The synthetic substance N-t-BOC-L-alanine-p-nitrophenyl ester was used as the substrate. Hydrolysis of the substrate was carried out at pH 6.5. Elastase activity was determined in μ-katals per 1 kg of bone tissue. One μ-katal was equal to 1 μmol of substrate hydrolyzed in 1 second of incubation.

5.Determination of in vivo antielastase activity (AEA) of antistressants

AEA *in vivo* визначали за формулою:

AEA = $\frac{b-c}{b-\text{a}} \cdot 100$, where

a – elastase activity in the femur of intact rats;

b – elastase activity in the femur of rats after stress;

c – elastase activity in the femur in stressed rats treated with anti-stress agents.

AEA was defined as the percentage decrease in elastase activity in the femur of rats after stress as a result of the action of an anti-stress agent.

6. Determination of antielastase efficacy (AEE) of an antistressant (AS)

AEE was determined by the formula:

AEE = $\frac{\text{AEA}}{C \cdot n}$, where

C – total dose of the drug in g/kg of live weight for all days of AS application;

n – number of days (doses) of AC use.

AEE was determined as the percentage decrease in elastase activity in vivo per 1 g/kg of antistressant.

Results and discussion

Table 1 presents the results of determining elastase activity in rat femoral homogenates on the 4th day after cold stress with 6-fold administration of antistress drugs. Stress causes a significant (by 45 %) increase in elastase activity. All used antistress drugs significantly reduce elastase activity, in almost all cases returning it to the control level, and when using the drug quercetin at a concentration of 1.2 %, it even reduces it below normal.

Table 2 presents the results of calculations of antielastase activity and antielastase efficacy of antistressors. These data show that quercetin preparations in doses of 0.6 % and 1.2 % differ little from the indicator for the EkSoVit preparation in a dose of 5 %. However, in terms of antielastase efficacy, the 0.6 % quercetin preparation exceeds the corresponding indicator for the EkSoVit preparation by almost 7 times and the indicator for the 1.2 % ascorbic acid preparation by 3 times.

Quercetin is a flavonoid, which belongs to the group of vitamins P, which have the highest antioxidant activity, exceeding ascorbic acid by several times. Activation of elastase

in the bone tissue of rats after stress indicates the development of an inflammatory process. A significant decrease in elastase activity under the influence of quercetin gives grounds to consider quercetin not only an antioxidant, but also an anti-inflammatory factor.

Table 1. The effect of antistressors on elastase activity in the femur of rats on the 4th day of stress (n=5)

№№ groups	Groups	Dose of AS for 6 days, g/kg of live weight	Elastase activity, μ-kat/kg of live weight
1	Intact	0	6,66±0,40
2	Stress, day 4	0	9,61±0,32 p<0,01
3	Stress, 4th day + EkSoVit, 2 %	0,430	8,16±0,56 p<0,05; p_1<0,05
4	Stress, 4th day + EkSoVit, 5 %	1,070	6,90±0,30 p>0,1; p_1<0,011
5	Stress, day 4 + Quercetin, 0.6 %	0,128	7,20±0,38 p>0,05; p_1<0,01
6	Stress, day 4 + Quercetin, 1.2 %	0,257	5,60±0,28 p>0,05; p_1<0,01
7	Stress, 4th day + Ascorbic acid, 1.2 %	0,257	7,20±0,47 p>0,05; p_1<0,01

Notes: p – compared to group 1; p_1 – compared to group 2.

Table 2. Antielastase activity and antielastase efficacy in rat femur on the 4th day of stress

№№ groups	Groups	Antielastase activity, Δ%/6 dose	Antielastase efficacy, Δ%/g/kg
3	EkSoVit, 2 %, 430 mg/kg	49,15±3,60	114,3±7,9
4	EkSoVit, 5 %, 1070 mg/kg	126,05±7,11	117,8±8,1
5	Quercetin, 0.6 %, 128 mg/kg	112,10±6,94	875,8±20,3
6	Quercetin, 1.2 %, 257 mg/kg	135,93±8,92	528,9±14,7
7	Ascorbic acid, 1.2 %, 257 mg/kg	81,69±5,37	317,8±11,8

Given the extremely high incidence of osteoporosis in people over 50 years of age [16], it can be considered advisable to use vitamin P preparations for the prevention and treatment of osteoporosis. The source of vitamin P is berries, fruits, vegetables [17, 18]. The daily requirement for vitamin P (50 mg) can be met by consuming the dietary supplement "Antistress – Vitamin P (quercetin)".

Conclusion

1. After stress, the activity of the proteolytic enzyme elastase increases in bone tissue.

2. The use of anti-stress agents with antioxidant action significantly prevents the activation of elastase.

3. The most effective anti-elastase drug was quercetin (vitamin P).

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

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