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Effects of estrogen intervention on the biomechanical characteristics of serum SOD, MDA, and middle cerebral artery in aged female rats

S.J. Wang[1], S.J. Zhao[1], Y.S. Wang[1], T. Yu[2], M. Luo[2]

[1] Department of Critical Care Medicine, the 1st Hospital of Jilin University, Changchun
[2] Department of Osteology, Sino-Japanese Friendship Hospital of Jilin University, Changchun (China)

Summary

Objective: This study aims to study the biological and biomechanical characteristics of the middle cerebral artery (MCA) in aged and estrogen-intervened aged rats, thereby providing biomechanical basis for clinics. *Materials and Methods:* Thirty 18-month-old Sprague Dawley (SD) rats, 30 18-month-old estrogen-intervened SD rats, and 30 four-month-old SD rats were studied. The estrogen-intervened rats were given estradiol benzoate on the fifth feeding day. Thirty-four days after the feeding, the serum of each rat was obtained. The radioimmunoassay was performed for the content determination of serum E2, ER, malondialdehyde (MDA), and superoxide dismutase (SOD). The tensile test was performed to evaluate the MCA of each rat. *Results:* Through the estrogen intervention, the serum contents of E2, ER, SOD, and MDA in old rats were restored to normal levels. The maximum stress, maximum strain, and elastic limit of the MCA in the aged estrogen-intervened rat group were greater than those of the non-intervened aged rat group, with a significant difference ($p < 0.05$). The elastic modulus in the aged estrogen-intervened rat group was less than that of the non-intervened aged rat group, with a significant difference ($p < 0.05$). *Conclusion:* E2 intervention can improve the flexibility, toughness, and compliance of MCA in aged rats.

Key words: Aged female rat; Estrogen; Middle cerebral artery; Mechanical properties.

Introduction

The role of estrogen intervention on the biomechanical properties and serum contents of superoxide dismutase (SOD) and malondialdehyde (MD) of the middle cerebral artery (MCA) of protocerebrum and metencephalon in aged female rats would have great significance in preventing cerebrovascular disease in aged women. Nerve epidemiological studies have shown that the stroke incidence of premenopausal women is specifically lower than the men with the same age [1]. However, the stroke incidence of postmenopausal women is significantly higher than those of premenopausal women [2]. A large number of studies, using exogenous estrogen replacement therapy (ERT) to treat menopausal syndrome, have found that ERT can lessen the relative risk of stroke as well as the stroke-related death rate [3]. Therefore, scholars have deduced that estrogen might have a strong neuroprotective function. Currently, the effects of the sex hormone level on the intracranial arterial structural components and biomechanical characteristics have aroused great concern from scholars domestically and abroad. Numerous studies have focused on the relationship of sex hormones to the brain blood vessels and nerve disease [4-13], Momoi *et al.* [14] performed estrogen intervention after removing the gonads of female and male rats and found that estrogen enabled the nitric oxide (NO) released from the microvasculature of the gonad-removed rats. The estrogen intervention effects were more obvious on the female rats. Thus, they derived that estrogen can improve the brain microvasculature by adjusting NO release and maintain the cerebral blood flow, thereby protecting the cerebral from ischemia. Duckles and Krause [15] simulated a rat cerebral ischemia model and then applied estrogen intervention. Large doses of estrogen can promote the release of cGMP and NO from the rat brain microtubule in the early stage of cerebral ischemia, dilated blood vessels, and increased cerebral blood flow. Won *et al.* [16] also simulated an MCA occlusion model and then applied estrogen intervention. Results also showed that estrogen can inhibit the over-expression of bcl-2 protein in the ischemic area and the activation of nuclear transcription factor, thereby reducing apoptosis and the inflammatory response. Few scholars have studied the effects of estrogen on the biomechanical features of intracranial arteries. Ibrahim *et al.* [17] studied the myogenic regulatory functions of MCA in normal Wistar Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SH RSP) with different genders. In the stress–strain relation figure of female MCA, MCA showed little expansion with the in-

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crease of pressure in the normal female WKY. By contrast, MCA significantly expanded with the increase of pressure in the SH RSP group. In the section-stress elastic modulus figure, the normal female WKY group was stronger than the male group but was significantly weaker than the male group in the SH RSP group.

In past research, estrogen intervention was mostly focused on the bioresearch in aged women and animals. Rare domestic and international reports exist regarding estrogen intervention on the biomechanical properties of MCA. In this article, the authors report the quantitative clarification of the different results of estrogen intervention on the biomechanical characteristics and serum superoxide dismutase (SOD) and malondialdehyde (MDA) contents in the MCA of aged female rat and non-intervened aged female rats. Biomechanical and biological indicators were used to determine the effects of estrogen intervention, providing biological and biomechanical basis for the prevention of cerebrovascular disease in aged females.

Materials and Methods

Animals and grouping

Studies have shown that the weight difference of rats less than nine grams could effectively prevent errors caused by weight [18]. The experimental animals used were 30 18-month-old clean, female Sprague Dawley (SD) rats (body weight 290 to 330 grams) and 30 four-month-old SD rats (body weight 290 to 325 grams), which were provided by the Changchun High-tech Medical Experimental Animal Center (license number SCXK (Ji) 2003-0004). The animal laboratory safety level was ABSL-2, and the level of pathogen-carrying animal was clean level. Breeding was conducted in a room temperature of 20 ± 2°C, with good ventilation, relative humidity of 55% to 70%, and natural light. The animals were caged with free food and water. The diet was purchased from the experimental animal diet factory in Shenyang, with product standard code of DB-21741-93. Sixty 18-month-old female SD rats were randomly divided into estrogen-intervened group (30 rats) and non-intervened aged group (30 rats). Thirty four-month-old female SD rats were set as the young control group. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jilin University.

Estradiol benzoate intervention

After the fifth feeding day, the intervention group rats were given estrogen estradiol benzoate (0. 2 mg/kg, Batch No: 061101) through intramuscular injection every other day. The young control group and aged non-intervened group rats were not subjected to any treatment, and all rats were fed for 34 days.

Specimen preparation

On the 34th feeding day, the eye blood from the orbit was obtained from 15 rats of each group. The blood stood for a while, six ml fresh blood were centrifuged, and then left in open air for another 15 minutes, and centrifuged at low speed (2,000 r/min) for 20 minutes. The supernatant was stored at -40°C for future use. Craniotomy was then performed under the ASX-1 surgical microscope. The MCA was located and cut down with S-5 sterile plastic-handle scalpel. Fifteen specimens of each group were wrapped with saline-soaked cloth, placed in a saline bath, and stored at 4°C environment for future use.

Measurement of the specimen geometry dimension

The CG reading microscope was used to measure the MCA sample's geometry as follows: length ten mm, diameter 1.0 mm to 1.02 mm for the young control group, length ten mm, diameter 1.0 mm to 1.02 mm for the estrogen-intervened aged female group, and length ten mm, diameter 1.0 mm to 1.02 mm for the non-intervened aged group.

The estradiol and estradiol receptor ELISA kit were used for the concentration determination of serum E2, ER, MDA, and SOD in each group. Microplate reader was used strictly in accordance with the estradiol and estradiol receptor ELISA kit instructions for the determination of serum E2 and ER concentrations in each group. The serum E2 and ER levels were measured using the radioimmunoassay (RIA) method. The MDA kit was used according to instructions, with thiobarbituric acid colorimetric method, in determining the serum MDA levels, which is expressed as MDA (nmol/ml)=(absorbance of measurement tube - absorbance of blank tube)/(absorbance of standard tube - absorbance of standard blank tube)×standard concentration (ten nmol/ml)×dilution times. The ultra SOD kit was used according to SOD kit instructions, with U3410 spectrophotometer and yellow purine oxidation enzyme (XO) method, for the SOD determination.

MDA determination

According to the MDA kit instructions, the thiobarbituric acid colorimetric determination method was used to measure the serum MDA content. Serum content (nmol/ml) = (absorbance of the sample tube - absorbance of the blank tube)/(absorbance of the standard pipe - absorbance of the standard blank)×concentration of the standard (ten nmol/ml)×dilution factor.

SOD determination

According to the SOD kit instructions, U3410 spectrophotometer and yellow purine XO method were used for the SOD determination.

Serum E2 and ER determination

The microplate reader and ELISA method were strictly used according to the estradiol and estradiol receptor ELISA kit instructions for the determination of serum E2 and ER concentrations. The serum E2 and ER levels were measured through RIA.

Longitudinal tensile tests of MCA

The CSS4500 automatic-control electronic universal testing machine was employed. Ten times of loading and unloading pretune treatments were performed for each sample according to reference [19]. The experimental ambient temperature was 36.5 ± 1.0°C. Each specimen was clamped in the test machine cartridge. The tensile test was conducted under a speed of two mm/min. The maximum stress, maximum strain, elastic limit, and stress–strain curve were determined. To maintain the humidity of the sample, leaching saline was performed to the specimen during the experiment. After the experiment, the computer automatically imported the experimental data and curve.

Statistical analysis

The SPSS16.0 software was used for the data analysis. Results were expressed as $X \pm s$. The experimental data were statistically analyzed using the one-way ANOVA method. The Scheffé's method was used to compare the groups. A $p < 0$ 05 was defined as statistically significant.

Table 1. — *Serum MDA and SOD assay results in each group.*

Group	MDA	SOD
Young control group	7.120.21	340.110.5
Estrogen-intervened group	6.870.15[a]	378.2 13.3[a]
Non-intervened group	8.660.48[ab]	29610.6[ab]

Note: Data are expressed as x±s, comparison among groups used Scheffé's method; [a]$p < 0.01$, *vs* young control group; [b]$p < 0.05$, *vs* estrogen-intervened group.

Table 2. — *Measurement results of serum E2 and ER contents of each group.*

Group	E2	ER
Young control group	53.11.64	20.311.43
Estrogen-intervened group	56.41.5[a]	20.61.3[a]
Non-intervened group	41.03.3[ab]	15.4 1.2[ab]

Note: Data were expressed as x±s, comparison among groups used Scheffé's method; [a]$p < 0.01$, *vs* young control group; [b]$p < 0.05$, *vs* estrogen-intervened group.

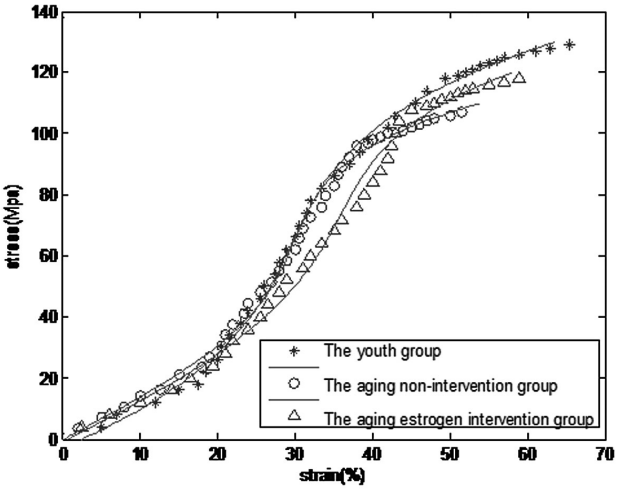

Figure 1. — Longitudinal tensile stress-strain curves of different groups' MCA.

Table 3. — *Experimental results of brain artery tension in each group.*

Index	Young control group	Non-intervened group	Estrogen-intervened group
Maximum stress (MPa)	129.90.76	106.1 9.4[a]	118.0 5.8[ab]
Maximum strain (%)	65.40.85	51.54.47[a]	59.0 1.90[ab]
Elastic limit strain under 16 mpa stress	15.11.64	12.60.81[a]	14.50.70[ab]
Modulus of elasticity under 13.3 mpa stress	57.41.10	61.9 1.09[a]	59.11.10[ab]
Modulus of elasticity under 16.0 mpa stress	61.3 6.7	64.90.96[a]	63.2 0.85[ab]
Modulus of elasticity under 16.0 mpa stress	63.91.20	66.71.24[a]	65.00.93[ab]

Note: Data were expressed as x±s, comparison among groups used Scheffé's method; [a]$p < 0.01$, *vs* estrogen-intervened group; [b]$p < 0.05$, *vs* young control group.

Results

MDA and SOD

The measurement results of the serum MDA and SOD contents are shown in Table 1. The serum MDA content of the non-intervened group was higher than those of the estrogen-intervened and young control groups, with a significant difference ($p < 0.05$). By contrast, the serum SOD content of the non-intervened group was lower than those of the estrogen-intervened and young control groups, with a significant difference ($p < 0.05$). The serum SOD content of the estrogen-intervened group was higher than those of the young control and non-intervened groups, with a significant difference ($p < 0.05$).

Serum E2 and ER contents

The serum E2 content of the estrogen-intervened group was higher than those of the non-intervened and young control groups, with a significant difference ($p < 0.05$). However, the serum ER content of the estrogen-intervened group had no significant difference with those of the young control group ($p > 0.05$, Table 2).

Brain artery tension and stress–strain function

The experimental results of the brain artery tension in each rat group are shown in Table 3, and the stress-strain curves of MCA in each group are shown in Figure 1.

The maximum stress, maximum strain, and elastic limit strain values of MCA in the aged estrogen-intervened group were greater than those of the non-intervened group, with a significant difference ($p < 0.05$). The values of the modulus of elasticity at 13.3, 16.0 and 22.5 kPa of MCA in the aged estrogen-intervened group were less than those of the aged non-intervened group, with a significant difference ($p < 0.05$).

The dependence relationships among variables were obtained from the measured data and the total deviation. The stress–strain function of each group is expressed as follows:

- Young control group: $\sigma(e) = -0.0094e^4 + 0.8612e^3 + 2.5144e^2$
- Aged non-intervened group: $\sigma(e) = -0.0001e^5 + 0.0008e^4 + 0.7319e^3 + 0.2024e^2$
- Aged estrogen-intervened group: $\sigma(e) = -0.0001e^5 + 0.0011e^4 + 0.7795e^3 + 0.6919e^2$

In Figure 1, the ordinate expresses the stress and the abscissa the strain. The strain of the brain artery walls in each group varied regularly under the stress. When the stress is 0 kPa to 16.0 kPa, the stress–strain curve exhibits an exponential relationship. The maximum stress, maximum strain, and elastic limit strain in the young control group were greater than those of the aged non-intervened and estrogen-intervened groups. When the stress exceeded 16.0 kPa, the strain of each group increased rapidly. The strain curve slope increased with stress, showing a non-linear relationship. A continuous force stretched occurred and extended at the wall fiber until reached the final fracture.

Discussion

The experimental results showed that the maximum stress, maximum strain, and elastic limit strain value of the cerebral artery in the aged estrogen-intervention group were greater than those of the aged non-intervention group, with a significant difference ($p < 0.05$). The estrogen intervention did change the mechanical properties of the cerebral artery in the aged female rats, which is in accordance with the expected results. The elastomeric components of the cerebral arterial wall include elastic, collagen, and smooth muscle fibers [20]. The thickness, hardness distribution, and spatial configuration type of the intracranial arteries are important factors for determining the vascular function and biomechanical properties [21]. Meng *et al.* [22] indicated that the structure of the cerebrovascular elastic tissue plays an important role in maintaining the tension and elasticity of the cerebral blood walls. Hayashi [23] comparatively analyzed the relationships of the elasticity and stiffness of the intracranial and the outer arteries and found that the modulus of the elasticity and stiffness of the arteries increased with the blood pressure and with the distance to the heart. Such increase is related to the difference of the ratios of wall stretch and collagen fibers, the number of smooth muscles, and the different arrangements of fibrous tissue structure. The reduction of arterial elasticity is considered as the early change of atherosclerosis, which is also a risk factor for plaque formation [24, 25]. As the female rats aged, arteriosclerosis appeared, leading to the content and arrangement direction changes of the arterial collagen and elastic fibers. Consequently, the displacement and strain of the MCA reduce under stress, which also reduces its elasticity and tenacity and increases its elastic modulus. The modulus of elasticity represents the arterial stiffness and hardness. The greater the modulus of elasticity, the smaller the expansion degree of the arterial wall and the worse the elasticity. The elasticity reduction in the aged female rats' brain artery, indicates its poor compliance. In this study, the aged female rats were subjected to estrogen intervention, which improved the elasticity and toughness of the aged rats' brain artery, reduced the elastic modulus of MCA in the aged female rats, and restored the MCA compliance of the cerebral artery in the aged female rats to some extent. The longitudinal tensile mechanical properties of the MCA in the aged estrogen estrogen-intervention group presented some recovery through the intervention, and the compliance also obtained some recovery. Therefore, estrogen intervention can increase the elasticity and compliance of the MCA in the aged female rats.

The elastic moduli of the cerebral artery in the aged female rats at the elastic stresses of 13.3, 16.0, and 22.5 kPa are greater than those of the aged estrogen-intervention group. The elastic modulus of the MCA increased with stress in each group. The elastic modulus represents the stiffness and hardness of an artery. The higher the elastic modulus, the greater the hardness of the arterial wall and the less the flexibility. In this experiment, the longitudinal stretching was applied to the cerebral arteries of the young, aged, and aged estrogen intervention rats [17]. Their mechanical properties were tested by applying an internal pressure to their arteries. Although different methods were used, the elastic modulus of the present experiment increased with stress similar to the result of reference [17]. Therefore, longitudinal stretching is feasible to be applied to the research of the mechanical properties of the arterial wall.

The SOD system is one of the important physiological mechanisms, effectively removing the excess free radicals. With the increase of age, this balance will gradually be destroyed, resulting in excess free radicals. Free radicals could cause DNA damage, leading to the mutation and tumor formation. Its superior ability to respond would oxidize a variety of cell substances, damaging biofilm and causing macromolecules, such as proteins and nucleic acids, to crosslink, which affect their normal functions. As an important antioxidant enzyme *in vivo*, SOD enables superoxide anion radicals to transform into the peroxide of hydrogen and oxygen ions, thereby reducing the lipid peroxidation and protecting the body tissues and cells from damage. Estrogen changes dramatically in females. The periodic secretion stops after 45 years to 50 years. Compared with the sexual maturity, estrogen decreases rapidly. The decline of the estrogen levels lead to the slow-down of the cholesterol metabolic rate, causing the acceleration of atherosclerosis, which in the long run would induce cerebral infarction. E2 can reduce the cerebral artery elasticin, plasma cholesterol, and atherosclerosis [1]. Estrogen has similar roles of neuroprotective agents, which could have a direct impact on the growth and function of the nerve cells [26]. In this study, the serum MDA content in the estrogen-intervened group significantly decreased. By contrast, the SOD activity of this group significantly increased compared with the non-intervened group. Hence, estrogen can increase the antioxidant capacity, inhibit the formation of lipid peroxidation products, and reduce oxygen-free radicals and lipid peroxidation product-pair injury, thereby protecting the rat brain tissue.

Blood vessels are affected not only by the blood pressure but also significantly by the longitudinal tension . Therefore, the vascular tissue reconstruction under longitudinal tension has gradually been taken seriously in recent years. Previous studies on the E2 estrogen intervention on people or animals

have focused on the biological views [14-16]. In this experiment, the maximum stress, maximum strain, elastic strain limit, and the stress-strain curve of the MCA longitudinal stretching in the young, aged, and E2 estrogen-intervened rats were obtained, different from the previous studies. The elastic modulus corresponding to 13.3, 16.0, and 22.5 MPa were also obtained. Moreover, the arterial stress-strain relationship expression was established through the regression analysis, which can quantitatively clarify and comparatively analyze the MCA mechanical properties of each group. Biomechanics indicators, serum SOD, and MDA were utilized to judge the effect of E2 intervention on the aged female rats, providing a biomechanical basis for the prevention of cerebrovascular disease in aged women. The design, experimental methods, experimental data, and processing methods presented in this paper are innovative.

The sampling and preservation methods used in each group during the experiment are the same. The preset processing method used is also the same to reduce the experimental data errors by controlling the experimental ambient temperature and speed. Considering the limited experimental sample and the individual differences among the animals, the experimental data have certain discreteness. However, these data can still provide a certain reference for the mechanism research of the clinical estrogen interventions for preventing the cerebrovascular disease in aged women.

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Address reprint requests to:
T. YU, M.D.
Department of Osteology
Sino-Japanese Friendship Hospital of Jilin University
No. 299 Xiantai Street
Changchun, 130031 (China)
e-mail: taoyucn@126.com