Effect of estrogen administration on middle cerebral arterial viscoelastic properties in aged female rats

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ABSTRACT

PURPOSE: To develop a model for studying cerebrovascular disease prevention in elderly women.

METHODS: Sixty 18-month-old Sprague Dawley (SD) rats were randomly divided into an estrogen administration group (EA, n=30) and a non-administration group (NA, n=30); thirty 4-month-old SD rats were allocated to a control group. The EA group received estradiol benzoate starting on the 5th day of a 34-day breeding period, and the serum levels of estradiol (E2), estrogen receptor (ER), and malondialdehyde (MDA) were measured. The MCA of each group was then sampled for viscoelastic experiments.

RESULTS: The serum levels of E2 and MDA in the EA group showed significant differences compared to those in the control group (p<0.05), while the difference in ER between the EA and control groups was not significant (p>0.05). The decrease in MCA stress at 7,200 s and the increase in strain at 7,200 s in the EA group showed no significant differences compared to the control group (p>0.05).

CONCLUSION: Estradiol administration inhibited the formation of lipid peroxidation products and restored middle cerebral arterial viscoelasticity in aged female rats.

Key words: Aging. Estrogens. Middle Cerebral Artery. Female. Rats.
Introduction

Numerous clinical studies have reported that the incidence of cardiovascular disease (CVD) in women begins to increase during menopause, indicating that estrogen deficit plays an important role in atherosclerosis and CVD. Estrogen replacement therapy used to treat menopausal symptoms reportedly decreased the relative risk of stroke, and that stroke-related mortality was also decreased. Much research has evaluated the relationships between sex hormones, cerebral vessels, and neurological diseases. Won et al. created a rat model of middle cerebral artery (MCA) occlusion, and found that estrogen administration (EA) could inhibit the overexpression of Bcl-2 protein and activation of nuclear transcription factors in ischemic areas, thereby reducing apoptosis and inflammatory reactions. Momoi et al. resected the gonads of male and female rats, and then performed EA. They found that estrogen could induce the microvascular system of gonadectomized rats to release NO, which was more obvious in female rats, and concluded that estrogen could regulate the release of NO, thus improving brain microvessel quality, maintaining cerebral blood flow, and exhibiting protective effects against cerebral ischemia. Only a few researchers have reported the effect of estrogen on intracranial arterial biomechanical characteristics. Ibrahim et al. studied myogenic regulatory function of the MCA in normal male and female WKY rats and those with spontaneous hypertensive stroke (WKY), and found that MCA in the normal female WKY group was not significantly increased to the extent of pressure expansion, while that in the Spontaneously Hypertensive Stroke Prone Rat (SH RSP) group was significantly increased; the vascular stiffness in the normal female WKY group was stronger than that in the male group, while that in the SH RSP group was significantly decreased compared to that in the male group.

One report discussed the effect of EA on MCA viscoelastic properties. Estradiol (E2) administration increased the elasticity, strength, and compliance of the MCA in aged rats. Therefore, it could be inferred that changes in sex hormone levels might show a correlation with intracranial viscoelastic properties; if EA could increase estrogen levels in aged female rats, it might restore viscoelastic properties. The purpose of this paper was to use viscoelastic and biological indicators to evaluate the effects of EA in aged rats, thus providing a basis for EA in preventing cerebrovascular disease in elderly women.

Methods

The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jilin University.

Sixty 18-month-old female Sprague Dawley (SD) rats (clean-level, body weight 290-299g) and 30 4-month-old SD rats (clean-level, body weight 291-300g) were obtained from Changchun High-tech Medical Laboratory Animal Center (license number SCXK (Ji) 2003-0004). The animals were bred at room temperature (20±1°C), with air circulation, relative humidity 55-70%, and a natural light cycle. The animals were caged with free food and water at Animal Biosafety Level 2, and feed was purchased from Shenyang Experimental animal feed factory, with the product standard code: DB-21741-93. 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 60 18-month-old female SD rats were randomly divided into an EA group (n=30), and a non-administration (NA) group (n=30); the 30 4-month-old female SD rats were used as a control group.

Estrogen administration (EA)

The rats received intramuscular injections of estradiol benzoate 0.2 mg/kg starting on the 5th day of breeding, once every other day. The control and NA groups received no treatment; all rats were bred for 34 days.

Specimen sampling

After 34 days of breeding, 6 ml of orbital blood was sampled from 15 rats in each group, left to stand at room air for 15 min, and centrifuged at low-speed (2,000 rpm) for 20 min; the supernatant was then preserved at -40°C for later use; the rats then underwent craniotomy using an ASX-1 operating microscope (Shanghai Anxin Optics Manufacturing Co., China); the MCA was identified and resected with an S-5 sterile scalpel (Huaian Joint Yikang Medical Products, Xuyi, Jiangsu Province, China), Each group was sampled for 15 specimens, which were wrapped with saline-soaked cloth, and stored in a saline bath at 4°C for future use.

Measurement of sample geometric size

The geometric sizes of 15 MCA specimens from each group were measured using a CG-type reading microscope (Third Optical Instrument Factory, Changchun, Jilin, China). MCA
specimens from the control, EA, and NA groups were all 10 mm long, with a diameter of 1.0-1.02 mm.

**Determination of E2 and estrogen receptor (ER) concentration**

A microplate reader (LABsystems Co., Finland) and enzyme-linked immunosorbent assay (ELISA) kits were used according to instructions to determine serum concentrations of E2 and ER. The serum levels of E2 and ER were detected by radioimmunoassay (RIA), with instrument sensitivity set for 1.1 pg/mL, intra-assay coefficient of variation (CV) ≤10%, and inter-assay CV ≤10%. The assay sensitivity T was 0.05 ng/mL, the intra-assay CV ≤10%, and inter-assay CV ≤10%; the sample measurements used the double-tube parallel method; readings were obtained from the instrument, and corresponding concentrations were read from a standard curve.

**Determination of malondialdehyde (MDA)**

Analysis was performed according to the instructions of the MDA kit, and the thiobarbituric acid assay was used to detect serum MDA level. MDA in degradation products of lipid peroxide was condensed with thiobarbituric acid, thus forming a red product with maximum absorption peak at 532 nm; absorption was then detected using a U3410 spectrophotometer (Hitachi, Japan); then, MDA content was calculated using the following formula (with homogenate concentration of 10%): serum MDA (nmol/ml) = (absorbance of measurement tube - absorbance of blank tube) (absorbance of standard tube - absorbance of standard blank tube) × standard concentration (IO nmol/ml) × dilution time of sample.

**Stress relaxation test**

Using a CSS4500 automatic electronic universal testing machine (Changchun Institute of test machine, Changchun, Jilin, China), each sample was preconditioned and treated as previously described. The tester was equipped with a thermostat that could be set from -30°C to 250°C; the ambient temperature of this experiment was set as 36.5±1.0°C; each sample was clamped in a chuck on the test machine, and strain was applied to the sample at a rate of 30%/min; when the stress in each group reached 16.0 kPa, the MCA strain of the control group reached 39.0%, the strain in the NA group reached 29.3%, and the strain in the EA group reached 39.0%; the strain values then remained constant. Stress decreased as time increased; saline was used to continuously moisten the samples during the experiment. After completion of 16.0 kPa stress relaxation tests, the samples were subjected to 22.5 kPa stress relaxation at a strain rate of 30%/min; when the stress reached 22.5 kPa, the MCA strain in the control group reached 48.2%, the strain in the NA group reached 34.8%, and the strain in the EA group reached 34.8%; the strain values then remained constant.

**Creep test on the MCA**

The clamping method and preconditioning treatment for the creep test were the same as for the stress relaxation tests. Stress at an increasing rate of 0.5 MPa/s was applied to the 3 groups. When the stress in each group reached 16.0 kPa, the MCA strain of the control group reached 39.0% and the strain in the NA group reached 29.3%; the stress then remained constant, and the strain was constantly increased as a function of time; after a preset test time was reached, the computer would automatically calculate the results. After the creep test with 16.0 kPa was completed, the test was performed with 22.5 kPa under constant stress, at an increasing stress rate of 0.5 MPa/s; when the stress in each group reached 22.5 kPa, the MCA strain in the control group reached 48.2%, the strain in the NA group reached 34.8%, and the strain in the EA group reached 34.8%, with the strain kept constant.

**Statistical analysis**

SPSS 11.0 software (SPSS, Chicago, IL, USA) was used for the paired t-test, with p<0.05 considered statistically significant.

**Results**

**E2 and ER**

The serum levels of E2 and ER in each group are shown in Table 1. The serum values of E2 and ER in the EA group were greater than the levels in the NA group, and the difference was statistically significant (p<0.05).

<table>
<thead>
<tr>
<th>No</th>
<th>E2</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>54.8±1.3^a</td>
<td>19.6±1.2^a</td>
</tr>
<tr>
<td>EI group</td>
<td>55.8±1.4^a</td>
<td>21.2±1.3^b</td>
</tr>
<tr>
<td>NI group</td>
<td>42.6±2.9^c</td>
<td>16.8±1.7^c</td>
</tr>
</tbody>
</table>

Note: The data were expressed as x±s, the intergroup comparison used the Sceffe method, ^p<0.05, vs the control group; ^p<0.05, vs the EI group. ^p<0.05, vs Aged rats-estrogen intervention group.
Serum MDA content

The serum MDA level in the EA group was less than the level in the NA group, and the difference was statistically significant (Table 2, p<0.05).

<table>
<thead>
<tr>
<th>No</th>
<th>Mda (mmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>7.08±0.26(^a)</td>
</tr>
<tr>
<td>EI group</td>
<td>6.96±0.31(^b)</td>
</tr>
<tr>
<td>NI group</td>
<td>8.52±0.41(^c)</td>
</tr>
</tbody>
</table>

Note: The data were expressed as \(\bar{x} \pm s\), the intergroup comparison used the Sceffe method, \(^a\)p<0.05, vs the control group; \(^b\)p<0.05, vs the EI group. \(^c\)p<0.05, vs Aged rats-estrogen intervention group.

MCA stress relaxation

The relaxation curves with an initial stress of 16 kPa on the MCA in different groups are shown in Figure 1, and those with stress of 22.5 kPa are shown in Figure 2.

When the initial stress on the MCA was set at 16 kPa, the 7.200s stress on the MCA in the control group, the NA group, and the EA group was decreased in comparison with the values at 0 s, by 4.1 kPa, 3.2 kPa, and 3.9 kPa, respectively; the 7.200s stress on the MCA in the EA group decreased more than in the NA group at 16 kPa initial stress (p<0.05; paired t-test). When the initial stress on the MCA was set at 22.5 kPa, the 7.200s stress on the MCA in the control group, the NA group, and the EA group was decreased in comparison with the values at 0 s, by 3.7 kPa, 2.8 kPa, and 3.5 kPa, respectively; the 7.200s stress on the MCA in the EA group decreased more than in the NA group at 22.5 kPa initial stress (p<0.05; paired t-test).

Figure 1 shows that the stress in each group decreased rapidly within the first 600 s, while the stress relaxation curves tended to be stable when 7.200s was reached; the stress relaxation curve of each group exhibited logarithmic changes.

Creep curves for the MCA

The creep curves with initial stress on the MCA of 16 kPa in different groups are shown in Figure 3, and those with stress on the MCA of 22.5 kPa are shown in Figure 4.
When constant stress on the MCA was set at 16 kPa, the 7.200s stress on the MCA in the control group, the NA group, and the EA group were increased in comparison with the values at 0 s, by 4.9%, 3.8%, and 4.6% (p<0.05; analysis of variance [ANOVA] and Scheffe’s method), respectively, while the 7,200 s stress on the MCA in the EA group increased more than in the NA group (p<0.05; paired t-test). When the constant stress on the MCA was set at 22.5 kPa, the 7.200s stress on the MCA in the control group, the NA group, and the EA group increased in comparison with the values at 0 s, by 4.1%, 3.8%, and 3.9% (p<0.05; ANOVA and Scheffe’s method), respectively; the 7.200s stress on the MCA in the EA group increased more than in the NA group (p<0.05; paired t-test).

The strain in each group increased rapidly within the first 600s, then slowed, and the creep curves tended to become stable at 7.200s; the creep curve for each group exhibited logarithmic changes, and the normalized creep function curve of each group also exhibited logarithmic changes.

**Discussion**

The results showed that the creep curve for each group exhibited logarithmic changes. Each group exhibited rapidly decreasing stress and rapidly increasing strain in the initial 1200s, after which the stress decreased and the strain increased slowly; when 7.200s was reached, the stress relaxation curve and the creep curve basically became stable. The deformation of the MCA did not increase in proportion to the increase in stress, while exhibiting slow logarithmic changes; this may play a protective role under conditions of both normal blood pressure and symptomatic emergent blood pressure elevation. This experiment demonstrated that changes in sex hormones influenced the elasticity characteristics of the MCA in animals; EA increased the amounts of decreased stress and increased creep in the MCA in aged rats change with time. Therefore, the amounts of decreased stress and increased creep in the MCA in the NA group at 7.200s were less than in the control group and EA group. Estrogen could increase the content of elastic fibers in the arterial wall and reduce the content of collagen fibers, thus producing direct effects on arterial remodeling14. Duckles and Krause15 showed that large doses of estrogen could promote rat cerebral microtubules to release cGMP and NO in the early stages of cerebral ischemia, thus dilating blood vessels and increasing cerebral blood flow. Momoi et al.7 found that estrogen could induce the microvascular system of gonadectomized rats to release NO, which was much more obvious in female rats. Estrogen regulated the release of NO, improved cerebral capillary quality, and maintained cerebral blood flow, thus preventing cerebral ischemia and cerebral artery atherosclerosis. Oxygen free radicals are produced by enzymatic and non-enzymatic systems, and can attack the polyunsaturated fatty acids in biological membranes and initiate lipid peroxidation, leading to the formation of lipid peroxide MDA and new oxygen free radicals. Oxygen free radicals not only induce cell damage through polyunsaturated fatty acids in biological membranes, but also cause cell damage through the decomposition of hydrogen peroxide products. High or low MDA reflects the level of lipid peroxidation in the body, and indirectly reflects the level of reactive oxygen species (ROS) level16,17. In vivo production of a large amount of ROS and lipid peroxidation would be accompanied by the inactivation and reduction of a large number of antioxidant defense enzymes18,19. Wang et al.9 showed that the serum levels of MDA in an aged NA group were significantly higher than in an EA group. Superoxide dismutase (SOD) is an active substance in the body that can eliminate harmful substances produced in the process of metabolism. SOD has a special anti-aging effect in the human body. This study showed that serum levels of MDA in the EA group were significantly higher than in the aged NA group. This indicated that E2 could reduce ROS and MDA, improve antioxidant ability, inhibit the formation of lipid peroxidation products, and reduce injury by oxygen free radicals and lipid oxidation products. It plays a protective role in rat MCA, and restores stress relaxation and creep properties in aged female rats.

Fukunari et al.12 reported that the contraction of spirally-arranged smooth muscle fibers could increase arterial axial tension, while the contraction of toroidally-arranged smooth muscle fibers could decrease arterial tension. Once the functions of smooth muscles are lost, they may be replaced by collagen fibers, and the mechanical properties may be altered. Meng et al.13 reported that the structures of cerebrovascular elastic tissues play important roles in maintaining the tension and elasticity of the cerebral blood vessel wall. The analysis showed that aged rats would develop atherosclerosis, and the contents and arrangements of arterial elastic fibers, collagen fibers, and smooth muscle fibers were changed; thus, the properties of stress relaxation and sustained-stress creep in the MCA in aged rats change with time. Therefore, the amounts of decreased stress and increased creep in the MCA in the NA group at 7.200s were less than in the control group and EA group. Estrogen could increase the content of elastic fibers in the arterial wall and reduce the content of collagen fibers, thus producing direct effects on arterial remodeling14. Duckles and Krause15 showed that large doses of estrogen could promote rat cerebral microtubules to release cGMP and NO in the early stages of cerebral ischemia, thus dilating blood vessels and increasing cerebral blood flow. Momoi et al.7 found that estrogen could induce the microvascular system of gonadectomized rats to release NO, which was much more obvious in female rats. Estrogen regulated the release of NO, improved cerebral capillary quality, and maintained cerebral blood flow, thus preventing cerebral ischemia and cerebral artery atherosclerosis. Oxygen free radicals are produced by enzymatic and non-enzymatic systems, and can attack the polyunsaturated fatty acids in biological membranes and initiate lipid peroxidation, leading to the formation of lipid peroxide MDA and new oxygen free radicals. Oxygen free radicals not only induce cell damage through polyunsaturated fatty acids in biological membranes, but also cause cell damage through the decomposition of hydrogen peroxide products. High or low MDA reflects the level of lipid peroxidation in the body, and indirectly reflects the level of reactive oxygen species (ROS) level16,17. In vivo production of a large amount of ROS and lipid peroxidation would be accompanied by the inactivation and reduction of a large number of antioxidant defense enzymes18,19. Wang et al.9 showed that the serum levels of MDA in an aged NA group were significantly higher than in an EA group. Superoxide dismutase (SOD) is an active substance in the body that can eliminate harmful substances produced in the process of metabolism. SOD has a special anti-aging effect in the human body. This study showed that serum levels of MDA in the EA group were significantly higher than in the aged NA group. This indicated that E2 could reduce ROS and MDA, improve antioxidant ability, inhibit the formation of lipid peroxidation products, and reduce injury by oxygen free radicals and lipid oxidation products. It plays a protective role in rat MCA, and restores stress relaxation and creep properties in aged female rats.

In this study, the properties of stress relaxation and creep were restored in the EA group, indicating that E2 plays an important role in increasing arterial flexibility, i.e., compliance. Wang et al.9 investigated the effects of EA on the biomechanical properties of the MCA in aged female rats using a 1D extension method; the results showed that the maximum stretch stress, maximum strain, and elastic limits of the MCA in
aged rats after EA were greater than those in the NA group, and the differences were significant (p<0.05); therefore, the conclusion was that E2 administration could improve elasticity, strength, and compliance of the MCA in aged rats. In this experiment, stress relaxation and creep methods were used to determine viscoelastic properties in MCA samples in young rats, aged rats, and aged rats with EA; 1D methods used in the literature were applied to study the effects of EA on the biomechanical properties of the MCA in aged female rats; although the research methods were different, the results showed that the decreased amount of 7.200s stress and the increased amount of 7.200s strain in the MCA in aged rats after EA were greater than in the NA group in this study, showing good consistency with a prior report.

In contrast with this study, a report by Wang et al. investigated mechanical properties of the MCA, such as elasticity and strength. The MCA is a viscoelastic material, and its inherent stress relaxation and creep properties exist in order to meet the needs of its physiological functions. This study investigated the effects of EA on the viscoelasticity of the MCA in aged female rats using stress relaxation and creep methods, to determine changes according to stress, strain, and time. This was more conducive to determining the biomechanical properties of the MCA. It was feasible to study EA in aged rats using indexes including stress relaxation and creep properties.

The artery has viscoelastic properties; elasticity has normally been used as an arterial compliance indicator, and therefore has certain limitations; it would be better to use viscoelastic properties to reflect and measure arterial compliance. This experiment determined variation patterns in MCA stress- and strain-time, as well as stress relaxation curves and creep curves, in 3 groups; the characteristics of this study differed from those of previous studies by obtaining variable patterns of MCA stress- and strain-time at 13.3 kPa, 16.0 kPa, and 22.5 kPa, and therefore had greater clinical relevance. This study quantitatively clarified and comparatively analyzed the viscoelastic properties of the MCA in each group, and used MCA viscoelastic parameters and serum MDA indicators to determine the effects of E2 administration in aged female rats; therefore, it provided a viscoelastic basis for preventing cerebrovascular disease in elderly women. Hence, the design and methods of this experiment were innovative.

The methods of sampling, sample preservation, and preconditioning among the groups were consistent; as a result, the data were reliable. Because of individual differences and limited specimen samples, the experimental data showed some dispersion, but still provide reference values for use of EA to prevent cerebral arterial disease in elderly women.

**Conclusion**

Estradiol administration inhibited the formation of lipid peroxidation products and restored middle cerebral arterial viscoelasticity in aged female rats.

**References**

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