Basic Investigation

Regulative Effects of Auricular Acupuncture, Moxibustion and Chinese Herbs on Immunologic Function in the D-Galactose-induced Aging Mouse

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Objective: To observe the regulative effect of auricular acupuncture, moxibustion and Chinese herbs on immunologic function in the D-galactose-induced aging mouse and to probe the feasibility and possible mechanisms for delaying aging, so as to provide experimental basis for acupuncture, moxibustion and Chinese herbs for delaying aging. Methods: Aging mouse model was established by subcutaneous injection of D-galactose, and treated by auricular acupuncture, auricular acupuncture plus moxibustion, and auricular acupuncture plus Chinese herbs respectively for 6 weeks and then serum interleukin-2 (IL-2) and IL-6 contents and splenic lymphocyte transformation rate were compared among the groups. Results: Compared with the young group, the serum IL-2 level and the splenic lymphocyte transformation rate significantly decreased and the IL-6 level significantly increased in the aging mouse. After treatment by auricular acupuncture, auricular acupuncture plus Chinese herbs, especially auricular acupuncture plus moxibustion, the serum IL-2 level and the splenic lymphocyte transformation rate significantly increased, and the IL-6 level significantly decreased as compared with the model group. Conclusions: Combined application of auricular acupuncture, moxibustion and Chinese herbs can improve the decline or dysfunction of immunological function in the aging organism to a certain extent.

Key Words: D-galactose-induced aging mouse; Auricular acupuncture; Moxibustion; Reinforcing kidney, strengthening spleen and removing blood stasis

Along with the aging of population and increase of senile diseases, anti-aging has become a key point of study in this century. Searching for the drugs and methods for delaying aging and reducing occurrence of senile diseases has important economic benefit and social significance. The present study observed the regulative effect of auricular acupuncture, moxibustion and Chinese herbs on immunologic function in the D-galactose-induced aging mouse and probed the feasibility and possible mechanisms for delaying aging so as to provide experimental basis for acupuncture, moxibustion and Chinese herbs for delaying aging.

MATERIALS

Grouping
Forty Kunming healthy mice (20 male and 20 female), aged 3-4 months, weighing 20 ± 2g, without lesion in both ears, supplied by the Center for Experimental Animals, Guiyang Medical University, were randomly divided into 5 groups with 8 mice in each: young control group, aging model group, model + auricular acupuncture group, model + auricular acupuncture + moxibustion group, and model + auricular acupuncture + Chinese herbs group. After adaptive feeding for one week, both modeling and treatment started and 6 weeks later they were killed.

Establishment of animal model
After one week of adaptive feeding, the mice were given by subcutaneous injection in the neck-back with D-galactose (100 mg/kg/day, Shanghai Second Reagent Factory, diluted into 7% with saline solution...
just before injection), once each day, for 6 consecutive weeks. Then serum SOD activity and MDA content were determined in both the aging model group and the young control group. The serum SOD activity and MDA content in the model group were significantly different from those in the young control group, indicating that the D-galactose-induced aging model establishment was successful.

METHODS
The mice in the young control group and the model group were grasped once very other day, and other 3 groups were treated with auricular acupuncture, moxibustion, and Chinese drugs respectively. Auricular points of the mouse were selected in reference to Atlas of Animal Auricular Points edited by Hua Xingbang. For the model + auricular acupuncture group, thumb-tack needles for subcutaneous embedding were alternately inserted into the auricular points Kidney, Spleen and Stomach on both ears and fixed, and kept for 12-20h, once every other day, for 6 weeks. For the model + auricular acupuncture + moxibustion group, the auricular acupuncture with the above-mentioned method and warming moxibustion (During the treatment, kept the mouse relatively peaceful, with no severe struggle and scream) were given at Zusanli (ST 36), 5 min each session, once every other day, for 6 weeks. For the model + auricular acupuncture + Chinese herbs group, the mice were treated with the auricular acupuncture with above method and Chinese herbs, modified Liuwei Dihuang Pill (六味地黄药丸) which was composed of Shudihuang (熟地黄 Radix Rehmanniae Preparation), Shanyao (山药 Rhizoma Dioscoreae), Shanzhuyu (山茱萸 Fructus Corni), Mudanpi (牡丹皮 Cortex Moutan Radicis), Fuling (茯苓 Poria), Zexie (泽泻 Rhizoma Alismatis), Huangqi (黄芪 Radix Astragali), and Danshen (丹参 Radix Salviae Miltiorrhiza) for 6 consecutive weeks. The pills were ground into powder and mixed with the forage in 10 folds of adults’ dosage, and the mice were freely access to the forage.

For the young control group and the model group, the mice were fed by normal forage each day under a same condition and freely assess to food and water.

Sampling
At the end of treatment for 6 weeks, the mice in all the groups were weighed and fixed at the palm for local disinfection. The skin above inguinal artery was open and the inguinal artery was separated and cut off, and the blood was collected in a plastic tube, which was centrifugalized at 3000 rpm, 4°C, for 10 min, and the serum was kept for determination of IL-2 and IL-6. And the spleen was taken and used for determination of the splenic lymphocyte transportation rate after the adhered tissues were removed.

Indexes and determination methods
Splenic lymphocyte transformation rate: The spleen was cut into small pieces and put into a disinfected mortar to be ground, which was washed with RPMI 1640 solution for 3 times, and added with lymphocyte separating medium, followed by centrifugation. The middle layer of the solution was sucked to make 3x10⁶/L splenic lymphocytes suspension, which was put into a small bottle containing RPMI 1640 culture solution with PHA and placed in an incubator at 37°C, 5% CO₂, for 72h. Then 2/3 supernatant was removed by sucking with a capillary pipette, and the low layer cell suspension was taken for centrifugation, at 2000 rpm, for 10 min, and the supernatant was removed. Then the sediment was blown and smashed with capillary dropping pipette and made into a cell suspension, and smeared, stained with Giemasa-Wright’s stain, the transformed lymphocytes in 200 cells were observed with an oil immersion objective and splenic lymphocyte transformation rate was calculated. Lymphocyte transformation rate = Transformed lymphocyte number/(Transformed lymphocyte number + Un-transformed lymphocyte number) 100%.
IL-2 and IL-6 contents: IL-2 and IL-6 contents were determined by double antibody Sandwich ELISA with the kits supplied by Shenzhen Jingmei Bioengineering LTD. Co.

**Statistical processing**

All data were expressed as mean ± standard deviation (X ±s), and t-test was used for comparison between two.

**RESULTS**

Comparison of serum IL-2, IL-6 and splenic lymphocyte transformation rate among the groups (see Table 1):

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-2 (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>Splenic lymphocyte transformation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>8</td>
<td>19.9±2.4***</td>
<td>112.9±18.5***</td>
<td>69.0±0.04***</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>4.3±2.9</td>
<td>413.2±48.97</td>
<td>36.5±0.05</td>
</tr>
<tr>
<td>AA</td>
<td>8</td>
<td>8.5±3.7*</td>
<td>246.9±52.4***</td>
<td>42.8±0.05*</td>
</tr>
<tr>
<td>AA + Mox</td>
<td>8</td>
<td>15.3±3.07***</td>
<td>161.7±59.53***</td>
<td>53.9±0.11***</td>
</tr>
<tr>
<td>A+CH</td>
<td>8</td>
<td>13.6±3.59***</td>
<td>185.7±34.01***</td>
<td>48.0±0.07*</td>
</tr>
</tbody>
</table>

Notes: Compared with the model (M) group, *P<0.05, **P<0.01, ***P<0.001. The auricular acupuncture (AA) group compared with the AA + moxibustion (Mox) group and the AA + Chinese herbs (CH) group, #P<0.05, ##P<0.01

**DISCUSSION**

Traditional Chinese Medicine (TCM) has a long history in observation on aging and in practice of delaying aging, and it has representative theories for recognition of aging causes, such as kidney-deficiency theory, spleen-kidney deficiency theory, blood stasis theory. In recent years, TCM has gradually a uniform recognition for aging, i.e., the viewpoint of weakened body resistance with blood stasis theory, holding that deficiency-weakness of zang- and fu-organs, particularly deficiency-weakness of both spleen and kidney is main cause of aging. Protracted deficiency must induce blood stasis, while blood stasis is an important sign of aging. Therefore, deficiency of both spleen and kidney with blood stasis is main cause of aging, and the method of “reinforcing kidney, strengthening spleen and removing blood stasis” is an ideal way for delaying aging. Based on this principle, combined use of auricular acupuncture, moxibustion and Chinese herbs with functions of reinforcing kidney, strengthening spleen and removing blood stasis can improve the decrease or disorder of immune function in the aging organism to a certain extent.

Based on “acupoints-viscera correlation theory”, auricular points are closely related with all channels and collaterals, and viscera. Acupuncture at auricular points can dredge channels and collaterals, regulate viscera, and promote circulation of qi and blood. The present study used acupuncture at auricular point Kidney for nourishing kidney essence, reinforcing the kidney and strengthening the body resistance; Acupuncture at Spleen, Stomach for supplementing qi and strengthening spleen. Combination of the 3 points exerts functions of reinforcing kidney and strengthening spleen, dredging channels and collaterals, and regulating yin and yang. Moxibustion is one of traditional treatment methods and it also is a health-preserving and health-care method praised highly by doctors of past ages. For example, health-preserving and health-care function of moxibustion is emphasized in Bianque Xinsu (扁鹊心书). Zusani (ST 36) is a traditional important point for strengthening body resistance and health-care, and acupuncture at Zusani (ST 36) has functions of strengthening spleen and stomach, strengthening resistance and reinforcing the kidney, dredging
channels and activating collaterals, and prolonging life. Modified *Liuwei Dihuang Pill* (六味地黄丸) is composed of *Liuwei Dihuang Pill* (六味地黄丸) plus Huangqi (黄芪 Radix Astragali) and Danshen (丹参 Radix Salviae Miltiorrhizae). *Liuwei Dihuang Pill* (六味地黄丸) functions reinforcing kidney and replenishing essence, Huangqi (黄芪 Radix Astragali) functions supplementing qi, strengthening spleen and invigorating yang, and Danshen (丹参 Radix Salviae Miltiorrhizae) functions activating blood circulation and removing blood stasis. The whole prescription has functions of supplementing kidney, strengthening spleen, reinforcing qi and invigorating vital essence, activating blood circulation and removing blood stasis, jointly exerting the functions of strengthening the body resistance, removing blood stasis and anti-aging. Combination of these methods can improve immune function of the aging organism, hence delaying aging.  

Aging is involved in multi-systems and organs of the organism, and it is a complicated course participated by multi-genes and multi-factors. Among them, immune system not only is an adaptive and response system of the organism to internal and external environments, but also thoroughly participates in whole aging course of the organism. During aging, the immune system like other systems declines in physiological function, so senile organism is easily suffered from immunity-closely related diseases, such as infective diseases, autoimmune diseases and tumors, etc. Study indicates that imbalance of T_{H1}/T_{H2} ratio leads to imbalance of ratio of cytokines in the body. Decreases of cytokines secreted by T_{H1}, such as IL-2 and IL-3, and increase of cytokines secreted by T_{H2}, such as IL-4, IL-6, are one of main manifestations of immune function disorder in the aging organism. IL-2 and IL-6 are respectively representative of cytokines secreted by T_{H1} and T_{H2}. IL-2 is the most main and strongest T cell growth factor in the organism, and plays an important role in immune response, immune regulation and anti-tumor immunity. Decrease of IL-2 level is an important cause leading to immune aging. IL-6 is a kind of inflammatory factor with multi-effectiveness related with decomposition of proteins, and an important factor for severe inflammatory response in the senile organism, and it can increase sick rate and death rate of many senile diseases. IL-6 is also a strong stimulating factor of bone absorption and an important cause for senile osteoporosis. IL-6 participates in occurrence of immune diseases and is one of causes for increase of autoimmune diseases, so, increase of IL-6 is also one of important causes inducing immune aging in the organism. Therefore, simultaneous observation on changes of IL-2 and IL-6 levels in the organism can reflect immune aging of the organism from the angle of cytokines.

In the present study, it is indicated that auricular acupuncture, moxibustion and Chinese herbs can increase splenic lymphocyte transformation rate, increase serum IL-2 level and decrease IL-6 level in the D-galactose-induced aging mouse, so as to delay immune aging to a certain extent.

REFERENCES

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(Translated by Wang Youjing 王友京)