Improvement of cerebral ATP and choline deficiencies by Shao-Yin-Ren Shi-Quang-Da-Bu-Tang in senescence-accelerated mouse prone 8

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Received 25 November 1998; received in revised form 30 November 1998; accepted 22 April 1999

Abstract

Shao-Yin-Ren Shi-Quang-Da-Bu-Tang (SDT) has been used traditionally to improve the systemic blood circulation and biological energy production in the body. The object of this study is to determine the effect of SDT extract on the decline of cerebral adenosine triphosphate (ATP) and choline content associated with learning and memory impairments in senescence-accelerated mice prone 8 (SAM P8). Twenty-four-week old mice were orally treated with SDT at 400 mg/kg body weight per day, and continued for 12 consecutive weeks. At the termination of the treatment, the body weight of SAM P8 was markedly lower than that of the equal aged senescence-resistant prone 1 (SAM R1), but this was conspicuously recovered to the level of SAM R1 by SDT treatment. SDT also significantly reduced the decline of cerebral weight (P < 0.05). By comparison with normal mice, a spontaneous decrease of cerebral ATP was observed in the SAM P8. Two- and 6-fold increases of cerebral ATP content were found in SAM R1 and SAM P8 by SDT administration, respectively. The cerebral choline content was significantly different between SAM R1 and SAM P8 aged 36-week old (P < 0.01). SDT remarkably restored the decrease of cerebral choline content in SAM P8 (P < 0.01). Taken together, these results demonstrate that SDT can reduce the decrease of cerebral weight, and restore the decline of cerebral ATP and choline content associated with an alteration of neuronal metabolism in SAM P8 brain. This suggests that pharmacological properties of SDT may participate in improvement of declined cerebral energy production and cholinergic neurotransmitter synthesis in senile dementia. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Shao-Yin-Ren Shi-Quang-Da-Bu-Tang; Cerebral cortex; ATP; Choline; SAM P8
1. Introduction

Shao-Yin-Ren Shi-Quang-Da-Bu-Tang (SDT) is a noted prescription among traditional medicines in Korea, and has been widely used for a variety of diseases such as stroke, palsy and amnesia in elderly patients (Lee, 1986). Regarding its traditional use, SDT has been known to enhance systemic blood circulation and biological energy production in the brain. Recently, Park (1992) suggested that SDT increases both cell-mediated and humoral immune responses in normal mice. In addition to the immunological response, SDT also affects various physical functions such as elevation of body weight and body temperature, improvement of exercise endurance time and better hair condition in hydrocortisone-treated or cold-conditioned rats (Chang et al., 1995). From the systemic and neurological activity of SDT, we attempted to show that SDT might improve the age-related progression of neuronal degeneration caused by the decreased cerebral energy production which had been identified in the normal and demented senescence brain (Meier-Ruge et al., 1991; Hoyer, 1996) by enhancing systemic blood circulation. However, the pharmacological activity and biochemical mechanism of SDT action on the brain associated with cerebroneuronal degeneration has not yet been scientifically clarified. With this background, a primary objective of the work reported here was to determine the pharmacological effect and biochemical mechanism of SDT on declined cerebral function in senescence-accelerated mouse prone 8 (SAM P8). The 6-month old SAM P8 used in the present study is known to begin to develop learning deficit and already have deteriorating active avoidance-learning ability (Nishiyama et al., 1994), and ‘short-term’ memory deficit shows at 8 months old in delayed discrimination learning (Ohta et al., 1994).

2. Materials and methods

2.1. Animals

Male SAM P8 aged 12 weeks and age-matched normal senescence-resistance prone 1 (SAM R1) were kindly supplied by the Drug Safety Research Center, Korea Research Institute of Chemical Technology (Daejun, Korea), and were allowed free access to a commercial diet (Samyang, Korea) and tap water. The mice were maintained under 12-h light–dark cycles at a temperature of 23 ± 2°C and relative humidity of 55–60% throughout both the adaptation (12 weeks) and experimental (12 weeks) periods. SDT was administered orally at 400 mg/kg body weight per day for 12 consecutive weeks. The control animals received orally an equal volume of water instead of the herbal medicine used in the experimental group. The administration volume was adjusted to 2 ml/kg body weight. At 12 weeks after beginning the experiment, all the animal were anesthetized with diethyl ether and sacrificed. The brain was removed immediately after the blood sample was obtained from the abdominal vein. The isolated brain was washed with ice-cold phosphate-buffered saline (PBS, pH 7.4) to remove blood contamination, and blotted on a filter, followed by measurement of the cerebral weight. The cerebral cortex was dissected as soon as possible under ice-cold conditions, and kept at −80°C until use. The collected blood was separated into serum by centrifugation at 3000 rpm for 5 min at 4°C (Beckman, Avanti 30).

2.2. Preparation of extracts

All plant materials used in this study were purchased from the Korea Medicine Herbs Association and from the most famous cultivating districts in Korea, and authenticated by both botanists and pharmacognosists at the Korea Institute of Oriental Medicine, and their voucher specimens (No. KIOM 97-3-7) have been deposited at the herbarium at the Korea Institute of Oriental Medicine. The ingredients consist of 41.25 g SDT composed of Ginseng radix (7.5 g), Astragali radix (3.75 g), Atractylodis macrocephalae rhizoma (3.75 g), Paeoniae radix (3.75 g), Angelicae gigantis radix (3.75 g), Cnidii rhizoma (3.75 g), Citri pericarpium (3.75 g), Glycyrrhizae radix (3.75 g), Polygoni multiflori, radix (3.75 g) and Cinnamomi cortex (3.75 g). These ingredients
correspond to parts of the following plants: Panax ginseng C. A. Meyer (Araliacea), Atractylodes membranaceus Bunge (Leguminosae), Paeonia albiflora Pall var. tricocarpa Bunge (Ranunculaceae), Angelica gigas Nakai (Umbelliferae), Angelica gigas Nakai (Umbelliferae), Cnidium officinale Makino (Umbelliferae), Citrus unshiu Markovich (Rutaceae), Glycyrrhiza uralensis Fischer (Leguminosae), Polygonum multiflorum Thunberg (Polygonaceae) and Cinnamomum cassia Blume (Lauraceae), respectively. An extract of the prescription was prepared by decocting the mixed herbs with 10 times (v:w) H2O for 1.5 h. After filtration, the residue was boiled for an additional 1 h. The filtrates were mixed together, and lyophilized by freezing drier (Labconco, Prezone), and kept at 4°C. The yield of extract was about 20% of a dried ingredient’s weight.

2.3. Measurement of cerebral ATP content

By using the microchemiluminescence system which was described previously (Jung and Endou, 1990), intracellular adenosine triphosphate (ATP) was analyzed. In all the protocols there were at least duplicate measurements of intracellular ATP content for any given mouse. Cerebral ATP was extracted by adding 500 μl 10% TCA–1 mM EDTA to 0.1 g tissue weight, and homogenized. To complete the extraction the samples were vibrated for 2 min by a vortex mixer (Thermolyne 37600). The media containing denatured tissues were centrifuged briefly at 4°C, and 10 μl of the supernatants were transferred to polystyrene cuvettes (BioOrbit) filled with 390 μl Tris–acetate buffer containing 0.5 mM EDTA (pH 7.75). The cuvettes were set into a luminometer (BioOrbit 1251, Sweden). After addition of 100 μl ATP monitoring reagent by autodispenser (BioOrbit 1243-200, Sweden), the light intensity was measured automatically for 10 s by a computer-driven system. The production of light intensity was in a linear fashion for 10−14–10−11 M ATP per tube in our assay system.

2.4. Cerebral choline determination

Cerebral choline content was measured by chemiluminescence, using a modification of the method previously described for seminal plasma and brain (Pacifici et al., 1991; Klein et al., 1992). Cerebral cortex was homogenized with a glass-Teflon pestle in 10 times (v/w) 7% PCA, and centrifuged at 3000 rpm (Beckman, Avanti 30). The supernatant was then neutralized with 2 M K2CO3, and potassium-perchlorate precipitate was removed by centrifugation. Each supernatant was diluted appropriately with 67 mM glycine buffer, pH 8.6. Each sample was assayed in duplicate. Twenty microliters of this dilution plus 380 μl glycine buffer were analyzed for choline in a luminometer (BioOrbit 1251, Sweden) by rapidly adding 100 μl of reagent. The reagent contained 1 ml of 2 mg/ml horse radish microperoxidase, 60 μl 10 mM luminol and 50 U choline oxidase in the glycine buffer. This was freshly prepared each day for use. The integrity of the light output between 5 and 25 s after adding the reagent was linear with choline concentration between 0 and 100 pmol.

2.5. Glucose measurement

The serum and tissue glucose were measured by using a clinical test kit, enzymatic glucose reagent (Trace, Melbourne, Australia) based on the hexokinase method. Each sample was analyzed in duplicate. The cerebral cortex was homogenized in 10 volumes of ice-cold PBS contained 0.1% Triton X-100 by using a glass-teflon homogenizer, and centrifuged briefly at 4°C to remove unbroken cell debris.

2.6. Reagents

Choline oxidase, microperoxidase, luminol and choline chloride were obtained from Sigma (St. Louis, MO). The ATP monitoring reagent and standard were purchased from BioOrbit (Sweden). All other chemicals were of the highest grade from commercial sources.

2.7. Statistical analysis

Values obtained in this study are expressed as mean ± S.E. Statistical comparison was made by one-factor analysis of variance or the Student–

3. Results

3.1. Change of body weight and cerebral weight

As shown in Fig. 1, the body weight of SAM P8 decreased through the period of this study compared to that of normal control animals, SAM R1. However, statistical significance was not observed between those groups. This might have resulted from a relative variation of body weight among the animals used. Oral administration of SDT at 400 mg/kg body weight per day for 12 weeks reduced the decrease in body weight in the SAM P8, but it did not affect a change of body weight in the SAM R1. Interestingly, this study observed that treatment with SDT conspicuously improved clinical observations, including behavioral activity and body-surface features in the SAM P8 (data not shown). Table 1 shows the change of cerebral weight between SAM R1 and P8 aged 36 weeks old. There was a significant difference of absolute brain weight between SAM R1 and P8 (P < 0.05), but not in relative cerebral weight to body weight. Importantly, SDT significantly increased both the absolute and relative cerebral weight in the SAM P8, but not in the SAM R1. These results demonstrate that SDT can restore an aging-related decrease of body and cerebral weight in SAM P8.

3.2. Glucose change

In order to estimate the effect of SDT on chemical sources used in cerebral energy production, the present study measured changes in serum and cerebral glucose content. As shown in Table 2, there was no significant difference of serum glucose content between SAM R1 and P8. We also could find no statistical significance in cerebral glucose content between SAM R1 and P8.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brain (g)</th>
<th>Body weight (g)</th>
<th>Brain/body weight (× 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAM R1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>0.45 ± 0.005</td>
<td>34.5 ± 1.89</td>
<td>1.32 ± 0.07</td>
</tr>
<tr>
<td>Treatment (n = 12)</td>
<td>0.46 ± 0.004</td>
<td>34.6 ± 1.01</td>
<td>1.33 ± 0.04</td>
</tr>
<tr>
<td><strong>SAM P8</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>0.40 ± 0.003*</td>
<td>31.0 ± 1.04</td>
<td>1.29 ± 0.02</td>
</tr>
<tr>
<td>Treatment (n = 12)</td>
<td>0.45 ± 0.004*</td>
<td>34.4 ± 1.14</td>
<td>1.32 ± 0.03*</td>
</tr>
</tbody>
</table>

* Cerebral weight of 36-week old SAM R1 and P8 treated with or without SDT for 12 weeks was measured, and expressed as mean ± S.E.
# P < 0.05 vs. control in SAM R1.
* P < 0.05 vs. control in SAM P8.
Table 2
Changes of serum and cerebral glucose content by treatment with SDT

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAM R1 (n = 9)</td>
<td>211.8 ± 15.85</td>
<td>200.2 ± 5.91</td>
</tr>
<tr>
<td>SAM P8 (n = 12)</td>
<td>198.5 ± 10.83</td>
<td>190.4 ± 8.32</td>
</tr>
<tr>
<td>Cerebral glucose (μg/mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAM R1 (n = 9)</td>
<td>6.68 ± 0.18</td>
<td>6.61 ± 0.29</td>
</tr>
<tr>
<td>SAM P8 (n = 12)</td>
<td>6.88 ± 0.20</td>
<td>7.79 ± 0.25</td>
</tr>
</tbody>
</table>

* The glucose content in the 36-week old SAM R1 and P8 treated with or without SDT for 12 consecutive weeks was measured, and expressed as mean ± S.E.

3.3. Cerebral ATP content

This study examined the effect of SDT administration on the cerebral energy turnover by determining a change of intracellular ATP content in SAM R1 and P8. As shown in Fig. 2, cerebral ATP content in the 36-week old SAM P8 (14.1 ± 0.9 × 10⁻⁹ mol/mg protein) was significantly lower than that in the age-matched SAM R1 (17.8 ± 0.9 × 10⁻⁹ mol/mg protein, P < 0.05). Oral administration of SDT significantly elevated the cerebral ATP content in both SAM R1 (P < 0.01) and P8 (P < 0.001). The increased rate in SAM P8 (6-fold) was higher than that in the SAM R1 (2-fold). The results obtained here clearly demonstrate the pharmacological activity of SDT, including that SDT increases the intracellular ATP content of the cerebral cortex which can be utilized as a major chemical energy source to maintain a variety of physiological functions. Its efficacy in elevating the cerebral ATP content is significantly high in brains associated with pathological conditions.

3.4. Cerebral choline content

In the present study, cerebral choline content was measured in the SAM R1 and P8 treated with or without SDT for 12 weeks, and the results are shown in Fig. 3. There was a significant difference in cerebral choline content between the 36-week old SAM R1 (1.37 ± 0.028 × 10⁻⁹ mol/mg protein) and P8 (1.03 ± 0.021 × 10⁻⁹ mol/mg protein, P < 0.01). Interestingly, the decreased content of cerebral choline in SAM P8 was almost completely restored to the level of SAM R1 by treatment with SDT. However, administration of SDT did not change the cerebral choline content of SAM R1. These results obviously demonstrate that cerebral choline content in the SAM P8 aged protein, P < 0.01). Interestingly, the decreased content of cerebral choline in SAM P8 was almost completely restored to the level of SAM R1 by treatment with SDT. However, administration of SDT did not change the cerebral choline content of SAM R1. These results obviously demonstrate that cerebral choline content in the SAM P8 aged protein, P < 0.01). Interestingly, the decreased content of cerebral choline in SAM P8 was almost completely restored to the level of SAM R1 by treatment with SDT. However, administration of SDT did not change the cerebral choline content of SAM R1. These results obviously demonstrate that cerebral choline content in the SAM P8 aged protein, P < 0.01). Interestingly, the decreased content of cerebral choline in SAM P8 was almost completely restored to the level of SAM R1 by treatment with SDT. However, administration of SDT did not change the cerebral choline content of SAM R1. These results obviously demonstrate that cerebral choline content in the SAM P8 aged
36 weeks old was markedly reduced compared to that in normal control mice, SAM R1. This decrease might be associated with the progressive development of learning and memory impairments in SAM P8.

4. Discussion

In the present study we show that the alteration of cellular metabolism is observed in the cerebral cortex of SAM P8 aged 36 weeks old: the cerebral ATP and choline content were, respectively, 22 and 25% lower in SAM P8 than in normal control animals, SAM R1. These alterations may represent an involvement of cerebral ATP and choline deficiencies in the development of learning and memory impairment in SAM P8 as suggested in the dementia brain (Bartus et al., 1982; Meier-Ruge et al., 1991; Kanfer et al., 1993; Hoyer, 1994; Roberts and Chich, 1995; Chandrasekaran et al., 1996). On the basis of these pathological findings, it was demonstrated that oral administration of SDT at 400 mg/kg body weight per day for 12 weeks significantly reduced the deficit of cellular ATP and choline content in the cerebral cortex of SAM P8. Therefore, these results demonstrate that SDT is able to counteract the decline of cellular ATP and choline content in the cerebral cortex of SAM P8, which is also manifested in the brain with learning- and memory-impaired senile dementia (Meier-Ruge et al., 1994a,b; Hoyer, 1996).

Important pathomorphological changes in SAM P8 brains are the spongiform degeneration of the brain stem and PAS-positive granular structure mainly in the hippocampus. Cortical atrophy is found, but the brain weight is not significantly different from the age-matched SAM R1. Recently, Akiyoshi et al. (1994) also reported β/A4 protein-like immuno-reactive granular structures occurring in the SAM P8 brain, and this shows a marked age-related increase. In addition, the spontaneous age-related deficit of learning and memory in the SAM P8 strain was also suggested as an important pathological change (Takeda et al., 1991). The deficit was detected at a relatively younger age and progressed with advancing age.

From this evidence, SAM P8 might be a useful animal model to clarify the pathological characteristics of neurodegeneration and to evaluate the pharmacological efficacy of medicine which can prevent and/or improve a progressive neuronal dysfunction such as senile dementia.

According to the pathophysiological significances of cellular processes for energy synthesis in the dementia brain (Meier-Ruge et al., 1991; Torre and Mussivand, 1993; Waldemar et al., 1994; Hoyer, 1996), the present study assayed the serum and cerebral glucose content to estimate redistribution of glucose by SDT treatment in the blood and cerebral cortex. As shown in Table 2, we could find no significant change in serum and cerebral glucose content by treatment of SDT. This might have resulted from the complex biochemical mechanisms associated with the relationship between glucose supply and utilization occurring in the brain under certain conditions. Further studies need to clarify the alteration of glucose uptake and its utilization rate in the cerebral cortex of SAM P8.

In the present study, we observed that in 36-week old SAM P8 associated with a spontaneous age-related deficit in learning and memory (Takeda et al., 1991; Nishiyama et al., 1994; Ohta et al., 1994), cerebral ATP content was found to be diminished by 22% as compared to the age-matched normal animal (Fig. 2). It is difficult to assume that this decrease is directly related to a reduced rate of ATP synthesis from glucose metabolism, because the results obtained here represent the cellular ATP content manifested by cerebral ATP turnover. However, we consider that a decrease of cerebral ATP content might be importantly related to abnormal maintenance of physiological functions, resulting in the development of progressive learning and memory deficiencies in SAM P8 as described in the brain with senile dementia (Duara et al., 1983; Erecinska and Silver, 1989). As shown in Fig. 2, the cerebral ATP content in both SAM R1 and P8 was markedly increased by treatment with SDT, and the increased rate in SAM P8 (6-fold) was much higher than that in SAM R1 (2-fold). On the basis of the results obtained by this study, even though
it is difficult to explain exactly the biochemical mechanism involved in the SDT-induced cerebral ATP increase, the fact that SDT elevates the cellular ATP content in the cerebral cortex of SAM P8 may suggest the possibility of memory-enhancing properties mediated in part by maintaining energy-required physiological functions and increasing the acetyl CoA production needed in acetylcholine (ACh) synthesis (Sims et al., 1981).

In addition, we found for the first time that cerebral choline content in SAM P8 declined to 25% of that in the normal control brain (Fig. 3). This pathological change was a similar phenomenon to the deficit of cerebral choline content in the Alzheimer’s brain (Coyle et al., 1983; Blusztajn et al., 1990; Nitsch et al., 1992; Winblad et al., 1993), suggesting that the decline in cerebral choline content may be associated with the development of progressive neurodegeneration characterizing learning and memory deficit in the SAM P8. Recently, Ehrenstein et al. (1997) suggested the choline leakage hypothesis for the loss of ACh in the brain with Alzheimer’s disease, indicating that β-amyloid causes leakage of choline across the cell membrane. This leads to a reduction in intracellular choline concentration and hence a reduction in ACh production. This suggestion indicates that loss of ACh caused by choline deficiency may be very sensitive to the initial rate of β-amyloid production. In view of this hypothesis, we conclude that the elevation of SDT-induced cerebral choline content may be associated with an increase of choline uptake from the blood into the brain and/or maintaining a membrane structure to prevent choline leakage, because the traditional use of SDT is based on the enhancement of systemic blood circulation and biological energy supply. This suggestion can also be supported by the result obtained in this study (Fig. 2), indicating that SDT increases cellular ATP content in the cerebral cortex of senescence-accelerated and normal mice.

Acetyl CoA is an intermediate molecule produced in the process of energy synthesis from glucose, and both acetyl CoA and choline are essential constituents for ACh synthesis in the neuronal cells (Sims et al., 1981; Klein et al., 1992). Although this study did not directly measure a change in cerebral acetyl CoA content with SDT treatment, the acetyl CoA content in the cerebral cortex of SAM P8 might be increased by the administration of SDT. This suggestion can be supported by the evidence demonstrating that SDT treatment dramatically increases the cerebral ATP content in SAM P8 (Fig. 2). On the basis of the increases of cerebral ATP and choline content with SDT treatment, we postulated a possible pharmacological mechanism of SDT for enhancement of the neuronal function in learning- and memory-deficient brains of SAM P8 (Fig. 4). SDT-induced cellular ATP increase can reduce the decline of energy-required physiological functions and maintain morphological changes of the cerebral structure. Moreover, this may also be related to an increase in acetyl CoA production. Taken together with the change of cerebral ATP content, it is also expected that restoration of cerebral choline by SDT plays a pivotal role in the increase of ACh synthesis. Also, SDT can counteract the decline in age-related neuronal functions through the enhancement of cerebral ATP and choline deficiency in SAM P8. However, as a sequel to this study, further investigation is required to clarify the detailed biochemical mechanism underlying the increase in SDT-induced cerebral ATP and choline content and the pharmacological effect on the regulation of cerebral neurotransmitter release related to learning and memory.

Fig. 4. A postulated mechanism of pharmacological action of SDT in the cerebroneuronal cells. Increase of cerebral ATP has a physiological significance for the maintenance of cellular function, and may related to elevation of acetyl CoA production. Taken together with acetyl CoA, choline restoration can be directly associated with improvement of memory loss resulting from enhanced ACh synthesis.
5. Conclusion

The present study maps the spontaneous degenerative changes of the cerebral cortex in the memory-impaired SAM P8, and discusses the improvement in cerebral ATP and choline content by SDT treatment. In the SAM P8 aged 36 weeks old, cerebral ATP and choline content were significantly lower than those in the age-matched SAM R1. Declines in cerebral ATP and choline content were dramatically counteracted by treatment of SDT. These findings suggest for the first time that cerebral ATP and choline reduction may be associated with the development of progressive learning and memory loss in SAM P8, and increases of SDT-induced cerebral ATP and choline content suggest possible pharmacological properties of SDT on the dementia brain associated with spontaneous degenerative neuronal changes.

Acknowledgements

This work was supported in part by research grants at Wonkwang University and the Korea Administration of Health and Welfare. The authors would like to thank Dr Bong Kyu Choi, Department of Pharmacology, Wonkwang University School of Medicine, and Dr Hitoshi Endou, Department of Pharmacology and Toxicology, Kyorin University School of Medicine, for helpful suggestions and encouragement.

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