Preventive Effects of Shichimotsu-koka-to on Cerebral Lesions in Stroke-Prone Spontaneously Hypertensive Rats

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Efffects of chronic administration of a Japanese herbal medicine, Shichimotsu-koka-to (SKT) on the incidence of cerebral stroke was investigated in stroke-prone spontaneously hypertensive rats (SHRSPs). SHRSP fed with SKT extract at 1.5g/kg/day from 8 through 23 weeks of age showed significantly lower incidence of stroke in our apparent observation, although the inherent age-dependent elevation of systolic blood pressure was not affected at all. Control SHRSPs at 23 weeks of age showed hemorrhages, calcification, spongiosis, reticulating colliquation and cavitary area in their brain. In SKT-treated SHRSPs, their cerebral lesions were decreased. These observations suggest that the SKT-induced prevention of stroke contributes to the prolongation of the life span of SHRSP that we have previously reported.

1. INTRODUCTION

Shichimotsu-koka-to (SKT) is a formula of Japanese herbal medicine (kampo medicine), the prescription of which was established by Otsuka[1] in 1956, since when it has traditionally been prescribed to treat the patients with essential and renal hypertension. In Dahl salt-sensitive rats which develop salt-induced hypertension, Hiwara *et al.*[2] have reported that SKT decreased their systolic blood pressure. And Matsui *et al.*[3] have reported that SKT had inhibitory effects of blood pressure rise in stroke-prone spontaneously hypertensive rat (SHRSP) loaded with a 1% solution of saline. We have previously reported that the chronic oral administration of SKT to SHRSPs significantly prolonged their life span without affecting their blood pressure and ameliorated renal lesions secondary to hypertension.[4,5] The SHRSP which develops essential hypertension from its youth spontaneously suffers from lesions secondary to hypertension, such as cerebral infarction and / or hemorrhage, renal dysfunction and cardiac fibrosis, which are similar to those in humans, and therefore has been regarded as the best animal model available for studying human circulatovascular disor-

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ders.[6] It may be acceptable that the prevention of stroke causes the prolongation of life span in chronically-SKT-administered SHRSPs. In the present study, we continuously observed the effects of SKT on the incidence of stroke by means of the external features.

2. MATERIALS AND METHODS

2.1 Formulation

SKT consists of seven crude drugs; Rehmanniae Radix, Angelicae Radix, Cnidii Rhizoma, Paeoniae Radix, Phellodendri Cortex, Uncariae Ramulus et Uncus and Astragali Radix, at the ratio of 3 : 3 : 3 : 3 : 2 : 4 : 3. All the constituents were purchased separately from Uchida Wakanyaku, Ltd. (Tokyo, Japan) and mixed at this ratio. 13.3kg of the total mixture was boiled in hot water (100℃) of 20-fold volume for 60 min. After filtration, the filtrate was concentrated under reduced pressure and then dried, in the same way as we have previously reported.[4,5] The yield of this extract was 19.9%.

2.2 Animal Experiment

Forty-two male SHRSPs / Izm provided from the Disease Model Cooperative Research Association (Kyoto, Japan) were used. Animals were divided into two groups, and one was treated with SKT (n=18) and the other served as a control (n=24). Groups of three rats were kept in a plastic breeding cage in air-conditioned room (23±2℃, 55±5% humidity), with lights on for 12 h a day (5:00-17:00). The oral administration of SKT extract which was dissolved once in hot water and cooled till room temperature, was started from 8 weeks of age. The drug mixture was always kept mixed by continuously stirring one with magnetic bars to prevent the deposit of the extract from the accumulation. The rats were allowed to freely obtain the drug mixture for the experimental period. The average intake of SKT was set to be about 1.5g/kg/d, based on the daily consumption. The control SHRSPs were given tap water, all the animals were fed a special diet (Funabashi SP, Funabashi Farm Co., Ltd., Funabashi, Japan), took food and water or SKT solution ad libitum. The above experimental condition was set up in the same way as we have previously reported.[4,5] During the experimental period, the incidence of stroke (convulsion, paralysis and / or lethargy) was apparently checked everyday, according to a previous report (Yamori's classification). The blood pressure was measured once two weeks by means of a tail-cuff method

using electrosphygmomanometer (Riken Kaihatsu, Tokyo, Japan). At 23 weeks of age, six animals of each group, including three rats observed with incidence of stroke and three unobserved rats, were sacrificed under anesthesia with ethyl ether, and the brain was immediately dissected out and fixed with 10% formalin neutral buffer solution. It was then cut on parietal and frontal lobe and embedded in paraffin blocks, which were cut into 3 μm thick slices and stained with hematoxylin eosin (H-E) in the continuously ongoing histopathological study. The dead rats before the schedule of dissection were treated in a similar way as occasional demands.

2.3 Statistics

The incidence of stroke and the survival rate were statistically examined by means of Fisher exact probability test and Kaplan-Meier method, respectively. A p value of less than 0.05 was regarded as statistically significant.

3. RESULTS

The chronic treatment with SKT did not affect the development of spontaneous hypertension in SHRSPs throughout the experimental period (Fig. 1-B). Blood pressures in both control SHRSP and SKT-treated SHRSP were over 200 mmHg after 10 weeks of age. However, the incidence rate of stroke was significantly decreased by treatment with SKT after 18 weeks of age (Fig. 1-A).

Four control SHRSPs and four SKT-treated SHRSPs died during the experimental period. No significant difference in the life span was seen between these two groups. The large hemorrhages on the surface or in the section were evident in all the dead rats. A control SHRSP died at 15 weeks of age showed a large hemorrhage and cavitary area around that. After the schedule of dissection, akinetic rats were appeared infarct area on surface. A moribund control SHRSP dissected at 29 weeks of age showed no large hemorrhages, but cavitary area, which was suggestive of reticulating colliquation.

Our histopathological examination revealed that control SHRSPs exhibited hemorrhages, calcification, spongiosis, reticulating colliquation and cavity in their brain at 23 weeks of age. There were remarkable degeneration and atrophy, but the ventricles still did not remain expanded as the dead rats. In SHRSPs treated with SKT, their cerebral lesions were significantly decreased as compared to those in control SHRSPs (data not shown, ongoing).

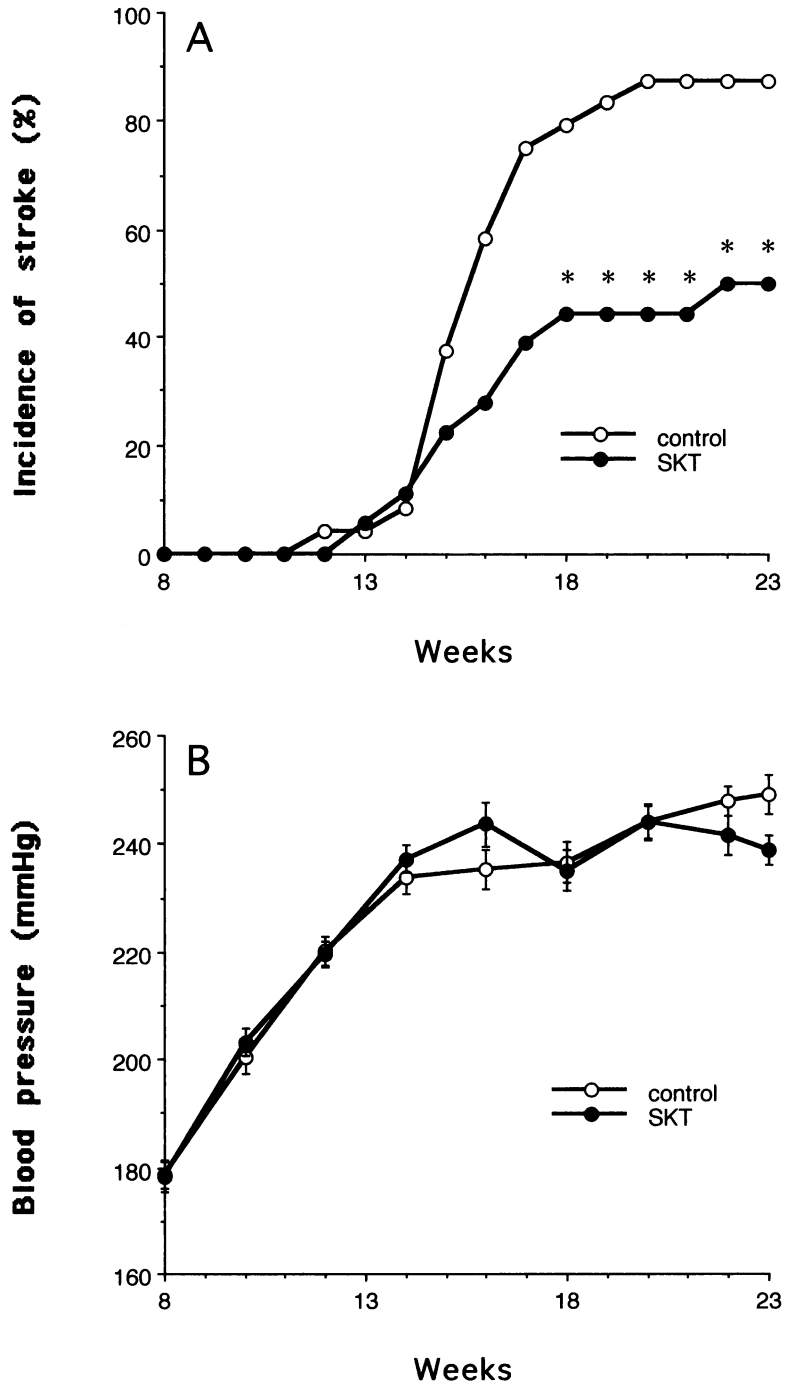

Fig. 1 Effects of SKT on the incidence of stroke (A) and blood pressure (B) in SHRSPs. SKT-treated animals were allowed to obtain the drug mixture freely from 8 weeks of age. Incidence of stroke was apparently checked everyday during the experimental period. Each data is represented as the percentage of the animal number within the groups (A) and as the mean ± S.E. of 14~24 animals (B). *; significantly different from control group ($P<0.05$), by Fisher exact probability test.

4. DISCUSSION

SKT reduced blood pressure in Dahl salt-sensitive rats[2] fed a high-salt (2% NaCl) diet and SHRSPs[3] loaded with 1% saline solution. However, the chronic treatment with SKT did not affect the development of spontaneous hypertension in SHRSPs throughout the present experimental period. We did not load with high salts in the present study. SKT may be somewhat selective to ameliorating the salt-inducing hypertension. The apparent incidence-rate of stroke was significantly decreased by treatment with SKT in SHRSPs. The control SHRSP died at 15 weeks of age showed a large hemorrhage and cavitary area around that. The moribund control SHRSP dissected at 29 weeks of age showed no large hemorrhages, but cavitary area and spongiosis. These findings correspond well to the fact that SHRSPs suffer from hemorrhage in their youth and infarction at their old age.[7] In the developing hypertension period, the blood vessels burst and lose blood by reason of sudden increasing of blood pressure. Meantime, in the period of chronic hypertension, various infarct damages are caused by chronic ischemia.

SHRSP develops hypertension from its youth and chronically suffers from uncompleted ischemia which make the repetition of hypoxia and reoxygenation.[8,9] It is widely accepted that free radicals produced by macrophage[10,11] and xanthine oxidase[12] cause damage in ischemia reperfusion. The infiltration of macrophages to remove the necrotic cell was noted in the ischemic organ. Much free radicals, superoxide anion and nitric oxide (NO), are synthesized and released by activated macrophages and neutrophils, and appear to be responsible for the cytotoxic function of these cells.[13,14] There were aggregates of either macrophages or glial cells in the brain of SHRSPs. During hypoxia, the conversion of xanthine dehydrogenase to the oxidase may occur, and produce superoxide anion on reoxigenation.[12] High concentrations of free radicals can lead to disruption of normal cellular function in the surrounding cells.[15] We have reported that the chronic administration of SKT to SHRSPs significantly prolonged their life span without affecting their blood pressure, decreased the xanthine oxidase activity in the cerebral cortex, and that SKT had strong free radical scavenging action.[4] It may be said that our present results are closely related with these effects which we have previously proved. And moreover, Sakuma et al.[16] have reported that SKT were effected as a NO donor, so as to enhance serum NOx levels. NO make relax and reduce blood pressure.[17] On the other hand, high levels of NO can cause a chemical reaction with superoxide anion to occur, whereby peroxynitrite anion and other cytotoxic radi-

cal species are generated.[13, 14] Excellence or evil? Details of the mechanism have not been clear yet.

In summary, we found that SKT has a strong protective effect against stroke in the brain of SHRSPs without affecting the development of hypertension, what is guessed to cause the prolongation of their life span. It was recognized that SHRSPs died of hemorrhage in the period of developing hypertension and died of infarction with akinesia or other lesions, such as those in kidney, in the period of chronic hypertension. It is possible that the preventive effects of SKT against lesions secondary to hypertension without heavy hemorrhage may also cause the betterment of survival rate in their elder ages. With observing the life span, the effects of SKT are being continuously investigated by histopathological and biochemical study.

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