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— A Histopathological Study —

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Stroke-prone spontaneously hypertensive rats (SHRSPs) are widely used for the study of cerebral stroke. In the previous study, we indicated that SKT-treated SHRSPs prolonged their life span without affecting their blood pressure. When the significant decrease of the incidence rate of stroke was appeared, we investigated by quantitatively studying cerebral hemorrhage and infarction on histopathological viewpoints. The number of hemorrhage and the mean size of infarct area in SKT-treated SHRSPs were significantly decreased as compared to those in control SHRSPs. These results are considered to be one of most important causes for their prolongation by SKT. Further, as SKT has strong radical scavenging action, the importance of properties as antioxidant is suggested.

1. INTRODUCTION

Cerebral stroke is a life-threatening disease, causing serious morbidity and mortality. The patients' prognosis is still poor. One who's blood pressure is high is loaded with a high risk of manifesting stroke, and therefore it is needed to search for such drugs that can effectively protect the incidence of stroke, in addition to antihypertensive agents such as calcium blockers, β-adrenergic antagonists, angiotensin converting enzyme inhibitors and angiotensin receptor antagonists.

Shichimotsu-koka-to (SKT) is a formula of Japanese traditional medicine (Kampo medicine), the prescription of which was first established in 1956 by Otsuka.[1] It has been clinically used in Japan to treat patients with essential and renal hypertensions. We have previously reported that chronic oral administration of SKT to stroke-prone spontaneously hypertensive rats (SHRSPs) which are vulnerable to stroke, such as cerebral hemorrhage and/or infarction, significantly prolonged their life span without apparently affecting their blood pressure,[2] signifi-

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cantly suppressed the development of renal lesions that are generally known to arise secondary to hypertension,[3] significantly decreased the apparent incidence rate of stroke after 18 weeks of age[4]. Prevention of stroke might have contributed greatly to the prolongation of their life span of SHRSPs. In this present study, when the significant decrease of the incidence rate of stroke was appeared, we investigated by quantitatively studying cerebral hemorrhage and infarction on histopathological viewpoints.

2. MATERIALS AND METHODS

2.1 Experiment

All animal manipulations were carried out in accordance with the ethical guidelines for experimental animals of the National Institute of Health Sciences, Japan. Forty-two male SHRSPs/Izm, which are an established strain to manifest stroke,[5] were provided from the Disease Model Cooperative Research Association (Kyoto, Japan). Animals were divided into two groups, one of which was treated with SKT extract (n=18) and the other served as a control (n=24). See Higuchi and Satake[4] for details of formulation and animal experiment including many not cited here for reasons of space.

Apparent incidences of stroke (convulsion, paralysis and/or lethargy) were observed during the experimental period, according to the previous report.[6] Because the chronic treatment with SKT significantly decreased the incidence rate of stroke after 18 weeks of age[4], at 23 weeks of age, the brain samples were obtained from six animals in both groups, including three rats showing apparent stroke and three rats without any clinical signs. These animals were sacrificed under anesthesia with ethyl ether, and the brain was immediately dissected out and fixed with 10 % neutrally buffered formalin. Transitional sections on parietal ($-4.3mm$ from bregma) and frontal ($+0.2mm$ from bregma) lobes were then made and embedded in paraffin blocks. They were cut into 3 μm thick sections and stained with hematoxylin eosin (H-E). The total number of cerebral hemorrhage sites was counted in the two above-mentioned sections for each animal under a microscope by means of a manual counter. Moreover, photographs of all the sections were taken (objective: 1×), and the photo images on the films were scanned with the resolution of 2700 dots per inch and stored on a Macintosh computer by using a photo film scanner. The infarct areas were picked up in the field of magnification of about 10× on the computer monitor screen with the aid of a photo-retouching software (Photoshop 6.0J, Adobe

Systems Inc., San Jose, CA, USA), and the total area of infarction was measured using an image analyzing software (NIH Image 1.59b, free software).[7] The obtained data were expressed both as total sizes analyzed in terms of the mean values, and as the five-graded scores ranked by seriousness of infarction. Furthermore, for the purpose of the description of the variation of each size, the sizes per lesion were represented.

2.2 Statistics

Values are expressed as *mean* ± *s.e.m.* Statistical analysis was made by one-way factorial analysis of variance (ANOVA) by using a statistical software (Statview 4.5J, Abacus Concepts, Inc., Berkeley, CA, USA). In the case that the data were in scores, Mann-Whitney's *U*-test was used. A p-value of less than 0.05 was regarded as statistically significant.

3. RESULTS

At 23 weeks of age, our histopathological examination revealed that control SHRSPs exhibited hemorrhages, calcification, spongiosis, reticulating colliquation, gliosis and large cysts in the brain. In contrast, in SHRSPs treated with SKT the number of hemorrhages was significantly decreased as compared to that in control SHRSPs (Fig. 1A). Distribution of damaged area was analyzed by regarding calcification, spongiosis, reticulating colliquation and large cysts as the infarct areas. The total number of the infarct areas was 14 in control SHRSPs, whereas it was only 2 in SKT- treated SHRSPs And the mean size of infarct area in SKT-treated SHRSPs was significantly decreased as compared to those in control SHRSPs (Fig. 1B). The variation of each size per lesion is represented in the histogram (Fig. 1C). In SKT-treated SHRSPs the mean size of the infarct areas per section was significantly smaller than that in control animals (p = 0.033, Fig. 1B). When the total infarct area per section were further ranked into five grades according to their pathological seriousness, it was found by statistical analysis that SKT-treated SHRSPs showed a significant decrease in the seriousness in the infarction, as compared with the control animals (p = 0.022, Fig. 1D). Aggregates of macrophages or glial cells were often found to exist in the brain of control SHRSPs. Those in SKT-treated animals were not clearly observed in marked contrast to control animals.

Although we did not observe severe edema, including accumulation of ascite and hydrothorax, in the schedule-sacrificed rats, either in control or SKT-treated, several moribund animals exhibited edema after the experimental period. Four control animals exhibited severe edema at the

age between 31 to 36, but only one SKT-treated SHRSP showed slight hydrothorax at 29 weeks of age.

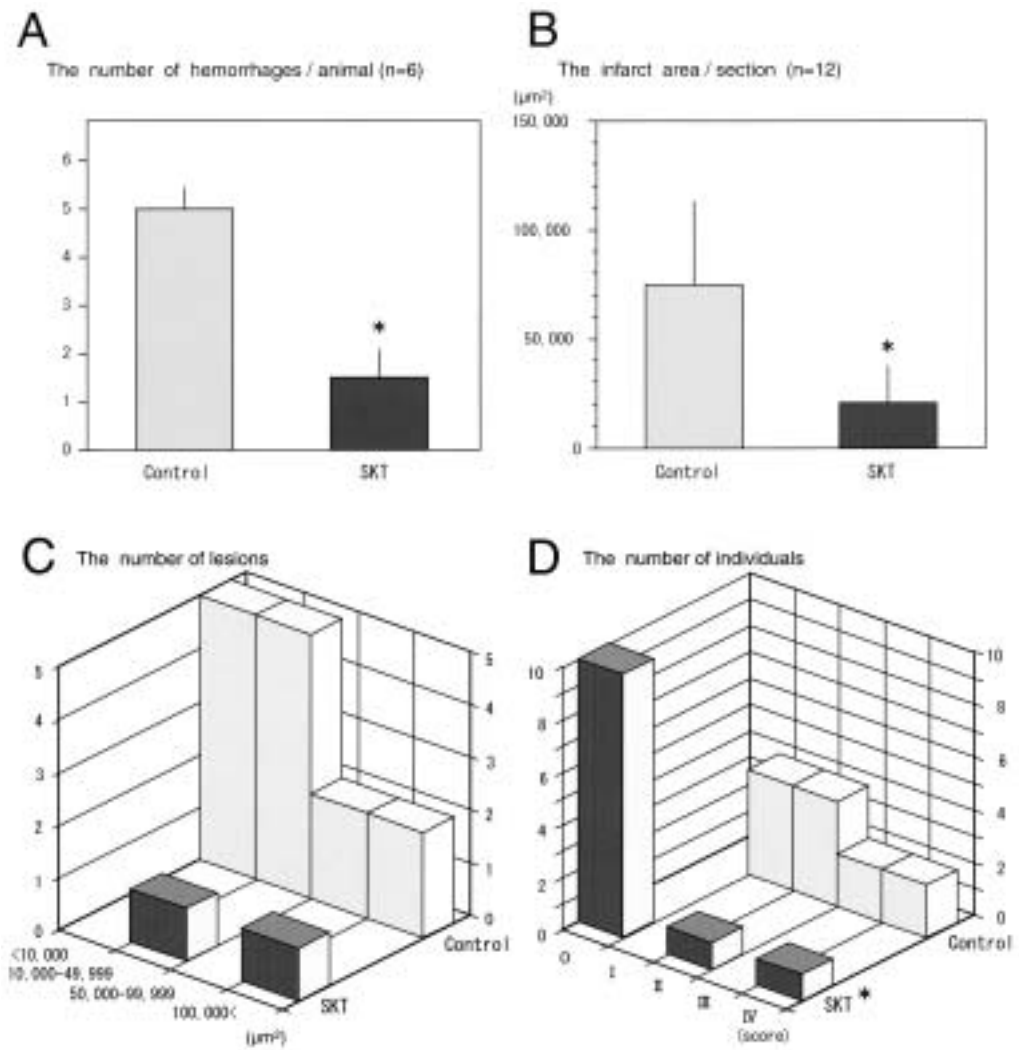

Fig. 1 Effects of SKT on the total number of the hemorrhage sites (A) and the total infarction area (B) in the brain of SHRSPs. Each value is represented as *mean* ± *s.e.m.* of 6 animals (A) and 12 sections (B). The total number of hemorrhages per two sections was counted on the photomicrograph by a manual counter. The infarct area was picked up and measured. The summarized infarct area is expressed both as the sizes per lesion (C) and as the ranked scores of the infarct area per section (D). The histograms show the variation of each size of infarct area per lesion (C) and the seriousness of the infarct area expressed as five grade evaluations (D). *; significantly different from control ($p< 0.05$, by one-way factorial analysis of variance (ANOVA) (A, B) and by Mann-Whitney's *U*-test (D)).

4. DISCUSSION

In the previous study, some SHRSPs died of severe hemorrhage in their youth during the period of developing hypertension.[4] SHRSP develops hypertension from its youth and then chronically suffers from ischemia, which might potentially cause repetition of hypoxia and reoxygenation.[8,9] SKT has been reported to decrease systolic blood pressure in Dahl salt-sensitive rats that have developed salt-induced hypertension.[10] However, here, an important finding in the previous study is that the prevention of spontaneous elevation of blood pressure did not seem to be the mechanism by which SKT prevented hemorrhage in SHRSP; SKT seems to have an protective action on peripheral vascular system against the pressure or other harmful chemicals.

The apparent high rate of incidence of stroke in SHRSPs was significantly reduced by the treatment with SKT. It is thus possible that the protection by SKT from stroke that we found in the present study is closely related to such peripheral actions that prevent damages in the cerebral vasculature. SKT significantly prevented hemorrhage in SHRSPs at 23 weeks of age. But, 4 SKT-treated SHRSPs died of large hemorrhage during the experimental period. It may be thought that serious hemorrhage, which causes mortality in acutely developing hypertension is not prevented by SKT. It is postulated that the heavy burst of vessel is not prevented by SKT, as blood pressure is not affected. Meanwhile, infarction of both total size and the grade of seriousness were significantly decreased. The narrowness of vessel is caused by hyperlipidemia, lipid-oxidation, inflammation, thrombus and endothelial dysfunction. Nitric oxide (NO), especially endothelium-derived NO, makes relax peripheral vasculature, can be scavenged by superoxide.[11] In SHRSP superoxide generation increases and NO availability reduces.[12] We have reported free-radical-scavenging action of SKT included polyphenols as constituents.[2] The long-term ischemic hypoxia is thought to reduce cerebrovascular endothelial function, which causes cerebral bleeding and infarction. In fine, it seems that SKT ameliorates endothelial function for one's scavenging action. Saito et al. have reported a scavenger administration results in maintenance of NO levels after stroke in SHRSP.[13] A number of antioxidants show that the effects of antithrombosis in SHRSP.[14] Furthermore, a recent research reports that polyphenols influence protein expression, that is a scavenging enzyme catalase expression is increased by polyphenols administration.[15] Generally, traditional herbal medicines may effect against clonic disease rather than acute disease. SKT may also effect clonic cerebrovascular dysfunction more than acute and serious bleeding.

In summary, we found that a Japanese traditional medicine SKT has a strong protective effect against occurrence of hemorrhage and development of infarction in the brain of SHRSPs without even affecting the development of hypertension. This striking finding is considered to be one of most important causes for the prolongation of their life span by SKT. It is possible that the preventive effects of SKT against other lesions, such as that in kidneys[3] may also contribute to improving the survival rate of the SHRSPs in their elder ages, which awaits further study.

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