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Reseach and Report

Yokukansan increases serum Brain-derived neurotrophic factor (BDNF) levels in Gunn rat

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Abstract

Brain-derived neurotrophic factor (BDNF) is expressed at high levels in the hippocampal dentate gyrus (DG), and decreased levels of BDNF have been implicated in the pathophysiology of schizophrenia (SCZ). We have previously reported that yokukansan (YKS), which is a traditional Japanese medicine, is effective for SCZ and promotes neurogenesis in the DG of Gunn rats, an animal model of SCZ. In this study, we investigated the effect of YKS on serum BDNF levels in Gunn rats. The results showed that YKS increased serum BDNF in this model, which may suggest that BDNF expression in the DG leads to increased neurogenesis. Our findings may help to explain the efficacy of YKS in treating SCZ.

Key words: brain-derived neurotrophic factor; yokukansan; schizophrenia;

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unconjugated bilirubin; rat

Introduction

Schizophrenia (SCZ) is a heterogeneous group of mental illnesses with a pathogenesis resulting from multiple factors, including genetic, biological and environmental ones. From the standpoint of the heterogeneity of SCZ, previous studies have indicated a close association between unconjugated bilirubin (UCB) and SCZ [1, 2]. On the basis of these findings, we suggested that elevated levels of UCB play an important role in SCZ etiology, and that Gunn rats, which exhibit a high concentration of UCB, are useful as an animal model of SCZ [3].

Brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain, regulates neuronal survival, differentiation and growth during development [4]. BDNF is also active during a critical developmental period and likely to influence the neuroplasticity of SCZ [5]. Therefore, researchers have indicated that BDNF is a key factor for the pathogenesis and treatment of this disease [6].

We previously reported that yokukansan (YKS), one of the traditional Japanese medicines known as "Kampo" medicines in Japan, is effective as an adjunctive therapy for treatment-resistant SCZ [7]. We also revealed that YKS promotes neurogenesis in the hippocampal dentate gyrus (DG) of Gunn rats [3].

BDNF is expressed at high levels in the DG [8]. The ability of BDNF to freely cross the blood-brain barrier [9] suggests that serum BDNF levels may reflect the BDNF levels in the DG. Therefore, in this study, we investigated whether YKS affects the serum BDNF levels in Gunn rats and normal rats.

Methods

Animals

Seven-week-old male homozygous (j/j) Gunn rats and male Wistar rats (Japan SLC Inc., Shizuoka, Japan) were used in this study. The rats were housed in plastic cages (39 × 27 × 18 cm) under standard conditions (temperature, 23 ± 2°C;

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humidity, 55 ± 5%; 12 h light/dark cycle [light phase from 0700 to 1900h]) and were given free access to food and water. One week before the experiment, the rats underwent a handling procedure once daily to reduce stress during the experiment. All procedures were performed with the approval of the Shimane University Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Japan.

Drugs

YKS (Tsumura & Co., Tokyo, Japan) is composed of 7 dried medical herbs (Table 1). Each plant material was authenticated by identifying the external morphology and marker compounds of plant specimens according to the methods of the Japanese Pharmacopoeia and the company's standard. The 7 medical herbs were mixed and extracted with purified water at 95°C for 1 h, and the extraction solution was filtered and concentrated under reduced pressure. The spray-drying technique was used to produce dried extract powder. The yield of the extract was about 15.9%. The rats were divided into 4 groups: a Wistar-control (WC) group, Wistar-YKS (WY) group, Gunn-control (GC) group, and Gunn-YKS (GY) group. The rats in the control groups (WC and GC) were given drug-free water ad libitum for 6 weeks, whereas those in the YKS-treated groups (WY and GY) were given water containing 0.6% YKS (corresponding to a dosage of 1 g/kg of body weight) for the same period.

Blood sampling

Twenty-four hours after the last administration, blood samples were collected into sampling tubes under deep intraperitoneal anesthesia with sodium pentobarbital (80 mg/kg body weight). The blood samples were centrifuged at 2000 g for 20 min. Serum was stored at –80°C until analysis. After the blood samples were collected, the rats were perfused transcardially with 500 ml of physiological saline, followed by 500 ml of 4% paraformaldehyde in 0.1 M

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phosphate buffer (PB; pH 7.3).

Serum BDNF determination by enzyme-linked immunosorbent assay (ELISA)

A commercial sandwich ELISA kit (Abnova, Taipei, Taiwan) was used to quantify the serum BDNF level according to the manufacturer's instructions. The plate was read in an ELISA-spectrophotometer reader with an absorbance wavelength of 405 nm. Standard curves were obtained from values generated from known concentrations of BDNF in provided kits. All assays were performed in triplicate.

Statistical analyses

Results were analyzed by one-way analysis of variance (ANOVA) and *post hoc* Bonferroni test to determine differences among groups. Values are expressed as the mean ± SEM. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. In the analyses, *P* values < 0.05 were considered statistically significant.

Results

The serum BDNF levels in the WC, WY, GC, and GY groups were 2.65 ± 0.15, 3.27 ± 0.40, 2.60 ± 0.16, and 4.07 ± 0.53 ng/ml, respectively (Figure 1). No significant difference was observed among the WC, WY, and GC groups. However, the serum concentration of BDNF in the GY group was significantly increased compared with that in the GC group ($P = 0.039$) .

Discussion

In the present study, we found that chronic YKS treatment increased the serum BDNF levels in Gunn rats. This finding could support our previous result that YKS promotes hippocampal neurogenesis in association with its anti-inflammatory action [3].

Several studies have indicated that BDNF has a crucial role for neurogenesis in the DG. For example, infusion of endogenous BDNF into the DG leads to increased neurogenesis [10]. Other studies have shown that BNDF is critically required for the increased neurogenesis following dietary restriction [11],

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antidepressant treatment [12] and environmental enrichment [13].

On the other hand, an extensive review has pointed out that BDNF has a close association between the nervous and immune systems and plays an important role in brain-related disorders [14]. In addition, serum BDNF levels are negatively correlated with inflammatory marker IL-6 and TNF-α in psychosis [15].

A recent meta-analysis study has shown that serum BDNF levels are not significantly different between naive SCZ patients and SCZ patients medicated with antipsychotics [16], while other studies have reported that serum BDNF levels are positively correlated with clozapine (CLZ) treatment [17, 18]. CLZ is the only effective antipsychotic for treatment-resistant SCZ, while we reported that YKS is effective as an adjunctive therapy for treatment-resistant SCZ [7]. Thus, there may be a common pharmacological characteristic between CLZ and YKS. Further work will be needed to investigate this possibility.

In conclusion, this study was the first to report that YKS increased the serum BDNF levels in an animal model of SCZ. These results may provide a clue to the effects of YKS in SCZ, and could contribute to the identification of candidate biomarkers and a better understanding of this disease.

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Crude drug name	Composition (g)
Atractylodes lancea rhizome	4.0
Poria sclerotium	4.0
Cnidium rhizome	3.0
Uncaria hook	3.0
Japanese angelica root	3.0
Bupleurum root	2.0
Glycyrrhiza	1.5

Table 1. Crude Drug Composition of YKS

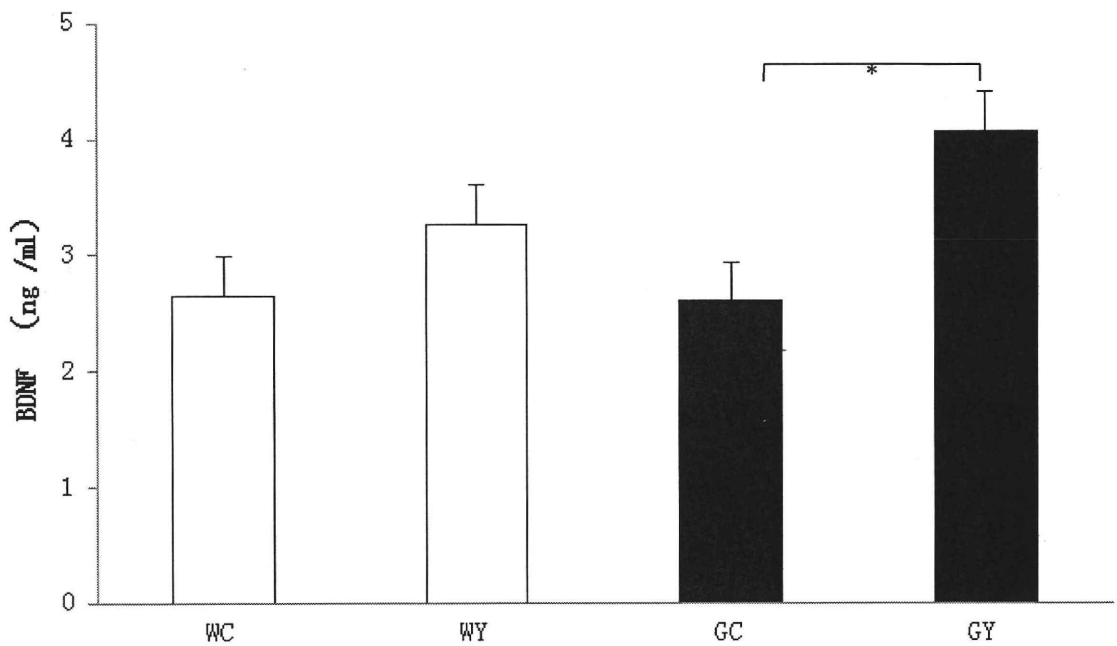

Figure 1. YKS increased serum BDNF levels in Gunn rats

Data are presented as the mean ± SEM (n = 4, respectively).*P <0.05, ANOVA followed by post hoc Bonferroni test.

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