The Improvement of Sleep by Oral Intake of GABA and *Apocynum venetum* Leaf Extract

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Summary The effects of two food materials, γ-aminobutyric acid (GABA) produced by natural fermentation and *Apocynum venetum* leaf extract (AVLE), on the improvement of sleep were investigated in humans. The electroencephalogram (EEG) test revealed that oral administration of GABA (100 mg) and AVLE (50 mg) had beneficial effects on sleep. GABA shortened sleep latency by 5.3 min and AVLE increased non-rapid eye movement (REM) sleep time by 7.6%. Simultaneous intake of GABA and AVLE shortened sleep latency by 4.3 min and increased non-REM sleep time by 5.1%. The result of questionnaires showed that GABA and AVLE enabled subjects to realize the effects on sleep. These results mean that GABA can help people to fall asleep quickly, AVLE induces deep sleep, and they function complementarily with simultaneous intake. Since both GABA and AVLE are materials of foods and have been ingested for a long time, they can be regarded as safe and appropriate for daily intake in order to improve the quality of sleep.
Key Words γ-aminobutyric acid (GABA), *Apocynum venetum*, sleep quality, sleep latency, non-REM sleep

Sleep has recently become a matter of public concern especially in industrialized countries. In Japan, a survey has revealed that over 20% of the general adult population has been suffering from insomnia (*1*). Since insomnia is a serious health problem, it is pharmacologically treated by drugs, for example, benzodiazepines and non-benzodiazepines such as zolpidem (*2*). However, these treatments always face risks such as side effects and drug dependence, and therefore gentle treatment without such drugs is required. Considering safety and security, it is one of the ideal treatments to take functional foods effective for sleep.

We focused on two materials of functional foods, γ-aminobutyric acid (GABA) and *Apocynum venetum* leaf extract (AVLE). GABA is a kind of amino acid (Fig. 1) contained in various foods and is known as an inhibitory transmitter of the central nervous system (*3*). GABA can be produced by natural fermentation as an ingredient of functional foods and oral administration of highly concentrated GABA has beneficial effects on the autonomic nervous system; that is to say, GABA can relieve anxiety (*4*), GABA is effective for reducing psychological stress (*5*), and GABA possesses a relaxation effect by increasing the total and parasympathetic nerve activity (*6*). Meanwhile, *Apocynum venetum* is an herb widely growing in the middle and northwestern region of China, and it is drunk as a traditional tea. Extract of its leaves (*Apocynum venetum* leaf extract: AVLE), which contains flavonoids such as hyperoside and isoquerci-

trin (Fig. 2), shows antidepressant effects (*7*) and anti-anxiety and antihypertensive activities (*8*). Since both GABA and AVLE have effects on psychological disorders, which are deeply involved in sleep quality (*9, 10*), the improvement of sleep can be expected from ingestion of them. In fact, GABA has been reported to help elderly people to sleep well (*11*), and AVLE has been recorded in 'Pharmacopoeia of People's Republic of China' and used as an herbal medicine to treat insomnia in China (*12*). However, there are few reports that quantitatively and objectively demonstrate the sleep-improving effect of GABA and AVLE.

In the present study, we investigated the effect of GABA and AVLE on sleep more thoroughly by using the electroencephalogram (EEG), which made it possible to quantitatively and objectively evaluate sleep. The effect of the simultaneous intake of GABA and AVLE on sleep was also investigated and a complementary effect of the combination of them was revealed.

MATERIALS AND METHODS

Ethical consideration. This study was approved by the Ethics Committee of Pharma Foods Co., Ltd. The participants were given an explanation of study procedures and potential risks of the study. Written informed consent was obtained from all participants prior to the initiation of study procedures. All of the procedures followed the code of the Declaration of Helsinki.

Samples. Participants took two gelatin capsules containing GABA (100 mg of GABA and 50 mg of dextrin) or AVLE (50 mg of AVLE and 100 mg of dextrin) or GABA (100 mg) and AVLE (50 mg) or a placebo

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(150 mg of dextrin). GABA was produced by natural fermentation using a specific strain of lactic acid bacteria (PharmaGABA® with 89% purity of GABA, Pharma Foods International Co., Ltd., Japan). AVLE was produced from leaves of *Apocynum venetum* (Venetron® with not less than 4% of hyperoside and isoquercitrin, Tokiwa Phytochemical Co., Ltd., Japan).

Subjects. The selection of subjects was based on a questionnaire known as the Pittsburgh Sleep Quality Index (PSQI) (*13*). Sixteen subjects (average age: 36.8 ± 8.9, 7 males and 9 females) whose PSQI scores were six or greater than six were selected from volunteers recruited for this study. They were suspected to possess some kind of sleep disorder because people with a PSQI score of six or greater than six are classified as poor sleepers (*14*).

EEG measurement. The EEG data were collected using a portable one-channel EEG device (SleepScope, Sleepwell Co., Ltd., Osaka, Japan) in a similar way as in the prior literature (*15*). The sampling rate was 128 Hz, and self-adhesive and disposable electrodes were used. Sleep was evaluated in the following four stages: wakefulness, rapid eye movement (REM) sleep, light non-REM sleep (stage N1 and N2), and deep non-REM sleep (stage N3), according to the criteria of the American Academy of Sleep Medicine, 2007 (*16*).

Study design and test procedure. This study was a single-blinded, placebo-controlled trial. The sixteen subjects were divided into four groups of four people each (group 1 to 4), and the average PSQI scores of the four groups were approximately the same. The study consisted of two test periods (1 wk each) and there was a wash-out period (1 wk) between the test periods. During the first test period, subjects of group 1, 2, 3, and 4 ingested GABA, AVLE, GABA and AVLE, and a placebo, respectively, 30 min before going to bed everyday. During the second test period, subjects ingested different samples from the first period, that is to say, subjects of group 1, 2, 3, and 4 took the placebo, GABA and AVLE, AVLE, and GABA, respectively. As a result, each sample group consisted of eight subjects. However, data for one

subject were omitted because of illness, and that of two others were also omitted because the EEGs were not successfully collected. As a result, the group of GABA, AVLE, GABA and AVLE, and placebo consisted of seven, eight, seven and seven subjects, respectively. On two consecutive nights 3 d before the first and the second test period, the subjects went to bed wearing EEG instruments for adaptation. On the night just before the test periods, subjects' EEGs were collected for baseline data, and on the last night of the test period, subjects' EEGs were also collected as data after administration. On the EEG measurement day, alcohol and drugs such as cold medicine and hypnotics were prohibited. In addition, foods and drinks such as coffee, tea and others that may affect natural sleep were also prohibited 2 h before going to bed. Subjects were requested to keep their lifestyle patterns constant during the test period. On the day just after the EEG measurement, subjects rated their own feeling with respect to ease of falling asleep, feelings on awakening, and satisfaction with sleep by using a visual analog scale (VAS). Subjects gave high scores if they felt positive effects of test samples as to those items. In addition to the VAS, the PSQI was used for evaluation of the sleep quality.

Statistical analysis. Data are expressed as the mean$\pm$SD of the amount of change before and after administration. In order to detect differences between the placebo group and the other groups, every result of the EEG test and questionnaires was analyzed by means of Steel's test. A probability value of less than 5% was considered to indicate a significant difference.

RESULT

The results of the EEG test

The results of the EEG test are shown in Fig. 3. GABA shortened sleep latency by 5.3 min after administration and showed a weak tendency toward difference (Fig. 3A, $p=0.13$). AVLE and the combination of GABA and AVLE shortened sleep latency by 0.9 min and 4.3 min, respectively, and there was no significant difference compared with the placebo (Fig. 3A). GABA, AVLE, and the combination of GABA and AVLE shortened deep non-REM sleep latency by 5.6 min, 3.1 min and 5.3 min, respectively, and no significant difference was observed (Fig. 3B). GABA showed an increase of non-REM sleep time of only 1.0% and there was no significant difference (Fig. 3C). AVLE significantly increased non-REM sleep time by 7.6% ($p=0.01$), and the combination of GABA and AVLE increased it by 5.1 min with a small trend
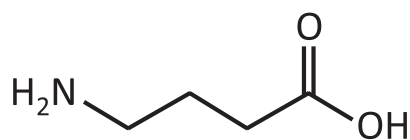

Fig. 1. γ-Aminobutyric acid (GABA).
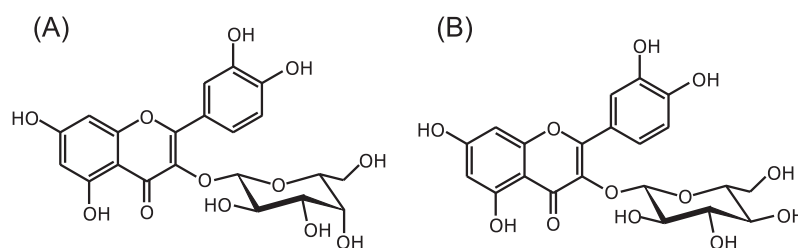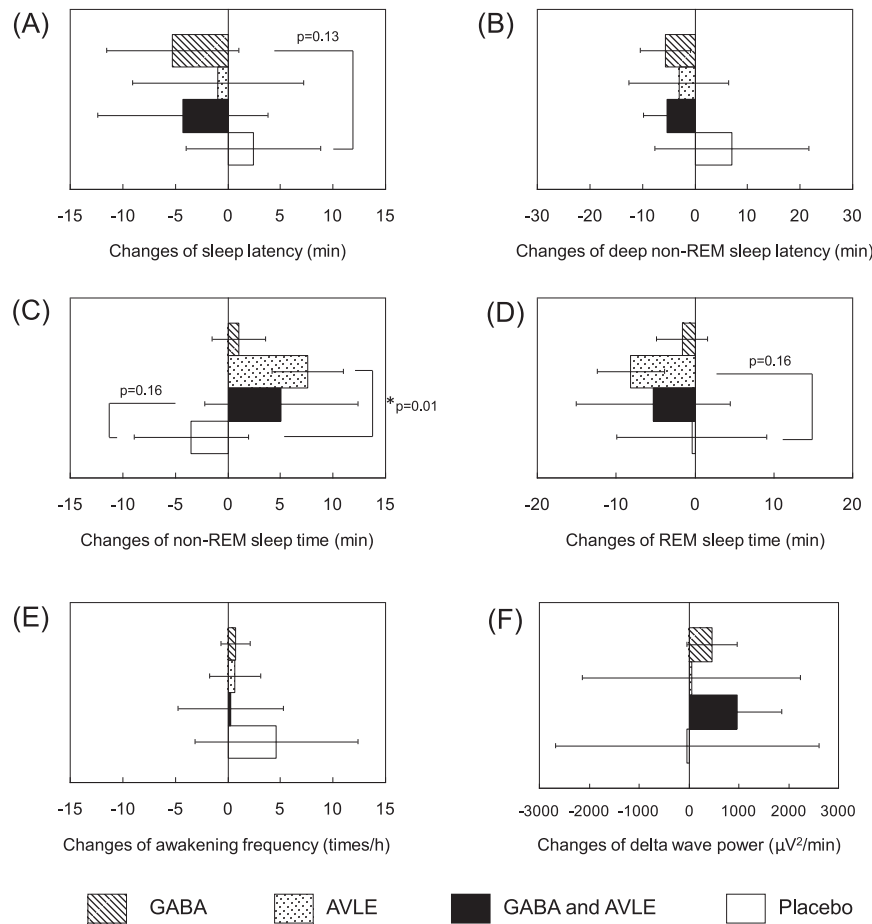

Fig. 2.   Hyperoside (A) and isoquercitrin (B).

Fig. 3.   The EEG test results. Values are means±SD of changes in sleep latency (A), deep non-REM sleep latency (B), non-REM sleep time (C), REM sleep time (D), awakening frequency (E), and delta wave power (F) before and after sample administration. * indicates significant difference compared with a placebo ($p<0.05$).

(Fig. 3C, $p=0.16$). GABA and the combination of GABA and AVLE showed a decrease of REM sleep time by 1.6% and 5.3%, respectively, and there was no significant difference (Fig. 3 D). AVLE decreased REM sleep time by 8.2% with a small trend (Fig. 3D, $p=0.16$). Awakening frequency was scarcely increased in any experimental group (GABA, AVLE, or the combination of GABA and AVLE), and no significant difference was observed (Fig. 3E). GABA, AVLE, and the combination of GABA and AVLE increased delta wave power by 466 μV^2/min, 50 μV^2/min and 973 μV^2/min, respectively, and no significant difference was observed (Fig. 3F).

The results of questionnaires

The results of the VAS questionnaires and PSQI are shown in Fig. 4. In every item on the VAS questionnaire, the VAS value was increased in every experimental group (GABA, AVLE and the combination of GABA and AVLE) compared with the placebo, but no significant difference was observed. The combination of GABA and AVLE improved satisfaction with sleep and showed a weak trend (Fig. 4A, $p=0.18$). The result of the PSQI revealed that AVLE improved subjective sleep quality and showed a tendency toward difference (Fig. 4D, $p=0.10$).

DISCUSSION

In this study, participants ingested 100 mg of GABA or 50 mg of AVLE 30 min before going to bed, and the EEG test revealed the effects of them. GABA greatly shortened sleep latency, while AVLE did so poorly. Although there was no significant difference, GABA also shortened non-REM sleep latency. These results suggested that GABA had an effect to help people to fall asleep quickly and easily. AVLE, on the other hand, significantly increased non-REM sleep time, which GABA hardly increased. This suggested that AVLE was effective for increasing deep sleep. However, the delta wave was hardly increased in the AVLE group. The delta wave is regarded as an indicator of deep sleep because it appears mainly during the deepest sleep (i.e., deep non-REM sleep) (*17*). Because the greater part of non-REM sleep appearing in AVLE group was light non-REM sleep (data not shown), the delta wave was not increased in spite of the increase in non-REM sleep time. Participants also ingested both GABA and AVLE simultaneously and this revealed the effect of the combination of GABA and AVLE. It shortened both sleep latency and non-REM sleep latency to the same degree as GABA. Meanwhile, it increased non-REM sleep time much more than
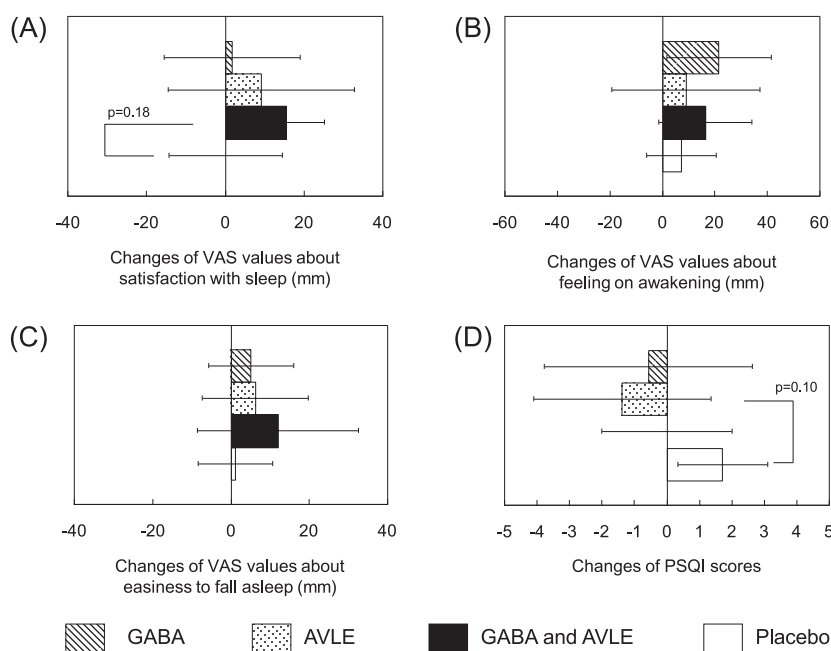
Fig. 4. The VAS questionnaire and PSQI results. Values are means±SD of changes in VAS values about satisfaction with sleep (A), feeling on awakening (B), ease of fallng asleep (C) and changes in PSQI scores (D) before and after sample administration.

GABA. It was suggested that the sleep latency-shortening effect of the combination of GABA and AVLE was mainly brought on by GABA, and the non-REM sleep time-increasing effect of it was mainly brought on by AVLE. Although no synergic effect of GABA and AVLE was clearly observed, they could exhibit their beneficial effects without disturbing each other.

Participants rated their own feeling about sleep after administration, and no deterioration was observed in either the GABA group or AVLE group for any item on the VAS questionnaire or PSQI. AVLE showed a decrease in PSQI score, and this meant that AVLE improved subjects' sleep quality. The same result was observed in the combination of GABA and AVLE group; that is to say, no item on the questionnaire was worsened and in particular satisfaction with sleep was well improved compared with the results for the placebo. These results suggested that participants felt their sleep better than before administration. From the above results, it can be said that GABA and AVLE are effective for sleep, and simultaneous intake of them complements their effects without interference. It is also said that the two food materials possess the potential to make people realize the improvement of their sleep by oral intake in humans.

In general, antianxiety drugs are also effective for the improvement of sleep because psychological disorders such as stress, depression and anxiety are deeply involved in sleep quality (9, 10). GABA is known to have effects of anti-stress and relaxation (3–6), and AVLE is also effective for relieving depression (7) and anxiety (8). Furthermore, there is a report that has shown the stress-reducing effect of GABA and AVLE by concurrent ingestion (18). Although serious psychological disorders were not discovered among the subjects in this

study, it was no wonder that they had stress or depression to some extent in the so-called high-stress modern society. It was suggested that the improvement of sleep quality by GABA and AVLE was through the relief of a poor psychological state. Although the mechanism of how oral intake of GABA affects the neurons is still not clear, the sleep-improving effect of GABA may possibly be explained from its role as an inhibitory transmitter in the central nervous system. AVLE, on the other hand, contains flavonoids such as hyperoside and isoquercitrin which are known to have antidepressant effects with oral administration (19). Both hyperoside and isoquercitrin are quercetin glycosides and they are deglycosylated by lactase phlorizin hydrolase in the intestine before absorption (20). The resulting aglycone (i.e., quercetin) is then absorbed and metabolized into quercetin glucuronides and glucuronyl sulfates of methylated quercetin, and the quercetin metabolites also show antidepressant effects with oral administration (21). There is a report that quercetin and its glucuronide metabolites affect the monoamine oxidase-A in brain mitochondria and attenuate the breakdown of serotonin (22). There is some possibility that the sleep-improving effect of AVLE is due to this protection against the decomposition of serotonin. Of course, the active ingredients for sleep in AVLE are still not identified and further studies should be conducted in order to reveal the mechanism of the sleep-improving effect of AVLE.

Regarding the difference in effects between GABA and AVLE, it may be described from the point of view of absorption and metabolism. GABA can be absorbed quickly. The blood level of GABA is highest 30 min after oral administration, and then it goes down and returns to the original level 1–2 h after administration

in humans (unpublished data). Because of fast absorption and fast return of blood level, GABA may show the effect mainly at the early stage of sleep, that is, the onset of sleep. Although the active ingredients for sleep improvement in AVLE are not clear, quercetin and its metabolites, metabolized forms of flavonoids such as hyperoside and isoquercitrin, may possibly be involved in the improvement of sleep. An experiment showed that the plasma concentration of quercetin was highest 15 min after oral administration of isoquercitrin, but those of quercetin metabolites were highest 2–3 h after administration and the blood level of it was maintained for 8–10 h (23). From the above, if flavonoids such as hyperoside and isoquercitrin are active ingredients in AVLE, it is reasonable that the sleep-improving effect appears several hours after administration and AVLE does not shorten sleep latency. GABA quickly shows the effect after administration, and therefore, it is suitable to take GABA and AVLE simultaneously.

In the present study, naturally fermented GABA and extract of *Apocynum venetum* leaf were used as the materials to be examined. They were applied to the study not as samples of single active ingredients but as food. Food is a mixture of various components and there is a possibility that the effect of food is different from that of a single active ingredient because of a synergistic effect or offsetting by other components in the food. From the point of view of food science, the present study has meaning in terms of evaluating the sleep-improving effect of food as a mixture by oral intake in humans using quantitative and objective means.

In conclusion, this study revealed that GABA and AVLE possessed beneficial effects on sleep. They could show the effects complementarily through simultaneous intake of them. They are regarded as safe because both GABA and AVLE are materials of foods and have been ingested for a long time. Therefore, the combination of GABA and AVLE is ideal and appropriate for daily intake for the improvement of sleep.

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