Effects of a Bovine Alpha S1-Casein Tryptic Hydrolysate (CTH) on Sleep Disorder in Japanese General Population

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Abstract: This study describes the effect of bovine alpha-S1 casein tryptic hydrolysate (CTH) in a representative sample of day-time workers from the general population of Japan with the occurrence of insomnia during the preceding six months. To investigate this issue, 32 subjects, aged between 25 and 40 years, were examined for the subjective sleep quality using the Japanese Pittsburg Sleep Quality Index (PSQI-J).

CTH significantly improves the PSQI total score of the treated subjects. It particularly improves the sleep quality after two weeks of treatment, decreases the sleep latency and the daytime dysfunction after four weeks of treatment.

Given the antistress properties of CTH, it seems possible to relate the detected improvement of sleep aspects to a reduction of stress following its' chronic administration.

In conclusion, being given its beneficial effects and its absence of negative side effects, it would be advantageous to use the CTH to improve chronic insomnia in the Japanese general population. However, further studies will be necessary in order to clarify the essential aspect of CTH properties in the sleep problems.

Keywords: Alpha-S1 casein tryptic hydrolysate, Sleep, PSQI, Human, Japan.

INTRODUCTION

Difficulties in falling asleep, in staying asleep, waking up too early every morning, are the main symptoms of insomnia. In the last years, numerous studies indicated that about 25-36% of adults in the general population suffer of transient or occasional insomnia [1-6]. Chronic insomnia was reported in 7.5-15% of adult people. It is well known that insomnia can lead to many adverse consequences at individual and societal levels. At the individual level, chronic sleep debt leads to daytime fatigue and tiredness, impairments in cognitive functions like lacks of concentration and memory loss [7, 8]. In the emotional area, stress, anxiety and depression are commonly reported by insomniac people [9-11]. Moreover, recent studies have concluded that sleep deficits can facilitate the development of chronic metabolic disorders such as obesity, diabetes, and hypertension [12, 13]. At the societal level, insomnia leads to impaired work performance, absenteeism, lost productivity, and increased work-related accidents [8], increased health care costs and reduced quality of life [14, 15].

Particularly in Japan, it has been reported that the prevalence of insomnia in the general population is comparable to that reported in Western countries studies [16-18]. In addition, it was reported from a recent survey that one in five Japanese and one in three elderly Japanese suffer from insomnia [19]. The respective prevalence rates of sleep problems were estimated to 26.4% for males and 31.1% for females [20] and that approximately 23.6% of adult Japanese male and female daytime workers experienced insomnia lasting more than 6 months and that insomnia was associated with multiple psychosocial factors as psychological stress and job stress [21-23]. Insomnia affects Japanese society [24-26] and it is becoming a serious social problem [27, 28], leading the Ministry of Health, Labor and Welfare in Japan to propose a plan called "Health Japan 21", which adopted sleep as one of the specific living habits needing improvement. Insomnia is considered to be chronic if it occurs on three nights a week for one month or longer.

The most commonly occurring forms of chronic insomnia are associated with stress, anxiety and mild depression [29, 30]. Stress and anxiety affect certain aspects of sleep with some consistency [31] and several alterations are described in the literature: changes of sleep/wake rhythm and modifications both in sleep duration and in sleep architecture [32].

Knowing that the most commonly occurring forms of chronic insomnia are associated with stress, anxiety and mild depression [9, 33, 10], we can postulate that treatments which reduce stress could be beneficial to people suffering from sleep disorders.

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In the 1960s, the benzodiazepines were introduced for the treatment of anxiety and insomnia. These medications were shown to have undesirable effects including amnesia, ataxia, tiredness, nausea, hypotension, leucopenia, hostility, confusion and depression [34], tolerance and dependence [35, 36]. Benzodiazepines are still in use today for the treatment of insomnia. They are less often prescribed now than in the past, however, because of those concerns regarding abuse and dependence, memory and movement impairment, next day "hangover" plus residual effects [37].

In the 1990s a newer class of medications, known as "non-benzodiazepine, benzodiazepine receptor agonists" were introduced for the treatment of insomnia [38, 39]. Although highly effective in promoting sleep and seeming to have better safety profiles, these drugs are however associated with residual side-effects.

An alternative approach is to focus on natural substances. Cow's milk has long been considered by folk wisdom as a tranquillizing beverage facilitating sleep. Since, it was reported that adults consuming a meal of cornflakes and milk exhibited a stronger tendency toward uninterrupted sleep [40] and it was shown that evening intake of lactalbumin may improve sleep and morning alertness [41].

Recently, it was discovered that a tryptic hydrolysate from bovine αS1-casein, containing a decapeptide [αS1-casein (f91–100) termed α-casozepine], displayed anxiolytic-like effects in rats [42, 43] and improves sleep in rats subjected to chronic mild stress [44]. In healthy human volunteers, this tryptic hydrolysate showed anxiolytic-like effects, without side effects, on the basis of hemodynamic parameters under mentally and physically stressful situations [45]. A recent study showed that a 30-day ingestion of αS1-casein tryptic hydrolysate decreased stress-related symptoms in women, particularly in digestive, cardiovascular, intellectual, emotional and social problems [46]. Thus, considering the deleterious link between stress, anxiety and sleep, we hypothesized that this αS1-casein hydrolysate might also prevent or reduce the effects of stressful environmental conditions on sleep. Good sleep quality has been associated with better physical health [47, 48] and greater psychological well-being [49, 50]. Therefore, factors that affect sleep quality could also influence the general well-being of individuals. Consequently, the aim of the present study was to investigate whether the anxiolytic effect of CTH (i.e. the tryptic hydrolysate of bovine αS1-casein) was accompanied by a proportional and concomitant improvement in sleep quality. For this purpose, a population of full-time job Japanese daytime working adults complaining for insomnia was given CTH over an extended period of 28 consecutive days. The evolution of sleep parameters were evaluated using the Japanese version of the Pittsburgh Sleep Quality Index (PSQI-J) [51, 52].

METHODS

Subjects

A total of 44 subjects from the pool of candidates of registered persons of Institute of General Health Development Co., Ltd. (Shiba Palace Clinic, Tokyo, Japan) participated in the present study.

The subjects were healthy Japanese men and women aged 25-40 and had reported sleeping disorders.

A medical doctor assessed medical history and sleeping disorders on the basis of an interview and a completed health and lifestyle questionnaire. The study was approved by a non-dependent ethics committee in accordance with the Helsinki Declaration of 1975 as revised in 1983 and corrected at the Edinburgh general meeting in 2000.

Following soul of the Welfare ministerial ordinance No.28 dated 27th March 1997 "Ministerial ordinance about General standard on Clinical test implementation for Pharmaceuticals", this test shall aim at reservation of a subject's human right's and safety and reliability of the test data.

All subjects gave their oral and written informed consent to participate.

Were included, only subjects who were conscious of sleeping disorders, worked more than 4 days a week and sensed stressful in jobs, can enter in self-diagnoses paper and other documents, came to appointed institution on the visit-to-the-hospital scheduled day, scored more than 5 in PSQI-J and relatively lower.

Were not included: those who are taking or who have taken within the past month hypnotic or sleep drugs, eat usually health food (teanin) considerably influencing the test result, under pregnancy or those who may be pregnant, and persons under breast-feeding, those who take more than 10 cups of green tea, coffee, tea and so on in a day totally (approx. 2L), those who are afraid to cause allergic symptoms (milk products), those who attend other clinical test, those who have a case history of critical liver obstacle, kidney obstacle and myocardial infarction, those who drink every day (quantity equivalent to one big bottle of beer (633 ml), one unit of Japanese sake (180 ml), one unit of white distilled liquor (180 ml) or more, those who scored high in HAD scale (more than 8 points in HAD anxiety subscale) and those who get under 4 points in PSQI-J total score .

In total, 44 male and female subjects signed the informed consent. Of the eligible subjects, 32 subjects (25 women and 7 men) of which 20 consumed the test product and 12 consumed the placebo, were included on the basis of their anxiety HAD-subscale score (cut-off point 7/8) and their PSQI total score (> 4 points, indicating sleep disorder). The Japanese version of HAD scale is a sensitive and specific tool for screening for psychological distress in Japanese patients. The validity and reliability of the Japanese version of HAD scale have been confirmed previously [53, 54]. The anxiety subscale of HAD also gave high sensitivity and specificity at the cut-off point 7/8. This result supports the previous report [55].

Tested Products

The tested products were alpha-s1 casein hydrolysate (CTH) (lactium[TM], supplied by INGREDIA, Arras, France) and placebo (CBC Co., Ltd., Japan).

The content per capsule of the two products was shown in the Table **1**.

Both products were administered in exactly the same capsule form containing either CTH or placebo.

Table 1.   Composition of CTH and Placebo Per Capsule

CTH	Placebo
Protein: 112.50 mg	Dextrin: 142.35 mg
Gelatin (protein): 53.11 mg	Gelatin (protein): 53.11 mg
Minerals: 22.50 mg	Moisture: 16.64 mg
Moisture: 17.39 mg	Titanium dioxide: 0.76 mg
α_{s1}-casein f91-100: 2.70 mg	Ash: 0.15 mg
Fat: 1.50 mg	
Titanium dioxide: 0.76 mg	
Lactose: 0.75 mg	

All the test products were consumed and therefore compliance for study substance intake was considered 100%. The subjects completed the forms and consumed the test substances as planned. No serious adverse events were reported.

Experimental Design

The study was designed as a double-blind, controlled, parallel study of five weeks in total, including a follow-up period of one week. In the follow-up week we evaluated if parameters returned to baseline values. This study, conducted in the Shiba Palace Clinic, Tokyo, Japan, was intended to carry out evaluation of efficacy on sleeping-disorder improvement of CTH using the PSQI-J.

Between Day 1 and Day 28, the four-week study period, each subject daily ingested one capsule 1 hour before going to bed together with cold or warm water.

The subjects completed the PSQI-J, before treatment intake (D0), after two weeks (D14), after four weeks (D28) and one week after the follow-up week (D35).

The PSQI is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Nineteen individual items generate seven component scores: sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores for these seven components (range of score 0-3) allows to determine a global score of subjective sleep quality.

Statistics

Only the data from subjects who completed the entire PSQI-J at the four sessions were used for the present analy-

sis. All raw data were delivered on paper and entered using a double data-entry.

The Statview statistical software package V5.0 (SAS institute Inc., USA) was used for statistical analysis. Comparisons between the parameters of the two groups were performed using the Mann-Whitney U-test. The repeated measures were investigated using the Friedman's test. If the Friedman indicated a treatment effect (P<0.05), comparisons between D0 and D14, D28 and D35 were performed using Wilcoxon rank test. Since the probability of a type I error is increased with multiple comparisons, we used the Bonferroni inequality formula to adjust our alpha level. We divided an initial type I error rate of 0.05 by 6 (the number of independent comparisons per group) and set our level of statistical significance at P=0.008.

RESULTS

PSQI Total Score

As shown in Table **2**, the Mann-Whitney U-test did not show significant differences between CTH and Placebo groups in PSQI-J total scores at D0 (U=85; NS), D14 (U=86.5; NS), D28 (U=98.5; NS) and D35 (U=99.5; NS). In CTH group, the Friedman's test showed a significant difference between the three test sessions ($\chi2_{(3df)}$=30.25; P<0.0001) and significant improvements in PSQI-J total scores were observed using Wilcoxon rank test at D14 (z=2.63; P=0.008), D28 (z=3.39; P=0.0007) and D35 (z=3.22; P=0.001) compared with D0. In Placebo group, the Friedman's test showed a significant difference between the three test sessions ($\chi2_{(3df)}$=16.43; P<0.0001), however no significant difference was observed at D14 (z=2.11; NS) and at D28 and D35, the PSQI-J total scores only tended to be lower than that of D0.

Sleep Quality

As shown in Table **3**, the Mann-Whitney U-test did not show significant differences between the sleep quality scores of CTH and Placebo groups at D0 (U=110.5; NS), D14 (U=108.5; NS), D28 (U=110; NS) and D35 (U=108.5; NS). In CTH group, the Friedman's test showed a significant difference between the three test sessions ($\chi2_{(3df)}$=25.31; P<0.0001) and significant improvements in sleep quality scores were observed using Wilcoxon rank test at D14 (z=3.61; P=0.0003), D28 (z=3.50; P=0.0005) and D35 (z=3.15; P=0.002) compared with D0. In Placebo group, the Friedman's test showed a significant difference between the three test sessions ($\chi2_{(3df)}$=11.48; P<0.009), however no significant difference was observed in sleep quality scores at

Table 2.   Effects of CTH on PSQI Total Score (Median with IQR Values)

Test Session Treatment	D0	D14	D28	D35
CTH (n=20)	9 (6.5-11.5)	7* (5-9)	5.5*** (3.5-7.5)	5.5** (4-7.5)
Placebo (n=12)	7.5 (6-8)	5.5 (4-7.5)	5^T (2.5-7)	5^T (3.5-6.75)

Wilcoxon rank test: [T]P<0.10; *P<0.05; **P<0.01; ***P<0.005 (*vs.* D0). Alpha level was adjusted using the Bonferroni inequality formula. IQR: interquartile range.

Table 3.   Effects of CTH on Sleep Quality (Median with IQR Values)

Test Session Treatment	D0	D14	D28	D35
CTH (n=20)	2 (2-2)	1*** (1-2)	1*** (1-1.5)	1** (1-2)
Placebo (n=12)	2 (2-2)	1.5 (1-2)	1 (1-2)	1.5 (1-2)

Wilcoxon rank test: **P<0.01; ***P<0.005 (*vs.* D0); Alpha level was adjusted using the Bonferroni inequality formula. IQR: interquartile range.

D14 (z=2.12; NS), D28 (z=2.33; NS) and D35 (z=2.12; NS) in comparison with that of D0.

Sleep Latency

As shown in Table **4**, the Mann-Whitney U-test did not show significant differences between the sleep latency scores of CTH and Placebo groups at D0 (U=103; NS), D14 (U=82.5; NS), D28 (U=88.5; NS) and D35 (U=86.5; NS). In CTH group, the Friedman's test showed a significant difference between the three test sessions ($\chi2_{(3df)}$=18.91; P<0.0003) and significant improvements in sleep latency scores were observed using Wilcoxon rank test at D28 (z=3.09; P=0.002) and D35 (z=2.68; P=0.008) compared with D0. No difference was observed at D14 (z=1.30; NS). In Placebo group, the Friedman's test showed a significant difference between the three test sessions ($\chi2_{(3df)}$=12.75; P<0.005), however no significant difference was observed in sleep latency scores at D14 (z=1.93; NS) and D35 (z=2.16; NS) in comparison with that of D0. The sleep latency score obtained at D28 tended to be lower than that of D0 (z=2.39; P=0.017).

Sleep Duration

The Mann-Whitney U-test did not show significant differences between the sleep duration scores of CTH and Placebo groups at D0 (U=76.5; NS), D14 (U=79.5; NS), D28 (U=94.5; NS) and D35 (U=100.5; NS). In CTH group, the Friedman's test showed that the sleep duration scores of the three test sessions tended to be different ($\chi2_{(3df)}$=7.44; P<0.06); however, even the sleep duration scores decreased at D28 (z=2.18; P=0.03) and D35 (z=2.14; P=0.03) compared with that of D0, the differences were not significant. In Placebo group, the Friedman's test did not show significant differences between the sleep duration scores of the three test sessions ($\chi2_{(3df)}$=0.60; P=0.90).

Habitual Sleep Efficiency

The Mann-Whitney U-test did not show significant differences between the sleep duration scores of CTH and Placebo groups at D0 (U=93; NS), D14 (U=102; NS), D28 (U=108; NS) and D35 (U=99; NS). In CTH group, the Friedman's test showed significant differences in habitual sleep efficiency scores of the three test sessions ($\chi2_{(3df)}$=11; P<0.01); however, even the sleep duration scores decreased at D28 (z=2.27; P=0.02) and D35 (z=2.27; P=0.02) compared with that of D0, the differences were not significant. In Placebo group, the Friedman's test did not show significant differences between the sleep efficiency scores of the three test sessions ($\chi2_{(3df)}$=3; P=0.39).

Sleep Disturbances

The Mann-Whitney U-test did not show significant differences between the sleep duration scores of CTH and Placebo groups at D0 (U=108; NS), D14 (U=115.5; NS), D28 (U=102; NS) and D35 (U=104; NS). In CTH group, the Friedman's test showed significant differences in sleep disturbances scores of the three test sessions ($\chi2_{(3df)}$=17.40; P<0.0006) and a significant improvement in sleep disturbances score was observed using Wilcoxon rank test at D35 (z=3.16; P=0.002). At 14 and D28, the Wilcoxon test showed that sleep disturbances scores tended to be improved compared with D0 (z=2.24; P=0.03 and z=2.45; P=0.01, respectively). In Placebo group, the Friedman's test did not show significant differences between the sleep disturbances scores of the three test sessions ($\chi2_{(3df)}$=5; P=0.17).

Daytime Dysfunction

The Mann-Whitney U-test did not show significant differences between the sleep duration scores of CTH and Placebo groups at D0 (U=111.5; NS), D14 (U=104.5; NS), D28 (U=105; NS) and D35 (U=110; NS). In CTH group, the Friedman's test showed significant differences in daytime

Table 4.   Effects of CTH on Sleep Latency (Median with IQR Values)

Test Session Treatment	D0	D14	D28	D35
CTH (n=20)	3 (1-3)	2 (1-3)	1* (1-2)	2* (1-2)
Placebo (n=12)	2 (1.5-3)	1 (1-2)	1 T (0-2)	1.5 (1-1.5)

Wilcoxon rank test: TP<0.10; *P<0.05 (*vs.* D0); Alpha level was adjusted using the Bonferroni inequality formula. IQR: interquartile range.

dysfunction scores of the three test sessions ($\chi2_{(3df)}$=11.26; P<0.01) and a significant improvement in daytime dysfunction score was observed using Wilcoxon rank test at D28 (z=2.89; P=0.004). At 14 and D35, the daytime dysfunction scores decreased compared with D0 (z=2.12; P=0.03 and z=1.89; P=0.06, respectively). However, the differences were not significant. In Placebo group, the Friedman's test showed significant differences between the daytime dysfunction scores of the three test sessions ($\chi2_{(3df)}$=7.80; P=0.05), however, at D14, D28 and D35, no significant improvement was observed in comparison with D0 (z=0.82; P=0.41; z=1.67; P=0.10 and z=2.12; P=0.03, respectively).

DISCUSSION

Sleep is a physical and mental resting state in humans. It is a vital biological function, necessary for physical and emotional well-being. Sleep problems, particularly insomnia, rapidly induce impairments in many areas of physical, intellectual and affective aspects of life [8]. Concerning the causes of insomnia, the attention was drawn toward hyperarousal [32] which is central to modern models of primary insomnia [56]. It is assumed that hyperarousal in various areas (i.e. cognitive, emotional, neuroendocrine or cardiovascular,…) can be induced in vulnerable individuals by acute psychological/psychosocial stressors [57, 58]. The insomnia would be connected with excessive and continual excitement of the central nervous system. A study reveals that a person suffering from insomnia is in permanent mode of reacting to stress [59].

Given that hyperarousal may be induced by stress, the purpose of this study was to investigate whether oral intake of αS1-casein hydrolysate would improve various aspects of sleep in stressed Japanese insomniac male and female subjects.

In several cultures, drinking a glass of warm milk before going to bed is believed to facilitate sleep. Indeed, even if many of these reports are only anecdotal, several milk components, like tryptophan (precursor of serotonin, a neurotransmitter involved in sleep induction), can act upon sleep and magnesium [60, 61]. More recently, a lot of bioactive peptides released during enzymatic digestion of milk proteins have revealed various interesting properties like calcium bio-transfer activity [62], opiate activity [63], immunomodulating activity [64, 65], anti-hypertensive activity [66] and anti-thrombotic activity [67]. CTH, a milk αS1-casein tryptic hydrolysate, has shown interesting properties (reduction of systolic blood pressure reaction and of cortisol increase) in healthy human volunteers facing mental and physical stress situations [45]. Moreover, this hydrolysate has shown an anxiolytic-like profile in rats, in two classical tests: the conditioned defensive burying (CDB) test and the elevated plus maze [43]. These properties of CTH seem to be in connection with a decapeptide, the αs1-casein-(f91-100) [42] that has been spatially modelled [68].

The results of the study provided evidence that the chronic administration of CTH during a 4-week period had significant effects on various aspects of sleep, as evaluated by the Japanese version of Pittsburgh Sleep Quality Index (PSQI-J) [52]. Opposite to placebo-treated subjects, who never reported significant improvements of their symptoms, the subjects treated with CTH showed a significant decrease of the PSQI-J total score after 14 days of treatment. This improvement was greater after 28 days of treatment, and was still perceptible one week after the cessation of the treatment. These effects reinforce the results obtained on some sleep parameters in rats [44].

Concerning the PSQI-J detailed components, the best improvement due to CTH was observed for the sleep quality score. Indeed, after two weeks of treatment, the seriousness of this symptom was significantly decreased, and this improvement remained for the three following weeks. The sleep latency was also significantly reduced after four weeks of CTH treatment, as for the daytime dysfunction. The sleep disturbances only indicated a statistical trend for improvement after a 28-day period of treatment and a significant improvement at D35.

The comparisons between the two groups with the test of Mann-Whitney did not show significant differences, probably because of the control product's placebo effect. Despite everything, the paired comparisons with the test of Wilcoxon show interesting effects of CTH on sleep disorders of the treated subjects.

At present, the exact mechanism of action of CTH on sleep disorders is not known. The tryptic hydrolysate has been shown to exhibit a benzodiazepine-like activity probably due to its affinity to the GABA-A receptor, without displaying side effects [42]. Given the anti-stress properties of CTH, it seems possible to relate the detected improvement of sleep aspects to a reduction of stress following the chronic administration of the treatment. Further studies will be necessary in order to clarify this essential aspect of CTH properties on sleep. While waiting, to replace the therapeutic agents currently used as aids to sleep by the Japanese, in other words the pharmacological hypnotic-medication and alcohol [69], it would be more advantageous to use CTH because of its beneficial effects and its lack of negative side effects.

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