Report

Assessment of Acute Adverse Events of Glycine Ingestion at a High Dose in Human Volunteers

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Abstract

We found in our previous study that glycine ingestion (3 g) before bedtime significantly produced subjective sleep effects in volunteers who had been continuously experiencing unsatisfactory sleep. Further, a series of published reports has shown that the safety of glycine is relatively high. The present study was conducted to assess acute adverse events or a daytime sleepiness effect after using a 3-fold higher dose (9 g) than the previous study. The results indicated that 9 g doses of glycine produced changes in several clinical test parameters, which were however within the range of physiological normal variation, and changes in blood level of some amino acids, which also were considered to be within or very close to the range of physiological normal variation. Digestive symptoms occurring only after the bedtime ingestion were observed; though these possibly were adverse events, they were not serious. Further, glycine did not produce daytime sleepiness. It could be concluded that 9 g of glycine produced neither acute serious adverse events nor a daytime sleepiness carry-over effect.

Keywords: amino acid, glycine, excessive ingestion, acute adverse events, daytime sleepiness

Ⅰ. Introduction

A previous study on volunteers who had been continuously experiencing unsatisfactory sleep indicated that glycine ingestion (3 g) before bedtime significantly produced subjective sleep effects [1]. With regard to its safety, no serious side effects have been noted either in healthy participants ingesting 30 g/day of glycine [2] or schizophrenic patients receiving oral glycine at doses between 30 to 60 g/day/person for 2-12 weeks [3-7]. Also, the pharmacokinetic parameters Tmax and Cmax of glycine in healthy volunteers (N = 9) ingesting glycine at doses from 3.6 to 5.4 g/person were calculated to be 40 min and 909 μmol/L, respectively [8]. Glycine is absorbed rapidly and eliminated within several hours after ingestion as are other amino acids. Based on such a series of published reports, it has been shown that the safety of glycine is relatively high.

Because of a new finding on the subjective effects of glycine on the sleep quality, the safety of glycine particularly with regard to acute adverse events and a daytime sleepiness carry-over effect were assessed using a 3-fold higher dose (9 g) than the previous study.

Ⅱ. Materials and Methods

Twelve healthy volunteers [6 females and 6 males, 25-38 years old (mean = 34), engaged in work during the daytime] participated in the study. These volunteers received instructions before the start of the study to maintain their

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usual lifestyle, to avoid ingestion of additional drugs or supplements as much as possible, and not to eat or drink after 21:00 on a day before blood and urine sampling.

The study protocol was an open trial for addressing the safety problems as quickly as possible if they occurred. As a control condition for the glycine ingestion condition, reduced malt sugar with the same flavor as glycine was ingested. The total period of the study (17 days) consisted of a pre-glycine ingestion period (7 days, including the reduced malt sugar ingesting period), a glycine ingestion period (7 days) and a post-glycine ingestion period (3 days). Reduced malt sugar was ingested before bedtime during the last 3 days of the pre-glycine ingestion period. Glycine was ingested for 7 days, the same as the ingestion period in the previous study [1], in 3 different ways to examine whether dosage timing has an influence on the occurrence of acute adverse events. For the first 2 days, 3 g of glycine was ingested 30 min after each meal (every-meal timing), for the following 2 days, 9 g of glycine was ingested 30 min after either breakfast or lunch (once-in-daytime timing), and for the last 3 days, 9 g of glycine was ingested within 1 hr before bedtime (once-at-bedtime timing). No dietary restriction was imposed. Blood and urine sampling were conducted at the start of the test period for the purpose of screening, on the day after the last reduced malt sugar ingestion (before glycine ingestion) and on the day after the last glycine ingestion (after glycine ingestion).

The following clinical laboratory data were collected for analysis. Levels of free amino acids in the blood were measured before and after glycine ingestion for comparison. Also, hematological examination [examination items: white blood cell (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb), hematocrit (Ht), platelet count (Plt) and WBC picture], biochemical examination [examination items: total bilirubin (T-Bil), direct bilirubin (D-Bil), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), choline esterase (ChE), glucose, total protein (TP), albumin (ALB), albumin/globulin ratio (A/G ratio), urea nitrogen (UN), creatinine (CREA), uric acid (UA), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (IP), total cholesterol (T-Cho), high density lipoprotein-cholesterol (HDL-Cho), low density lipoprotein-cholesterol (LDL-Cho) and triglyceride (TG)] and urinalysis [examination items: pH, specific gravity, urinary protein, glucose, occult blood, urobilinogen, bilirubin, urinary sediment, urinary creatinine (urinary CREA) and urinary urea nitrogen (urinary UN)] were also conducted before and after glycine ingestion for comparison. In addition to these laboratory examinations, the participants kept diaries describing their subjective symptoms everyday during the study period, and clinical interviews by the supervising physician were carried out 4 times: at the start of the study, just before and after the glycine ingestion period, and in the post-glycine ingestion period. The medical procedures including the clinical interview and blood and urea sampling were performed at the Kawagoe clinic of BML, Inc., while measurement of laboratory data was conducted at the Kawagoe laboratories of BML, Inc.

The presence of a sleepiness carry-over effect in the daytime was assessed with the questionnaire for fatigue complaints ("Jikaku-sho Shirabe") every day during the test period. This questionnaire ("Jikaku-sho Shirabe") for participants' self-rating assesses daytime experience of sleepiness, languidness, mood instability, blur, and unpleasant sensations [9].

The data obtained before and after glycine ingestion were analyzed with a paired t-test, while for the assessment of daytime sleepiness, three-way [glycine ingestion / ingestion conditions / ingestion period] ANOVA was performed. Statistical significance was set to be p value <0.05.

The study protocol was approved by the Institutional Review Boards of Ajinomoto Co., Inc. and of Tomisaka Clinic. Written informed consent for participating in the study was obtained from all of the participants after they were given an explanation of the study and its potential risks. All of the procedures were carried out in accordance with "Good Clinical Practice", the Helsinki Declaration, and related laws.

Ⅲ. Results and Discussion

Table 1 shows blood amino acids levels determined before and after ingestion of glycine. Amino acids observed

to have significant differences in blood level before and after glycine ingestion were lysine, histidine, phenylalanine, tryptophan, valine, isoleucine, leucine, asparagine, glutamic acid, glutamine, serine, glycine, alanine, tyrosine, cystine, ornithine, and β-alanine. These changes were, however, within or very close to the physiological normal range, which was determined based on statistically processed data obtained from healthy normal volunteers at the Kawagoe laboratories of BML, Inc. Large deviation from the physiological normal range was not observed. These medical judgments were supported by a supervising physician.

Table 2 shows results of hematological examination, biochemical examination and urinalysis. The items exhibiting significant changes between before and after glycine ingestion were WBC, MCV, MCH, albumin, A/G ratio, Na, and UN. These changes were, however, within the physiological normal ranges, which were determined at the Kawagoe laboratories of BML, Inc. These medical judgments were supported by a supervising physician.

Table 3 shows adverse events. Only one of the female participants had menstruation during the testing, and the irregular bleeding in Table 3 corresponded to her menstrual period. Adverse events considered to have a possible causal association with ingestion of glycine were digestive symptoms such as soft stool and abdominal pain. These events were not serious, and were observed only in a case of bedtime timing. The continued ingestion of glycine (9 g) was possible. The cause of these digestive symptoms may be that excessive amino acid (9 g dose of glycine) ingested in comparatively empty digestive organs produced a transient high osmotic pressure state. These medical judgments were supported by a supervising physician.

Fig. 1 shows results of the assessment of daytime sleepiness. Three-way ANOVA shows no significant effects of any of the three factors glycine ingestion, ingestion conditions, and ingestion period on daytime sleepiness. The

Table 1 Changes in blood amino acids levels determined before and after glycine ingestion

	normal range (n mol/mL)	Before glycine ingestion (Mean±SD)	After glycine ingestion (Mean±SD)	statistical difference
Lysine	125.7 ~ 281.9	237.88 ± 76.60	202.51 ± 48.91	*
Histidine	63.0 ~ 105.2	103.37 ± 16.12	86.63 ± 10.34	**
Phenylalanine	43.5 ~ 79.8	78.92 ± 7.41	67.91 ± 10.36	**
Tryptophan	36.2 ~ 79.3	61.09 ± 6.83	53.16 ± 7.07	**
Valine	156.2 ~ 360.4	248.99 ± 58.31	213.01 ± 37.89	*
Isoleucine	37.0 ~ 100.4	72.35 ± 16.83	60.61 ± 10.90	**
Leucine	74.2 ~ 169.1	146.94 ± 31.58	120.61 ± 20.03	**
Methionine	15.5 ~ 38.6	29.60 ± 4.36	26.65 ± 4.05	
Threonine	74.2 ~ 216.1	145.44 ± 41.86	118.95 ± 24.85	
Aspartic acid	trace ~ 7.2	4.50 ± 1.74	4.12 ± 1.43	
Asparagine	43.8 ~ 90.6	58.76 ± 7.42	51.81 ± 8.35	*
Glutamic acid	12.2 ~ 82.7	58.71 ± 20.00	46.45 ± 17.29	*
Glutamine	418.0 ~ 739.8	621.33 ±103.73	527.55 ± 63.31	**
Serine	91.5 ~ 186.4	132.80 ± 17.46	174.69 ± 32.55	**
Proline	71.3 ~ 373.0	163.67 ± 49.44	145.89 ± 52.24	
Glycine	140.4 ~ 427.3	261.20 ± 43.87	395.76 ± 74.09	***
Alanine	258.8 ~ 615.2	459.74 ±116.69	400.94 ± 90.21	*
Tyrosine	38.4 ~ 89.4	75.53 ± 11.74	61.81 ± 9.41	***
Arginine	31.8 ~ 149.5	85.28 ± 25.33	73.35 ± 25.29	
Taurine	48.4 ~ 128.2	111.13 ± 14.56	94.85 ± 18.75	
Citrulline	17.9 ~ 48.0	32.10 ± 8.34	29.01 ± 5.89	
α-Amino-*n*-butyric acid	8.1 ~ 31.0	26.69 ± 5.51	25.39 ± 4.14	
Cystine	4.7 ~ 34.8	29.26 ± 8.47	34.08 ± 8.45	**
Ornithine	42.6 ~ 141.2	91.17 ± 26.61	75.76 ± 14.88	*
Hydroxyproline	trace ~ 18.8	12.33 ± 7.01	12.42 ± 0.50	
3-Methylhistidine	trace ~ 8.2	5.12 ± 5.32	2.55 ± 0.73	
Ethanolamine	trace ~ 10.5	4.19 ± 1.08	3.40 ± 0.51	
β-Alanine	trace ~ 11.8	3.35 ± 0.74	2.50 ± 0.59	*
1-Methylhistidine	<9.1	3.40 ± 0.28	14.55 ± 10.25	
β-Amino-*iso*-butyric acid	<5.9	3.81 ± 1.23	3.76 ± 1.39	

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, paired t-test

Table 2 Changes in clinical laboratory examination data before and after glycine ingestion

	normal range	Before glycine ingestion (Mean±SD)	After glycine ingestion (Mean±SD)	statistical difference
WBC	3500 ~ 9700 /μL	5612.50 ±1630.29	5116.67 ±1484.31	*
RBC	438 ~ 577 ×10000/μL	462.75 ± 36.06	453.25 ± 49.22	
Hb	13.6 ~ 18.3 g/dL	13.97 ± 1.46	13.86 ± 1.57	
Ht	40.4 ~ 51.9 %	43.59 ± 3.51	42.96 ± 4.20	
MCV	83 ~ 101 fL	94.33 ± 4.01	95.00 ± 4.22	*
MCH	28.2 ~ 34.7 pg	30.18 ± 2.09	30.63 ± 2.07	*
MCHC	31.8 ~ 36.4 %	32.00 ± 1.10	32.22 ± 1.02	
Plt	14.0 ~ 37.9 ×10000/μL	24.45 ± 2.79	25.54 ± 5.03	
WBC picture				
Baso	0 ~ 2 %	0.67 ± 0.40	0.73 ± 0.37	
Eosino	0 ~ 7 %	2.57 ± 1.66	2.91 ± 2.16	
Lympho	18 ~ 50 %	34.78 ± 8.56	36.02 ± 11.64	
Mono	1 ~ 8 %	5.83 ± 1.76	5.29 ± 1.19	
Neut	42 ~ 74 %	56.17 ± 9.33	55.06 ± 13.32	
EBL	0 /100WBC	0.00 ± 0.00	0.00 ± 0.00	
TP	6.5 ~ 8.2 g/dL	7.38 ± 0.33	7.17 ± 0.42	
ALB	3.7 ~ 5.5 g/dL	4.43 ± 0.22	4.64 ± 0.37	**
A/G ratio	1.30 ~ 2.00	1.51 ± 0.16	1.87 ± 0.32	***
AST(GOT)	10 ~ 40 U/L	18.67 ± 3.11	19.92 ± 4.12	
ALT(GPT)	5 ~ 45 U/L	18.92 ± 10.01	20.08 ± 12.57	
LDH	120 ~ 245 U/L	165.58 ± 12.15	160.42 ± 14.48	
ALP	104 ~ 338 U/L	187.83 ± 43.39	186.17 ± 52.14	
γ-GTP	16 ~ 73 U/L	25.42 ± 10.51	25.67 ± 11.17	
ChE	3500 ~ 8000 U/L	4987.67 ±1044.39	4967.42 ±1180.23	
T-Bil	0.2 ~ 1.0 mg/dL	0.68 ± 0.21	0.89 ± 0.57	
D-Bil	0.0 ~ 0.4 mg/dL	0.20 ± 0.07	0.28 ± 0.19	
CREA	0.65 ~ 1.09 mg/dL	0.75 ± 0.10	0.76 ± 0.10	
urinary CREA		1.89 ± 0.87	1.68 ± 0.86	
UN	8 ~ 20 mg/dL	12.13 ± 3.31	14.44 ± 4.10	**
urinary-UN		8.33 ± 3.47	8.92 ± 3.32	
UA	7.0< mg/dL	5.25 ± 1.14	5.05 ± 1.29	
T-Cho	150 ~ 219 mg/dL	188.08 ± 26.72	185.50 ± 21.13	
HDL-Cho	40 ~ 80 mg/dL	65.92 ± 15.24	64.25 ± 14.10	
LDL-Cho	70 ~ 139 mg/dL	115.17 ± 28.79	111.67 ± 21.22	
TG	50 ~ 149 mg/dL	79.00 ± 41.06	76.33 ± 40.50	
Na	135 ~ 145 mEq/L	139.75 ± 1.42	138.75 ± 1.36	*
K	3.5 ~ 5.0 mEq/L	4.08 ± 0.21	4.12 ± 0.17	
Cl	98 ~ 108 mEq/L	102.42 ± 1.88	102.83 ± 1.70	
Ca	8.2 ~ 10 mg/dL	9.39 ± 0.22	9.36 ± 0.44	
IP	2.5 ~ 4.5 mg/dL	3.65 ± 0.41	3.64 ± 0.57	
blood glucose (hungry)	70 ~ 109 mg/dL	87.67 ± 7.82	86.58 ± 8.54	
urinary protein	(−)or(+−)	(−) n=12	(−) n=12	
urinary glucose	(−)or(+−)	(−) n=12	(−) n=12	
specific gravity	1.008 ~1.034	1.021 ± 0.008	1.021 ± 0.008	
urinary pH	4.8 ~ 7.5	5.83 ± 0.54	6.00 ± 0.64	
urinary urobilinogen	(+−)	(+−) n=12	(+−) n=12	
urinary bilirubin	(−)	(−) n=12	(−) n=12	
urinary occult blood	(−)	#3,#4	#3	
urinary sediment	(−)	−	−	

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, paired t-test
#: participant's number

Table 3 Adverse events observed

Partici-pant No.	Period of symptom	Symptom reported by subject	Specific time of symptom	Measures taken (treatment of symptom etc.)	Ingestion of test food (continued/ discontinued)	Outcome	Date and hour of outcome	Severity	Serious-ness	Causal link with glycine
3	Whole study	Irregular bleeding from genital organ	from June 9 onward	None	Continued	No recovery	–	Mild	No	None
4	Whole study	Stomach ache (like pressure was applied)	from 3 weeks before starting the study (since June 9)	Since June 23, a capsule of Gastol fine granules was ingested every morning & night	Continued	No recovery	–	Inter-mediate	No	None
	glycin ingestion	Soft stool	Bowel movement the morning after ingesting 3 capsules at bedtime from June 27 to 29 (every morning)	None	Continued	Recovered	June 30, morning	Mild	No	Yes
6	pre-glycine	Diarrhea (soft stool only)	Starting from several days before June 23	None	Continued	Recovered	June 21	Mild	No	None
7	glycin ingestion	Abdominal pain	After ingestion of 3 capsules at bedtime to the next morning on riseing from June 27 to 29 (each time)	None	Continued	Recovered	June 30, morning	Mild	No	Yes
	glycine ingestion	Soft stool	Bowel movement the morning after ingesting 3 capsules at bedtime from June 27 to 29 (every morning)	None	Continued	Recovered	June 30, morning	Mild	No	Yes
9	post-glycine	Abdominal pain	July 1	None	Continued	Recovered	July 1, AM	Mild	No	None
10	pre-glycine	Cold	June 19, 20	June 19, one Pavulon tablet after each meal. June 20, one Pavulon tabulet in the morning	Continued	Recovered	June 20	Inter-mediate	No	None
	post-glycine	Diarrhea (waterly stool)	In the morning and at noon (2 times) on July 1 Once in the morning on July 2	None	Continued	Recovered	July 2, daytime	Mild	No	None

Study period: pre-glycineingestion period June 16-June 22, 2005, glycine ingestion period June 23-June 29, 2005, post-glycine ingestion period June 30-July 3, 2005

present results suggest that 9 g of glycine produced neither daytime sleepiness on the ingestion day nor a carry-over effect to the next day.

The results of the present study are summarized as follows: 1) 9 g of glycine produced changes in several variables of clinical laboratory tests which were however within the range of physiological normal variation and also produced changes in the blood level of some amino acids which were however within or very close to the range of physiological normal variation. 2) Digestive symptoms occurring only during the once-at-bedtime timing were possible adverse events, but were not serious. 3) 9 g of glycine did not produce daytime sleepiness.

Based on the present results using a 3-fold higher dose (9 g) of glycine than the dose which produced significant effect on subjective sleep feeling and a series of published reports on the safety of glycine [2-7], it may be concluded that 3 g of glycine would produce neither acute serious adverse events nor a carry-over effect to daytime sleepiness.

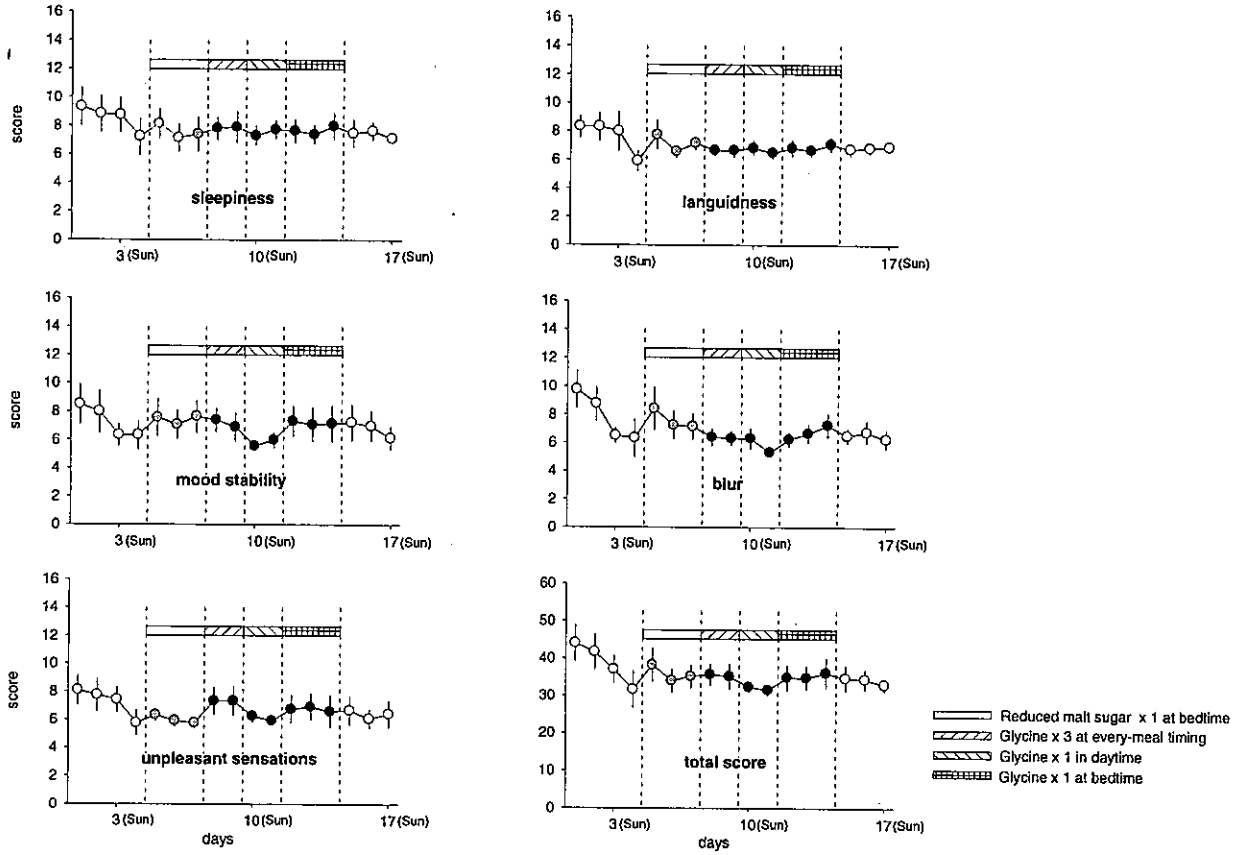

Fig. 1 Effects of glycine (9 g) on daytime sleepiness as seen in scores of the questionnaire for fatigue complaints ("Jikaku-sho Shirabe")
Data expressed as mean scores of questionnaires ± standard error.

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