

## Regular Article

# Pyridoxamine: A novel treatment for schizophrenia with enhanced carbonyl stress

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**Aim:** The aim of this clinical trial was to obtain proof of concept for high-dose pyridoxamine as a novel treatment for schizophrenia with enhanced carbonyl stress.

**Methods:** Ten Japanese schizophrenia patients with high plasma pentosidine, which is a representative biomarker of enhanced carbonyl stress, were recruited in a 24-week, open trial in which high-dose pyridoxamine (ranging from 1200 to 2400 mg/day) was administered using a conventional antipsychotic regimen. Main outcomes were the total change in Positive and Negative Syndrome Scale score and the Brief Psychiatric Rating Scale score from baseline to end of treatment at week 24 (or at withdrawal).

**Results:** Decreased plasma pentosidine levels were observed in eight patients. Two patients showed marked improvement in their psychological symptoms. A patient who harbors a frameshift mutation in the *Glyoxalase 1* gene also showed

considerable reduction in psychosis accompanied with a moderate decrease in plasma pentosidine levels. A reduction of greater than 20% in the assessment scale of drug-induced Parkinsonism occurred in four patients. Although there was no severe suicide-related ideation or behavior, Wernicke's encephalopathy-like adverse drug reactions occurred in two patients and were completely suppressed by thiamine supplementation.

**Conclusion:** High-dose pyridoxamine add-on treatment was, in part, effective for a subpopulation of schizophrenia patients with enhanced carbonyl stress. Further randomized, placebo-controlled trials with careful monitoring will be required to validate the efficacy of high-dose pyridoxamine for these patients.

**Key words:** advanced glycation end-products, carbonyl stress, pentosidine, pyridoxamine, schizophrenia.

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**S**CHIZOPHRENIA IS A devastating disorder characterized by positive symptoms, including auditory hallucinations and delusions, and negative symptoms, including blunted affect, anhedonia, and cognitive deficits. The lifetime prevalence ranges from approximately 0.3% to 0.7%, and disease onset frequently occurs during puberty or early adolescence. Adequate psychosocial support as well as

psychopharmacologic treatment is needed to achieve and maintain recovery. Although many studies have been conducted to clarify the underlying mechanism of the disease, the main cause and pathophysiology of schizophrenia still remains elusive since there is considerable heterogeneity in symptoms, and long-term clinical courses differ substantially from patient to patient.

Carbonyl stress is an abnormal metabolic state resulting from either increased production of reactive carbonyl compounds (RCO) or decreased detoxification of RCO.<sup>1</sup> Advanced glycation end products (AGE) are generated as a consequence of elevated carbonyl stress, and numerous experimental studies in animals and humans implicate increased AGE in a variety of diseases, including diabetes mellitus,<sup>2-4</sup> chronic kidney disease,<sup>1</sup> cardiovascular diseases<sup>5</sup> and cognitive impairment with or without dementia.<sup>6-8</sup> Pyridoxamine, one of the three forms of vitamin B<sub>6</sub>, is capable of scavenging certain RCO, thereby inhibiting the formation of AGE and alleviating their unfavorable physiological effects. The other forms of vitamin B<sub>6</sub>, pyridoxine and pyridoxal, lack this therapeutic benefit. However, pyridoxamine is synthesized from both pyridoxal and pyridoxine *in vivo*, so that depleting pyridoxamine to combat enhanced carbonyl stress eventually leads to decreases in both pyridoxal and pyridoxine.

We previously reported that enhanced carbonyl stress is associated with a subpopulation of patients suffering from schizophrenia.<sup>9</sup> In addition, a close relationship between schizophrenia and carbonyl stress was also found in a larger cohort,<sup>10</sup> and a drug-naïve patient with high pentosidine levels has been reported.<sup>11</sup> Clinical features of patients with enhanced carbonyl stress are similar to those with treatment-resistant schizophrenia, and negative correlations between psychological symptoms of schizophrenia and serum pyridoxal levels have been reported.<sup>12</sup> Likewise, a recent study suggests that pyridoxal levels could be a possible biomarker to predict the clinical course in a subset of schizophrenia patients.<sup>13</sup> Clozapine is the most effective agent among various antipsychotics for patients with treatment-resistant schizophrenia.<sup>14,15</sup> However, the use of clozapine is restricted because of serious and lethal adverse effects, including granulocytopenia,<sup>16</sup> which require strict and frequent monitoring of white blood cell counts. Thus, clozapine treatment causes problems for both patients as well as health-care providers.

In contrast, clinical interventions, such as pyridoxamine supplementation, aimed at reducing carbonyl stress, may provide a safe and effective treatment for schizophrenia patients with enhanced carbonyl stress. To test this hypothesis, we conducted a 24-week, open-label clinical trial with high-dose pyridoxamine supplementation in schizophrenia patients. In this study, two patients showed marked improvement in clinical symptoms, and one patient showed considerable improvement in psychological symptoms accompanied by a moderate decrease in plasma pentosidine levels. Additionally, four participants showed improvements in drug-induced Parkinsonism and two patients experienced Wernicke's encephalopathy, which was completely alleviated following thiamine supplementation.

## METHODS

### Subjects

Inclusion criteria for this clinical trial were patients with schizophrenia or schizoaffective disorder aged 20–65 years who were hospitalized in Matsuzawa Metropolitan Hospital. Diagnoses were made by at least two experienced psychiatrists according to the DSM-IV-TR. Patients with diabetes mellitus or chronic kidney disease were excluded, since both diseases occasionally induce increased plasma pentosidine levels. Other exclusion criteria included: organic mental diseases, substance dependence, past history of substance dependence, comorbid malignant tumors, pregnancy, modified electroconvulsive therapy within 3 months, prescription to theophylline, choline theophylline, aminophylline and levodopa (which are contraindicated with pyridoxamine), and severe physical conditions that made study participation inappropriate. Eligible patients exhibited enhanced carbonyl stress defined as elevated plasma pentosidine levels according to a previous report<sup>9</sup> (>55.2 ng/mL; defined above 2 SD of healthy control subjects). In this study, serum pyridoxal levels could not be used as an inclusion criterion because the number of patients fulfilling both high pentosidine and low pyridoxal were so few that sufficient participants could not be recruited. After patient selection, 10 eligible patients were identified for this clinical trial.

### Study design

A 24-week, open-label design was adopted. Pyridoxamine was initiated with a dose of 1200 mg/day

supplementing the existing antipsychotic regimen, with the responsible doctor setting the daily dose (1200, 1800, or 2400 mg/day) during the trial. The dose was determined according to phase I clinical trials. To avoid degradation, we stored pyridoxamine in powder form in a light-blocking box and dissolved it in water and syrup (to reduce sourness) for three daily oral administrations. Treatment adherence and the general condition of all participants were carefully monitored throughout the trial period. All participants provided written informed consent and study protocols were approved by the Institutional Review Board of Tokyo Metropolitan Matsuzawa Hospital. The trial was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry, number UMIN000006398.

### Efficacy and safety assessment

Clinical efficacy and safety assessment as well as measurements of plasma pentosidine and the three forms of vitamin B<sub>6</sub> were carried out every 2 weeks for the first month and every 4 weeks for the following 5 months. Primary outcomes were improvements in the Brief Psychiatric Rating Scale (BPRS) and the Positive and Negative Syndrome Scale (PANSS) from baseline assessments scored by the same rater during the trial. The Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS) and Columbia-Suicide Severity Rating Scale were assessed to determine the severity of extrapyramidal symptoms (EPS) and the occurrence of suicide-related adverse events. We also recorded body-weight, blood pressure, and pulse rate, and measured blood count, coagulation, liver and kidney function, blood sugar, hemoglobin A1c, thyroid hormone, urine test, and electrocardiography at each visit.

### Measurement of pentosidine

Fresh plasma and serum was obtained from participants, and pentosidine was measured using high-performance liquid chromatography (HPLC), as described previously.<sup>17</sup> In brief, plasma samples were lyophilized, hydrolyzed in 100 µL 6 N hydrochloric acid for 16 h at 110°C under nitrogen, neutralized with 100 µL 5 N sodium hydroxide and 200 µL of 0.5 M sodium phosphate buffer (pH 7.4), filtered through a 0.5-µm filter, and diluted with

phosphate-buffered saline. A sample (corresponding to 25 µg of protein) was injected into an HPLC system and fractionated on a C18 reverse-phase column. Eluent was monitored at excitation-emission wavelengths of 335/385 nm using a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan). Synthetic pentosidine was used to obtain a standard curve. Three forms of vitamin B<sub>6</sub> (pyridoxine, pyridoxal, and pyridoxamine) were measured in serum samples by HPLC according to a previously described method. These measurements were conducted at SRL (Tokyo, Japan) or LSI Medience Corporation (Tokyo, Japan), which are private clinical laboratory test companies. As serum pyridoxamine and pyridoxine were below detection levels, we used serum pyridoxal levels to quantify serum vitamin B<sub>6</sub>. Other parameters (glycohemoglobin A1c, creatinine) were measured in blood samples. The glomerular filtration rate was estimated using the abbreviated Modification of Diet in Renal Diseases study equation.

### Genotype of *Glyoxalase 1* gene

Glyoxalase 1 (GLO1) is the key enzyme in the detoxification system of carbonyl compounds and dysfunction of this enzyme, due to genetic mutations, such as frameshift mutations or the Glu111Ala *GLO1* genotype (rs4746), contribute to increased carbonyl stress and high plasma pentosidine levels. We conducted genotyping of *GLO1* for all patients as described previously.<sup>9</sup> *GLO1* genotypes are shown in Table 1.

### Statistical analysis

All statistical analyses were performed using PRISM (GraphPad Software, San Diego, CA, USA). Comparisons of means and standard errors of data were performed using unpaired *t*-tests or Mann-Whitney *U*-tests (both two-tailed). Fisher's exact tests were used for categorical variables. Significance was defined as  $P < 0.05$ .

## RESULTS

Half of the patients fulfilled the rigid definition of treatment-resistant schizophrenia by Kane<sup>18</sup> and all participants showed poor social function. Baseline demographic and clinical characteristics of the participants are summarized in Table 1.

**Table 1.** Demographics of participants

Patients	A	B	C	D	E	F	G	H	I	J
Age	64	38	61	63	42	62	50	41	33	42
Sex	M	F	F	F	F	M	F	M	F	F
Pentosidine (ng/mL)	227.9	302.8	89.8	185.2	143.5	89.0	383.2	139.3	75.8	109.0
Pyridoxal (ng/mL)	2.1	6.5	5.0	10.1	2.5	3.5	6.4	4.6	12.2	7.3
HbA1C	4.9	5.0	5.0	4.6	5.2	5.2	4.7	4.8	4.9	4.9
Creatinine (mg/dL)	1.01	0.89	0.57	0.48	0.78	0.70	0.51	0.80	0.69	0.49
Smoking status	Current smoker	Never	Never	Never	Current smoker	Never	Never	Never	Never	Never
GLO1 genotype	T 27NfsX15	Glu/Glu	Glu/Glu	Glu/Glu	Glu/Glu	Glu/Glu	Glu/Glu	Glu/Glu	Glu/Ala	Glu/Glu
Clinical variables										
Family history of psychosis	Yes	No	No	No	No	Yes	Yes	No	No	Yes
Educational status (years)	9	12	12	12	12	18	12	12	12	16
Social function <sup>†</sup>	Hospitalized	Hospitalized	Hospitalized	Hospitalized	Poor	Poor	Poor	Poor	Poor	Poor
Onset (years old)	15	19	15	26	16	20	28	20	22	24
Duration of illness (years)	49	19	46	37	26	42	22	21	11	18
Number of previous hospitalizations	5	4	16	14	5	4	5	4	2	11
Total duration of hospitalization (years)	46	16	20	20	4	3	3	1	1	6
Antipsychotics (mg/day, CP equivalent)	2532	2732	1005	530	2505	441	1255	3214	2552	2636

<sup>†</sup>'Poor' defined as not having continuous work at least 1 year prior to trial.  
CP, chlorpromazine.

Trial results are summarized in Table 2. Each letter from A to J indicates an individual schizophrenia patient listed in order of participation. Patient F dropped out of the trial at day 121 because of worsening physical conditions. Thus nine patients completed the trial. Of the participants, patients C and J showed marked improvements from baseline assessments of psychotic symptoms demonstrated by BPRS and the PANSS Negative, General, and Total subscale scores (Table 2). They also both showed significant improvement in terms of rapport, expressing emotions, and frequency of participation in daily care programs. Although patient C could not walk alone unless a care-giver supported her at the beginning of the trial, she became highly motivated to participate in the walking rehabilitation program

during the trial, ultimately succeeding in walking by herself. Drastic improvements in the daily activities of these patients had not been observed in previous hospitalizations before the start of this trial. Behavioral changes were confirmed by primary care-givers, including nurses. The effective dose of pyridoxamine in patients C and J was 1200 mg/day. Patient C deteriorated immediately after the trial, whereas patient J maintained an improved psychological condition beyond the study end-point. Improvements in rapport and emotional expressions were also observed in other patients. Patient A, who harbors a *GLO1* frameshift mutation and exhibited low pyridoxal levels (which indicates a vulnerability to carbonyl stress), also showed moderate improvement of clinical symptoms assessed by BPRS and

total PANSS score accompanied with a considerable reduction in plasma pentosidine levels (Table 2). The clinical courses of patients A, C, and J are summarized in Fig. 1. Intriguingly, four patients (patients C, G, I, and J) demonstrated a >20% reduction in DIEPSS scores from baseline evaluations, indicating improvement of EPS (Table 2). Patient F also showed a marked reduction in his DIEPSS score. However, his improvement might have been caused by discontinuation of antipsychotics due to worsening physical conditions.

Decreases in plasma pentosidine were observed in eight patients, and the overall reduction (including patient F) was 26.8% (Table S1), a finding in contrast with a previous report by Katsuta *et al.*, which found a reduction of 3.0% in 137 schizophrenia patients.<sup>13</sup> Patient J showed remarkable increase of plasma pentosidine levels; however, the precise mechanism of elevated pentosidine concentration remains to be elucidated. Serum pyridoxal concentrations increased in all patients and the overall rate of increase was 345.3-fold from baseline (Table S1). In the clinical assessment, the overall reduction rates for the PANSS Positive, Negative, General, and Total subscales and the BPRS scores were 6.9%, 5.8%, 9.9%, 8.1%, and 10.8%, respectively, which did not reach statistical significance, probably due to the small number of participants (Table S1).

All adverse events occurring in this trial are summarized in Table 3. No severe suicide-related ideation or behavior was observed during the trial, as assessed by the Columbia-Suicide Severity Rating Scale. We decreased the antipsychotic doses in patients B, D, and H during the trial because of

oversedation or exacerbation of EPS. Unfortunately, severe adverse drug reactions occurred in two patients at a dose of 1800 mg/day, where clinical symptoms and course were very similar to Wernicke's encephalopathy (Fig. 2a,b upper panels). However, the patients recovered completely following thiamine supplementation (Fig. 2a,b lower panels). After observing these adverse reactions, we measured serum thiamine levels and conducted head magnetic resonance imaging (MRI) of the other participants, but observed no abnormal findings. We also produced a new consent form explaining these adverse reactions, and obtained consent from patients and guardians for trial continuation. Additionally, we conducted a preventive intervention consisting of oral thiamine administration for all participants. No further adverse events occurred after preventive administration of thiamine.

## DISCUSSION

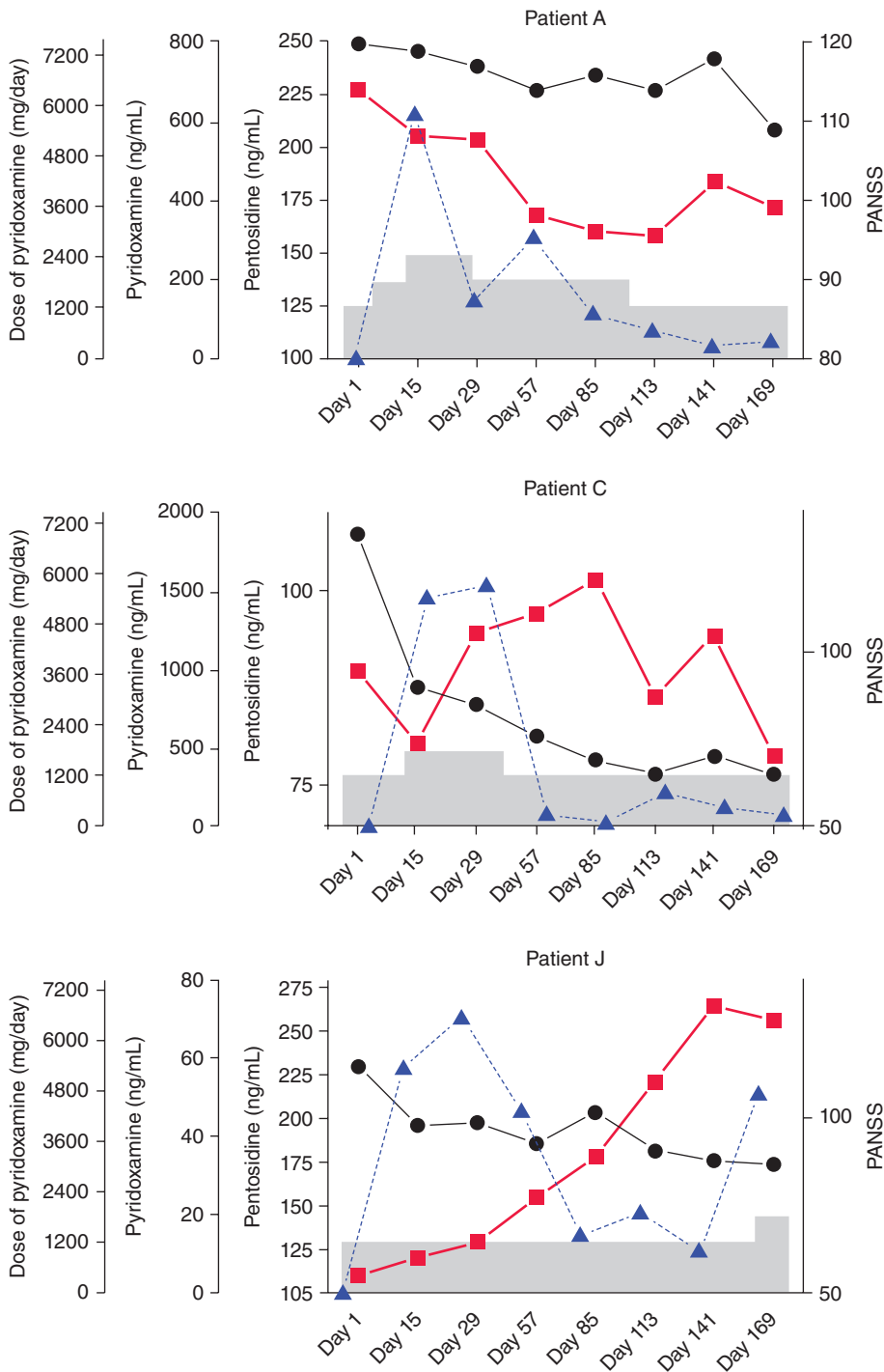
In this trial, we tested the hypothesis that pyridoxamine treatment improves clinical symptoms in a subset of schizophrenia patients with increased carbonyl stress. Previously, we found that most schizophrenia patients with high plasma pentosidine are classified as having treatment-resistant schizophrenia.<sup>9</sup> Of 10 total participants, two patients showed marked improvements in psychotic symptoms, especially the PANSS Negative and General subscales, and one patient with a *GLO1* frameshift mutation showed considerable improvement accompanied by a moderate reduction of plasma pentosidine levels. In addition, four participants exhibited improved

**Table 2.** Summary of clinical parameters

Patients	A	B	C	D	E	F	G	H	I	J
Pentosidine	-24.7	-32.8	-12.1	-15.0	19.6	-14.3	-79.6	-5.7	-32.8	135.2
PANSS Positive	-20.7	17.2	-33.3	18.8	25.0	-57.9	12.5	-16.1	5.3	-3.9
PANSS Negative	-5.6	20.8	-60.5	12.2	-8.3	40.0	35.0	-6.7	-4.4	-36.4
PANSS General	-5.5	6.1	-53.6	13.7	22.6	-3.7	-4.7	-5.8	-15.2	-26.8
PANSS Total	-9.2	11.8	-51.5	13.9	17.5	-6.1	8.9	-8.9	-6.7	-24.4
BPRS	-15.0	13.0	-52.6	8.8	18.4	-27.9	2.1	-14.5	2.4	-21.3
DIEPSS	0.0	-14.3	-43.8	14.3	0.0	-100.0	-20.0	10.0	-33.3	-85.7

Numeric values represent rates of change (%), which are calculated by (week 24 – baseline) / baseline × 100. Negative values indicate decreases in pentosidine levels or improvement in clinical assessments. Boldface is used for variables that show >10% improvement. For patient F, values at the time of discontinuation are used instead of week 24.

BPRS, Brief Psychiatric Rating Scale; DIEPSS, Drug-Induced Extrapyrarnidal Symptoms Scale; PANSS, Positive and Negative Syndrome Scale.



**Figure 1.** Clinical courses of patients (a) A, (b) C, and (c) J, who showed great improvements in their psychological symptoms. (—●—) Positive and Negative Syndrome Scale (PANSS) score, (—■—) plasma pentosidine levels, (---▲---) serum pyridoxamine concentrations, and (■) daily dose of pyridoxamine are shown in the figure.

drug-induced Parkinsonism. Two patients experienced Wernicke's encephalopathy but completely recovered following thiamine supplementation.

Since 1973, several clinical studies have been conducted to evaluate the efficacy of vitamin B<sub>6</sub>

treatment for schizophrenia; however, results have been inconclusive.<sup>19–25</sup> Previous studies used pyridoxine or pyridoxal as the vitamin B<sub>6</sub> therapeutic, and used dosages much lower than ours. Additionally, the participants in past trials were not as well

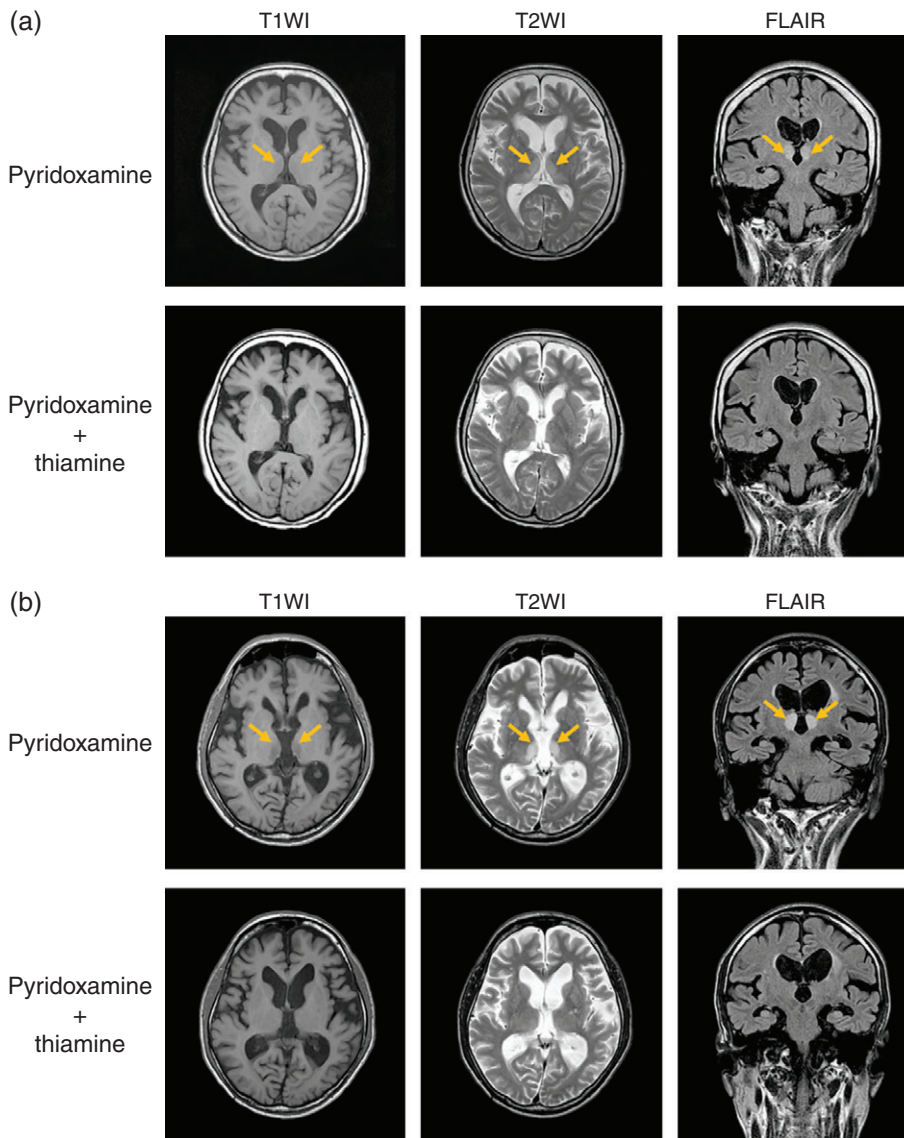
**Table 3.** Summary of adverse events

Adverse events	Incidence	Seriousness	Outcome	Causal relation
Wernicke-like encephalopathy	2	Serious	Disappearance	Causal
Seizure	1	Serious	Disappearance	None
Substupor	1	Serious	Disappearance	Not likely
Loss of consciousness	1	Serious	Disappearance	Possible
Subileus	1	Serious	Disappearance	None
Pneumonia	1	Serious	Disappearance	Possible
Oversedation	4	Nonserious	Disappearance 3, remission 1	Not likely = 3, None = 1
Increased urinary white blood cells	4	Nonserious	Disappearance 3, remission 1	None
Common cold	3	Nonserious	Disappearance	None
Elevated creatine kinase	3	Nonserious	Disappearance	None
Worsening of EPS	2	Nonserious	Disappearance	Possible = 1, None = 1
Bradycardia	2	Nonserious	Disappearance 1, remission 1	None
Elevated lactate dehydrogenase	2	Nonserious	Disappearance	None
Eosinophilia	2	Nonserious	Disappearance	None
Asthmatic attack	1	Nonserious	Disappearance	None
Aggravation of asteatotic dermatitis	1	Nonserious	Remission	None
Sciatica	1	Nonserious	Remission	None
Unspecific T wave abnormality	1	Nonserious	Continued	None
Influenza virus infection	1	Nonserious	Disappearance	None
Insomnia	1	Nonserious	Disappearance	None
Thought disturbance	1	Nonserious	Disappearance	None
Agitation	1	Nonserious	Disappearance	None
Leukocytosis	1	Nonserious	Disappearance	None
Neutrophilia	1	Nonserious	Remission	None
Monocytosis	1	Nonserious	Disappearance	None
Lymphopenia	1	Nonserious	Remission	None
Hypnatremia	1	Nonserious	Disappearance	None
Hyponatremia	1	Nonserious	Continued	None
Hyperfibrinogenemia	1	Nonserious	Disappearance	None
Hyperammonemia	1	Nonserious	Continued	None
Hyperglycemia	1	Nonserious	Disappearance	None
Bacteriuria	1	Nonserious	Remission	None
Increased urinary squamous cells	1	Nonserious	Disappearance	None

EPS, extrapyramidal symptoms.

characterized as the patients in our study, who were selected based on plasma levels of pentosidine, a representative carbonyl stress biomarker. To our knowledge, this is the first clinical trial to test the hypothesis that a therapeutic strategy aimed at reducing carbonyl stress yields symptomatic improvements for schizophrenia patients with high pentosidine. Supporting this hypothesis, we found that eight patients showed decreased plasma pentosidine levels, with one patient showing clinical improvements associated with a moderate reduction in pentosidine levels, and a second showing significant improvements in psychological symptoms and

a slight decrease in plasma pentosidine. A third patient also showed significant psychological improvements not associated with decreased plasma pentosidine, suggesting that pyridoxamine may also have uncharacterized benefits unrelated to its function in relieving carbonyl stress. Because glutamate decarboxylase and aromatic-L-amino-acid decarboxylase are pyridoxal-phosphate-dependent enzymes, high-dose pyridoxamine may affect metabolism and biosynthesis of neurotransmitters, including dopamine, serotonin, and gamma aminobutyric acid (GABA). Indeed, pyridoxine treatment increased the protein levels of glutamic acid decarboxylase (GAD)



**Figure 2.** (a) Head magnetic resonance imaging (MRI) of patient C. Top: Bilateral medial thalamus lesions are shown in horizontal sections of T2-weighted image (T2WI) and T1-weighted image (T1WI). The lesions were also confirmed in a coronal section of fluid-attenuated inversion recovery (FLAIR) image. Yellow arrows indicate the lesions. Bottom: After thiamine treatment, the abnormal intensities almost completely disappeared. (b) Head MRI of patient F. Top: MRI image scanned at onset. Similar to patient C, bilateral medial thalamic lesions were observed in case F. Yellow arrows indicate the lesions. Bottom: MRI image scanned after thiamine treatment. Thiamine administration markedly improved the abnormal intensities.

67, an enzyme for GABA synthesis in the mouse dentate gyrus.<sup>26</sup> This report suggests that pyridoxine treatment upregulated the GABAergic system *in vivo*. Thus, it is speculated that pyridoxine converted from high-dose pyridoxamine augments the GABA function leading to improvement in clinical symptoms. Changes in dopamine in the central nervous system (CNS) induced by high-dose pyridoxamine may be associated with improvements in negative symptoms, daily activity, and drug-induced Parkinsonism, while pyridoxamine-induced alterations in CNS GABA synthesis may induce sedation in some patients, reducing the required dosages of antipsychotics. The observed improvements in EPS are also

consistent with the results of previous trials investigating the effect of vitamin B<sub>6</sub> on tardive dyskinesia.<sup>27,28</sup> However, precise molecular mechanisms underlying these clinical changes still remain unclear and should be addressed in future studies.

As mentioned above, Wernicke's encephalopathy-like reactions occurred in two patients during treatment. A previous study using a rodent model reported that high-dose pyridoxal caused death due to convulsions.<sup>29</sup> Additionally, clinical studies have indicated that high doses of pyridoxine caused neurotoxicity related to peripheral neuropathy.<sup>30,31</sup> Another study noted that pyridoxine treatment caused dizziness in a chronic schizophrenia



patient.<sup>19</sup> We propose that administration of high-dose pyridoxamine may cause Wernicke's encephalopathy-like reactions through conversion of pyridoxamine to pyridoxal, which entraps amino compounds, such as thiamine, by carbonyl-amine chemistry. These adverse drug reactions can be prevented with simultaneous prescription of thiamine, and Wernicke's encephalopathy was not observed after thiamine supplementation in our trial.

Our current study had a small sample size and was not a randomized, placebo-controlled trial. Thus, although our results suggest that pyridoxamine supplementation is a promising therapy for treatment-resistant schizophrenia associated with increased carbonyl stress, a larger randomized, placebo-controlled study will be required to confirm the validity of our findings.

## Conclusion

High-dose pyridoxamine was effective in alleviating psychotic symptoms in a subset of schizophrenia patients with enhanced carbonyl stress. Thiamine should be coadministered to prevent Wernicke's encephalopathy. Further studies will be needed to validate the clinical efficacy and investigate the pharmacological actions of pyridoxamine.

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## DISCLOSURE STATEMENT

We state that there is a conflict of interest. This work was supported by PROJECT PM Co., Ltd. The

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## AUTHOR CONTRIBUTIONS

Drs M.I., M.M., M.A., and T.M. designed the trial. Drs M.M., M.I., T.T., K.I., and K.T. collected the clinical data. Drs M.I., M.M., M.A., and T.M. conducted the literature search, designed the figures and tables, and analyzed and interpreted the data. Drs T.D., T.I., K.T., A.K., Y.H., T.Y., Y.O.N.A., and S.W. interpreted the data. Dr S.U. provided special advice on statistical analysis. Drs M.I., M.M., M.A., and T.M. revised the first draft and completed the final manuscript. All authors approved the final manuscript.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Clinical and safety assessments during the trial.