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Neurological effects of glufosinate poisoning with a brief review

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Herbicides containing glufosinate ammonium are widely used in many countries including Japan. Many Japanese cases of accidental and suicidal poisoning by glufosinate have been reported since 1989. We report a case of a 64-year old man who ingested glufosinate in an attempted suicide. The patient suffered mental disturbances and hematological changes together with gastrointestinal effects shortly after ingesting the poison, and later developed generalized convulsions, impaired respiration and circulatory failure. During recovery he exhibited loss of short-term memory (retrograde and anterograde amnesia). Neurotoxicity is a characteristic of glufosinate poisoning, although the mechanism is not clear. From the analysis of clinical symptoms of previously published cases, glufosinate toxicity appears to arise both from the active ingredient and the surfactant in the formulation.

Keywords: glufosinate poisoning; neurotoxicity; surfactant active agents; consciousness disturbance; herbicide

Introduction

Glufosinate ammonium (GLA; ammonium-DL-homoalanin-4-yl-(methyl)phosphinate) is a rigid analogue of glutamate-containing phosphino amino acid, which is the active ingredient of non-selective herbicides. They are marketed worldwide under the following trade names: BASTA (Hoechst Japan Ltd., Tokyo, Japan), Ignite, Challenge, and Harvest.[1] GLA is contained in BASTA at a concentration of 18.5%. The BASTA herbicide formulation also contains surfactant active agents, ethylene glycol, coloring, and water. As the use of GLA-containing herbicides has increased, poisoning from accidental and suicidal ingestion have also been increased. Suicide attempts by BASTA ingestion have been reported in Japan since 1989, and have occasionally been fatal. We report a case of GLA poisoning with neurological symptoms, and discuss some Japanese cases.

Case report

A 64-year old man (body weight: 60 kg) was admitted to hospital after attempting suicide. His past medical history included diabetes mellitus and hypertension which was treated with insulin and anti-hypertensive drugs. He had also been hospitalized for alcoholism for 2 months in 1989 but was otherwise in good health. About 4.00 a.m. on July 18, 1993, the patient intentionally ingested about 180 ml of BASTA herbicide containing 33.3 g GLA (555 mg kg^{-1} body weight). About 2 h later he was found with vomiting, diarrhea and impaired consciousness. On admission to hospital, he was immediately intubated for airway maintenance and received gastric lavage followed by administration of charcoal, diuretics, and a purgative. He was mechanically ventilated by a respirator and an indwelling urinary catheter was inserted. The patient was transferred to the intensive care unit for further treatment and hemodynamic monitoring. His level of consciousness was assessed at 11 points on the Glasgow Coma Scale (GCS) scores; he could respond weakly to voice. Physical and laboratory evaluations showed that the blood pressure was 70/0 mmHg. The 0 mmHg in the diastolic blood pressure means not measurable; hemic murmur was not detectable by stethoscopy and heartbeat was not detectable by palpation, indicating that the patient was in a state of shock. The central venous pressure (CVP) measured with a respirator was 9 cmH_2O (normal range, 4–8 cmH_2O). The body temperature was 35.4°C and the pulse was 110 beats per min. The hemoglobin level was 12.5 g dl^{-1} and red cell count was 3.75×10^6 cells μl^{-1}. The blood gas analysis revealed mild metabolic acidosis. Table 1 shows the daily values of blood and urine tests. The glufosinate ammonium was quantitatively determined by a rapid and simple paper chromatographic method.[2] Blood and urine ammonia levels were not measured. The blood pressure was markedly lowered, so massive amounts of intrave-

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nous fluids were administered with a lactated Ringer solution including dopamine, diuretics and electrolytes. Five hours after receiving this intravenous infusion, the blood pressure increased to 122/60 mmHg. The patient had slight edema of the face and dorsum of the hands during the following night.

On the second day, the patient frequently exhibited tremor, generalized convulsive movements of the whole body and GLA was positive in the urine. The first seizure occurred about 29 h after ingestion. Direct hemophoresis (DHP) was performed for 6 h and thiopental sodium and diazepam were administered. The urinary GLA level decreased and the convulsions slowly resolved. He was drowsy all day. On the third day, edema was noted over the whole body, especially in the eyelids and the hands. GLA level was negative in the blood and the urine. On the fourth day, he was still on the ventilator and exhibited irregular and impaired respiration and the edema persisted. On the seventh day, he was extubated and could talk. On the ninth day, he was transferred to the general medical care and started to eat. Although a computed tomographic (CT) cerebral scan at 24 h showed a normal appearance, a magnetic resonance imaging (MRI) scan demonstrated slight changes induced by ischemia in the white matter of the lateral regions of the brain (Figure 1). However, no evidence of hippocampal lesions was seen by CT and MRI.

The patient was referred for further treatment to the Shinjo Hospital of Neuropsychiatry after 18 days without any sequelae. However, retrograde and anterograde amnesia for one week beginning the day before ingestion appeared and was accompanied by confabulation. He required psychiatric treatment for a year before he was discharged home.

Discussion

GLA selectively inhibits the activity of glutamine synthetase by which glutamate is combined with ammonia.[3–5] This enzyme plays an important role

Table 1 Blood and urine laboratory data in the patient after BASTA poisoning

Hospital days	*1 (7/18)*	*2 (19)*	*3 (20)*	*4 (21)*	*5 (22)*	*19 (8/5)*	*Normal ranges*[8]
White blood cells[1]	13.4	17.7	18.4	15.8	16.2	4.8	3.9–9.8
Lactate dehydrogenase[2]	372	321	399	600	571	214	109–193
Cholinesterase[3]	2.8	2.5	3.0	2.6	2.4	6.9	5.5–17.2
Urea nitrogen[4]	21.9	11.8	11.3	16.6	14.1	9.3	9.0–19.0
Uric acid[4]	2.8	2.8	1.3	1.6	1.7	5.1	3.0–7.0
Glucose[4]	326	235	147	341	287	182	70–120
Glufosinate[5] (blood)	n.d.	n.d.	–	n.d.	n.d.	n.d.	
Glufosinate[5] (urine)	n.d.	+[6],±[7]	–	n.d.	n.d.	n.d.	

[1]x10^3 μl^{-1}. [2]unit 1^{-1} . [3]unit ml^{-1}. [4]mg dl^{-1}. [5]minimum detectable amount on the chromatogram, 40 μg/ml^{-1}. [6]9.00 a.m. on the second day. [7]6.00 p.m. on the second day. [8]minimum to maximum. n.d., not determined. ( ), months and days. –, negative. ±, not positively detectable but not negative. +, positive

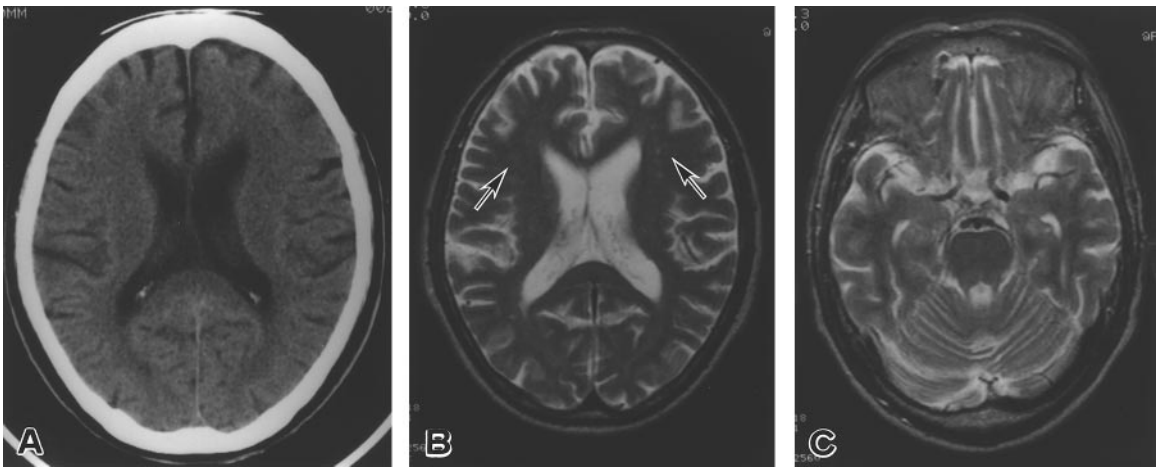

Figure 1 Computed tomographic scan (**A**) and magnetic resonance imaging (**B**, **C**) of the bilateral cerebellum 24 h after BASTA ingestion. CT (**A**) showed no lesions in the white matter of cerebral hemispheres. However, MRI (0.5T) (**B**) (approximately same level as (**A**)) revealed small and slightly high signal intensity lesions at paraventricular area in the white matter (arrows) on axial T2-weighted images (TR/TE 4000/100)

in catabolism of ammonia and the regulation of nitrogen metabolism. If this enzyme activity is inhibited, ammonia accumulates in cells and photosynthesis is inhibited. Only a few reports on the effects of GLA on animals are available in the published literature.[6,7] According to these reports, the oral LD_{50} values for the formulation of GLA are nearly identical in rats, mice and rabbits, ranging from 1500 mg kg^{-1} to 2000 mg kg^{-1} body weight. When taking the GLA formulation of more than 40 mg kg^{-1} in the subchronic feeding studies, the activity of glutamine synthetase and the levels of related metabolites in some tissues were slightly changed. Although GLA is a phosphorus-containing amino acid, no inhibition of cholinesterase activity was found in the brain of female rats at 7.5 days after application of GLA.[7] However, the present case showed the apparently reduced cholinesterase levels for 5 days after ingestion of GLA. Also, Ishizawa *et al*.[8] demonstrated that the cholinesterase activities of red blood cells and serum were reduced in seven of 16 patients by GLA. Therefore, GLA may play an important role as a cholinesterase inhibitor in GLA poisoning as well as organophosphate poisonings.

There were no neoplastic or non-neoplastic lesions related to GLA administration. With regards to reproductive toxicity, although the resorption rate and number of dead fetuses were increased in rats and rabbits administered a large amount of GLA by oral gavage (100 and 20 mg kg^{-1} per day, respectively), maternal metabolism and fetal development were not affected. Therefore, GLA was not considered teratogenic, mutagenic, or carcinogenic in these animals. However, we have recently found that GLA is teratogenic in mice and rats in whole embryo culture, and that GLA specifically induces apoptosis in the neuroepithelium of developing embryos.[9,10]

In the present case of BASTA poisoning, the gastrointestinal symptoms (vomiting and diarrhea) developed in the early stage. Afterwards, the clinical symptoms were characterized by neurological symptoms such as seizure, convulsions and impaired respiration. This case showed also loss of memory from 6–7 h (around 9.00 p.m. on July 17) before ingesting BASTA to the complete recovery at the consciousness level 7 days after ingestion (July 24), which was regarded as retrograde and anterograde amnesia. This clinical picture resembles previously reported cases.[11] Koyama[12] recently reviewed 31 cases of BASTA poisoning reported from Japan including this one. Six patients (19.4%) died by the fourth hospital day, and the estimated amount of ingested BASTA herbicide ranged from 20–500 ml (3.7–92.5 g GLA). The amount of BASTA ingestion was 50–500 ml in 25 survivals, and 20 (the number of days till death, 3 days), 80 (3 days), 220 (2 days), 300 (1 days), 500 ml (3 days) and unknown (1 day) in six fatal cases. Therefore, the death did not seem to be dose-related or time-related. The direct cause of death seemed to be circulatory disturbance, especially the cardiac insufficiency by the large amount of surfactant active agents in BASTA. Likewise, the amount of BASTA ingestion was 20–90 ml in 20 patients without loss of memory, while 50–500 ml in five patients (of three cases, less than 100 ml) with loss of memory. Hashimoto *et al*.[13] reported a case of a 66-year-old patient who attempted suicide by drinking 80 ml of BASTA formulation, who died 50 h later. It was unknown whether the cause of death was due to GLA or other components of the formulation.

In the present case, the urinary GLA level decreased on the second day and was negative on the third day. On the other hand, Hirose *et al*.[14] reported that the concentration of GLA in urine was higher than that in blood samples simultaneously drawn in four patients. GLA excretion in urine continued up to 5 days after ingestion, when GLA could no longer be detected in blood. This may be due to differences in the sensitivity for GLA between paper chromatography and gas chromatography.

The early symptoms shortly after ingestion were mainly gastrointestinal such as nausea and vomiting (41.9%), and diarrhea and abdominal pain (9.7%). Hematological analysis revealed increased white-cell count (51.5%) and increased levels of GOT, GPT and LDH (48.4%). In the course of time, impaired respiration (67.7%), and neurological defects such as mental status change (74.2%), tremor, fever $>38^{\circ}C$ (35.5%) and convulsions (35.5%) developed.[12] In three out of four other cases, convulsions first appeared after the blood GLA level decreased below detectable limits.[13] Mental status alteration was observed even in those who ingested relatively small amounts of BASTA (50 ml). After recovery, nine cases (36.0%) from 25 survivors showed retrograde amnesia.[8] This has also been seen in 14.7% of a separate case series of 34 cases treated at the Tsukuba Poisoning Treatment Center. However, although no defects in the hippocampus region were detected, a decrease in electrical activity without a seizure discharge was seen on the electroencephalogram.[15]

The present case showed neurological changes and symptoms. MRI demonstrated slight changes induced by ischemia in the white matter of the lateral regions of the brain. It is thought that the areas of ischemia seen on the MRI pictures may be related to the circulatory imbalance during the acute phase of poisoning. However, two cases reviewed by Koyama[12] had loss of memory for 7–10 days after intoxication, although no neurological defects were detected by CT or MRI. Therefore, we could determine conclusively whether the slight

changes in the white matter were directly or indirectly due to GLA-intoxication.

The neurological symptoms have not been observed in poisoning by other herbicides, and may be characteristic of GLA. Although patients ingesting varying amounts of GLA showed different symptoms, no pattern can be established between the amount ingested and a predictable toxic syndrome. This cannot be explained by the action of GLA alone. Metabolites and other formulation components such as glutamate, ammonia, and surfactants should be considered. The timing of ingestion with meals may affect absorption of GLA from the intestinal tract and thus the entry of GLA into the blood and brain. The concentration of protein in the diet may also be important in determining toxicity.

Excessive release of glutamate, neuronal excitatoxicity, is known to mediate neuronal cell death and produce degeneration of brain cells,[16,17] because the hippocampus contains a class of receptors for the excitatory amino acids. The biochemical and physiological effects of GLA have been examined in animal experiments *in vivo*.[6,7] Marked inhibition of glutamine synthetase activity was found in the liver, kidneys and brain. Glutamate levels were increased in the liver, but showed a dose-dependent decrease in the brain. In one ingestion case an increase in glutamate and a decrease in glutamine was seen in the serum.[18] Thus, the effects of GLA are similar between experimental animals and human poisoning. It is uncertain whether glutamate is the primary cause of the neurological effects in GLA poisoning. It is possible that since GLA is a structural analogue of glutamate, it may induce convulsions and memory impairment by interfering with the neurotransmitter function of endogenous glutamate.

Ammonia is partly derived *in vivo* from amino acids by deamination in protein metabolism, and utilized in the pathway of glutamate to glutamine.[19] There was no increase in the ammonia level in tissues even in a chronic toxicity feeding study. This may be due to the absorption of ammonia as the cation in the salt form of GLA administered. Ammonia may not be involved in the toxicity of GLA in mammals.[6] However, the accumulation of ammonia via the inhibition of glutamine synthetase in the GLA-treated animals cannot be denied. In two cases of BASTA poisoning (Mito Saiseikai General Hospital) the serum level of ammonia was increased, which may affect the central nervous system (CNS).[20]

Surface active agents, in general, are only moderately toxic.[21–23] However, they may play an important role in the toxicity of formulated herbicide ingestion. Surfactants can induce permeability of blood vessels, leading to whole body edema due to exudation of fluid, decrease in cardiac function, pulmonary edema, and shock. Circulatory failure has been seen in BASTA poisoning, which suggests that the surfactants in the formulation play some role in clinical toxicity.[24] The central nervous system is protected by the blood brain barrier and the glial cells. GLA by itself is less toxic to mammals than plants because of its inability to cross the brain barrier and its rapid clearance by the kidneys.[25] However, in experimental animals the internal absorption rate of GLA in a formulation containing surfactant was 25–30% higher than the absorption rate of GLA when given alone. We speculate whether the CNS effect of relatively small doses may be due to surfactant enhanced access to the brain.

In conclusion, poisoning by GLA-containing herbicides may result from both the active ingredient and the surfactant. The clinical symptoms can be classified into two categories. Initial symptoms are characterized by gastrointestinal symptoms, which may be mainly associated with the mucosal irritant effect of the surfactant. The symptoms developing later are characterized by neurological effects and circulatory failure with a delay of about one day. These may be due to the effects of GLA on the CNS and the surfactant on the cardiovascular system.

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References

1 Hoerlein G. Glufosinate (phosphinothricin), a natural amino acid with unexpected herbicidal properties. *Reviews of Environmental Contamination and Toxicology* 1994; **138:** 73–145.

2 Suzuki A, Kawana M. Rapid and simple method for identification of glufosinate-ammonium using paper chromatography. *Bulletin of Environmental Contamination and Toxicology* 1989; **43:** 17–21.

3 Wild A, Sauer H, Rühle W. The effect of phosphinothricin (glufosinate) on photosynthesis. I. Inhibition of photosynthesis and accumulation of ammonia. *Zeitschrift für Naturforschung* 1987; **42c:** 263–269.

4 Sauer H, Wild A, Rühle W. The effect of phosphinothricin (glufosinate) on photosynthesis. II. The causes of inhibition of photosynthesis. *Zeitschrift für Naturforschung* 1987; **42c:** 270–278.
5 Johnson CR, Boettcher BR, Cherpeck RE, Dolson MG. Design and synthesis of potent inhibitors of glutamine synthetase. 1. Cyclic analogs of phosphinothricin. *Bioorganic Chemistry* 1990; **18:** 154–159.
6 Ebert E, Leist K-H, Mayer D. Summary of safety evaluation toxicity studies of glufosinate ammonium. *Food and Chemical Toxicology* 1990; **28:** 339–349.
7 Hack R, Ebert E, Ehling G, Leist K-H. Glufosinate ammonium - some aspects of its mode of action in mammals. *Food and Chemical Toxicology* 1994; **32:** 461–470.
8 Ishizawa J, Tsujikawa A, Kuroki Y, Oosashi N. Glufosinate poisoning. *Pharmaceutics Months* 1992; **34:** 1920–1922. (in Japanese).
9 Watanabe T, Iwase T. Developmental effects of glufosinate ammonium on mouse embryos in culture. *Teratogenesis, Carcinogenesis, and Mutagenesis* 1996; **16:** 287–299.
10 Watanabe T. Apoptosis induced by glufosinate ammonium in the neuroepithelium of developing mouse embryos in culture. *Neuroscience Letters* 1997; **222:** 17–20.
11 Koyama K, Andou Y, Saruki K, Matsuo H. Delayed and severe toxicities of a herbicide containing glufosinate and a surfactant. *Veterinary and Human Toxicology* 1994; **36:** 17–18.
12 Koyama K. Acute oral poisoning caused by a herbicide containing glufosinate. *Japanese Journal of Toxicology* 1995; **8:** 391–398. (in Japanese).
13 Hashimoto H, Yamamoto R, Sumino K, Koizumi N. A case of glufosinate poisoning. *Japanese Journal of Toxicology* 1994; **7:** 47–50. (in Japanese with English abstract).
14 Hirose Y *et al*. A clinical study of acute BASTA herbicide intoxicated patients: Correlations of the blood and urine glufosinate concentration and the development of clinical symptoms. *Japanese Journal of Toxicology* 1996; **9:** 301–308. (in Japanese with English abstract).
15 Koyama K *et al*. A case of BASTA® poisoning. *Intensive & Critical Care Medicine* 1993; **5:** 1311–1314. (in Japanese).
16 Olney JW. Neurotoxicity of excitatory amino acids. In: *Kainic acid as a tool in neurobiology*. EG. McGeer and JW Olney (ed.). New York: Raven, 1978, pp. 95–121.
17 Collingridge GL, Lester RAJ. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacology Reviews* 1989; **40:** 143–210.
18 Yoshida I *et al*. A case of glufosinate ammonium poisoning. *Japanese Journal of Toxicology* 1997; **10:** 75–79. (in Japanese with English abstract).
19 Miflin BJ, Lea PJ. Ammonia assimilation. In: *The biochemistry of plants; a comprehensive treatise*. PK Stumpf and EE Conn (eds.). New York: Academic Press, 1980. Vol. 5, pp. 169–202.
20 Taguchi M, Watanabe S, Inomata S. Two cases of glufosinate poisonong. *Japanese Journal of Toxicology* 1992; **5:** 193–194. (in Japanese) (Abstract).
21 Arena JM. Poisonings and other health hazards associated with use of detergents. *Journal of the American Medical Association* 1964; **190:** 168–170.
22 Swisher RD. Exposure levels and oral toxicity or surfactants. *Archives of Environmental Health* 1968; **17:** 232–246.
23 Koyama K, Goto K, Yamashita M. Circulatory failure caused by a fungicide containing iminoctadine and a surfactant: A pharmacological analysis in rats. *Toxicology and Applied Pharmacology* 1994; **126:** 197–201.
24 Hirose Y *et al*. Glufosinate poisoning. *Journal of Japanese Association for Acute Medicine* 1992; **3:** 88–91. (in Japanese).
25 Kishore GM, Shah DM. Amino acid biosynthesis inhibitors as herbicides. *Annual Review of Biochemistry* 1998; **57:** 627–663.