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Seizure as the main presenting manifestation of three patients with acute glufosinate-ammonium poisoning

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Glufosinate-ammonium herbicides are the most widely used broad-spectrum, non-selective herbicides in the world. Glufosinate-ammonium is a structural analogue of glutamate (Glu) which can irreversibly inhibit the activity of glutamine synthetase (GS) and Glu decarboxylase in plants, thereby blocking the synthesis of glutamine (Gln) from Glu and ammonia (Hoerlein, 1994). This causes the plants to die because of the nitrogen metabolism disorder and subsequent intracellular accumulation of ammonia. In humans, the characteristic features of glufosinate-ammonium herbicide poisoning include gastrointestinal symptoms and neurotoxicity (Watanabe and Sano, 1998). Currently, there are no antidotes for glufosinate-ammonium herbicide poisoning, and thus supportive care is the key treatment.

In recent years, the damage of herbicides to the central nervous system (CNS) has attracted extensive attention (Tong et al., 2022). Emergency departments often encounter many urgent cases (Song and Lu, 2022; Wang et al., 2022, 2023; Qin et al., 2023), including herbicide poisoning. Among the various herbicides, glufosinate-ammonium herbicides are the most common seizurogenic pesticide class. Therefore, it is crucial for medical staff to be well-prepared to promptly and effectively identify glufosinate-induced neurotoxicity and provide appropriate treatment. In this paper, we describe three patients in our hospital who presented neurological symptoms after glufosinate-ammonium herbicide poisoning. Then we discuss the prediction and examination methods for neurotoxicity in glufosinate-ammonium herbicide poisoning patients.

Table 1 shows the detailed clinical characteristics of the three patients on admission. All of them developed nausea and vomiting immediately after ingesting glufosinate-ammonium. Although they received timely treatments such as gastric lavage and prokinetic and fluid rehydration in local hospitals, their condition did not improve significantly. Finally, they were transferred to our hospital for further treatment.

All patients experienced multiple seizures, and the neurological presentations are listed in Table 2. The duration from drinking glufosinate-ammonium to seizure onset ranged from 12 to 30 h (mean time: 23 h). Patient 1 had two generalized tonic-clonic seizures, the second of which was less severe than the first but still met the criteria for status epilepticus (myoclonic seizures >5 min). Patient 2 had multiple myoclonic seizures and remained lethargic throughout the hospital stay. Patient 3 developed seizures 12 h after the administration of 500 mL 10% (volume fraction) glufosinate-ammonium, which was the fastest neurotoxic response. His neutrophil-to-lymphocyte ratio (NLR) and blood ammonia levels increased faster than those of the other two patients, but his head computed tomography (CT) showed no abnormalities. However, it is noteworthy that Patient 3 had the longest time interval between ingestion and arrival at the emergency department.

To support the general condition of the patients, we closely monitored their vital signs and administered conventional treatments including gastric lavage, purgation, gastric-acid suppression by proton pump inhibitors (PPI), liver protection, and blood volume and

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Table 1 Clinical characteristics of the patients on admission

Variables	Patient 1	Patient 2	Patient 3
Age (years)	29	62	59
Sex	Female	Male	Male
Reason for ingestion	Suicidal	Suicidal	Suicidal
Time elapsed from ingestion to ED arrival (h)	0.33	0.67	3.50
Symptoms on admission	Nausea and vomiting	Dizziness, vomiting, and diarrhea	Vomiting, diarrhea, and lethargy
Previous medical history	Good health, no special problems	Good health, no special problems	Hypertension, controlled
Herbicide intake	300 mL (concentration unknown)	800 mL (20%, volume fraction)	500 mL (10%, volume fraction)
Four vital signs			
Temperature (°C)	36.9	38.0	38.8
Pulse rate (beats/min)	84	91	66
Respiration (breaths/min)	20	21	18
Blood pressure (mmHg)*	93/58	185/78	129/70
GCS at the ED	15	13	14
White blood-cell count ($\times10^9$ L^{-1})	5.70	12.00	22.39
Neutrophil count ($\times10^9$ L^{-1})	5.10	10.80	21.20
Lymphocyte count ($\times10^9$ L^{-1})	0.54	0.62	0.58
NLR	9.44	17.42	36.55
Total protein (g/L)	61.5	56.5	55.4
Albumin (g/L)	38.7	34.2	33.9
ALT (U/L)	118	15	12
AST (U/L)	12	26	12
Lactic acid (mmol/L)	0.6	8.3	6.7
Blood glucose (mmol/L)	6.8	7.4	7.0
Initial serum ammonia (μmol/L)	Not performed	24	105

* Data are expressed as systolic/diastolic blood pressure. ED: emergency department; GCS: Glasgow Coma Scale; NLR: neutrophil-to-lymphocyte ratio; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table 2 Neurological presentation of the patients

Patient	Onset time of seizures (from taking herbicide) (h)	Seizure type and frequency	Head computed tomography (CT) (from taking herbicide)
1	26	Two generalized tonic-clonic seizures	Not performed
2	30	Myoclonic seizures, multiple	Day 1: low-density lesion in the left insula (considered ischemic); Day 6: no progress
3	12	Myoclonic seizure, generalized tonic-clonic seizures, multiple	Day 2: normal

electrolyte resuscitation. Furthermore, when necessary, tracheal intubation and blood purification were employed. Considering the severe agitation of the patients, we used sedatives (olanzapine, haloperidol, valproate, or levetiracetam) and hemoperfusion to realize effective and continuous toxicant clearance, thereby improving their condition. The prognosis of the three patients was as follows. (1) Patient 1 was no longer agitated after 48 h of hemoperfusion, and was discharged after 6 d in a clinically stable condition. (2) Patient 2 experienced reduced neurotoxicity after 72 h of hemoperfusion, but was still confused. Therefore, we continued to treat him with sedatives and discharged him after his condition improved. (3) Patient 3 was in the most critical condition. His neurological symptoms were controlled after 4 d of continuous renal replacement therapy (CRRT) combined with hemoperfusion. He was only discharged after 46 d of anti-inflammatory treatment for aspiration pneumonia. In addition, Patient 3 was reevaluated by electroencephalography (EEG) one month after discharge, showing unremarkable results.

Severe effects from acute glufosinate-ammonium herbicide poisoning include mental change, seizures,

and respiratory arrest, which usually appear after a latent period of 4–60 h (Hori et al., 2003). Among our patients, the average time for seizures to occur was 23 h, which should serve as a reminder to clinicians that neurotoxicity might occur later in patients with glufosinate-ammonium herbicide poisoning than is typical for other substances. However, the relationship between the precise pathophysiological mechanisms that occur in CNS and the chemical constituents of glufosinate-ammonium herbicides is controversial and has not been well elucidated. Some researchers have proposed that the surfactant in the herbicide is more cytotoxic to neuronal cells than glufosinate (Song et al., 2012), which requires further research.

Glu is the primary excitatory neurotransmitter that binds to *N*-methyl-D-aspartate (NMDA) receptors. Moreover, ammonia is a highly toxic substance, and its accumulation can cause neuronal oxidative stress, leading to cell death. Under physiological conditions, ammonia and Glu can form Gln under the action of GS to maintain homeostasis of the nervous system. Given that glufosinate-ammonium acts as a GS inhibitor, exposure to glufosinate-ammonium can destroy the Glu-Gln cycle, and consequently increase Glu and blood ammonia. The following review of relevant literature summarizes the mechanisms leading to neurotoxicity, which are illustrated in Fig. 1.

(1) Previous studies have shown that glufosinate and its metabolites bind to the NMDA receptors that are highly expressed in the hippocampus.

(2) After the destruction of the Glu-Gln cycle, Glu levels increase. Then the Glu binds to the NMDA receptor, which produces neuroexcitatory toxicity (Zhou et al., 2013).

(3) When blood ammonia levels rise, the gaseous form, NH_3, readily crosses the blood-brain barrier. At the same time, NH_4^+ can enter the brain via Na^+-K^+-$2Cl^-$ cotransporter isoform 1 (NKCC1), which competitively impairs the potassium buffering of astrocytes and increases extracellular potassium concentration, resulting in neurological dysfunction and seizures. Meanwhile, excessive NH_4^+ and K^+ depolarize the neuronal γ-aminobutyric acid (GABA) reversal potential (E_{GABA}) by driving the overactivation of NKCC1. Eventually, the inhibitory neurotransmission in the cortex is impaired (Rangroo Thrane et al., 2013).

(4) Blood ammonia can increase Ca^{2+} influx and trigger Glu release. At the same time, Ca^{2+} binds to calmodulin (CaM) and activates nitric oxide synthase (NOS), resulting in increased formation of nitric oxide (NO). NOS activates guanosine cyclase (GC), increases cyclic guanosine monophosphate (cGMP) content, promotes NMDA receptor activation, and produces excitotoxicity (Hermenegildo et al., 2000).

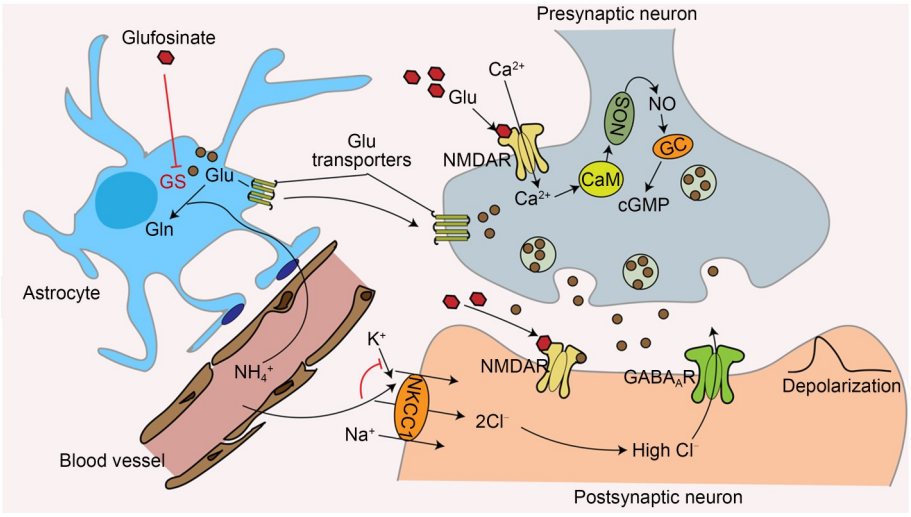

Fig. 1 Mechanism of neurotoxicity after glufosinate-ammonium poisoning. Glufosinate-ammonium and its metabolites can not only bind to NMDAR, but also activate NMDAR by increasing Glu content. The elevated blood ammonia can also further activate NMDAR, and damage inhibitory neurotransmission in cerebral cortex by over-activating NKCC1. NMDAR: *N*-methyl-D-aspartate receptor; Glu: glutamate; Gln: glutamine; GS: glutamine synthetase; GC: guanosine cyclase; NKCC1: Na^+-K^+-$2Cl^-$ cotransporter isoform 1; NO: nitric oxide; NOS: nitric oxide synthase; CaM: calmodulin; cGMP: cyclic guanosine monophosphate; $GABA_AR$: γ-aminobutyric acid sub-type A receptor.

Glufosinate-ammonium herbicide poisoning can lead to moderate-to-severe neurotoxicity through the mechanisms described above. Consistent with this, all of our patients affected by glufosinate-ammonium herbicide presented neurological symptoms during the latent period. Studies have shown that initial serum ammonia can be used as a biochemical marker of neurotoxicity associated with glufosinate-ammonium herbicide poisoning (Cha et al., 2018). We measured blood ammonia in only two patients, and found that it was significantly increased in Patient 3, who also had the most severe neurotoxic symptoms and the longest hospital stay. However, the limitation of our study was that the sample size was so small that we were unable to verify the role of blood ammonia in glufosinate-ammonium herbicide poisoning. Previous studies are predominantly characterized by their single-center, retrospective design, along with limited patient cohorts and varied inclusion criteria, which hinder the derivation of definitive conclusions. Given that the onset of neurotoxicity in our study occurred within the initial 12 h post-ingestion, it is imperative to employ shorter categorization intervals. This adjustment is essential for accurately tracking noteworthy variations in ammonia levels, thereby enhancing clinicians' ability to predict the prognosis of glufosinate-ammonium herbicide poisoning in human cases. In addition, a recent study revealed that NLR has an advantage in predicting neurotoxicity. The use of NLR might help in readily and rapidly predicting development of neurotoxicity in clinical settings after glufosinate-ammonium herbicide poisoning (Kim et al., 2022).

Although seizure is not a risk factor for mortality in patients with acute glufosinate-ammonium herbicide poisoning (Park et al., 2018), and the above-described patients with glufosinate-ammonium herbicide poisoning were stable after appropriate treatment, the occurrence of seizure still is an overwhelming event that requires immediate attention. Seizures have the potential to cause respiratory muscle spasms and obstructed airways, which can lead to respiratory infection and even respiratory congestion. Notably, one of our patients was infected with Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and developed pulmonary cavities. Fortunately, he was discharged after 46 d of anti-infective treatment in our hospital. Therefore, early prevention and monitoring are particularly important.

With regard to imaging examinations, we performed head CT on Patients 2 and 3, but no related abnormalities were found. We initiated a neurology consultation on the brain CT findings of Patient 2, conducted bedside EEG monitoring, and conducted a follow-up brain CT scan 5 d later (with results similar to the previous scan). These steps were taken to systematically eliminate the possibility of other intracranial abnormalities that could potentially trigger the onset of seizures. At the same time, this reminds us that glufosinate-ammonium poisoning may not cause organic impairment of the nervous system, but lead to functional destruction, which would cause no change in head CT scans. However, it is worth mentioning that Park et al. (2020) performed brain fluorodeoxyglucose-positron emission tomography (FDG-PET) scans on patients with neurological symptoms after glufosinate-ammonium herbicide poisoning and found glucose hypometabolism in the inferior frontal and temporal lobes in the brain, which not only indicated that the neurotoxicity of glufosinate-ammonium might be associated with glucose metabolism, but also demonstrated that FDG-PET scans might be an effective imaging modality for predicting neurotoxicity.

Overall, additional research is required to gain a better understanding of the mechanism underlying glufosinate-induced neurotoxicity. There is a pressing need to identify relevant predictors, detection indicators, and novel treatment methods. Additionally, it is crucial to investigate the neurotoxicity of surfactants, especially to determine whether they affect the nervous system independently or act synergistically with glufosinate-ammonium.

Data availability statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Ping WANG studied the concept and prepared the first draft of the manuscript. Congying SONG and Xuan LU made critical revisions to the article. Yuanqiang LU acquired funding, and participated in reviewing and editing the manuscript. All authors have read and approved the final manuscript, and

therefore, have full access to all data relevant to the study and take responsibility for the integrity and security of such data.

Compliance with ethics guidelines

Ping WANG, Congying SONG, Xuan LU, and Yuanqiang LU declare that they have no conflict of interest.

This research was approved by the First Affiliate Hospital, School of Medicine, Zhejiang University, Hangzhou, China (No. 2022624). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this paper.

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