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BRIEF COMMUNICATION

Hyperammonemia following glufosinate-containing herbicide poisoning: A potential marker of severe neurotoxicity

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Glufosinate-ammonium (GLA) is the active ingredient of certain widely used non-selective contact herbicides (''e.g.,'' Basta®). Although it is thought to be much less toxic to humans than to plants, deliberate ingestion of GLA could still lead to serious effects (''e.g.,'' neurotoxicity) or even death. Three cases presented with delayed-onset neurotoxicity including stupor, delirium, seizures, coma, and amnesia after ingesting large amount of Basta. Considering that GLA could irreversibly inhibit glutamine synthetase (GS) in plants, we performed serial measurements of serum ammonia in those patients and revealed marked hyperammonemia in all of them. All patients recovered with the sequelae of persistent amnesia after receiving intensive care and hemodialysis. We speculated that the occurrence of hyperammonemia might at least be partially related to GS inhibition in humans. Moreover, hyperammonemia could serve as a potential marker of severe neurotoxicity, especially prolonged amnesia, following massive ingestion of GLA-containing herbicides. The possible dose-response relation between GLA exposure and serum ammonia level, however, needs more investigations.

Keywords Glufosinate poisoning; Herbicide; Hyperammonemia; Neurotoxicity

Introduction

Glufosinate was derived from bialaphos, a novel tripeptide herbicide discovered in the 1970s. Because of its excellent non-selective herbicidal activity and relative low toxicity to humans, herbicides containing ammonium salt of glufosinate (GLA) are marketed worldwide.

In Taiwan, the sale of herbicide formulations containing GLA, either 13.5% or 18.5% w/v, is rapidly increasing. Acute poisonings related to GLA were increasingly reported to the Taiwan National Poison Control Center in recent years.[1] Acute GLA poisonings are associated with various neurotoxicities including drowsiness, stupor, coma, seizures, and prolonged amnesia as well as life-threatening respiratory suppression and apnea.[2] Currently, there is no available marker that can predict the development of severe effects following acute GLA exposure. We present three patients who developed hyperammonemia after ingesting large amount of 18.5% w/v formulation of GLA-containing herbicide (Basta®) and discuss the potential role of hyperammonemia in severe GLA poisonings.

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Case reports

Case 1

A 39-year-old previously healthy woman attempted suicide by ingesting 300 ml Basta. She soon developed vomiting and was sent to a local hospital. She stated that she only ingested Basta and the toxic exposure was confirmed by identifying the pesticide container brought to the emergency department (ED). After receiving gastric lavage and activated charcoal, she was referred to our ED 3 h post-ingestion. On arrival, her blood pressure was 159/94 mmHg, pulse 101/min and body temperature 36.6°C. Physical examinations, chest X-ray, electrocardiography (ECG) and laboratory data were all unremarkable (Table 1). The patient appeared stable and was kept in an observation unit at the

Table 1. Baseline laboratory data of three patients with severe Basta® poisoning.

Laboratory data	Case 1	Case 2	Case 3
White blood cell count (4500–11 000 × 10^9/L)	6400	10 900	24 700
Differential count (neutrophil/lymphocyte)	70%/23%	76%/18%	91%/3%
Hemoglobin (g/dL; male 14–18, female 12–16)	13.9	15.5	14.9
Platelet count (150 000–350 000 × 10^9/L)	247 000	294 000	289 000
C-reactive protein (0–0.5 mg/dL)	0.1	–	0.2
Serum sodium (135–147 mEq/L)	141	141	131
Serum potassium (3.4–4.7 mEq/L)	3.8	3.7	4.2
Blood urea nitrogen (7–20 mg/dL)	8	13	24
Serum creatinine (0.5–1.5 mg/dL)	0.6	1.2	1.9
Alanine transaminase (0–40 IU/L)	18	16	25
Aspartate transaminase (5–45 IU/L)	20	18	43
Serum ammonia (5–69 μg/dL)	–	42	104
Serum lactate (3–12 mg/dL)	10.4	–	36.8
Arterial blood gases			
pH	7.40	7.36	7.25
$PaCO_2$	39.7	40.3	38.4
PaO_2	174	88	78
HCO_3	24.9	23.1	16.9

ED. Toxicological screening on initial presentation including urine basic drugs, benzodiazepines, and plasma cholinesterase measurement were all negative. Analysis of serum or urine GLA level, however, was not done due to inadequate laboratory facility.

Twenty-three hours post-ingestion, she became drowsy with a Glasgow Coma Scale (GCS) of 9 [eye (E) 2, verbal (V) 2, motor (M) 5]. Her vital signs remained stable. Follow-up arterial blood gases showed pH 7.27, $PaCO_2$ 63.7 mmHg, PaO_2 156 mmHg and HCO_3^- 29.8 mEq/L. Notably, her serum ammonia level was increased at 171 μg/dL (reference range 5–69 μg/dL). Thirty hours post-ingestion, she was unresponsive. Supportive measures including noninvasive positive pressure ventilation were instituted, followed by 6 h of hemodialysis. After receiving the above-noted treatments, she appeared more responsive with GCS of 11 ($E_3V_3M_5$) and her serum ammonia level decreased to 133 μg/dL. Another session of hemodialysis was performed 50 h post-ingestion in an attempt to remove GLA as much as possible. She became fully awake on day 3; follow-up serum ammonia level also declined gradually (Fig. 1). Serial blood biochemistry tests including liver enzymes, renal function, and electrolytes were all normal.

On day 4, she could not recall what happed on the day prior to the poisoning event, why she was confined in the hospital, and what she had experienced after hospitalization. A detailed evaluation of the patient's medical history, physical examination and cognitive function as well as brain imaging study (magnetic resonance imaging, MRI) was performed to rule out other possible causes of memory loss. Both retrograde and anterograde amnesia attributable to GLA poisoning were then diagnosed. On day 8, she was discharged with the sequelae of amnesia. Telephone follow-up of the patient disclosed the persistence of amnesia for nearly 2 years.

Case 2

A 41-year-old previously healthy male attempted suicide by ingesting 300 ml Basta. He was immediately sent to a local hospital for gastrointestinal decontamination and was referred to our ED 4 h later. On arrival, he was alert and oriented and had stable vital signs. The diagnosis of Basta poisoning was confirmed by identifying the pesticide container brought to the ED. The patient denied co-ingesting other toxicants. Physical examinations, chest X-ray, ECG, and all laboratory data, including serum ammonia (42 μg/dL) were within normal limits (Table 1). Initial toxicological screening for basic drugs, benzodiazepines, and plasma cholinesterase was unremarkable.

Eleven hours post-ingestion, he manifested delirium with visual hallucination. Supportive measures including fluid and electrolyte replacement and physical restraint were instituted; however he was found comatose with GCS of 7 ($E_3V_1M_3$) 20 h post-ingestion. Follow-up serum ammonia level was 122 μg/dL and arterial desaturation with SaO_2 of 70% was noted. Endotracheal intubation with assisted ventilation was commenced. Hemodialysis for 4 h was arranged and he was oriented 36 h post-ingestion. Follow-up clinical evaluation of the patient found that he was unable to recall what happed immediately preceding the poisoning event as well as what had occurred to him after hospitalization. Detailed neurological examinations including assessment of cognitive function were then performed, which suggested the diagnosis of both retrograde and anterograde amnesia. Brain MRI was also arranged to rule out other possible causes of amnesia, but was unyielding. Amnesia attributable to GLA poisoning was documented 48 h post-ingestion.

On day 3, he was successfully weaned from the ventilator and serum ammonia level gradually declined (Fig. 1). Other

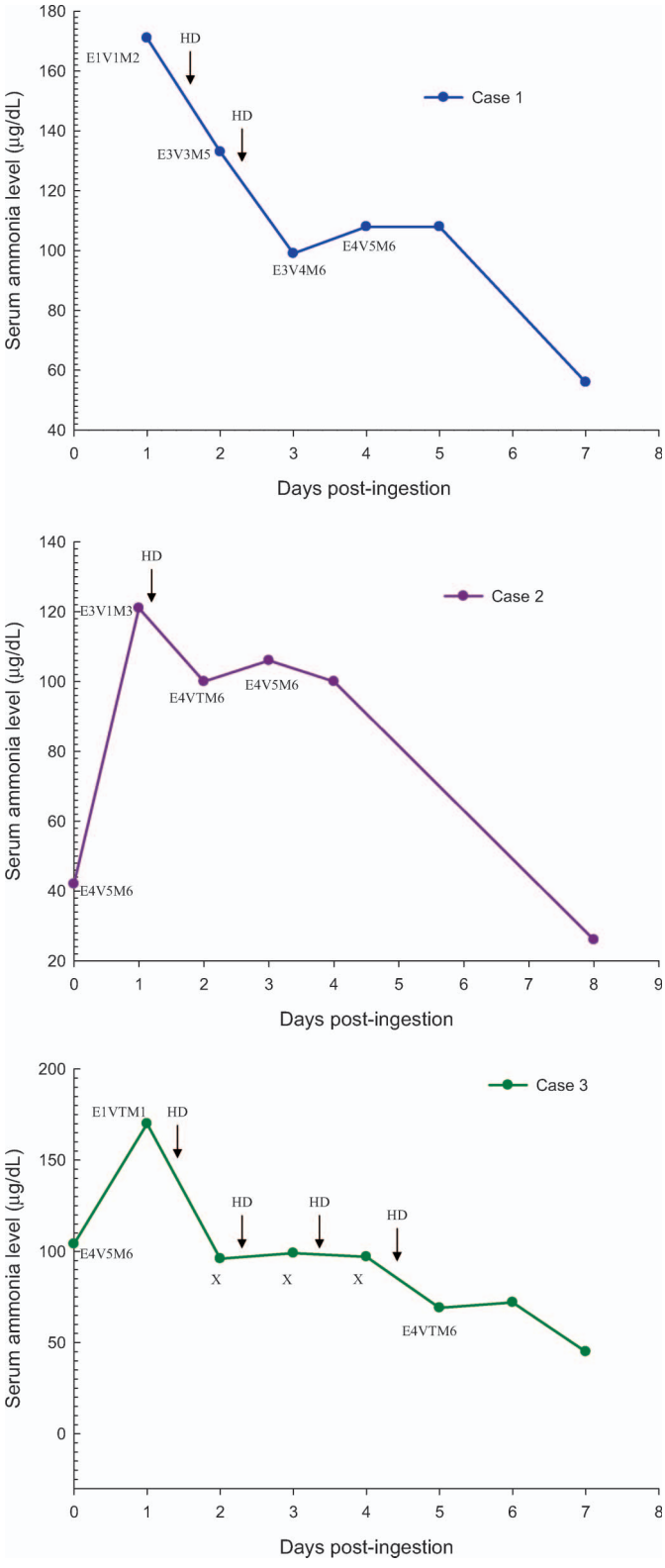

Fig. 1. Serum ammonia levels (reference range 5–69 μg/dL) and relevant Glasgow Coma Scores (GCS) following the ingestion of glufosinate-containing herbicide in three patients. *HD: hemodialysis; T in GCS: endotracheal intubation; and X: time points when GCS was not measured because the patient was receiving intravenous sedation.

than hyperammonemia, serial blood biochemistry tests including serum electrolytes, liver enzymes, and renal function tests were all within normal limits. On day 10, he was discharged with persistent amnesia, which lasted for 5 months as revealed by telephone follow-up.

Case 3

A 69-year-old male with history of major depression and renal cell carcinoma status post left nephrectomy attempted suicide by ingesting 900 ml Basta. The diagnosis was confirmed by identifying the pesticide container and the patient stated that Basta was the only substance ingested. He soon developed nausea and was sent to a primary care facility. After receiving gastrointestinal decontamination, he was referred to our ED 2.5 h post-ingestion.

On arrival, his blood pressure was 170/90 mmHg, pulse 88/min, respiratory rate 20/min and body temperature 36.7°C. He looked restless. Neurological examinations did not reveal focal neurologic signs. Laboratory data disclosed leukocytosis, slightly elevated creatinine of 1.9 mg/dL, and serum ammonia level of 104 μg/dL (Table 1). Arterial blood gas analysis under 3 L/min oxygen supplement further revealed pH of 7.25, $PaCO_2$ 38.4 mmHg, PaO_2 78 mmHg and HCO_3^- 16.9 mEq/L.

Six hours post-ingestion, he became drowsy and had much secretion, necessitating endotracheal intubation with assisted ventilation. He was unresponsive ($E_2V_TM_4$) 12 h post-ingestion when follow-up serum ammonia level was 170 μg/dL. Sixteen hours post-ingestion, he developed an episode of seizure, which subsided after receiving intravenous diazepam. However, he subsequently developed recurrent tonic-clonic seizures, which necessitated the treatment with continuous midazolam. Four sessions of 4-h hemodialysis were then performed in the next 4 days in an attempt to remove remaining GLA because of recurrent seizures after discontinuation of midazolam therapy.

His hospital course was complicated with ventilator-associated pneumonia that developed 5 days after hospitalization. Although he suffered transient hypotension, inotropic agents were not required because his blood pressure never dropped below 80 mmHg and serial serum lactate levels were largely normal, ranging from 10.8 to 14.1 mg/dL (reference range 3–12 mg/dL). On day 6, he was awake; however he was disorientated to time, place and persons. He was successfully weaned on day 11. Nineteen days post-ingestion, he could follow verbal orders. Nevertheless, he could not recall what happed on the day prior to GLA poisoning event and why he was confined in the hospital. Retrograde and anterograde amnesia with confabulation were documented by detailed clinical evaluations of the patient. He was discharged on day 28 with persistent amnesia. Telephone follow-up of the patient disclosed that he remained amnesic about the poisoning event 1 year after discharge.

Initial toxicological screening of the patient was positive for benzodiazepine and venlafexine, both of which were the patient's regular anti-depressive medications. The patient denied taking excessive amount of the aforementioned medications and the statement was supported by pill counting of the patient's remaining medications.

Discussion

We reported three patients who had both hyperammonemia and delayed-onset severe neurotoxicity including stupor, delirium, seizures, coma, and prolonged amnesia following the ingestion of large amount of Basta. In all three patients, clinical history and the results of toxicological screening suggested that Basta was the only toxic substance ingested. Furthermore, GLA alone was likely to be responsible for the toxic effects observed in these patients.[3,4]

In addition to GLA, Basta also contains 30% surfactant, ''i.e.,'' sodium polyoxyethylene alkylether sulfate, and 10% propylene glycol as a solvent.[5] The surfactant tended to cause cardiovascular suppression following deliberate ingestion,[6] while the acute toxicity of propylene glycol is low.[7] None of our patients manifested significant cardiovascular toxicity. Even in case 3, who was exposed to very large amount of Basta, his blood pressure was relatively stable and serum lactate levels were largely normal. Therefore, surfactant was unlikely to play a role in causing those patients' severe toxicity. In the absence of profound shock, propylene glycol was also unlikely to contribute to the neurotoxicity observed in our patients.[3] On the contrary, GLA was known to be associated with delayed-onset neurotoxicity.[2]

The mechanisms of GLA-related neurotoxicity remain unclear but have been proposed to be caused by GLA itself, its metabolites or imbalance between glutamate and glutamine caused by GLA.[2,4,8] Given the findings in our patients and previous studies,[2,9,10] we speculated that hyperammonemia might partly contribute to GLA-related neurotoxicity as well; or it could at least serve as a potential marker of delayed-onset severe neurotoxicity, especially prolonged amnesia, following the ingestion of large amount of GLA-containing herbicide.[4,11]

The etiology and magnitude of hyperammonemia following GLA exposures in humans remain poorly defined. Glufosinate, an analog of glutamate, irreversibly inhibits glutamine synthetase (GS) in plants, thereby blocking the synthesis of glutamine from glutamate and ammonia. Subsequent intracellular accumulation of ammonia causes tissue necrosis and death of the plant shoot.[12] Although there is some similarity between mammalian and plant GS,[13] only a few studies regarding the effects of glufosinate on mammals were available.[14] In those studies, increased hepatic ammonia levels, an effect partially attributable to mammalian GS inhibition, were found at lethal or sublethal dose of GLA exposure.[14] In humans, previous report suggested that the toxic effects of GLA would become severe when more than 100 ml of Basta was ingested.[9] However, hyperammonemia following exposure to GLA or its precursor Bialaphos has been reported only twice.[4,11]

In the brain, GS regulates the level of ammonia and converts neurotoxic glutamate to harmless glutamine; whereas in the liver, it is one of the enzymes responsible for the detoxification of ammonia.[15] Although GS inhibition following GLA exposures generally would not lead to hyperammonemia or severe neurotoxicity, ingestion of massive amount of GLA, as occurred in our patients and previously reported cases,[4,11] might result in significant GS inhibition, which could then contribute to the development of hyperammonemia.

Theoretically, absorption of the ammonium ion of GLA might also increase patients' serum ammonia level. GLA absorption in human gastrointestinal tract was thought to be rapid with peak serum levels achieved within 3 h.[16] We measured serum ammonia level for both case 2 and case 3 upon their arrival, but found mild elevation in case 3 only. Therefore, we believe that the delayed elevation of serum ammonia levels in our patients was more likely to be primarily from GS inhibition.

Mammals have alternative pathways to metabolize ammonia,[14,15] therefore the magnitude of hyperammonemia after the ingestion of large amount of GLA is expected to be less severe than that seen in patients with liver failure or urea cycle disorders. Significant elevations of serum ammonia level however might still occur following massive ingestion of GLA, and might partly contribute to delayed-onset neurotoxicity.[2,9]

In addition to a possible contributory role in GLA-related neurotoxicity, hyperammonemia can also serve as a potential marker of severe neurotoxicity following massive ingestion of GLA.[2,9] To our knowledge, hyperammonemia has not been reported among patients with GLA exposure who did not manifest severe neurotoxicity. Given the findings of our patients and those from previously reported cases,[4,11] the occurrence of hyperammonemia in the absence of liver failure or other causes of hyperammonemia thus should alert the treating physicians about the possibility of developing delayed-onset severe neurotoxicity, especially prolonged amnesia, among GLA-exposed patients. More close observation and more aggressive treatments (''e.g.,'' hemodialysis) at an earlier stage are indicated to prevent sudden and unexpected life-threatening manifestations and/or prolonged amnesia.

All of our patients received hemodialysis. Although the management of GLA poisonings remains supportive, hemodialysis had previously been introduced in GLA-poisoned patients, and the timing of therapy initiation spanned from 5 to 48 h post-ingestion.[2,4,17,18] Furthermore, hemodialysis had been proposed to be effective in removing GLA from the blood given its favorable toxicokinetic properties.[19] However, it is now demonstrated that the clearance of GLA by the kidney is 1.6–1.8 times more than that by hemodialysis,[16] and there is no solid evidence that hemodialysis can improve the morbidity or mortality of GLA-poisoned patients. Thus, unless hemodialysis is

instituted in patients with renal failure or in the early phase of severe GLA poisoning, it seems unlikely that hemodialysis will be an effective treatment in GLA-poisoned patients, as evidenced by the persistent amnesia in our patients despite receiving aggressive hemodialysis. Prospective studies are required to better evaluate the effectiveness of hemodialysis and its best timing in the treatment of severe GLA poisonings.

There were several weaknesses of this report. First, serum ammonia level was generally measured only after the development of certain severe neurotoxicity. Therefore, the finding of hyperammonemia in our patients did not really alert the attending physicians to the possibility of delayed-onset severe neurotoxicity and promptly initiated aggressive treatments (''e.g.,'' hemodialysis). Second, because serum ammonia levels were not measured on a regular and/or frequent basis, we were unable to comment on the exact time course of hyperammonemia and the correlation between the rapidity of developing hyperammonemia and clinical severity. Finally, although the data of our patients suggested a possible dose-response relation between hyperammonemia and GLA-related neurotoxicity, ''e.g.,'' level of unconsciousness and the duration of amnesia, such a relation could not be accurately assessed in a retrospective analysis of only three patients.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of this paper.

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