Toxicokinetics of $\text{dl}$-Glufosinate Enantiomer in Human BASTA® Poisoning

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We found that glufosinate ($\text{dl}$-GLUF) was distributed in the spinal fluid in glufosinate poisoning. A 50-year-old Japanese man (weighing 67 kg) attempted to commit suicide by ingesting about 100 ml of BASTA® (containing $\text{dl}$-GLUF 18.5 g; ratio of $\text{d}$-GLUF to $\text{l}$-GLUF: 1 : 1). He was transported to our hospital, where severe respiratory depression was seen 26 h after ingestion, and management with artificial ventilation was initiated. The $\text{d}$-GLUF concentration 1 h after ingestion was 191.1 µg/ml, almost the same as that of $\text{l}$-GLUF 193.5 µg/ml, but by 3 h after ingestion, these levels had sunk to 60.3 µg/ml and 52.3 µg/ml, respectively, with the concentration of $\text{l}$-GLUF lower than that of $\text{d}$-GLUF. Later, at 27 and 35 h after ingestion, the $\text{d}$-GLUF level was still higher than the $\text{l}$-GLUF level, and the total amounts of urinary excretion were 2835 mg for $\text{d}$-GLUF and 2298 mg for $\text{l}$-GLUF, each variable thus showing a difference between the enantiomers. Cerebrospinal fluid taken from the patient 27 h after poison ingestion revealed the presence of $\text{dl}$-GLUF on CG-MS analysis, and quantitative HPLC analysis of the enantiomers indicated that the $\text{d}$-GLUF concentration was 0.48 µg/ml, and the $\text{l}$-GLUF concentration 0.12 µg/ml. The levels in blood collected at the same time were: $\text{d}$-GLUF, 1.44 µg/ml, and $\text{l}$-GLUF, 0.35 µg/ml. Also, the cerebrospinal fluid contained about one-third of the blood levels of both $\text{dl}$-GLUF enantiomers. He was discharged without any sequelae after 11 d of hospitalization.

Key words  glufosinate; enantioselective analysis; cerebrospinal fluid; HPLC; GC-MS

Herbicides containing $\text{dl}$-homoalanin-4-yl(methyl)phosphinic acid (registered agricultural chemical name, Glufosinate; $\text{dl}$-GLUF) are non-hormonal, non-selective foliage-applied herbicides that were registered in 1984.1) In cases of severe poisoning due to the ingestion of $\text{dl}$-GLUF, the typical course is that an asymptomatic latency period lasting between 4 and 60 h is followed by respiratory depression of acute onset, and the patient’s life is heavily dependent on initiation of artificial ventilation with appropriate timing. It is possible to predict the onset of this delayed respiratory depression from the serum concentration of $\text{dl}$-GLUF and the time interval since its ingestion.2) For such reasons, $\text{dl}$-GLUF has been singled out by the Japanese Society for Clinical Toxicology as one poison for which it advocates qualitative and quantitative analysis in clinical cases.3–5) Regarding the toxicokinetics of $\text{dl}$-GLUF in the body, Hirose et al.5) and Honda et al.6) have already reported its toxicokinetic parameters in the body in cases of attempted suicide by BASTA fluid ingestion, but we have been interested in the fact that $\text{dl}$-GLUF is a racemate. The reason for this is that it is known that the enantiomers of some racemic pharmaceuticals have different pharmacological actions, and it is thought that analysis of the kinetics of the enantiomers in the body is of importance.7) As we have reported,8) we therefore developed a method of enantioselective analysis of $\text{dl}$-GLUF in biological samples, and applied this technique in one case in which BASTA fluid was ingested for the purpose of suicide. This is the only case so far in which the blood elimination kinetics of $\text{dl}$-GLUF enantiomers have been reported.8) In this case, although $\text{d}$-GLUF exhibited the elimination kinetics of a one-compartment model, $\text{l}$-GLUF showed those of a two-compartment model, and the elimination half-life of $\text{l}$-GLUF was longer than that of $\text{d}$-GLUF.

This study describes our analysis, carried out on another case of attempted suicide by BASTA fluid ingestion, in which we were able not only to analyze the toxicokinetics of $\text{dl}$-GLUF enantiomers, but also to confirm their distribution to the cerebrospinal fluid.

Case Report A 50-year-old Japanese man (weighing 67.0 kg) attempted to commit suicide by ingesting about 100 ml of BASTA® (containing $\text{dl}$-GLUF 18.5 g, with $\text{d}$-GLUF and $\text{l}$-GLUF present in the ratio of 1 : 1). On discovering evidence of nausea and vomiting, and of fecal incontinence, his younger brother called an ambulance, and the subject was brought to our hospital an hour after ingesting the poison. On admission, he complained of throat pain and exhibited a normal level of consciousness. His blood pressure was 116/68 mmHg, heart rate 100 beats/min, and no neurological symptoms were evident. The results of blood examinations and renal function tests were within normal limits (serum creatinine, 0.7 mg/dl). The contents of the stomach were aspirated through a nasal tube, but turned out to be scanty, and so neither gastric lavage nor administration of activated charcoal was performed. Intravenous fluid infusion was conducted to maintain the daily urine volume above 2000 ml. Diuretics were not used. Eighteen and a half hours after ingestion, the respiration rate slowed, but the patient was fully conscious. At the 26th hour after ingestion, his respiration was seen to be seriously inhibited. He was sedated by continuous infusion of midazolam (5.8 mg/h), and ventilatory support was initiated. After 4 d of artificial ventilation, midazolam was discontinued, and he was extubated. He has shown no neurological symptoms since that time, and his clinical course was uneventful. He was discharged without any sequelae after 11 d of hospitalization. His serum creatinine and urea nitrogen were within the normal range throughout his hospitalization.

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MATERIAL AND METHODS

**Enantioselective Analysis** The selective quantification of the dl-GLUF enantiomers was done by the HPLC method we established, which uses precolumn derivatization with chiral reagents. The serum specimen was diluted 1:15 by the process of deproteinization, the cerebrospinal fluid specimen was diluted 1:2 by the same process, and the urine sample was diluted 1:15, 1:150, 1:1500 or 1:15000 by that process according to the concentration of GULP that it contained. Then, samples were derivatized to diastereomers with (+)-1-(9-fluorenyl) ethyl chloroformate (FLEC, Sigma Chemical, St. Louis, MO, U.S.A.) and were subjected to HPLC analysis under the following conditions.

**HPLC Conditions** The HPLC instrument (SCL10AVP; Shimadzu, Kyoto, Japan) was adjusted so that the mobile phase of 10 mM ammonium acetate (pH 5.0)/acetonitrile at a ratio of 77/23 had a flow rate of 0.8 ml/min. This mobile phase was retained for 10 min, and then changed to 50/50 until the 16th minute, to 20/80 until the 19th minute, and finally back to 77/23 at the 28th minute. Infused samples were separated on an Inertsil ODS 2 column (4.6 mm × 150 mm, 5 μm particle size; GL Sciences Inc., Tokyo, Japan) at 40 °C. Fluorescence detection (model RF-10AXL, Shimadzu) was carried out at an excitation wavelength of 260 nm and an emission wavelength of 305 nm. Quantitation was performed by the absolute calibration curve method using analysis software (Class VP; Shimadzu).

**GC-MS Analysis** Using a slightly improved form of the GC-MS method that we established, we carried out identification of the dl-GLUF in the cerebrospinal fluid as follows: after the addition of dl-2-amino-3-phosphonopropionic acid (Aldrich Chemical Co., Milwaukee, WI, U.S.A.), and with deproteinized cerebrospinal fluid sample as the internal standard solution, dl-GLUF in the sample was solid-phase extracted using an Isolute® HAX 100 mg cartridge (International Solvent Technology Ltd., Mid Glamorgan, U.K.), subjected to tert-butyldimethylsilyl (t-BDMS) derivatization using N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and catalyzed by dimethyl formamide. Then, the t-BDMS derivative of dl-GLUF was analyzed under the conditions stated below.

**GC-MS Conditions** A Shimadzu GC 17A gas chromatograph/QP 5050A mass spectrometer was employed with a Shimadzu Class 5000 computer system. Chromatographic conditions for these analyses were: a DB-1 fused-silica capillary column (15 m × 0.25 mm I.D., 0.25 μm film thickness; J&W Scientific, Folsom, CA, U.S.A.); helium carrier gas at 1 ml/min; GC oven temperature programme, 80 °C (hold 2 min), 15 °C/min to 300 °C (hold 5 min); injector temperature, 300 °C; split/splitless injector, splitless mode for 2 min, and injection volume, 1 μl. The mass spectrometer was operated in the electron impact (EI) mode. EI ionization was employed at 70 eV with an electron multiplier set at 1200 V in full scan operation mode for peak identification. Manifold temperature was 280 °C.

**Toxicokinetic Analysis** The patient sera used for analyses were collected 1, 3, 27 and 35 h after the poison was ingested, and the collection of cerebrospinal fluid was carried out 27 h after the ingestion. In our earlier report analyzing the kinetics of the elimination of dl-GLUF enantiomers from the blood we stated that d-GLUF is eliminated by the 1-compartment model, and l-GLUF by the 2-compartment model, so we calculated the toxicokinetic parameters in accordance with these models. However, since it was judged that, in the present patient, there were limits to the drawing of blood and that there would consequently be insufficient blood for calculating the kinetic parameters, these calculations were not performed.

The amounts of dl-GLUF secreted into the urine collected at 1, 3, 26.5, 50.5, 74.5 and 98.5 h after poison ingestion was calculated, and these amounts were totaled to obtain the overall amount of dl-GLUF contained.

RESULTS AND DISCUSSION

The serum dl-GLUF enantiomer concentrations and the time elapsed since ingestion are shown in Fig. 1.

One hour after ingestion, the concentration of d-GLUF was 191.1 μg/ml, almost the same as that of l-GLUF (192.5 μg/ml). In a preliminary experiment, we had confirmed that the BASTA fluid that the patient swallowed contained these two enantiomers in equal proportions. It was thus suggested that there is no difference in the absorption of the two enantiomers from the digestive tract. However, the d-GLUF level measured 3 h after ingestion was 60.3 μg/ml and that of l-GLUF, 52.3 μg/ml, so that the former had now become greater; and 27 and 35 h after ingestion, the d-GLUF level was still higher than the l-GLUF level.

In our earlier report, the patient who drank 100 ml of BASTA as in the present case exhibited fast elimination of d-GLUF in the first 24 h according to the one-compartment model of elimination kinetics, whereas the l-GLUF elimination followed the two-compartment model. Thus, the elimination half-life of l-GLUF was longer than that of d-GLUF. We theorized, on the basis of the longer elimination half-life of l-GLUF, that this substance was involved in the delayed central nervous symptoms. However, in the present subject, it cannot be said that the elimination half-life of l-GLUF was longer. For a study of the difference between the elimination half-lives of the dl-GLUF enantiomers, it will be necessary to accumulate data on more such patients.

In the previously reported case, the AUC for l-GLUF was 1278 μg/ml, and that for d-GLUF, 1593 μg/ml, which is 1.2 times the l-GLUF figure. The AUC for the present patient was not calculated, but the concentration of d-GLUF was always higher, which suggests that there is no inconstancy here. In addition, the total amounts of urinary excretion 74.5 h after poison ingestion for d-GLUF and l-GLUF were 2835 mg and 2298 mg, respectively, so that more d-GLUF was excreted as compared with l-GLUF.

![Fig. 1. Changes in Serum Concentrations of dl-GLUF Enantiomers in a Patient Who Ingested Basta Fluid](image-url)
was excreted in the urine. Over 98.5 h after ingestion, the concentrations of both D-GLUF and L-GLUF were below the detection limit (5 ng/ml in a specimen\(^8\)). Finally, the total excretion of D-GLUF by 26.5 h was 95.9% and that of L-GLUF, 96.3%, and these figures correspond well with the 97% total urinary excretion of DL-GLUF reported in an analysis of both enantiomers by Hirose et al.\(^5\) for the first 24 h after BASTA ingestion. A question that arises is, what happened to the L-GLUF that was not recovered from the urine, although the same amount was absorbed from the digestive tract? In the rat, DL-GLUF is slightly metabolized to DL-3-methylphosphinicopropionic acid (MPPA)\(^1\); and we have confirmed the presence of MPPA in the serum of a patient who ingested BASTA fluid.\(^6\) It may be that L-GLUF is involved in the metabolism to MPPA. Alternatively, an idiosyncratic distribution of L-GLUF to certain tissues may be occurring.

In order to ascertain whether the patient had any encephalitis, we collected cerebrospinal fluid from him 27 h

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**Fig. 2.** Typical Mass Chromatogram (Total Ion Mode (A) and SIM Mode (B)) of \(t\)-BDMS Derivatives of an Extract from the Cerebrospinal Fluid of a Patient with Acute GLUF Poisoning, and Its EI Mass Spectra of \(t\)-BDMS Derivatives of GLUF (C)

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**Fig. 3.** Separation of the \(\alpha\)-GLUF Enantiomers

A: Chromatogram of the FLEC derivatives of \(\alpha\)-GLUF and \(\beta\)-GLUF in a standard enantiomer solution containing 0.4 \(\mu\)g/ml of each. B: Typical chromatogram of the FLEC derivative of cerebrospinal fluid from a patient with acute \(\alpha\)-GLUF poisoning. The cerebrospinal fluid was diluted 1:2 in the process of deproteinization. The concentrations of the two enantiomers in the cerebrospinal fluid were calculated to be: \(\alpha\)-GLUF, 0.48 \(\mu\)g/ml; and \(\beta\)-GLUF, 0.12 \(\mu\)g/ml.
after BASTA ingestion. We also carried out an analysis of GC-MS using the remainder of the cerebrospinal fluid, and found that there was some DL-GLUF in it (Fig. 2). t-BDMS-derivatized DL-GLUF gives the base ion at \( m/z \) 466. Also, by SIM mode chromatography using the \( m/z \) 466 ion, the peaks of DL-GLUF were observed at their corresponding retention times. In addition, it was confirmed by the full scan mass fragment that these peaks were those of DL-GLUF. With this method, simultaneous analysis of MPPA in the cerebrospinal fluid is possible, but it was not detected in this fluid. (The detection limit of MPPA in samples is 1 ng/ml\(^9\).)

The present study is the first report to clarify the transfer of DL-GLUF enantiomers to the cerebrospinal fluid in man. Figure 3 presents HPLC chromatograms obtained using this cerebrospinal fluid and showing selective separation of the DL-GLUF enantiomers. Chromatogram (A) shows the peaks of D-GLUF and L-GLUF obtained from analysis of a standard solution of 0.4 \( \mu g/ml \) of each enantiomer; (B) is a chromatogram of the patient’s cerebrospinal fluid diluted 1 in 2 by the process of deproteinization, and quantifies the D-GLUF at 0.48 \( \mu g/ml \) and the L-GLUF at 0.12 \( \mu g/ml \). In our earlier study,\(^8\) in which we analyzed the contents of DL-GLUF enantiomers in the serum, we speculated that it was the L-GLUF that migrated from the blood into the tissues. However, as a result of the present analysis, we realized that both D-GLUF and L-GLUF enter the cerebrospinal fluid. Also, the concentrations in the blood collected at the same time as the cerebrospinal fluid were 1.44 \( \mu g/ml \) of D-GLUF and 0.35 \( \mu g/ml \) of L-GLUF, so that high proportions of both enantiomers were present: approximately one-third of the levels in the blood collected simultaneously. The molecular weight of GLUF is small, 198.2, but, since it is a highly polarized compound, it is believed not to pass readily through the blood-brain barrier.\(^10\) It is considered unlikely that one-third of the concentrations of both DL-GLUF enantiomers are always transferred into the cerebrospinal fluid. It is more probable that there is some specific transport system at work in such circumstances.

It is considered on the basis of clinical observation that the central nervous symptoms due to DL-GLUF show that its action on this system causes reciprocation between inhibition and excitation. DL-GLUF is known to inhibit glutamine synthetase activity in vitro\(^13\); and it has been suggested that it acts on the metabolic pathway of glutamic acid and the glutamic acid receptors in the brain.\(^10\) In the present case, 26 h after poison ingestion, when most of the DL-GLUF enantiomers had been excreted in the urine, respiratory depression took place. The delayed central nervous symptoms were perhaps caused by the small amount of GLUF that had entered the brain from the blood. Our work from this point on is to collect more cases of this type in which the concentrations of D-GLUF and L-GLUF in the blood and cerebrospinal fluid have been measured over time and to determine the detailed kinetic parameters from those data. Further, using those findings, it may be possible to clarify aspects of the toxicokinetics of DL-GLUF that are not yet understood.

REFERENCES