Five Cases of Aconite Poisoning: Toxicokinetics of Aconitines

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Abstract

Aconite poisoning was examined in five patients (four males and one female) aged 49 to 78 years old. The electrocardiogram findings were as follows: ventricular tachycardia and ventricular fibrillation in case 1, premature ventricular contraction and accelerated idioventricular rhythm in case 2, AIVR in case 3, and nonsustained ventricular tachycardia in cases 4 and 5. The patient in case 1 was given percutaneous cardiopulmonary support because of unstable hemodynamics, whereas the other patients were treated with fluid replacement and antiarrhythmic agents. The main aconitine alkaloid in each patient had a half-life that ranged from 5.8 to 15.4 h over the five cases, and other detected alkaloids had half-lives similar to the half-life of the main alkaloid in each case. The half-life of the main alkaloid in case 1 was about twice as long as the half-lives in the other cases, and high values for the area under the blood concentration-time curve and the mean residence time were only observed in case 1. These results suggest that alkaloid toxicokinetics parameters may reflect the severity of toxic symptoms in aconite poisoning.

Introduction

Aconite is a well-known toxic plant of the genus Aconitum in the Ranunculaceae family. Aconite contains aconitine alkaloids such as aconitines, benzoylconines and aconines, and the aconitines (aconitine, hypaconitine, jesaconitine, and mesaconitine) are the causative agents of aconite poisoning, since the toxicity of aconitines is much stronger than that of other alkaloids (1). The LD₅₀ value of aconitine in mice is reported to be 1.8 mg/kg when given orally (1), and the lethal dose of aconitine for humans is estimated to be 1–2 mg (2,3). Similar cases have been reported in Europe and the United States, although aconite-poisoning cases are relatively rare in these regions (12–14). Various toxic symptoms, such as nausea, vomiting, numbness and palsy of the extremities, and arrhythmia, are observed in aconite poisoning, with arrhythmia being the most typical symptom. Aconite poisoning often results in death due to cardiac arrest caused by a fatal arrhythmia such as ventricular fibrillation (VF), and therefore an understanding of arrhythmias caused by aconite poisoning is of importance.

There have been many pharmacological studies of aconitines (1,15–21), and the toxicity of these molecules is known to be due to their action on sodium channels in excitable membranes (16–19). In contrast, the toxicokinetics of aconitines in humans have only been examined in two reports: a study of the distribution of aconitine alkaloids by Ito et al. (22) and measurement of the half-life of aconitine by Moritz et al. (14).

Establishment of pharmacokinetics (toxicokinetics) parameters in aconite poisoning would be useful for improvement of clinical treatment of poisoning cases. Such parameters include half-life, area under the blood concentration-time curve (AUC), clearance (CL), volume of distribution, and mean residence time (MRT). These parameters are typically used in dosage regimen design: the half-life may be used to determine the interval between doses, and CL can be used to achieve a target AUC that is appropriate in terms of efficacy and toxicity; CL is used, for example, in the formula-based calculation of the dosage of the anticancer agent carboplatin (23).

We have developed a new method for rapid analysis of aconitines in serum and urine, using liquid chromatography–mass spectrometry (LC-MS) (24). The method was applied to the determination of aconitines in serum collected from five patients with aconite poisoning who were admitted to the Critical Care and Emergency Center at Iwate Medical University. In this paper, we report the toxicokinetics parameters of aconitines calculated using serial serum concentrations from the five patients.
Methods

Patients
A summary of background data for the five patients with aconite poisoning is shown in Table I. The patients comprised four males and one female, with ages ranging from 49 to 78 years old and a mean age (± standard deviation) of 61.4 ± 11.9 years old. One of the patients had ingested aconite leaves and four patients had eaten roots. The electrocardiogram findings were as follows: ventricular tachycardia (VT) and Vf in patient 1, premature ventricular contraction (PVC) and accelerated idioventricular rhythm (AIVR) in patient 2, AIVR in patient 3, and non-sustained ventricular tachycardia (NSVT) in patients 4 and 5. All the patients recovered from the poisoning, with case 1 requiring additional percutaneous cardiopulmonary support (PCPS) because of unstable hemodynamics.

Reagents
Aconitine, mesaconitine, and hypaconitine were purchased from Wako Pure Chemical Industries (Osaka, Japan);jesaconitine was purchased from Sanwa Shoyaku (Utsunomiya, Japan); and methyllycaconitine was purchased from Sigma-Aldrich (St. Louis, MO) and used as an internal standard (I.S.). Formic acid of high-performance liquid chromatography (HPLC) grade and acetonitrile of LC–MS grade were purchased from

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<th>Table I. Profiles of Patients with Aconite Poisoning</th>
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* Abbreviations: ECG, electrocardiogram; VT, ventricular tachycardia; DF, defibrillation; Vf, ventricular fibrillation; PCPS, percutaneous cardiopulmonary support system; PVC, premature ventricular contraction; AIVR, accelerated idioventricular rhythm; and NSVT, nonsustained ventricular tachycardia.

Figure 1. Selected ion-monitoring chromatograms of aconitines. Selected ions for aconitine: m/z 646; hypaconitine: m/z 616; jesaconitine: m/z 676; mesaconitine: m/z 632; and methyllycaconitine (I.S.): m/z 683. Blank serum (A); spiked serum (1.0 ng/mL) (B); patient sample (case 1): 7 h after ingestion (C-1); and patient sample (case 1): 62 h after ingestion (C-2).
Kanto Kagaku (Tokyo, Japan). Other reagents were of analytical reagent grade and were purchased from Wako Pure Chemical Industries.

Sample preparation and equipment
One milliliter of serum was mixed with 2.5 ng of I.S. and 2 mL of 0.025% (v/v) ammonia solution. The mixture was applied to an Extrelut NT3 column (Merck, Darmstadt, Germany) and the column was left to stand for 15 min at room temperature. Aconitines were then eluted with 15 mL of diethyl ether. The eluate was evaporated to dryness under a stream of nitrogen gas in a heat block at 40°C and dissolved in 0.2 mL of the mobile phase. The serum samples were stored at --80°C and measurements were made within three days after sample collection.

LC was performed on a Waters 2690 instrument (Waters, Milford, MA). Separation was achieved using an XTerra RP18 column (150 x 2.1-mm i.d., 3.5-μm particle size, Waters) protected by a guard column (10 x 2.1-mm i.d.) containing the same packing. The injection volume was set to 20 μL, and the mobile phase was a mixture of 0.1% (v/v) formic acid in acetonitrile and 0.1% (v/v) formic acid in aqueous solution (24:76, v/v), with a flow rate of 0.2 mL/min. The column temperature was maintained at 40°C.

MS was performed on a Micromass ZMD 4000 instrument (Waters) operating in the electrospray ionization positive ion mode. The MS parameters were as follows: capillary potential, 3.0 kV; cone voltage, 60 V; ion source temperature, 120°C; drying gas temperature, 350°C; and nitrogen gas flow rate, 350 L/h. Measurements were carried out in the selected ion monitoring mode, and the mass-to-charge values of the monitoring ion were 646 for aconitine, 616 for hypaconitine, 676 for jesaconitine, 632 for mesaconitine, and 683 for methyllycaconitine (I.S.). The monitoring ion reflects the protonated alkaloid and formed the main peak for each alkaloid. No interfering endogenous substances were observed in blank serum samples.

Toxicokinetics parameters of aconitines
Concentrations of serum aconitines were plotted logarithmically against time after ingestion, and a serum concentration-time curve was obtained. The time of ingestion of aconite is shown as 0 h, and the time after ingestion was obtained from the patient or their family. Regression analysis of these data was performed for each patient, using at least three concentration-time data points in the terminal log-linear phase. The elimination velocity constant (K\text{el}) was calculated as K\text{el} = \text{slope}, where the slope is that of the regression line. Serum half-lives for each alkaloid were calculated using the following equation: half-life = 0.693/K\text{el}. AUC (from 0 to infinity) was calculated using the logarithmic trapezoidal method with extrapolation to infinity, and MRT was calculated as MRT = AUMC/AUC, where AUMC is the area under the moment curve from 0 to infinity.
Results

Aconitine, jesaconitine, and mesaconitine were detected in serum in cases 1, 2 and 5, and aconitine and jesaconitine were found in case 4. Jesaconitine was the main alkaloid (that is, the alkaloid with the highest serum concentration) in cases 1, 2, 4, and 5. In case 3, the main alkaloid was mesaconitine, and hypaconitine was detected only in case 3. Chromatograms for blank serum, spiked serum and a patient sample (case 1) are shown in Figure 1. Logarithmic serum concentrations of aconitines were plotted against time after ingestion for each case (Figure 2). The log concentration-time curves showed good linearity from 20 h after ingestion in case 1, from 8 h after ingestion in case 2, from 6 h after ingestion in case 3, from 12 h after ingestion in case 4, and from 5 h after ingestion in case 5.

The toxicokinetics parameters for aconitines obtained from the log concentration-time curves are shown in Table II. In case 1, the $K_e$ values for aconitine and jesaconitine (the main alkaloid) were 0.039 h$^{-1}$ and 0.045 h$^{-1}$, respectively, and the half-lives were 17.8 h and 15.4 h, respectively. In case 2, the $K_e$ values for aconitine, jesaconitine (the main alkaloid) and mesaconitine were 0.186 h$^{-1}$, 0.119 h$^{-1}$, and 0.249 h$^{-1}$, respectively, and the half-lives were 3.7 h, 5.8 h, and 2.8 h, respectively. In case 3, the $K_e$ values for aconitine and mesaconitine were 0.087 h$^{-1}$ and 0.120 h$^{-1}$, respectively, and the half-lives were 8.0 h and 5.8 h, respectively. In case 4, the $K_e$ for jesaconitine (the main alkaloid) was 0.106 h$^{-1}$, and the half-life was 6.5 h. In case 5, the $K_e$ values for aconitine and jesaconitine (the main alkaloid) were 0.086 h$^{-1}$ and 0.085 h$^{-1}$, and the half-lives were 8.1 h and 8.2 h, respectively.

In case 1, the AUCs for aconitine and jesaconitine (the main alkaloid) were 51.1 ng·h/mL and 28.6 ng·h/mL, respectively, and the MRTs were 23.6 h and 22.6 h, respectively. In case 2, the AUCs for aconitine, jesaconitine (the main alkaloid) and mesaconitine were 3.1 ng·h/mL, 33.5 ng·h/mL and 13.0 ng·h/mL, respectively, and the MRTs were 10.8 h, 12.8 h and 9.7 h, respectively. In case 3, the AUCs for aconitine and mesaconitine (the main alkaloid) were 24.4 ng·h/mL and 5.4 ng·h/mL, respectively, and the MRTs were 14.8 h and 11.9 h, respectively. In case 4, the AUC for jesaconitine (the main alkaloid) was 6.9 ng·h/mL, and the MRT was 17.2 h. In case 5, the AUCs for aconitine and jesaconitine (the main alkaloid) were 2.7 ng·h/mL and 26.1 ng·h/mL, respectively, and the MRTs were 10.9 h and 11.5 h, respectively.

Discussion

In this study, we determined toxicokinetics parameters (half-life, AUC, MRT) for aconitines in five patients with aconite poisoning. Ito et al. (22) have studied the distribution of aconitine alkaloids in an autopsy case, and Moritz et al. (14) determined the half-life of aconitine in a poisoning case, but the current study is the first report of the toxicokinetics parameters of aconitines in patients with aconite poisoning. The half-lives of the main alkaloid ranged from 5.8 h to 15.4 h in the five patients, and the half-lives of other detected alkaloids were similar to the half-life of the main alkaloid in each case. The half-life of hypaconitine was not calculated, but it is likely that this value would have been similar to that of other alkaloids in the same case because all aconitines detected in each case had similar half-lives and aconitine alkaloids have closely related molecular structures.

After a period of four times the half-life, 93.75% of the original amount of an agent has been eliminated from the body. Therefore, our results suggest that the main alkaloid is eliminated from the body over a period of 23–62 h. However, it has been reported that aconitines can be detected in urine until 7 days after ingestion, but that they are not detectable in serum 24 h after ingestion (10). Similarly, aconitines were detectable in urine in our cases after they became undetectable in serum (data not shown). Aconitines are distributed in high concentration to the liver and kidney (22), and therefore it appears that aconitines mainly migrate to the liver and kidney after absorption, and are then eliminated gradually from the body.

The most important pathway appears to be via the kidney, given the detection of high concentrations of aconitines in urine (8,10) and the continuous detection of aconitines in urine after intoxication (10). Therefore, deterioration in renal function may lead to a delay in excretion of aconitines, and a corresponding increase in half-life. Among our patients, the half-life of the main alkaloid in case 1 was about 2 times longer than those in the other cases, but the renal function of the patient in case 1 was considered to be normal, since clinical data for serum creatinine and blood urea nitrogen were in the normal range. These results suggest that the prolonged half-life in case 1 was not due to deterioration of renal function. However, it is of note that cardiac output in case 1 was lower than that in the other cases, due to hemodynamic instability, and the patient required PCPS; therefore, the prolonged half-life in case 1 may have been due to decreased cardiac output, rather than to deterioration of renal function.

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* Abbreviations: $K_e$, elimination velocity constant; AUC, area under the blood concentration-time curve; and MRT, mean residence time.

1 Main alkaloid: the alkaloid with the highest serum concentration among the detected aconitines.
The AUC of the main alkaloid was larger in cases 1, 2, and 5 compared to cases 3 and 4, whereas the MRT of the main alkaloid showed a tendency to prolong in cases 1 and 4 compared with the other cases. Therefore, only case 1 showed high values for both AUC and MRT. Because AUC reflects the amount of exposure and MRT reflects the time of exposure, these results suggest that a larger concentration of aconitines were acting on sodium channels for a longer time in case 1, compared to the other cases. In animal experiments, it has been reported that the toxic symptoms in aconite poisoning are dependent on the dose of aconitines (25), and our findings suggest that the extent of toxic symptoms is affected not only by amount of exposure to aconitines but also by time of exposure.

The AUC values in cases 3 and 4 were low compared with the other cases. The patient in case 4 was the only one to ingest leaves of the plant. The content of aconite alkaloids varies according to the part of plant, growing regions, the season (26-33), and the level of aconitines in roots was about 10 species (26-33), and the content in roots is higher than that of stems and leaves; the level of aconitines in roots was about 10 times that in stems (30), and about 300 times that in leaves (29). Therefore, our results suggest that the AUC in case 4 reflects the lower alkaloid content of leaves. The patient in case 3 had ingested roots, but the AUC in this patient was low compared to other cases of root ingestion. Furthermore, in case 3 the main alkaloid differed from that in the other cases, and this was also the only one in which hypaconitine was detected and in which jesaconitine was not detected. The levels of aconitine, hyaconitine, jesaconitine, and mesaconitine in aconite vary according to the part of plant, the growing region, the aconite species, and the season (26-33); therefore our results suggest that the alkaloid level and composition in case 3 was influenced by one or more of these variables.

Overall, the calculated toxicokinetics parameters may give an indication of the severity of toxic symptoms in aconite poisoning; however, a mechanistic study and calculation of toxicokinetics parameters in a larger numbers of patients are needed for full establishment of the relationship between toxic symptoms and toxicokinetics.

References

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