

Development of an enzyme immunoassay system for mesaconitine and its application to the disposition study on mesaconitine

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Abstract

For the purpose of quantitative determination of mesaconitine, an extremely toxic and major alkaloid in aconite tuber, a highly sensitive enzyme immunoassay (EIA) was developed as follows; Firstly, a hapten [*N*-desethylaconitine *N*-glutarate (DEAG)] was synthesized by introduction of a glutaryl group to the nitrogen atom of aconitine after elimination of an *N*-ethyl group. Subsequently it was coupled with bovine serum albumin (BSA) and β -galactosidase (β -Gal) to give an immunogen (DEAG-BSA) and a labeled antigen (DEAG- β -Gal), respectively. After immunization of female albino rabbits with DEAG-BSA for six months to elicit a polyclonal antiserum (As-DEAG), the experimental conditions were optimized for a highly sensitive EIA. At 10^3 -fold dilution of DEAG- β -Gal and 2.5×10^4 -fold dilution of As-DEAG, the assay exhibited a linear range of 0.005–5 ng/tube for mesaconitine. Under the same conditions, the antiserum had a 24.4 % cross-reaction with aconitine and virtually no reaction with benzoylmesaconine.

Next, the EIA method was applied to the disposition study on mesaconitine to monitor the plasma concentration after oral administration of mesaconitine at 1 mg/kg to rats, its low bioavailability being suggested from $C_{\max}$ and AUC values obtained.

Key words aconite tuber, aconitine, benzoylmesaconine, enzyme immunoassay, mesaconitine, pharmacokinetics.

Abbreviations As-DEAG, antiserum prepared by immunization of a DEAG-BSA conjugate; BSA, bovine serum albumin; DCC, *N,N'*-dihexylcarbodiimide; DEA, *N*-desethylaconitine; DEAG, *N*-desethylaconitine *N*-glutarate; EIA, enzyme immunoassay; DMF, *N,N*-dimethylformamide; FAB MS, fast atom bombardment mass spectrometry; β -Gal, β -galactosidase; LC/MS/MS, liquid chromatography/mass spectrometry/mass spectrometry; NHS, *N*-hydroxysuccinimide; NMR, nuclear magnetic resonance.

Introduction

The roots of aconite, such as *Aconitum carmichaeli* DEBX. and *A. japonicum* THUNB., and so on, are one of the most important Chinese medicines, and contain a series of neuro and cardiotoxic diterpenoid alkaloids, such as mesaconitine (**1**), aconitine (**6**), and hypaconitine. There are many reports that ingestion of aconite plant extracts can lead to severe or even fatal toxic effects, commonly known as herb-induced aconitine poisoning, and sometimes, they are viciously used as poisons for homicide or suicide.^{1–3)} On the other hand, these alkaloids, es-

pecially mesaconitine, have shown many valuable pharmacological activities as well, such as analgesic, antinociceptive, and vasorelaxing effects.^{4–17)} So if these alkaloids present in the traditional prescription are carefully monitored, they can save lives rather than take lives. Bearing this consideration in mind, it is indispensable to develop an analytical method to monitor the exact quantity of these compounds in biological fluids. Until now, various techniques have been established for determination of aconitine type alkaloids, such as capillary electrophoresis, LC/MS/MS, and capillary liquid chromatography/frit FAB-MS.^{18–24)} However, a time-consuming pretreatment is inevitable, together with lay-

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ing the costly instrumentation for analysis of the biological samples. In comparison with the above-mentioned analytical methods, the enzyme immunoassay (EIA) method outstrips all others, due to its appealing advantages of specificity, simplicity and sensitivity.

We have developed highly sensitive and selective EIA for aconitine, and studied disposition of aconitine by using this analytical method,²⁵⁾ in which two spacers consisting of four and fourteen carbon chains have been introduced to C-8 of aconitine by an ester-exchange reaction to make haptens. However, the designing of the most suitable haptens remains to be a critical step for production of efficient antibodies. In particular, the different position of the spacer attachment has been proven to have a profound influence on selectivity of EIA. In the current experiment, the heterocyclic nitrogen atom incorporated in the highly fused ring systems attracted our attention. Aconitine and mesaconitine differ from each other in an alkyl group on the tertiary amine (a methyl group for mesaconitine and an ethyl group for aconitine), in which a spacer was attached to the nitrogen atom after removal of the alkyl group. The resultant EIA showed high sensitivity to mesaconitine, which is the most potent toxic and has the highest pharmacological activities among aconitine alkaloids.^{8, 26)} We investigated the performance of the EIA and its application.

Materials and Methods

Apparatus: Ultraviolet spectra were recorded by a Shimadzu UV-2200 spectrophotometer. ¹H and ¹³C-NMR and 2D NMR experiments were run by a JNM-LA 400 WB Lambda (Joel) NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Fluorometry was measured with a Shimadzu RF-5000 spectrofluorometer.

Chromatography: Thin-layer chromatography was carried out with precoated silica gel plates (0.25 mm thickness, Kieselgel F₂₅₄, Merck, Darmstadt, FRG), and detected under UV light and by spraying with H₂SO₄-10% anisaldehyde reagent followed by heating. Column chromatography was performed on silica gel, Sephadex LH-20 or Sepharose 6B for different purposes.

Chemicals: Aconitine, the goat antiserum to rabbit IgG, BSA, complete and incomplete Freund's adjuvants were purchased from Sigma-Aldrich Inc. β -Galactosidase

from *Escherichia coli* was a product of Roche Co. Unless otherwise stated, all the other organic and inorganic chemicals were obtained from Nacalai Tesque Inc. Besides, buffer A was designated for a 0.02 M phosphate buffered saline (pH 7.0) containing 0.1% BSA, 0.1% NaN₃ and 0.001% MgCl₂, while buffer C was designated for a 0.02 M phosphate buffer saline (pH 7.0) containing 0.1% NaN₃ and 0.001% MgCl₂. Nunc-immuno tubes (Nalge-Nunc International) were used exclusively throughout the EIA experiment.

Synthesis of N-desethylnaconitine (DEA, 2): According to the method of Tsuda *et al.*^{27,28)} an acetone solution (0.9 ml) of 60 mg aconitine was treated with the same amount of KMnO₄ in 0.9 ml of acetone-H₂O (1:1) for 10 min at room temperature. Subsequently, the reaction was quenched by addition of H₂SO₃ to decompose an excess of KMnO₄. After evaporation of acetone under reduced pressure, the remaining water layer was adjusted to pH 8-9 with 1N NH₄OH and extracted 3 times with CHCl₃. The CHCl₃ extract was subjected to silica gel chromatography eluting with NH₃·H₂O saturated CHCl₃ to afford a colorless substance (yield 75.6 %). ¹H NMR (400 MHz, CDCl₃): δ 8.03 (2H, dd, *J*=7.8, 1.5 Hz, H-Bz-3 and -7), 7.58 (1H, t, *J*=7.8 Hz, H-Bz-5), 7.46 (2H, t, *J*=7.8 Hz, H-Bz-4 and -6), 4.88 (1H, d, *J*=5.4 Hz, H-14), 4.49 (1H, d, *J*=5.4 Hz, H-15), 4.07 (1H, d, *J*=7.1 Hz, H-6), 3.83 (1H, dd, *J*=9.9, 5.6 Hz, H-3), 3.75, 3.32, 3.29, 3.16 (3H each, s, OCH₃ $\times$ 4), 3.62, 3.58 (1H each, d, *J*=9.3 Hz, H_{a,b}-18), 3.36 (1H, d, *J*=5.4 Hz, H-16), 3.22 (1H, dd, *J*=5.8, 8.8 Hz, H-1), 2.84 (1H, t, *J*=9.2 Hz, H-9), 2.78 (1H, s, H-17), 1.36 (3H, s, OAc-CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 81.4 (C-1), 34.8 (C-2), 71.3 (C-3), 43.8 (C-4), 50.8 (C-5), 83.3 (C-6), 43.4 (C-7), 91.5 (C-8), 47.2 (C-9), 40.8 (C-10), 49.3 (C-11), 34.7 (C-12), 74.1 (C-13), 79.0 (C-14), 78.8 (C-15), 89.8 (C-16), 55.8 (C-17), 76.7 (C-18), 40.9 (C-19), 61.2, 59.2, 57.7, 55.8 (C-OCH₃ $\times$ 4) 172.2 (C₈-OOCCH₃), 21.4 (C₈-OOCCH₃), 166.1 (C-Bz-1), 129.8 (C-Bz-2), 129.6 (C-Bz-3 and 7), 128.7 (C-Bz-4 and 6), 133.4 (C-Bz-5).

Synthesis of N-desethylnaconitine N-glutarate (DEAG, 3): An anhydrous CHCl₃ solution (3.5 ml) of 30.0 mg DEA was mixed with 11.1 mg of glutaric anhydride. After stirring at room temperature for 4 h, CHCl₃ was evaporated in vacuum and the reaction mixture was subjected to silica gel column chromatography eluting with CHCl₃-MeOH (49:1) to afford a colorless substance

(yield 90.2%). ^1H NMR (400 MHz, CDCl_3): δ 8.02 (2H, d, $J=7.6$ Hz, H-Bz-3 and -7), 7.57 (1H, t, $J=7.6$ Hz, H-Bz-5), 7.45 (2H, t, $J=7.6$ Hz, H-Bz-4 and -6), 4.87 (1H, d, $J=4.1$ Hz, H-14), 4.47 (1H, d, $J=4.4$ Hz, H-15), 4.04 (1H, m, H-17), 3.75, 3.30, 3.24, 3.17 (3H each, s, $\text{OCH}_3 \times 4$), 3.58 (2H, d, $J=6.6$ Hz, $\text{H}_{a,b}$ -18), 2.87 (1H, overlapped, H-9), 2.75 (1H, s, H-17), 1.36 (3H, s, OAc-CH_3). ^{13}C NMR (100 MHz, CDCl_3): δ 80.2 (C-1), 32.8 (C-2), 72.1 (C-3), 43.5 (C-4), 51.2 (C-5), 83.3 (C-6), 43.5 (C-7), 90.8 (C-8), 46.1 (C-9), 40.5 (C-10), 49.3 (C-11), 35.3 (C-12), 74.1 (C-13), 78.7 (C-14), 78.5 (C-15), 90.2 (C-16), 56.2 (C-17), 76.7 (C-18), 40.9 (C-19), 61.2, 58.9, 58.1, 55.5 ($\text{C-OCH}_3 \times 4$), 172.0 ($\text{C}_8\text{-OOCCH}_3$), 21.3 ($\text{C}_8\text{-OOCCH}_3$), 166.0 (C-Bz-1), 129.7 (C-Bz-2), 129.6 (C-Bz-3 and 7), 128.7 (C-Bz-4 and 6), 133.3 (C-Bz-5).

N-hydroxysuccinimide ester of N-desethylaconitine N-glutarate (DEAG, 3a): A free COOH group of the glutaryl moiety was activated by an NHS method as usual. A DMF solution (2 ml) of DEAG (29.1 mg) was mixed with 9.2 mg of NHS for 30 min in an ice bath. After dropwise addition of 16.5 mg DCC in 1 ml of DMF, the reaction mixture was stirred at room temperature for 24 h. The reaction was monitored by formation of white precipitates of *N,N'*-dicyclohexylurea and further confirmed by appearance of a new spot due to **3a** and disappearance of that of **3** on TLC plates. With respect to the high instability of **3a**, it was not further purified and used directly for coupling with BSA and β -Gal after centrifugation to remove the precipitates.

Synthesis of a DEAG-BSA conjugate: A grossly 39.8 μmol compound **3a** was added dropwise to 3 ml of a phosphate buffer solution (0.05 M, pH 7.5) of BSA (52.9 mg) and the reaction mixture was gently stirred for 24 h at 4°C . The resultant turbid solution was dialyzed exhaustively against phosphate buffer (0.05 M, 0.03 M and 0.01 M) and distilled water for a total of eleven days. Subsequently, the BSA conjugate in the dialysis tube was lyophilized and kept at -20°C until use.

Preparation of a polyclonal antiserum (As-DEAG): Three female albino rabbits, around 2 kg each, were subcutaneously injected with an emulsion prepared by 2 mg DEAG-BSA in 2 ml of saline and the same volume of complete Freund's adjuvant. After two weeks, the rabbits were boosted with a half amount of DEAG-BSA in 1 ml of saline emulsified with 1 ml of incomplete Freund's adjuvant at two weeks interval for three months

and once per three weeks for another three months. Ten days after the last injection, blood was collected from the abdominal veins of the rabbits. After standing at room temperature for 2 h, coagulum was removed by centrifugation at $94 \times g$ for 20 min. The antisera were labeled As-DEAG and stored at -20°C until use.

Synthesis of a DEAG- β -Gal conjugate: A DMF solution containing grossly 0.063 μmol of **3a** was added dropwise to 3.4 mg of β -Gal in 1 ml of phosphate buffer (0.05 M, pH 7.5). The reaction mixture was stirred at 4°C for 48 h and applied to the top of a Sepharose column (1.5 i. d. $\times$ 35 cm) eluting with buffer A to collect fractions at 1.4 ml/tube. The fractions were monitored by UV absorbance at 280 nm and the enzymatic activity. The fractions that could catalyze the hydrolysis of 4-methylumbelliferyl β -D-galactoside were kept at 4°C until use. The labeled antigen was designated to be DEAG- β -Gal in accord with the hapten.

Measurement of β -D-galactosidase activity: Twenty-five μl of 10^3 -fold diluted DEAG- β -Gal was incubated with 150 μl of the substrate, 0.1 mM 4-methylumbelliferyl β -D-galactoside, for 30 min at 30°C . The reaction was quenched by addition of 3 ml of 0.1 M glycine-NaOH buffer (pH 10.3) and the released product, 7-hydroxy-4-methylumbelliferone, was measured fluorometrically at wavelengths of 365 nm for excitation and 448 nm for emission.

The binding curve of As-DEAG and DEAG- β -Gal: Fifty μl of $10\sim 10^5$ -fold diluted As-DEAG in buffer A were mixed with 50 μl of buffer A and 25 μl of 10^3 -fold diluted DEAG- β -Gal. After incubation at room temperature for 2 h, 20 μl of 10^2 -fold diluted normal rabbit serum and 50 μl of 10-fold diluted goat antiserum to rabbit IgG were added to the reaction mixture and allowed to stand at 4°C overnight. After addition of 1 ml of buffer A, the reaction mixture was centrifuged at $94 \times g$ for 20 min at 4°C . The supernatant was discarded and the immunoprecipitate was washed with buffer A once again. After decantation, 150 μl of 4-methylumbelliferyl β -D-galactoside was added and the enzymatic activity was measured as mentioned above. B_0/T values were calculated as the percentage of the bound enzyme activity (B_0) in the precipitate to the total enzyme activity (T).

The standard curve of mesaconitine: 2.5×10^4 -fold diluted As-DEAG in 50 μl of buffer A, 50 μl of serially

diluted mesaconitine (5×10^{-4} –50 ng/tube) or 50 μ l of buffer A as a blank, and 25 μ l of 10^3 -fold diluted DEAG- β -Gal were incubated at room temperature for 2 h. Afterward, 20 μ l of 10^2 -fold diluted normal rabbit serum and 50 μ l of 10-fold diluted goat antiserum to rabbit IgG were added to the reaction mixture, which was allowed to stand at 4 °C overnight. The following steps were identical with those mentioned above. After measurement of fluorescence, B/B_0 values were calculated as the ratio of the enzyme activity of the labeled antigen bound to the antiserum in the presence of various concentrations of mesaconitine (B) to that in the absence of mesaconitine (B_0).

Measurement of cross-reactivity with aconitine and benzoylmesaconine: Using the same experimental conditions as mentioned above, the standard curves of aconitine and benzoylmesaconine were measured with that of mesaconitine running in parallel. From the curves, the I_{50} value (at $B/B_0=0.5$) of each compound was determined and the cross reactivity was calculated as the percentage of I_{50} of mesaconitine to that of aconitine or benzoylmesaconine.

Assessment of precision (inter-day and intra-day assays): Variations of in the intra- and inter-day assays were calculated using mesaconitine at high, moderate and low concentrations.

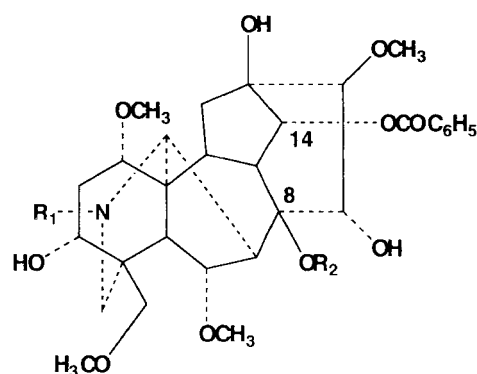
Measurement of plasma concentration of mesaconitine after intravenous and oral administration: Seven-week old male Wistar rats about 220 g each, were fasted one day before the experiment. After oral administration of mesaconitine at a dose of 1 mg/kg to rats, blood was collected by heparinized capillaries at 5 min, 15 min, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after administration.

Results and Discussion

The enzyme immunoassay is a simple and selective analytical technique based on the specific binding of the antibodies to the antigen or the hapten. For analysis of small molecules, numerous studies have demonstrated the importance of designing of haptens in development of a highly efficient EIA. In a previous study, we prepared two haptens possessing a short or long spacer of 4 or 14 carbons, respectively, at C-8 of an aconitine molecule *via* an ester-exchange reaction.²⁵ Two kinds of antisera have shown quite different recognition toward

aconitine alkaloids, one is highly selective for aconitine and another binds both aconitine and mesaconitine. The EIA developed by using the former antiserum showed high sensitivity toward aconitine in a linear range of 0.1 pg/tube – 1 ng/tube. On the other hand, aconitine is unique for an ethylated-nitrogen-containing heterocycle fused with rings A and B of the diterpenoid skeleton simultaneously (Fig. 1). If the nitrogen atom bears a methyl group instead, the compound turns out to be mesaconitine. It is of interest to investigate the effect of spacer attachment at the nitrogen atom on the performance of EIA. With this consideration, the ethyl group was readily removed from the nitrogen atom to give *N*-desethylaconitine (DEA, **2**) by reacting with KMnO_4 in acetone- H_2O (Fig. 2). The secondary amine of DEA (**2**) was further derived by formation of an amide bond with glutaric anhydride. The structures of both DEA (**2**) and DEAG (**3**) were confirmed by comparison of 1D NMR data with that of aconitine. Next, the remaining COOH group in the glutaryl spacer was activated by the conventional *N*-hydroxysuccinimide (NHS) method and coupled with BSA and β -Gal, respectively, to provide an immunogen (DEAG-BSA, **4**) and a labeled antigen (DEAG- β -Gal, **5**). After immunization of three rabbits with DEAG-BSA for six months, the antisera (As-DEAG) were harvested.

Firstly, the binding activity of As-DEAG was investigated using 10^3 -fold diluted DEAG- β -Gal. As-DEAG



	R ₁	R ₂
Mesaconitine (1)	CH ₃	COCH ₃
Aconitine (6)	CH ₂ CH ₃	COCH ₃
Benzoylmesaconine (7)	CH ₃	H

Fig. 1 Structures of mesaconitine (**1**), aconitine (**6**) and benzoylmesaconine (**7**)

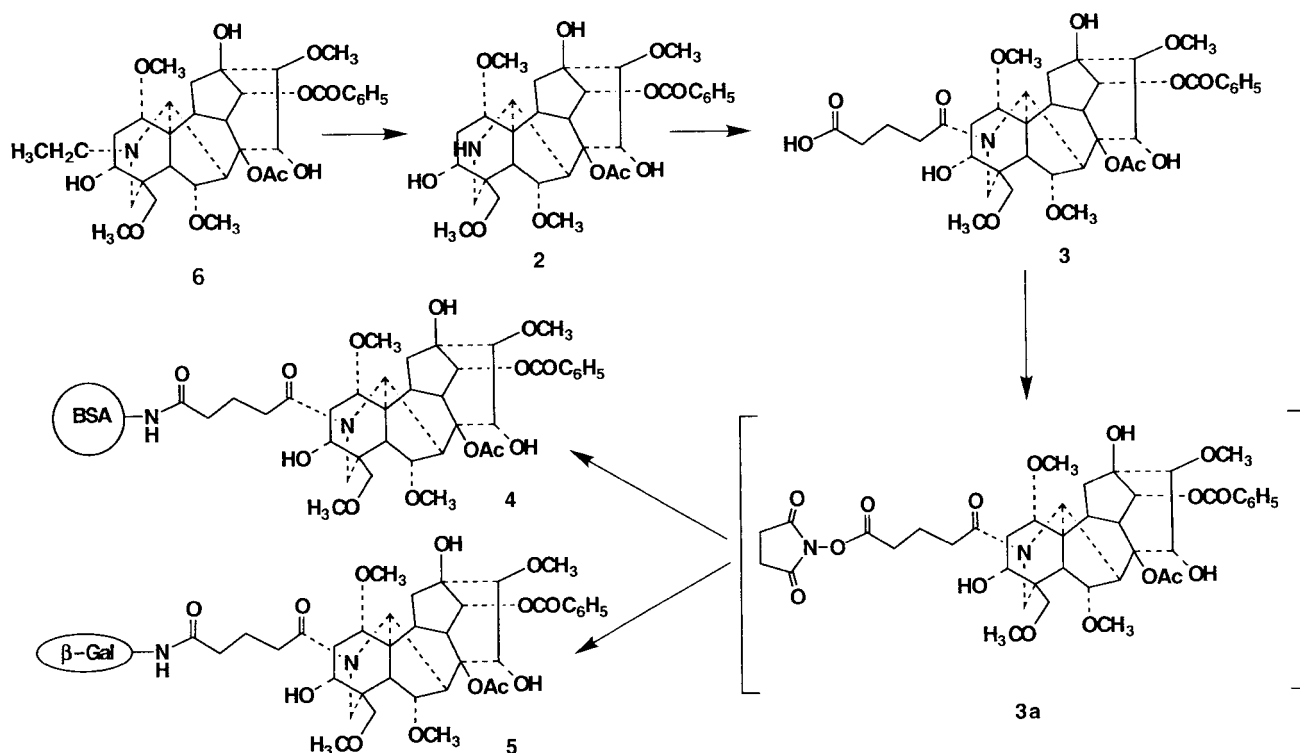


Fig. 2 Synthetic scheme of a hapten (DEAG, 3), an immunogen (DEAG-BSA, 4), and labeled antigen (DEAG-β-Gal, 5)

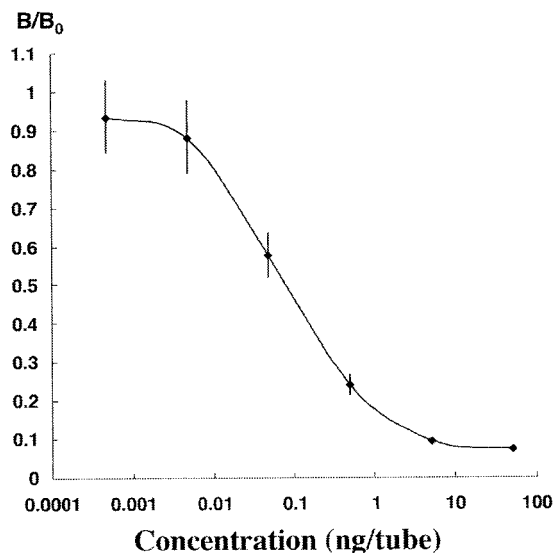


Fig. 3 A standard curve of mesaconitine using 2.5×10^4 -fold diluted As-DEAG and 10^3 -fold diluted DEAG-β-Gal.

Table I Cross-reactivity of aconitine and benzoylmesaconine with mesaconine in the presence of an antiserum As-DEAG

Compound	Cross-reactivity (%)
Mesaconitine	100
Aconitine	24.4
Benzoylmesaconine	0

showed the highest binding capacity of 78.9 % at 10^3 -fold dilution. Even at 10^5 -fold dilution, the binding activity remained at 47.3 %. This indicated that the polyclonal antibodies in the antiserum were of high titer. Fig. 3 shows a standard curve of mesaconitine using 2.5×10^4 -fold diluted antiserum As-PEAG, where a linear relationship was observed between ratios of B/B₀ and concentrations of mesaconitine in a range of 0.005-1 ng/tube.

Next, we examined the selectivity of As-DEAG toward aconitine and mesaconitine. The anti-serum recognized more favorably mesaconitine than aconitine, which showed a cross reactivity equivalent to 24.4 % of mesaconitine as shown in Table I. Another compound, benzoylmesaconine (7) bearing a methyl group on the nitrogen atom as that of mesaconitine but a free hydroxy group at C-8, instead of an acetoxy group, could no longer be recognized by the antigenic binding sites of antibodies. The result was in good agreement with the notion that the antibodies could best recognize the farthest part from the spacer of the hapten, as has been observed in the previous experiment where benzoyl-mesaconine and benzoylaconine showed weak cross-reactivity using the anti-sera prepared with haptens synthesized by an es-

ter-exchange reaction of an acetoxy group at C-8 in the aconitine molecule.²⁵⁾

The relatively broad specificity of the anti-serum As-DEAG against mesaconitine and aconitine may be applicable to the pharmacokinetic or dispositional studies of either mesaconitine or aconitine, though the sensitivity is rather lower in the case of aconitine. In addition, approximate monitoring of both alkaloids could be possible after administration of aconite-containing prescriptions. Combination of the present and previous (aconitine-specific) anti-sera will provide further accurate quantification of the respective alkaloids in the body fluid.

Finally, in order to achieve the highest sensitivity toward mesaconitine, the experimental conditions for EIA were optimized. The conditions were established to be 2.5×10^4 -fold dilution for As-DEAG and 10^3 -fold dilution for DEAG- β -Gal. Under the experimental conditions as shown in "Materials and Methods," the EIA had a linear range of 0.005–5 ng/tube with I_{50} values of 35.8–45.8 pg/tube for mesaconitine.

From the above observation, we concluded that the EIA system prepared at present was of high sensitivity toward mesaconitine. To study the precision of this EIA system, intra-day and inter-day assays were performed. Under similar conditions, the EIA showed I_{50} values (concentrations at $B/B_0 = 0.5$) of 39.7 ± 1.4 pg for the intra-day assays and 40.1 ± 4.5 pg for inter-day assays, respectively (Table II). Thus it was expected that good precision could be achieved if the experimental conditions are carefully maintained, though the inter-day SD is much larger than that of intra-day assay.

After the establishment of EIA, the method was applied to disposition studies on mesaconitine. Following

oral administration of mesaconitine to rats at a dose of 1 mg/kg, the plasma concentration reached the maximum at 2 h and then out of the detection after 8 h (Fig.4). The pharmacokinetic parameters were calculated as shown in Table III. The AUC value (1060 ng min/ml) and C_{max} value (6.01 ng/ml) were close to those of aconitine (1600 ng min/ml and 3.3 ng/ml, respectively) after oral administration at the same dose, suggesting similar disposition of two compounds. However, the intravenous injection caused death at a dose of 0.01 mg/kg. Though absolute bioavailability for mesaconitine could not be calculated, its low bioavailability was supposed from our previous pharmacokinetic data for aconitine intravenously injected at a dose of 0.02 mg/kg. In addition, this disposition study further demonstrated the feasibility of the EIA approach.

和文抄録

附子に含まれる aconitine と並ぶ猛毒なアルカロイドである mesaconitine の定量の目的で、高感度酵素免疫測定法 (EIA) を開発した。すなわち、最初に aconitine の *N*-エチルグループを除去した後、二級窒素原子にグルタル酸基をアミドとして導入することによりハプテンを合成した。次いでこのハプテンと *N*-ヒドロキシスクシニイミドを反応させ活性エステルを合成した後、仔ウシ血清アルブミン (BSA) あるいは β -ガラクトシダーゼ (β -Gal) と反応させ、それぞれ免疫原 (DEAG-BSA)、標識抗原 (DEAG- β -Gal) を作製した。DEAG-BSA を白色ウサギに 6 ヶ月間投与し、抗血清 (As-DEAG) を得た後、高感度 EIA を確立するために諸条件を検討した。 10^3 倍希釈した DEAG- β -Gal と 2.5×10^4 倍希釈した As-DEAG 抗血清を用いた場合、mesaconitine に対して 0.005–5 ng /

Table II A comparison of I_{50} values (pg/tube) and standard deviations between inter-day and intra-day assays

Assays	$I_{50} \pm SD$ (pg/tube)
Intra-day assay	39.7 ± 1.4
Inter-day assay	40.1 ± 4.5

I_{50} , a concentration of mesaconitine at $B/B_0 = 0.5$ in a standard curve

Table III Pharmacokinetic parameters of mesaconitine after oral administration to rats.

Dose	C_{max} (ng/ml)	t_{max} (min)	AUC_{0-420} (ng min/ml)
1.0 mg/kg	6.01 ± 2.04	126 ± 4.8	1060 ± 203

Each point represents the mean $\pm$ S.D. (n=3).

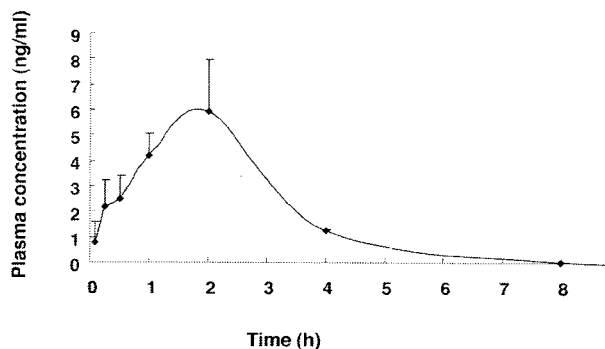


Fig. 4. Plasma concentration of mesaconitine after oral administration at 1 mg/kg to Wistar rats

tube の濃度範囲で直線性が示された。同条件下、この抗血清は aconitine と 24.4% の交差反応を示したが、benzoylmesaconine に対しては交差反応を示さなかった。

この EIA を mesaconitine の薬物動態研究に応用した結果、1 mg/kg の mesaconitine を経口投与した場合の血清中の濃度測定が可能であり、猛毒性アルカロイドである mesaconitine の動態パラメーターを初めて得ることができた。

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