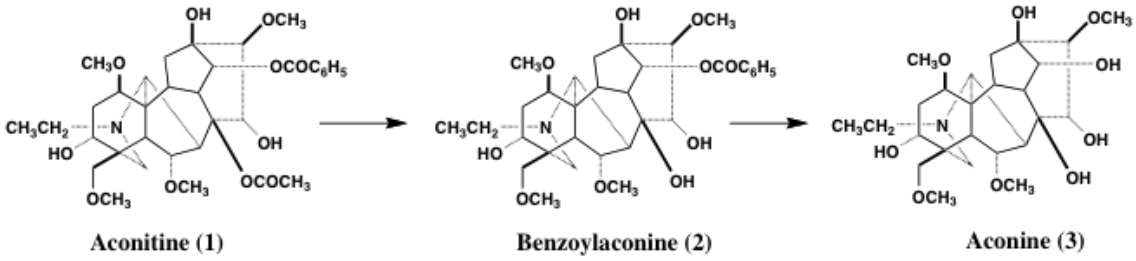
Aconitine



Metabolic processes of aconitine

代謝実験

代謝動物　ラット

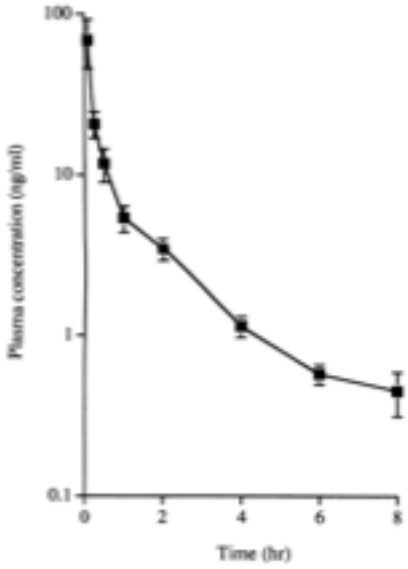


Fig. 1.　Plasma concentration-time curve of aconitine (**1**) after intravenous administration of 0.02 mg aconitine /kg b.w. to rats

Quantitative determination of aconitine (1) in plasma after intravenous administration to rats

Aconitine (**1**, 0.1 mg) was dissolved in DMSO (250 ml) and diluted with saline to a total volume of 5 ml. A portion of the solution was injected through tail vein to rats at a dose of 0.02 mg aconitine/kg b.w. The blood samples were repeatedly collected through

tail vein at 5, 15 and 30 min, and then 1, 2, 4, 6 and 8 hr after intravenous administration. The samples were centrifuged at 1,100 × *g*, 15 min and 4°C to give the respective plasma. The original plasma or 10-fold diluted ones (5 ml) were used for EIA to determine aconitine levels. The standard calibration curve ranging from 0.1 ~ 1000 pg aconitine/tube was prepared in the presence of the plasma from conventional rats. [Tazawa *et al.*, *Biol. Pharm. Bull*., **26**, 1289-1294 (2003)]

Procedure of enzyme immunoassay (EIA)

Samples or standard solutions containing various amounts of aconitine (**1**) were incubated with an antiserum (50 ml) and a 10^3-fold diluted *b*-Gal conjugate (25 ml) at room temperature for 2 h. Then, 10-fold diluted goat anti-rabbit Ig G (20 ml) and 100-fold diluted normal rabbit serum (20 ml) were added to the reaction mixture. The mixture was kept overnight at 4°C. After addition of buffer A (1 ml), the resulting mixture was centrifuged at 1,100 × *g* for 15 min at 4°C. The supernatant was discarded, and the precipitates were washed with buffer A followed by incubation with 01 mM 4-methylumbelliferyl *β*-D-galactoside for 30 min at 30°C. The reaction was stopped by the addition of 100 mM glycine–NaOH buffer, pH 10.3, (4 ml). The fluorescence intensity of a product (4-methylumbelliferone) was spectrofluorometrically measured at wavelengths of 365 nm (excitation) and 448 nm (emission). [Tazawa *et al.*, *Biol. Pharm. Bull*., **26**, 1289-1294 (2003)]

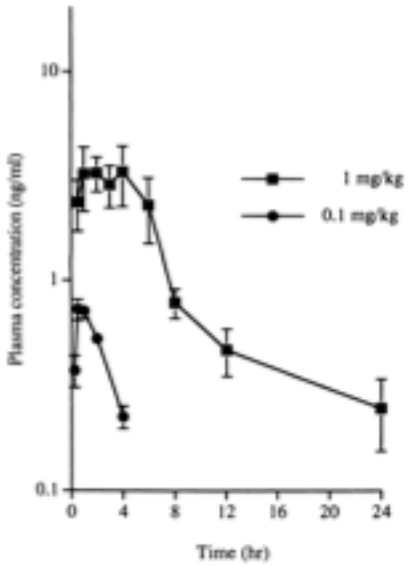


Fig. 2. Plasma concentration-time curves of aconitine (**1**) after oral administration of 0.1 and 1.0 mg aconitine/kg b.w.

Quantitative determination of aconitine (1) level in plasma after oral administration to rats

Aconitine (**1**, 0.01 mg/ml) dissolved in DMSO-H_2O (1:19) was orally administered to four rats at a dose of 0.1 mg/kg b.w. The blood samples were repeatedly collected through tail vein at 5, 15 and 30 min, and then 1, 2, 4, 6 and 8 hr after oral administration, and centrifuged at 1,100 × *g,* for 15 min at 4°C to give the respective plasma. The original plasma (5 ml) was subjected to EIA for quantitative determination of aconitine. [Tazawa *et al.*, *Biol. Pharm. Bull*., **26**, 1289-1294 (2003)]

Table 1. Parameters after intravenous administration of aconitine at 0.005 mg/kg to Wistar rats.

Parameter	Dose (mg/kg)
	0.02
A (ng/ml)	56±12
α (min^{-1})	0.049±0.007
B (ng/ml)	3.4±0.9
β (min^{-1})	0.004±0.001
t1/2α (min)	16±2
t1/2β (min)	246±91.3
Vc (l/kg)	0.41±0.09
Vdss (l/kg)	1.7±0.4
CLtot(ml/min kg)	10±2
$AUC_{0\text{-}4800}$ (ng min/ml)	2055±294.3

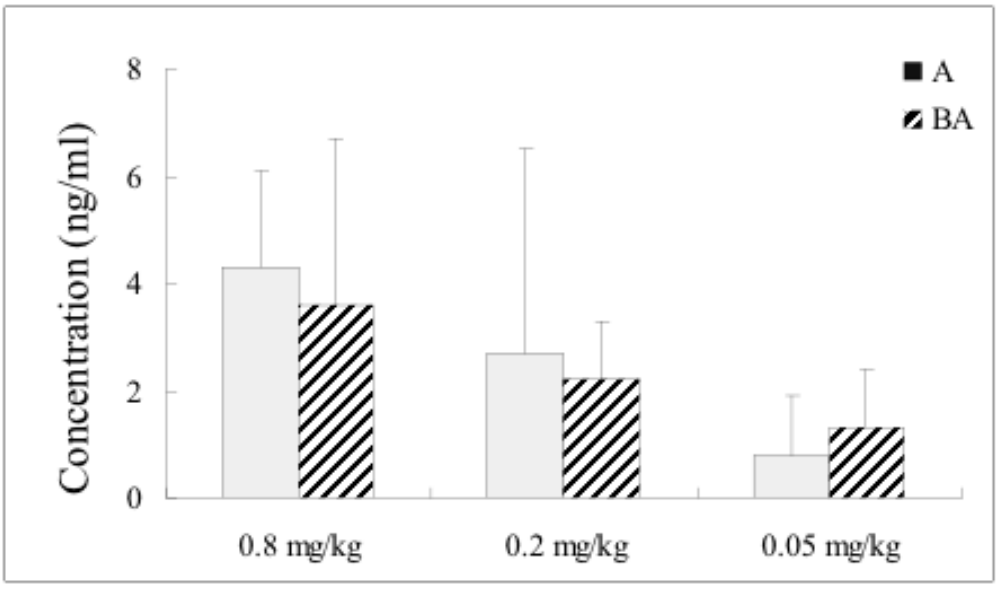
Each value represents mean±S.E. (n=5–6) [Tazawa *et al.*, *Biol. Pharm. Bull.*, **26**, 1289-1294 (2003)]

Table 2. Parameters for aconitine after oral administration to Wistar rats.

Dose	t_{max} (min)	C_{max} (ng/ml)	AUC (ng min/ml)
1.0 mg/kg	150±52	3.3±0.6	1600±270
0.1 mg/kg	45±9	0.73±0.08	130±4

Each point represents the mean±SD (n=4) [Tazawa *et al.*, *Biol. Pharm. Bull.*, **26**, 1289-1294 (2003)]

I



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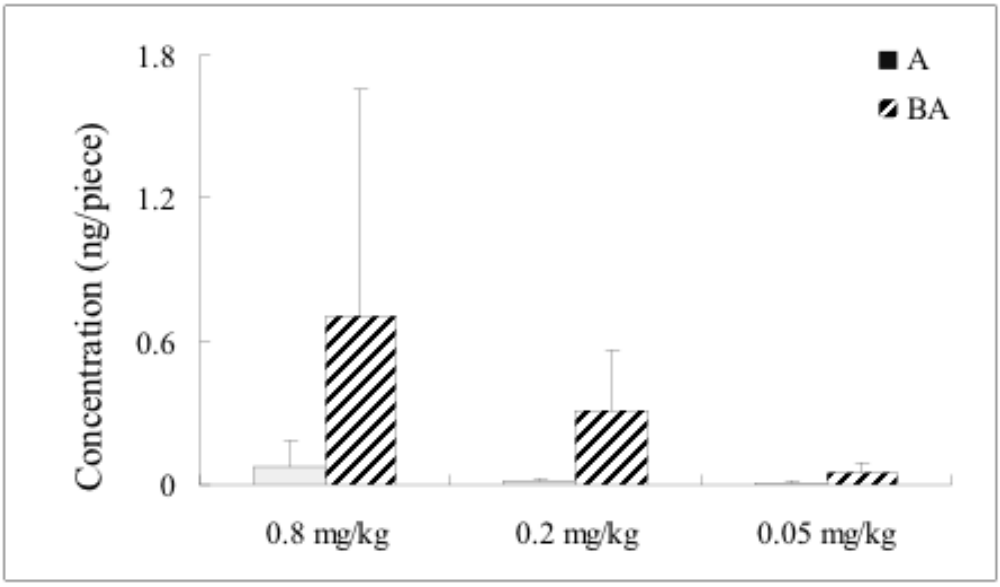

Fig. 1. The concentrations of aconitine (**1**) and benzoylaconine (**2**) 1 h after oral administration of **1** in sera (I) and in spinal cord (II) samples

A, aconitine (**1**); BA, benzoylaconine (**2**)

Disposition of aconitine (1) after oral administration in rats

One hour after oral administration of aconitine (**1**) at doses of 0.8, 0.2, 0.05 mg/kg in rats, under general anesthesia, the blood and spinal cord (L1-L4) were collected, and their aconitine (**1**) and benzoylaconine (**2**) contents were quantitatively determined by the respective enzyme immunoassay systems. [Zuo *et al*., *J. Nat. Med*., **60**, 313-321 (2006)]

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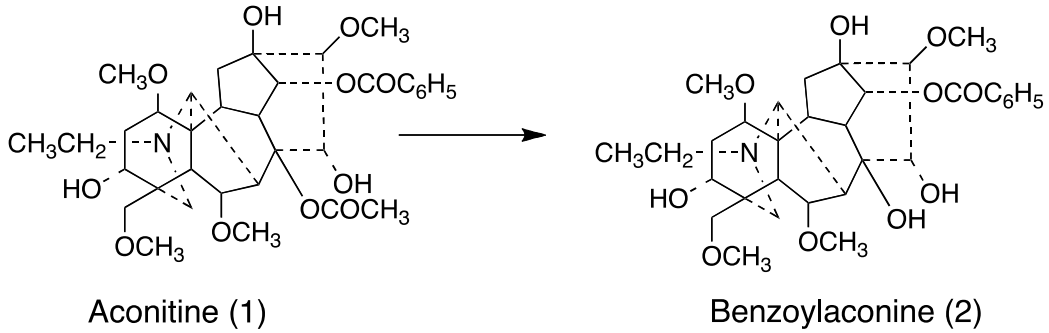
1) Kawata Y., Ma C. M., Meselhy M. R., Nakamura N., Wang H., Hattori M., Namba T., Satoh K., and Kuraishi Y.: Conversion of aconitine to lipoaconitine by human intestinal bacteria and their anti-nociceptive effects in mice. *J. Trad. Med.,* **16**, 15-23 (1999).

2) Tazawa T., Zhao H., Li Y., Meselhy M. R., Nakamura N., Akao T. and Hattori M.: A new enzyme immunoassay for aconitine and its application to quantitative determination of aconitine levels in plasma. *Biol. Pharm. Bull*., **26**, 1289-1294 (2003).
3) Zuo F., Zhao J., Nakamura N., Gao J. J., Akao T., Hattori M., Oomiga Y., and Kikuchi Y.: Pharmakokinetic stydy of benzoylmesaconine in rats using an enzyme immunoassay system. *J. Nat. Med*., **60**, 313-321 (2006).

Aconitine（追加）



【化合物】Aconitine、SHEN-FU 注射粉末投与後の *Aconitum* alkaloid の血中濃度の測定

【対象】動物（ヒト）

【測定機器】LC-MS/MS

【代謝実験】

"SHEN-FU" injectable powder were applied to 18 healthy volunteersby intravenous drop infusion. Six volunteers were involved in each experiment. The blood samples were collected at intervals after intravenous drop infusion. The pharmacokinetics demonstrated that the concentrations of aconitine, mesaconitine, and hypaconitine were at very low levels with < 0.2, < 0.2, and < 0.7 ng/mL or under detection limit for all) and the content of benzoylmesaconine was highest. [Fan Zhang et al., *J. Chromato. B,* **873**, 173–179 (2008).]

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【参考文献】

Fan Zhanga, Ming-hai Tanga, Li-juan Chena, Rui Li, Xian-huoWang, Jun-guo Duan, Xia Zhao, Yu-quanWei, Simultaneous quantitation of aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine and benzoylhypaconine in human plasma by liquid chromatography–tandem mass spectrometry and pharmacokinetics evaluation of "SHEN-FU" injectable powder. *J. Chromato. B,* 873, 173–179 (2008).