

New enzyme immunoassay systems for quantitative analysis of mesaconitine and benzoylmesaconine

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[Purpose]

The crude drug aconite ("bushi" in Japan) is derived from the roots of certain *Aconitum* species (Ranunculaceae), which are widely distributed both in China and in Japan. The drug is frequently used as a component in various traditional Chinese medical prescriptions for the purposes of analgesia, anti-inflammatory and vasorelaxing *etc*. On the other hand, there are many reports that ingestion of Aconitum alkaloids can lead to severe or even fatal toxic effects. For the purpose of quantitative determination of mesaconitine, an extremely toxic and major alkaloid in Aconitum tuber, and its hydrolysate benzoylmesaconine in the biological samples, the highly sensitive enzyme immunoassay (EIA) systems were developed, respectively.

[Methods]

Firstly, the haptens were synthesized by introduction of the glutaryl group to the nitrogen atom of aconitine and benzoylaconine after elimination of an *N*-ethyl group, respectively. Subsequently they were coupled with bovine serum albumin (BSA)- and β -galactosidase (β -Gal) to give the immunogens and enzyme-labeled antigens, respectively. After immunization of female albino rabbits with BSA-conjugate for six months to elicit the polyclonal antisera respectively.

[Results and Conclusion]

Two kinds of β -Gal-labeled antigens with antisera showed high sensitivity in range of 0.005-5ng/ml of mesaconitine and 1-1000ng/ml of benzoylmesaconine, respectively. Under the same conditions, the antiserum had the quite weak cross reactivities with other related alkaloids in Aconitum tuber. In the presence of the plasma, the coefficients of variance (CV) were 3.5% and 11.2% of mesaconitine, and were 6.4% and 23.8% of benzoylmesaconine for intra- and inter-day assays, respectively. Therefore, the EIA systems for quantitative determination of mesaconitine and benzoylmesaconine in the biological samples were valid, and applicable to their pharmacokinetic studies.