# E—4

## Development of an Enzyme Immunoassay for Quantitative Analysis of 12-*O*-Acetylphorbol 13-decanoate

○趙　静[1]，中村憲夫[1]，高　江静[1]，赤尾光昭[2]，服部征雄[1]，楊　秀偉[3]
富山医科薬科大学・和漢薬研究所[1]，同・薬学部[2]，北京大学[3]

【目的】12-*O*-Acetylphorbol 13-decanoate from the seeds of ***Croton tiglium*** was found to inhibit HIV-induced cytopathic effect (CPE) on MT-4 cells without activation of protein kinase C (PKC). In view of its significant implications for chemotherapy of AIDS, we aimed to develop a sensitive, specific, and efficient analytical method-an enzyme immunoassay, for its quantification.

【方法】Two haptens with spacers of different lengths at C-20 of 12-*O*-acetylphorbol 13-decanoate were prepared by reacting with succinic anhydride or adipoyl chloride. Then they were coupled with bovine serum albumin (BSA) as well as $\beta$-galactosidase via an *N*-hydroxysuccinimide ester method to give immunogens and labeled antigens, respectively. Next, two sets of antisera were generated by subcutaneous injections of each phorbol-BSA conjugates into female albino rabbits. Subsequently, the EIA was performed based on a double-antibody principle throughout the experiment.

【結果・考察】The linear working range of the EIA could reach as low as 0.05-50 ng/tube, while spacer heterogeneous combinations of the antisera and labeled antigens showed higher sensitivities than homogeneous combinations. Besides, the antisera exhibited low cross-reactivities with related phorbol derivatives and no cross-reactivities at all with isophorbol derivatives. This result provided a solid foundation for the ongoing pharmacokinetic studies of 12-*O*-acetylphorbol 13-decanoate.