LEUKOCYTE MIGRATION INHIBITION INDUCED BY THE COMBINATION OF DRUG AND A LIVER CONSTITUENT IN PATIENTS WITH DRUG-INDUCED HEPATITIS

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Summary

The leukocyte migration inhibition test in agarose medium was performed in 23 cases of clinically diagnosed drug-induced hepatitis. When the test antigen was the combination of soluble phase of a liver homogenate fractionated by Sephadex G-100 which should have contained liver specific antigen and the offending drug the leukocyte migration was inhibited in 86% of cases. Whereas none of 12 cases of drug allergy without hepatic injury showed a positive result with the same combination of antigens.

Other organ homogenate-muscle and kidney-never gave positive results when mixed with the offending drugs in cases of drug-induced hepatitis.

It was concluded that in hypersensitivity type drug-induced hepatitis cell-mediated immunity might be established to the complex of liver specific antigen and the drug.

Key Words: leukocyte migration inhibition test, liver specific antigen, drug-induced hepatitis, drug allergy.

Introduction

There are two categories of hepatic injury caused by drug administration. The first is hepatic injury caused by the direct cytotoxic effect of the drug on the liver\(^1\) and the second is hepatic injury which is caused by hypersensitivity reaction of the host to the drug\(^9\). In both cases the detailed mechanism still remains controversial. In the latter type of drug-induced hepatitis, the reaction of the host to the drug is considered to be mainly cell-mediated immunity\(^5\) in which the sensitized lymphocyte plays a central role\(^6\).

The differential diagnosis of viral hepatitis and the drug-induced hepatitis is often difficult. Serum enzyme determination or liver biopsy are not decisive. In suspected cases of drug-induced hepatitis it is also difficult to discriminate the causative drug from other drugs administered simultaneously. In recent years in vitro methods to detect the specific cell-mediated immunity such as the lymphocyte transformation test\(^3\), macrophage migration inhibition test\(^1\), and the leukocyte migration inhibition test\(^1\) have been applied for the diagnosis of the drug-induced hepatitis as well as for drug hypersensitivity and proved useful.

Because most of the drugs are small molecules, they need some carrier to become a
sensitizing antigen and may be regarded as haptens\textsuperscript{6,14-16}).

In this study the leukocyte migration inhibition test in agarose medium was applied for detecting the cellular hypersensitivity against the offending drug and the fractionated soluble phase of a liver homogenate which should have contained liver specific antigen\textsuperscript{17} was used as a test antigen mixed with drug. The purpose of this study is to investigate their significance in the diagnosis of drug-induced hepatitis.

Methods

The patients included 29 cases of drug-induced hepatitis in which the diagnosis was made by clinical evidences which included the history of drug administration antecedent to liver injury, improvement of liver injury after discontinuance of drug administration, clinical symptoms such as fever, jaundice, pruritus, and laboratory findings of eosinophilia or leukocytosis. Serological tests for viral hepatitis were negative in all cases. The histological findings included each of hepatitic, cholestatic, and mixed form.

Twelve cases of drug allergy without hepatic injury were studied in the same way. Their clinical symptoms were limited to skin manifestation, fever or leukopenia. Twenty normal subjects were also chosen as controls to test their response to the drugs.

The leukocyte migration inhibition test (LMIT) in agarose medium was performed following the method of Clausen\textsuperscript{18}. Twenty milliliters of heparinized blood was drawn from the peripheral vein by plastic syringe during the convalescent period of the disease. Another 2 ml of 6% dextran (T-250) in saline was drawn in the same syringe and the syringe was placed in upright position for one hour at room temperature. The leukocyte rich plasma was collected in sterile plastic tube and then centrifuged for 10 minutes at 1,000 rpm. The sedimented leukocytes were washed in Hanks' BSS for three times and then suspended in the TC-medium 199 supplemented by 10% horse serum, streptomycin 100 \( \mu \text{g/ml} \), and penicillin 100 IU/ml, at a cell concentration of \( 2.1 \times 10^8/\text{ml} \). The leukocyte suspension was placed in a number of culture tubes. Each tube contains 28 \( \mu \text{l} \) of the leukocyte suspension. Antigens were then added. After incubation for 30 minutes at 37\textdegree \text{C} in 5% CO\textsubscript{2}, 95% air saturated with water vapour 4 aliquots of leukocyte suspension from single culture tube were transferred to the wells punched out in a agarose gel plate. The diameter of the well was 2.3 mm. The volume each aliquot of 1.5 \( \times 10^6 \) leukocytes was 7 \( \mu \text{l} \). The agarose gel plate consisted of 1% agarose in a single strength TC-medium 199 with 10% horse serum, streptomycin 66 \( \mu \text{g/ml} \), penicillin 66 IU/ml. The plates were incubated for another 20 hours at 37\textdegree \text{C} in 5% CO\textsubscript{2}, 95% air, saturated with water vapour. The migration area was measured on a slide projector after fixation in 10% formalin solution and staining with Giemsa solution. The migration index was expressed as a percent of the mean migration area of the leukocytes cultured with test antigen to that of control culture.

Single antigen or mixtures of three test antigens i.e. the suspected offending drug, autologous serum and the fractionated soluble phase of a liver homogenate were added as follows. 1) 2 \( \mu \text{l} \) of distilled water was added in 28 \( \mu \text{l} \) of leukocytes suspension as a control, 2) 1 \( \mu \text{l} \) of autologous serum plus 1 \( \mu \text{l} \) of distilled water were added in 28 \( \mu \text{l} \) of leukocytes suspension, 3) a single dosage of the test drug was dissolved in 180 ml of distilled water then 1 \( \mu \text{l} \) of this drug solution plus 1 \( \mu \text{l} \) of distilled water were added in 28 \( \mu \text{l} \) of leukocytes suspension, 4) 1 \( \mu \text{l} \) of the same drug solution plus 1 \( \mu \text{l} \) of autologous serum were added in