Title: ASSAYS FOR THE DETECTION OF ANTIBODIES DIRECTED AGAINST HALOTHANE-INDUCED LIVER NEOANTIGENS IN THE SERA OF PATIENTS WITH HALOTHANE HEPATITIS

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Introduction. Previous studies have demonstrated the presence of antibodies to novel halothane-induced liver neoantigens in sera from patients with halothane hepatitis, and have suggested that these neoantigens may play an immunopathological role in development of the patients' liver damage. The antibodies have been shown recently by immunoblotting to recognize several distinct halothane-induced polypeptide neoantigen fractions (100kDa, 76kDa, 59kDa, 57kDa, 54kDa) in liver microsomes that have been covalently modified by the reactive trifluoroacetyl halide (CF_3COX) metabolite of halothane [1]. It appears that the patients' antibodies do not recognize simply the trifluoroacetyl (TFA) hapten, since antibody binding to the neoantigens is inhibited only partially by the hapten derivative TFA-lysine, even at very high concentrations, and since the patients' sera differ in patterns of neoantigen recognition [1]. These findings indicate that the carrier proteins and not solely the covalently bound TFA hapten play a crucial role in the overall expression of the patients' immune response.

A sensitive and specific method for the detection of these antibodies is of potential diagnostic importance for two reasons. First, their presence indicates that a patient has been previous sensitized to halothane and therefore may be susceptible to an allergic reaction on further exposure to this agent. Second, these same antibodies have been shown recently to react with liver microsomal neoantigens produced by the structurally related oxidative acyl halide metabolite (CHF_2OCF_2COX) of enflurane and therefore suggests the potential for a cross-sensitization reaction after the administration of enflurane [2].

In the present study, an enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies in the sera of halothane hepatitis patients has been evaluated. This procedure, which is a modification of a previously described method[3], has now been more completely evaluated utilizing a larger population of patients. The assay is based upon the use of TFA-rabbit serum albumin (TFA-RSA) as antigen.

Methods. Human sera were collected from patients with a clinical diagnosis of halothane hepatitis and from several different control groups. The ELISA procedure, in short, involved the coating of wells of microtiter plates with TFA-RSA or RSA (control for reaction against albumin), blocking with casein buffer, adding human sera (1:100 dilution), washing with casein buffer, adding rabbit horseradish peroxidase (HRP) conjugated IgG, blocking with casein buffer, and adding HRP substrate. The reaction was stopped by the addition of H_2SO_4 and the optical density (O.D.) was determined at 492 nm. O.D. values obtained from the RSA wells were subtracted from those of the TFA-RSA well to give corrected results. A positive reaction was defined as a serum sample producing a corrected TFA-RSA value of three standard deviations above the values obtained for normals.

Group	Number	% Positive
Halothane Hepatitis	44	59
Multiple Halothane Exposures	11	0
Normals	21	0
Primary Biliary Cirrhosis	17	17
Chronic Active Hepatitis	13	46

Results. Antibodies reacting with TFA-RSA were detected in the serum of 59% of the halothane hepatitis patients. This is an improvement from the 33% (2 of 6 halothane hepatitis patients) positive responses found in the original study using TFA-RSA as an antigen [3]. Normal patients not previously exposed to halothane and patients that had received multiply halothane exposures without developing hepatitis gave negative results. In contrast, sera of patients with other types of hepatic disease gave positive reactions ranging from 17-46%.

Discussion. Although the sensitivity of the ELISA procedure using TFA-RSA as antigen for the detection of antibodies in the sera of halothane hepatitis patients has been improved significantly, the finding of a significant number of positive responses with sera of patients with other forms of liver disease was not expected. This finding clearly shows that the assay in its present form is not adequately specific and illustrates the importance of testing a diverse group of controls when developing an ELISA procedure. We plan to improve both the sensitivity and specificity of the ELISA assay by utilizing recently purified liver microsomal TFA-neoantigens as antigens in place of TFA-RSA.

References.

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