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Detection of autoantibody to aldolase B in sera from patients with troglitazone-induced liver dysfunction

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Abstract

Troglitazone is a thiazolidinedione antidiabetic agent with insulin-sensitizing activities that was withdrawn from the market in 2000 due to its association with idiosyncratic hepatotoxicity. To address the suspected autoantibody production associated with troglitazone, we investigated autoantibodies in sera from patients with type II diabetes mellitus with troglitazone-induced liver dysfunction. Two female patients (47- and 70-year-old) ceased taking troglitazone (400 mg/day) after 23.5 and 16 weeks, respectively, due to increased serum ALT. Using two-dimensional electrophoresis and amino acid sequence analyses, aldolase B was identified as an autoantigen that reacted with antibodies in sera from both patients. The titer of anti-aldolase B remained high for several weeks after stopping troglitazone administration. The mean reactivity of autoantibodies to aldolase B determined by ELISA with sera of patients with chronic hepatitis ($n = 40$) and liver cirrhosis ($n = 40$) was significantly higher ($p < 0.05$ and $p < 0.001$, respectively) than with sera of healthy subjects ($n = 80$). These findings suggest that liver injury may cause the appearance of autoantibodies to aldolase B which may then aggravate the hepatitis. In addition, the anti-aldolase B titer might indicate the severity of liver dysfunction.

Keywords: Autoantibody; Aldolase B; Troglitazone; Thiazolidinediones; Liver dysfunction

1. Introduction

Adverse drug reactions can be classified into two basic types, reactions that occur directly and can be predicted from the pharmacology of the drug and, in contrast, idiosyncratic reactions which are induced dose-independently and are infrequent and unpredictable. Many idiosyncratic drug reactions have an immunological (hypersensitivity) basis, whereas some are due to a metabolic abnormality of the host (Ju and Uetrecht, 2002; Pirmohamed et al., 1998; Pohl et al., 1988). The liver is an important target for the toxic effects of drugs

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because of its essential role in the metabolism of xenobiotic substances. Idiosyncratic drug-induced hepatitis has been assumed to be mediated by immunogens formed by covalent interaction of a reactive drug metabolite with cellular macromolecules (Ju and Uetrecht, 2002; Park et al., 1998). The bioactivated immunogens may not only lead to an immune response directed against the haptenic epitope and the neoantigen, but also against autoantigenic determinants, which is characterized by the formation of autoantibodies (Pohl et al., 1988). A number of hepatotoxic drugs have been reported to produce autoantibodies. For instance, anti-protein disulfide isomerase, anti-microsomal carboxyesterase, anti-calreticulin, anti-ERp72, anti-GRP78, anti-GRP94 and anti-CYP2E1 in halothane hepatitis (Bourdi et al., 1996; Gut et al., 1993; Kenna et al., 1993; Pumford et al., 1993), anti-CYP2C9 in tienilic acid-induced hepatitis (Homberg et al., 1984; Robin et al., 1996), anti-CYP1A2 in dihydralazine-induced hepatitis (Bourdi et al., 1990), and anti-CYPs in aromatic anticonvulsant-induced hypersensitivities (Leeder et al., 1992). However, it is not known whether the autoantibodies are the cause or consequence of the progression of hepatotoxicity. Studies to clarify the possible involvement of autoantibodies in drug-induced hepatitis have been limited, since the appearance of autoantibodies can be seen usually only in human (Descotes, 2000).

Troglitazone (Noscal®, Sankyo, Tokyo, Japan or Rezulin®, Parke-Davis, Morris Plains, NJ) was an early member of the thiazolidinedione chemicals developed for type II diabetes. It has a novel mechanism of action on lowering the blood glucose level by increasing glucose uptake by skeletal muscles, decreasing hepatic glucose production, and sensitizing target tissues to insulin (Fujiwara et al., 1995, 1988; Ciaraldi et al., 1990). However, a rare type of hepatic injury has been reported to be associated with troglitazone therapy. During clinical trials, 1.9% of patients experienced increases in ALT levels greater than three times the upper normal limit (Watkins and Whitcomb, 1998). Fulminant hepatic failure in some patients was reported to occur after long-term troglitazone treatment (more than 4 weeks) (Gitlin et al., 1998; Kuramoto et al., 1998; Neuschwander-Tetri et al., 1998; Shibuya et al., 1998). The hepatic toxicity of troglitazone was not observed in any experimental animals tested including monkey, which has a similar metabolic profile to human (Rothwell et al., 2002; Watanabe et al., 1999). Although the mechanism by which troglitazone causes liver dysfunction in certain individuals is not yet clear, it is thought to be idiosyncratic. There is no report so far of whether a metabolic idiosyncrasy or immunological idiosyncrasy causes this hepatotoxicity.

In the present study, we identified aldolase B as an autoimmune antigen which reacted against antibodies in sera of patients with troglitazone-induced liver dysfunction. The titer of the aldolase B autoantibody remained high for several weeks after stopping troglitazone administration. In addition, we also investigated the formation of the aldolase B autoantibodies in patients with chronic hepatitis and liver cirrhosis as compared with healthy subjects.

2. Materials and methods

2.1. Materials

Biotinylated anti-human IgG, biotinylated anti-rabbit IgG, and a VECTASTAIN ABC kit were purchased from Vector Labolatories Inc. (Burlingame, CA). Prestained SDS–polyacrylamide gel electrophoresis (PAGE) standard of low molecular weight range and prestained isoelectric point (p*I*) marker for two-dimensional PAGE were from Bio-Rad (Hercules, CA). 3,3′-Diaminobenzidine tetrahydrochloride (DAB) and 3,3′,5,5′-tretramethylbenzidine (TMB) liquid substrate system were from Sigma (St. Louis, MO). HRP conjugated anti-human IgA, IgG, IgM, kappa, lambda was from DakoCytomation (Glostrup, Denmark). Immobilon-P membrane was from Millipore (Bedford, UK). Ampholine was from Amersham Biosciences (Buckinghamshire, UK). Purified human aldolase B protein was previously prepared by Haimoto et al. (1989). Recombinant human aldolase B was a generous gift from Prof. Dean R. Tolan (Boston University, Boston, MA). Other chemicals were of the highest grade commercially available.

2.2. Patients

This study was approved by the Ethics Committee of Kanazawa University, Nagoya First Red-Cross Hospital, and Fukui Prefectural Hospital, Japan. The two patients (A and B) gave written informed consent. Serum ALT was periodically measured throughout the time of monitoring. Patient A was a 47-year-old Japanese female with diabetes mellitus. She had been prescribed insulin (36 U/day) for 3 years. Because of inadequate control of the blood sugar level, administration of insulin was stopped and troglitazone therapy (400 mg/day) was started in 1998. Sixteen weeks after the start of troglitazone therapy, the serum ALT level started to increase (32 IU/L). Since the serum ALT level had prominently increased to 229 IU/L at 23.5 weeks, troglitazone therapy was stopped (Fig. 1A). The serum ALT levels gradually decreased (183 IU/L, week 24; 113 IU/L, week 26; 24 IU/L, week 30; 12 IU/L, week 75). Patient B was a 70-year-old Japanese female with diabetes mellitus, hyperlipidemia, and essential hypertension. The patient had been prescribed glibenclamide (10 mg/day), pravastatin (10 mg/day), and celiprolol (100 mg/day). In 1998, because of inadequate control of the blood sugar level, troglitazone therapy was started at