Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 551–555, January 1987
Medical Sciences

Human anti-endoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis are directed against a human liver cytochrome P-450 that hydroxylates the drug

(metabolism/tienilic acid/immunotoxicology)

PH. BEAUNE*, P. M. DANSETTE†, D. MANSUY†‡, L. KIFFEL*, M. FINCK†, C. AMAR†, J. P. LEROUX*, AND J. C. HOMBERG§

*Laboratoire de Biochimie, Institut National de la Santé et de la Recherche Médicale U75, CHU-Necker, 156 rue de Vaugirard, 75730 Paris Cedex 15, France; †Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris V, UA 400 Centre National de la Recherche Scientifique, 45 rue des Saints Pères, 75270 Paris Cedex 06, France; and §Laboratoire Central d'Immunologie et d'Hématologie, Hopital Saint-Antoine, 184 rue du Faubourg Saint Antoine, 75571 Paris Cedex 12, France

Communicated by Allan H. Conney, September 23, 1986 (received for review January 10, 1986)

ABSTRACT **"Anti-liver/kidney microsome" (anti-LKM) autoantibodies have been found in the serum of patients with cryptogenic chronic hepatitis and with immunoallergic drug-induced hepatitis, such as those induced by halothane or by tienilic acid (called anti-LKM_2 in this case). So far the nature of the human microsomal macromolecules recognized by these antibodies has not been determined. Here we show, by using immunoblot techniques, that among the macromolecules present in human adult liver microsomes, one protein called cytochrome P-450-8 is specifically recognized by most sera of patients containing anti-LKM_2 antibodies but not by control serum. Human fetal liver microsomes that do not contain cytochrome P-450-8 are not recognized by the anti-LKM_2 antibodies. It is also shown that anti-cytochrome P-450-8 antibodies as well as human serum containing anti-LKM_2 antibodies specifically inhibit the hydroxylation of tienilic acid by human liver microsomes. These results indicate that anti-LKM_2 antibodies appearing in patients with hepatitis and concomitant administration of tienilic acid are directed against a cytochrome P-450 isoenzyme that catalyzes the metabolic oxidation of this drug. This suggests a possible mechanism for the appearance of anti-organelle antibodies in a drug-induced hepatitis.**

Some drugs have been found to induce immunoallergic hepatitis in humans as well as the appearance, in human serum, of circulating antibodies that are directed against cell organelles (1, 2). By using immunofluorescence techniques, it was shown that some of these antibodies specifically recognized as yet unidentified macromolecules of the endoplasmic reticulum of rat liver or kidney cells (3). For this reason, they were called "anti-liver/kidney microsome" (anti-LKM)¶ antibodies.

These autoantibodies should be very useful tools for the determination of the detailed molecular mechanisms of hepatitis. In that context, it was shown that the anti-LKM_1 antibodies that are present in the sera from several patients recovering from severe hepatitis after halothane treatment interacted specifically with hepatocytes from halothane-treated rabbits (4), leading eventually to their destruction by lymphocytes.

An important step toward understanding the molecular mechanism of formation of these anti-organelle antibodies and, perhaps, of the associated drug-induced hepatitis should be the determination of the nature of the as yet unidentified cell macromolecules against which these antibodies are directed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The present report describes the determination of the nature of a protein against which an anti-LKM antibody is directed. It shows that serum containing anti-LKM_2 antibodies from patients with hepatitis and concomitant administration of tienilic acid recognizes specifically a protein of human liver microsomes called cytochrome P-450-8. It also shows that this cytochrome P-450 is mainly responsible for the hydroxylation of tienilic acid in human liver microsomes. These results provide evidence for the existence of circulating antibodies against a cytochrome P-450 in humans and suggest a possible mechanism for the production of anti-organelle antibodies in some individuals with drug-induced hepatitis.

MATERIALS AND METHODS

Chemicals. All chemical reagents were of the highest quality available. Affinity sorbent (glutaraldehyde activated) was from Boehringer Mannheim, electrophoresis reagents were from Serva (Heidelberg), protein A-Sepharose was from Pharmacia, nitrocellulose sheets were from Bio-Rad, peroxidase-conjugated immunoglobulins were from Dako Immunoglobulin (Copenhagen), and anti-human IgG rabbit immunoglobulin was from Dako.

[^{14}C]Tienilic acid (label in the keto group, 25 Ci/mol; 1 Ci = 37 GBq) was synthesized by CEA Saclay (5); 5-hydroxytienilic acid was prepared as described (6).

Human Liver Microsomes. Human liver microsomes were prepared from a liver (HF97) from a female renal transplant donor after accidental death. Documentation of medical history and medication received was unavailable. The liver was removed, perfused, and chilled on ice within 15 min after death. Small portions (about 10 g) were frozen in liquid nitrogen and stored at −80°C. Microsomal fractions were prepared as described (7, 8) and stored at −80°C. Human fetal liver microsomes were a gift of T. Cresteil (9). Their cytochrome P-450 contents were determined by the method of Omura and Sato (10) and protein concentration was measured colorimetrically (11).

Human Sera. Twenty serum samples containing anti-LKM_2 antibodies (3) were obtained from patients suffering from hepatitis; 19 of them were treated with tienilic acid

Abbreviation: anti-LKM, anti-liver/kidney microsome.

‡To whom correspondence should be addressed.

¶Depending upon their characteristic fluorescence pattern on hepatocytes and on proximal renal tubule cells, two classes of anti-LKM antibodies were defined. They were called anti-LKM_1 and anti-LKM_2. Anti-LKM_1 antibodies were found in patients with cryptogenic hepatitis and cryptogenic cirrhosis and in patients with halothane-induced hepatitis. Anti-LKM_2 antibodies have so far only been detected in patients suffering from tienilic acid-induced hepatitis (1–3).

either concomitantly or previously in France or the United States. Their anti-LKM_2 titers by immunofluorescence (3) varied from 1:100 to 1:10,000. The titers of patients CAI and JOR were 1:2000 and 1:500, respectively.

Twenty control serum samples that did not contain anti-LKM_2 antibodies were obtained from blood donors or patients; 15 of these samples were from individuals who had never taken tienilic acid. Their bilirubin and glutamic-pyruvic transaminases were within the normal range except for 2 samples (bilirubin, 28 and 30 μM; transaminase, 125 and 740 units/liter, respectively). The patient with the transaminase value of 740 units/liter had a viral B hepatitis. Five of the control sera were from patients without hepatitis, but these subjects were taking 250 mg of tienilic acid per day for >1 year. Their sera contained 5–15 μM tienilic acid, and they were dialyzed against 0.9% NaCl to remove tienilic acid that could have interfered in the inhibition studies. We have verified that identical dialysis of serum from patient CAI did not modify the results obtained with this serum.

Preparation of Purified Human Liver Cytochrome P-450, Epoxide Hydrolase, and Anti-Cytochrome P-450-8 and Anti-P-450-5 Antibodies. Cytochromes P-450-5, -8, and -9 and epoxide hydrolase were purified from adult human liver microsomes by reported techniques (7, 8). Antibodies were raised in rabbits against each of these purified proteins. None of the three purified cytochromes P-450 crossreacted with antibodies directed to the other two proteins (7–9).

Immunoblot Analyses. Human liver microsomes or purified human liver proteins were subjected to electrophoresis (12) on $NaDodSO_4$/polyacrylamide gels (7.5%). After completion of electrophoresis, the separated proteins were electrophoretically transferred to nitrocellulose paper according to previously described procedures (7, 8, 13, 14). Nitrocellulose was successively saturated by serum albumin and fetal calf serum and incubated with either specific anti-cytochrome-P--450 antibodies (7, 8) or the serum to be tested. In the case of human serum, nitrocellulose was incubated 30 min at room temperature with rabbit immunoglobulins raised against human immunoglobulins (Dako). Finally, nitrocellulose sheets were incubated with peroxidase-conjugated swine immunoglobulins raised against rabbit immunoglobulins. Peroxidase was revealed by 4-chloro-1-naphthol and H_2O_2 as reported (7, 8).

Tienilic Acid Hydroxylation by Human Liver Microsomes. Microsomes (0.18–0.24 nmol of cytochrome P-450) were diluted in 100 μl of sodium phosphate buffer (0.1 M, pH 7.4). Incubation was started by adding 50 μl of the same buffer containing a NADPH-generating system (0.15 μmol of NADP, 1.5 μmol of glucose 6-phosphate, 0.75 μmol of $MgCl_2$, 0.2 unit of glucose-6-phosphate dehydrogenase) and the substrate (15 nmol of tienilic acid, ^{14}C on the keto group, 5–25 Ci/mol). After 30 min at 37°C, 300 μl of methanol containing 3 nmol of unlabeled 5-hydroxytienilic acid was added to the incubation mixture to stop the reaction. Precipitated proteins were centrifuged at 2000 × *g* and the supernatant fraction was transferred to a new tube and concentrated under a N_2 flow to 100 μl. The solution was kept at 4°C until analysis by HPLC. Formation of 5-hydroxytienilic acid was quantified by reverse-phase HPLC on an Altex Ultrasphere ODS column (150 × 4.6 mm) eluted at 1 ml/min by a gradient from 0.1 M ammonium acetate buffer (pH 4.6) to 50% CH_3CN in H_2O in 20 min with UV detection at 310 nm. Fractions were collected in 3-ml propylene tubes every 0.5 min and radioactivity was quantified after addition of 2 ml of Picofluor 30 (Packard) in a Packard Tricarb 300 scintillation counter. The amount of 5-hydroxytienilic acid was computed by summing the radioactivity under the UV peak. Reproducibility was good (±10% for triplicates). Control activity was 10 nmol formed in 30 min/nmol of cytochrome P-450.

The inhibitory effects of the various antibodies or sera were studied by preincubation (20 min at 4°C) of the human liver microsomes with various dilutions of antibodies or sera in 100 μl of phosphate buffer.

Other Enzymatic Assays. (*S*)-Mephenytoin hydroxylase and demethylase activities were measured by the method of Meier *et al.* (15). Ethoxycoumarin deethylase activity was measured by a modification of the method of Aitio (16). Incubation was made in a final volume of 150 μl with 0.33 mM substrate for 10 min at 37°C. Inhibition experiments with antibodies or sera were performed by preincubating them with microsomes as described for tienilic acid hydroxylation.

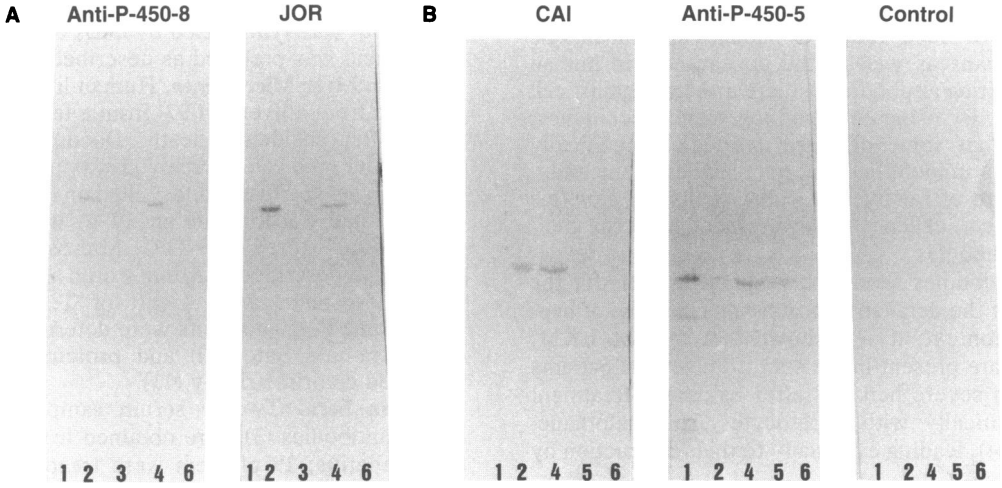


FIG. 1. Immunoblot analyses of human liver microsomes, purified human liver cytochromes P-450, and epoxide hydrolase with specific anti-cytochrome P-450 antibodies or by sera from patients with or without anti-LKM_2 antibodies. (*A*) Electrophoresis was performed on purified cytochrome P-450-5 (4 pmol; lanes 1), purified cytochrome P-450-8 (3.4 pmol; lanes 2), purified cytochrome P-450-9 (6.7 pmol; lanes 3), adult human liver microsomes (5.0 μg of protein; lanes 4), and purified epoxide hydrolase (0.6 μg of protein; lanes 6). Visualization of the immunoreaction was made by treatment of nitrocellulose with anti-P-450-8 (rabbit serum diluted 1:100 containing anti-cytochrome P-450-8) or JOR [serum diluted 1:100 from a patient (JOR) containing anti-LKM_2 antibodies]. (*B*) Lanes 1, purified cytochrome P-450-5 (4.7 pmol); lanes 2, cytochrome P-450-8 (3.9 pmol); lanes 4, adult human liver microsomes (8 μg of protein); lanes 5, fetal human liver microsomes (12 μg of protein); lanes 6, epoxide hydrolase (0.4 μg of protein). Visualization of the immunoreaction was made by treatment with CAI (serum of patient CAI containing anti-LKM_2 diluted 1:100), anti-P-450-5 (anti-cytochrome P-450-5 rabbit serum diluted 1:100), or control (control human serum diluted 1:100 not containing anti-LKM_2 antibodies).

Table 1. Ability of sera of patients with or without anti-LKM_2 antibodies to recognize specifically cytochrome P-450-8 and to inhibit 5-hydroxylation of tienilic acid in human liver microsomes

Serum	No. of sera recognizing cytochrome P-450-8 in immunoblot analysis*	No. of sera inhibiting tienilic acid hydroxylation†
Containing anti-LKM_2 antibodies‡	12/20	11/11
Control from patients taking tienilic acid§	0/5	0/5
Control from patients not taking tienilic acid§	0/15	0/11

*Considered as positive when they behaved as patients JOR and CAI.

†Considered as positive when the ID_{50} value was <50 μl of serum per nmol of cytochrome P-450 (in fact, most "positive" sera had ID_{50} values of <20 μl of serum per nmol of cytochrome P-450; see Fig. 4). Considered as negative when the half-inhibition was not reached even with 250 μl of serum per nmol of cytochrome P-450; see Fig. 4.

‡All of these sera were from patients suffering from hepatitis. Nineteen of these patients were treated with tienilic acid either concomitantly or previously (in France or the United States). Their anti-LKM_2 titers by immunofluorescence (3) varied from 1:100 to 1:10,000 (CAI, 1:2000; JOR, 1:500).

§Sera not containing anti-LKM_2 from blood donors or patients.

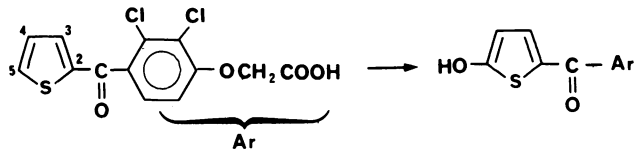
RESULTS

To determine the macromolecule(s) of human liver microsomes that could be recognized by the anti-LKM_2 antibodies appearing in the serum of patients treated with tienilic acid and suffering from hepatitis, adult and fetal liver microsomes were analyzed by electrophoresis and immunoblotting either with human serum containing anti-LKM_2 antibodies or with specific antibodies raised in rabbits against some purified microsomal human liver proteins. For comparison, four proteins purified from human liver microsomes—epoxide hydrolase and three cytochrome P-450 isoenzymes called P-450-5, P-450-8, and P-450-9, which exemplify three families of human liver cytochrome P-450 (7, 8)—were subjected to immunoblot analysis under identical conditions.

As shown in Fig. 1, two sera containing anti-LKM_2 antibodies from patients JOR and CAI with hepatitis and concomitant administration of tienilic acid recognized purified cytochrome P-450-8 and only one major component of adult human liver microsomes producing a major comigrating band. Both sera failed to react with the other purified human liver microsomal proteins used in this study, cytochromes P-450-5 and -9 and epoxide hydrolase (Fig. 1). Specific recognition of cytochrome P-450-8 by these sera was confirmed by the following results: (*i*) neither serum recognized any component of fetal liver microsomes (Fig. 1*B*) that were shown to be devoid of cytochrome P-450-8 (9); (*ii*) the immunoblot recognition patterns of human liver microsomes or of purified proteins with these two sera were almost identical to those obtained with anti-cytochrome P-450-8 antibodies (Fig. 1*A*, immunoblot analyses with anti-cytochrome P-450-8 and with serum JOR). In contrast, the recognition pattern obtained with anti-cytochrome P-450-5 antibodies was very different, this antibody reacting only with purified cytochrome P-450-5 and with a major component of adult and fetal human liver microsomes having the same electrophoretic characteristics (Fig. 1*B*). Accordingly, fetal human liver microsomes have been reported to contain cytochrome P-450-5 (9).

These results show that sera from patients JOR and CAI contain antibodies that specifically recognize one component of human liver microsomes, the cytochrome P-450-8 isoenzyme. Control sera from volunteers or patients that did not contain anti-LKM_2 antibodies did not recognize any purified protein used in this study or any component of adult or fetal human liver microsomes (Fig. 1*B* Control). Analogous immunoblot analyses were performed with 40 sera from patients or volunteers. Twenty of the serum samples were from patients suffering from hepatitis and contained anti-LKM_2 antibodies. As shown in Table 1, 12 of these 20 sera recognized cytochrome P-450-8 and gave the same clear pattern of recognition in immunoblot as sera from patients JOR and CAI. Among the 20 control sera devoid of anti-LKM_2 antibodies, 15 were from blood donors or patients having not taken tienilic acid and 5 were from patients having taken tienilic acid for at least 12 months and having no sign of hepatitis. All of the control sera failed to recognize cytochrome P-450-8 and did not give a pattern similar to that of patients JOR and CAI.

The major metabolite of tienilic acid in humans is derived from hydroxylation of the thiophene ring in position 5 (6) (Scheme 1).



Scheme 1

We found that this hydroxylation also occurs in human liver microsomes and is linked to the covalent binding of reactive metabolites to microsomal proteins (P.M.D., C. Smith, M.F., C.A., Ph.B., and D.M., unpublished data). It was thus tempting to determine if cytochrome P-450-8, specifically recognized by anti-LKM_2 antibodies appearing in patients with hepatitis and concomitant administration of tienilic acid, was involved in tienilic acid hydroxylation. Thus, adult human liver microsomes (from the same human liver used for immunoblot analyses) were incubated with tienilic acid and NADPH in the presence or absence of various sera. Fig. 2 shows that 5-hydroxylation of tienilic acid by human liver microsomes is greatly inhibited by anti-cytochrome P-450-8 antibodies (60%) as well as by a serum of a patient containing anti-LKM_2 antibodies (JOR) (80%). In contrast, anti-cytochrome P-450-5 antibodies and a control serum that did not

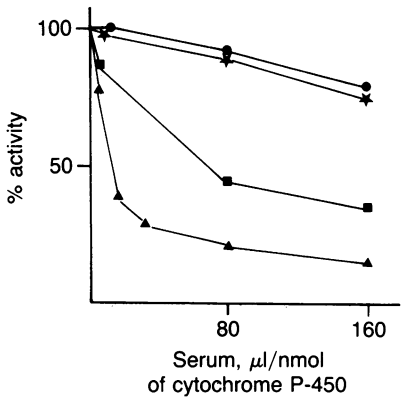


FIG. 2. Inhibition of human liver microsome-dependent 5-hydroxylation of tienilic acid by anti-human liver cytochrome P-450 antibodies or by serum containing or not containing anti-LKM_2 antibodies. The control samples (●) formed 10 nmol of 5-hydroxytienilic acid per nmol of cytochrome P-450 in 30 min. ★, Anti-P-450-5; ■, anti-P-450-8; ▲, JOR.

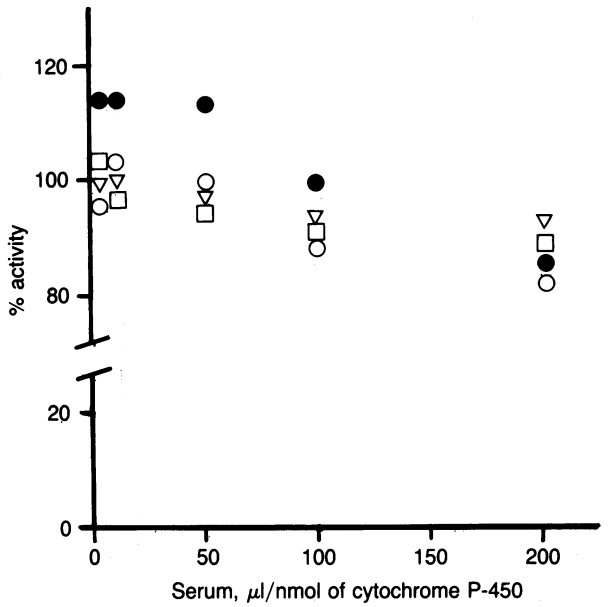


FIG. 3. Effects of anti-cytochrome P-450 antibodies and of sera containing anti-LKM_2 antibodies on the deethylation of 7-ethoxycoumarin by human liver microsomes. Control activity, 1.3 ± 0.1 nmol of 7-hydroxycoumarin formed per min/nmol of cytochrome P-450. ▽, Serum containing anti-LKM_2 antibodies (JOR); □, control serum not containing anti-LKM_2 antibodies; ○, anti-cytochrome P-450-8 antibodies; ●, anti-cytochrome P-450-5 antibodies.

contain anti-LKM_2 antibodies exhibited only a very small inhibitory effect toward the 5-hydroxylation of tienilic acid.

The observed inhibition of tienilic acid hydroxylation by human serum containing anti-LKM_2 antibodies is not due to a general nonspecific inhibitory effect of these sera on microsomal cytochrome P-450-dependent activities since the sera of patients JOR and CAI were unable to inhibit ethoxycoumarin deethylation, a classical microsomal reaction that does not seem to depend on cytochrome P-450-8 (17) (Fig. 3).

Hydroxylation of (*S*)-mephenytoin in the *para* position of its aromatic ring [(*S*)-mephenytoin 4-hydroxylation] appears to be dependent on cytochrome P-450-8 (17). Therefore, the effects of anti-LKM_2 antibodies on microsomal hydroxylation of mephenytoin were tested. Sera from patients CAI and JOR inhibited the (S)-mephenytoin 4-hydroxylation by >90% in microsomes of an adult human liver [ID_{50} values of 5 and 20 μl of serum per nmol of cytochrome P-450, respectively (data not shown; P.M.D., U. T. Meier, D.M., and U. Meyer, unpublished data)], whereas control sera had no effect. These results further confirmed that anti-LKM_2 antibodies specifically recognize cytochrome P-450-8 and indicate that tienilic acid hydroxylation by human liver microsomes depends on cytochrome P-450-8.

As shown in Table 1, all of the sera from patients containing anti-LKM_2 antibodies that could be tested were found to inhibit 5-hydroxylation of tienilic acid with ID_{50} values between 2 and 25 μl of serum per nmol of cytochrome P-450 (the mean ID_{50} value for these 11 sera tested was 16 μl/nmol of cytochrome P-450) (Fig. 4). All of the 20 tested control sera that did not contain anti-LKM_2 antibodies, either from patients or volunteers who took or did not take tienilic acid, gave no or very low inhibitory effects toward the 5-hydroxylation of tienilic acid (<50% inhibition was always obtained even after addition of 250 μl of serum per nmol of cytochrome P-450) (Fig. 4).

DISCUSSION

Taken together, these results show that a great number of sera containing anti-LKM_2 antibodies from patients with hepatitis and concomitant administration of tienilic acid specifically recognize cytochrome P-450-8, the human liver isoenzyme that seems mainly responsible for metabolic hydroxylation of tienilic acid. The ability of human serum to recognize cytochrome P-450-8 in immunoblot analyses of human liver microsomes and to inhibit tienilic acid hydroxylation is related to the presence of anti-LKM_2 antibodies. This ability is not due simply to the administration of tienilic acid since sera from patients having taken this drug, but which did not contain anti-LKM_2 antibodies, failed to recognize cytochrome P-450-8 and to inhibit tienilic acid hydroxylation (Table 1). To our knowledge, evidence for the existence in humans of circulating antibodies raised against a cytochrome P-450 has not been reported previously. This is also an indication that antibodies appearing in the sera of patients suffering from drug-induced hepatitis could be directed against a human liver cytochrome P-450 that is responsible for the oxidative metabolism of the administered drug. Since preliminary results have shown that 5-hydroxylation of tienilic acid in human liver microsomes is accompanied by the formation of reactive metabolites that bind to microsomal proteins (P.M.D., C. Smith, M.F., C.A., Ph.B., and D.M., unpublished data), it is tempting to propose a mechanism for anti-LKM_2 antibody production after tienilic acid administration (Scheme 2): (step 1) hydroxylation of the drug mainly by cytochrome P-450-8, (step 2) formation of reactive metabolites during this process and covalent binding of them to liver proteins and particularly to cytochrome P-450-8, (step 3) immunological response and production of antibodies against this cytochrome. The aforementioned results are consistent with step 1 and show the presence of antibodies against cytochrome P-450-8 proposed in step 3. Studies on step 2 are necessary. Almost all sera containing anti-LKM_2 antibodies

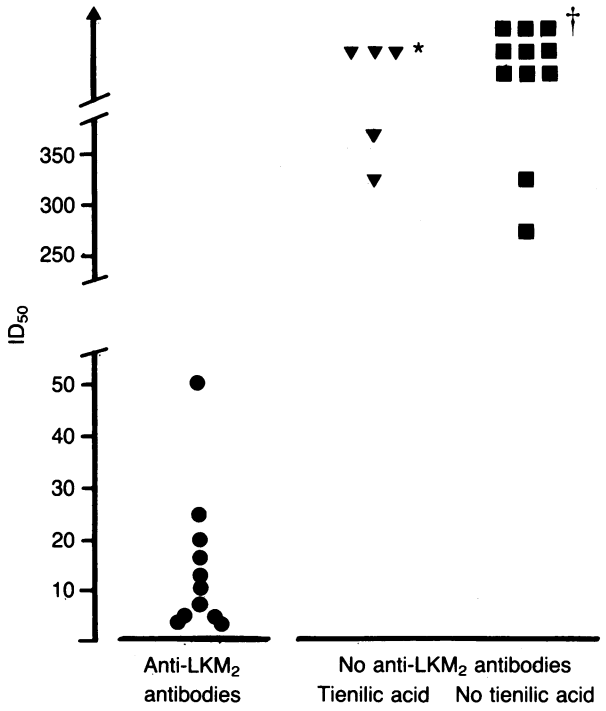
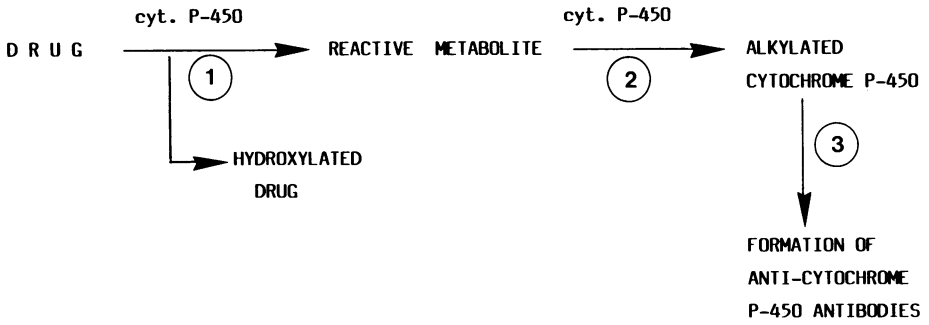


FIG. 4. Effects of different human sera containing or not containing anti-LKM_2 antibodies on the 5-hydroxylation of tienilic acid by human liver microsomes. ●, Sera containing anti-LKM_2 antibodies; sera not containing anti-LKM_2 antibodies: ▼, from patients taking tienilic acid; ■, from patients not taking tienilic acid. *, ID_{50}: amounts of serum (μl/nmol of cytochrome P-450) leading to 50% inhibition of hydroxylation. †, For most control sera, 50% inhibition was never reached even with 300 μl of serum per nmol of cytochrome P-450. These points correspond to sera giving <30% inhibition with 300 μl/nmol of cytochrome P-450.



Scheme **2**

used in this study were from patients treated with tienilic acid, which was found as a good substrate of cytochrome P-450-8, but our results suggest that other compounds capable of being oxidized by cytochrome P-450-8 into reactive metabolites could also lead to the appearance of anti-LKM_2 antibodies. In this proposed mechanism for production of anti-LKM_2 antibodies after tienilic acid administration, the steps between the irreversible modification of cytochrome P-450-8 after covalent binding of reactive metabolites of tienilic acid and the appearance of antibodies against the cytochrome remain unclear. In that regard, it was recently shown that halothane was oxidatively metabolized into a reactive species, trifluoroacetyl chloride, which bound covalently to a liver cytochrome P-450 (18). A trifluoroacetyl chloride adduct, which could be the trifluoroacetylated cytochrome P-450, appeared on the rat hepatocyte membrane (19). Moreover, evidence was provided that sera from several patients recovering from severe hepatitis after halothane treatment contained specific anti-organelle autoantibodies, called anti-LKM_1 in that case (1, 4, 20). These antibodies were found to interact specifically with hepatocytes from halothane-treated rabbits and to activate their destruction by lymphocytes (4). These literature data suggest a possible mechanism for antibody production against a liver cytochrome P-450 after administration of a drug. This cytochrome, originally present in the endoplasmic reticulum of the hepatocyte, could be alkylated by a reactive metabolite and migrate onto the hepatocyte membrane surface. At this level, the modified protein could be recognized by the immune system, starting the production of antibodies that could recognize not only the hapten derived from the reactive metabolite but also the native protein.

Naturally, much work is necessary to verify these hypotheses and to establish the mechanism of formation of anti-endoplasmic reticulum autoantibodies after administration of certain drugs to humans. It remains to be established whether anti-LKM_2 antibodies are responsible for the hepatitis that occurs in ≈ 1 in every 10,000 patients treated with tienilic acid. However, the above results together with those published on the mechanism of halothane-induced hepatitis (4, 18, 19) constitute a first approach to a detailed understanding of the molecular mechanism of some drug-induced immunoallergic hepatitis.

P.M.D., D.M., M.F., and C.A. thank Laboratoire ANPHAR-ROLLAND (France) for providing tienilic acid and for continuous financial and scientific support during this work.

1. Homberg, J. C., Abuaf, N., Helmy-Khalil, S., Biour, M., Poupon, R., Islam, S., Darnis, F., Levy, V. G., Opolon, P., Beaugrand, M., Toulet, J., Danan, G. & Benhamou, J. P. (1985) *Hepatology (N.Y.)* **5**, 722–727.
2. Mackay, I. R. (1985) *Hepatology (N.Y.)* **5**, 904–906.
3. Homberg, J. C., Andre, C. & Abuaf, N. (1984) *Clin. Exp. Immunol.* **55**, 561–570.
4. Neuberger, J., Mieli-Vergani, G., Tredger, J. M., Davis, M. & Williams, R. (1981) *Gut* **22**, 669–672.
5. Herbert, M. & Pichat, L. (1976) *J. Labelled Compd. Radiopharm.* **12**, 437–453.
6. Mansuy, D., Dansette, P. M., Foures, C., Jaouen, M., Moinet, G. & Bayer, N. (1984) *Biochem. Pharmacol.* **33**, 1429–1439.
7. Wang, P. P., Beaune, P., Kaminsky, L. S., Dannan, G. A., Kadlubar, F. F., Larrey, D. & Guengerich, F. P. (1983) *Biochemistry* **22**, 5375–5382.
8. Beaune, P., Flinois, J. P., Kiffel, L., Kremers, P. & Leroux, J. P. (1985) *Biochim. Biophys. Acta* **840**, 364–370.
9. Cresteil, T., Beaune, P., Kremers, P., Celier, C., Guengerich, F. P. & Leroux, J. P. (1985) *Eur. J. Biochem.* **151**, 345–352.
10. Omura, T. & Sato, R. (1962) *J. Biol. Chem.* **237**, 1375–1376.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
12. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
13. Towbin, H. T., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
14. Guengerich, F. P., Wang, P. P. & Davidson, N. K. (1982) *Biochemistry* **21**, 1698–1706.
15. Meier, U. T., Kronbach, T. & Meyer, U. A. (1985) *Anal. Biochem.* **151**, 286–291.
16. Aitio, A. (1978) *Anal. Biochem.* **85**, 488–491.
17. Shimada, T., Misono, K. S. & Guengerich, F. P. (1986) *J. Biol. Chem.* **261**, 909–921.
18. Satoh, H., Gillette, J. R., Davies, H. W., Schulick, R. D. & Pohl, L. R. (1985) *Mol. Pharmacol.* **28**, 468–474.
19. Satoh, H., Fukuda, Y., Anderson, D. K., Ferrans, V. J., Gillette, J. R. & Pohl, L. R. (1985) *J. Pharmacol. Exp. Ther.* **233**, 857–862.
20. Walton, B., Simpson, B. R., Strunin, L., Doniach, D., Perrin, J. & Appleyard, A. J. (1976) *Br. Med. J.* **1**, 1171–1172.