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Effect of Corticosteroids on the Expression of Cytochromes P450 and on Cyclosporin A Oxidase Activity in Primary Cultures of Human Hepatocytes

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SUMMARY

Prednisone, prednisolone, and methylprednisolone are currently administered in association with cyclosporin A in the postoperative treatment of transplant patients. The aim of this work was to evaluate the effects of these corticosteroids on the expression of several forms of cytochromes P450 (P450), including P450 1A2, 2D6, 2E1, and 3A, and on cyclosporin A oxidase activity in human liver. For this purpose, human hepatocytes prepared from lobectomies were maintained in culture in a serum-free medium, in collagen-coated dishes, for 96–144 hr, in the absence or presence of 50–100 μM corticosteroids, rifampicin, or dexamethasone. To mimic more closely the current clinical protocol, hepatocyte cultures were also co-treated with corticosteroids and cyclosporin A or ketoconazole (a selective inhibitor of P450 3A). Cyclosporin A oxidase activity, intracellular retention of cyclosporin A oxidized metabolites within hepatocytes, accumulation of P450 proteins and corresponding messages, and *de novo* synthesis and half-lives of these P450 were measured in parallel in these cultures. Our results, obtained from seven different hepatocyte cultures, showed that 1) dexamethasone and prednisone, but not prednisolone or methylprednisolone, were inducers of P450 3A, at the level of protein and mRNA accumulation, as well as of cyclosporin A oxidase activity, known to be predominantly catalyzed by these P450; 2) although corticosteroids are known to be metabolized in human liver, notably by P450 3A, partial or total inhibition of this P450 by cyclosporin or ketoconazole, respectively, did not affect the inducing efficiency of these molecules; 3) corticosteroids did not affect the half-life of P450 3A or the accumulation of other forms of P450, including 1A2, 2D6, and 2E1; 4) chronic treatment of cells with cyclosporin did not affect P450 3A accumulation; 5) corticosteroids were all competitive inhibitors of cyclosporin A oxidase in human liver microsomes, with K_i values of 61 ± 12, 125 ± 25, 190 ± 38, and 210 ± 42 μM for dexamethasone, prednisolone, prednisone, and methylprednisolone, respectively; and 6) chronic treatment of cells with corticosteroids did not influence the excretion of oxidized metabolites of cyclosporin from the cells. These results support most of clinical reports dealing with mutual interactions between cyclosporin A and corticosteroids.

CsA is widely used as an immunosuppressant in organ transplantation (1). We and others have demonstrated (2–5) that this drug is predominantly metabolized in human liver by one or several forms of the P450 3A subfamily,[1] most likely P450 3A4. It was suspected and subsequently shown (7) that drugs that induce or inhibit P450 3A forms affect the metabolism of CsA and eventually its pharmacological effect. These drug interactions might reduce the serum concentration of CsA, with the risk of graft rejection, or increase it, with the risk of nephrotoxicity (8, 9). Clinically, CsA is administered in association with prednisone, prednisolone, or methylprednisolone. These corticosteroids are used chronically at low dose to complement the immunosupressive action of CsA and occasionally at high dose (bolus) to treat acute graft rejection. A number of reports have been published concerning the interaction between CsA and corticosteroids, with conflicting results and conclusions. Some authors observed an increased serum concentration of CsA after administration of high or low doses of corticosteroid (10–13). These results were interpreted as reflecting an inhibitory effect of the corticosteroid on the hepatic metabolism of CsA. In contrast, other investigators observed an increased clearance of CsA, using an HPLC assay instead of radioimmunoassay, suggesting an inducing effect of corticosteroids on the hepatic metabolism of CsA (14).

Steroids, including corticosteroids, are metabolized by P450 in animal and human liver. Regioselective and stereospecific

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[1] P450 recommended nomenclature (6) is used throughout this paper. The human P450 3A subfamily appears to comprise at least five genes, encoding proteins whose primary sequences exhibit at least 82% similarity. Polyclonal antibodies directed against P450(CsA oxidase) are, therefore, expected to cross-react with all members of the subfamily. We shall accordingly use the term P450 3A to designate the form(s) of P450 detected in Western blot, immunoprecipitated by anti-P450 3A antibodies, and involved in CsA oxidase activity.

ABBREVIATIONS: CsA, cyclosporin A; SDS, sodium dodecyl sulfate; P450, cytochrome(s) P450; HPLC, high performance liquid chromatography.

hydroxylation of these molecules in positions 2, 6, 7, 15, 16, and 21 has been studied in detail and shown to be catalyzed by several forms of P450 from families 1, 2, and 3 in rats (15, 16) and 3 and 4 in humans (17–19). Besides dexamethasone, which has been characterized as a typical inducer of P450 3A in rats, prednisolone and methylprednisolone have been reported to be inducers of these P450 in cultures of rat hepatocyte (20, 21). However, there has been no extensive study on the effects of these corticosteroids on the expression and/or activities of P450 from human liver.

We have shown that primary cultures of human hepatocytes could be used as an alternative *in vitro* model to investigate the effect of drugs on P450 gene expression or to assess drug metabolism in human liver (7, 22, 23). During a previous investigation, preliminary results with corticosteroids indicated that these compounds behaved as inducers and inhibitors of CsA oxidase in such cultures (7). The aim of the present work was to extend our investigation further, to establish the molecular basis of the interaction between CsA and prednisone, prednisolone, or methylprednisolone, using primary cultures of human hepatocytes to mimic the various clinical protocols of coadministration of these drugs. Here we report the effects of these molecules on CsA oxidase activity, intracellular retention of CsA metabolites, accumulation of P450 3A proteins and mRNA, and *de novo* synthesis and half-lives of these P450 determined in these cultures.

Materials and Methods

Drugs and Materials

CsA and [*mebmt-β-*^{3}H] CsA (specific radioactivity, 11 Ci/mmol) from Amersham (Amersham, England) were generously supplied by Sandoz Ltd (Reuil Malmaison, France, and Basel, Switzerland). Prednisone and prednisolone were from Houdé (Paris, France), methylprednisolone from Upjohn (Paris, France), rifampicin from Merrel-Dow, Lepetit (Paris, France), and dexamethasone from Merck Sharp and Dohme-Chibret (Paris, France). Ham F-12 and Williams E. culture media, culture medium additives, dimethylsulfoxide, NADPH, and horseradish peroxidase-labeled antibodies were from Sigma (Saint Louis, MO). Acetonitrile used for the HPLC analysis of CsA was from Merck (Darmstadt, Germany). Fetal calf serum was from GIBCO (Paisley, Scotland). Bovine serum albumin (fraction V) for Western and Northern blots was from Boehringer-Mannhein France (Meylan, France). Nitrocellulose and Zeta probe filters were from Bio-Rad (Richmond, CA). [4,5^{3}H]Leucine (130 Ci/mmol) and [5-γ-^{32}P]ATP (3000 Ci/mmol) were from Amersham. T4 polynucleotide kinase was from Appligene (Illkrich, France).

Human Liver Samples

Patient FH6 was a 55-year-old female who became a renal donor after cerebral anoxia owing to acute heart failure. Patient 61289 was a 60-year-old male who became a kidney donor after rupture of a cerebral aneurysm. He had been treated with captopril. These livers could not be used for transplantion owing to an elevated steatosis; their use for scientific research was accordingly authorized by the French National Ethics Committee. Patient FT2 was a 57-year-old male who underwent a right hepatic lobectomy for metastatic gastric carcinoma. Treatment with 5-fluorouracil was stopped 1 month before surgery, but the patient was receiving piracetam (1–2 g/day) at the time of operation. Patient FT7 was a 77-year-old female who underwent left lobectomy for a hepatocellular carcinoma. She had no cirrhosis and was receiving no medication. Patient FT10 and FT12 were 32- and 55-year-old females, respectively, who underwent elective resection for hepatic angiomas. Patient FT21 was a 65-year-old male who underwent left lobe plus segment IV resection for metastatic rectal carcinoma. Patient HTL27 was a 54-year-old female who underwent left lobectomy for metastatic colorectal cancer. She was not receiving any medication before surgery. Patient HTL28 was a 60-year-old female who underwent left lobe resection for metastatic cancer of the ileum. Patient HTL37 was a 54-year-old female who underwent right lobectomy for metastatic breast cancer.

Preparation and Primary Cultures of Human Hepatocytes

Samples from patients FH6, FT2, FT10, FT12, FT21, HTL28, and HTL37 were used for hepatocyte preparation. After washing of the tissue with Eurocollins (2.05 g/liter $NaHCO_3$, 35 g/liter glucose, pH 7.3), hepatocytes were prepared as described before, using collagenase perfusion (7, 22, 23). Yield and viability, determined using the trypan blue exclusion test, were between 0.9 and 2.8 × 10^9 cells and 71 and 95%, respectively. Four million cells in 3 ml of culture medium were placed in 60-mm plastic dishes precoated with 50 μg of rat tail collagen. The culture medium consisted of a 1:1 mixture of Ham F-12 and Williams E, supplemented as recommended by Isom and Georgoff (24). During the first 4 hr, medium was supplemented with 5% fetal calf serum, to favor the plating. The medium was then changed and thereafter renewed every 24 hr in the absence of serum. Cultures were maintained at 37° in a humid atmosphere of air and 5% carbon dioxide.

Preparation of Human Liver Microsomes

Samples from patients 61289, HTL27, and HTL28 were used to prepare liver microsomes, as described previously (22). Microsomes and cell lysates were also prepared from three and two plates of hepatocytes cultures, respectively, as described before (22). Protein concentration was determined by the bicinconinic acid method, according to the protocol provided by the manufacturer (Pierce Chemical Co, Rockford, IL); bovine serum albumin (Pierce Chemical Co) was used as the standard.

Metabolism of CsA in Primary Cultures of Human Hepatocytes

Conditions for induction. Two protocols were used to test the inducer effect of corticosteroids. In protocol I, human hepatocytes were maintained in culture for 96–144 hr in the absence or presence of 50 μM rifampicin, a typical inducer of the P450 3A subfamily in humans (7), or 50 or 100 μM prednisone, prednisolone, methylprednisolone, or dexamethasone. The medium and the inducer, when present, were renewed every 24 hr. These molecules were added as a 1000× solution in dimethylsulfoxide; untreated cultures received the same amount of solvent (0.1%). At the end of the induction treatment, the medium was renewed in the absence of the inducer and in the presence of 5 μM CsA and 0.5 μCi of [^{3}H]CsA, and the cells were maintained in culture for 24 hr under normal conditions. At 2, 4, 6, and 24 hr, 100-μl aliquots of extracellular medium were collected, immediately mixed with an equal volume of acetonitrile, and stored at −20° until further analysis. At the same time, cells were washed with phosphate-buffered saline and scraped in a 1:1 mixture of water and acetonitrile. A lysate was prepared by sonication (100 W) for 45 sec on ice and was stored at −20° until further analysis. In protocol II, conditions were as in protocol I, except that 5 μM CsA or ketoconazole was present during the induction period.

Conditions for inhibition. Two different protocols were used to test the inhibitory effect of corticosteroids. In protocol III, the inhibitory effect was evaluated on young cultures, that is, under conditions reflecting the *in vivo* P450 3A status of the patient (half-life of P450 3A is on the order of 40 hr; see below). Twelve-hour-old cultures were exposed to 5 μM CsA and 0.5 μCi of [^{3}H]CsA, in the absence or presence of 100, 200, or 300 μM corticosteroids. In protocol IV, the inhibitory effect was evaluated on P450 3A4 preinduced by rifampicin. For this purpose, hepatocytes were maintained in culture for 72–96 hr in the presence of 50 μM rifampicin. At this time, medium was renewed in the absence of the inducer and cells were exposed to 5 μM CsA and 0.5 μCi of [^{3}H]CsA, in the absence or presence of 5, 25, or 100 μM corticosteroids. Aliquots of extracellular medium and cell lysate were collected and prepared at 2, 4, 6, and 24 hr.

CsA Oxidase Activity of Human Liver Microsomes

Liver microsomes (500 μg) were resuspended in 500 μl of 0.1 M potassium phosphate buffer, pH 7.4, in the presence of 2.5–10 μM CsA, in the absence or presence of 0–200 μM corticosteroids; 0.5 μCi of [^{3}H] CsA was used as radiotracer. After a 3-min incubation at 37°, the reaction was initiated by the addition of 1 mM NADPH and quenched 15 min later (under conditions of linear kinetics) by mixing with an equal volume of acetonitrile. After centrifugation, 100 μl of supernatant were analyzed by HPLC. CsA oxidase activity was expressed as nanomoles of total metabolites produced per minute per milligram of protein. Inhibition by the corticosteroids was characterized by Lineweaver-Burk plot analysis. Inhibitory constant K_i was determined from the plot of apparent K_m versus inhibitor concentration.

HPLC Analysis of CsA and Metabolites

CsA and metabolites were separated and quantitated by the HPLC procedure described in a previous paper (7). Briefly, 100-μl aliquots of 1:1 mixture of extracellular medium or cell lysate and acetonitrile were loaded on a Beckman (San Ramon, CA) Ultra-Sepharose ODS column (5 mm, 4.6 mm × 25 cm), protected with a precolumn of same phase, and were eluted at 70° with a mobile phase consisting of a mixture of water and acetonitrile, whose composition was programmed according to the following steps: 1) water/acetonitrile, 41:59, from 0 to 5 min; 2) linear acetonitrile gradient from 59 to 62% from 5 to 7 min; 3) water/acetonitrile, 38:62, from 7 to 14 min; 4) linear acetonitrile gradient from 62 to 82% from 14 to 20 min; and 5) water/acetonitrile, 18:82, from 20 to 35 min. Under these conditions, CsA eluted at 23 min and metabolites eluted as three main groups collected between 2 and 8 min, 8 and 12 min, and 12 and 20 min, consisting of dihydroxy, monohydroxy, and *N*-demethylated derivatives, respectively, as shown previously (2, 7). Radioactivity of the effluent from the HPLC column was analyzed in a LB 506-CI radioactivity monitor (Berthold, Wildbad, Germany), after mixing, in a 1:3 ratio, with scintillation cocktail (Quickszint Flow 302; Zinsser, Maidenhead, England). Radioactivity peaks were integrated with a Copam PC 286 C 100 computer and converted to molar concentrations of CsA and metabolites. In most cases, CsA oxidase activity was expressed as the amount of total metabolites produced versus time. In some experiments, this activity was evaluated as the molar ratio of di- to monohydroxy derivatives. Relative uncertainty of ±15% was estimated from measurements in triplicate on several different preparations.

Immunoquantitation of P450

P450 from the 1A, 2D, 2E, and 3A subfamilies were quantitated by Western blot using polyclonal or monoclonal antibodies, as described previously (22). In this study, 100 μg of cell lysate, prepared from hepatocytes in primary culture after various treatments, were subjected to electrophoresis on SDS-10% polyacrylamide gels before transfer to nitrocellulose. Blots were developed using diaminobenzidine and hydrogen peroxide as the horseradish peroxidase substrates. The relative amount of P450 3A was estimated from densitometric analysis of the blot with a scanner (Shimadzu, Japan); purified P450 (CsA oxidase) was used as a standard.

Determination of *De Novo* Synthesis of P450 3A

The level of *de novo* P450 3A synthesis was determined as described previously (22). Briefly, hepatocytes maintained in primary culture in the absence or presence of various inducers were labeled for 3 hr with 10 μCi/plate of [4,5-^{3}H]leucine, in a leucine-free culture medium. After washing of the cells, lysate was prepared from two plates. The *de novo* synthesis of total protein was evaluated by measuring the radioactivity of the trichloroacetic acid precipitate of an aliquot of cell lysate. The level of *de novo* synthesis of P450 3A was determined by a radioimmunoassay, with the following steps: 1) immunoprecipitation of an aliquot of cell lysate with specific anti-P450 3A antibodies; 2) resolution of the immunoprecipitate on a SDS-10% polyacrylamide gel; 3) slicing (2 mm) of the gel; and 4) determination of the radioactivity associated with the slices containing P450 3A. The *de novo* synthesis of P450 3A was evaluated as the percentage of radioactivity associated with P450 3A, with respect to the radioactivity associated with total protein in the same aliquot of cell lysate. Relative uncertainty of ±25% was estimated from measurements in triplicate on several different preparations.

Half-life Evaluation of P450 3A

Hepatocytes were induced for 72 hr with 50 μM rifampicin, and cells were pulse-labeled for 4 hr with 20 μCi/plate of [4,5-^{3}H]leucine, in a leucine-free medium. After several washes, to eliminate the inducer, the medium was renewed, in the absence or presence of 50 μM rifampicin or 100 μM corticosteroids, and the chase was continued for 96 hr. At 24, 48, 72, and 96 hr, cultures were washed, cells were harvested, and lysate was prepared from two plates. The radioactivity associated with P450 3A was determined by the radioimmunoassay described above. The half-life of P450 3A was calculated from linear regression analysis of the plot of logarithm of radioactivity associated with P450 3A per plate versus time.

Preparation of Poly(A) RNA from Cell Cultures and Northern Blot Analysis

Total RNA was isolated from 15 plates, and poly(A) RNA was purified by one cycle of absorption and elution from oligo(dT)-cellulose, as described (22). Four micrograms of poly(A) RNA were size fractionated on a 1.2% agarose gel, under denaturing conditions, and were blotted onto a Zeta Probe filter (BioRad, Richmond, CA). Oligonucleotide 87–27, complementary to nucleotides 132–161 of P450 3A4 mRNA (25) (T_l = 78°), was labeled with [γ-^{32}P]ATP (3000 Ci/mmol) and T4 polynucleotide kinase and used as a probe. Hybridization was performed in 7% SDS, 1% bovine serum albumin, 0.5 M sodium phosphate, pH 7.4, 1 mM EDTA, at 55° for 18 hr. Washes were performed in 5% SDS, 0.5% bovine serum albumin, 40 mM sodium phosphate, pH 7.4, at 55° for 1 hr. The blot was exposed overnight to Kodak X-Omat AR film, with an amplifying screen, at −70°. Control experiments to assess the quality of poly(A) RNA preparations were carried out using glyceraldehyde phosphate dehydrogenase cDNA as a probe.

Results

Primary cultures of human hepatocytes constitute a particularly useful experimental model that allows direct examination of the inducing or inhibitory effects of compounds that cannot ethically be tested *in vivo*. In the present work, these cultures were prepared from hepatic tissue collected either from healthy subjects (organ donors) or from patients with hepatocellular carcinoma, liver metastasis from extrahepatic tumors, or angioma. Previously published comparative studies demonstrated that, in such cultures, no essential difference was apparent in terms of electron microscopy, P450 induction, or drug metabolism (7, 22, 23, 26).

Corticosteroids as inducers of P450 3A and CsA oxidase in primary cultures of human hepatocytes. Twelve hours after plating, human hepatocytes were maintained in culture for 96 hr in the absence or in the presence of 50 μM

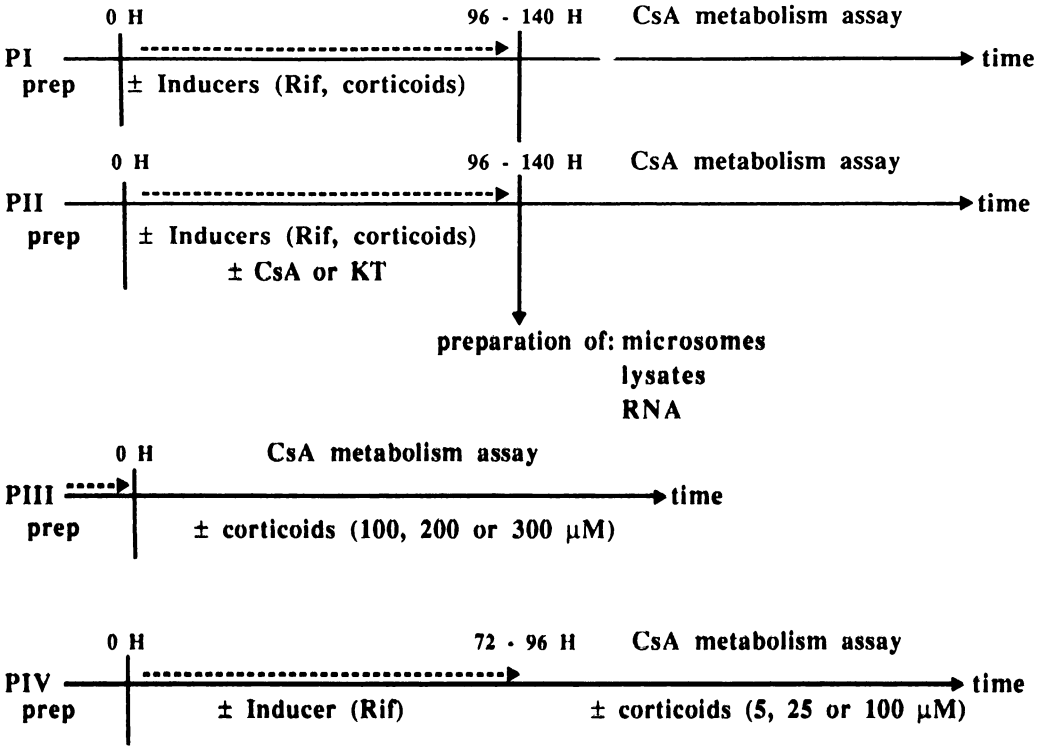

Fig. 1. Experimental protocols used in this work. Protocols I and II were used to test the inducer effects of corticosteroids on P450 expression and CsA oxidase activity. Protocols III and IV were used to test the inhibitory effects of corticosteroids on CsA oxidase activity. *Rif*, rifampicin; *KT*, ketoconazole; *prep*, time of preparation of the culture.

rifampicin or of 50 or 100 μM corticosteroids,[2] including prednisone, prednisolone, methylprednisolone, or dexamethasone (protocol I) (Fig. 1). At the end of the treatment, some cells were harvested for preparation of microsomes, RNA, and cell lysate after 3 hr of pulse-labeling with 10 μCi/plate of [4,5-^{3}H] leucine (for measurement of the *de novo* synthesis), whereas some were maintained in culture for determination of the CsA oxidase activity. The effects of the corticosteroids on the CsA oxidase activity, on the accumulation of both P450 3A protein and mRNA, and on the *de novo* synthesis of P450 3A could, therefore, be evaluated in parallel. In these experiments, rifampicin and dexamethasone were used as reference compounds, rifampicin because it is the best inducer of P450 3A (7) and dexamethasone because it has been shown previously to be an inducer of P450 3A1 in rats (20, 21) and of P450 3A in human hepatocytes (7). The results obtained with culture FT10, but representative of cultures HTL28, FT12, and FH6, are shown in Figs. 2 and 4. CsA oxidase activity, determined from the amount of oxidized metabolites of CsA released into the extracellular medium 4 hr after addition of CsA to the culture, was clearly increased in cells treated with rifampicin, dexamethasone, or prednisone, with respect to untreated, prednisolone-treated, or methylprednisolone-treated cells (Fig. 2A). The accumulation of P450 3A in these cultures, revealed by Western blot analysis of cell lysates, is shown in Fig. 2B. The induction factor of this P450 was 7.6, 4.6, 3.5, 1.5, and 1.6 with rifampicin, prednisone, dexamethasone, prednisolone, and methylprednisolone, respectively. Fig. 2 shows that CsA oxidase activity and P450 3A were correlated in these hepatocytes; the greatest increase was obtained with rifampicin, followed by dexamethasone and prednisone, whereas neither prednisolone nor methylprednisolone affected these parameters. This is in close agreement with the previous finding that, in human liver, oxidation of CsA is predominantly catalyzed by one or several P450 from the 3A subfamily (2–5). However, the magnitude of the increase of P450 3A accumulation was larger than that of the increase of CsA oxidase activity. This disagreement could result from several contributions, including 1) imbalance between *de novo* synthesized P450 3A apoproteins and the heme pool, 2) steric hinderance on the endoplasmic reticulum due to high accumulation of P450 3A, resulting in impaired electron transfer from the reductase to the P450, 3) induction of several forms of P450 3A that contribute to CsA activity to varying extents, or 4) a basal level of CsA oxidase activity in uninduced cells, resulting in part from the contribution of non-3A P450.

As it appears from Western blots presented in Fig. 3, the accumulation of the other forms of P450 tested in this work, including P450 1A2, 2D6, and 2E1, was not modified significantly in these cultures.

The comparative effects of rifampicin and corticosteroids on *de novo* P450 3A synthesis, determined by a radioimmunoassay, and P450 3A4 mRNA accumulation, determined with a P450 3A4-specific oligonucleotide under conditions of protocol I (Fig. 1), are shown in Fig. 4. In Northern blots, *CYP3A* probes were previously shown to reveal two mRNAs, at 2.2 and 3 kilobases,

[2] Steroids, including corticosteroids, produce both specific pharmacological effects and side effects. The former are likely to occur at very low concentrations, both *in vivo* and in hepatocyte cultures (i.e., <1 μM). The latter, notably interactions with drug metabolism systems like P450, occur at higher concentrations. In this paper, we are interested in these side effects. This is the reason why concentrations used here may appear rather high, in comparison with those giving rise to the corticoid specific effects. At these concentrations, corticoids were not toxic to the cells, as evidenced from measurement of *de novo* protein synthesis and other tests carried out in this work, and were soluble in the culture medium. Other investigators (20, 21) have used similar ranges of concentrations to evaluate the effect of these compounds on the expression of P450 in rat liver *in vivo* and in hepatocyte cultures. Accordingly, the terms "induction" or "inducer" used in this paper strictly refer to the corticosteroid-mediated increased expression of P450 3A; this process should not be confused with the classical glucocorticoid-induced expression of genes through the glucocorticoid receptor-dependent mechanism.

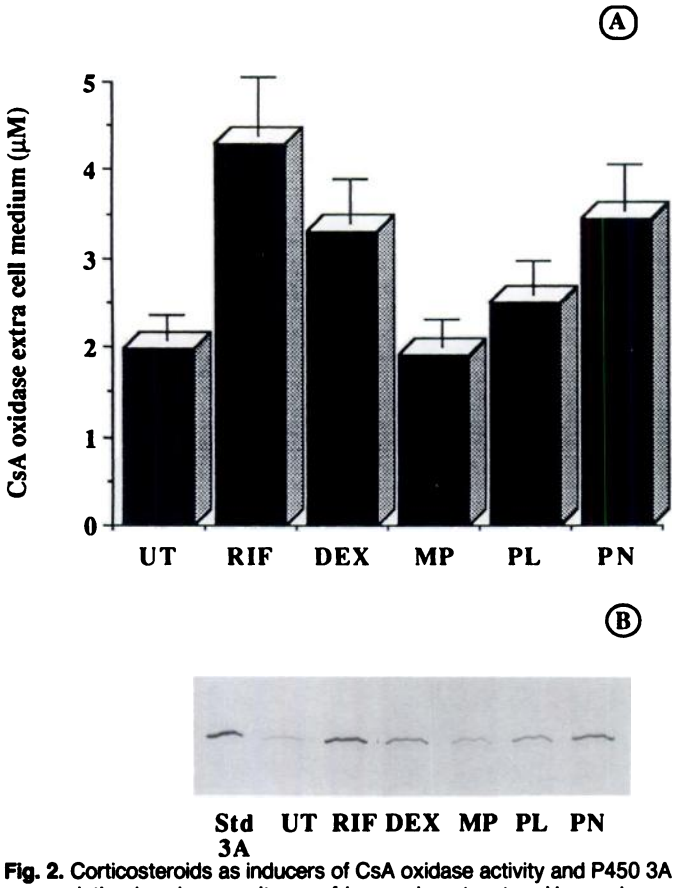

Fig. 2. Corticosteroids as inducers of CsA oxidase activity and P450 3A accumulation in primary cultures of human hepatocytes. Human hepatocytes were maintained in culture for 96 hr in the absence (*UT*) or presence of 100 μM dexamethasone (*DEX*), methylprednisolone (*MP*), prednisolone (*PL*), or prednisone (*PN*) or 50 μM rifampicin (*RIF*), under conditions of protocol I. At this time, cells were treated as described for A and B. A, Medium was renewed in the absence of inducer but in the presence of 5 μM CsA and 0.1 μCi of [^{3}H]CsA. CsA oxidase activity was then determined by HPLC analysis of extra- and intracellular media at various time intervals between 2 and 24 hr. The amount (in μM) (±10%) of oxidized CsA metabolites released in the extracellular medium after 4 hr is reported here for the various treatments. B, Cell lysates were prepared and 100 μg of protein were analyzed by Western blot, using anti-P450 3A6 polyclonal antibodies. *Std 3A*, 1 pmol of purified P450 3A6. These results obtained with culture FT10 are representative of cultures HTL28, FT12, and FH6.

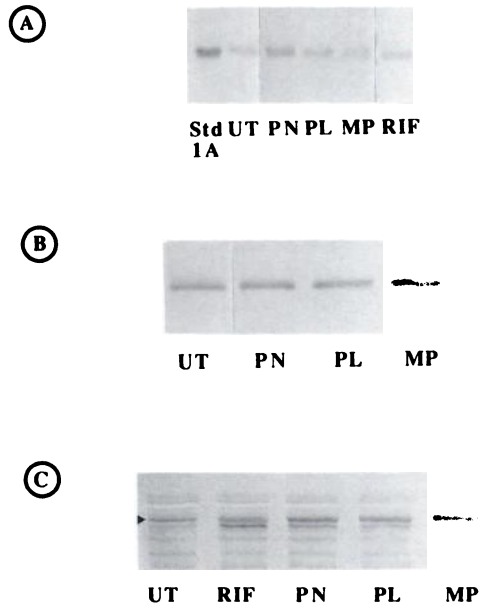

Fig. 3. Effect of corticosteroids on P450 1A2, 2D6, and 2E1 accumulation in primary culture of human hepatocytes. Human hepatocytes were maintained in culture for 96 hr in absence (*UT*) or presence of 100 μM methylprednisolone (*MP*), prednisolone (*PL*), or prednisone (*PN*) or 50 μM rifampicin (*RIF*), under conditions of protocol I. At this time, microsomes were prepared and 10 (1A2) or 50 μg (2D6 and 2E1) of protein were analyzed by Western blot, using anti-P450 1A2 (Mab 28) (A), 2D6 (B), or 2E (C) polyclonal antibodies. These results obtained with culture HTL28 are representative of cultures FT12, FT21, and FH6. On the Western blot of P450 2E1 (C), note that anti-P450 2E1 antibodies slightly cross-react with P450 3A.

that differed in the length of their 3′ noncoding region (25). As Fig. 4 shows, *de novo* P450 3A synthesis and accumulation of specific messages were closely correlated. Here again, rifampicin was the strongest inducer, followed by dexamethasone and prednisone, whereas prednisolone and methylprednisolone had no effect. Glucocorticoids are known to affect the expression of genes in a number of ways. Besides transcriptional and post-transcriptional effects, alteration of the post-translational processing of proteins has been shown (27). We, therefore, raised the question of whether these molecules could affect the half-life of P450 3A in our culture system, using the leucine-chase assay designed for this purpose. Neither of these compounds significantly affected the half-life of P450 3A, which was 44 hr in untreated cultures and 32, 55, 44, and 28 hr (±10 hr) in rifampicin-, prednisolone-, prednisone-, and methylprednisolone-treated cells, respectively. In comparison, the half-life of the pool of hepatocyte proteins was 120 hr (±20 hr) under these conditions and was not affected by the inducers. Thus, chronic treatment of human hepatocytes in culture with dexamethasone or prednisone results in a significant induction of P450 3A protein, mRNA, and related CsA oxidase activity, whereas prednisolone and methylprednisolone are not inducers. This induction process probably results from a pretranslational effect, because both P450 3A message accumulation and *de novo* synthesis were increased in parallel with the protein accumulation. It should be noted that, in all cultures examined in this study, rifampicin was a stronger inducer of P450 3A than was dexamethasone or prednisone. In addition, all cultures tested here exhibited homogeneous behavior, i.e., they all responded to dexamethasone and prednisone but not to prednisolone or methylprednisolone, in terms of P450 3A increased expression.

Steroid hormones and corticoids are biotransformed in the liver through P450-mediated hydroxylation reactions (15, 16). In fact, P450 3A has been shown to be involved in the 6β-hydroxylation of steroids and corticosteroids (17–19). We, therefore, questioned whether the metabolism of corticoids could interfere with their inducing capacity. For this purpose, hepatocytes were induced for 96–144 hr with corticoids, under conditions where P450 3A was partly (5 μM CsA) or totally (5 μM ketoconazole) inhibited (protocol II) (Fig. 1). Ketoconazole was used in these experiments because this compound was demonstrated to be a strong ($K_i < 1$ μM) and selective inhibitor of P450 3A from human liver (28). This protocol was designed to be relevant to clinical conditions, in which CsA and corticoids are usually coadministered. Comparative analysis at the level of P450 3A protein and mRNA accumulation (data not shown) indicated that neither CsA nor ketoconazole affected the inducing efficacy of the corticosteroids or of rifampicin. This

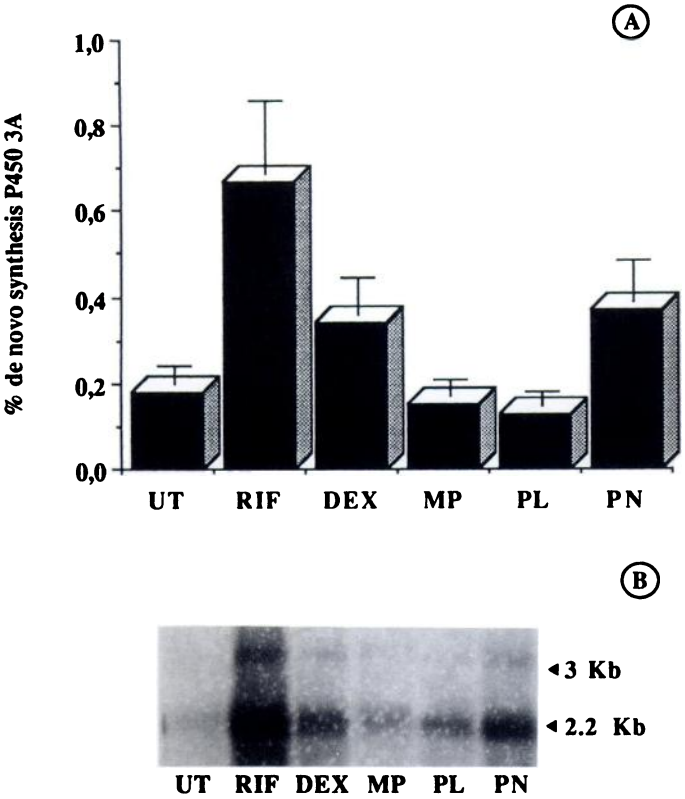

Fig. 4. Corticosteroids as inducers of P450 3A *de novo* synthesis and mRNA accumulation in primary cultures of human hepatocytes. Human hepatocytes were maintained in culture for 96 hr in the absence (*UT*) or presence of 100 μM dexamethasone (*DEX*), methylprednisolone (*MP*), prednisolone (*PL*), or prednisone (*PN*) or 50 μM rifampicin (*RIF*), under conditions of protocol I. At this time, cells were treated as described for A and B. A, Cells were labeled for 3 hr with 10 μCi of [4,5-^{3}H]leucine, in a leucine-free culture medium, and a lysate was prepared. An aliquot of 100 μl of lysate was immunoprecipitated with anti-P450 3A6 antibodies, and the radioactivity associated with *de novo* synthesized P450 3A was determined from SDS-polyacrylamide gel electrophoresis of the immunoprecipitate. Relative *de novo* synthesis of P450 3A was expressed as percentage of total protein synthesis, determined from trichloroacetic acid precipitation of a 15-μl aliquot of cell lysate. B, Poly(A)RNA was extracted and 4 μg were analyzed by Northern blot, using a P450 3A4-specific oligonucleotide radiolabeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. These results obtained with culture FT10 are representative of culture FH6.

suggests that either the metabolism of these molecules is very slow or it does not affect their capacity to induce P450 3A. In addition, these experiments show that chronic treatment of human hepatocytes with CsA does not affect the accumulation of P450 3A.

Corticosteroids as inhibitors of CsA oxidase activity in primary cultures of human hepatocytes and in human liver microsomes. Two different protocols were used to investigate the inhibitory effect of corticoids on the metabolism of CsA. In protocol III (Fig. 1), the CsA oxidase activity was determined over a period of 24 hr, on 12-hr-old cultures, in the absence or presence of increasing concentrations of corticoids. Because P450 3A half-life was found to be in the range of 40 hr in these cultures, these conditions allowed us to evaluate the effects of these compounds on the "natural" P450 3A, reflecting the P450 3A status of the patient from whom hepatocytes had been prepared. In protocol IV (Fig. 1), the same experiments were repeated with cultures that had been treated for 72–96 hr in the presence of rifampicin, to induce P450 3A. Results obtained with culture FH6, but representative of cultures FT12 and HTL37, are shown in Fig. 5, *upper* (protocol III) and *lower* (protocol IV).[3] In all cases, corticosteroids were found to be inhibitors of CsA oxidase activity, calculated from the amount of metabolites released in the extracellular medium. However, this inhibition was weak, in comparison with that observed with well known inhibitors such as ketoconazole (7, 28). Significant inhibitory effects became apparent for concentrations higher than 100 μM in untreated cells (Fig. 5, *upper*) or higher

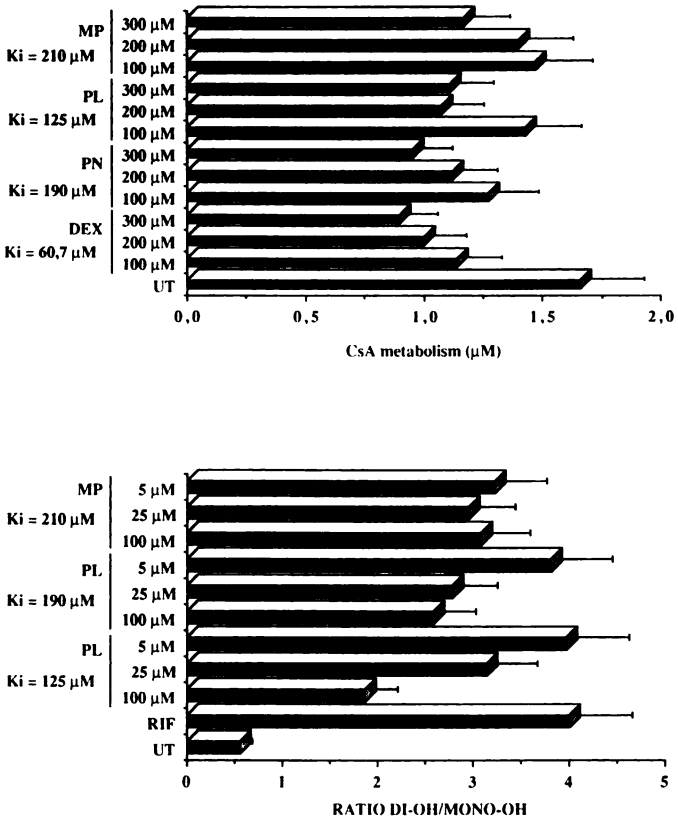

Fig. 5. Corticosteroids as inhibitors of CsA oxidase activity in primary cultures of human hepatocytes. *Upper*, CsA oxidase activity was determined, as indicated in the legend to Fig. 2, in freshly plated hepatocytes, in the absence (*UT*) or presence of 100, 200, or 300 μM methylprednisolone (*MP*), prednisolone (*PL*), prednisone (*PN*), or dexamethasone (*DEX*), under conditions of protocol III. The amount (in μM) (±10%) of oxidized CsA metabolites released in the extracellular medium after 4 hr is reported here for the various treatments. *Lower*, human hepatocytes were maintained for 96 hr in the absence (*UT*) or presence of 50 μM rifampicin (*RIF*). At this time, CsA oxidase activity was determined, as indicated in the legend to Fig. 2, in the RIF-induced cultures, in the absence or in the presence of 5, 25, or 100 μM methylprednisolone (*MP*), prednisone (*PN*), or prednisolone (*PL*), under conditions of protocol IV. *UT*, CsA oxidase activity in uninduced culture in the absence of corticosteroids. Due to a high level of metabolism of CsA in these cultures, the ratio of dihydroxy to monohydroxy derivatives of CsA, reflecting the production of second-generation metabolites (dihydroxy derivatives), is presented here after 4 hr. These results obtained with culture FH6 are representative of cultures FT12 and HTL37.

[3] The experiments presented in Fig. 5B have been carried out on rifampicin-induced hepatocytes. The CsA oxidase activity was so high in these cells that measurement of the total amount of oxidized metabolites (our standard assay) was not adequate to visualize inhibitory effects. Under these conditions, the ratio of dihydroxy to monohydroxy derivatives was used as a suitable marker of CsA oxidase activity, because previous investigations (2) have shown that dihydroxylated metabolites of CsA are produced from monohydroxy derivatives by the same P450.

than 25 μM in rifampicin-treated cells. The rank order of decreasing inhibitory effect was dexamethasone, prednisolone, prednisone, and methylprednisolone. This was similar under both protocols III and IV, except that the amplitude of the effect was larger in rifampicin-treated cells, where the level of P450 3A was increased. Some authors suspected that corticosteroids could interfere with the metabolism of CsA by blocking the release of oxidized metabolites from hepatocytes (14). Analysis of the intracellular medium of cultures chronically treated for 96 hr with rifampicin or corticoids, 4 and 24 hr after incubation with CsA, is presented in Fig. 6. These results show that the amounts of intracellular metabolites were much smaller than those of their extracellular counterparts at both 4 and 24 hr. In addition, the amounts of intracellular metabolites decreased with time in cells treated with corticoids or with rifampicin, which is not an inhibitor. These experiments suggest that the inhibitory effect of corticoids is not related to a defect in excretion of metabolites from the cells. This inhibitory effect of corticoids was further characterized by Lineweaver-Burk plot analysis of CsA oxidase activity in human liver microsomes (data not shown). Dexamethasone, prednisolone, prednisone, and methylprednisolone were found to be competitive inhibitors, with K_i values of 61 ± 12, 125 ± 25, 190 ± 38, and 210 ± 42 μM, respectively. These values are in good agreement with those determined in a preliminary study, except for prednisolone, for which a K_i of 210 μM had been previously determined (7). These relatively high K_i values explain the weak inhibition observed in culture (Fig. 5) at the concentrations tested in this work.

Discussion

Despite prednisone, prednisolone, and methylprednisolone having been used in clinical practice for more than 20 years, and now being coadministered with CsA for the treatment of transplant patients, relatively little data on their effects on the expression of drug-metabolizing enzyme systems from animal and human liver have been reported. The aim of this work was, therefore, to evaluate the effects of these molecules, as well as that of dexamethasone for comparison, on the expression of several forms of P450, including P450 3A, and on CsA oxidase in human liver. For this purpose, primary cultures of human hepatocytes, previously shown to retain liver-specific P450 gene expression (7, 22, 23), have been used. Our results from seven different hepatocyte cultures (prepared from seven different patients) demonstrated the following points: 1) dexamethasone and prednisone are inducers of P450 3A, increasing the accumulation of mRNA, protein, and CsA oxidase activity; 2) neither of the tested molecules affect the half-life of P450 3A or the accumulation of P450 1A2, 2D6, and 2E1; 3) these molecules are competitive inhibitors of CsA oxidase in human liver microsomes, with K_i values of 61–210 μM; and 4) they do not affect the excretion of oxidized metabolites from the cells.

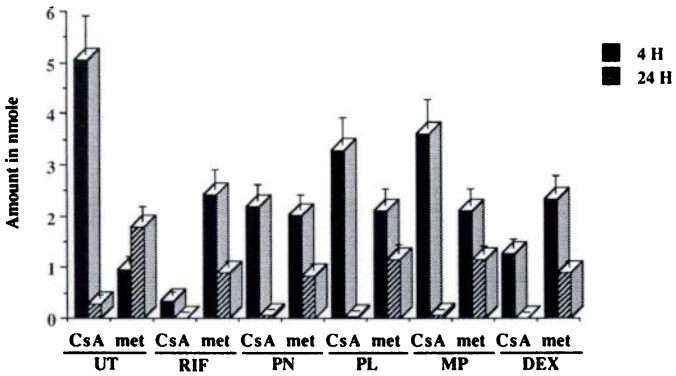

Fig. 6. Effect of chronic treatment with corticosteroids on the intracellular content of CsA and of oxidized CsA metabolites in human hepatocytes. Human hepatocytes (FT21) were maintained in culture for 140 hr in the absence (*UT*) or the presence of 100 μM dexamethasone (*DEX*), methylprednisolone (*MP*), prednisolone (*PL*), or prednisone (*PN*) or 50 μM rifampicin (*RIF*), under conditions of protocol I. At this time, cells were exposed for 4 or 24 hr to 5 μM CsA, and CsA and its oxidized metabolites were quantitated by HPLC analysis of extra- and intracellular media. Intracellular content of both CsA and oxidized metabolites (in nmol) (± 10%) is shown here for the various treatments.

Dexamethasone and pregnenolone 16α-carbonitrile have been recognized for some time as inducers of P450 from animal liver microsomes. Guzelian and co-workers (20) first demonstrated that these compounds constituted a "new class" of inducers, in that they stimulated the expression of P450 3A genes in rat liver. These observations were extended to other steroids, notably prednisolone and methylprednisolone, in rat liver and rat hepatocytes in culture (21). These authors concluded that synthesis of P450 3A was a specific glucocorticoid-responsive liver function, involving a novel mechanism, that was readily distinguishable from the classical glucocorticoid receptor pathway. Our observations are only in partial agreement with those made using rat liver. Although dexamethasone appears to be the best glucocorticoid inducer of P450 3A in both rat and human hepatocytes, methylprednisolone and prednisolone were not inducers in human hepatocytes, whereas in rats they exhibited inducing efficacy 62% and 43%, respectively, that of dexamethasone (prednisone was not tested by these authors) (21). Such interspecies differences between rats and humans, concerning the specificity and efficiency of P450 3A inducers, are not new. Previously documented examples include rifampicin, a strong inducer in humans and not in rats, and pregnenolone 16α-carbonitrile, for which the opposite is observed (29). The finding that dexamethasone and prednisone are inducers of P450 3A in human hepatocytes needs further comment. A high level of P450 3A was previously observed in critically ill patients receiving dexamethasone (30). However, phenobarbital and phenytoin, two drugs that were shown to be inducers of P450 3A in our culture system, were coadministered with dexamethasone in all these patients. It was, accordingly, not possible to conclude unequivocally that P450 3A was induced by this corticoid *in vivo*. Our results confirm this point.

Prednisone is known to be converted to prednisolone through a reductive pathway in human liver (31). Our finding that prednisone but not prednisolone was an inducer in primary culture of human hepatocytes suggests either that this conversion did not occur in our culture system or that it occured at a very low rate, in comparison with the timing of P450 3A induction. Whatever the explanation, these results suggest that clinically relevant induction of P450 3A (CsA oxidase) by prednisone, prednisolone, or methylprednisolone is unlikely. This is in good agreement with clinical reports on the effects of these compounds on the metabolism of CsA (8, 9). Indeed, there is only one paper that reports a modest inducing effect of corticoids on CsA metabolism (14). These authors measured CsA levels, by HPLC, in the serum of patients receiving CsA in association with a low maintenance dose of prednisone (20 mg/day), before and after a bolus of methylprednisolone (250

mg, intravenously). They found increased clearance of CsA (from 5.5 to 6.6 ml/min/kg) after the high dose of methylprednisolone. Their conclusion that this observation resulted from an inducing effect of methylprednisolone on the metabolism of CsA is unlikely, in view of the present results. Indeed, we had no evidence of induction of either P450 3A or CsA oxidase activity in several different preparations of human hepatocytes treated with methylprednisolone. Several explanations could be proposed for this disagreement. 1) Our *in vitro* culture system did not emulate the *in vivo* situation. This is unlikely, because P450 3A genes responded not only to rifampicin but also to dexamethasone and prednisone in this culture system. 2) Coadministration of CsA and corticosteroids is known to result in decreased metabolism of both drugs, by mutual competitive inhibition (see below). In this case, corticosteroids would accumulate sufficiently in the liver to induce P450 3A. This is again unlikely, in view of our results obtained using protocol II, in which the inducing effect of corticosteroids was shown to be unaffected by the presence of CsA or of ketoconazole, a selective inhibitor of P450 3A (28). 3) High doses of methylprednisolone would inhibit or slow the conversion of prednisone to prednisolone, allowing this compound to accumulate sufficiently within hepatocytes to induce P450 3A. This latter hypothesis remains to be tested *in vivo*. In a preliminary study of these molecules as inducers of P450 3A, we observed a weak inducing effect of prednisolone in one of three hepatocyte cultures tested. This observation could not be reproduced in the present work, at the level of either protein or mRNA. However, similar observations were made recently with other putative weak inducers, suggesting that interindividual variability in the response of human *CYP3A* genes to these molecules should be considered as an additional factor (32).

In primary cultures of rat hepatocytes, we demonstrated that troleandomycin, a substrate with high affinity for P450 3A1, was able to induce this isozyme by increasing its half-life (from 14 to 60 hr), presumably by binding tightly to the protein active site and, thus, blocking its degradation (33). Such a stabilizing effect was suspected to occur with corticoids and we, therefore, investigated the effect of these molecules on the half-life of P450 3A in our human hepatocyte culture system. In contrast to the rat experiments with troleandomycin, neither of the corticoids tested in this work affected this parameter significantly. These molecules are, therefore, not likely to interfere with the P450 3A degradation process in human hepatocytes.

Numerous authors reported that corticosteroids, including prednisolone, prednisone, and methylprednisolone, were able to increase the serum half-life of CsA and to decrease its clearance *in vivo* in humans (8–13). Our results support these observations. Dexamethasone, prednisolone, prednisone, and methylprednisolone were all characterized in this work as competitive inhibitors of CsA oxidase from human liver microsomes; the K_i values were 61, 125, 190, and 210 μM, respectively. This inhibitory effect occured both in freshly isolated hepatocytes (constitutive P450 3A) and after induction of P450 3A by rifampicin (protocols III and IV). In addition, neither impairment of CsA metabolite excretion from hepatocytes by these molecules nor decreased levels of P450 3A after chronic treatment with CsA were demonstrated. These observations raise the question of whether glucocorticoids are substrates of P450 3A. Several reports from the literature suggest that this could be the case. 1) Steroids, including corticosteroids, have been shown to be hydroxylated at various positions by P450 from liver microsomes. In particular, hydroxylation at position 6β of these molecules was shown to be catalyzed predominantly by the P450 3A forms in humans (17–19). 2) CsA was reported to interfere with corticosteroid metabolism in humans by increasing the half-lives of the corticosteroids and, in parallel, decreasing their clearance (12, 34–36). 3) Rifampicin, phenytoin, and phenobarbital, all known as inducers of P450 3A in human hepatocytes (7, 29), were reported to accelerate the metabolism of corticosteroids (36, 37). Together, these observations and the results reported here strongly suggest that corticosteroids could be metabolized, at least in part, by P450 3A in human liver. However, direct studies of the metabolism of these compounds by liver microsomal P450 will be necessary to confirm this point.

The Michaelis-Menten equations indicate that, in the case of competitive inhibition, the magnitude of the inhibition depends on the term $K_m/K_i \times I/S$, where K_m and K_i are the dissociation constants for substrate and inhibitor and I and S are the concentrations of inhibitor and substrate, respectively. In practice, the dose of CsA ranges from 120 to 900 mg/day, depending on the clinical protocols, whereas the dose of corticosteroids is, on average, 20 mg/day for maintenance treatment and 250 mg/day to 1.5 g/day as a bolus dose during acute rejection episodes. The actual concentrations of these molecules within liver cells are not known with certainty. They depend not only on the dose administered but also on several other factors, including adsorption and tissue distribution. The fact that the K_m of CsA (5 μM) is much lower than the K_i of corticosteroids suggests that, in the case where both drugs are present at similar concentrations within the liver cells, the competition for the P450 3A active site should favor CsA. This could be what was observed in renal transplant patients who developed cushingoid features, despite the use of low doses of corticosteroids, while receiving CsA (12). However, when high doses of corticosteroids (0.5–1.5 g/day, intravenously), such as those currently administered in rejection episodes, are given, the moderate affinity of corticosteroids for the enzyme active site could be compensated for by their higher concentrations in the liver. This might explain the increased half-life or decreased clearance of CsA repeatedly observed after bolus treatments of patients with corticosteroids (10). This inhibitory effect may not be limited to CsA but may affect all molecules that are oxidized by P450 3A. At present, these include erythromycin, midazolam, nifedipin, diltiazem, ethynylestradiol, progesterone, cortisol (7), tamoxifen (38), and lovastatine (39). Similarly, there is a risk of corticosteroid-related side effects in patients needing steroids while receiving one of these drugs.

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References

1. Borel, J. F. Cyclosporin A. *Triangle* **20**:97–105 (1981).
2. Combalbert, J., I. Fabre, G. Fabre, I. Dalet, J. Derancourt, J. P. Cano, and P. Maurel. Metabolism of cyclosporin A. IV. Purification and identification of the rifampicin-inducible human liver cytochrome P450 (cyclosporin A oxi-

dase) as a product of P450IIIA gene subfamily. *Drug Metab. Dispos.* **17**:197–207 (1989).
3. Kronbach, T., V. Fischer, and U. A. Meyer. Cyclosporine metabolism in human liver: identification of a cytochrome P-450IIIA gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. *Clin. Pharmacol. Ther.* **43**:630–635 (1988).
4. Shaw, P. M., T. S. Barnes, D. Cameron, J. Engeset, W. T. Melvin, G. Omar, J. C. Petrie, W. R. Rush, C. P. Snyder, P. H. Whiting, C. R. Wolf, and M. D. Burke. Purification and characterization of an anticonvulsant-induced human cytochrome P-450 catalysing cyclosporin metabolism. *Biochem. J.* **263**:653–663 (1989).
5. Aoyama, T., S. Yamano, D. J. Waxman, D. P. Lapenson, U. A. Meyer, V. Fischer, R. Tyndale, T. Inaba, W. Kalow, H. V. Gelboin, and F. J. Gonzalez. Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. *J. Biol. Chem.* **264**:10388–10395 (1989).
6. Nebert, D. W., D. R. Nelson, M. J. Coon, R. W. Estabrook, R. Feyereisen, Y. Fujii-Kuriyama, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, J. C. Loper, R. Sato, M. R. Waterman, and D. J. Waxman. The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol.* **10**:1–14 (1991).
7. Pichard, L., I. Fabre, G. Fabre, J. Domergue, B. Saint-Aubert, G. Mourad, and P. Maurel. Cyclosporin A drug interactions: screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metab. Dispos.* **18**: 595–606 (1990).
8. Wadhwa, N. K., T. J. Schroeder, A. J. Pesce, S. A. Myre, C. W. Clardy, and M. R. First. Cyclosporine drug interactions: a review. *Ther. Drug Monit.* **9**:399–406 (1987).
9. Baciewicz, A. M., and F. A. Baciewicz. Cyclosporine pharmacokinetic drug interactions. *Am. J. Surg.* **157**:264–271 (1989).
10. Klintmalm, G., and J. Säwe. High dose methylprednisolone increases plasma cyclosporin levels in renal transplant recipients (Letter). *Lancet* **1**:731 (1984).
11. Thiel, G., F. Harder, R. Loertscher, M. Brünisholz, J. Landmann, F. Brunner, F. Follat, M. Wenk, and M. Mihatsch. Cyclosporine alone or in combination with prednisone in cadaveric renal transplantation. *Transplant. Proc.* **16**:1187–1190 (1984).
12. Öst, L., G. Klintmalm, and O. Ringdén. Mutual interaction between prednisolone and cyclosporine in renal transplant patients. *Transplant. Proc.* **17**:1252–1255 (1985).
13. Brown, M. W., and M. A. Forwell. Rejection reaction after stopping prednisolone in kidney-transplant recipients taking cyclosporine (Letter). *Lancet* **314**:183 (1986).
14. Ptachcinski, R. J., R. Venkataramanan, G. J. Burckart, T. R. Hakala, J. T. Rosenthal, B. J. Carpenter, and R. J. Taylor. Cyclosporine-high-dose steroid interaction in renal transplant recipients: assessment by HPLC. *Transplant. Proc.* **19**:1728–1729 (1987).
15. Waxman, D. J., A. Ko, and C. Walsh. Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J. Biol. Chem.* **58**:11937–11947 (1983).
16. Swinney, D. C., D. E. Ryan, P. E. Thomas, and W. Levin. Regioselective progesterone hydroxylation catalyzed by eleven rat hepatic cytochrome P-450 isozymes. *Biochemistry* **26**:7073–7083 (1987).
17. Waxman, D. J., C. Attisano, F. P. Guengerich, and D. P. Lapenson. Human liver microsomal steroid metabolism: identification of the major microsomal steroid hormone 6β-hydroxylase cytochrome P450 enzyme. *Arch. Biochem. Biophys.* **263**:424–436 (1988).
18. Ged, C., J. M. Rouillon, L. Pichard, J. Combalbert, N. Bressot, P. Bories, H. Michel, P. Beaune, and P. Maurel. The increase in urinary excretion of 6β-hydroxycortisol as a marker of human hepatic cytochrome P450IIIA induction. *Br. J. Clin. Pharmacol.* **28**:373–387 (1989).
19. Waxman, D. J., D. P. Lapenson, T. Aoyama, H. V. Gelboin, F. P. Gonzalez, and K. Korzekwa. Steroid hormone hydroxylase specificities of eleven cDNA expressed human cytochrome P450. *Arch. Biochem. Biophys.* **290**:160–166 (1991).
20. Schuetz, E. G., S. A. Wrighton, J. L. Barwick, and P. S. Guzelian. Induction of cytochrome P450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16α-carbonitrile regulate *de novo* synthesis of a common form of cytochrome P450 in cultures of adult rat hepatocytes and in the liver *in vivo*. *J. Biol. Chem.* **259**:1999–2006 (1984).
21. Schuetz, E. G., and P. S. Guzelian. Induction of cytochrome P450 by glucocorticoids in rat liver. II. Evidence that glucocorticoids regulate induction of cytochrome P450 by a nonclassical receptor mechanism. *J. Biol. Chem.* **259**:2007–2012 (1984).
22. Diaz, D., I. Fabre, M. Daujat, B. Saint Aubert, P. Bories, H. Michel, and P. Maurel. Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. *Gastroenterology* **99**:737–747 (1990).
23. Pichard, L., G. Gillet, I. Fabre, I. Dalet-Beluche, C. Bonfils, J. P. Thénot, and P. Maurel. Identification of the rabbit and human cytochromes P450IIIA as the major enzymes involved in the *N*-demethylation of diltiazem. *Drug Metab. Dispos.* **18**:711–719 (1990).
24. Isom, H. C., and I. Georgoff. Quantitative assay for albumin-producing liver cells after simian virus 40 transformation of rat hepatocytes maintained in chemically defined medium. *Proc. Natl. Acad. Sci. USA* **81**:6378–6382 (1984).
25. Bork, R. W., T. Muto, P. H. Beaune, P. K. Srivastava, R. S. Lloyd, and F. P. Guengerich. Characterization of mRNA species related to human liver cytochrome P450 nifedipine oxidase and regulation of catalytic activity. *J. Biol. Chem.* **264**:910–919 (1989).
26. Cano, J. P., G. Fabre, P. Maurel, N. Bichet, Y. Berger, and P. Vic. Interindividual variability and induction of cytochromes P450 and UDP-glucuronyl transferases in human liver microsomes and primary cultures of human hepatocytes, in *Molecular Aspects of Glucuronidation*, (G. Siest, J. Magdalou, and B. Burchell, eds.), Vol. 173. Editions INSERM, Paris, 249–260 (1988).
27. Karlsen, K., A. K. Vallerga, J. Hone, and G. L. Firestone. A distinct glucocorticoid hormone response regulates phosphorylation maturation in rat hepatoma cells. *Mol. Cell. Biol.* **6**:574–585 (1986).
28. Maurice, M., L. Pichard, M. Daujat, I. Fabre, H. Joyeux, J. Domergue, and P. Maurel. Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture. *FASEB. J.* **6**:752–758 (1992).
29. Daujat, M., L. Pichard, I. Fabre, T. Pineau, G. Fabre, C. Bonfils, and P. Maurel. Induction protocols for cytochromes P450IIIA *in vivo* and in primary cultures of animal and human hepatocytes. *Methods Enzymol.* **206**:345–353 (1991).
30. Molowa, D. T., E. G. Schuetz, S. A. Wrighton, P. B. Watkins, P. Kremers, G. Mendez-Picon, G. A. Parker, and P. S. Guzelian. Complete cDNA sequence of a cytochrome P450 inducible by glucocorticoids in human liver. *Proc. Natl. Acad. Sci. USA* **83**:5311–5315 (1986).
31. Uribe, M., S. W. Schalm, and W. H. Summerskill. Oral prednisone for chronic active liver disease: dose-responses and bioavailability studies. *Gut* **19**:1131–1135 (1978).
32. Pichard, L., I. Fabre, J. Domergue, H. Joyeux, and P. Maurel. Effect of FK506 on human hepatic cytochromes P450: interaction with cyclosporin A. *Transplant. Proc.* **23**:2791–2793 (1991).
33. Watkins, P. B., S. A. Wrighton, E. G. Schuetz, P. Maurel, and P. S. Guzelian. Macrolide antibiotics inhibit the degradation of the glucocorticoid-responsive cytochrome P450p in rat hepatocytes *in vivo* and in primary monolayer culture. *J. Biol. Chem.* **261**:6264–6271 (1986).
34. Ost, L. Impairement of prednisolone metabolism by cyclosporine treatment in renal graft recipients. *Transplantation (Baltimore)* **44**:533–535 (1987).
35. Ost, L. Effect of cyclosporin on prednisolone metabolism. *Lancet* **2**:451 (1984).
36. Langhoff, E., S. Madsen, H. Flachs, K. Olgaard, J. Ladefoged, and E. F. Hvidberg. Inhibition of prednisolone metabolism by cyclosporine in kidney-transplanted patients. *Transplantation (Baltimore)* **39**:107–109 (1985).
37. Gambertoglio, J. G., N. H. G. Holford, J. E. Kapusnik, R. Nishikawa, M. Saltiel, P. Sanik-Lizak, J. L. Birnbaum, T. Hau, and W. J. C. Hamend, Jr. Disposition of total and unbound prednisolone in renal transplant patients receiving anticonvulsivants. *Kidney Int.* **25**:119–123 (1984).
38. Jacolot, S., I. Simon, Y. Dreano, P. Beaune, C. Riche, and F. Berthou. Identification of the cytochrome P450 3A family as the enzyme involved in the *N*-demethylation of tamoxifen in human liver microsomes. *Biochem. Pharmacol.* **41**:1911–1919 (1991).
39. Wang, R. W., P. H. Kari, A. Y. H. Lu, P. E. Thomas, F. P. Guengerich, and K. P. Vyas. Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. *Arch. Biochem. Biophys.* **290**:355–361 (1991).

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