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SHORT COMMUNICATION

In Vivo Induction of Cytochrome P450 CYP3A Expression in Rat Leukocytes using Various Inducers*

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ABSTRACT. Although the induction of cytochromes P450 3A (CYP3A) is relatively well characterized in liver, its inducibility in an easily available tissue such as the peripheral leukocytes is not known. The purpose of this study was, therefore, to determine if CYP3A is inducible *in vivo* in peripheral leukocytes. Microsomes from rat leukocytes and liver were examined for CYP3A protein expression using Western blotting with a rabbit polyclonal antibody against rat CYP3A. Although CYP3A was not detected in control leukocytes, *in vivo* treatment with known CYP3A inducers (dexamethasone, clotrimazole, phenobarbital, pregnenolone-16α-carbonitrile) resulted in CYP3A leukocyte levels of 0.2–0.8 pmol/mg protein. This leukocyte induction was approximately 1000-fold lower than in induced liver. Interestingly, there was an apparent linear relationship between leukocyte and liver CYP3A contents (r^2 = 0.748, n = 29). These results not only demonstrate for the first time that CYP3A is inducible in rat leukocytes after *in vivo* treatment with various CYP3A inducers, but also suggest that peripheral leukocytes could be used to assess induction *in vivo*. BIOCHEM PHARMACOL 51;11:1579–1582, 1996.

KEY WORDS. cytochromes P450; CYP3A; induction; leukocyte; noninvasive test; Western blot

Cytochromes P450 belong to a superfamily of heme monoxygenases involved in the metabolism of numerous endogenous and foreign compounds [1, 2]. Members of all but three of the 14 P450 families found in mammals are microsomal proteins that are predominantly expressed in liver and, to a lesser degree, in other organs and tissues [3]. Enzymes of the CYP3A subfamily are not only involved in the metabolism of important endogenous compounds such as steroids [4], bile acids [5], and retinoic acid [6], but can also metabolize a large number of foreign substances including procarcinogens [7] and pharmaceuticals (e.g. immunosuppressive agents, antibiotics and anticancer drugs) [4, 8, 9].

Because of marked interindividual variability in CYP3A expression in humans [7] and frequent drug interactions involving CYP3A isoenzymes, there is a need to monitor the level of these P450s in the liver. Although noninvasive tests using CYP3A substrates administered *in vivo* are presently available to assess CYP3A levels, these tests are not totally satisfactory because the probes may sometimes be metabolized by other enzymes [10]. A possible alternative to test substrates could be the direct evaluation of a given P450 in easily available extrahepatic cells, such as peripheral blood cells, as previously suggested for monocytes [11]. Leukocytes are known to express P450s, such as CYP1A1 [12, 13], CYP2E1 [14], and CYP4F3 [15], constitutively and also in response to inducing agents [11, 12, 14]. However, no such data are presently available for P450s of the CYP3A subfamily, the major P450 subfamily in humans.

The present work was, therefore, undertaken to study the expression of CYP3A proteins in leukocytes and in the liver of rats in response to appropriate inducers administered *in vivo*, as well as to examine the possible correlation between leukocyte and liver CYP3A contents.

MATERIALS AND METHODS

Dexamethasone, clotrimazole, and pregnenolone-16α-carbonitrile were purchased from Sigma (St. Louis, MO, U.S.A.), phenobarbital from Rhône-Poulenc Rorer (Paris), protease inhibitor cocktail tablets from Boehringer, and bicinchoninic acid (BCA) and micro BCA protein assay reagents from Pierce (Rockford, IL). Triacetyloleandomycin (TAO) was a kind gift from Pfizer (Karlsruhe, Germany). The biotinylated donkey antirabbit antibody, the streptavidin-horseradish peroxidase conjugate, and the che-

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miluminescence detection system used in Western blots were from Amersham (Little Chalfont, U.K.).

Male and female rats (Sprague-Dawley, 200–250 g, C.E.R.J., Saint Berthevin) were treated intraperitoneally with clotrimazole, dexamethasone, pregnenolone-16α-carbonitrile (one injection/day of 100 mg/kg suspended in corn oil, 1 or 3 days), or phenobarbital (one injection/day of 120 mg/kg/day in water, 4 days). Control rats received the same volume of corn oil or normal saline. One day after the final treatment, heparin (5000 U, i.p.) was administered 40 min before killing the animals by exposure to 100% nitrogen, and 10–12 mL of blood withdrawn by cardiac puncture into heparinized glass tubes.

For the isolation of leukocytes, 1 volume of heparinized blood (pooled from 3 rats) was adjusted to 4 volumes of lysis buffer (38.8 mM NH_4Cl, 2.5 mM $KHCO_3$, 0.1 mM EDTA, pH 8) [16] containing 0.1 mM phenylmethylsulfonylfluoride, and incubated for 10 min at 4°C. The nucleated cells were centrifuged at 1200 × *g* and washed in the above buffer. For preparation of leukocyte microsomes, the cells were homogenized in potassium phosphate buffer (0.1 M, pH 7.4) containing EDTA (1 mM) and a protease inhibitor cocktail (1 tablet/25 mL, Boehringer) with a tissue homogenizer (Polytron PT 10-35) 4 times for 15 sec at level 6. The homogenate (approximately 15 mg protein/mL) was centrifuged at 10,000 × *g* (20 min) and 150,000 × *g* (1 hr). The microsomal pellet was resuspended in 0.4 mL of the above homogenizing medium containing 20% glycerol, which was sufficient for 3–4 immunoblots. Liver microsomes were prepared as described [17]. Protein concentration was determined according to Smith *et al.* [18].

Microsomal proteins were resolved using SDS-polyacrylamide gel electrophoresis [19] and transferred to a nitrocellulose membrane [20]. The polyclonal antibody used against CYP3A (P450p) was prepared as previously described [21, and references therein] and further purified [22]. The blotted membrane was blocked (12 hr, 4°C) in Tris buffered saline (TBS) containing 10% nonfat dry milk, incubated (1 hr, room temperature) with the anti-CYP3A antibody, washed with TBS containing 1.5% Tween 20, and incubated (1 hr, room temperature) with a secondary biotinylated antibody (donkey antirabbit, 1:1250) and a streptavidin-horseradish peroxidase conjugate (1:1250). All dilutions were in TBS containing 1.75% nonfat dry milk. The Amersham chemiluminescent detection system was used, and signals were recorded on a photographic film and analyzed with a Joyce-Loebel Chromoscan 3. Standard curves relating signal intensity (total CYP3A proteins) and amounts of CYP3A determined as TAO metabolite complex [23] were established, using liver microsomes from dexamethasone-treated rats and were observed to be linear from 0.1 to 1 pmol CYP3A.

RESULTS

Basal levels of liver CYP3A microsomal proteins determined by Western blotting with a polyclonal antibody against rat CYP3A are presented in Fig. 1 (lane 1). Our antibody recognized 2 CYP3A bands with molecular weights of approximately 51 kDa. In contrast to liver, control leukocyte microsomes did not show any CYP3A-specific band, even at high protein concentrations (lanes 10–14).

After *in vivo* treatment with dexamethasone, CYP3A proteins were strongly induced in liver microsomes, as expected (lanes 2–4). Leukocyte microsomal CYP3A proteins were also markedly induced following *in vivo* dexamethasone treatment (lanes 5–9), as shown by a distinct CYP3A band with an apparent molecular weight similar to the upper band of the control liver microsomes (lane 1) and the strong dexamethasone-induced band in liver microsomes (lanes 2–4).

Other well-known hepatic CYP3A inducers, such as clotrimazole, phenobarbital, and pregnenolone-16α-carbonitrile, were also effective inducers in leukocytes, inducing proteins with mobility similar to those induced by dexamethasone (data not shown). As presented in Fig. 2, there was an apparent linear relationship between the CYP3A contents in leukocyte and liver microsomes in the range of 0.2 to 1 nmol CYP3A/mg liver microsomal protein. It should be noted, however, that the CYP3A levels in induced leukocytes (expressed in pmol/mg) were about 1000-fold less than in liver (expressed in nmol/mg). Phenobarbital (diamonds) and pregnenolone-16α-carbonitrile (triangles) appeared to be somewhat less effective inducers than clotrimazole (squares) and dexamethasone (circles). There was apparently no marked difference between males (closed symbols) and females (open symbols) with regard to CYP3A induction, although the number of females ($n = 4$) is too low to draw any firm conclusion.

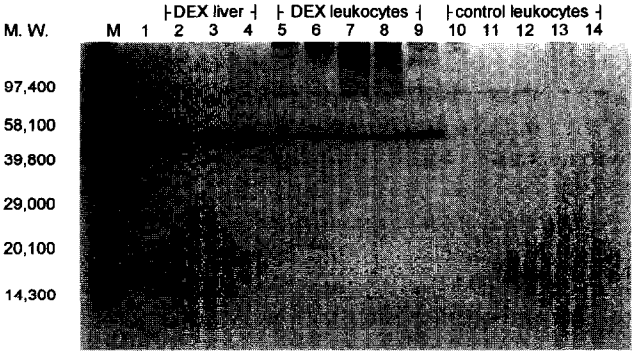

FIG. 1. Detection of CYP3A proteins in leukocyte and liver microsomes of male dexamethasone-treated and control rats. Pools of 3 animals treated for 3 days with dexamethasone (DEX) were used for each lane. Lane M, molecular weight markers; 1, liver microsomes of untreated rats (20 μg protein); lanes 2–4, liver microsomes of DEX-treated rats (0.2, 0.35, 0.5 μg protein); lanes 5–9, leukocyte microsomes of DEX-treated rats (70, 150, 150, 110, 200 μg protein); lanes 10–14, leukocyte microsomes of untreated rats (250 μg protein). Conditions for SDS-gel electrophoresis and Western blotting are described in Materials and Methods.

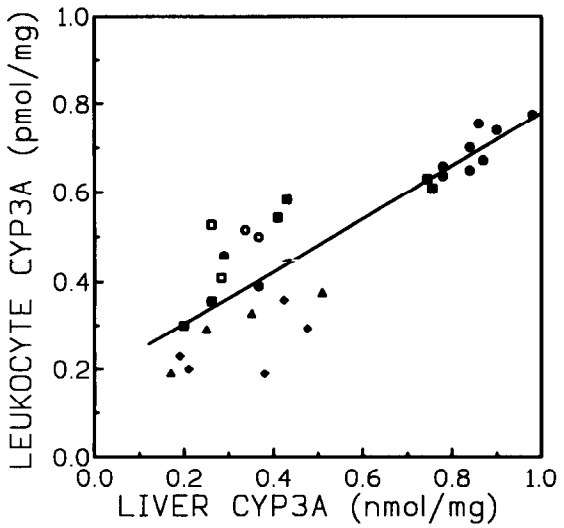

FIG. 2. Correlation between rat leukocyte and liver microsomal CYP3A contents, determined by Western blot densitometry. Signal intensity was converted to amount of CYP3A using a standard curve relating densitometry measurements and TAO metabolite complex formation as described in Materials and Methods. Concentrations are expressed as pmol/mg microsomal protein for leukocytes, and as nmol/mg microsomal protein for liver. Pools of 3 rats were used for each data point. Open symbols, females; filled symbols, males. Treatments: clotrimazole (squares); dexamethasone (circles); phenobarbital (diamonds); pregnenolone-16α-carbonitrile (triangles). Linear regression: r^2 = 0.748, n = 29, $P < 0.0001$.

DISCUSSION

These results demonstrate, for the first time, that CYP3A is inducible *in vivo* in rat leukocytes. As expected, CYP3A levels in induced leukocytes were about 1000-fold less than in liver, as was already observed with other extrahepatic cell types [3]. The approximately linear relationship between leukocyte and liver CYP3A contents, within the experimental concentration range, suggests that dexamethasone, pregnenolone-16α-carbonitrile, clotrimazole, and phenobarbital can activate the expression of CYP3A proteins by apparently similar mechanisms in both tissue types.

The nondetection of CYP3A in leukocytes from control animals may be due either to physiological absence or to an abundance below the sensitivity limit of our detection system (≈ 0.15 pmol/mg). Because the antibody used in our studies recognizes many rat CYP3A isoenzymes, such as CYP3A1, CYP3A2, cDEX, and CYP3A18 (unpublished observation), it is, thus, unlikely that isoenzymes of the CYP3A subfamily would have escaped detection in control leukocytes. Contrary to control rats, human polymorphonuclear leukocytes appear to express CYP3A isoenzymes [24], although this is still controversial [25]. It is, therefore, possible that rats and humans differ in their basal leukocyte expression of CYP3A, because species differences in the expression of these isoenzymes have been reported for liver [26].

Although the present data clearly demonstrated the induction of CYP3A in peripheral leukocytes *in vivo*, the identification of the specific CYP3A isoenzyme(s) involved was not possible. In the liver of male rats, CYP3A2 [27], cDEX [28], and CYP3A18 [29] are constitutively expressed, and CYP3A1 seems to be absent [27]. CYP3A1, cDEX, and CYP3A18 are inducible by dexamethasone and pregnenolone-16α-carbonitrile [27–30]. Thus, it is likely that the induced CYP3A observed in leukocytes includes one or more of these P450 isoenzymes, apparently corresponding to the upper band of control male liver microsomes, and the noninduced lower band may be due to male-specific CYP3A2 [27]. Work to identify the CYP3A isoenzyme inducible in leukocytes is in progress.

If human leukocytes are also responsive to *in vivo* treatment with CYP3A inducers, as was demonstrated here in rats, it would be possible to determine liver CYP3A levels indirectly, using easily available leukocytes from peripheral blood. This test would complement other noninvasive tests of liver CYP3A enzymes already available [10]. Such noninvasive tests become particularly attractive because important and numerous compounds are metabolized by this enzyme system [1–9], and prior knowledge of an individual capacity to metabolize compounds *via* these enzymes could have clinical or epidemiological applications.

In conclusion, we have demonstrated that CYP3A is inducible in rat leukocytes following *in vivo* treatment with appropriate inducers, and that an apparently linear correlation with liver induction exists. It is suggested that peripheral leukocytes may serve as an indicator of liver CYP3A induction *in vivo*.

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