Induction of Cytochrome P450 3A (CYP 3A) by E 5110, a Non-steroidal Anti-inflammatory Agent (NSAID), and Typical CYP 3A Inducers in Primary Cultures of Dog Hepatocytes

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First, the effect of E 5110, a non-steroidal anti-inflammatory agent (NSAID), on cytochrome P450 subfamilies in dog hepatocyte culture was examined. E 5110 has been shown to cause a drug interaction in dogs and humans via induction of cytochrome P450 3A (CYP 3A). When dog hepatocytes were cultured for 72 h in the presence of 2–30 μM E 5110, the activity levels of ethoxycoumarin O-deethylase (ECOD) and testosterone 6β-hydroxylase (6β-OH-T) rose dose-dependently. Subsequent Western blot analysis of microsomes from hepatocyte cultures indicated the presence of higher amounts of CYP 2B and 3A proteins than those of the control. Next, the P450 inducing potency of E 5110 was compared with those of phenobarbital, rifampicin, phenytoin and carbamazepine, which induce CYP 3A in human subjects and human hepatocyte cultures. E 5110 was found to be nearly as effective as phenytoin, but less potent than rifampicin, on the basis of 6β-OH-T induction.

Key words dog hepatocyte culture; cytochrome P450; cytochrome P450 3A; E 5110; induction

Induction or inhibition of hepatic drug metabolizing enzymes sometimes seriously influences the drug blood level and modifies the therapeutic effect or produces unwanted side-effects.1–3 Since patients are often treated with multiple drugs, it is important to predict the risk of drug interaction using in vitro systems, for example, the hepatocyte primary cultures of experimental animals. Various attempts at using rat hepatocytes have resulted in only limited success because drug metabolizing activity drops sharply during culture, and the response to enzyme inducers are thus weak.4,5 We have previously shown that a primary culture of dog hepatocytes can retain a relatively high cytochrome P450 level for several days with significant elevation in the presence of enzyme inducers.6 The major inducible P450 subfamilies in dog hepatocytes were CYP 2B and 3A by phenobarbital, 3A by rifampicin, and 1A by β-naphthoflavone and omeprazole. Similar results have been described for human subjects and human hepatocyte cultures.7–9 In 1991, Nakamura et al.10 reported that orally administered E 5110, a non-steroidal anti-inflammatory agent (NSAID), induced hepatic CYP 2B and 3A in dog, and accelerated the urinary excretion of 6β-hydroxycortisol in healthy volunteers, suggesting the induction of CYP 3A in humans. Subsequent clinical studies revealed that E 5110 reduced the therapeutic effect of prednisolone or cyclosporin.11,12 Thus, an instance of drug interaction in dogs was reproduced in humans. In this study, we confirmed that several chemicals, which induce CYP 3A in human subjects and cultured human hepatocytes, do induce CYP 3A in dog hepatocyte cultures. Furthermore, E 5110 was found to induce CYP 3A in a primary culture of dog hepatocytes, suggesting that CYP 3A-related drug interaction in humans can be predicted by using an animal in vitro system.

MATERIALS AND METHODS

Hepatocytes were isolated from male beagle dogs, 8–12 months old, weighing 6–10 kg and fed laboratory chow (Japan Clea Co., Tokyo), by a collagenase perfusion method as described previously.6 The viability of the cells according to a trypan blue exclusion test was 90%. The culture of hepatocytes, treatment of the cultures with inducers, enzyme assay and Western blotting all followed reported procedures.6,13 Testosterone hydroxylase was assayed by HPLC according to Imaoka et al.14 E 5110, N-methoxy-3-(3,5-di-tert-butyl-4-hydroxybenzylidene)pyrrolidin-2-one, was a gift from Dr. Horie, Eisai Res. Labs, Eisai Co., Tsukuba, Japan. Polyclonal antibodies to rat CYP 2B1 and 3A215 were purchased from Daiichi Pure Chem. Co., Ltd. (Tokyo). The P450 isozyme content was estimated by densitometric analysis of the blot with a Shimadzu dual-wavelength flying-spot scanner (Shimadzu Co., Kyoto, Japan).

RESULTS AND DISCUSSION

When dog hepatocytes were cultured with E 5110, a several-fold increased activity of testosterone 6β-hydroxylase (6β-OH-T), a marker enzyme of CYP 3A,16,17 was detected compared with the control. As shown in Table 1, the stimulatory effect of E 5110 was dose-dependent, and the maximal effect was observed at about

<table>
<thead>
<tr>
<th>E 5110 (μM)</th>
<th>ECOD (nmol/min/mg protein)</th>
<th>6β-OH-T (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.40 ± 0.04</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.79 ± 0.09</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.96 ± 0.07</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>1.08 ± 0.07</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>1.13 ± 0.11</td>
<td>0.31 ± 0.04</td>
</tr>
</tbody>
</table>

The hepatocytes were cultured for 72 h with various concentrations of E 5110, and ECOD and 6β-OH-T activities in a 9000 × g supernatant fraction were determined. The initial levels (at culture 0 time) of ECOD and 6β-OH-T were 0.70 ± 0.09 and 0.25 ± 0.04 nmol/min/mg protein, respectively. Values are expressed as mean ± S.E. from 3 dogs.

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Antibodies

P4502B1

P4503A2

Fig. 1. Western Blotting of Microsomal Proteins Prepared from Cultured Dog Hepatocytes with Polyclonal Antibodies to CYP 2B and 3A
Lane 1, 2, untreated cultured hepatocytes; lane 3, 4, 100 μM phenobarbital treated cultured hepatocytes; lane 5, 6, 30 μM rifampicin treated cultured hepatocytes; lane 7, 8, 30 μM E 5110 treated cultured hepatocytes; lane 1—8, 20 μg microsomal protein was used.

Table 2. Effect of Various Chemicals on ECOD and 6β-OH-T in Primary Culture of Dog Hepatocytes

<table>
<thead>
<tr>
<th>Inducers</th>
<th>ECOD (nmol/min/mg protein)</th>
<th>6β-OH-T (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.40 ± 0.04</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>100 μM phenobarbital</td>
<td>1.50 ± 0.10</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>30 μM rifampicin</td>
<td>0.53 ± 0.10</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>30 μM carbaamine</td>
<td>1.27 ± 0.06</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>30 μM phenytoin</td>
<td>1.58 ± 0.20</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>2 μM clotrimazole</td>
<td>1.00 ± 0.11</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>30 μM clotrimazole</td>
<td>0.41 ± 0.10</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>100 μM omeprazole</td>
<td>0.51 ± 0.02</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>100 μM clotibre</td>
<td>0.39 ± 0.02</td>
<td>0.08 ± 0.00</td>
</tr>
</tbody>
</table>

The hepatocytes were cultured for 72 h with various inducers, and ECOD and 6β-OH-T activities were determined. Means and S. E. from 3 independent experiments are presented.

30 μM. Concomitant rise in ethoxyquinol O-deethylase (ECOD) activity indicates the co-induction of CYP 2B and 3A in dog hepatocyte cultures similar to in vivo. • Western blot analysis of microsomes from cultured dog hepatocytes indicated the induction of the specific P450 subfamily; according to densitometry, the amount of CYP 3A increased to about 2.2-fold that of the control, and enrichment of CYP 2B was also observed (Fig. 1). CYP 1A was not influenced either by E 5110 or rifampicin (data not shown). Thus, the in vitro and in vivo results correlated well in terms of CYP 2B and 3A induction. Next, the effect of E 5110 on 6β-OH-T activity was compared with various chemicals which are known to induce or not to induce hepatic CYP 3A in humans. As shown in Table 2, E 5110, phenytoin, phenobarbital and clotrimazole (2 μM) stimulated 6β-OH-T activity approximately 3-fold, while rifampicin stimulated it 5-fold. An additional blotting with 5 μg microsomal protein showed that the contents of CYP 3A increased 2.2- and 3.5-fold by treatment with 30 μM E 5110 and rifampicin, respectively (data not shown). Phenobarbital, phenytoin, rifampicin and carbaamine have been shown to cause drug interactions in humans, and to induce CYP 3A in human hepatocyte cultures. Omeprazole induces CYP 1A in humans, while clotibre, which is known to enhance the proliferation of peroxisomes in rodents, induces CYP 4A. Antifungal clotrimazole enhanced the drug metabolizing activity at a low dosage (2—10 μM) but it inhibited the activity at a high concentration (30 μM). Therefore, the response to inducers should be carefully examined using low and high concentrations of chemicals.

CYP 3A is important from the viewpoint of drug interaction because it is the major P450 in humans and is involved in the metabolism of various drugs. Antituberculosis rifampicin stimulated the biosynthesis of CYP 3A and enhanced the metabolic elimination of cyclosporin or dexamethasone, by which the blood levels of these drugs decreased. Likewise, chronic treatment with an anticonvulsant phenytoin increased the clearance of prednisolone, antipyrine or theophylline and reduced their therapeutic efficacy or worsened the symptoms. Dosage adjustment of the target drug may be undertaken, but the risk of toxic consequence should be carefully monitored when phenytoin is withdrawn.

Although primary cultures of human hepatocytes have provided much valuable information related to P450 induction, a wide range of variety exists in human preparations owing to disease states, drug treatments, postmortem time, or time after tissue dissection. These qualitative variations seriously influence cell function and enzyme activities, and make it difficult to compare individual data on a quantitative basis.

The present study indicates that a well-controlled primary culture of dog hepatocytes could serve as an alternative for predicting, at least in part, drug interaction with small amounts of chemicals. It should be emphasized that the development of such an in vitro system could be effective in reducing the number of experimental animals needed.

REFERENCES