SELECTIVE AND POTENT INHIBITION OF HUMAN CYP2C19 ACTIVITY BY A CONFORMATIONALLY TARGETED ANTIPEPTIDE ANTIBODY

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ABSTRACT:

A conformationally targeted anti-peptide antibody was produced by immunizing a rabbit with a cyclized peptide corresponding to a loop region of human CYP2C19 (residues 250–261). In an enzyme-linked immunosorbent assay, the antibody bound strongly to recombinant CYP2C19 and poorly to recombinant CYP2C8, CYP2C9, and CYP2C18. In immunoblotting studies, the antibody bound strongly to recombinant CYP2C19 and weakly to recombinant CYP2C8. No binding to recombinant CYP1A2, CYP2C9, CYP2C18, CYP2D6, CYP2E1, and CYP3A4 was detected. In immunoinhibition experiments, the anti-peptide antibody targeted against CYP2C19 potently inhibited (S)-mephenytoin 4'-hydroxylase activity of human hepatic microsomal fraction (>90%). It had no appreciable effect on ethoxysorafin O-deethylase (CYP1A2), tolbutamide methyl-hydroxylase (CYP2C9), dextromethorphan O-demethylase (CYP2D6), 4-nitrophenol hydroxylase (CYP2E1), or testosterone 6β-hydroxylase (CYP3A4) activity of human hepatic microsomal fraction. However, large amounts of purified IgG fractions were able to inhibit up to 35% of paclitaxel 6α-hydroxylase (CYP2C8) activity. In conclusion, we have demonstrated that an anti-peptide antibody targeted against residues 250 to 261 of human CYP2C19 selectively and potently inhibited CYP2C19 activity of human hepatic microsomal fraction.

The superfamily of P450 monooxigenases have been classified on the basis of primary amino acid sequence similarity into families and subfamilies, with enzymes belonging to families 1 to 3 playing an important role in the metabolism of numerous xenobiotics (Nelson et al., 1996). In humans, the CYP2C subfamily is made up of four closely related enzymes, CYP2C8, CYP2C9, CYP2C18, and CYP2C19 (Goldstein and de Morais, 1994). These enzymes display greater than 88% sequence similarity, with the enzyme sequence of CYP2C19 sharing a 91, 93, and 96% resemblance with that of CYP2C8, CYP2C18, and CYP2C9, respectively (Romkes et al., 1991). Genetic polymorphisms within the CYP2C subfamily have been recorded, the most established being human CYP2C19, an enzyme that is predominantly involved in the 4' hydroxylation of the anticonvulsant agent (S)-mephenytoin (Wedlund et al., 1984; Wrighton et al., 1993). The incidence of the poor metabolizer phenotype is approximately 18 to 25% of Asians (Goldstein et al., 1997). As a result of this polymorphism, the half-life, efficacy, and toxicity of many exogenous compounds is affected in poor metabolizer individuals. Therefore, the determination of the quantitative contribution of CYP2C19 to the metabolism of specific drugs is a vital step in the development of new drug products.

Immunoinhibition of enzyme activity by conformationally targeted antibodies has been shown previously to provide an effective method in determining the contribution of individual P450 enzymes to the metabolism of drugs (Schulz-Utermoehl et al., 2000). Consequently, in this study, an anti-peptide antibody was raised against a cyclic peptide corresponding to a surface loop region of CYP2C19 (residues 250–261) which, based on secondary structure alignment, is predicted to correspond to the proinhibitory region of CYP2D6 identified previously (Schulz-Utermoehl et al., 2000). The ability of the resultant antibody to bind selectively to human CYP2C19 and inhibit its activity was assessed.

Materials. Human P450 enzymes expressed in insect cells were purchased from Gentest Corporation (Woburn, MA). The expression of human P450 enzymes in Escherichia coli membranes was as described by Pritchard et al. (1998). A pooled sample of human liver microsomal fraction (batch number 1029) was obtained from In Vitro Technologies (Baltimore, MD). (S)-(−)-mephenytoin and (±)-4-hydroxymephenytoin were obtained from Ultrafine Chemicals (Manchester, UK). All other chemicals were purchased from Sigma (Vallenbaek Strand, Denmark) or Merck (Darmstadt, Germany), and were of analytical grade.

Peptide Synthesis, Immunization, and Ig (IgG) Preparation. The peptide KGCEHQESMDINPRC was synthesized on an Applied Biosystems 431A peptide synthesizer (Perkin-Elmer Cetus Instruments, Birkerod, Denmark) as...
The CYP2C subfamily, together with the CYP3A subfamily, com-
prises more than half of the P450 enzymes in human liver and plays an
important role in the elimination of xenobiotics (Shimada et al.,
1996). The amount of recombinant CYP1A2, CYP2C9,
CYP2C19, and CYP2C18 (Fig. 2). No binding to CYP2D6 was
detected (Fig. 2).

Previous attempts by other investigators to produce an anti-peptide
antibody that binds specifically to, and potently inhibits the activity
of, an individual CYP2C enzyme has proved quite difficult to date.
Anti-peptide antibodies based on epitope mapping of an inhibitory
monoclonal antibody (designated 2F5) to CYP2C5 were recently
raised against a linear peptide corresponding to residues 252–263 of
human CYP2C19. However, although the antibody was form-specific,
it was unable to inhibit CYP2C18-catalyzed diazepam N-demethyl-
ylation of recombinant CYP2C18 expressed in E. coli. The reasons for
the lack of inhibition are unclear, but may be due to low affinity of the
antibody for the native protein.

The use of cyclic peptides as immunogens was shown previously to
be superior to linear peptides for the production of antibodies that bind
strongly to their target enzyme and inhibit its activity (Schulz-Utermoehl
et al., 2000). In this study, the antibody raised against a cyclic peptide
corresponding to residues 250 to 261 of human CYP2C19 was
further analyzed to its antigenic epitope. The epitope for antibodies pro-
duced in this study against human CYP2C19 was identified by cyclic
peptides as it is shown in Fig. 1.

**Results and Discussion**

In this study, anti-peptide antibodies raised against a cyclic peptide
corresponding to residues 250 to 261 of human CYP2C19 were
successfully produced. It was found that all three rabbits produced
anti-peptide antibodies that selectively recognized their target enzyme
and were able to significantly inhibit (60–95%) its activity in human
liver microsomal fraction. Only the result of the most potent proin-
hibitory antibody is discussed here.

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1994). Although the members of the human CYP2C subfamily are
highly similar, they can still be individually distinguished on immu-
noblots due to their different relative mobilities on SDS-polyacryl-
amide gels (Goldstein and de Morais, 1994). In this study, the anti-
body produced against human CYP2C19 recognized its target enzyme
in human hepatic microsomal fraction (Fig. 1). It also bound strongly
to recombinant CYP2C19 and more weakly to recombinant CYP2C8
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in human hepatic microsomal fraction (Fig. 1).

**Enzyme-Linked Immunosorbent Assay and Immunoblotting.**
These were carried out according to the methods described previously (Schulz-Utermoehl et al., 2000; T. Schulz-Utermoehl, R.J. Mountfield, R.P. Bywater, K. Madsen, P.N. Jørgenson and K.T. Hansen, submitted).

**P450 Assays.** The measurement of P450-mediated activity of human he-
patic microsomal fraction was carried out as described previously (T. Schulz-Utermoehl, R.J. Mountfield, R.P. Bywater, K. Madsen, P.N. Jørgenson and K.T. Hansen, submitted).

**Antiserum dilution**

<table>
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**Microtiter plates were coated with 1 µg/well of microsomal protein from insect cells expressing CYP2C8 (●), CYP2C9 (▲), CYP2C18 (●), CYP2C19 (●), or CYP2D6 (×). A range of dilutions of antibody was then added to each series of wells, and antibody binding was determined as described under Experimental Procedures. Each point represents the mean of two determinations.**

CYP2C18, CYP2D6, and CYP3A4 used was sufficient for detection by immunoblotting using their respective antibodies (data not shown, other forms not tested). The binding specificity of the antibody to closely related forms of P450 enzymes was examined further in enzyme-linked immunosorbent assays using microsomal fraction from insect cells expressing individual members of the CYP2C subfamily. The antibody bound strongly to recombinant CYP2C19 and in comparison, there was very weak binding to recombinant CYP2C8, CYP2C9, and CYP2C18 (Fig. 2). No binding to CYP2D6 was detected (Fig. 2).

**Fig. 1. Binding of an anti-peptide antibody targeted against human CYP2C19 to recombinant (r) human P450 enzymes.**

The CYP2C subfamily, together with the CYP3A subfamily, com-
prises more than half of the P450 enzymes in human liver and plays an
important role in the elimination of xenobiotics (Shimada et al.,
1994). Although the members of the human CYP2C subfamily are
highly similar, they can still be individually distinguished on immu-
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**Fig. 2. Relative binding of an anti-peptide antibody targeted against human CYP2C19 to various recombinant P450 enzymes.**

Microtiter plates were coated with 1 µg/well of microsomal protein from insect cells expressing CYP2C8 (●), CYP2C9 (▲), CYP2C18 (●), CYP2C19 (●), or CYP2D6 (×). A range of dilutions of antibody was then added to each series of wells, and antibody binding was determined as described under Experimental Procedures. Each point represents the mean of two determinations.
addition of NADPH and ethoxyresorufin, paclitaxel, tolbutamide, phenytoin, dextromethorphan, or testosterone 6-β-hydroxylase activity of human hepatic microsomal fraction (with no serum added) was 11.5, 18.2, 40.2, 9.3, 92.8, 190.2, and 1100 pmol/min/mg protein, respectively.

It has been shown previously that both purified CYP2C9 and CYP2C19 expressed individually in E. coli were able to efficiently catalyze the metabolism of tolbutamide (Lasker et al., 1998). However, the finding in this study that the antibody targeted against CYP2C19 did not inhibit tolbutamide methyl-hydroxylation of human hepatic microsomal fraction suggests that CYP2C19 plays only a very minor or no role in the metabolism of tolbutamide in human liver. CYP2C9 appears to be the principal enzyme responsible for the biotransformation of this compound in human liver. Consistent with this is the lack of any correlation found between relative apoprotein levels of CYP2C9 and tolbutamide methyl-hydroxylation activity in human liver (Edwards et al., 1998). The discrepancy between the metabolism of tolbutamide in human liver and by E. coli expressed enzymes is probably due to the fact that the ratio of the levels of CYP2C9 to CYP2C19 in human liver averages around 13:1 (Lasker et al., 1998), implying that any contributions by CYP2C19 to this reaction in human hepatic microsomal fraction is actually relatively minor in comparison to catalysis by CYP2C9.

Specific proinhibitory anti-peptide antibodies targeted against the other major human P450 enzymes have already been produced in this laboratory, and together with this antibody targeted against CYP2C19, will serve as important tools in the identification and quantification of the role of a particular P450 enzyme in the metabolism of currently used drugs and those in development. In conclusion, it has been shown that a novel anti-peptide antibody targeted against human CYP2C19 that binds with a high degree of selectivity to its target protein and can potently inhibit its activity was successfully produced.

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