INTRODUCTION

For more than four decades cytochrome P450 (CYP) has been the subject of intense investigation, largely as a result of its catalytic diversity. Not only is CYP responsible for the metabolism of tens of thousands of xenobiotics (e.g. drugs, industrial chemicals, environmental pollutants, plant products and toxins), but the enzyme is essential also for the biosynthesis and catabolism of a broad range of endogenous compounds, including bile acids, biogenic amines, eicosanoids, fatty acids and steroid hormones.

Of greatest relevance to this review article, however, is the role of CYP in xenobiotic metabolism. Humans are exposed to an array of xenobiotics capable of exerting a broad range of pharmacological and toxicological effects. In most instances CYP-mediated biotransformation serves as a detoxification mechanism since the metabolites formed generally possess less biological activity than the parent compound. CYP-mediated biotransformation additionally facilitates the elimination of typically lipophilic xenobiotics and the newly introduced functional group may serve as an acceptor for conjugating enzymes (e.g. UDP-glucuronosyltransferase, sulphotransferase, glutathione transferase), enhancing renal clearance further.
The route to lifelong and appropriate blood pressure control

**PRESCRIBING INFORMATION:**

**Composition:**
Each tablet contain 5 mg/10 mg felodipine in an extended release formulation

**Indication:**
Hypertension, Angina pectoris

**Dosage:**
Initial dose 5 mg, maintain on 5-10 mg once daily

**Adverse reactions:**
In common with other vasodilatations, felodipine may cause flushing, headache, palpitations, dizziness and fatigue in some patients. Most of these reactions are dose dependent and appear at the start of treatment or after a dose increase. Should such reactions occur they are usually transient and diminish in intensity with time.

**Interactions:**
Enzyme inhibitors have been shown to cause an increase in felodipine plasma levels. Enzyme inducers will cause a decrease in plasma levels of felodipine.

Further information available on request
Hoechst Marion Roussel (Thailand) Ltd.
193 Lake Rajada Bldg., 20th Floor
Ratchadaphisek Road, Klong Toey,
P.O. Box 960, Prakanong, Bangkok 10110
Tel: 264-0520 / Fax: 264-0992

Hoechst Marion Roussel
The Health Care Division of Hoechst
Trental 400
CONTROLLED RELEASE TABLET
active ingredient: Pentoxifylline

improves cerebral perfusion and mental function

Patients suffering from cerebrovascular disease are the victims of inadequate blood flow due to obstructive arterial processes as well as platelet adhesion and aggregation.

An acute reduction of cerebral blood flow may result in transient ischaemic attacks or even in stroke leading to reversible or irreversible neurological deficits.

A chronic decrease in cerebral blood flow, however, may be manifested by disorders of mental function, behavioural changes and somatic disorders.

Haemorheological and antiplatelet medication has the objective to prevent recurrent ischaemic attacks and to improve cognitive and mental function.

Trental 400 exerts neuropharmacological effects and improves the nutritive microcirculation by

- restoring red cell deformability
- decreasing blood viscosity
- inhibiting platelet aggregation

Trental 400 the haemorheological and antiplatelet approach in the treatment of cerebrovascular disease

Prescribing Information: Active Ingredient: Pentoxifylline 400 mg. Indications: Conditions associated with cerebrovascular insufficiency, such as impairment of concentration and memory, vertigo, sleep disturbances, headache, tinnitus, low drive, sequelae of cerebrovascular accidents. Peripheral occlusive arterial disease, and circulatory disorders of ischaemic, diabetic, inflammatory or functional origin, neurotic disorders, lower leg ulcer and gangrene. Dosage: 1 tablet, 2-3 times daily, after meals. To be swallowed whole with some liquid. Contraindications: Hypersensitivity to pentoxifylline, severe haemorrhage, acute inflammatory infection, pregnancy, massive retinal haemorrhage. Side effects: GI disturbances, headache, cutaneous hyperirritability, dizziness, bleeding (eg. skin, mucosa etc), thrombocytopenia, flushing, tachycardia, angina pectoris, hypotension. Interactions: Hypotensive agents.

Reference:

Further information available on request.
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P.O. Box 960, Prakanong, Bangkok 10110 Tel: 264 0520 / Fax: 264 0492

Hoechst Marion Roussel
The Health Care Division of Hoechst

Hoechst
Although metabolism of xenobiotics by CYP is normally associated with inactivation of the parent compound, it is now recognised that certain compounds may be converted to highly reactive intermediates which are capable of interacting with cellular macromolecules. Indeed, considerable research over the last two decades has demonstrated that CYP-catalysed metabolic activation is a prerequisite for the toxicity, mutagenicity and carcinogenicity of many foreign compounds. Thus, interindividual variability in CYP activity assumes importance as both a determinant of pharmacokinetics and response to clinically-used drugs and in the development of carcinogenesis or other toxicities following exposure to environmental chemicals.

**General aspects of cytochrome P450**

CYP has been shown to be ubiquitous, being found in all living organisms and in almost all tissues. Mammalian CYP can be classified simply into two classes based on intracellular location; microsomal CYP, which is bound to the membrane of the endoplasmic reticulum, and mitochondrial CYP, which is located on the inner mitochondrial membrane. Mitochondrial CYP is quite distinct from its microsomal counterpart in that it utilises an iron sulphur protein (adrenodoxin) and a flavoprotein, NADPH-adrenodoxin reductase, as the electron donor enzymes. Unlike microsomal CYP, the mitochondrial enzyme is fairly selective in the choice of substrates, being involved primarily in steroid synthesis. Although CYP biotransformation results from the insertion of a single atom of atmospheric oxygen, different reactions may arise depending on the nature of the substrates and the intermediates formed. These reactions include hydroxylation, epoxidation, deamination, dealkylation, sulphoxidation, dehalogenation, and occasionally reduction.
It is now well established that CYP is not a single enzyme, but in fact exists as a gene superfamily where each gene encodes a separate isoform. The multiplicity of CYP was first postulated on the basis of species differences in metabolism and the selective induction of drug metabolism by a range of xenobiotics.\(^1\)\(^2\) Subsequent evidence, including selective inhibition of drug metabolism by certain chemicals, differing patterns of perinatal development in drug metabolism, and genetically determined deficiencies in the metabolism of some substrates, all supported the existence of multiple forms of CYP.\(^3\) Advances in chromatographic techniques greatly facilitated the isolation and purification of individual CYP isoforms from animal and human tissues providing direct evidence of enzyme heterogeneity.\(^4\) However, knowledge of CYP multiplicity, function and regulation has expanded enormously over the last decade or so with the application of recombinant DNA techniques. Since the first complementary deoxyribonucleic acid (cDNA) for a phenobarbitone-inducible rat CYP was isolated by Fujii-Kuriyama in 1982,\(^5\) more than 480 CYP genes have been described.\(^6\) Of 74 gene families so far described, 14 families exist in all mammals. The mammalian enzymes within the CYP1, CYP2 and CYP3 gene families are responsible primarily for the metabolism of xenobiotics. Members of the CYP4 family are responsible mainly for the metabolism of fatty acids while other mammalian CYP gene families encode enzymes which are involved in steroid biosynthesis. Human CYP isoforms in families one to four are shown in Table 1. It is this multiplicity which is responsible for the extremely broad substrate specificity characteristic of CYP.


**Table 1.1 Human xenobiotic metabolising cytochromes P450**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Tissue</th>
<th>Chromosomal location</th>
<th>Selected model substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>mainly extrahepatic tissues</td>
<td>15q22-qter</td>
<td>benzo[a]pyrene and other polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>liver</td>
<td>15q22-qter</td>
<td>caffeine, heterocyclic arylamines phenacetin, theophylline, acetanilide</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>liver</td>
<td>19q13.1-13.2</td>
<td>coumarin, diethylnitrosamine</td>
</tr>
<tr>
<td>CYP2A7</td>
<td>liver</td>
<td>19q13.1-13.2</td>
<td></td>
</tr>
<tr>
<td>CYP2B6</td>
<td>liver</td>
<td>19q12-q13.2</td>
<td>cyclophosphamide</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>liver</td>
<td>10q24.1-24.3</td>
<td>tolbutamide, phenytoin</td>
</tr>
<tr>
<td>CYP2C9/10</td>
<td>liver</td>
<td>10q24.1-24.3</td>
<td>tolbutamide, phenytoin, naproxen, ibuprofen, diclofenac, tienetic acid, S-warfarin (7-hydroxylation),</td>
</tr>
<tr>
<td>CYP2C17</td>
<td>liver</td>
<td>10q24.1-24.3</td>
<td></td>
</tr>
<tr>
<td>CYP2C18</td>
<td>liver</td>
<td>10q24.1-24.3</td>
<td>S-mephenytoin, hexobarbital, omeprazole</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>liver</td>
<td>10q24.1-24.3</td>
<td>S-mephenytoin, hexobarbital, omeprazole</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>liver</td>
<td>22q13.1</td>
<td>bufuralol, debrisoquine, s鹗artene, perhexiline, dextromethorphan</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>liver</td>
<td>10q24.1-24.3</td>
<td>chloroxazone, dimethylbarbitosuline, ethanol, 4-nitrophenol, halothane</td>
</tr>
<tr>
<td></td>
<td>intestine</td>
<td>10q24.1-24.3</td>
<td>ethanol, 4-nitrophenol, halothane</td>
</tr>
<tr>
<td></td>
<td>leukocyte</td>
<td>10q24.1-24.3</td>
<td>enturane</td>
</tr>
</tbody>
</table>
Table 1.1 (cont).

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Tissue</th>
<th>Chromosomal location</th>
<th>Selected model substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2F1</td>
<td>lung</td>
<td>19</td>
<td>3-methylindole</td>
</tr>
<tr>
<td>CYP3A3</td>
<td>liver</td>
<td>7q22.1</td>
<td>aflatoxin B1, alfentanil, androsteindione, benzo[a]pyrene, cyclosporine, erythromycin, estradiol, nifedipine, quinidine, testosterone, triazolam, terfenadine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>tract</td>
<td>7q22.1</td>
<td>cyclosporine, nifedipine, testosterone placenta</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>liver</td>
<td>7q22.1</td>
<td>aflatoxin B1, testosterone, progesterone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3-sulphate</td>
</tr>
<tr>
<td>CYP3A7</td>
<td>liver</td>
<td>7q22.1</td>
<td>aflatoxin B1, testosterone, progesterone (fetal)</td>
</tr>
<tr>
<td>CYP4A9</td>
<td>kidney</td>
<td>1</td>
<td>fatty acids</td>
</tr>
<tr>
<td>CYP4B1</td>
<td>lung</td>
<td>1p12-p34</td>
<td></td>
</tr>
</tbody>
</table>

**Structure and membrane topology**

Individual eukaryotic CYP isoforms are integral to the endoplasmic reticulum or the mitochondrial membrane. The integral membrane nature of CYP isoforms is important for both effective electron transfer from the electron donor enzyme system and metabolism of lipophilic substrates. Microsomal CYP isoforms are synthesised primarily on membrane-bound polyribosomes before insertion into the lipid bilayer of the endoplasmic reticulum. The insertion of CYP and other intrinsic membrane proteins...
requires highly specific cellular mechanisms. Although more than 220 primary structures have been reported, there is structural homology of certain segments of all members of the CYP gene superfamily. Such homologous segments include the heme-binding cysteine residue near the carboxy-terminal and, in the case of the microsomal enzymes, a highly hydrophobic segment at the amino-terminal region. The hydrophobic amino-terminal region of CYP isoforms is generally accepted as a signal recognition site that not only directs insertion of these proteins into the membrane but also functions as a "halt-transfer signal" which anchors the enzyme to the membrane. The remainder of the enzyme resides on the cytoplasmic side of the membrane. It remains controversial whether the amino-terminal segment exists in a hair-pin loop configuration (model A, Fig.1) or as a single membrane-spanning region (model B, Fig.1). However, the available data tends to support model B in which CYP is anchored to the membrane by a single amino-terminal transmembrane helix of 20-30 amino acids with the globular part of the protein, where the substrate and the electron donor enzyme bind, residing in the cytosol.

Figure 1 Proposed membrane topologies for microsomal cytochromes P450.
A major goal of CYP structure determination is to elucidate the substrate binding domain(s) which in turn should allow direct prediction of substrate specificity. Several approaches have been applied to CYP structure determination, including chemical modification with substrate analogues or mechanism based inhibitors, site-directed mutagenesis, molecular modelling and sequence alignment with the crystal structure of CYP101. However, a consensus view for the substrate binding sites of mammalian CYP isoforms has not yet been established. A major impediment to CYP structure elucidation is the lack of a crystal structure for any eukaryotic CYP isoforms. Most of the three-dimensional models for mammalian CYP rely on the structure of bacterial CYP101, the only tertiary CYP structure available, despite the fact that there is only minimal sequence identity between CYP101 and mammalian CYP and this bacterial enzyme is not membrane bound. The crystal structure of bacterial CYP102 has also been determined recently and this enzyme exhibits greater sequence homology to mammalian CYP than does CYP101. It is possible that the three-dimensional structure of CYP102 may provide a better model for the mammalian enzymes.

Approaches used for assessment of cytochrome P450 function and activity

It is now well established that various CYP isoforms differ in terms of substrate specificity and regulation. Differences in the activities and the relative proportion of individual isoforms therefore become important determinants of interindividual differences in pharmacological effects of drugs, susceptibility to adverse drug reactions and probably chemical carcinogenesis. Identification and characterisation of the substrate specificity and regulation of individual CYP isoforms is essential for the elucidation of the
biological processes underlying such relationships. In the case of new drugs, drug interactions and other factors influencing drug plasma concentrations can be predicted readily if the major isoforms responsible for the metabolism of those drugs have been identified. Several approaches have been employed to determine the contribution of individual isoforms in the metabolism of drugs, toxicants and carcinogens. These approaches range from biochemical, immunological, chemical and heterologous cDNA expression techniques. Given that each technique has advantages and disadvantages, the most reliable conclusions should be derived on the basis of data obtained from a battery of approaches, not a single one.

(a) Reconstitution of purified cytochrome P450 isoforms

The purification of CYP isoforms from animals and human tissues and reconstitution of activity of these purified proteins has provided valuable information concerning the catalytic specificity of some isoforms. This approach has been used successfully to characterise the substrate specificities of human CYP1A2, CYP2E1, CYP2A6, CYP3A4/5, and CYP2D6. However, the use of purified proteins for studying CYP has a number of limitations. Purification of CYP isoforms is difficult as they are membrane bound proteins; isolation of these proteins requires the use of detergent. Removal of excess detergent from the purified protein prior to analysis of the catalytic activity is critical since coupling of the electron donor enzyme with CYP is markedly inhibited by the presence of detergent. Purity of protein is sometimes difficult to demonstrate as distinct isoforms of CYP often display marked similarity on SDS-polyacrylamide gel electrophoresis. Indeed, many CYP isoforms differ by only a few amino acids but, as indicated earlier, those differences can have a profound
effect on catalytic activities. It is also noteworthy that a number of CYP isoforms do not appear to retain full activity following purification. Thus, while of use in defining substrate specificity, problems are association with the use of purified enzymes.

(b) Immunological approaches

Antibodies directed against purified CYP isoforms have provided invaluable information concerning CYP. The immunorelatedness of CYP is suggestive of structural similarities of enzymes. Immunoinhibition studies provide information on the functional role of CYP isoforms since the extent to which a specific CYP isoform contributes to the total reaction in microsomes can be predicted from the degree of inhibition observed with a particular antibody. Antibodies can also be used in isolating the cDNAs encoding specific CYP isoforms by recombinant DNA techniques. A variety of immunological techniques including immunoblot analysis (Western blotting), immuno-diffusion assays, enzyme-linked immunosorbent assays (ELISA) and immunohistochemistry permit determination of expression, distribution and content of a specific isoform or subfamily of CYP in a tissue or organ. Specificity appears to be a major problem confronting the use of antibodies. Given the high similarity of primary structure within some CYP subfamilies, an antibody raised against one isoform is likely to cross-react with other isoforms regardless of the purity of the enzyme used for immunisation. Cross-reactivity may also occur between different CYP subfamilies. For example, a monoclonal antibody raised against fish CYP1A1 has been demonstrated recently to cross-react with human CYP2E1. Generation of anti-peptide antibodies directed to specific regions of CYP isoforms is one of the approaches used to circumvent the problem of cross-reactivity. Anti-peptide
antibodies which cause inhibition of the enzyme activity are also of value for the identification of functionally important regions of CYP.\(^{(21)}\)

\textit{(c) Chemical approaches}

The use of chemicals as selective substrates or selective inhibitors of CYP offers an advantage over other approaches given potential applications both \textit{in vitro} and \textit{in vivo}. Regulation of CYP activity in humans by environmental and genetic factors can be investigated by administration of a substrate selective for a particular CYP isoform. Inhibitor probes are particularly useful for determining the contribution of a particular CYP isoform to a reaction under consideration. Clearly, xenobiotic inhibitors have advantages over antibodies in terms of availability. Selective substrates may also serve as competitive inhibitors of the metabolism of another compound by the same isoform. Competitive inhibition of xenobiotic metabolism by a selective substrate frequently provides information about isoform(s) potentially involved in that reaction. However, the competitive inhibition of a reaction by another xenobiotic does not necessarily indicate that the reaction is catalysed by the same isoform which responsible for the metabolism of that xenobiotic. For example, quinidine is a potent inhibitor of CYP2D6 although it is, in fact, metabolised by isoforms of CYP3A subfamily.\(^{(22)}\) Once an isoform-specific substrate has been identified correlation studies between the activity under consideration with the activities of selective substrates across a bank of human liver microsomal samples can additionally be used to indicate the isoform(s) potentially involved in that reaction.

A number of compounds have been proposed as selective substrates or selective inhibitors of CYP isoforms (or subfamilies) (Table 2) and many of these compounds may be used as substrates to study the
Table 2  Proposed model substrates and inhibitors of human cytochrome P450 isoforms

<table>
<thead>
<tr>
<th>CYP isoform</th>
<th>Substrate</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>caffeine (3-demethylation)</td>
<td>a-naphthoflavone</td>
</tr>
<tr>
<td></td>
<td>phenacetin</td>
<td>furafylline</td>
</tr>
<tr>
<td>2A6</td>
<td>coumarin</td>
<td></td>
</tr>
<tr>
<td>2C9/10</td>
<td>phenytoin</td>
<td>sulphaphenazole</td>
</tr>
<tr>
<td></td>
<td>tolbutamide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>warfarin</td>
<td></td>
</tr>
<tr>
<td>2C19</td>
<td>S-mephenytoin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>omeprazole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>proguanil</td>
<td></td>
</tr>
<tr>
<td>2D6</td>
<td>bufuralol</td>
<td>quinidine</td>
</tr>
<tr>
<td></td>
<td>debrisoquine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dextromethorphan</td>
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<tr>
<td></td>
<td>metoprolol</td>
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<tr>
<td></td>
<td>sparteine</td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td>chlorzoxazone</td>
<td>diethylidithiocarbamate</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-nitrophenol</td>
<td></td>
</tr>
<tr>
<td>3A3/4 and 3A5</td>
<td>erythromycin</td>
<td>troleandomycin</td>
</tr>
<tr>
<td></td>
<td>dapsone</td>
<td>gestodene</td>
</tr>
<tr>
<td></td>
<td>lignocaine</td>
<td>naringenin</td>
</tr>
<tr>
<td></td>
<td>nifedipine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>midazolam</td>
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</tr>
</tbody>
</table>
**in vivo** activity of human CYP. However, some (e.g. amino-pyrene, antipyrine, metronidazole etc.) are not isoform (or subfamily) specific and therefore the predictive value of these probes for the metabolism of other drugs is limited. When xenobiotics are used **in vivo** it is essential that administration (usually oral) should be simple and the compounds and their metabolites are free of serious adverse effects. Ideally sampling techniques should be simple (e.g. urine or blood collection). Intrinsic metabolic clearance of the substrate probe should be used as the **in vivo** measure of enzyme activity, although other measures (e.g. urinary metabolic ratio, CO₂ breath test) may be used if they correlate with intrinsic clearance. Obviously, the interpretation of data obtained from substrate and inhibitor studies is dependent upon the specificity of the chemical probes. Thus, it is essential that the selectivity of chemical probes need to be established unambiguously.

**Heterologous cDNA-expression**

Recent advances in recombinant DNA techniques have enabled the isolation of genes or cDNAs encoding particular CYP isoforms and their expression in heterologous host cells. Heterologous DNA expression systems have proved to be useful tools for both the characterisation of substrate specificity of individual CYP isoforms and for structure-function relationship analysis based on chimeric proteins. These systems eliminate the necessity for the laborious purification of CYP from animal and human tissues and have the obvious advantage of being able to assure purity of the proteins, particularly members of closely related genes which are difficult to isolate by classical protein purification procedures. Heterologous expression systems also provide a source of constitutive CYP isoforms of low abundance. The expression systems developed so far include bacteria, yeast, insect and mammalian cells. Each system has advantages and disadvantages. Choice
of an expression system depends on the purposes of the research, amount of the expressed protein required and personal experience. Although high levels of CYP can be obtained from bacterial and yeast expression systems with relatively low cost, the catalytic activity of expressed proteins may be limited by insufficient levels of endogenous NADPH-cytochrome P450 oxidoreductase or cytochrome b5. For example, CYP can be obtained at high levels from insect cells using the baculovirus expression system but the efficiency of this system is limited by an insufficiency of de novo synthesis of heme in the insect cells. This problem may, however, be circumvented by using fresh cells and growing cells in a hemin supplemented culture medium. Stable or transient expression of CYP in mammalian cells offers the advantage of expression in a higher eukaryotic cell which allows more meaningful investigation of post-transcriptional regulation. However, mammalian cell systems are difficult to scale-up and permit only low or moderate production of CYP. Mammalian promutagen testing systems containing stably expressed CYP isoforms have been developed recently. Integration of a gene or a cDNA of CYP into the cellular DNA of the target cell allows the desired genotoxic end-points to be measured directly. These systems provide advantages over the use of exogenous CYP since a longer exposure time and a lower dose of the test chemical generally simulates normal environmental human exposure levels.

Role of cytochromes P450 in drug response, drug toxicity, chemical carcinogenesis and cancer.

It is now clear that interindividual variation in CYP activities, particularly those subject to genetic polymorphism, will lead to variation in drug response and toxicity in humans. Individuals who
metabolise drugs at a slower rate than normal may suffer from adverse effects due to accumulation of the parent drug and/or production of toxic metabolites by alternative pathways. For example, it was found that of twenty patients who developed irreversible peripheral neuropathy while being treated with perhexiline, ten were poor metabolisers of debrisoquine. Less commonly, defects in drug metabolising enzymes could also be responsible for poor drug response if a pharmacologically active metabolite cannot be formed. This seems to be the case with codeine where the O-demethylated metabolite, morphine, has a much more pronounced analgesic effect than does codeine itself.

A role of CYP in the development of human cancer is intuitively obvious given almost all carcinogens are not toxic per se but elicit their effect after bioactivation by CYP to electrophilic intermediates which can modify cellular macromolecules. However, in contrast to experimental animals, an association of cancer risk with changes in CYP composition has been established less rigorously in humans. Initial evidence for such an association in humans emerged from the work of Kellermann et al. In these studies, individuals were classified into a trimodal distribution based on their CYP1A1 inducibility in mitogen-activated lymphocyte cultures. Smokers of the high CYP1A1 inducibility phenotype were more prone to develop lung cancer than low inducibility individuals. Although these results were controversial, the association between CYP1A1 inducibility and human lung cancer was subsequently confirmed. The induction of CYP1A1 mRNA in lung by cigarette smoke and the increase in formation of benzo[a]pyrene DNA adducts within pulmonary tissues from cigarette smokers all support the view that the level of CYP1A1 expression in human lung is important in the etiology
of lung cancer. Hence, interindividual differences in the regulation and expression of the \( \text{CYP1A1} \) gene may result in differences in cancer susceptibility.

Several \( \text{CYP1A1} \) RFLPs have been reported. Of these RFLPs, \( \text{Msp I} \) RFLP, a polymorphism in the 3'-flanking region of the \( \text{CYP1A1} \) gene, has generated considerable interest. Genotyping of \( \text{CYP1A1} \) alleles associated with the presence or absence of the \( \text{Msp I} \) site in the 3'-region is carried out by PCR followed by digestion with \( \text{Msp I} \). Genotype A is a predominant homozygote where the \( \text{Msp I} \) site is absent, genotype C is a homozygous rare allele having a \( \text{Msp I} \) site derived from one base substitution of thymidine with cytosine and genotype B is the heterozygote with both alleles. Kawajiri \textit{et al.}^{(34)} demonstrated an apparent association between genotype C and an increased incidence of lung cancer in a Japanese population. Consistent with these observations, a recent three-generation family study of Eastern Mediterraneans revealed that the high \( \text{CYP1A1} \) inducibility phenotype cosegregated with the \( \text{Msp I} \) polymorphism in the \( \text{CYP1A1} \) gene.\(^{(35)} \) In contrast, studies carried out in Norwegians, Caucasian Americans and Black Americans showed no correlation between the \( \text{Msp I} \) polymorphism in the \( \text{CYP1A1} \) gene and lung cancer.\(^{(36)} \) Ethnic differences in allelic frequency of the \( \text{Msp I} \) polymorphism were observed. The susceptible genotype (i.e. genotype C) was about 10 times less frequent in Caucasians than in Japanese.\(^{(36,37)} \) Recent studies carried out in a Japanese population also demonstrated that the \( \text{Msp I} \) polymorphism is strongly associated with a nucleotide mutation which gives rise to an amino acid substitution (viz. replacement of isoleucine by valine) adjacent to the heme-binding site of \( \text{CYP1A1} \) and this mutant allele has also been shown to have a link to lung cancer susceptibility.\(^{(38,39)} \) Further cDNA-expression studies revealed that the valine-substituted \( \text{CYP1A1} \) variant
exhibited about two-fold higher activity and mutagenicity towards benzo[a]pyrene compared to that of the isoleucine-substituted CYP1A1.\(^{(37)}\) However, additional studies involving larger and additional ethnic populations will be required before any firm conclusions regarding the association of lung cancer and the \(Msp\) I polymorphism can be drawn. Furthermore, expression of the CYP1A1 gene is regulated by interactions between the cis-acting elements in the 5'-flanking region and several trans-acting factors, including the ligand binding subunit of the Ah receptor and Arnt protein. Induction of CYP1A1 therefore involves the products of a number of different genes and characterisation of all of these genes is necessary for understanding the relationship between CYP1A1 inducibility and human cancer.

There have been several studies in which attempts were made to demonstrate associations between CYP2D6 phenotype and human cancers. Ayesh \textit{et al.}\(^{(40)}\) demonstrated that the extensive debrisoquine metaboliser phenotype could be associated with an increased incidence of bronchiogenic lung carcinomas in smokers. In agreement with this finding, a 6- to 11-fold increased risk of lung cancer in the extensive debrisoquine metaboliser phenotype has been demonstrated in a recent case-control study carried out in Blacks and Caucasians.\(^{(41)}\) However, conflicting results have been reported where only slightly or non-statistically significant increased risks in extensive metaboliser phenotype subjects were observed.\(^{(42-44)}\) The development of CYP2D6 genotyping assays allows more precise classification of individuals compared to phenotyping, which cannot distinguish precisely the heterozygotes from homozygotes amongst the extensive metaboliser group. No difference in genotype frequencies between lung cancer patients and a control group was reported.
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Life and work environmental conditions are often causes of alterations of the lacrimal film.

Tears Naturale® Mimics Natural Tears

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Human Tears</th>
<th>Tears Naturale®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucin, Aqueous, Lipids</td>
<td>Duasorb® System®</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Tonicity</td>
<td>287-312</td>
<td>291</td>
</tr>
<tr>
<td>Low Viscosity</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mucin</td>
<td>Produced by Conjunctival Goblet Cells</td>
<td>Mucomimetic</td>
</tr>
<tr>
<td>Potassium</td>
<td>17 mmol/L</td>
<td>16 mmol/L</td>
</tr>
</tbody>
</table>

Dual Effect
Corrects both aqueous and mucin deficiencies.

Unique Duasorb® System Mimics Natural Mucin
Patented polymeric system mimics the action of natural mucin Creates a strong, stable, more natural tear film layer to protect the corneal surface

Formulated for Maximum Comfort
- Low viscosity
- Isotonic
- Same pH as natural tears

Long Lasting Effect
Provides long-lasting wetting of corneal surface—90 minutes or longer.

Easy-to-use
Multiple-dose convenience is a plus for patients with busy lifestyles.

2. Alcon Duraglide For
but in this study only one mutant allele was screened and matched controls were not included. Recent studies which screened all known CYP2D6 inactivating mutations demonstrated that the frequency of the poor metaboliser genotype is decreased among lung cancer patients.\(^{(46)}\)

Although there is some evidence for an association of the debrisoquine polymorphism with lung cancer, the experimental evidence to establish biological plausibility for such an association is limited. A recent report\(^{(47)}\) which demonstrated that cDNA-expressed CYP2D6 is capable of activating a procarcinogen found in tobacco smoke, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), has provided some new support. It is unlikely, however, that CYP2D6 is the major enzyme involved in the metabolism of this compound. An association between bladder cancer and the debrisoquine extensive metaboliser phenotype has also been proposed,\(^{(49)}\) but corroboration evidence is again lacking.

REFERENCES


13. Distlerath LM, Reilly PE, Martin MV, Davis GG, Wilkinson GR and Guengerich FP. Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in


21. Edwards RJ, Murray BP, Marruy S, Singleton AM, Davies DS and Boobis AR. An inhibitory monoclonal anti-protein antibody and an anti-peptide antibody share an epitope on rat cytochrome P450 enzymes CYP1A1


