Relevance of induction of human drug-metabolizing enzymes: pharmacological and toxicological implications

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- 1 Human drug-metabolizing systems can be induced, or activated, by a large number of exogenous agents including drugs, alcohol, components in the diet and cigarette smoke, as well as by endogenous factors.
- 2 Such perturbation of enzyme activity undoubtedly contributes to both intra- and inter-individual variation both with respect to the rate and route of metabolism for a particular drug. Induction may, in theory, either attenuate the pharmacological response or exacerbate the toxicity of a particular drug, or both.
- 3 The clinical impact of enzyme induction will depend upon the number of different enzyme isoforms affected and the magnitude of the inductive response within an individual, and also on the therapeutic indices of the affected substrates.
- 4 The toxicological implications will be determined either by any change in the route of metabolism, or by a disturbance of the balance between activation and detoxication processes, which may be isozyme selective.

Keywords enzyme induction human liver drug metabolism cytochrome P450 glucuronidation toxicity pharmacodynamics pharmacokinetics clearance

Introduction

The exposure of mammals to lipophilic substances leads to an adaptive response of the drug-metabolising systems which involves the accumulation of specific mRNAs and the increased expression of the associated enzymes. Some groups of enzymes are under co-ordinate regulatory control whereas other enzymes may undergo more selective induction.

Most studies of enzyme induction have been carried out in rats, rabbits and mice, and have usually been conducted with high mass doses of drugs given chronically. Enzyme induction, like enzyme inhibition, is a dose-dependent phenomenon with reasonably steep dose-response relationships and therefore clear-cut no-effect levels [1]. Indeed, for the most part, enzyme induction is usually observed with drugs given at relatively high doses or after exposure to chemicals, such as dioxin, which have extra-ordinarily long tissue half-lives. This is in keeping with the theory that the

regulatory system for drug-metabolizing enzymes has evolved to protect against the accumulation of foreign compounds to levels which may cause toxic effects.

Induction of drug metabolism has been noted in man after administration of therapeutic doses of drugs. However, the number of drugs which can produce such an effect is perhaps more limited than was first anticipated. Rifampicin, barbiturates, phenytoin and carbamazepine are all well established inducers that produce clinically significant changes in drug metabolism [2, 3]. More recently a small number of drugs, including omeprazole [4] and simvastatin [5] have been found to cause statistically significant changes in the pharmacokinetics of substrates for specific P450 isoforms, but the broader clinical importance of these observations still needs to be established. Ethanol and cigarette smoke cause relatively selective induction of the cytochrome P450 isoforms, CYP2E1 and CYP1A respectively [6], which may have therapeutic and toxicological implications for certain drugs.

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Detection of enzyme induction

The first indication that a drug is an enzyme-inducing agent usually occurs during preclinical evaluation of drug metabolism or during the toxicokinetic analysis of long-term toxicity and carcinogenicity testing in animals. In order to establish the significance of induction for a new therapeutic agent, the essential information required is:

- the dose-response relationship for the inductive effect
- the exposure (duration of dosing) required for induction
- the level of induction assessed by rigorous kinetic studies ($V_{\max}$, K_m)
- qualitative aspects for the induction of different enzymes, particularly with respect to:
 - (i) the P450 enzymes
 - (ii) glucuronyl transferase
 - (iii) glutathione-S-transferase

Such information is invaluable since a comprehensive and direct measurement of enzyme activity can be made in animals which obviously is not possible in human studies. The most important factor in extrapolating the findings to man is a consideration of the relative exposure of patients taking therapeutic drug doses to the minimum exposure required in animals to obtain measurable enzyme induction. This is dependent on the enzyme being considered since minor isoforms in the rat, such as CYP1A1, show over a 100-fold induction as determined by protein levels and functional activity, while the major constitutive forms, such as CYP3A2, show only a two- to three-fold increase [7]. Additional factors which need to be considered include species differences in drug disposition and in the response to certain classes of enzyme inducing agents. It is also important to note that induction of metabolism might be masked by concomitant induction of the synthesis of α_1 -acid glycoprotein which would cause an increase in plasma binding and elevation of the total plasma drug level.

A clear lack of induction in more than one species, taken together with an absence of any effect of repeat dosing on the pharmacokinetics of the drug itself in volunteers, might be sufficient to argue against the need for any further investigation in man, depending on the clinical application of the drug and other data regarding safety. In such cases, follow-up data should always be obtained from pharmacovigilance studies and population kinetics. If, however, the drug is found to be an enzyme inducer in any of the usual species used for preclinical evaluation, then some further studies may be required in man. *In vitro* systems, using either human hepatocytes or lymphoblastoid cell lines that express specific human CYP enzymes, have proved exceptionally useful for the early detection of potential drug interactions which involve enzyme inhibition. The establishment of test systems to assess enzyme induction in such cell lines may prove more challenging because of the complexity of the regulatory machinery controlling gene expression, and because of the multiple mechanisms by which induction can occur (see below). This particularly applies to heterologous expression systems where it is difficult to ensure that all nucleotide sequences involved in promotion and activation are incorporated within the transfected unit. Nevertheless, such systems have great potential not only to detect enzyme induction but also to investigate the molecular mechanisms involved.

The more traditional approach involves the use of probe drugs such as antipyrine and caffeine [3] (Table 1). Human experience, together with recent advances in molecular biology, have provided a framework for the human cytochrome P450 superfamily, which has allowed the functional characterization of individual enzymes with respect to therapeutic agents and probe drugs. When extrapolating from changes in the kinetics of a probe drug, we can only legislate for changes in the enzyme for which the probe drug is a selective marker, i.e. one cannot expect a probe drug to be a universal marker of all phase I and phase II biotransformations that a therapeutic agent may undergo.

Table 1 Examples of *in vivo* probes for drug metabolising enzymes

Enzyme	Probe drug	Reference
CYP1A2	Caffeine	[8]
CYP2C9	Tolbutamide	[9]
CYP2C19	Mephenytoin	[10]
	Proguanil	[11]
CYP2D6	Sparteine	[12]
	Dextromethorphan	[13]
CYP2E1	Chlorzoxazone	[14]
CYP3A4	Erythromycin (breath test)	[15]
	Midazolam	[16]
	6 β -Hydroxycortisol	[17]
Microsomal epoxide hydrolase	Carbamazepine-10,11-epoxide	[18]
N-acetyl transferase	Sulphadimidine	[19]
	Isoniazid	[19]
	Caffeine	[20]
Glucuronyl transferase	Oxazepam	[21]

Mechanism of enzyme induction

The levels of several hepatic cytochrome P450 enzymes are controlled by the concentration of substrates in the liver. Enzymes can be controlled at the pre-translational, translational and post-translational levels (Figure 1). The cytochrome P450 enzymes are under diverse transcriptional control according to studies in rodents [22]. Practically every mammalian P450 has its own regulatory circuit. Certain P450 enzymes are present only at low levels and are induced by foreign compounds, while others are expressed constitutively at relatively high levels. For example, the level of CYP2E1 can fluctuate constitutively approximately 20-fold in the rat, but the maximum capability for induction is about 50-fold [23]. Translational and post-translational regulation has been less well studied, and so far has only been recognised for CYP2E1 [24, 25], and possibly CYP3A1 [26]. Levels of CYP2E1 return to normal very rapidly following induction in animal models (within 24 h in Sprague-Dawley rats induced with ethanol [27]); the short half-life of this P450 isoform is attributed to a structural peculiarity that targets the protein for rapid degradation, possibly by enhanced phosphorylation [28]. All agents which increase CYP2E1 degradation also cause phosphorylation of the enzyme on Ser-129, while substrates which prevent this phosphorylation also prevent degradation [29]. Phosphorylation of Ser-129 triggers a rapid loss of haem [29].

The important questions with respect to mechanisms of induction are as follows:

- 1 Can we predict whether a chemical will be an inducer?
- 2 What is the selectivity of the inducer both with respect to the P450 isoforms, and also whether it is a multifunctional inducing agent, i.e. can it induce more than one enzyme including phase I and phase II enzymes? (Figure 2).

The molecular mechanism of induction has been more clearly defined with respect to environmental chemicals rather than therapeutic drugs, although the basic principles are the same. Polycyclic aromatic hydrocarbons typified by TCDD (dioxin) are effective inducers of CYP1A1 and CYP1A2 which are under similar regulatory control [30]. Induction of CYP1A1 involves interaction of the inducer with a hydrophobic cytosolic receptor, termed the Ah (aromatic hydrocarbon) receptor and translocation of the ligand-receptor complex to the nucleus followed by *de novo* protein synthesis [31]. The human Ah receptor has recently been characterized [32]. It is a ligand-activated transcription factor which acts in a superficially similar way to the steroid hormone receptors. Recent cloning experiments of cDNA for the

Figure 1 Mechanisms by which enzymes may be induced.

Figure 2 Classification of inducers of drug metabolizing enzymes into monofunctional, those inducing only members of a specific family of enzymes, and multifunctional, which act on more than one enzyme system.

Ah receptor revealed that it is a basic helix-loop-helix transcription factor [33] rather than a 'zinc finger' protein that characterizes the steroid receptors.

The CYP1A1 gene has one or more segments of DNA upstream from its transcription start sites called Ah-receptor regulatory elements (AhRE). Binding of the Ah-receptor to the AhRE activates transcription of the CYP1A1 gene. The Ah receptor in its active form actually consists of a heterodimer comprising the ligand binding domain (ALBD) and the Ah-receptor nuclear transporter (ARNT). In the absence of ligand the ALBD is associated with heat shock protein HSP-90. Upon ligand binding to the ALBD, the HSP-90 dissociates and ARNT binds yielding the receptor complex capable of interacting with AhRE [34] (Figure 3). It is interesting to note that the Ah-receptor has an important physiological function, possibly during foetal development. A recent study which constructed an Ah-receptor deficient mouse line by homologous recombination demonstrated that 50% of mice died shortly after birth, and those that survived showed immune system impairment and bile duct fibrosis, as well as non-inducibility with dioxin [35].

Receptors for the induction of other CYP enzymes, including those of the CYP2 and CYP3 families, which are responsible for the metabolism of the vast majority of drugs, have not been defined. A number of P450 genes in the CYP2 family are inducible by barbiturates. However, although the induction of P450 genes by phenobarbitone has been extensively studied, and several possible mechanisms of regulation proposed [36], a phenobarbitone receptor has not been identified. In both bacteria and rodents, a repressor binding site within a 15-17 base pair sequence in the 5'-flanking region of phenobarbitone-inducible genes (the so-called 'Barbie box') has been identified [37]. More recently, this element has been shown to exist in essentially all eukaryotic and prokaryotic genes that are barbiturate inducible [38]. It has been shown that activation of the bacterial cytochrome P450 CYP102 gene by phenobarbitone is due to release of inhibition by a repressor protein [39].

The catalytic activity of cytochrome P450 enzymes can be altered directly by substrates and other low

Figure 3 Proposed mechanism of induction of CYP1A1. The inducing ligand enters the cell cytoplasm and displaces the heat shock protein, HSP-90, from its binding site on the ligand binding domain (ALBD) of the Ah receptor. This allows the nuclear transporter (ARNT) to associate with ALBD to form the Ah receptor-ligand complex. Translocation of this complex into the nucleus allows it to bind to the Ah receptor response element (AhRE) upstream of the CYP1A1 gene leading to enhanced transcription of the gene.

molecular weight compounds. Indeed, there are now a number of low molecular weight inhibitors, such as furafylline, quinidine and sulphaphenazole, which are regarded as diagnostic inhibitors of specific enzymes.

Conversely, certain P450 enzymes can be post-translationally activated. The activity of the CYP3 isoforms is increased in the presence of 7,8-benzoflavone; the activation is due to the simultaneous presence of

the substrate and activator in the active site [40] which may create a more efficient environment for oxygen activation by the exclusion of water from the active site.

The mechanisms by which expression of glucuronyl transferases (UDPGTs) is regulated have not been elucidated [41], although it is recognised from animal studies that a wide range of compounds can effect induction in a selective fashion. Multiple isoforms of UDPGT have been identified [42], and clearly, as has been shown for the different P450 isoforms, the mechanisms of induction may vary with the different isoforms. In some instances UDPGTs may be induced co-ordinately with particular cytochrome P-450 enzymes [3]. Whether this bifunctional induction is caused by common properties amongst certain chemicals or represents a concerted mechanism at the level of gene regulation is not known. In man, where it has proved difficult even to demonstrate induction, very little is known about induction of UDPGT. Indirect evidence suggests that rifampicin, phenobarbitone and the other anticonvulsants all induce UDPGT, but it is not known which of the different isoforms can be induced [3]. More recently, studies with the novel anticonvulsant lamotrigine, which is eliminated mainly by glucuronidation, have shown that its half-life is reduced from 29 h to 15 h by concomitant administration of the aromatic anticonvulsants (carbamazepine, phenytoin, phenobarbitone and primidone) [43, 44]. In terms of clinical practice, this has been translated into a two-fold higher starting dose and a higher maintenance dose of lamotrigine in patients already taking one of the enzyme-inducing anticonvulsants [45].

Studies of UDPGT-1, which glucuronidates bilirubin, in patients with Crigler-Najjar syndrome and Gilbert's syndrome are providing some insights into how mutations in the structural and regulatory regions may affect enzyme activity. For example, structural mutations in the gene result in Crigler-Najjar syndromes types 1 and 2 which are characterized by an absence or severe deficiency, respectively, of bilirubin glucuronidation [46]. More recently, in patients with Gilbert's syndrome, a condition characterised by mild hyperbilirubinaemia, it has been shown that two extra bases (TA) in the TATAA element of the 5'-promoter region of the gene results in reduced expression of the UDPGT-1 [47]. Such investigations in naturally occurring diseases should provide important insights into the mechanisms of expression and induction of UDPGT. In this respect, it is interesting to note that the clearances of paracetamol [48] and lamotrigine [49] are decreased in patients with Gilbert's syndrome, indicating that they are metabolised (at least partly) by UDPGT-1.

Pharmacokinetic considerations

The pharmacological response to a drug may be a function of either the peak concentration achieved ($C_{\max}$) if there is a threshold for activity, or, more usually, of total exposure, as measured by the area under the plasma concentration-time curve (AUC). More precisely,

the response is a function of exposure to unbound drug (AUC_u), since only drug that is not bound to plasma proteins is considered free to act at its target site. AUC_u is directly related to the dose of the drug, but is also dependent on the rate of free drug clearance (CL_u) from the bloodstream. Assuming complete absorption:

$$\text{AUC}_u = \frac{\text{dose}}{\text{CL}_u} \quad (1)$$

Therefore, to maintain a pharmacological response, the dose must be adjusted to any change in total free drug clearance (CL_u), which is the sum of both renal clearance (CL_{uR}) and metabolic clearance (CL_{uM}) of unbound drug:

$$\text{CL}_u = \text{CL}_{uM} + \text{CL}_{uR} \quad (2)$$

For a drug cleared by a single metabolic pathway, and in the absence of renal clearance, its clearance (CL_{int}) is a function of the Michaelis-Menten parameters, K_m and $V_{\max}$, for the enzyme that mediates the metabolic change; then for unbound drug:

$$\text{CL}_{u\text{int}} = \frac{V_{\max}}{K_m} \quad (3)$$

where CL_{uint} is the intrinsic clearance of unbound drug. Assuming the 'well-stirred' model of clearance, this will equate to systemic unbound clearance for low extraction drugs given i.v. or orally, and for high extraction drugs, given orally.

On induction of this enzyme the change in clearance (R) may be defined as:

$$R = \frac{V_{\max(I)} \cdot K_m}{K_{m(I)} \cdot V_{\max}} \quad (4)$$

where I refers to the induced parameters.

Since induction should affect only the amount of enzyme present, it is reasonable to assume that the affinity of the enzyme is unaltered. Thus,

$$K_{m(I)} = K_m \quad (5)$$

Therefore,

$$R = \frac{V_{\max(I)}}{V_{\max}} \quad (6)$$

and represents the dose adjustment required to compensate for the altered clearance. In practice, few drugs are cleared by a single enzyme and their clearance is the sum of all the contributing metabolic pathways (again assuming negligible excretion of unchanged drug and in the absence of organ blood flow limitation). In this case,

$$\text{CL} = \sum_i^n \frac{V_{\max}}{K_m} \quad (7)$$

or

$$\text{CL} = \frac{V_{\max A}}{K_{mA}} + \frac{V_{\max B}}{K_{mB}} + \frac{V_{\max C}}{K_{mC}} \dots \text{etc.} \quad (8)$$

where A, B, C represent individual enzymes involved in the overall clearance of the drug and clearance is the sum of the fractional clearances (f) associated with each

individual isozyme. Thus, for enzyme i

$$f_i \cdot \text{CL}_u = \frac{V_{\max i}}{K_{m i}} \quad (9)$$

If only this single enzyme is induced then

$$R = \frac{V_{\max i(I)}}{V_{\max i}} \quad (10)$$

and the new induced unbound clearance $[\text{CL}_u(I)]$ will be given by

$$\text{CL}_u(I) = \text{CL}_u + (R - 1)f_i \cdot \text{CL}_u \quad (11)$$

Thus the dose adjustment required, which is equivalent to the ratio of the pre- and post-induction clearances, is a function of the change in $V_{\max}$ after induction (R) and the fractional clearance through the pathway

$$\frac{\text{CL}_u(I)}{\text{CL}_u} = 1 + (R - 1)f_i \quad (12)$$

Figure 4 shows hypothetical curves for the effect of enzyme induction on drug clearance at different fractional clearances of the affected pathway. The curves clearly indicate that dosage adjustment is only required when the clearance of the drug by the affected pathway is, or becomes, a significant fraction of total drug clearance. Thus, a doubling of the dose is required if a pathway is induced threefold only if that pathway initially accounted for 50% or more of the total drug clearance. In the more common case, in which the clearance associated with a particular pathway is only 20% of total clearance, then more than a six-fold induction would be required to necessitate altering the dose. Moreover, a need to change the dose is not anticipated even if metabolic clearance increased 20-fold, if this clearance usually only contributes about 1%.

In order to carry out any quantitative assessment of the implications of enzyme induction, and in particular

Figure 4 Hypothetical curves indicating the effect of enzyme induction, R , on the clearance of a drug by a pathway that contributes various proportions (f_i) of the total clearance. The ratio of the induced to control unbound clearances $[\text{CL}_u(I)/\text{CL}_u]$ indicates the dose adjustment required to maintain plasma concentrations at their pre-induced level.

the changing relationship between dose and effect, we first need to know the change in the amount of enzyme caused by induction, i.e. $V_{\max}$. This information can be obtained in two ways, by pharmacokinetic analysis of model drugs and by direct measurement of protein levels in liver biopsy samples using Western blot analysis.

If response is simply related to exposure (as measured by AUC_u) and toxicity does not accrue from any disproportionate change in $\text{Cu}_{\max}$, then the dose can be adjusted according to the change in clearance. This simple argument makes a number of assumptions concerning blood flow, which may influence clearance of drugs given by the intravenous route though, according to the 'well-stirred' model, should leave clearance of orally-administered drugs unaltered. In addition, there may be induction of extrahepatic enzymes such as those in the gut wall which make a substantial contribution to the clearance of a number of lipophilic drugs, in particular, those that undergo first pass elimination. Indeed, drugs that do undergo a high first pass effect will often show the greatest alterations in their plasma concentrations following enzyme induction. A 5% increase in CL_{int} for a drug that already undergoes 90% clearance on first pass would be translated into a 50% decrease in AUC . This point is emphasised by the drug felodipine, a calcium channel blocker that undergoes extensive pre-systemic clearance under normal circumstances. When taken concomitantly with phenobarbitone, carbamazepine or phenytoin by epileptic patients, the AUC of felodipine was reduced by over 95% compared with controls; less than 1% of the oral dose was systemically available [50].

These arguments are based on mean data and do not take into account inter-individual variation in enzyme induction or drug response. For example, with carbamazepine which causes a dose-dependent increase in the 6 β -hydroxylation of cortisol [51], a marker of CYP3A activity [17], inter-individual variability in induction at the same dose does occur. Thus, at daily carbamazepine doses of 400 mg ($n=6$), 600 mg ($n=15$) and 800 mg ($n=7$), the variability in CYP3A activity was seven, 11- and two-fold, respectively [51]. Such variability can be ignored if we assume that it was adequately covered by the pre-induction regime (i.e. the drug is efficacious for the target population). If the variation in induction is normally distributed then a dose adjustment corresponding to the mean change in clearance and its 95% confidence intervals should be efficacious in the majority of the population, but may now produce a safety hazard in those patients with an initial low clearance who show minimum enzyme induction.

In the case of toxic metabolite formation almost the opposite arguments apply. The relative change in the clearance by the bioactivation pathway is independent of the initial clearance by that pathway in terms of the rate of bioactivation. Thus, the rate of reactive metabolite formation will increase as a function of changes in $V_{\max}$ which may be sufficient to overwhelm bioinactivation processes if they are not also induced. Furthermore, if the reactive metabolite is normally only a minor (<5%) metabolite, then the total dose of toxic metabolite will

increase substantially and could therefore overcome any threshold for drug toxicity. The exaggerated effects of enzyme induction on the bioavailability of drugs with a high first pass metabolism (see example of felodipine above) will not, of course, extend to potential toxicity from metabolites, since the extreme changes in plasma levels are the result of fairly modest alteration in oral clearance to the (toxic) metabolite.

Enzyme induction and drug efficacy

Exposure to an enzyme inducing agent during chronic drug administration may lead to a loss of drug efficacy because of increased drug clearance. Clinically, the most important interaction of this type is that between the oral contraceptive steroids and rifampicin, which resulted in a number of unwanted pregnancies. Most new drugs are screened for their potential to interact in this way and the problem becomes exacerbated when the new drug is a suspect teratogen.

The potential for such an interaction will be determined by a number of factors including:

- the therapeutic index and efficacy of the drug;
- the fractional metabolic clearance of the drug;
- inducibility of the relevant enzymes;
- the potency of the enzyme inducer;
- the contribution of extra-hepatic metabolism/induction;
- the cytochrome P450 status of the individual;

In the case of the rifampicin–oral contraceptive interaction at least four of the criteria are fulfilled. The oral contraceptive is given at the minimum effective dose to avoid the risk of thrombosis. The oestrogen component of the pill, ethinyloestradiol, is cleared by a combination of oxidation and glucuronidation, and analysis of urinary and biliary metabolites indicates an oxidation:glucuronidation ratio of 2.4:1 [52, 53]. The data shown in Table 2 are consistent with induction of both oxidation, which was assessed by changes in antipyrine clearance and 6 β -hydroxycortisol excretion, and glucuronidation which is known to be induced by anticonvulsants and rifampicin [3]. In an *in vitro* system, using liver biopsies from patients, it was found that rifampicin caused a fourfold increase in steroid oxidation [55] which is similar to the threefold increase in cortisol 6 β -hydroxylation noted *in vivo* [56]. It should be noted that the database is small and that there is no estimate

of inter-individual variation in response. Nevertheless, current clinical recommendations are that for epileptic patients requiring contraception, the dose of oestrogen should be increased two- or threefold, while those patients on rifampicin should use an alternative form of contraception.

Cigarette smoking does not affect the availability of ethinyloestradiol [57], although Michnovicz *et al.* [58] reported a marked increase in oestradiol 2-hydroxylation in smokers with a consequent decrease in circulating oestradiol levels. Different P450 enzymes are involved in the metabolism of the two oestrogens: oestradiol 2-hydroxylation is catalysed by a number of P450 isoforms including CYP1A, while ethinyloestradiol is catalysed largely by CYP3A4 [59]. In addition, cigarette smoking does not influence the glucuronidation of ethinyloestradiol. Cigarette smoking does not diminish oral contraceptive efficacy but does enhance toxicity by a synergistic effect on blood coagulation.

Omeprazole has been reported to be an inducer of CYP1A1 and CYP1A2 in human hepatocytes [60] and enterocytes [61], but whether this effect occurs *in vivo* and is clinically significant is controversial. *In vivo* studies in volunteers have shown that omeprazole can induce hepatic CYP1A2 but not CYP3A4 [4, 62]. The extent of enzyme induction was dependent on the dose and the S-mephenytoin phenotype which determines the rate of clearance of omeprazole itself. However, even with a high dose (40 mg), the increase in CYP1A2 activity was <50%. In a separate study, in which phenacetin and paracetamol were used as probe drugs, there was no evidence for induction of either oxidation or glucuronidation by omeprazole *in vivo*, but the maximum dose was 20 mg day⁻¹ [63]. Finally, it was found that omeprazole (20–60 mg day⁻¹) caused a small but significant increase in CYP1A activity in duodenal biopsy samples while, in contrast UDP-glucuronyl-transferase activity towards 4-methylumbelliferone was unaffected [61]. Cigarette smoking was also associated with selective induction of oxidation.

Therefore, an interaction between omeprazole and the oral contraceptive is only likely to occur in an individual with an initial high clearance of the oestrogen, who is a poor metabolizer of mephenytoin taking high doses of omeprazole. From a pharmacological point of view, the chance of an interaction is thus remote, but in terms of drug licensing the possibility of an interaction cannot be excluded.

Interactions between the established anticonvulsants are well documented and are complex at both the

Table 2 The effect of enzyme-inducing drugs and cigarette smoking on the relative bioavailability of the contraceptive steroids ethinyloestradiol and norethisterone (data taken from [54])

Inducer	Relative bioavailability of ethinyloestradiol (% of control)	Relative bioavailability of norethisterone (% of control)
Rifampicin	58	58
Phenytoin	51	58
Carbamazepine	58	60
Cigarette smoking	100	100

Table 3 Induction and inhibition of human drug-metabolizing enzymes by anticonvulsant drugs

Drug	Induction	Inhibition	Reference
Phenytoin	CYP2C9, CYP3A4		[3]
	Glucuronyl transferase		[65]
Carbamazepine	CYP3A4		[66]
	Glucuronyl transferase		[65]
Valproic acid		Glucuronyl transferase	[67]
		Microsomal epoxide hydrolase	[68]
Valpromide		Microsomal epoxide hydrolase	[68]
Oxcarbazepine			
Vigabatrin			
Lamotrigine		? Microsomal epoxide hydrolase	[69]

pharmacodynamic and pharmacokinetic level [64]. The pharmacokinetic interactions involve both induction and inhibition of phase I and phase II metabolism (Table 3). The problem may be exaggerated in the case of phenytoin where zero-order pharmacokinetics cause a steep dose-response relationship. Against this, one has to consider that the drugs are carefully titrated in patients being switched from monotherapy to polytherapy. Some of the newer antiepileptic drugs such as gabapentin and vigabatrin are more promising in this respect in that, because of their pharmacokinetic features (well absorbed but excreted unchanged in urine), their potential for drug interactions appears minimal.

The arguments outlined above are based solely on hepatic drug clearance. Rifampicin decreases the bioavailability of cyclosporine (33% to 9%) to a greater extent than would be predicted from the increase in hepatic metabolism; this is due to an additional induction of intestinal P450 enzymes that appears greater than the induction of hepatic metabolism [70].

Toxicological implications of enzyme induction

The relationship between drug metabolism and drug toxicity is outlined in Figure 5. Exposure to more than one drug, or chemical, may lead to toxic effects that are more than the simple sum of the effects of the individual compounds. Moreover, such synergistic toxicity may accrue without any apparent change in the plasma levels

Figure 5 Scheme outlining the relationship between drug metabolism and drug toxicity.

of the individual compounds involved. In theory, drug toxicity could result from a variety of factors including:

- enhanced bioactivation;
- decreased detoxication;
- decreased bioinactivation;
- an alteration in the balance between bioactivation and bioinactivation.

Proof that induction of bioactivation may overwhelm defence mechanisms comes largely from animal studies, where there is evidence for a massive perturbation of drug metabolism. In many instances a clear cut effect is only observed at doses of toxin which begin to saturate the drug-metabolizing systems. For man, evidence comes from retrospective clinical studies taken together with *in vitro* metabolic studies and, where appropriate, molecular biology investigations. Again in such studies it is essential that both qualitative and quantitative aspects of induction are considered.

Paracetamol (acetaminophen) overdose

Paracetamol (acetaminophen) toxicity is a useful model (Figure 6) to consider the possible contribution of enzyme induction to (i) the threshold dose for toxicity, and (ii) the prognosis of an individual being treated for overdose.

Clinical experience indicates that chronic alcohol consumption can lower the dose-threshold for the acute hepatotoxicity associated with acetaminophen overdose [71, 72]. At least three factors may contribute to this effect. First, alcohol is known to induce CYP2E1, one of at least three cytochrome P450 enzymes that can catalyse the bioactivation of paracetamol to *N*-acetyl-*p*-benzoquinoneimine (NAPQI), without concomitant induction of the major detoxication pathways of paracetamol [73, 74]. The role of CYP2E1 in the bioactivation of paracetamol was further emphasised by recent experiments with HepG2 cells where cytotoxicity was seen only when the cells were transfected with CYP2E1 cDNA [75]. Secondly, the malnourishment associated with alcoholism can cause depletion of glutathione, which is essential for the bioinactivation of NAPQI, and glucose, required for glucuronidation [76]. Thirdly, there may be a convergence of toxicological pathways, initiated independently by paracetamol and alcohol, at

Figure 6 The relationship between the metabolism and toxicity of paracetamol: implications of enzyme induction.

the level of calcium homeostasis or prostanoid synthesis [77]. In the induced state, it has been estimated that the concentration of CYP2E1 in the centrilobular region of the liver, which is the most susceptible area to paracetamol-induced necrosis, may be as high as 0.1 mM [78]. A clinical study has recently shown that chronic alcoholism adversely affects the prognosis of paracetamol overdose, and is characterised by massive elevations of aspartate transaminase [79]. Interestingly, patients on enzyme-inducing anticonvulsants (which induce CYP3A) are also more susceptible to paracetamol toxicity [80] despite the fact that this class of drugs induce glucuronidation as well as oxidation [81].

Induction of CYP2E1

CYP2E1 is involved in the metabolism of certain drugs including paracetamol, ethanol and fluorinated volatile

anesthetics. It is also responsible for the bioactivation of many low molecular weight toxins and carcinogens including benzene, styrene, *N*-nitrosamines and a number of halocarbons [82, 83].

Induction of CYP2E1 has been demonstrated in a number of species, including man, and has been found to involve pre-translational (transcriptional activation and mRNA stabilisation), translational and post-translational regulation (discussed above), and may therefore be induced independently of other phase I and phase II drug-metabolizing enzymes. In experimental animals ethanol does not induce glucuronidation [84, 85].

Ethanol intake is associated with an increase (twofold) in CYP2E1 protein content in human liver biopsy samples [86] and a three-fold elevation of mRNA [25]. The 6-hydroxylation of chlorzoxazone is thought to be mediated in man principally by CYP2E1. Studies in alcoholic patients (daily intake 333 ± 191 g of alcohol)

showed a 73% increase in the mean plasma clearance of chlorzoxazone compared with controls (weekly intake <100 g), whereas cigarette smoking was without effect [87]. Severe obesity is associated with increased 6-hydroxylation of chlorzoxazone consistent with the induction of CYP2E1 caused by this condition [88]. Acute administration of alcohol can, on the other hand inhibit drug oxidation.

Population studies have shown that the *per capita* consumption of alcohol is related to the prevalence of, and mortality from, cirrhosis, although at the individual level it is difficult to demonstrate such a relationship [89]. Only 10–30% of individuals who abuse alcohol develop serious forms of liver disease, such as hepatitis, fibrosis and cirrhosis, while up to 30% of alcoholics have completely normal liver histology [90].

The main enzymes involved in the metabolism of alcohol are the alcohol dehydrogenases and CYP2E1. Although both enzyme systems can effect the oxidation of alcohol, a significant role for CYP2E1 in the pathogenesis of alcohol-induced liver disease has been postulated because of the discovery of antibodies directed against antigens derived from the ethoxyl radical [91]. This radical is a putative metabolite of P450-mediated ethanol oxidation [92], but cannot arise through the dehydrogenase pathway. Consistent with this hypothesis, it has been found that a restriction fragment length polymorphism (RFLP), which is associated with a 10-fold higher transcriptional activity than the more common wild type allele [93], is a significant factor in predisposing individuals to alcoholic liver disease [94]. An increase in transcription rate, coupled with the post-transcriptional stabilisation resulting from exposure to alcohol, may well be sufficient to overcome the detoxication mechanisms for the bioinactivation of the ethoxyl radical and thus promote toxicity by antigen formation.

Biotransformation of the fluorinated volatile anaesthetics results in the formation of products which can cause either renal or hepatic toxicity. Halothane hepatitis, which is now regarded as a model for immune-mediated hepatotoxicity [95], is believed to be caused by the formation of neoantigens derived from oxidative defluorination of the drug, a pathway metabolized mainly by CYP2E1 [96]. Thus, it is theoretically possible that a combination of induction by ethanol and a genetic polymorphism which increases transcription would constitute an increased risk factor for halothane hepatitis. The release of inorganic fluoride from anaesthetics such as methoxyflurane can cause a high urine output syndrome leading to dehydration and in some cases renal failure. Inorganic fluoride ions inhibit chloride transport in the ascending limb of the loop of Henle [97]. The extent of defluorination, and the potential for nephrotoxicity, is largely related to chemical structure of the drug involved [98]. Thus, methoxyflurane is extensively metabolized (40%) and is characterized by high serum levels of inorganic fluoride, while enflurane (3%) and isoflurane (1%), which rarely cause nephrotoxicity, undergo lower rates of metabolism. There is growing evidence that CYP2E1 is the major enzyme responsible for the metabolism of these class of

drugs. In accordance with this, it has been shown that chronic isoniazid therapy induces the metabolism of enflurane and isoflurane, markedly increasing the peak plasma fluoride concentrations [99, 100], whereas barbiturates and phenytoin have no effect [101]. However, barbiturates do induce the metabolism of methoxyflurane which is catalysed by a number of enzymes, including members of the CYP2C sub-family.

The degree of metabolism and thus the serum levels of inorganic fluoride, however, cannot be the sole reason for nephrotoxicity since sevoflurane, which is also associated with high inorganic fluoride levels, rarely causes nephrotoxicity [102]. The explanation for this may be the high rate of intra-renal metabolism by CYP2E1, 2A6 and 3A4 (in diminishing order of activity) of methoxyflurane but not of sevoflurane [103].

Tacrine transaminitis

Tacrine, a centrally acting anticholinesterase introduced for the treatment of Alzheimer's disease, has been shown to have an adverse effect on liver function tests [104]. Two notable features of the transaminitis are (i) that it occurs in only 20–50% of patients, and (ii) that in a large number of patients it is reversible despite continued treatment or repeated exposure.

In vitro experiments, using human liver microsomes, have shown that tacrine undergoes metabolic activation to chemically reactive and cytotoxic quinone-methide metabolites [105]. The hepatic metabolism of tacrine, including bioactivation, is catalysed exclusively by CYP1A2 [106] which may offer an explanation for individual susceptibility, since there is wide individual variation in expression of this highly inducible enzyme.

It has been suggested that the hepatic injury associated with tacrine is immune-mediated and that the reduction in susceptibility on its continued administration may represent some form of immune tolerance. An alternative explanation would be that the reactive metabolite can induce the 'electrophile counter-attack' response [107] or that CYP1A may be down-regulated by reactive oxygen species, such as H₂O₂ [108]. Although glutathione conjugation can detoxify the quinone methide, there was no association between tacrine toxicity and the glutathione transferase M1 (GST M1) genotype [109].

Anticonvulsant toxicity

In common with other drugs used in the treatment of epilepsy, carbamazepine is a proven teratogen both in rodents and in man [110]. The drug is also associated with an idiosyncratic hypersensitivity syndrome which may affect a number of bodily systems, the most frequently affected being the skin and the liver. Both of these types of reaction have been attributed to metabolites rather than the parent compound, and it has been speculated that an arene oxide (epoxide) is in both cases the causative agent [11, 112].

The principal pathways of metabolism of carbamazepine

pine include glucuronidation (direct detoxication), 10,11-epoxidation followed by hydrolysis yielding a dihydrodiol (indirect detoxication) and aromatic hydroxylation [113]. In addition, direct evidence for the *in vivo* bioactivation of carbamazepine has been obtained in the rat by characterization of glutathionyl dihydro alcohols derived from arene oxide intermediates; these are excreted exclusively in bile and, therefore, have not as yet been identified as human metabolites. *In vitro* studies indicate that both 10,11-epoxidation and bioactivation are catalysed by the same enzyme, CYP3A4, which undergoes dose-dependent induction in patients undergoing treatment with carbamazepine [114]. Thus the availability of the reactive metabolite will be a function of the relative rates of glucuronidation, oxidation and bioactivation (epoxide hydrolase, glutathione transferase). It has been postulated that susceptible individuals have a genetically determined deficiency in the enzyme microsomal epoxide hydrolase [111, 115]. However, functional and molecular analysis has rendered this hypothesis untenable [116–119]. Furthermore, it has been shown that GST M1 genotype is not a major determinant of toxicity [120].

A large proportion of the patients with severe carbamazepine hypersensitivity that we have studied were receiving multiple therapy which included valproic acid. The question arises as to whether induction or inhibition may exacerbate the toxicity. Valproate, which is an inhibitor of glucuronidation [121], has a small effect on the total clearance carbamazepine but does cause a larger increase in the extent of 10,11-epoxidation [122] indicating a shift in the balance between oxidation and glucuronidation. Furthermore, in an animal model, we have found that inhibition of direct glucuronidation is associated with an increase in glutathione conjugation (bioactivation) (unpublished data). These data indicate that several factors, including induction, together with immunological factors [123] may determine individual susceptibility.

Dapsone-induced methaemoglobinaemia

Dapsone, a drug used in the treatment of leprosy, dermatitis herpetiformis and *Pneumocystis carinii* pneumonia, can cause dose-dependent methaemoglobinaemia, and occasionally, idiosyncratic white cell toxicity [124]. The methaemoglobinaemia is thought to be due to P450-mediated activation of dapsone to the hydroxylamine metabolite. The hydroxylamine is taken up by red cells where it is co-oxidised with haemoglobin to form nitroso-dapsone and methaemoglobin. The nitroso intermediate may be reduced back to the hydroxylamine by intracellular glutathione while the methaemoglobin is reduced back to haemoglobin by both NADH- and NADPH-dependent methaemoglobin reductase enzymes so that a futile cycle exists [125]. Initial studies suggested that dapsone was metabolized by CYP3A4 to the hydroxylamine [126], although more recent studies have shown that CYP2C9 and CYP2E1 are also involved [127, 128]. In the treatment of leprosy, dapsone is usually administered with rifam-

picin, a potent P450 inducer, which would be expected to increase activation to the hydroxylamine. This has, in fact, been confirmed by Occhipinti *et al.* [129] who showed that rifampicin (2.4 g day⁻¹ for 10 days) significantly increased the formation clearance of dapsone hydroxylamine by fourfold and methaemoglobinaemia by 1.7-fold.

Induction of detoxication enzymes

Dietary composition is a major determinant of cancer risk in man and in experimental animals. Prochaska *et al.* [130] have developed mutant hepatoma cell lines which can distinguish selective (monofunctional) inducers of protective phase II enzymes from (bifunctional) inducers that also increase CYP enzymes and thereby pose the risk of carcinogen activation. Consumption of vegetables, especially crucifers, reduces the risk of developing cancer. Sulforaphane, which has been isolated from broccoli, is a potent inducer of quinone reductase and glutathione transferase in several mouse tissues [131].

Oltipraz (4-methyl-5-pyrazinyl-3H-1, 2-dithiole-3-thione) and several other dithiolethiones protect against the acute toxicities of many xenobiotics and are effective inhibitors of experimental carcinogenesis. These effects are mediated in part by induction of glutathione-S-transferase, NAD(P)H: quinone reductase and glucuronyl transferase. The induction of these enzymes results from enhanced transcription and is mediated entirely through a 41 bp enhancer unit [132].

Physiological implications

Phenobarbitone treatment of rats leads to increased glucuronidation of the thyroid hormones, which is followed by functional adaptation of the thyroid gland, with respect to size and activity [133]. Subclinical depression of thyroid hormone concentrations has been reported in epileptics [134] and in patients taking rifampicin [135], although the incidence of clinical hypothyroidism in such patients is low.

Enzyme-inducing agents can also perturb the metabolism of steroids and fat-soluble vitamins. Clinically relevant changes in bone structure, caused by induction of vitamin D metabolism, have been associated with long-term anticonvulsant therapy [3].

Conclusions

Human drug-metabolizing enzymes can be induced by various exogenous agents, including a limited number of drugs, cigarette smoke, alcohol and dietary factors. Such perturbation of enzyme activity undoubtedly contributes to intra-individual and inter-individual variation in the rate and route of the metabolism of xenobiotics. This may lead to an attenuation of

pharmacological response, and dose adjustment will be required when a drug with a low therapeutic index is co-administered with a potent inducing agent of the major pathways of metabolism of the therapeutic agent.

The toxicological implications of induction will be a consequence of any change in the balance between detoxication and toxic metabolite production, and will be most apparent for isozyme-selective inducers of phase I or phase II metabolism.

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