Deuterium isotope effects in studies of drug metabolism

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Substitution of one or more carbon-bonded hydrogen atoms in a drug molecule with deuterium imposes negligible steric effects and influences physio-chemical properties minimally. Yet the resultant increased bond stability may cause dramatic changes in biological properties, particularly by retarding certain pathways of its metabolism. Allan Foster describes the potential of deuteration in modifying drug action. A specific biological action of a drug may be enhanced, reduced or prolonged; its metabolism may be diverted along a pathway which promotes formation of highly active metabolites, or avoids the formation of toxic metabolites. So why have deuterium isotope effects (DIEs) not been exploited? Cost of toxicology testing and clinical trials may underline the pharmaceutical industry's reluctance to synthesize deuterated analogues. Nonetheless, deuteration remains a promising possibility for future drug design.

The majority of drugs and xenobiotics, following administration to, or ingestion by, humans and animals, are metabolized, often rapidly and extensively[1]. Metabolism, which can occur in many organs of the body but takes place mainly in the liver, has been regarded as a defence mechanism whereby exogenous substances are converted into more polar derivatives that are excreted more rapidly than the parent compound. In the case of drugs, this defence mechanism can be counterproductive in that metabolism may limit plasma levels and half-lives and hence efficacy. Also, the usual, but not invariable, consequence of drug metabolism is deactivation, since metabolites usually have an affinity for the target (receptor, enzyme, membrane) lower than that of the parent drug, or may have properties which limit access to, and therefore interaction with, the target. Moreover, for some drugs, the metabolites have a biological activity different from that of the parent drug or may be toxic or carcinogenic.

Control of the metabolism of a drug can give information on the mode of action and the role of metabolism in the expression of biological activity. In turn, this information could indicate the importance of metabolism control as a parameter in the design of more efficacious drugs. It is in this general context that deuterium isotope effects are now considered.

Hydroxylation and the effect of deuteration

Of the wide variety of drug metabolism pathways which have been identified, perhaps the most important are those mediated by the cytochrome P-450 group of enzymes[2] (mono-oxygenases, mixed function oxidases) which are haem proteins commonly found as clusters of membrane-bound isoenzymes in the endoplasmic reticulum of many types of cell, and are particularly prevalent in liver cells. These enzymes utilize molecular oxygen and NADPH to effect, *inter alia,* the overall reaction:

$$\geqslant C\text{-}H \rightarrow \geqslant C\text{-}OH + H_2O.$$

For aromatic compounds the reaction usually involves the initial formation of an arene oxide and subsequent rearrangement into a phenol. However, for aliphatic compounds and moieties, hydrogen abstraction occurs first to give a radical $\geqslant C\cdot$ which is then hydroxylated. Deuterium isotope effects (DIEs) would be expected for this latter type of reaction.

In a reaction in which the cleavage of a C–H bond is partially or wholly rate-determining, then the reaction of the corresponding C–D analogue will be retarded because the difference in mass between hydrogen and deuterium results in the zero-point energy (lowest ground state vibrational level) for C–D being 1.2–1.5 kcal mole^{-1} lower than that of

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k_D) of the rate constants for the protium and deuterium forms defines the primary observed isotope effect. The situation for enzyme reactions is complex in that the maximal velocity of most such reactions is dependent on several rate-contributing or partially rate-limiting steps. Thus, an enzyme reaction could involve the following sequence of events:

$$E + A \rightleftharpoons EA + B \rightleftharpoons EAB \rightleftharpoons {}^*EAB \rightleftharpoons {}^*EPQ \rightleftharpoons EPQ \rightarrow P + EQ \rightarrow Q + E$$

where E is the enzyme, A and B are substrates or substrate and cofactor, P and Q are the products, and * connotes an activated complex. Northrop[3] has described a family of DIEs associated with various segments of this sequence. That associated with the *EAB ⇌ *EPQ segment is the intrinsic DIE. (^Dk) which is analogous to the DIEs for chemical reactions. Although the magnitude of ^Dk may be large (10–15) it will usually be masked to an extent depending on the character of the preceding and succeeding steps in the overall sequence shown above, and the observed DIE. ($^Dv = k_H/k_D$) may be quite low (1–5); ^Dv values in metabolism studies are usually determined from the relative rates (k_H/k_D) of disappearance of the protium and deuterium forms, or the relative rates of appearance of the corresponding metabolites. Thus, Lu *et al.*[4] found a ^Dk value of 12 for the de-*O*-ethylation of 7-ethoxycoumarin (Fig. 1. [i]) and its d_2-derivative (Fig. 1. [ii], by purified cytochrome P-450 (phenobarbital induction) and P-448 (methylcholanthrene induction) from rat liver microsomes, but ^Dv values of approximately 3.8 and 2, respectively.

The replacement of one, or a few hydrogens in an aliphatic moiety of a drug molecule by deuterium will have negligible steric consequences or influence on physico-chemical properties and hence the deployment of DIEs in drug metabolism studies is attractive. However, it should be emphasized that it is the magnitude of the observed DIE (^Dv) which will determine the extent to which a particular metabolism pathway is retarded and the consequent effect on biological activity.

There are numerous uses[5] of deuterium-labelling in drug metabolism studies other than for DIEs, and mass spectrometry is a key technique when deuterium-labelled compounds are used. For example, the change in metabolism profile caused by specific deuteration of a drug can be readily detected and

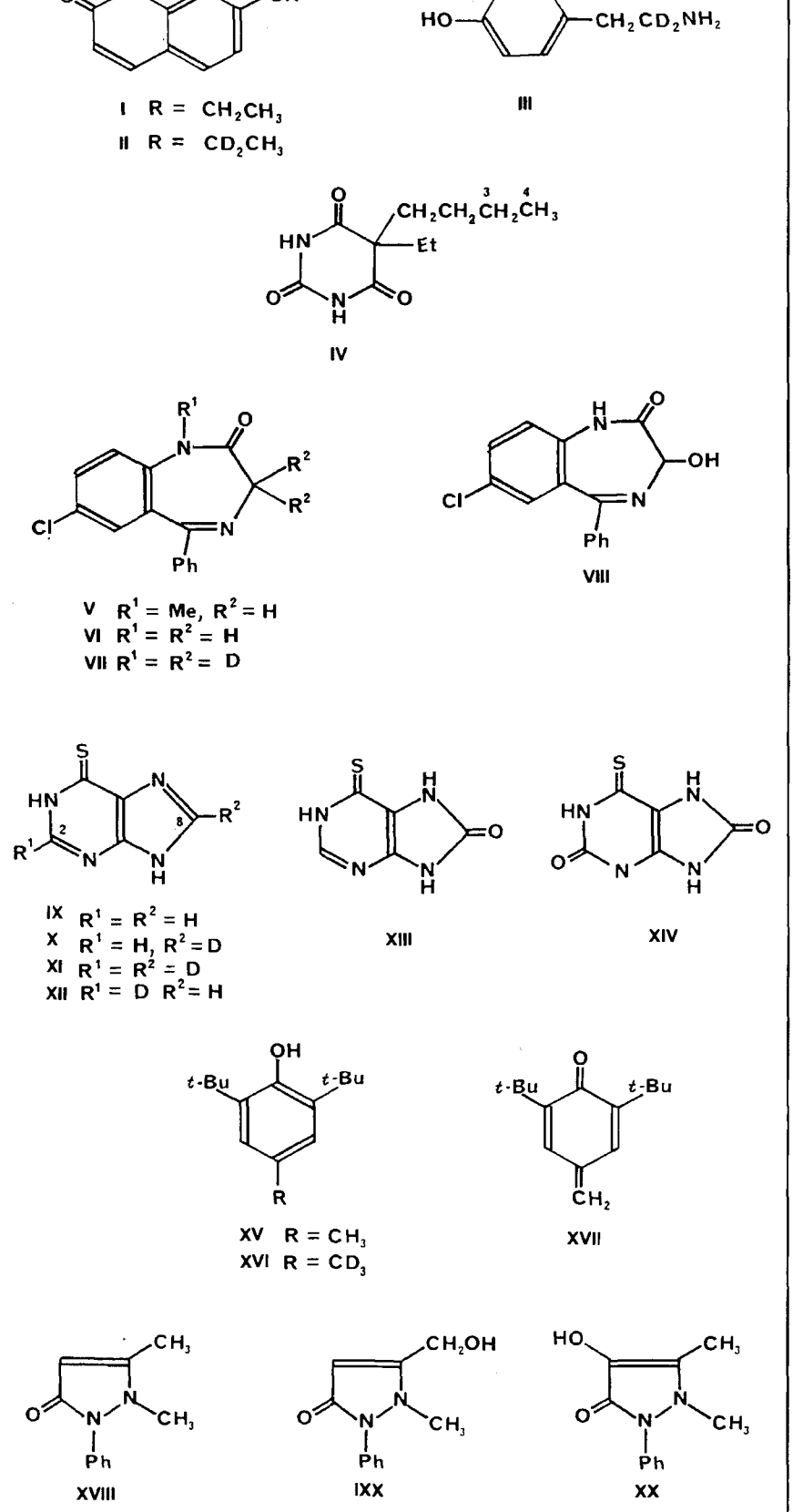


Fig. 1. Structures of drugs the metabolism of which is influenced by deuterium labelling.

or *in-vivo* metabolism.

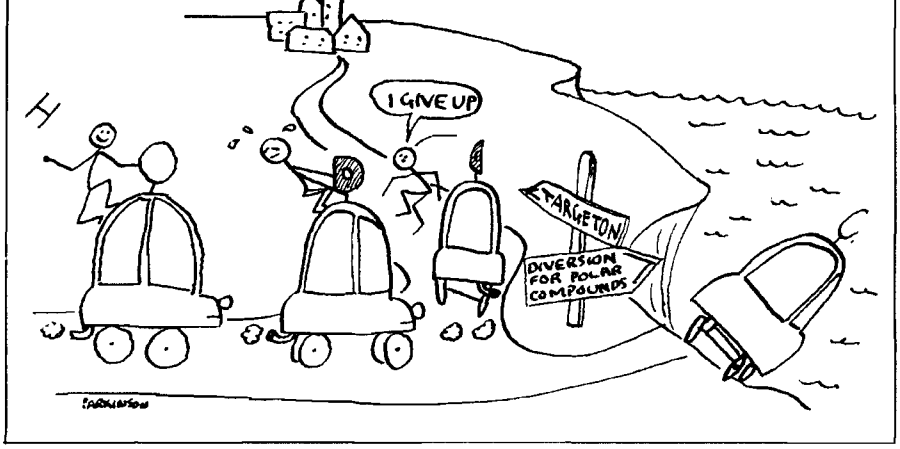
Modification of the biological activity of drugs by deuterium isotope effects

One of the earliest attempts to modify the biological properties of a drug by specific deuteration and thereby elucidate the mode of action was described by Belleau *et al.*[6]; dideuteration of the adrenergic *p*-tyramine (Fig. 1. [iii]) doubled the duration of the effect on arterial pressure and nictitating membrane contractions following i.v. administration to the cat. Subsequently, a DIE of approximately 2.4 was found for the oxidative deamination of δ_2-*p*-tyramine (Fig. 1. [iii]) with monoamine oxidase from rat liver mitochondria[7].

Barbiturates were also an early subject of study. Thus, whereas trideuteration at position 4 of the butyl group of butethal (Fig. 1. [iv]) had no effect on biological activity, dideuteration at position 3 doubled the sleep time in mice[8] because 3-hydroxylation of the butyl group, which is a major metabolism pathway, was thereby retarded. A ^{D}v of approximately 1.6 was found in *in-vitro* metabolism experiments.

The foregoing examples, which showed that relatively small DIEs could modify the *in-vivo* properties of a drug, illustrate the enhancement or prolongation of drug activity by specific deuteration. The opposite effect, namely reduction in the duration of drug action has also been reported[9]. *N*-Desmethyldiazepam (Fig. 1. [vi]) is the major metabolite of diazepam (Fig. 1. [v]) and is further metabolized by 3-hydroxylation to give oxepam (Fig. 1. [viii]) which accumulates in the brains of diazepam-treated mice and is responsible for the prolonged anticonvulsant action of the drug. Dideuteration at position 3 (Fig. 1. [vii]) reduced the duration of the anticonvulsant action from 20→5 hours and *in-vitro* experiments using mouse liver microsomes revealed an approximately 7.5-fold reduction in the extent of 3-hydroxylation.

The use of specific deuteration in an attempt to retard the formation of toxic products of metabolism can be illustrated with the anaesthetic methoxyflurane ($CH_3OCF_2CHCl_2$) which is metabolized by two pathways involving hydroxylation of the methyl and dichloromethyl groups. The former pathway gives $CHCl_2COOH$ and releases fluoride ion which can cause renal dysfunction. McCarty *et al.*[10] found a 33% decrease in the urinary excretion of fluoride for perdeuterated methoxy-



flurane ($CD_3OCF_2CDCl_2$), but the serum fluoride levels and renal dysfunction after anaesthesia of rats for 2 h were still unacceptable[11].

The occurrence of a DIE in an *in-vitro* system does not necessarily mean that biological properties will be altered *in vivo*. A major metabolic detoxification pathway for the antitumour agent 6-mercaptopurine (Fig. 1. [ix]) is via 8-hydroxy-6-thiopurine (Fig. 1. [xiii]) to thiouric acid (Fig. 1. [xiv]) thought to be mediated by xanthine oxidase. A significant DIE (3.5) was found for the action of this enzyme *in vitro* on 8-d_1-6-mercaptopurine (Fig. 1. [x]) but not for the 2-d_1-derivative (Fig. 1. [xii]) suggesting (see above) a different balance of rate-limiting and/or partially rate-determining steps[12]. A DIE of 3.8 was found for the 2,8-d_2-6-mercaptopurine (Fig. 1. [xi]). These DIEs were not fully reflected in *in-vivo* experiments. Thus, following i.p. administration of the 2,8-d_2-derivative to rats, 2.2–3.7 times as much unchanged drug was excreted in the urine and 54–70% of thiouric acid in comparison with 6-mercaptopurine (Fig. 1. [ix]) reflecting retardation of the detoxification metabolism pathway in the dideuterated compound. However, although the potency of the 2,8-d_2-6-mercaptopurine (Fig. 1. [xi]) against the adenocarcinoma Ca755 in mice was increased 3–5-fold, the 8-d_1-derivative (Fig. 1. [x]), which also had a significant DIE *in vitro*, had the same potency as 6-mercaptopurine.

Most *in-vivo* studies of DIEs in drug metabolism have involved analysis of urine for metabolites and unchanged drug. An alternative approach was used recently[13] in a study of butylated hydroxytoluene (BHT; Fig. 1. [xv]). This compound, which is a widely used antioxidant, causes lung damage in mice and the covalent binding in lung tissue is probably mediated by the reactive quinone methide metabolite (BHT-QM; Fig. 1. [xvii]). Trideuteration of the methyl group in BHT (Fig. 1. [xvi]) reduced the pulmonary toxicity and markedly reduced the level of quinone methide in lung tissue thereby reflecting the DIE observed in *in-vitro* experiments.

Metabolic switching

When a drug is metabolized by two or more alternative pathways, a possible consequence of deuteration is metabolic switching, i.e. suppression of one pathway and accentuation of an alternative pathway. The term was introduced by Horning *et al.*[14] who found that the metabolism of antipyrine (Fig. 1. [xviii]), after i.p. injection into rats, and as reflected by the urinary metabolites, was switched from oxidation of the C-3 methyl group (Fig. 1. [xix]; normal major pathway) to de-*N*-methylation (normal minor pathway) on trideuteration of the former group. An even more marked effect was observed *in vitro*. Using the 10 000 g supernatant of homogenized rat liver, the ratio of the products of hydroxylation of the C-3 methyl group (Fig. 1. [xix]) and position 4 (Fig. 1. [xx]) was only slightly changed (1.3–1.6) when the *N*-methyl group was trideuterated, but dramatically (to < 0.1) when the C-3-methyl group was trideuterated.

Numerous other examples of metabolic switching of cytochrome P-450-mediated reactions as a result of DIEs have been reported (although not always recognized) in the past decade.

Conclusions and future trends

In their excellent 1975 review, Blake *et al.*[15] commented, 'At the present time, there are no drugs on the market that contain deuterium in the molecule . . . for use in humans or for products such as insecticides, the advantages to be gained by specific or general deuteration in modifying biological activity and/or duration of action must significantly outweigh the additional costs associated with the synthesis of deuterated analogues. For drugs intended for use in humans there will be a substantial additional cost, namely, that associated with preclinical toxicology and clinical trials. It seems very unlikely that the regulatory authorities associated with the pharmaceutical industry would regard a deuterated drug designed to have a biological activity significantly different from that of the parent protium form as other than a new drug.

The examples noted above, which represent a not unreasonable cross-section, show that the *in-vivo* biological activity of a drug can be modified as a consequence of altering the metabolism profile through a DIE but none of the *in-vivo* effects reported in the literature to date can be regarded as truly dramatic. However, the end of the story may not yet have been reached. Lu *et al.*[4] reported recently that, whereas the DIE for the de-*O*-ethylation of d_2-7-ethoxycoumarin (Fig. 1. [ii]) by liver microsomes from phenobarbital-treated rats was ~3, a much larger value (~6) was found for human liver microsomes. This, apparently, is the first report of the use of human liver microsomes in a study of DIEs in drug metabolism and is of potential importance for evaluating the real scope for using a DIE to influence metabolism, since DIEs associated with animal liver microsomes, apart from possible species differences, may be misleadingly small. Further, since microsomes are fragments of the endoplasmic reticulum, usually of liver cells, the metabolism they mediate may be quantitatively, if not qualitatively, different from that which occurs in intact hepatocytes. The use of animal, especially rodent, hepatocytes in drug metabolism studies is well established and it has now been shown[16] that human hepatocytes, when co-cultured with rat epithelial cells, retain their full metabolizing capability for many hours and can be deployed in drug metabolism studies. It will be of interest to see what use can be made of this new type of metabolizing system for evaluating DIEs and metabolic switching as parameters in drug design.

The phenomenon of metabolic switching is likely to be of growing interest in the future. The example noted above

types of structural change could be used. The deliberate deployment of metabolic switching as a parameter in drug design remains to be explored. Two promising areas involve the accentuation of the metabolism pathways which generate the active metabolites from prodrugs and, where overall metabolism cannot be retarded, the deflection of metabolism away from pathways leading to metabolites with toxic properties or other undesirable biological activity towards innocuous pathways.

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Transynaptic mechanisms in the action of antidepressant drugs

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Antidepressant drugs act on different neuronal systems and pre- and postsynaptic sites. Integrated transynaptic events are considered to be involved in those adaptive changes which seem to be operative after a prolonged administration. The authors explain how these long-term effects, rather than the acute pharmacological actions, are most likely to represent the biochemical mechanism underlying the delayed onset of antidepressant therapeutic efficacy. Among the possible mechanisms responsible for the adaptation of central aminergic neurons, interactions between serotonergic and noradrenergic systems, chemico-physical properties of the membranes and the modulatory actions of hormones and cotransmitters are considered.

It is now generally accepted that the mechanism of action of antidepressant drugs cannot be attributed to their acute pharmacological actions since agents possessing many of these effects lack antidepressant activity. Antidepressant therapy is associated with a lag phase of 15–20 days before the onset of a beneficial activity. Thus, in order to elucidate the mode of action of antidepressants, only those effects elicited after long-term treatment should be considered.

Pre- and postsynaptic biochemical modifications induced by long-term antidepressant treatment

Chronic antidepressant administration is associated with a number of adaptive changes in central monoaminergic systems which can occur both pre- and post-synaptically. The findings reported below summarize the most significant data to appear in the literature in the last few years. (For detailed information see Refs 1–4.)

Radioligand binding studies have revealed that chronic administration of antidepressants produces changes in sensitivity of presynaptic α-adrenoceptors and a decrease in the number of [^{3}H]imipramine ([^{3}H]IMI) sites located presynaptically on serotonergic (5HT) terminals. Central noradrenergic transmission is reduced by chronic antidepressants as indicated by (1) a decrease in the activity of tyrosine hydroxylase; (2) a reduction in the firing rate of noradrenergic cell bodies in the locus coerules; (3) lower cortical levels of normetanephrine (NMN), the *O*-methylated metabolite or norepinephrine (NE); (4) a reduction in 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG) content. However increase or no major changes have been also reported on this NE metabolite. This apparent discrepancy may be due to several factors such as the different methodology used in the measurement of NE turnover, the administration of antidepressants with multiple sites of action, non comparable time of treatment and washout period, different brain areas investigated.

Effects elicited by long-term antidepressant administration at the postsynaptic level include changes in the