CHIRALITY AND PHARMACOKINETICS: AN AREA OF NEGLECTED DIMENSIONALITY?[#]

Andrew J. Hutt*

Department of Pharmacy, King's College London, UK

SUMMARY

Drug stereochemistry has, until relatively recently, been an area of neglected dimensionality with the development of the majority of synthetic chiral drugs as racemates. This situation has changed in recent years as a result of advances in the chemical technologies associated with the synthesis, analysis and preparative scale resolution of the enantiomers of chiral molecules. As a result of the application of these technologies the potential significance of the differential pharmacodynamic and pharmacokinetic properties of the enantiomers present in a racemate have become appreciated.

Many of the processes involved in drug disposition, i.e. absorption, distribution, metabolism and excretion, involve a direct interaction with chiral biological macromolecules, e.g. transporters, membrane lipids and enzymes, and following administration of a racemate the individual enantiomers frequently exhibit different pharmacokinetic profiles and rarely exist in a 1:1 ratio in biological fluids. The magnitude of the differences between a pair of enantiomers observed in their pharmacokinetic parameters tends to be relatively modest in comparison to their pharmacodynamic properties. However, the

[#] Based on a presentation at the 57th Indian Pharmaceutical Congress, Hyderabad, December 2005.

* Author for correspondence:
Andrew J. Hutt
Department of Pharmacy
King's College London
Franklin-Wilkins Building
150 Stamford Street
London, SE1 9NH, UK
e-mail: andrew.hutt@kcl.ac.uk

observed stereoselectivity may be either amplified or attenuated depending on the organisational level, e.g. whole body, organ or macromolecular, the particular parameter represents. Differences in parameters involving a direct interaction between a drug enantiomer and a biological macromolecule, e.g. intrinsic metabolite formation clearance and fraction unbound, tend to be largest, and comparison of parameters reflecting the whole body level of organisation, e.g. half-life, clearance, volume of distribution, may well mask significant stereoselectivity at the macromolecular level.

In spite of the recent interest in drug chirality relatively limited pharmacokinetic data are available for the enantiomers of a number of commonly used racemic drugs. Factors influencing the stereoselectivity of drug disposition include: formulation and route of administration; *in vivo* stereochemical stability, both chemical and enzymatic; drug interactions, both enantiomeric and with a second drug; disease state; age; gender; race; and pharmacogenetics. As a result of such factors estimation of pharmacokinetic parameters, development of complex pharmacokinetic models and plasma-concentration-effect relationships based on 'total' drug concentrations following administration of a racemate are of limited value and potentially useless.

KEY WORDS

drug chirality, racemic drugs, pharmacokinetic parameters, factors influencing stereoselective drug disposition

INTRODUCTION

Drug stereochemistry has, until relatively recently, been an area of neglected dimensionality with the development of the majority of synthetic chiral drugs as racemic mixtures, which by the 1980s accounted for approximately 25% of all marketed pharmaceuticals /1/. This situation has changed in recent years as a result of advances in the chemical technologies associated with the synthesis, analysis and preparative scale resolution of the enantiomers of chiral molecules (see for example /2-7/). As a result of the application of these technologies the potential significance of the differential pharmaco-

dynamic and pharmacokinetic properties of the enantiomers present in a racemate have become appreciated, particularly with respect to safety issues /8/, by the regulatory authorities /9/, and in some instances exploited, by the pharmaceutical industry.

Stereoisomers are compounds which differ in the three dimensional spatial arrangement of their constituent atoms and may be divided into two groups, enantiomers and diastereoisomers. Enantiomers are stereoisomers which are non-superimposable mirror images of one another and are therefore pairs of compounds related as an object to its mirror image in the same way that the left and right hands are related. Such molecules are said to be chiral from the Greek *chiros* meaning handed. In terms of the compounds of interest in pharmacology the most frequent, but not the only, cause of chirality results from the presence of a tetraco-ordinate carbon atom in a molecule to which four different atoms or groups are attached (Fig. 1). The presence of one such stereogenic centre in a molecule gives rise to a pair of enantiomers, the presence of n such different centres yields 2^n stereoisomers and half that number of pairs of enantiomers. Those stereoisomers which are not enantiomeric, i.e. not mirror image related, are said to be diastereomeric. These two types of stereoisomer differ in that other than the direction of rotation of the plane of plane polarized light enantiomers are identical in their physicochemical properties, whereas pairs of diastereoisomers differ in their physical properties. As a result of their identical properties the separation, or resolution, of a pair of enantiomers was until recently fairly difficult, whereas diastereoisomers may, in principle at least, be separated relatively easily.

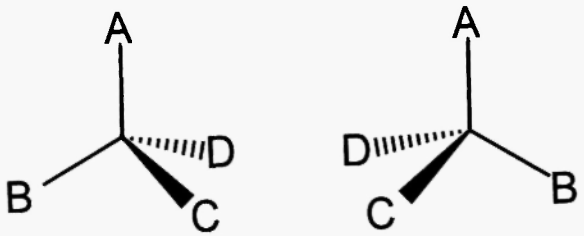

Fig. 1: Three dimensional representation of a pair of enantiomers.

BIOLOGICAL DISCRIMINATION OF STEREOISOMERS

At a molecular level biological environments are highly chiral being composed of 'handed' biopolymers, such as proteins, glycolipids and polynucleotides, formed from the chiral building blocks of nature, i.e. L-amino acids and D-carbohydrates. The macromolecular structures of these biopolymers may also exhibit chirality as a result of helicity, helical structures having either a left or right handed turn, in the same way that a spiral staircase, or screw thread, may have a left or right handed turn. For example, the DNA double-helix and the protein α-helix both have a right handed twist.

As nature has expressed a preference in terms of its stereochemistry it is hardly surprising that drug targets, be they enzymes, receptors or ion channels, exhibit stereoselectivity with respect to drug action, particularly as many of the natural substrates and ligands for such systems, e.g. neurotransmitters, hormones, endogenous opioids, etc., are single stereoisomers. In addition, many of the processes involved in drug disposition, i.e. absorption, distribution, metabolism and excretion, involve a direct interaction with chiral biological macromolecules, e.g. transporters, membrane lipids, plasma proteins and enzymes, and thus, following administration of a racemate the individual enantiomers present frequently exhibit different pharmacokinetic profiles and rarely exist in the 1:1 racemic ratio in biological fluids.

That enantiomers could exhibit different biological activities was established at the turn of the last century. In 1886 Piutti reported that the tastes of (+)- and (-)-asparagines were sweet and bland, respectively, and this observation is regarded by some as the first report of stereoselectivity with respect to a receptor /10/. Between 1904 and 1909 the British pharmacologist Cushny demonstrated that (-)-hyoscyamine had twice the activity of atropine (racemic hyoscyamine); that (-)-adrenaline had twice the potency of the racemate as a vasoconstrictor, and that the (-)-enantiomer was 12- to 15-fold more potent than the (+)-isomer with respect to their action on sympathetic vessels /11/. With respect to metabolism, Pasteur had shown in 1858 that the mould *Penicillum glaucum* could utilize the (+)- but not the (-)-enantiomer of tartaric acid as a carbon source, and Fischer, in 1894, demonstrated that maltose and emulsin exhibited differential hydrolysis on the diastereomeric α- and β-methyl-D-glucosides, but had no

activity with respect to their corresponding L-enantiomers /12/. Between 1886 and 1909 a number of reports appeared indicating the preferential "digestion and destruction" of one of a pair of enantiomers, using a variety of crude enzyme preparations and mainly racemic amino acids and synthetic peptides as substrates /11/. In addition to the above *in vitro* studies the concept that the stereoselectivity of drug action could be related to stereoselectivity in metabolism started to emerge. For example, Cushny reported that (-)- and (+)-hyosine appeared to be equivalent in some actions except that the (+)-enantiomer maintains its action longer, probably due to being excreted more slowly. Similarly Gottlieb reported the slightly greater potency of (-)-cocaine in "depressing nerve fibres" and that the (+)-enantiomer was more rapidly "destroyed", an excess of (-)-cocaine being excreted in urine. Such was the development of these ideas that Cushny in 1926, in what was probably the first book concerned with stereochemistry and pharmacology, *Biological Relations of Optically Isomeric Substances*, commented that "difference in action of optical isomers is due to less of one than the other reaching the site of action owing to different amounts being intercepted on the way" /11/.

In order to rationalise the observed differences in pharmacodynamic activity between enantiomers Easson and Stedman in 1933 /13/ proposed their so-called "three point-fit" model for the drug receptor interaction (Fig. 2). According to this model the more potent enantiomer is involved with a minimum of three intermolecular interactions with complementary sites on the receptor, whereas the less potent enantiomer may interact at two sites only. Ogston /14/ proposed a similar three point attachment model to rationalize the observed stereoselectivity in the enzymatic transformation of symmetrical prochiral substrates in 1948. The Ogston /14/ and Easson and Stedman /13/ models are essentially 'static' models based on the assumption that the 'selectand' (the drug or substrate) may approach the 'selector' macromolecule (the biological target, receptor, enzyme) from one direction, i.e. the substrate has essentially restricted access to the 'active' site. More recently these concepts have been extended to a more dynamic model, the so-called 'rocking tetrahedron' model, by Sokolov and Zefirov /15/. In this model, which is primarily associated with the metabolism of prochiral substrates to yield chiral products, the substrate binds to the biological target via a two site interaction and the conformational flexibility of the enantiotopic groups in

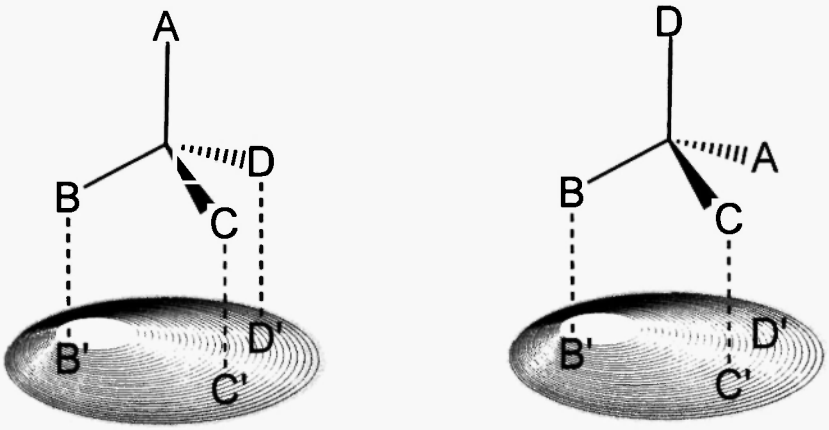

Fig. 2: Easson-Stedman model of chiral discrimination on interaction of a pair of enantiomers with a biological macromolecule /13/. The enantiomer on the left takes part in three complementary interactions with the biological target whereas that on the right interacts at two sites only. Alternative orientations of the enantiomer on the right to the target surface are possible but only two interactions may take place at one time.

relation to the enzyme catalytic site is the determinant of the stereoselectivity of the observed transformation (Fig. 3). The lower the conformational flexibility of the bound substrate, due for example to the presence of bulky enantiotopic groups, the greater the degree of stereoselectivity observed in product formation. In contrast, increased flexibility of the enantiotopic groups results in a reduction of stereoselectivity with respect to the product /15/.

The concepts associated with this model may be illustrated by a consideration of the metabolic oxidation of the prochiral substrates cumene /16/ and methoxychlor /17/ (Fig. 4). Examination of the metabolism of these compounds, using appropriately isotopically labelled chiral substrates, indicated that the observed product stereoselectivity was essentially due to the chirality of the enzyme active site. In the case of cumene the ω-oxidation of the prochiral methyl groups to yield the enantiomers of 2-phenylpropanol was concluded to be due to a small degree of incomplete equilibrium between alternative orientations of the substrate in the enzyme-substrate complex /16/. In contrast, the stereoselective demethylation of the prochiral methoxy groups in methoxychlor arises as a result of reduced conformational

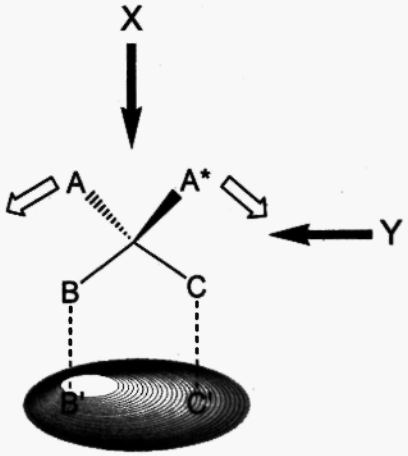

Fig. 3: The 'rocking tetrahedron' model of Sokolov and Zefirov /15/. The prochiral substrate interacts with the biological target at two sites (B-----B' and C-----C'), the enantiotopic groups A and A* occupy overlapping volumes and have a degree of conformational flexibility (indicated by the open arrows in the figure). The stereoselectivity of the transformation depends upon the orientation of the enantiotopic groups, A and A*, to the catalytic site and their conformational flexibility in the enzyme-substrate complex, reaction from sites X or Y being of potentially low and high stereoselectivity respectively.

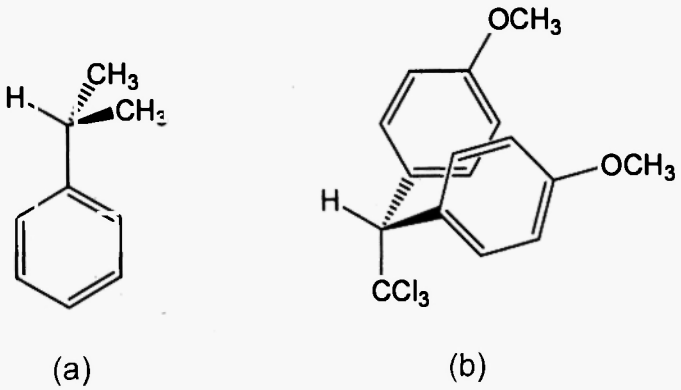

Fig. 4: Structures of the prochiral substrates cumene (a) and methoxychlor (b) /16,17/.

flexibility, due to slow interchange of the bulky methoxyphenyl groups, and a large degree of incomplete equilibrium in the enzyme-substrate complex /17/.

The three point attachment model of Easson and Stedman /13/ has also been challenged by recent studies by Mesecar and Koshland /18/ during investigations on the enzyme isocitrate dehydrogenase. Examination of X-ray structures of the complexes formed between the enzyme and D- and L-isocitrate, in the presence and absence of magnesium ions, revealed three common binding sites for both isocitrate enantiomers, and a fourth site was required to account for the observed selectivity.

From the above it is apparent that chiral recognition remains an area of considerable scientific interest /19/. Current ideas concerning chiral recognition involve complex formation between the biological macromolecule, the selector, and the substrate or selectand, such that there is a diastereoisomeric relationship between the selector and the two possible selectands /19/. However, formation of a diastereoisomeric complex alone is not sufficient for chiral recognition. An additional requirement of interactions between eight centres, i.e. four bonding interactions, is required in the absence of other constraints, such as a restriction in the direction of approach of the selectand to the selector, where six interactive sites, a three point attachment, may be sufficient /20,21/, i.e. the Easson-Stedman /13/ and Ogston /14/ models may be regarded as specific cases of the four location model.

PHARMACODYNAMIC CONSIDERATIONS

A number of possible scenarios may arise with respect to the pharmacodynamic properties of a pair of enantiomers, and a brief summary is presented in Table 1. There are relatively few examples of drugs in which the pharmacodynamic activity is restricted to a single enantiomer with the other being totally devoid of activity. In the majority of cases a pair of enantiomers will differ either quantitatively or qualitatively in terms of their activity. Similarly there are few examples where the beneficial activity resides in a single enantiomer and the adverse effects, or toxicity, are associated with the other.

The differential pharmacodynamic activity of drug stereoisomers has given rise to additional terminology, thus the stereoisomer with the greater affinity or activity is termed the *eutomer* and that with the

TABLE 1

Stereoisomerism and pharmacodynamic complexities

Activity resides in a single enantiomer:

The antihypertensive (*S*)-α-methyldopa /67/; the ACE inhibitor imidapril, the *S,S,S*-stereoisomer being greater than 10^6-fold more potent than its enantiomer /68/.

Both enantiomers have similar pharmacodynamic profiles:

The antihistamine promethazine; the antiarrhythmic flecainide.

Both enantiomers are marketed with different therapeutic indications:

Dextropropoxyphene, an analgesic and the antitussive levopropoxyphene; dextromethorphan, antitussive and levomethorphan, opioid analgesic.

Enantiomers have opposite effects at the same biological target:

The (+)-(3*S*,4*R*)- and (-)-(3*R*,4*S*)-enantiomers of picenadol exhibit agonist and antagonist activity at the μ-opioid receptor, respectively, the racemate being a partial agonist /69/; (*R*)- and (*S*)-sopromidine have agonist and antagonist properties at H_2-receptors, the racemate being a partial agonist /70/.

One enantiomer antagonizes the adverse effects of the other:

(*R*)-Indacrinone, a loop diuretic, causes elevation of uric acid, whereas the *S*-enantiomer is uricosuric /71,72/.

Required activity resides in one or both enantiomers, the adverse effects being predominantly associated with one of them:

(*S*)-Ketamine is between 2- and 4-fold more potent than the *R*-enantiomer as an anaesthetic/analgesic agent, the post anaesthesia emergence reactions (hallucinations, vivid dreams, agitation) are predominantly associated with (*R*)-ketamine; the *S*-enantiomer has been marketed in Germany /73/. The antitubercular drug ethambutol exists in three stereoisomeric forms, a pair of enantiomers and a *meso* form, the activity of the (+)-*S,S*-enantiomer being 500- and 12- fold greater than that of the (-)- and *meso* forms, respectively; use of the racemic mixture resulted in ocular neuropathy, the three stereoisomeric forms being essentially equipotent; use of (+)-(*S,S*)-ethambutol resulted in an improved risk/benefit ratio /74/.

A racemic mixture provides a superior therapeutic effect than either enantiomer individually:

The sympathomimetic agent dobutamine increases the force of myocardial contraction with no increase in heart rate or blood pressure, the peripheral vasodilator ((+)-enantiomer) and vasoconstrictor ((-)-enantiomer) effects cancel out /75/.

lower affinity or activity the *distomer* /22/. The ratio of the two activities, a measure of the stereoselectivity of the system under examination, is termed the *eudismic ratio*, the magnitude of which varies; values of 100- to 1000-fold are not uncommon, with the compound under investigation, and between receptor systems for compounds which interact with more than one receptor. The eutomer/ distomer designations of stereoisomers refer to a single biological activity of the drug, and for drugs which act at multiple sites the eutomer for one activity may be the distomer for the other, or the isomers may be equipotent.

PHARMACOKINETIC CONSIDERATIONS

Stereoselectivity in drug disposition, as pointed out above, has been appreciated for a number of years and is of particular significance for those processes which depend on a direct interaction between enantiomers and a chiral macromolecule, i.e. an enzyme during metabolism or a transporter system during absorption, distribution and excretion.

Absorption

Enantioselectivity has little impact on passive processes that are dependent on the physicochemical properties of a molecule (i.e. pK_a, lipid solubility, molecular size), such as diffusion across biological membranes, the major mechanism associated with absorption, distribution and renal excretion. In contrast differentiation may occur between diastereoisomers as a result of their differential solubility. For example, the aqueous solubility of D-ampicillin is greater than that of the L-diastereoisomer /23/. However, stereoselectivity in drug absorption has been observed for compounds which are substrates for carrier systems, e.g. the L-enantiomers of Dopa /24/ and methotrexate /25,26/ and the L-epimer of the cephalosporin, cephalexin, are preferentially absorbed in comparison to their D-stereoisomers /27/. In the case of the latter compound the L-epimer is also more susceptible to enzyme mediated hydrolysis such that only D-cephalexin can be detected in plasma /27/.

P-glycoprotein mediated efflux of (*S*)-talinolol has been suggested to account for the lower plasma concentrations of the *S*- compared to

the *R*-enantiomer /28/. An alternative explanation, based on slight stereoselective cytochrome P450 (CYP) 3A4 mediated metabolism, has been proposed for the difference in talinolol enantiomer disposition following oral administration /29/.

Distribution

Drug binding to plasma proteins exhibits stereoselectivity (Table 2) and thus the composition of the free fraction influences tissue distribution. Enantioselectivity in binding may also vary with the protein, for example the enantioselectivity of propranolol binding is *S*>*R* for α_1-acid glycoprotein (AGP) and *R*>*S* for human serum albumin (HSA). In whole plasma the binding to AGP predominates and the free fraction of (*R*)-propranolol exceeds that of the *S*-enantiomer /30/. In addition drug protein binding may also give rise to enantiomeric interactions and influence drug disposition. Following administration of the individual enantiomers of disopyramide there are no differences in the pharmacokinetic parameters, whereas following administration of the racemate the *S*-enantiomer has a lower total and renal clearance, volume of distribution and shorter half-life compared to (*R*)-disopyramide /31/. These differences arise due to enantioselective, concentration dependent interactions in plasma protein binding.

Drug-lipid interactions have also been reported to influence the distribution of both acidic and basic drugs. For example, recent evidence has indicated that some basic compounds, e.g. verapamil and disopyramide, preferentially accumulate in tissues with a high content of phosphatidylserine, an acidic phospholipid, which may be stereoselective /32/. Drug distribution may also be associated with metabolism. The *R*-enantiomers of a number of 2-arylpropionic acid non-steroidal anti-inflammatory drugs (NSAIDs) undergo chiral inversion to yield their cyclooxygenase inhibiting *S*-enantiomers /33,34/. The initial step in the reaction sequence involves the formation of an acyl-coenzyme-A (acyl-CoA) intermediate which is stereoselective for the (*R*)-2-arylpropionic acids, which then undergo epimerization of the profen moiety to yield a mixture of both the *R*- and *S*-epimers of the acyl-CoA derivatives. Hydrolysis of these epimers results in the liberation of the free acids (Fig. 5). Acyl transfer of the profen moiety from the acyl-CoA to glycerol may also occur *in vivo* and *in vitro*, e.g. ibuprofen /35/ and fenoprofen /36/, and results in the formation of

TABLE 2

Stereoselectivity in pharmacokinetic parameters following administration of racemic drugs to man

Drug	Route of administration	Enantiomer	Clearance	Renal clearance	Volume of distribution	Half-life (h)	Fraction unbound (%)
Bupivacaine	IV	*R*	0.40 l.min^{-1}	-	84 l	3.5	6.6
		S	0.32 l.min^{-1}	-	54 l	2.6	4.5
		*R**	7.3 l.min^{-1}	-	1576 l	-	-
		*S**	8.7 l.min^{-1}	-	1498 l	-	-
Carvedilol	oral	*R*	0.8 / l.min^{-1}	-	302 l	5.3	0.45
		S	1.26 l.min^{-1}	-	487 l	5.1	0.63
Etodolac	oral	*R*	22 ml.h^{-1}.kg^{-1}	-	0.21 l.kg^{-1}	6.6	0.47
		S	288 ml.h^{-1}.kg^{-1}	-	1.6 l.kg^{-1}	4.3	0.85
Ifosfamide	IVI	*R*	0.060 l.h^{-1}.kg^{-1}	-	0.61 l.kg^{-1}	7.1	-
		S	0.072 l.h^{-1}.kg^{-1}	-	0.63 l.kg^{-1}	6.0	-
Hexobarbitone	oral	*R*	136 l.h^{-1}	1.47 l.h^{-1}	-	6.7	-
		S	21 l.h^{-1}	0.13 l.h^{-1}	-	2.8	-
Ketorolac	IM	*R*	19.0 ml.h^{-1}.kg^{-1}	-	0.075 l.kg^{-1}	3.6	-
		S	45.9 ml.h^{-1}.kg^{-1}	-	0.135 l.kg^{-1}	2.4	-
Mephobarbitone	oral	*R*	170 l.h^{-1}	-	716 l	3.1	36.5
		S	1.5 l.h^{-1}	-	105 l	50.5	43.9
Metoprolol	oral	*R*	1.7 l.h^{-1}.kg^{-1}	74.8 ml.min^{-1}	7.6 l.kg^{-1}	2.7	-
		S	1.2 l.h^{-1}.kg^{-1}	69.7 ml.min^{-1}	5.5 l.kg^{-1}	3.0	-

Drug	Route of administration	Enantiomer	Clearance	Renal clearance	Volume of distribution	Half-life (h)	Fraction unbound (%)
Mexiletine	Oral	*R*	8.6 ml.min^{-1}.kg^{-1}	0.61 ml.min^{-1}.kg^{-1}	6.6 l.kg^{-1}	9.1	19.8
		S	8.1 ml.min^{-1}.kg^{-1}	0.72 ml.min^{-1}.kg^{-1}	7.3 l.kg^{-1}	11.0	28.3
	Oral	*R*	7.9 ml.min^{-1}.kg^{-1}	-	5.3 l.kg^{-1}	8.1	-
		S	8.8 ml.min^{-1}.kg^{-1}	-	6.0 l.kg^{-1}	8.4	-
Nitrendipine	IV	*R*	1.6 l.min^{-1}	-	3.7 l.kg^{-1}	4.0	-
		S	1.5 l.min^{-1}	-	3.9 l.kg^{-1}	4.3	-
	Oral	*R*	6.6 l.min^{-1}	-	-	7.5	-
		S	3.1 l.min^{-1}	-	-	7.7	-
Nivaldipine	Oral	*R*	110 ml.min^{-1}.kg^{-1}	-	-	2.1	-
		S	39.5ml.min^{-1}.kg^{-1}	-	-	1.5	-
Prenylamine	Oral	*R*	4.0 l.min^{-1}	1.3 ml.min^{-1}	-	8.2	-
		S	20.5 l.min^{-1}	4.0 ml.min^{-1}	-	24	-
Propranolol	IV	*R*	1.21 l.min^{-1}	-	4.82 l.kg^{-1}	3.5	-
		S	1.03 l.min^{-1}	-	4.08 l.kg^{-1}	3.6	-
	Oral	*R*	6.9 l.min^{-1}	-	-	4.3	-
		S	4.6 l.min^{-1}	-	-	4.8	-
Reboxetine	IV	*R,R*	0.027 l.h^{-1}.kg^{-1}	-	0.39 l.kg^{-1}	10.6	-
		S,S	0.071 l.h^{-1}.kg^{-1}	-	0.92 l.kg^{-1}	9.42	-
	Oral	*R,R*	41.6 ml.min^{-1}	-	50.9 l	14.8	-
		S,S	99.3 ml.min^{-1}	-	114 l	14.4	-

Table 2 con inued

Drug	Route of administration	Enantiomer	Clearance	Renal clea ance	Volume of distribuiion	Half-l fe (h)	Fr ction unbound (%)
Salbutamol	IV	*R*	0.62 l·h^{-1}·kg^{-1}	-	2.0 l·kg^{-1}	2 0	-
		S	0.39 l·h^{-1}·kg^{-1}	-	1.8 l·kg^{-1}	2 9	-
	Oral	*R*	0.17 l·h^{-1}·kg^{-1}	-	-	-	-
		S	0.19 l·h^{-1}·kg^{-1}	-	-	-	-
	IV	*R*	46.8 l·h^{-1}	-	-	2.5	-
		S	14.7 l·h^{-1}	-	-	4.7	-
	Ora.	*R*	-	-	-	2 9	-
		S	-	-	-	6 0	-
	Inhala ion	*R*	-	-	-	2 0	-
		S	-	-	-	4.5	-
Sotalol	Ora	*R*	12.4 l·h^{-1}	-	2 0 l·kg^{-1}	7.9	-
		S	11.7 l·h^{-1}	-	2 0 l·kg^{-1}	8 2	-
Terodiline	Ora	*R*	-	-	391 l	98	-
		S	59 ml·h^{-1}·kg^{-1}	-	443 l	86	-
Thiopental**	IV	*R*	0.30 l·min^{-1}	-	139 l	9.6	-
		S	0.23 l·m·n^{-1}	-	114 l	9.0	-
	IVI	*R*	0.10 l·m·n^{-1}	-	313 l	14.6	-
		S	0.08 l·m·n^{-1}	-	273 l	14.7	-
Tocainide	IVI	*R*	11.1 l·h^{-1}	-	136 l	9.3	-
		S	6.3 l·h^{-1}	-	134 l	17.1	-

Drug	Route of administration	Enantiomer	Clearance	Renal clearance	Volume of distribution	Half-life (h)	Fraction unbound (%)
Verapamil	IV	*R*	0.80 l.min^{-1}	-	2.74 l.kg^{-1}	4.1	-
		S	1.40 l.min^{-1}	-	6.42 l.kg^{-1}	4.8	-
	Oral	*R*	1.72 l.min^{-1}	-	-	-	-
		S	7.46 l.min^{-1}	-	-	-	-
Warfarin	Oral	*R*	1.9 ml.h^{-1}.kg^{-1}	-	129 ml.kg^{-1}	47.1	-
		S	2.0 ml.h^{-1}.kg^{-1}	-	70.5 ml.kg^{-1}	24.4	-

The reported parameters presented are average values.

IV = intravenous injection; IVI = intravenous infusion; IM = intramuscular injection.

*Values of unbound enantiomer clearance and volume of distribution.

** Thiopental doses: IV 0.25-0.5 g; IVI 12.5-86.9 g, duration of infusion between 31 and 285 hours.

Adapted from data presented in references [76-79].

Fig. 5: Mechanism of chiral inversion of the *R*-enantiomers of the 2-arylpropionic acid NSAIDs. Following formation of the acyl-coenzyme-A thioester the profenyl moiety undergoes epimerization to yield a mixture of both the *R*- and *S*-profenyl-CoA thioesters. Once formed the thioesters may undergo hydrolysis yielding the two enantiomers of the drug, or the acyl group may be transferred to glycerol to yield 'hybrid' triacylglycerides [31-33].

hybrid triacylglycerols and the stereoselective accumulation of the profen moiety in tissue. Johnson *et al.* /37/ have reported, in abstract form, the quantification of serum 'hybrid' triacylglycerols following administration of racemic ibuprofen to man; the mean areas under the serum concentration time curves (AUCs) of the triglycerols ranged from 3.5-9.0% of the total ibuprofen AUCs, with an enantioselectivity of 1.7-fold in favour of the *R*-enantiomer derivatives.

Stereoselective tissue distribution may also occur as a result of interactions with tissue uptake transporter systems and storage mechanisms. For example: the blood-brain barrier clearance of (*R*)-baclofen is four-fold greater than that of the *S*-enantiomer /38/; the stereoselective efflux of (-)- compared to (+)-*E*-10-hydroxynortriptyline from cerebrospinal fluid of depressed patients /39/; the *S*-enantiomers of the β-adrenoceptor antagonists, propranolol and atenolol, undergo selective storage and secretion by adrenergic nerve terminals in cardiac tissue /40/; active and passive uptake of L- and D-methyldopa into rabbit aqueous humour, respectively /41/.

Metabolism

Drug metabolism frequently exhibits marked stereoselectivity which may be associated with substrate binding and the stereochemistry of the enzyme active site. Alternatively, stereoselectivity may be associated with catalysis due to differential orientation, or reactivity, of potential target groups with respect to the catalytic site. Metabolic stereoselectivity can be associated with: the substrate, involving the preferential transformation of one of a pair of enantiomers; the product, resulting in the preferential formation of a particular stereoisomer of the metabolite; or both the substrate and the product, involving the selective metabolism of one enantiomer of the substrate to yield preferentially one of a number of possible diastereomeric products /42,43/. Examples of stereoselectivity in metabolism and the stereochemical outcome of the transformations are presented in Table 3.

Excretion

Renal excretion is the net result of both passive and active processes including glomerular filtration, active secretion and passive and active reabsorption. Apparent stereoselectivity in renal clearance,

TABLE 3

Stereoselectivity in metabolic transformations

Prochiral to chiral transformations:

Sulphoxidation of cimetidine to preferentially yield the (+)-enantiomer of cimetidine sulphoxide; enantiomeric composition in urine (+):(-): 71-75:29-25 /80,81/. Aromatic oxidation of phenytoin to yield (*S*)-4-hydroxyphenytoin in >90% enantiomeric excess /82/.

Chiral to chiral transformations:

Cytochrome P450 (CYP) 2C9 mediated oxidation of (*S*)-warfarin to yield (*S*)-7-hydroxywarfarin /83/.

Chiral to diastereoisomer transformations:

Stereoselective glucuronidation of oxazepam /60/.

Chiral to achiral transformations:

Sulphoxidation of omeprazole, mediated by CYP 3A4, to yield the corresponding sulphone derivative; intrinsic metabolite formation clearance from (*S*)-omeprazole ten-fold greater than that from the *R*-enantiomer /84/.

Chiral inversion:

Inversion of chirality of the inactive *R*-enantiomers of the 2-arylpropionic acid NSAIDs to their active, cyclooxygenase inhibiting *S*-enantiomers, e.g. ibuprofen, fenoprofen /33,34/, and (*S*)-2-aryloxypropionic acid herbicides to their *R*-enantiomers, e.g. haloxyfop /85/.

particularly with respect to glomerular filtration, may arise as a result of selectivity in plasma protein binding. Active tubular secretion is thought to be responsible for the differential clearance of the enantiomers of a number of basic drugs but the differences between enantiomers tend to be relative small, *ca* 1- to 3-fold, for example enantioselectivity: prenylamine, *R*, 1.3 ml/min; *S*, 4.0 ml/min /44/; chloroquine, (+) unbound clearance 824 ml/min; (-) unbound clearance 519 ml/min /45/; disopyramide, *R*, unbound clearance 6.26 ml/min/kg; *S*, 8.75 ml/min/kg /46/; diastereoselectivity: quinine and quinidine 24.7 and 99 ml/min, respectively /47/.

Enantiomeric interactions may also occur with those agents which undergo active secretion, e.g. administration of increasing amounts of (*R*)-ofloxacin to the cynomolgus monkey results in a reduction in both the total and renal clearance of the *S*-enantiomer /48/ which is thought to be due to competitive inhibition of the organic cation transport system. Interactions with other drugs may also exhibit enantioselectivity; for example, probenecid stereoselectively reduces the renal clearance of (-)- but not (+)-sultopride on administration of the racemic drug to rats /49/.

Pharmacokinetic parameters

In comparison to the magnitude of the differences between enantiomers in terms of their pharmacodynamic parameters, those associated with their pharmacokinetic parameters tend to be relative modest, frequently one- to three-fold (see for example the data presented in Table 2). However, pharmacokinetic parameters may be divided into three levels of organization representing: (1) the whole body, e.g. half-life, systemic clearance and volume of distribution; (2) organ, e.g. hepatic and renal clearance; and (3) macromolecular, e.g. fraction unbound to plasma proteins and intrinsic metabolite formation clearance /50/. Those parameters representing the whole body are determined by multiple organ parameters, which in turn are determined by the macromolecular parameters. As a result, the stereoselectivity observed in pharmacokinetic parameters may be either amplified or attenuated with each level of organization. It is therefore possible that a comparison between enantiomers in parameters which represent the whole body level of organization may well mask significant stereoselectivity at the organ and/or macromolecular level /50/. For example, the ratio of the half-lives, half-life being a hybrid parameter dependent on both systemic clearance and volume of distribution, of the enantiomers of propranolol (*R/S*) is approximately ~1 and ~0.9 following intravenous and oral administration, respectively. The corresponding ratios for the values of clearance and volume of distribution are 1.17 and 1.18, which essentially cancel out. However, examination of the ratio for fraction unbound yields a value of 1.15, and that for metabolite formation clearance for the major metabolite, 4-hydroxypropranolol, is 2.51 /40,50/. Similarly the ratio (*R/S*) for the half-life of the enantiomers of warfarin is 1.93, which is essentially determined by the difference between the enantiomers in

volume of distribution (*R/S*, 1.83) as the ratio of the clearance values (*R/S*) is 0.95. Examination of the corresponding ratios (*R/S*) in the fraction recovered as the 7- and 8-hydroxy metabolites yields values of ~0.16 and ~152, respectively /50/.

As a result of stereoselectivity in drug disposition, the plasma profiles of the enantiomers of a drug administered as a racemate frequently differ, and an examination of plasma concentration-effect relationships, or the determination of pharmacokinetic parameters based on 'total' drug present in biological samples is essentially meaningless or 'sophisticated' nonsense /51,52/.

This situation is exemplified by an examination of the pharmacokinetic properties of the enantiomers of modafinil following oral administration of the racemate. Modafinil is an anti-narcoleptic drug used for the treatment of excessive sleepiness, fatigue and lack of concentration associated with obstructive sleep apnea/hyponea syndrome /54-56/. The drug undergoes stereoselective metabolism such that the clearance of the (+)-*S*-enantiomer (1.4-1.8 $ml.min^{-1}.kg^{-1}$) is approximately three-fold greater than that of (-)-(*R*)-modafinil (0.43-0.65 $ml.min^{-1}.kg^{-1}$). This difference in enantiomeric clearance, together with the similar volumes of distribution (*ca* 0.50 $l.kg^{-1}$), results in a terminal half-life for (*R*)-modafinil ($t_{½}$, 10.0-16.0 h) similar to the value obtained based on the determination of 'total' drug plasma concentrations ($t_{½}$, 10.0-17.0 h) /57,58/, such that at 10 h post oral administration of the racemate the measured 'total' drug plasma concentration is essentially due to the *R*-enantiomer /59/. As a result of these pharmacokinetic differences the drug has been re-evaluated as a single enantiomer product /54,55,59/, undergoing the chiral switch process (see below), and the sponsoring company, Cephalon, has recently (April 2007) received an approvable letter from the US FDA for its new drug application for armodafinil, the single *R*-enantiomer.

In addition to the complexities outlined above relatively little information is available with respect to the influence of route of administration, formulation and biological factors on the disposition and action of the enantiomers of the large number of agents currently available as racemates. Examples of what may occur are summarised in Table 4.

RACEMIZATION *IN VIVO*

In addition to the 2-arylpropionic acid NSAIDs /33,34/, cited above, other compounds are known to be stereochemically unstable and undergo racemization *in vivo*, e.g. oxazepam /60/ and more importantly the teratogen thalidomide /61/. The stereochemical stability of the latter compound is of particular significance as it is frequently cited as an example of a compound where the use of a single enantiomer would have prevented the tragedy of the 1960s. A study carried out in the late 1970s using mice as a test species indicated that the teratogenic activity resided solely in the *S*-enantiomer. However, the situation with thalidomide is more complex, as previous studies, in a more sensitive test species, New Zealand White rabbits, indicated that both enantiomers were teratenogenic /62/. In addition, the drug is known to be stereochemically unstable in biological media *in vitro* /63/ and following administration of (*R*)- and (*S*)-thalidomide to man *ca* 25% and 43% of the total AUC respectively are due to the alternative enantiomer /61/. Thus stereochemical pharmacokinetic studies, in combination with animal studies, indicate that the situation with thalidomide is by no means as clear as some of the literature would imply and that the drug is not a good example to cite in favour of single stereoisomer compounds.

As a result of the considerations outlined above, together with potential adverse reaction and drug safety issues /8/, drug stereochemistry has become the subject of considerable debate and an issue for both the pharmaceutical industry and regulatory agencies.

DRUG REGULATION AND STEREOSELECTIVE PHARMACOKINETICS

The major regulatory authorities have examined the issues associated with drug stereochemistry and a number have issued guidelines and/or published policy statements. To date none of the agencies has an absolute requirement for the development of single isomer products, the choice of material being left to the compound sponsor. However, the pre-clinical safety evaluation of a chiral New Chemical Entity (NCE) should include pharmacodynamic, pharmacokinetic and appropriate toxicological investigations on both enantiomers and the racemate. It is obviously necessary to examine the stereochemical stability of the drug *in vivo* to establish whether chemical and/or

TABLE 4

Factors influencing the stereoselectivity of drug action and disposition

FACTOR	DRUG	COMMENT
1. Route of administration		
	Verapamil	Concentration-effect relationships based on 'total' drug plasma concentrations indicate an enhanced effect following intravenous compared to oral administration; associated with stereoselective first-pass metabolism of the more active *S*-enantiomer /86/
	Nitrendipine	Clearance of *S*-enantiomer (1.51 ± 0.25 l.min^{-1}) similar to that of (*R*)-nitrendipine (1.62 ± 0.25 l min^{-1}) following IV administration; bioavailability of (*S*)- greater than that of (*R*)-nitrendipine following tablet (*S*-, $13.4 \pm 5.6\%$; *R*-, $7.9 \pm 4.0\%$) or osmotic pump formulations (*S*-, $11.1 \pm 2.6\%$, *R*-, $6.1 \pm 1.2\%$) due to stereoselective first-pass metabolism /87/.
	Salbutamol	Following IV administration of the racemate, clearance of the more active *R*-enantiomer greater than that of (*S*)-salbutamol (*R*-, 0.62 l h^{-1}.kg^{-1}; *S*-, 0.39 l.h^{-1}.kg^{-1} /88/, *R*-, 46.8 l.h^{-1}; *S*-, 14.7 l.h^{-1} /89/) Oral availability of *R*- and *S*-enantiomers from 9-30% and 69-71%, respectively /88,89/, due to stereoselective first-pass metabolism. Following inhalation the bioavailability of (*R*)- and (*S*)-salbutamol are 21% and 60%, respectively; inhalation, together with oral administration of charcoal, bioavailability of both enantiomers equivalent (~20%); data indicative of lack of presystemic metabolism in the lung and extensive oral absorption following inhalation /89/.

FACTOR	DRUG	COMMENT
2. Formulation		
	Ibuprofen	Administration of the racemic drug as a controlled release (CR) formulation resulted in an increase in half-life of both enantiomers compared to a tablet (T) formulation (CR: *S*-, 8.4 h; *R*-, 8.8 h; T: *S*-, 2.4 h; *R*-, 1.5 h), due to absorption rate limited elimination; reduction in the plasma concentration ratio (*S*/*R*) following the CR formulation (CR: 1.8 ± 0.4, range 1.2-2.6; T: 2.5 ± 0.86, range 1.3-4.2) /90/.
	Verapamil	Enantiomeric ratio (*R*/*S*) of the maximum plasma concentrations (C_{max}) and area under the plasma concentration versus time curves (AUC) significantly lower following immediate release (IR) than sustained release (SR) formulations (Cmax, IR, 4·52; SR, 5·83; AUC, IR, 5·04; SR, 7·75); variation associated with concentration and/or input rate related saturable first pass metabolism of (*S*)-verapamil /91/.
3. Drug interactions		
	Warfarin	Most extensively examined drug with respect to stereoselectivity in drug interactions, some agents, e.g. phenylbutazone, sulphinpyrazone, metronidazole, cotrimoxazole, tricrynafen and quinalbarbitone, selective for the more active *S*-enantiomer; others, e.g. cimetidine, enoxacin, rifampicin, and clofibrate selective for (*R*)-warfarin, whereas others show no selectivity, e.g. amiodarone /76/.
	Verapamil	Stereoselective reduction in clearance of the *S*-enantiomer following administration with cimetidine, resulting in an increased negative dromotropic effect on atrioventricular conduction /92/.
	Hexobarbitone	Stereoselective increase in clearance following administration with rifampicin; *S*-enantiomer, 6-fold increase; *R*-enantiomer, 39-fold increase /93/.

Table 4 continued

FACTOR	DRUG	COMMENT
4. Ageing		
	Ibuprofen	Stereoselective increase in free fraction (young [Y], 0.48 ± 0.10%; elderly [E], 0.64 ± 0.20%), reduced total unbound clearance (Y, 15.9 ± 2.2 l.min^{-1}; E, 11.5 ± 4.1 l.min^{-1}) and 25-30% reduction in metabolic clearance via both oxidative and glucuronidation pathways for (*S*)-ibuprofen; *R*-enantiomer exhibits no significant differences with age /94/.
	Flurbiprofen	Non-stereoselective reduction in clearance (~38%) and corresponding increase in half-life of both enantiomers; non-stereoselective reduction in metabolite formation clearance via both glucuronidation (~55%) and oxidation (~48%) in the elderly /95/.
	Hexobarbitone	Stereoselective decrease in clearance with age; *S*-enantiomer two-fold greater clearance in young compared to elderly; *R*-enantiomer, no age effect /95/.
5. Disease		
	Nimodipine	Bioavailability of *R*- and *S* enantiomers increased 3- to 4- and 17-fold, respectively, due to reduced first pass metabolism in cirrhotic patients.
	Ibuprofen	Plasma concentrations of (*S*)-ibuprofen lower than those of the *R*-enantiomer in cirrhotic patients; ratio of area under the plasma concentration time curve (*S*/*R*) 0.94 in cirrhotic patients compared to 1.3 in healthy volunteers /97/.
6. Gender		
	Mephobarbital	Oral clearance of *R*-enantiomer significantly greater in young men compared to young or elderly women, or elderly men, *S*-enantiomer no significant differences between groups /98/.

FACTOR	DRUG	COMMENT
7. Pharmacogenetics		
	Metoprolol	Enantiomeric ratio (*S*/*R*) of the area under the plasma concentration versus time curve decreases from 1.37 in extensive metabolisers (EMs) to 0.90 in poor metabolisers (PMs) of debrisoquine; the 'total' plasma concentration effect relationship shifts to the right in PMs compared to EMs /99/.
	Fluoxetine	Oral clearance of both enantiomers similar (*R*-, 40 $l.h^{-1}$; *S*-, 36 $l.h^{-1}$) in EMs of debrisoquine; reduced in PMs to 17 $l.h^{-1}$ and 3 $l.h^{-1}$ for the *R*- and *S*-enantiomers, respectively; plasma concentrations increase by *R*-enantiomer, ~2.5- and *S*-enantiomer, ~11-fold in PMs compared to EMs /100/.
	Mephenytoin	Oral clearance of (*S*)-mephenytoin reduced from 4.7 $l\ min^{-1}$ in EMs to 0.029 $l.m\ n^{-1}$ in PMs; *R*-enantiomer clearance 0.03 and 0.02 $l.min^{-1}$ in EMs and PMs, respectively /50/.
8. Race		
	Propranolol	Oral clearance of both enantiomers greater in Black compared to White subjects; stereoselective for the *R*-enantiomer /101/.

biochemical mediated inversion of configuration occurs, e.g. the 2-arylpropionic acid NSAIDs, oxazepam, thalidomide. Such data, the extent and rate of inversion/racemization, will have considerable impact on the decision to proceed with the development of a single enantiomer or racemic mixture. When inversion of chirality does occur the stereoisomer formed is treated as a metabolite and, if biologically active, will contribute to the interpretation of the pharmacodynamic and toxicological data.

With the development of single enantiomer products from previously marketed racemates, the chiral switch process, the regulatory bodies permit appropriate bridging studies such that data for the single enantiomer may be linked to the original racemate documentation. The extent of the bridging studies required will depend on the properties of the compound under investigation but with respect to pharmacokinetic studies a comparison of the pharmacokinetic profile of the single enantiomer following administration as such and as a component of the racemate is required. For example, the bioavailability of the *S*-enantiomer of the selective serotonin reuptake inhibitor citalopram, and the active demethylated metabolite, were shown to be bioequivalent following administration of the racemate and the single enantiomer, escitalopram.

STEREOSELECTIVITY AND FORENSIC SCIENCE

Determination of the enantiomeric composition of a drug and/or metabolite in a biofluid provides evidence of the nature of the material taken, i.e. single enantiomer or racemate, and thus increases the information content of the sample. Analysis of the stereochemical composition of amphetamine in the urine of drug abusers taking part in a maintenance therapy programme enabled identification of those subjects using illicit material, assumed to be racemic, together with the prescribed dexamphetamine /64/. Similarly the urinary excretion of (*R*)-methylamphetamine has the potential to distinguish between drug abuse and those using a nasal inhaler, as Vicks inhalers in the USA contain the single *R*-enantiomer of the drug /65/.

The illicit recreational use of 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) is currently a topic of considerable concern due to toxicity, and a number of deaths have been reported. The stereoselective disposition of MDMA has been reported following oral

administration of a low dose (40 mg) of the racemate to healthy volunteers /66/. The oral clearance of the more active *S*-enantiomer was significantly greater than that of (*R*)-MDMA (CL_{oral}: *R*-, 55 ± 32 l/h; *S*-, 131 ± 76 l/h) resulting in a shorter half-life (*R*-, 5.8 ± 2.2 h; *S*-, 3.6 ± 0.9 h), and the urinary recovery of the unchanged drug was greater for the *R*-enantiomer (urinary recovery of the unchanged drug over 24 h: *R*-, 21.4 ± 11.6%; *S*-, 9.3 ± 4.9%). As the pharmacokinetic properties, and hence plasma profiles, of the individual enantiomers differed, the enantiomeric composition of the drug in plasma was evaluated as a means of estimation of the time elapsed between ingestion of the racemate and sampling. Mathematical modelling of the data yielded predicted times in good agreement with actual sampling times but some overlap between the predictions at adjacent times was observed /66/; however, the developed model was sufficiently accurate to predict within a 6 hour range. Such an approach has the potential to provide useful information within certain limitations /66/.

CONCLUDING REMARKS

As a result of stereoselectivity in drug disposition, pharmacokinetic parameters and concentration-effect relationships based on the measurement of 'total' drug plasma concentrations following administration of a racemate provide limited and potentially misleading data. It is now over 20 years since the publication of the article by the late Professor Ariens which referred to the generation of such data as "sophisticated nonsense" /51/. A number of years ago it was considered unacceptable to calculate pharmacokinetic parameters based on the determination of total radioactivity following administration of a radiolabelled drug. Similarly, as a result of advances in stereospecific analytical methodology, it is now, for new compounds at least, considered equally unacceptable to determine pharmacokinetic parameters based on the 'total' drug concentrations following administration of a racemate. However, even in the first decade of the 21st century, for a number of widely used racemic drugs the only readily available pharmacokinetic information continues to be based on flawed data, generated using outdated methodologies, where greater than 50% of the potentially available information was routinely discarded. That we continue to rely on such information is a sad indictment of the 'state-of-the-art'.

REFERENCES

1. Ariëns EJ, Wuis EW, Veringa EJ. Stereoselectivity of bioactive xenobiotics. A pre-Pasteur attitude in medicinal chemistry, pharmacokinetics and clinical pharmacology. Biochem Pharmacol 1988; 37: 9-18.
2. Bojarski J, Aboul-Enein HY, Ghanem A. What's new in chromatographic enantioseparations. Curr Anal Chem 2005; 1: 59-77.
3. Cox GB, ed. Preparative Enantioselective Chromatography. Oxford: Blackwell, 2005.
4. Francotte E, Lindner W, eds. Chirality in Drug Research. Weinheim: Wiley-VCH, 2006.
5. Maier NM, Franco P, Lindner W. Separation of enantiomers: needs, challenges, perspectives. J Chromatogr A 2001; 906: 3-33.
6. Nelson TD, Welch CJ, Rosen JD, et al. Effective use of preparative chiral HPLC in a preclinical drug synthesis. Chirality 2004; 16: 609-613.
7. Grabowski EJJ. Enantiopure drug synthesis: from methyldopa to imipenem to efavirenz. Chirality 2005; 17: S249-S259.
8. Shah RR, Midgley JM, Branch SK. Stereochemical origin of some clinically significant drug safety concerns: lessons for future drug development. Adverse Drug React Toxicol Rev 1998; 17: 145-190.
9. Branch, S. International regulation of chiral drugs. In: Subramanian G, ed. Chiral Separation Techniques. A Practical Approach, 2nd Ed. Weinheim: Wiley-VCH, 2001; 319-342.
10. Holmstedt B. The use of enantiomers in biological studies: an historical review. In: Holmstedt B, Frank H, Testa B, eds. Chirality and Biological Activity. New York: Alan R Liss, 1990; 1-14.
11. Cushny AR. Biological Relations of Optically Isomeric Substances. London: Bailliere, Tindall and Cox, 1926.
12. Mason SF. Molecular optical activity and the chiral discriminations. Cambridge: Cambridge University Press, 1982; 220-225.
13. Easson LH, Stedman E. Studies on the relationship between chemical constitution and physiological action V. Molecular dissymmetry and physiological activity. Biochem J 1933; 27: 1257-1266.
14. Ogston AG. Interpretation of experiments on metabolic processes using isotopic tracer elements. Nature 1948; 162: 963.
15. Sokolov VI, Zefirov NS. Enantioselectivity in two-point binding: rocking tetrahedron model. Dokl Akad Nauk SSSR 1991; 319: 1382-1383.
16. Sugiyama K, Trager WF. Prochiral selectivity and intramolecular isotope effects in the cytochrome P-450 catalyzed ω-hydroxylation of cumene. Biochemistry 1986; 25: 7336-7343.
17. Ichinose R, Kurihara N. Intramolecular deuterium isotope effect and enantiotopic differentiation in oxidative demethylation of chiral [monomethyl-D_3] methoxychlor in rat liver microsomes. Biochem Pharmacol 1987; 36: 3751-3756.
18. Mesecar AD, Koshland DE. A new model for protein stereospecificity. Nature 2000; 403: 614-615.

19. Bentley R. Diastereoisomerism, contact points, and chiral selectivity: a four point saga. Arch Biochem Biophys 2003; 414: 1-12.
20. Topiol S. A general criterion for molecular recognition: implications for chiral interactions. Chirality 1989; 1: 69-79.
21. Topiol S, Sabio M. Interactions between eight centres are required for chiral recognition. J Am Chem Soc 1989; 111: 4109-4110.
22. Lehmann PAF, Rodrigues de Miranda JF, Ariens EJ. Stereoselectivity and affinity in molecular pharmacology. In: Jucker E, ed. Progress in Drug Research, Vol. 20. Basel: Birkhäuser Verlag, 1976; 101-142.
23. Doyle FP, Fosker GR, Naylor JHC, Smith H. Derivatives of 6-aminopenicillanic acid. Part I α-aminobenzylpenicillin and some related compounds. J Chem Soc 1962: 1440-1444.
24. Wade DN, Mearick PT, Morris JL. Active transport of L-dopa in the intestine. Nature 1972; 242: 463-465.
25. Hendel J, Brodthagen H. Enterohepatic cycling of methotrexate estimated by use of the D-isomer as a reference marker. Eur J Clin Pharmacol 1984; 26: 103-107.
26. Itoh T, Ono K, Koido K-I, Li Y-H, Yamada H. Stereselectivity of the folate transporter in rabbit small intestine: studies with amethopterin enantiomers. Chirality 2001; 13: 164-169.
27. Tamai I, Ling H-Y, Timbul S-M, Nishikido J, Tsuji A. Stereospecific absorption and degradation of cephalexin. J Pharm Pharmacol 1988; 40: 320-324.
28. Wetterich H, Spahn-Langguth H, Mutschler E, Terhaag B, Rosch W, Langguth P. Evidence for intestinal secretion as additional clearance pathway of talinolol enantiomers: concentration and dose dependent absorption in vitro and in vivo. Pharm Res 1996; 13: 514-522.
29. Zschiesche M, Lemma GL, Klebingat K-J, et al. Stereoselective disposition of talinolol in man. J Pharm Sci 2002; 91: 303-311.
30. Walle UK, Walle T, Bai SA, Olanoff LS. Stereoselective binding of propranolol to human plasma, α_1-acid glycoprotein and albumin. Clin Pharmacol Ther 1983; 34: 718-723.
31. Giacomini KM, Nelson WL, Pershe RA, Valdivieso L, Turner-Tamiyasu K, Blaschke TF. In vivo interaction of the enantiomers of disopyramide in human subjects. J Pharmacokin Biopharm 1986; 14: 335-355.
32. Hanada K, Akimoto S, Mitsui, K, Mihara K, Ogata H. Enantioselective tissue distribution of the basic drugs disopyramide, flecainide and verapamil in rats: role of plasma protein and tissue phosphatidylserine binding. Pharm Res 1998; 15: 1250-1256.
33. Hutt AJ, Caldwell J. The metabolic chiral inversion of 2-arylpropionic acids - a novel route with pharmacological consequences. J Pharm Pharmacol 1983; 35: 693-704.
34. Caldwell J, Hutt AJ, Fournel-Gigleux S. The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. Biochem Pharmacol 1988; 37: 105-114.

35. Williams K, Day R, Knihinicki R, Duffield A. The stereoselective uptake of ibuprofen enantiomers into adipose tissue. Biochem Pharmacol 1986; 35: 3403-3405.
36. Sallustio BC, Meffin PJ, Knights KM. The stereospecific incorporation of fenoprofen into rat hepatocyte and adipocyte triacylglycerols. Biochem Pharmacol 1988; 37: 1919-1923.
37. Johnson JL, Brater DC, Hall SD. Formation of "hybrid" glycerolipids in normal volunteers. Clin Pharmacol Ther 1995; 57: 213.
38. Van Bree JBMM, Heijligers-Feijen CD, De Boer AG, Danhof M, Breimer DD. Stereoselective transport of baclofen across the blood-brain barrier in rats as determined by the unit impulse response methodology. Pharm Res 1991; 8: 259-262.
39. Bertilsson L, Otani K, Dahl M-L, Nordin C, Aberg-Wistedt A. Stereoselective efflux of (*E*)-10-hydroxynortriptyline enantiomers from the cerebrospinal fluid of depressed patients. Pharmacol Toxicol 1991; 68: 100-103.
40. Walle T, Webb JG, Bagwell EE, Walle UK, Daniell HB, Gaffney TE. Stereoselective delivery and actions of beta receptor antagonists. Biochem Pharmacol 1988; 37: 115-124.
41. Wainer IW, Chen J, Ducharme F, et al. Stereochemical aspects of metabolism and tissue distribution. In: Crommelin DJA, Midha KK, Nagai T, eds. Topics in Pharmaceutical Sciences 1993. Stuttgart: Medpharm Scientific, 1994; 223-246.
42. Jenner P, Testa B. The influence of stereochemical factors on drug disposition. Drug Metab Rev 1973; 3: 117-184.
43. Low LK, Castagnoli N. Enantioselectivity in drug metabolism. In: Clarke FH, ed. Annual Reports in Medicinal Chemistry, Vol. 13. New York: Academic Press, 1978; 304-315.
44. Gietl Y, Spahn H, Knauf H, Mutschler E. Single and multiple-dose pharmacokinetics of (*R*)-(-)- and (*S*)-(+)-prenylamine in man. Eur J Clin Pharmacol 1990; 38: 587-593.
45. Ofari-Adjei D, Ericsson O, Lindstrom B, Hermansson J, Adjepon-Yamoah K, Sjoqvist F. Enantioselective analysis of chloroquine and desethylchloroquine after oral administration of racemic chloroquine. Ther Drug Monit 1986; 8: 457-461.
46. Le Corre P, Gibassier D, Sado P, Le Verge R. Stereoselective metabolism and pharmacokinetics of disopyramide enantiomers in humans. Drug Metab Dispos 1988; 16: 858-864.
47. Notterman DA, Drayer DE, Metakis L, Reidenberg MM. Stereoselective renal tubular secretion of quinidine and quinine. Clin Pharmacol Ther 1986; 40: 511-517.
48. Okazaki O, Kurata T, Hakusui H, Tachizawa H. Species-related stereoselective disposition of ofloxacin in the rat, dog and monkey. Xenobiotica 1992; 22: 439-450.
49. Kamizono A, Inotsume N, Fukushima S, Nakano M, Okamoto Y. Inhibitory effects of procainamide and probenecid on renal excretion of sultopride enantiomers in rats. J Pharm Sci 1993; 82: 1259-1261.

50. Levy RH, Boddy AV. Stereoselectivity in pharmacokinetics: a general theory. Pharm Res 1991; 8: 551-556.
51. Ariëns EJ. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. Eur J Clin. Pharmacol 1984; 26: 663-668.
52. Evans AM, Nation RL, Sansom LN, Bochner F, Somogyi AA. Stereoselective drug disposition: potential for misinterpretation of drug disposition data. Br J Clin Pharmacol 1988; 26: 771-780.
53. Ferraro L, Fuxe K, Tanganelli S, Fernandez M, Rambert FA, Antonalli T. Amplification of cortical serotonin release; a further neurochemical action of the vigilance-promoting drug modafinil. Neuropharmacology 2000; 39: 1974-1983.
54. Roth T, White D, Schmidt-Nowara W, et al. Effects of armodafinil in the treatment of residual excessive sleepiness associated with obstructive sleep apnea/hypopnea syndrome: a 12-week, multicenter, double-blind, randomized, placebo-controlled study in nCPAP-adherent adults. Clin Ther 2006; 28: 689-706.
55. Dingles DF, Arora S, Darwish M, Niebler GE. Pharmacodynamic effects on alertness of single doses of armodafinil in healthy subjects during a nocturnal period of acute sleep loss. Curr Med Res Opin 2006; 22: 159-167.
56. Wong YN, Simcoe D, Hartman LN, et al. A double-blind, placebo-controlled, ascending-dose evaluation of the pharmacokinetics and tolerability of modafinil tablets in healthy male volunteers. J Clin Pharmacol 1999; 39: 30-40.
57. Wong YN, King P, Simcoe D, et al. Open label, single-dose pharmacokinetic study of modafinil tablets: influence of age and gender in normal subjects. J Clin Pharmacol 1999; 39: 281-288.
58. Gorman SH. Determination of the d- and l-enantiomers of modafinil in human plasma utilizing liquid-liquid extraction and high-performance liquid chromatography. J Chromatogr B 1999; 730: 1-7.
59. Hirshkowitz M, Black JE, Wesnes K, Niebler G, Arora S, Roth T. Adjunct armodafinil improves wakefulness and memory in obstructive sleep apnea/ hypopnea syndrome. Resp Med 2007; 101: 616-627.
60. Sisenwine SF, Tio CO, Hadley FV, Liu A-L, Kimmel HB, Ruelius HW. Species-related differences in the stereoselective glucuronidation of oxazepam. Drug Metab Dispos 1982; 10: 605-608.
61. Eriksson T, Bjorkman S, Roth B, Fyge A, Hoglund P. Stereospecific determination, chiral inversion in vitro and pharmacokinetics in humans of the enantiomers of thalidomide. Chirality 1995; 7: 44-52.
62. Fabro S, Smith RL, Williams RT. Toxicity and teratogenicity of optical isomers of thalidomide. Nature 1967; 215: 269.
63. Reist M, Carrupt P-A, Francotte E, Testa B. Chiral inversion and hydrolysis of thalidomide: mechanisms and catalysis by bases and serum albumin, and chiral stability of teratogenic metabolites. Chem Res Toxicol 1998; 11: 1521-1528.
64. Tetlow VA, Merrill J. Rapid determination of amphetamine stereoisomer ratios in urine by gas chromatography-mass spectroscopy. Ann Clin Biochem 1996; 33: 50-54.

65. Fitzgerald RL, Ramos JM, Bogema SC, Poklis A. Resolution of methamphetamine stereoisomers in urine drug testing: urinary excretion of *R*-(-)-methamphetamine enantiomer data. J Anal Toxicol 1988; 18: 49-51.
66. Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA, Hutt AJ. Stereospecific analysis and enantiomeric disposition of 3,4-methylenedioxymethamphetamine (Ecstasy) in humans. Clin Chem 1999; 45: 1058-1069.
67. Gillespie L, Oates JA, Cront JR, Sjoerdsma A. Clinical and chemical studies with α-methyldopa in patients with hypertension. Circulation 1962; 25: 281-291.
68. Kubota H, Numami K, Hayashi K, et al. Studies on angiotensin converting enzyme inhibitor VI. Synthesis and angiotensin converting enzyme inhibitory activities of the dicarboxylic acid derivative of imidapril and its diastereoisomers. Chem Pharm Bull 1992; 40: 1619-1622.
69. Carter RB, Dykstra LA. Quantitative analysis of the interaction between the agonist and antagonist isomers of picenadol (LY 150720) on electric shock titration in the squirrel monkey. Eur J Pharmacol 1985; 106: 469-476.
70. Schunack W, Schwarz S, Gerhard G, Buyuktimkin S, Elz S. Chiral agonists of histamine. In: Ganellin CR, Schwartz JC, eds. Frontiers in Histamine Research. Oxford: Pergamon, 1985; 39-46.
71. Vlasses PH, Irvin JD, Huber PB, et al. Pharmacology of enantiomers and (-)-*p*-OH metabolite of indacrinone. Clin Pharmacol Ther 1981; 29: 798-807.
72. Tobert JA, Cirillo VJ, Hitzenberger G, et al. Enhancement of uricosuric properties of indacrinone by manipulation of the enantiomer ratio. Clin Pharmacol Ther 1981; 29: 344-350.
73. Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. Anesth Analg 1998; 87: 1186-1193.
74. Blessington B. Ethambutol and tuberculosis, a neglected and confused chiral puzzle. In: Aboul-Enein HY, Wainer IW, eds. The Impact of Stereochemistry on Drug Development and Use. New York: Wiley-Interscience, 1997; 235-261.
75. Ruffolo RR. Chirality in α- and β-adrenoceptor agonists and antagonists. Tetrahedron 1991; 47: 9953-9980.
76. Tucker GT, Lennard MS. Enantiomer specific pharmacokinetics. Pharmacol Ther 1990; 45: 309-329.
77. Eichelbaum M, Gross AS. Stereochemical aspects of drug action and disposition. In: Testa B, Meyer UA, eds. Advances in Drug Research, Vol. 28. London: Academic Press, 1996; 1-64.
78. Hutt AJ. Drug chirality: stereoselectivity in the action and disposition of anaesthetic agents. In: Adams AP, Cashman JN, Grounds RM, eds. Recent Advances in Anaesthesia and Intensive Care, Vol. 22. London: Greenwich Medical Media, 2003; 31-64.
79. Hutt AJ. Drug chirality and its pharmacological consequences. In: Smith HJ, ed. Introduction to the Principles of Drug Design and Action, 4th Ed. Boca Raton, FL: CRC Press, 2006; 117-183.
80. Cashman JR, Park SB, Yany Z-C, et al. Chemical, enzymatic and human enantioselective *S*-oxygenation of cimetidine. Drug Metab Dispos 1993; 21: 587-597.

81. Kuzel RA, Bhasir SK, Oldham HG, et al. Investigations into the chirality of the metabolic sulfoxidation of cimetidine. Chirality 1994; 6: 607-614.
82. Poupaert JH, Cavalier R, Claasen MH, Dumont PA. Absolute configuration of the major metabolite of 5,5-diphenylhydantoin, 5-(4′-hydroxyphenyl)-5-phenylhydantoin. J Med Chem 1975; 18: 1268-1271.
83. Rettie AE, Korzekwa KR, Kunze KL, et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P450: a role for P450 2C9 in the aetiology of (*S*)-warfarin drug interactions. Chem Res Toxicol 1992; 5: 54-59.
84. Äbelo A, Andersson TB, Antonsson M, Naudot AK, Skånberg I, Weidorf L. Stereoselective metabolism of omeprazole by human cytochrome P450 enzymes. Drug Metab Dispos 2000; 28: 966-972.
85. Bartels MJ, Smith FA. Stereochemical inversion of haloxyfop in the Fischer 344 rat. Drug Metab Dispos 1989; 17: 286-291.
86. Echizen H, Vogelgesang B, Eichelbaum M. Effects of d,l-verapamil on atrioventricular conduction in relation to its stereoselective first-pass metabolism. Clin Pharmacol Ther 1985; 38: 71-76.
87. Soons PA, Breimer DD. Stereoselective pharmacokinetics of oral and intravenous nitrendipine in healthy male subjects. Br J Clin Pharmacol 1991; 32: 11-16.
88. Boulton DW, Fawcett JP. Enantioselective disposition of salbutamol in man following oral and intravenous administration. Br J Clin Pharmacol 1996; 41: 35-40.
89. Ward JK, Dow J, Dallow N, Eynott P, Milleri S, Ventresca GP. Enantiomeric disposition of inhaled, intravenous and oral racemic-salbutamol in man - no evidence of enantioselective lung metabolism. Br J Clin Pharmacol 2000; 49: 15-22.
90. Avgerinos A, Noormohammadi A, Hutt AJ. Disposition of ibuprofen enantiomers following the oral administration of a novel controlled release formulation to healthy volunteers. Int J Pharmaceut 1991; 68: 97-103.
91. Karim A, Piergies A. Verapamil stereoisomerism: enantiomeric ratios in plasma dependent on peak concentrations, oral input rate, or both. Clin Pharmacol Ther 1995; 58: 174-184.
92. Mikus G, Eichelbaum M, Fischer C, Gumulka S, Klotz U, Kroemer HK. Interaction of verapamil and cimetidine: stereochemical aspects of drug metabolism, drug disposition and drug action. J Pharmacol Exp Ther 1990; 253: 1042-1048.
93. Smith DA, Chandler MHH, Shedlofsky SI, Wedlund PJ, Blouin RA. Age-dependent stereoselective increase in the oral clearance of hexobarbitone isomers caused by rifampicin. Br J Clin Pharmacol 1991; 32: 735-739.
94. Tan SC, Patel BK, Jackson SHD, Swift CG, Hutt AJ. Influence of age on the enantioselective disposition of ibuprofen in healthy volunteers. Br J Clin Pharmacol 2003; 55: 579-587.
95. Patel BK, Jackson SHD, Swift CG, Hutt AJ. Disposition of flubiprofen in man: influence of stereochemistry and age. Xenobiotica 2003; 33: 1043-1057.
96. Chandler MMH, Scott SR, Blouin RA. Age associated stereoselective alterations in hexobarbital metabolism. Clin Pharmacol Ther 1988; 43: 436-441.

97. Li G, Treiber G, Maier K, Walker S, Klotz U. Disposition of ıbuprofen in patients with liver cirrhosis. Stereochemical considerations. Clin Pharmacokin 1993; 25: 154-163.
98. Hooper WD, Qing MS. The influence of age and gender on the stereoselective metabolism and pharmacokinetics of mephobarbital in humans. Clin Pharmacol Ther 1990; 48: 633-640.
99. Lennard MS, Tucker GT, Silas JH, Freestone S, Ramsay LE, Woods HF. Differential stereoselective metabolism of metoprolol in extensive and poor debrisoquin metabolisers. Clin Pharmacol Ther 1983; 34: 732-737.
100. Fjordside L, Jeppesen U, Eap CB, Poweel K, Baumann P, Brosen K. The stereoselective metabolism of fluoxetine in poor and extensive metabolizers of sparteine. Pharmacogenetics 1999; 9: 55-60.
101. Johnson JA, Burlew BS. Racial differences in propranolol pharmacokinetics. Clin Pharmacol Ther 1992; 51: 495-500.