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Invited Review

Racemates and enantiomers in drug development

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Summary

Drug stereoisomerism is increasingly being recognised as an issue having clinical, research and regulatory implications. Differences in the pharmacodynamic and pharmacokinetic properties of stereoisomers, molecules differing only in the spatial arrangement of certain atoms or groups at asymmetrical centres, is well documented. More than 400 drugs are administered as racemates. Racemic drugs usually contain 50% or more of an 'inactive' isomer that is presumed harmless. Measurements of such drugs in biological fluids have, nevertheless, been based on techniques unable to differentiate between enantiomers. Since the initial 50 : 50 enantiomeric ratio of racemic drugs may rapidly change to unknown ratios in the body, the value of plasma levels and pharmacokinetic parameters obtained with racemates is highly questionable. Numerous examples of racemic drugs possessing stereoselective pharmacokinetic or pharmacodynamic differences can be cited. Mechanisms by which isomers differ – intrinsic activity, potency, disposition – also vary and numerous stereo- selective drug-drug interactions have been reported. Drug stereoisomerism is likely to be the focus of greater attention in the future in the areas of clinical practice, research and drug regulation. This paper lists what constitutes a full description of a drug's stereostructure and the resultant therapeutic and toxicological implications.

Introduction

Substances with the same elementary chemical composition but different physical properties, isomers, were a problem for chemists until the theory of molecular structure was developed from the 1860s onwards. A particular puzzle was the case of optical isomers, substances which appeared to be identical chemically and physically, except that, one form rotated the plane of polarized light to the right, and another to the left, while a third form seemed to be optically inactive, with no effect on polarized light. The phenomenon of isomerism was discovered by the isolation of two almost identical substances from the tartars deposited by maturing wine. The major product (+)-tartaric acid, was found to be dextrorotatory to polarized light, whereas the minor product, racemic or paratartaric acid, proved to be optically inactive. Mitscherlich (1820), whose law of isomorphism correlated the similarity of crystal shapes with an analogy in chemical composition, reported that the sodium ammonium

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salts of (+)-tartaric acid and of racemic acid are completely isomorphous and are identical in all respects, except in optical activity.

Early last century the general view proposed by Biot (1812) that rotation of plane polarized light by a quartz crystal depended on the direction in which the crystal was viewed, whereas in organic materials, individual molecules were implicated in rotation, led Pasteur (1848) to conclude that the individual molecules of (+) and (−)-tartaric acid are structurally 'dissymmetric', related as non-superposable mirror image forms. Later, Pasteur's term 'dissymetrie' for enantiomorphism (Greek enantios morphe, opposite shape), became generally supplanted by 'chirality' from the familiar analogy of the mirror-image relation between the left and the right hand. Pasteur was able to physically separate the isomers of tartaric acid 145 years ago (Pasteur, 1848). The understanding of the three-dimensional geometry associated with isomerism waited a further 26 years for Van't Hoff (1874) and Le Bel (1874) to independently put forward the stereochemical theory of isomerism, based on the concept that carbon and other atoms had four valencies, and that with four different associated groups, it was possible to have two mirror image, non-superimposable molecules centred around an asymmetric carbon atom.

Over the ensuing years, many investigators attempted to understand the importance of those findings, but it was the 'lock and key' theory of Fischer (1894) who suggested that the molecules had to fit into a certain geometric space, that provided the first understanding of stereospecificity of biological action and the concept of receptors. Ever since, the relationship between drugs and their receptors has been characterised as analogous to that between keys and locks, or even hands and gloves. Later, the stereospecific action of adrenaline and atropine were reported. In comparing the pharmacological activity of enantiomers containing a single centre of asymmetry, Easson and Stedman (1933) advanced a three-point attachment model to account for the observed selectivities. A further 20–30 years elapsed before detailed stereoselectivity in kinetics for synthetic opiates (Beckett, 1956) and the phenylethylamines (Beckett and Brookes, 1970) was discovered. Then, apparently, quite suddenly, in the early 1980s, stereochemistry was cast headlong into the world after more than a century's gestation period. Of the different types of stereoisomeric drugs, chiral drugs are the most intriguing, the most important and sometimes the most neglected.

One of the limitations in correlating physical parameters with biologic activity is that selectivity of optical enantiomers for receptors would not be predicted by simple correlative techniques. True enantiomers, for which chirality is detected as optical asymmetry at a single atom, show no differences in physiochemical properties in the absence of a dissymetric surface and thus parameters that predict hydrophobicity or electronic arrangements do not differ between optical isomers. If more than a single centre of asymmetry is present in the molecule (a diastereomer), then physical properties will differ. This is the basis for derivatization of enantiomers to form diasteromers for optical isomer separation. Differences in the pharmacodynamic and pharmacokinetic properties of stereoisomers, molecules differing only in the spatial arrangement of certain atoms or groups at asymmetrical centres, have been described in recent years. More than 400 drugs are administered as racemates (Mason, 1984). Many of these drugs were developed and marketed in the 1960s, but technologies capable of separating isomers of a drug frequently were not developed until the 1970s.

Chirality in Bioactive Agents

Morphologically, nature shows remarkable symmetry, however, at a molecular level it is highly asymmetric (this is to a large extent due to chirality inherent in the carbon atom, one of the main constituents of biological molecules) with the predominance for the synthesis of one isomer in preference to another. This may be due to small energy differences in their stability leading to isomeric enrichment in the natural pool of chemicals (Kondepudi and Nelson, 1985; Mason, 1986). For example (−)-morphine is obtained

from opium, the juice of the poppy, *Papaver somniferum*, while (+)-digitoxin is obtained from the dried leaf of the foxglove plant, *Digitalis purpurea.* In contrast to nature, and without isomeric starting materials or specific chiral synthesis, the chemical synthesis in the laboratory of drugs containing a centre of asymmetry generally yields an optically inactive racemate (50:50 mixture of enantiomers). It has not, however, been possible, until recently, to easily separate the enantiomers. The progress in synthetic enantioselective organic chemistry and in separation techniques has, in recent years, resulted in an improved knowledge of the therapeutic properties of a number of enantiomeric drug substances. In consequence, for many years, over 25% of drugs throughout the world have been used as racemates (Mason, 1984). (Racemic drugs, usually contain 50% or more of an 'inactive' isomer that is presumed harmless.) Why, therefore, has there been this sudden upsurge in interest? Probably, the main reason has little to do with medicine per se, but due to a greater ability of the chemist to actually separate the enantiomers, initially in sufficiently large quantities to undertake pharmacological studies, and to allow measurements for pharmacokinetics analysis, but, later to be able to undertake a larger scale of manufacturing (Campbell, 1990a). Using techniques such as chiral starting materials, chiral reagents, chiral catalysts, chromatographic separation and enzymatic reactions, most enantiomers can now be synthesised, or separated cost effectively, to allow the full development of a single enantiomer. Similarly, using newer analytical techniques with both HPLC and GLC, such as chiral reagents, chiral stationery phases, chiral mobile phases, capillary zone electrophoresis, and possibly chiral detection (Allenmark, 1988, 1989; Drayer, 1988a), it is now relatively easy to separate and accurately measure low levels of enantiomers in body fluids when they are co-administered.

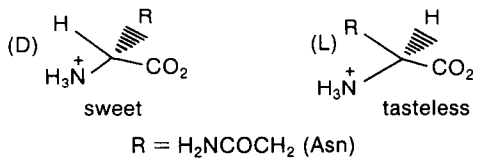

Fig. 1. Difference in our taste perception of enantiomer-discriminating action by the receptor proteins (modified from Allenmark, 1990).

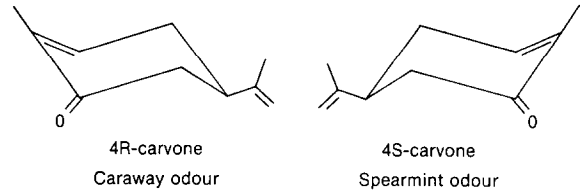

Fig. 2. Difference in our smell perception of enantiomer-discriminating action by receptor proteins to isomers differing only in their steric enantiomorphism (Russell and Hills, 1971).

Enantiomers in a Biological System

The ability, in many cases, of our bodies to distinguish between two enantiomers of a compound was recognised back in 1971, and some well known examples are found in the different smells and tastes of the optical antipodes of the structurally rather simple organic compounds (Russel and Hills, 1971) (Figs 1 and 2). This difference in our perception of enantiomers is thought to be largely a result of the direct enantiomer-discriminating action by the receptor proteins, which means that the chiral binding site of the receptor is preferentially occupied by one of the enantiomers due to a stronger binding interaction (Allenmark, 1990). Thermodynamically, this happens when there is a difference between the optical antipodes in the free energy of association with the proteins, i.e., $\Delta\Delta G \neq 0$. The free energy change, ΔG, associated with a drug-receptor interaction is related to the equilibrium constant, K, for the interaction by the equation:

$$\Delta G = -2.303RT \log K$$

under physiological conditions ($T = 310$ K) this is approximated (in kcal mol^{-1}) by:

$$\Delta G = -1.4 \log K.$$

The observed equilibrium constant thus provides a direct measurement of ΔG. For example, a

drug binding with a K_d of 10^{-4} M requires $(-1.4) \times (-4) = 5.6$ kcal mol^{-1} to dissociate from the receptor (Andrews, 1986).

While such direct effects are conceptually rather simple and easy to comprehend, the situation is often more complex when it comes to different actions of drug enantiomers. Drugs are often considered a single chemical substance, an assumption that is mostly, or always wrong, because chemical purity is an ideal condition that is never attained. Chemicals are often available with different degrees of purity, depending upon use.

Removing impurities from a chemical inevitably increases the price of the substance (Sørensen, 1990). Therefore, as a general rule, the quality chosen for a chemical substance is

TABLE 1

Examples of different pharmacological action of enantiomers

Compound	Effect
Estrone	(+)-form: estrogenic hormone (−)-form: inactive
Thyroxine	(*S*)-form: thyroid hormone (*R*)-form: antihypercholesterinic
Penicillamine	D-form: antiarthritic L-form: extremely toxic
Thalidomide	(*R*)-form: sedative (*S*)-form: teratogenic
Ketamine	(+)-form: dissociative anaesthesia (−)-form: CNS-stimulator
Bupivacaine	(±) form: both isomers are local anaesthetics but only the (−) isomer has an additional vasoconstrictory action, thus prolonging its local anaesthetic action
Timolol	(*S*)-form: correct choice as a β-adrenergic blocker in hypertension, angina pectoris and some arrhythmias (*R*)-form: exerts more localised β-adrenoceptor action, thus improving safety of ocular timolol therapy in glaucoma
Indacrinone	(+)-form: diuretic action and uric acid retention (−)-form: uricosuric effect (antagonizes the side-effect caused by the (+) isomer). The racemic 1:1 proportion of the isomers is far from optimal, a ratio of 1:4, or even 1:8, is preferable if racemates are considered acceptable
Picenadol	(+)-form: opioid agonist action (−)-form: competitive antagonistic action
Hydroxy-*N*-methyl-morphinan	(+)-form: morphine like analgesic action (−)-form: effective antitussive
Ibuprofen (propionic derivatives)	(−)-form: undergoes metabolic chiral inversion to pharmacologically active (+)-form of profens. The (−)-form can be considered as a prodrug of its (+)-form-antipode (+)-form: pharmacologically active with no chiral inversion (see Fig. 3)
Prilocaine (local anaesthetic)	(*S*)-form: is only slowly hydrolysed (*R*)-form: is rapidly hydrolysed forming toluidine which causes methemoglobinemia. This is an example of a stereospecific biotoxication.

Note: Biologic chirality should be distinguished from optical rotation [dextrorotatory *d* (+) or levorotatory *l* (−)] or the *R*, *S* classification of the Cahn-Prelog-Ingold convention (1956). The *d,l* designation relates to rotation of polarized light, which depends on electronic properties around the chiral atom, while the *R,S* classification depends on mass assignments around the chiral atom. Usually, but not always, biologic activities of homologous structures correlate with the direction of light rotation or a particular *R* or *S* notation (i.e., all the *d* (+) enantiomers in a series of congeners are more active than the *l* (−) enantiomers if one compound in the series shows this preference).

sufficiently good for the purpose for which it is being used. Drug substances are mainly pure compounds, their quality being stipulated by the health authorities in the form of specifications or pharmacopeial standards. Many synthetic drug substances contain one or more chiral atoms, resulting in the presence of stereoisomeric forms of the compound. These drug substances have mostly been marketed as a mixture of the stereoisomers due to the technical problems involved in the manufacture of a single enantiomeric form of a compound. Recent progress in enantioselective synthesis and separation techniques, however, has facilitated the study of pharmacokinetics, pharmacodynamics and toxicological properties of potential drug substances (Ariens, 1988).

Pharmacodynamics, involving the induction of effects by interaction of bioactive molecules with the specific site of action, receptors, shows a high degree of stereospecificity, such bioactive molecules include therapeutic agents, pesticides, agrochemicals, etc. The interaction is based on a chemical (thereby implicitly steric), complementarity of drug molecules and specific receptor sites. Distinguishing the isomers as active or inactive is too simplistic. Enantiomers, or diastereoisomers may differ only in activity, but in many cases they actually differ in action and may even behave antagonistically (Ariens, 1984). For a particular (e.g., therapeutic) action, the more active isomer is named the eutomer, and the less active the distomer. The ratio of activity, the eudismic ratio, is an indication of the degree of stereoselectivity (Lehmann, 1976). If the isomers produce different types of action, they are indicated as eutomers or distomers in relation to that particular action. Therefore, chemically, and particularly biologically, enantiomers have to be considered as different compounds, often with greater differences than two homologous agents (Ariens, 1986) (Table 1). In the circumstances outlined in the various examples given in Table 1, complex situations can arise. The 1 : 1 ratio of the enantiomers at the moment of application, in the organism, changes with time. Interaction between the enantiomers also occurs. Sometimes a mixture of four isomers is involved (Ariens, 1986). For the activities of chiral drugs, eudismic ratios in the order of 100 or more are not uncommon. The enantiomers may have similar action, but may differ in affinity for and/or their intrinsic activity on the receptors (e.g., adrenergic agents (Bowman and Rand (1980)). A review of a number of published examples reveals that chiral recognition in pharmacodynamic processes can occur at the binding step, at the activation step, or at both steps (Testa, 1990). An example is provided by the effect of dobutamine enantiomers on α-adrenoceptors in the rat aorta. Both enantiomers were partial agonists, the intrinsic activity of (−)- and (+)-dobutamine being 0.60 and 0.03, respectively. The (−) enantiomer was also 6-times more potent than the distomer. Both enantiomers, however, displayed identical affinity in two functional test models (Ruffolo et al., 1981). In this example, chiral recognition is thus limited to the activation step. No doubt medicinal chemists, pharmacologists, pharmacists, physicians and health authorities in general know this.

The terms 'active' and 'inactive' isomers are misleading (see review by Williams and Lee, 1985). Enantio-specificity in pharmacokinetics arises because enantioselectivity in one or more of the processes of drug absorption, distribution, metabolism and excretion. Stereoselectivity is not an issue with drugs that are passively absorbed by lipid diffusion. Differences in the absorption of enantiomers may arise if they undergo active transport, e.g., DOPA, methotrexate or if they differ in their effects on local blood flow, e.g., some local anaesthetics (Wade et al., 1973; Hendel and Brodthagen, 1984; Tucker, 1990).

Distribution and stereoselective plasma binding have been demonstrated for drugs bound to both albumin (mostly acidic compounds) and to α_1-acid glycoproteins (bases). In most cases the magnitude of the difference varies up to about 1.5. For those drugs which are highly protein bound such as warfarin, phenprocoumon and ibuprofen, a small change in binding can result in a large change in drug clearance (Yacobi et al., 1976). There are suggestions that the more active *S*-isomers of propranolol might be selectively bound to α_1-acid glycoproteins (Walle et al., 1983). *S*-Warfarin is the more potent enantiomer

and is more highly bound to human serum albumin (Sellers and Koch-Weser, 1975). There are two high-affinity binding sites of most drugs to human serum albumin, the warfarin site (or site I) and the benzodiazepine and indole site (site II) (Fehske et al., 1981; Jahnchen and Muller, 1983). Drugs that bind to site II do so in a highly stereoselective manner. For example the essential amino acid L-tryptophan binds to this site with an affinity about 100-times greater than that of D-tryptophan (McMenamy and Oncley, 1958). Similar to this, several chiral benzodiazepine derivatives also interact with this site in a highly stereoselective manner (Jahnchen and Muller, 1983). For example, the d-enantiomer of oxazepam hemisuccinate binds 90% serum albumin compared with only 45% for the levorotatory form (Muller and Wollert, 1975a). Also, the *R* enantiomer of 3-methyldiazepam binds to human serum albumin to a much greater extent than does the *S* form (82% vs 47%) (Alebic-Kolback et al., 1979). Like most of the nonsteroidal anti-inflammatory agents, ibuprofen is marketed as a racemate. The *R* isomer of ibuprofen is inactive and selectively taken up by fat. Protein binding characteristics of the two isomers are also different. At high concentrations, one isomer may displace the other from binding sites. This, however, is not solely a distribution phenomenon since metabolic formation of coenzyme A-thioester is involved (Hutt and Caldwell, 1983).

PROPIONIC ACID DERIVATIVES (IBUPROFEN)

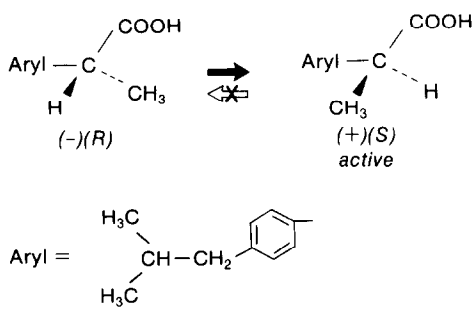

Fig. 3. The unidirectional chiral inversion of ibuprofen is an example of enantioselective pharmacokinetics from the inactive to pharmacologically active form. The anti-inflammatory, analgesic and antipyretic activities of ibuprofen arise from the ability of its *S* (+)-enantiomer to inhibit the synthesis of prostaglandins (Adams et al., 1976). The *R*-enantiomer can be considered as a prodrug of its *S* antipode. Hutt and Caldwell (1983) have reviewed the literature describing this unusual metabolic process.

Stereoselective differences in intrinsic activity and receptor interactions are apparent with adrenergic agents such as labetalol. Labetalol possesses two chiral centres and is a mixture of four isomers that differ in α- and β-receptor blocking activity. The *S,R* isomer contributes most of the α-receptor blocking activity; the *R,R* isomer contributes most of the β-receptor blocking activity and is only a weak α-receptor blocker (Miller, 1989).

Metabolism of enantiomeric drug substances have been exemplified in the case of the antiarrhythmic drug propranolol, a beta-adrenergic blocking agent, the (−) isomer is 100-times more active than the (+) isomer. In addition, the oral clearance of (+) propranolol is 40 to 50% higher than that of (−)-propranolol, thus establishing that propranolol's hepatic metabolism is stereospecific. As a result, racemic propranolol is two to 3 fold more potent after oral than after i.v. doses at equal plasma concentrations (Coltart and Shand, 1970).

This could be an additional factor for variable bioavailability. Up to 24-fold differences between minimum and maximum concentrations within persons receiving 40 mg three times a day are reported (Vervloet et al., 1977). Significant stereoselective hepatic extraction has been also demonstrated for metoprolol and this has been related to the oxidation phenotype. In the study conducted by Lennard et al. (1983) subjects were phenotyped as extensive metabolisers (EM) or poor metabolisers (PM) of debrisoquine. In the EM group, metoprolol was a medium to high clearance drug and the systemic availability of the active *S* enantiomer was 1.4-times that of the less active *R* enantiomer. In the PM group, metoprolol was a low clearance drug, and the systemic availabilities of the enantiomers were equal (see Fig. 4). The manifestation of the extent of enantioselectivity may also be markedly dependent upon the route of drug elimination. Stereoselectivity in the active renal secretion (excretion) of some basic drugs accounts largely for differences

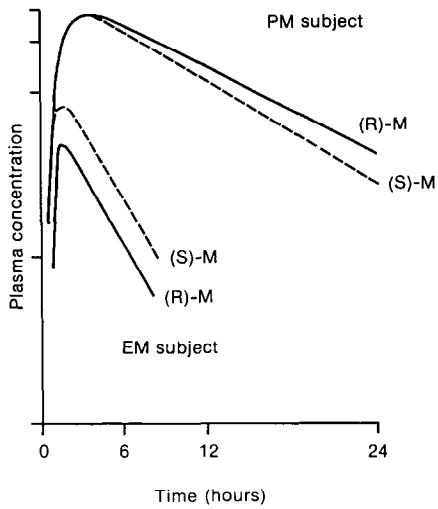

Fig. 4. Log plasma concentration-time curves for *S*-metoprolol (- - - - -) and *R*-metoprolol (———) after oral administration of 200 mg racemic (±)-metoprolol to extensive metaboliser (EM) and poor metaboliser (PM) of debrisoquine (modified from Lennard et al., 1983).

in the renal clearance of the isomers. For example, the renal clearance of metoprolol has been found to be stereoselective for the less active *R* enantiomers (Lennard et al., 1983). Low clearance drugs (e.g., warfarin) with stereoselective differences in protein binding might also be expected to demonstrate stereoselective renal clearance. A dilemma posed for therapy by drugs administered as racemic mixtures is that the enantiomers should be quantitated individually for the following reasons:

(a) The kinetics of the drug enantiomers often differ, leading to interindividual variations in the plasma ratios of the enantiomers;
(b) The pharmacological properties of drug enantiomers are dramatically different; one isomer may be predominantly responsible for the desired therapeutic action and the other for the side effects;
(c) Enantiomers may act as competitive antagonists; may have opposite effects, and have different plasma protein binding.

Measurements of such drugs in biological fluids have nevertheless been based on techniques unable to differentiate between enantiomers. Since the initial 50:50 enantiomeric ratio of racemic drugs may rapidly change to unknown ratios in the body, the value of plasma levels and pharmacokinetic parameters obtained with racemics is highly questionable as such. It is now, however, possible for the first time, to undertake detailed investigations into the stereospecificity and stereoselectivity of kinetics and metabolism (Jamali, 1988; Jamali et al., 1988, 1989; Spahn et al., 1988; Campbell, 1990a; Evans et al., 1990; Tucker and Lennard, 1990).

The important question is whether the enantioselectivity of processes like absorption, distribution, metabolism and excretion is large enough to be of therapeutic significance. To illustrate this the total body clearance values for the individual enantiomers of some drugs are shown in Table 2. The more rapidly cleared enantiomer to less rapidly cleared enantiomer ratio varies from 1.2 to 14. In addition, there is often large interindividual variation in the ratios of the plasma concentration of the drug enantiomers. Examples of pharmacokinetic differences posed by racemic drugs for therapeutic drug monitoring and undesired action are illustrated in Figs 5 and 6.

It is known that for the biological activity of certain agents, e.g., surfactants, chelators, heavy metal carriers, inhalation anaesthetics, radioopaque agents, alkylating agents, etc., chirality is unimportant. If meaningful structure-activity re-

TABLE 2

Total body clearance values for some drug enantiomers in man after racemic drug administration

	Average total body clearance (ml min^{-1})		More rapidly cleared isomer
i	(d) isomer	(l) isomer	less rapidly cleared isomer
Propranolol	1200	1000	1.2
Phenobarbitol	40	32	1.3
Disopyramide	604	401	1.5
Disopyramide	604	401	1.5
Verapamil	800	1400	1.8
Acenocoumarol	35	496	14.0

After Drayer (1988a).

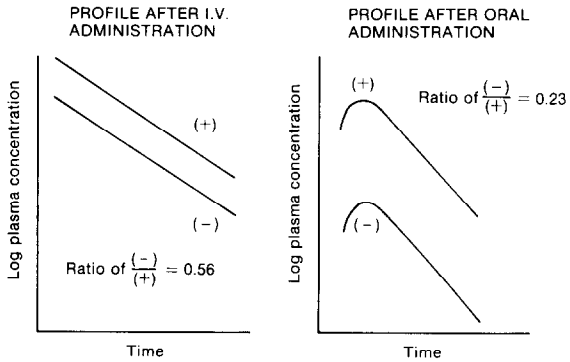

Fig. 5. The (−) enantiomer of the Ca^{2+} channel-blocker verapamil, is mainly responsible for the negative dromotropic activity on atrioventricular conduction. After intravenous dosing of racemic verapamil, the average (−) to (+) enantiomer plasma concentration ratio is 0.56 compared with 0.23 seen after oral administration of equal doses of racemic (±) verapamil. Therefore, after oral administration, the same plasma concentration of total verapamil consists of a two to three times smaller proportion of the more active (−) enantiomer. This is due to stereoselective first pass biotransformation with preferential elimination of the (−) enantiomer. Therapeutic drug level monitoring for verapamil with conventional analytical methods that do not resolve and quantitate the individual enantiomers can be misleading (modified and taken from Walle and Walle, 1986).

lationships are to be established, as long as the three dimensional structures of receptors remain unknown, it becomes indispensable to establish

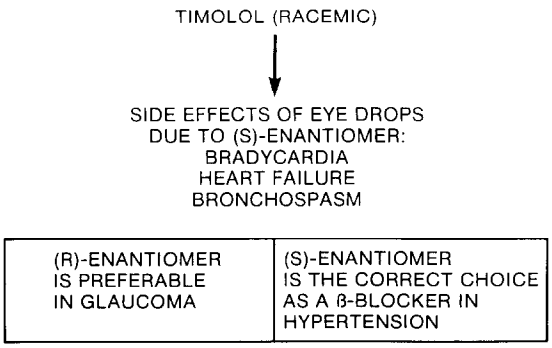

Fig. 6. Timolol has potent β-adrenergic antagonistic activity, whereas the *R* enantiomer is only weak in this respect. This indicates that the *S* enantiomer is the correct choice as a β-blocker in hypertension, and some arrhythmias. The *R* enantiomer exert more localised β-adrenoceptor action, thus improving saftey for the treatment of glaucoma (Richards and Tattersfield, 1985; Karhuvaara et al., 1989).

the 3-fold relation between (i) optical rotation, (ii) relative biological potency, and (iii) absolute configuration.

A recent survey (Lehmann, 1986) of the active principles included in the US Pharmacopoeia XX established that approx. 53 were stereoisomerically pure, compared with about 150 which were not. Of the latter, only some 25 were specified as such! It has been estimated that as much as 90% of the β-adrenergic agents, anti-epileptics, and oral anticoagulants are racemic mixtures, whereas about 50% of the antidepressants and antihistamines are so described (Ariens, 1984). The therapeutic implications of the administration of racemic drugs to patients are profound and are becoming increasingly widely recognised. There are also significant implications for the licensing authority and of course, for the industry itself. In the synthesis of chiral agents, the supposedly inactive isomers are by-products which, at least in the past, have not been easy to avoid or eliminate. Traditional methods for resolving racemates are often difficult, inefficient and expensive. Scientists are responding to the challenge by developing methods for stereospecific asymmetric synthesis of a desired enantiomer and for direct chromatographic resolution of racemates using chiral stationary phases. The aim of drug synthesis is thus to produce as pure a chemical as possible with the desired action.

Enantioselective Drug Analysis

For a profound study of the fate of both enantiomers in the body, it is necessary to determine them side by side in the same sample. Separation of enantiomers traditionally has been considered one of the most difficult problems in separation sciences: in a symmetric environment enantiomers have identical physical and chemical properties, with the exception of the rotation of plain polarized light. Therefore, separation generally requires an asymmetric medium. Consequently, to ensure the safety of new drugs the pharmaceutical community must be able to measure and control the stereochemical composition of drug substances. In this effort, three key areas must be

considered each of which presents specific technical problems (Doyle and Wainer (1985):

(1) Manufacturing, in which problems of physical, preparative-scale separations may be involved;
(2) Quality control (or regulatory analysis), in which analytical questions of purity and stability predominate;
(3) Pharmacological studies of plasma disposition and drug efficacy, in which ultrasensitive methods may be required.

Traditional methods of stereochemical analysis often proved to be inadequate for solving problems in these three areas. For example, the method published in the current US Pharmacopeia (USP XXII) for determining the isomeric purity of dextroamphetamine sulphate tablets requires preparation of an analytical solution of the drug containing 130 mg/10 ml. In this procedure, the equivalent of more than 25 tablets must be ground and triturated with 1 ml methanol. Following this step is a cumbersome deravitisation procedure, physical collection of the entire sample, and measurement of the optical rotation-all for an analysis accurate to only $\pm 12\%$ (USP XXII, 1990).

Although chromatographic techniques can be used for the isolation of the enantiomers and for the qualitative and/or quantitative analysis of the enantiomers in bulk substances, in pharmaceutical preparations, or in biological fluids. Other important methods to obtain enantiomers on a preparative scale are (Jacques et al., 1981):

(1) Isolation of natural products. Nature is still one of the most important sources of enantiomerically pure compounds, e.g., amino-acids, sugar derivatives, quinine, penicillins;
(2) Fractional crystallisation of diastereomeric salts;
(3) Asymmetric synthesis;
(4) Kinetic techniques.

If enzymes or other chiral compounds are allowed to react with the racemates, the possible differences in reaction rate in each of the enantiomers can be used for the resolution. As for enantioselective analysis, chromatographic techniques meet the high analytical demands best, although other methods are known as well (optical rotation). Polarimetry is still the simplest and most universal technique available to determine optical purity. It requires, however, (a) knowledge of the specific rotation of an optically pure sample of the compound and (b) relatively large amounts of substance are required (mg range). On the other hand, since the result and effect of an intrinsic property of the asymmetric molecules (viz., to rotate the plane of polarized light in either direction) is measured, the applicability is unrestricted. Some chiral substances yield a small optical rotation and in such a situation the sensitivity of the method is low, i.e., the change in optical rotation as a function of concentration is small. It must thus be decided, in the individual case, whether the test for optical rotation can be applied to determine the chiral purity of the drug substance or whether the test is more suited as a qualitative identification test.

IR spectrophotometry

IR spectrophotometry has become the principle identification test in specification and pharmacopeial standards. The spectra of enantiomers and a racemic compound most often diverge due to the fact that the environment of one molecule in the crystal of the enantiomer differs from that of the corresponding molecule in the racemic compound (Sørensen, 1990). The differences are primarily seen in the finger print region, 1400–700 cm^{-1}, and if the molecules form intermolecular hydrogen bonds also in the region of about 3000 cm^{-1}. The spectrum of a racemic mixture will be identical with the spectrum of the enantiomer.

X-ray powder diffraction

When a monochromatic X-ray beam interferes with the atom in a crystal, the beam is diffracted in directions that are determined by the dimensions of the crystal unit cell (Sørensen, 1990). A single crystal must be rotated in the X-ray beam in order to fulfil the conditions for diffraction, in contrast to a randomly oriented crystalline powder that displays all the conditions for diffraction at the same time. X-ray powder diffraction is the

optimal identification test with respect to crystalline substances. Thus, the X-ray diffractograms of an enantiomer and a racemic compound will always be distinctly different. Crystals of an *R* and an *S* isomer, respectively, are as mentioned above, mirror images of each other and will therefore give identical X-ray diffractograms, in contrast to a single crystal diffraction measurement where it is possible to determine the absolute configuration of a chiral molecule.

Nuclear magnetic resonance (NMR) spectroscopy

Enantiomeric 'signals' are usually made to appear separately in an NMR spectrum through interaction of the enantiomers with a chiral agent in the solution: a chiral solvent or a chiral shift reagent (Feitsma and Drenth, 1988).

Differential scanning calorimetry (DSC)

A quantitative analysis of the melting process performed in a differential scanning calorimeter (DSC) can, under certain conditions, be applied to analyse the chiral purity of the drug substance. The method is based on the principle that the substance containing impurities will show a wider melting range and a lower melting point than the pure substance (USP XXII, 1990). The method is nonspecific and determines the total amount of impurities having structures related to the principle components. When combined with one or more analytical methods which can determine the amount of impurities present (apart from the enantiomer) the DSC will reveal the amount of enantiomer as the difference between the two sets of data. Purity determination by DSC is limited to substances containing a small amount of impurities, normally 0–3% (Sørensen, 1990).

Enantioselective immunoassays or radioreceptor assays

In these assays the antibodies or receptors usually have a very high affinity to one of the enantiomers, implying that only one of the two is determined (Feitsma and Drenth, 1988).

Isotope labelling

This method may be applied to the determination of enantiomeric purity in two quite different ways. In one of these the labelled racemate is added to an enantiomeric mixture of unknown purity (isotope dilution method). In the other one a labelled pure enantiomer is used (Feitsma and Drenth, 1988).

Use of pseudoracemates

During the synthesis one of the enantiomers is isotopically labelled. The enantiomers can then be determined separately, eg, by mass spectrometry. Strictly speaking, however, an enantiomeric relationship no longer exists: the molecules differ in physical property, especially molecular weight, an isotope effect may occur (Feitsma and Drenth, 1988).

Chromatographic techniques

The development of chiral stationary phases (CSP_s) for gas and liquid chromatography is a dynamic field. Although the chromatographic resolution of stereoisomers is not a recent development, past successes have happened primarily on the diastereomeric approach, rather than on the CSP approach. In the diastereomeric approach, the enantiomeric analyte is derivatised with a reagent that is itself asymmetric and that produces products (diastereomers) that are chemically and physically distinct from each other. The diastereomeric products are then resolved using conventional (achiral) chromatographic techniques. Conversely, in the CSP approach the stationary phase has a chiral component, i.e., the enantiomers are resolved directly (Doyle and

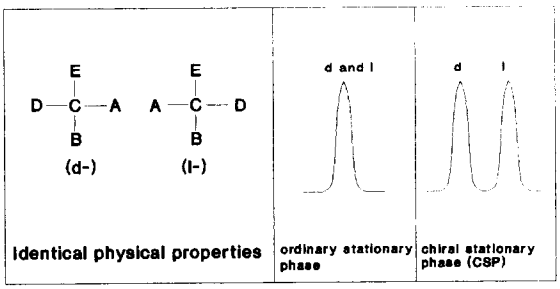

Fig. 7. Stereochemical properties and chromatographic behaviour – on both conventional and chiral stationary phases – of enantiomers.

Wainer, 1985). This distinction between the two approaches is illustrated in Fig. 7. Techniques for direct optical resolution by chromatography are still the focal point of considerable research activity and have been the subject of a number of reviews and monographs (Van der Greef et al., 1987; Allenmark, 1988; Krstulovic, 1989; Lough, 1989). Innovations in the field of stationary phase design have led to new or improved columns of great interest for future application to chiral drug monitoring and related areas. An important group of stationary phases is obtained from chiral biopolymers (carbohydrates, proteins) which have been chemically modified and immobilised to the support or column by chemical or physical means. Although chromatographers have spent much time and effort in their design and development of chiral stationary phases, their applicability to the determination of enantiomers in biological samples has, so far, been of limited value (Allenmark, 1990). The stability and efficiency of the columns are low and often only nonaqueous mobile phases may be used, which is a drawback when working with biological materials. Therefore, the use of chiral reagents to form diastereomeric compounds prior to the chromatographic separation seems at present to be more attractive.

In spite of the problems mentioned with chiral stationary phases much progress has been made in this field recently and it is to be expected that better phases will become available in the future.

Registration

Currently, there is no compendium that lists all drugs given as racemates. The licensing authorities are, at present, discussing the requirements of the necessary documentation for marketing the chiral drug substance, whether enantiomeric or racemic. The EEC guidelines (CPMP, 1988) mention that, in the case of a racemic drug substance already on the market, an application for marketing one of the enantiometers must supply full documentation, thus regarding it, as a completely new substance. In order to market a new racemic drug substance the manufacturer must present the documentation covering both the mixture of isomers and the individual enantiomers. There are, therefore, several questions which need to be addressed in the future development of chiral drugs (Campbell, 1990b).

(1) In future, should all chiral drugs be developed as single enantiomers?
(2) What are the minimum requirements to develop a racemic?
(3) What other types of chiral development are possible?

It is important that these questions are answered with forethought, sensitive understanding, now that the technology to resolve enantiomers is available, it should not be used indiscriminately to increase the efforts of drug development without significantly improving therapy and the risk benefit ratio.

It has been reported that an FDA task force is examinaing the issue of stereoisomerism in drug development and is considering related regulatory issues, such as the requirements for marketing an enantiomer when the drug is already marketed as a racemic. To date the FDA has not produced guidelines for full stereochemical drug development, although information on the synthesis, manufacture and control of chiral molecules has been circulated, with particular reference to, batch to batch variation and impurity levels of the unwanted isomers (FDA, 1987). Following detailed discussion with the Pharmaceutical Manufacturers Association of America (PMA), who have produced a carefully considered draft position paper (Grundfest, 1989) and other expert scientists, it is believed that guidelines will be available in the near future.

It has been realized at the Food and Drug Administration, how difficult it is with respect to time and expense to plan drug development when the FDA concerns relating to approval requirements for stereoisomeric compounds are not clearly stated. To address this issue before a problem arises and to be responsive to inquiries, the Center for Drug Evaluation and Research (CDER) has had in place, since early January 1989, a Stereoisomer Committee, charged with determining what requirements, if any, should be imposed upon a sponsor developing a stereoisomeric compound (Weissinger, 1989).

The following suggests some reason why a

company may develop a racemate in preference to an enantiomer, despite the additional work (Campbell, 1990a):

(a) Too costly separation of enantiomers;
(b) Eutomer racemises in solution;
(c) Activities are different but complementary;
(d) Distomer is totally inverted to eutomers;
(e) Distomer is shown to be totally inactive, but separation is unnecessarily expensive;
(f) If the development of an enantiomeric is excessively long for an urgently needed drug, e.g., cancer, AIDS, etc.

The clinical consequences of stereoselective differences can be important because

(1) So many racemates are currently marketed
(2) Many racemates, in particular, cardiovascular drugs, have narrow therapeutic indexes
(3) Much of the observed intrasubject and intersubject variability in drug effect may be related to differences in disposition of drug isomers.

Failure to account for enantiomeric differences may explain conflicting results of some studies. Thus, stereoisomerism has implications for study design. The consequences of stereoselective differences depend on factors such as the activity of the parent drug, any metabolites that are formed and whether the affected metabolic pathway is the sole pathway, or one of several pathways for elimination.

Those drugs in which the isomers exhibit marked differences in pharmacodynamic response (e.g., verapamil) or pharmacokinetic profiles are of greatest concern. Identifying factors that alter the relative steady state plasma concentration of enantiomers is of primary importance. Such factors can include route of administration, dose, existence of the stereoselective drug-drug or drug-disease interactions, and genetic differences. Interspecies differences may be marked, making extrapolation of animal data to humans difficult. For example, studies quantifying the oral clearance of D-hexobarbital relative to that of L-hexobarbital have produced opposite findings in humans and certain animal species, whereas plasma binding of *R*,*S*-propranolol favours the *S* enantiomer in both dogs and man, the stereoselectivity of metabolism is opposite in these two species (Silber et al., 1982). Such reversed stereoselectivity between animal species and man in chiral drug oxidation has also been seen for other drugs (Wilkinson and Shand, 1975). The reason for such species differences is poorly understood but has to be considered whenever extrapolations from animals to man are considered. Numerous stereoselective drug-drug interactions have been reported, e.g., interaction between warfarin and cimetidine and between enoxacin and warfarin (Miller, 1989).

Manipulation of the enantiomeric ratio or the use of only one enantiomer of a drug may allow separation of toxicity and efficacy, and this may lead to a significant increase in therapeutic ratio and a more rational approach to therapeutics (Williams and Lee, 1985). Separation of individual enantiomers in biological fluids after administration of racemic drugs is no longer an insurmountable problem. Our understanding of the behaviour of the individual enantiomers of racemic drug in the body should greatly improve as should the value of pharmacokinetics in the elucidation of mechanisms of drug actions and prediction of pharmacologic-toxicologic effects. In future, it appears that drug stereoisomerism is likely to be the focus of greater attention in clinical practice, research, and drug regulation.

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