REVIEW ARTICLE

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Impact of Chirality I: A Look in the Mirror from a Pharmacokinetic Perspective

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Over the last decade, the field of chirality has witnessed revolutionary changes with increased emphasis placed on delineation of enantioselective pharmacology, pharmacokinetics and pharmacodynamics. Indeed, there are numerous examples in the literature, which confirm the benefits of recognizing chirality in a drug entity. Based on the current data, therefore, it is important not to ignore chirality of the molecule during the drug development process. Inspite of lack of special regulatory requirements, it appears to be prudent to evaluate very early in the development, the pharmacologic, pharmacokinetic, pharmacodynamic, and toxicologic profiles of the racemate and the individual enantiomers to justify the development of any one form of the chiral drug for human use. In this context, the present article emphasis the impact of chirality in the pharmacokinetic disposition of racemic drugs.

More than sixteen years have elapsed from the time Prof. Ariens wrote in European Journal of Clinical Pharmacology, where he questioned the practice of collecting pharmacokinetic and pharmacodynamic data of racemic drugs without paying attention to chirality.[1] Since that time, the role and therapeutic consequences of chirality in xenobiotics has been increasingly brought to lime light[2-5]. Today, it has almost become a common knowledge that considerations to chirality in a drug molecule are critical in the decision making process of developing a chiral drug candidate[5]. Indeed, the failure to recognize chirality while describing the drug disposition and pharmacokinetic characteristics including pharmacodynamics can result in far reaching untoward effect as exemplified by the toxicity associated with thalidomide[3] and benoxaprofen[4]. The intent of this paper is to provide a comprehensive overview on the role and impact of chirality in the pharmacokinetic disposition of racemic drugs.

BASIC CONSIDERATIONS

Chirality and Stereoisomers:

Many naturally occurring medicinal compounds as well as those man-made exhibit the property of chirality. The term "Chiral" originates from "Chiros", a greek word meaning handed. In simple terms, chirality is a unique structural feature rendering the co-existence of at least two non-superimposable mirror image forms. The non-superimposable forms possess identical physical properties and are described by terms such as stereoisomers, enantiomers, enantiomorphs, and optical antipodes. In most instances, chirality in medicinal compounds is associated with a tetravalent carbon atom attached to four functionally different ligands. When a compound contains two chiral carbon atoms, existence of four possible stereoisomers is possible (general rule for calculating stereoisomers is 2^n, where n=number of chiral carbon atoms in the compound).

Optical Descriptors and Absolute Configuration:

The existence of a chiral center or asymmetry is often associated with the ability of a compound to show optical activity, the inherent property of the compound to rotate the plane of polarized light. Based on the direction of rotation of the polarized light each enantiomer is assigned the appropriate optical descriptor notation: "d" for dextro-rotatory (+) or "l" for levo-rotatory (-). Although optical descriptors help in the identity of the

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stereoisomers, they do not reveal the configuration of a compound.

By definition, absolute configuration denotes the actual physical orientation of the atoms in space. The determination of absolute configuration is cumbersome at times and involves the use of techniques such as X-ray crystallography and enantiospecific synthetic transformations. Once the orientation is established, the labeling of the stereoisomers can be approached by the rules proposed by Cahn et al (1956)[6] and the stereoisomers are denoted by symbols "R" (rectus) or "S" (sinister). Typically, the Cahn-Ingold-Prelog rule, prioritizes the four substituents attached to the chiral carbon atom such that the higher atomic number precedes lower atomic number. After the prioritization of the substituents, the chiral molecule is viewed down the bond from the chiral center to the lowest priority substituent and the direction of the path (top priority substituent-2nd highest priority-3rd highest priority) is assessed to be clockwise (R) or counterclockwise (S).

Living Body and Enantiorecognition Mechanisms:

The living body, comprising several chiral biological macromolecules, provides the necessary environment to elicit stereoselective interactions with chiral drugs. Therefore, a racemic compound when placed in the living body is not recognized as a single compound, but as a mixture of two independent drug molecules with different affinities for metabolizing enzymes and target receptors leading to different pharmacology, pharmacokinetic, and pharmacodynamic profiles. Enantiorecognition mechanisms of interaction of chiral drugs with the living body may be explained by the simple receptor/enzyme three-point attachment model first proposed by Easson-Stedman (1933)[7]. Recently, the classical theory of Easson-Stedman has been revisted and refined[8]. The salient features of the refined hypotheses are: (i) the stereo-electronics of the pharmacophore plays a major role rather than the specific functionality of the binding molecule and (ii) the occupancy of all binding sites is not essential to trigger the activation of enzymes or receptors[8].

ENANTIOSELECTIVE PHARMACOKINETICS

Absorption and Distribution:

The passage of drugs across the membrane matrices is a passive process and therefore, no apparent stereoselectivity is anticipated in the passive absorption process of chiral drugs. However, if the absorption is by an active transport or a receptor-mediated process, it may favor the transport of one enantiomer over the other. In other words, the two enantiomers may exhibit differences in the absorption characteristics when actively transported across the biological membrane. Typical examples of chiral drugs which undergo active absorption process include those of dopa[9], methotrexate[10], and cephalexin[11] (Table 1). The active transport of one enantiomer does not necessarily impose any restrictions on the passive diffusion of its antipode. For example, L- and D-dopa are absorbed to the same extent despite the much more rapid active transport of L-dopa[9]. Recently, a small difference in the absorption rates of the enantiomers of ethotoin has been shown to account for the stereoselective disposition of the drug[12].

In some instances, typically for local anaesthetics such as mepivacaine and bupivacaine, an indirect cause for stereoselective absorption has been observed[13,14]. Owing to their inherent pharmacological activity, mepivacaine or bupivacaine can significantly alter the regional blood circulation and consequently show stereoselective effect in the systemic absorption and the duration of anaesthetic activity of the enantiomers[13,14]. The occurrences of other mechanisms have been reported to account for differences in absorption of drug enantiomers. For instance, the bioavailability of S-(-)-propranolol is greater when administered along with R-(+)-antipode than given alone, indicating that the presence of R-(+)-propranolol enhances the availability of the more pharmacologically active S-(-)-enantiomer[15]. Likewise, the presence of (-)-terbutaline promotes the intestinal permeability and the blood flow thereby increasing the absorption of (+)-terbutaline during co-administration of equal amounts of the two enantiomers[16].

Distributive phase involves (i) partitioning into various body sites, a process in which stereoselectivity is unlikely to be observed, and (ii) binding to chiral macromolecules in plasma and tissues, a process documented to show stereoselectivity. Although stereoselective protein binding of several chiral drugs has been thoroughly investigated, limited information is available regarding the binding of drug enantiomers to tissue components.

Stereoselectivity has been described in the plasma protein binding of acidic drugs which involves albumin binding[17-22] (Table 1) and basic drugs which involves

TABLE 1: EXAMPLES OF STEREOSELECTIVITY IN THE PHARMACOKINETIC PROCESSES

	Pharmacokinetic Process	Substrate(s)	Stereoselective Effect	Reference	No
1.	Absorption				
		DOPA	L-(active transport)	Wade *et al.* 1973	9
		Methotrexate	L-(active transport)	Hendel and Brodthagen, 1984	10
		Cephalexin	L-(active transport)	Tamai *et al.* 1988	11
2.	Protein Binding				
a.	Acidic Drugs	Warfarin	(R;+) > (S;-)	Yacobi and Levy, 1979	17
		Pentobarbitone	(R) > (S)	Cook *et al.* 1987	18
		Ibuprofen	(+) > (-)	Evans *et al.* 1989	19
		Flurbiprofen	(S) > (R)	Knadler *et al.* 1989	20
		Mephobarbital	(R) > (S)	O'Shea and Hooper, 1990	21
		Carbenicillin	(S) > (R)	Itoh *et al.* 1996	22
b.	Basic Drugs				
		Verapamil	(-) > (+)	Bhatti *et al.* 1995	23
			(S) > (R)	Lima *et al.* 1985	24
		Disopyramide	(S;-) > (R;+)	LeCorre *et al.* 1988	25
				Hasselstrom *et al.* 1991	26
		Mexiletine	(S;+) > (R;-)	Kowk *et al.* 1994	27
		Chloroquine	(-) > (+)	Ofori-Adjei *et al.* 1986	28
		Hydroxy-chloroquine	(S) > (R)	McLachlan *et al.* 1993	29
		Ketolorac	(R) > (S)	Hayball *et al.* 1994	30
		Bupivacaine	(S;-) > (R;+)	Burm *et al.* 1994	31
3.	Tissue Binding				
a.	Adrenergic Nerve Cells Uptake	Propranolol	(-) > (+)	Walle *et al.* 1988	34
		Atenolol	(-) > (+)	Webb *et al.* 1988	35
		Ibuprofen	(R) > (S)	Williams *et al.* 1986	36
b.	Adipose Tissue Uptake	Fenoprofen	(R) > (S)	Sallustio *et al.* 1988	37
		Ketoprofen	(R) > (S)	Carabaza *et al.* 1996	38
c.	Salivary Uptake	Tocainide	(R) > (S)	Pillai *et al.* 1984	39
d.	Cardiac Tissue Uptake	Propranolol	(S;-) > (R;+)	Kawashima *et al.* 1976	40
				Takahashi *et al.* 1988	41
		Timolol	(S;-) > (R;+)	Tocco *et al.* 1976	42
e.	Liver Tissue Uptake				
		Mexiletine	(S;+) > (R;-)	Igwemezie *et al.* 1991	43
		Bupivacaine	(R;+) > (S;-)	Rutten *et al.* 1993	44
f.	Brain/lung Tissue Uptake	Bupivacaine	(R;+) > (S;-)	Rutten *et al.* 1993	44
g.	Fetal Uptake	Labetaolol	RR > (SS; SR; RS)	Dorudian *et al.* 1995	45
h.	Red Bood Cell	Verapamil;	(S) > (R)	Mehvar and Robinson, 1995	46

Table 1 (Contd..)

	Pharmacokinetic Process	Substrate(s)	Stereoselective Effect	Reference	No
	Uptake	norverapamil			
		Pimobendan	(-) > (+)	Chu *et al.*.1995	47
i.	Androgen Receptor Binding	Casodex	(R) > (S)	Mukherjee *et al.* 1995	48
j.	Cerebrospinal Fluid Uptake		(S;+) > (R;-)	Bannwarth *et al.* 1995	49
k.	Kidney Uptake		(S) > (R)	Stahl *et al.* 1993	50
4.	Chiral Inversion	NSAIDS	(R) to (S)		
		Ibuprofen		Baillie *et al.* 1989	52
		Fenoprofen		Geisslinger *et al.* 1990	53
				Rubin *et al.*1985	54
		Benoxaprofen		Bopp *et al.* 1979	55
		Ketoprofen		Skeith and Jamali, 1992	56
		CS670 (Oxocyclo-hexylidenemethyl propionic acid)		Asami *et al.* 1996	57
		Suprofen		Shinohara *et al.* 1991	58
5.	Pre-Systemic Hepatic Extraction	Propranolol	(R;+) > (S;-)	Walle *et al.* 1984	34
		Methylphenidate	(S;-) > (R;+)	Srinivas *et al.* 1987	65
				Srinivas *et al.* 1993	66
		Verapamil	(-) > (+)	Vogelgesang *et al.* 1984	67
		Metoprolol	(R;+) > (S;-)	Lennard *et al.* 1983	68
				Jonkers *et al.* 1991	69
		Nivaldipine	(-) > (+)	Tukuma *et al.* 1987	70
		Acebutolol	(R) > (S)	Foster *et al.* 1993	71
		Carvedilol	(R) > (S)	Tenero *et al.* 1996	72
		Zileuton	(R;+) > (S;-)	Braeckman *et al.* 1992	73
		Nicardipine	(+) > (-)	Iwaoka *et al.* 1995	74
		Salbutamol	(S;+) > (R;-)	Boulton and Fawcett,	75
		Nisoldipine	(+) > (-)	1996	76
				Heinig *et al.* 1994	
6.	Renal Excretion				
		Disopyramide	(+) > (-)	Lima *et al.* 1985	24
				LeCorre *et al.* 1988	25
		Chloroquine	(S;-) > (R;+)	Ofori-Adjei *et al.* 1986	28
		Pindolol	(+) > (-)	Hsyu and Giacomini, 1985	81
		Carprofen glucuronide	(+) > (-)	Iwakawa *et al.* 1989	82
		Oxprenolol glucuronide	(R) > (S)	Laethem *et al.* 1993	83
7.	Biliary Excretion				
		Ketoprofen glucuronides	(S) > (R)	Foster *et al.* 1989	88

binding to a-acid glycoprotein binding[23-31] (Table 1). With the notable exceptions of tryptophan and oxazepam[32,33], the magnitude of stereoselective binding affinity of either acidic or basic drugs rarely exceed a factor of two. Nevertheless, small equilibrium shifts in the binding constants of highly protein bound drugs (e.g. ibuprofen, ketoprofen, warfarin) can be critical to the respective kinetic and dynamic profiles, since the unbound fraction is readily available for metabolizing enzymes and also, to trigger pharmacodynamic response by interacting with the target receptors. Furthermore, due to stereoselectivity in protein binding, differences can arise in the total clearances between the enantiomers (e.g. phenobarbital)[18]. On the other hand, stereoselective protein binding can compensate the opposite stereoselectivity observed in clearances of unbound enantiomers, thereby producing identical plasma profiles for the total enantiomers (e.g. disopyramide)[25,26]. Finally, opposite stereoselectivity may be observed in the binding of an enantiomeric substrate to two different plasma proteins (e.g. propranolol)[34].

Only limited data are available in the literature concerning the tissue distribution of enantiomeric drugs (Table 1). A classic example is provided by the stereoselective uptake of (-)-propranolol[34] and (-)-atenolol[35] into the adrenergic nerve cells. Preferential uptakes of R-ibuprofen[36], R-fenoprofen[37], and R-ketoprofen[38] by the adipose tissue have been demonstrated. Stereoselective uptake of R-(-)-tocainide into saliva has been proposed[39]. In addition, preferential uptake of the S-enantiomers of propranolol[40,41], and timolol[42] into cardiac tissue; mexiletine[43] and bupivacaine[44] into the liver tissue and bupivacaine into the brain and lung tissues[44] have been reported. The distribution of labetolol in fetal tissue has been shown to be stereoselective compared to the maternal distribution[45]; S-enantiomers of verapamil and norverapamil are preferentially taken up by human red blood cells[46]; and S-enantiomer of pimobendan is preferentially bound to androgen receptor[47]. The uptake of casodex into cerebrospinal fluid[48], and of ibuprofen[49] and carvedilol[50] into the kidney has been shown to be stereoselective. Although the synovial fluid distribution of ibuprofen favors the S-enantiomer, stereoselective uptake mechanism was discounted since ibuprofen enantiomers show differences in the *in vivo* plasma protein binding[51].

Chiral Inversion:

The R-enantiomers of several non-steroidal anti-inflammatory drugs (examples: ibuprofen, fenoprofen, ketoprofen, benoxaprofen, and suprofen)[52-58], via a thio-ester intermediate, undergo a facile uni-directional inversion into the respective S-antipodes. Reviews on this topic indicate that the magnitude of chiral inversion varies profoundly between the substrates and species examined[59,60]. However, there is some ambiguity regarding the possible *in vivo* site(s) for chiral inversion. For example, liver has been implicated in the chiral inversion of ibuprofen[61,62]; whereas, the involvement of gastrointestinal tract is described for the chiral inversion of benoxaprofen[63]. From a human clinical view point, the chiral inversion of R-nonsteroidal antiinflammatory agents to the respective S-enantiomers can have a significant therapeutic relevance since the pharmacological activity resides in the S-(-)-enantiomers. Therefore, occurrence of chiral inversion complicates the assessment of correlation between the dose of the racemate and clinical response[64]. Furthermore, chiral inversion can have a significant impact on other routes of metabolism. Other clinical situations such as poly-pharmacy and renal/ hepatic impairment may enhance the extent of chiral inversion and warrant attention from a safety point of view.

Pre-Systemic Hepatic Extraction:

The stereoselective differences in the pharmacokinetics of many orally administered racemic drugs (e.g. methylphenidate, verapamil, propranolol, metoprolol, nivaldipine, carvedilol, zileuton, acebutolol, salbutamol, nisoldipine)[34,65-76] (Table 1) are due to enantiomeric differences in pre-systemic hepatic extraction. The extent of stereoselective hepatic extraction is determined by the affinities of the individual enantiomers for hepatic enzymes and protein binding. Several metabolic processes such as hydrolysis, ring oxidation, dealkylation, and glucuronidation have been documented to affect preferentially clearance of one enantiomer over the other. In addition, the first pass clearance or overall metabolism can be significantly affected by age as exemplified by the enantiomers of verapamil[77,78], mephobarbital[79], and hexabarbital[80].

The therapeutic consequences due to stereoselective pre-systemic hepatic extraction are numerous. Firstly, the potential for the inter-individual

variability may hamper the individualization of the clinical response with the administered dose of the racemate. Secondly, the opportunity for stereoselective drug interactions with other orally administered racemic or single-enantiomer drugs may result in serious side effects or adverse reactions. Thirdly, although the drug is administered as a racemate, there is no control on the systemic input of the two enantiomers, which is critical for drugs having a small therapeutic window or for those drug substrates that are subject to polymorphic metabolism. Fourthly, the occurrence of exaggerated pharmacological response due to the stereoselective formation of a first-pass metabolite cannot be undermined. Finally, the plasma concentration-response curves following oral and intravenous administration may be both qualitatively and quantitatively different.

Renal and Biliary Clearances:

Renal clearance may involve one or more processes such as glomerular filtration, active secretion, passive and active reabsorption including renal metabolism. Glomerular filtration, unlike secretory or absorptive processes, may not show enantioselectivity. However, enantioselectivity in glomerular filtration may occur as a consequence of the differences in the protein binding of the enantiomers. The renal clearance of drugs such as disopyramide[25,26] pindolol[81], and chloroquine[28] reflects the involvement of an active secretory process. Recently, glucuronides of carprofen and oxprenolol have been shown to be excreted in urine with considerable enantiomeric distortion[82,83].

The stereoselective pharmacokinetics of homochlorcyclizine[84], hydroxychloroquine[85], and tosufloxacin[86] has been attributed to differences in the renal clearances (i.e. urinary excretion rates) between the enantiomers. In addition to the stereoselective first pass metabolism, renal excretion of acebutolol accounts for the differences in the pharmacokinetics of the acebutolol enantiomers[87].

The transport of drugs or metabolites in bile may involve passive or active transport mechanisms. Due to the difficulties in obtaining the data, the assessment of bile transport of the isomers need to be carefully examined. For example, the stereoselectivity in the transport of drug enantiomers to bile may be a direct reflection of the differences in the plasma levels of the enantiomers. The biliary transport of ketoprofen, in cholecystectomy patients, appears to be consistent with an active process since the concentrations of the enantiomers in the bile were several folds greater than those in the plasma[88]. The biliary elimination of disopyramide and its metabolite, mono-N-deisopropyldisopyramide, has been observed to show opposite stereoselectivity as compared to either metabolic or renal clearance[89].

Enantiomer-Enantiomer Drug Interactions:

Several mechanisms of pharmacokinetic interactions between enantiomers are known to occur, which include: (i) a competition for the plasma protein binding sites, for example ibuprofen[19], disopyramide[90], flurbiprofen[91]; (ii) a preferential substrate stereoselectivity in metabolism, for example, methylphenidate,[66] p-chloroamphetamine[92], propoxyphene[93], and propafenone[94]; (iii) a competition for the active transport processes like tubular re-absorption, for example, terbutaline[16]. Another indirect mechanism for pharmacokinetic interaction is exemplified by racemic propranolol, where the pharmacokinetic parameters of the R- and S-enantiomers are significantly influenced by the pharmacodynamic effect (alteration in the liver blood flow) of the S-propranolol[95-97].

CONCLUSIONS

As illustrated in this article, the chiral nature of the substrate has the potential to account for a substantial alteration in the pharmacokinetics of the chiral drug. Therefore, it is very critical to assess the impact of chirality in the pharmacokinetic dispostion including metabolism aspects, prior to selecting a chiral drug candidate for drug development. If no enantioselective pharmacokinetic data are available for a racemic drug, it may be prudent to examine the pharmacokinetics of inidividual enantiomers to reconfirm the original choice of the chiral molecule.

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