Chiral Inversion of Drugs: Coincidence or Principle?

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Abstract: 2-Arylpropionic acid derivatives are probably the most frequently cited drugs exhibiting the phenomenon that is best known as chiral inversion. One enantiomer of drug is converted into its antipode either in the presence of a solvent or more often in inner environment of an organism. Mechanistic studies of the metabolic chiral inversion were carried out for several drugs from NSAIDs, and a model of this inversion was suggested and subsequently confirmed. The chiral inversion of NSAIDs has been intensively studied in the context of the pharmacological and toxicological consequences. However, the group of NSAIDs is not the sole group of drugs in which the inversion phenomenon can be observed. There exist several other drugs that also display chiral inversion of one or even both of their enantiomers. These drugs belong to different pharmacotherapeutic groups as monoamine oxidase inhibitors, antiepileptic drugs, drugs used in the treatment of hyperlipoproteinemia or drugs that are effective in the treatment of leprosy. Moreover, some chiral or prochiral drugs are metabolized to give chiral metabolites that undergo chiral inversion too, which can have direct impact on pharmacological properties or toxicity of the drug.

As the process of chiral inversion is affected by several factors, so the intensity of chiral inversion of individual substances and at different conditions can differ considerably. Interspecies differences and types of tissue are reported to be the main factors that were recognized to play the key role in the process of chiral inversion. Some of more recent studies have revealed that several other factors, such as the route of administration or interaction with other xenobiotics, can influence the enantiomeric conversion, too.

Chiral inversion does not seem to be a phenomenon connected with only several drugs from some unique group of 2-arylpropionic acid derivatives: it is also observed in drugs with rather different chemical structures and is much more frequent than it can be realized.

Key Words: Chirality, drug metabolism, inversion, enantiomer, stereoisomer.

INTRODUCTION

Symmetry or asymmetry is one of the basic features of geometric shapes with two or more dimensions. Molecules, three-dimensional structures, thus exist in symmetric or asymmetric forms. When molecules have the same structural formulas but differ from each other only in the way the atoms or groups are oriented in space, they are termed as stereoisomers. The substructural feature that gives rise to asymmetry is called the chiral center. One of the most common chiral centers is a tetravalent carbon atom with four different ligands attached to it. Enantiomers are optically active stereoisomers with one chiral center, whereas a diastereoisomer commonly contains at least two chiral centers [1].

Chirality is a prominent feature of most biological macromolecules. When a chiral drug enters an organism, its individual stereoisomeric forms are discriminated in their fate within the organism *via* intrinsic asymmetry of transporters, enzymes, receptors and other biomolecules. The stereoisomers of a drug generally differ in pharmacodynamic action, as well as in pharmacokinetic behavior. Chiral discrimination in drug biotransformation causes probably the most important differences in pharmacokinetic properties of a mixture of stereoisomers. The ratio of stereoisomers is changed mainly by stereospecificity and stereoselectivity of biotransformation enzymes. From the biochemical point of view, the terms stereoselectivity and stereospecificity have different meanings. While the former describes the preference of only one stereoisomer form to be the specific substrate for a given enzyme, the latter indicates the capability of enzymes to preferentially generate only one enantiomeric product [2]. The enzymes stereoselectivity and stereospecificity significantly change the ratio of drug enantiomers as well as metabolite(s) enantiomers in biological systems.

The enzymes can also change enantiomeric composition of drug and/or its metabolites by inversion of one stereoisomer to its antipode with no other change in the molecule. This process is called chiral inversion. Chiral inversion is always mediated by enzymes contrary to chemical isomerization, which is based on configurational instability of stereoisomers. Isomerization is a chemical process that goes on by various mechanisms. When an isomerization produces a racemate it is termed as racemization. Configurational stability depends largely on the structure and the conditions, and it can vary especially with solvent, pH and temperature [3].

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In humans, chiral inversion *via* enzymatic action was first demonstrated with ibuprofen [4, 5]. In *in vivo* studies, high plasma concentrations of *S*-ibuprofen were found after administration of the *R*-enantiomer. The inversion of ibuprofen was estimated to reach 57-71% of the dose [6]. Later it was proved that several other 2-arylpropionic acid (APA) derivatives, the so-called profens, can undergo metabolic chiral inversion [1]. Considerable attention has been paid to the mechanism of the inversion reaction and to its pharmacological and toxicological consequences. Nowadays, the mechanism of chiral inversion of profens is relatively well understood, and the enzymes responsible for it have been isolated and characterized.

It is widely believed that the APAs are the only drugs whose enantiomers are inverted enzymatically in organism, and chiral inversion is often considered to be confined to the closed group of these compounds. But is it true? Many studies have reported that chiral inversion of other drugs not related to profens also takes place in biological systems. These results indicate that chiral inversion could be more common than is generally accepted. But, can all chiral drugs undergo chiral inversion?

The aim of this review is to answer both questions. The data from studies of chiral inversion of various drugs have been collected and evaluated. The factors affecting the results of chiral inversion studies are discussed, and a plausible mechanism, pharmacological and toxicological significances are emphasized.

1. CHIRAL INVERSION OF 2-ARYLPROPIONIC ACID DERIVATIVES

The APAs are a subset of non-steroidal, anti-inflammatory drugs (NSAIDs), which are widely used in the treatment of pain, fever, and inflammation. These compounds exist in two enantiomeric forms due to the presence of an asymmetric carbon atom *alpha* to the carbonyl function. A characteristic feature of these therapeutic agents is their inhibition of cyclooxygenases (COXs), key enzymes in prostaglandin biosynthesis. Most APAs are administered as racemates. An important pathway in the metabolism of 2-arylpropionic acids is the chiral inversion in which the *R*-(–)-enantiomers, which are inactive in terms of COX inhibition, are inverted to the COX-inhibiting *S*-(+)-enantiomer [4, 5, 7, 8]. Both enantiomers of profens also diffuse to the brain where they exert their antipyretic and analgesic functions [9].

1.1. Mechanisms of Inversion

The molecular mechanism of chiral inversion of profens involves three steps:

1. Stereoselective activation of *R*-(–)-APA by formation of the acyl-CoA thioester in the presence of coenzyme A (CoA), ATP and Mg^{2+};
2. Enzymatic epimerization of the *R*-thioester to the *S*-(+)-thioester;
3. Release of the free active *S*-(+)-enantiomer by hydrolysis of the thioester [7, 10-12], Fig. (**1**).

The rate of inversion is substrate and species dependent [15]. Berry and Jamali [16] demonstrated that the liver is the most important organ in the development of this mechanism. Stereoconversion also takes place in the intestine, kidney, lung, muscle, the brain and fat [17, 14].

The chiral inversion may be of toxicological significance, because the intermediate acyl-CoA thioester can modulate lipid metabolism. In addition, the acyl-CoA thioester can inhibit mitochondrial β-oxidation of fatty acids, favoring the development of microvesicular steatosis [18], it can be incorporated into triglycerides, altering plasma membranes and second messenger mechanisms [19], and it can lower serum lipid levels [20]. Consequently, the rate of chiral inversion of *R*-profens may be affected by compounds that influence the intracellular concentrations of CoA [14].

1.2. Enzymes

The identity of the enzymatic systems involved in this biochemical pathway is beginning to be understood and the tissue and cellular distribution characterized. Chiral inversion has been investigated in cellular homogenates, subcellular fractions, whole isolated cells, and whole perfused organs. Activities of these enzymes are found in most subcellular compartments such as mitochondrial, microsomal and cytosolic fractions. Thus, long-chain acyl-CoA synthetases are mainly localized in microsomes [21] whereas epimerases are present either in mitochondrial or cytosolic fractions with a higher specific activity in cytosol [22,8]. Long-chain acyl-CoA hydrolases are abundant in the cytosol [23,24].

Long-chain acyl-CoA synthetases (EC 6.2.1.3, ACSs) catalyze the formation of acyl-CoA thioesters from fatty acids, ATP and CoA. In addition to the role of ACS in the

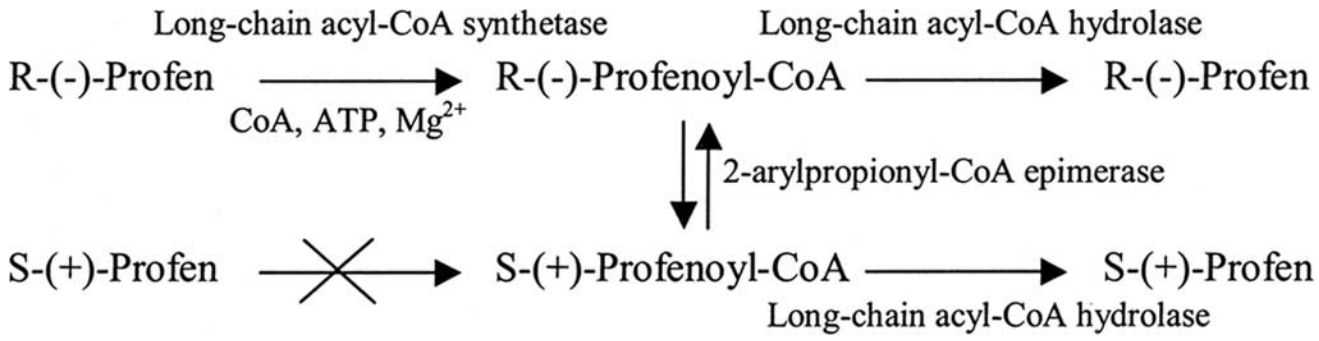

Fig. (1). Mechanism of inversion of APAs (according to [8, 13, 14]).

uptake of long-chain fatty acids and in their activation to acyl-CoA, evidence is now available to suggest that these enzymes are able to accept xenobiotic carboxylic acids as a substrate [25, 21, 26]. Some of the APAs are subject to a substantial unidirectional inversion of the inactive *R*-enantiomer to the active *S*-enantiomer. The molecular basis for this mechanism invokes the stereoselective formation of the acyl-CoA thioester of the APAs. It is presumed that the key enzyme catalyzing the stereoselective formation of the *R*-profenoyl-CoA in an Mg^{2+}- and ATP-dependent process is a long-chain ACS [21, 17, 26]. The unidirectional formation of the respective adenylates with *R*-(–)-enantiomers is the stereoselective step of inversion.

In the following reaction, the resulting *R*-profenoyl-CoA thioester is epimerized *via* a cytosolic and mitochondrial enzyme, the 2-arylpropionyl-CoA epimerase (ACE). The molecular biology of the epimerization pathway is largely unknown. To clarify this mechanism, the sequence of the ACE was identified, and the enzyme cloned and expressed [8]. The ACE was isolated from the cytosolic and mitochondrial fraction of rat liver and has been characterized biochemically [22]. The purified enzymes catalyze the epimerization of various 2-arylpropionyl-CoAs with some degree of stereochemical differentiation. Reichel *et al.* [27] raised polyclonal antibodies against the epimerase and analyzed the relationship between the inversion activity in various tissues and the tissue distribution of the protein in guinea pigs and rats. Significant amino acid sequence similarity was found between the rat epimerase and carnitine dehydratases. In addition to its obvious importance in drug metabolism, the homology of the epimerase with carnitine dehydratases from several species suggests that this protein, which up to now has only been characterized as having a role in drug transformation, takes part in lipid metabolism [8].

Hydrolysis of the fatty acyl-CoA thioesters proceeds in a non-stereoselective manner [28]. Long-chain acyl-CoA hydrolases (EC 3.1.2.2, ACH) catalyze hydrolysis of these thioesters [23]. A long-chain acyl-CoA hydrolase was obtained from rat liver microsomes [29] and mitochondria [30] and purified. Two long-chain acyl-CoA hydrolases, referred to as ACH1 and ACH2, were purified from the liver cytosol of rats [31]. Both enzymes were active toward fatty acyl-CoAs with the chain lengths of C12-16, but ACH1 had relatively broad specificity, as acyl-CoAs with C8-18 were good substrates. Several properties of ACH1 and ACH2 are distinct from those of mitochondrial and microsomal hydrolases. Immunoblot analysis suggested the presence of the enzymes also in extrahepatic tissues, especially in the brain and testis (ACH1), and in the heart and kidney (ACH2). Yamada *et al.* [24] purified this enzyme, referred to as ACH1, from the rat brain cytosol. Northern blot analysis showed that ACH mRNA was expressed constitutively in the rat brain and testis, whereas its expression in the liver was inducible by treatment with the peroxisome proliferator. This study demonstrated the molecular diversity of ACH and suggested the presence of tissue-specific mechanisms to regulate the ACH gene expression. The fact that thioesterase activity is very high in the brain suggests that this may be important for the central analgesic activity of NSAIDs of the aryl propionate class.

These data taken together certify the central role of ACS as a controller of the extent and stereochemical outcome of APA epimerization reactions, although the epimerase is responsible for inversion [32]. When epimerization is observed *in vivo*, its rate and extent vary among animal species, but it always appears to be the slowest in humans. Wechter [33] summarizes and comments on the current understanding of both the biochemical and clinical implications of the chiral inversion of *R*-aryl propionic acid class NSAIDs to the respective *S*-enantiomers in humans.

1.3. Pharmacological Consequences

An unusual phenomenon with 2-arylpropionic acids is the unidirectional metabolic chiral inversion of the *R*-enantiomer to the *S*-form [33-37]. A common structural feature of APA NSAIDs is the sp^3-hybridised tetrahedral chiral atom within the propionic acid side chain moiety. Stereospecificity can be observed in pharmacokinetic processes, particularly in those that utilize a carrier protein, receptor or enzyme. In addition, stereoselectivity occurs in pharmacodynamic processes, and the differences between enantiomers can be either qualitative or quantitative. Data on the inversion mechanism suggest that the *R*-enantiomers may be stereospecifically activated *via* their respective adenylates to CoA thioesters, whereas the *S*-enantiomers are not substrates for this adenylate formation [22, 27, 38-41].

Inhibition of prostaglandin synthesis *in vitro* shows that activity of APAs resides almost exclusively in the *S*-(+)-isomers. However, this stereoselectivity of action is not manifest *in vivo*, due to the unidirectional metabolic inversion of the chiral center from the inactive *R*-(–)-isomers to the *S*-(+)-antipodes [42]. The stereochemistry of the chiral center of these acids also influences other aspects of their disposition, including the oxidative metabolism of the aryl/arylalkyl moiety, glucuronidation of the carboxyl-group and plasma protein binding.

The pharmacokinetics in terms of absorption, distribution, metabolism, protein binding and elimination may be different for the 2 enantiomers, leading to interindividual variability in clinical response and drug toxicity [43]. When no chiral inversion was determined, it was assumed that either there was no chiral inversion or that the elimination of the drugs preceded the inversion, as it is the case e.g. with tiaprofenic acid in man [44].

On the basis of the extent and direction of chiral inversion, all APAs can be divided into five groups:

(I) Unidirectional chiral inversion

(II) Unidirectional or no chiral inversion

(III) Unidirectional, bi-directional or no chiral inversion

(IV) Bi-directional or no chiral inversion

(V) No chiral inversion

1.4. Group I: Unidirectional Chiral Inversion

Ibuprofen is the most extensively studied example of unidirectional chiral inversion in different animal species and man [4, 5, 7, 11, 12, 28, 45-50]. It represents a classical example of a drug where stereochemical considerations

are essential for understanding its biological properties. Ibuprofen exhibits marked stereoselectivity in its pharmacokinetics.

Ibuprofen is most often administered orally, but it has also been administered topically, intraocularly, intravenously, intramuscularly and rectally. Longer residence time in the gastro-intestinal tract may allow more pre-systemic inversion [51, 52, 53]. Some 75% of the dose in healthy volunteers was recovered in urine over 24 h as ibuprofen, 2-hydroxyibuprofen and carboxyibuprofen, both free and conjugated with glucuronic acid. Analysis of the stereochemical composition of the urinary excretion products indicated that 68% of the dose of *R*-ibuprofen had undergone chiral inversion. Metabolism *via* glucuronidation and both routes of oxidation (hydroxy and carboxy metabolites) showed enantioselectivity for *S*-ibuprofen [54]. Taurine conjugation represents only a minor biotransformation pathway for ibuprofen [55].

As the anti-inflammatory, anti-thrombotic and analgesic effects of most chiral NSAIDs are attributed to the *S*-enantiomers, the *R*-enantiomers are considered as useless and potentially harmful ingredients. However, in the case of ibuprofen the *R*-enantiomer acts as a prodrug because it is bio-inverted to *S*-ibuprofen. Nevertheless, it has been suggested that by marketing stereochemically pure *S*-NSAIDs, it may be possible to circumvent potential toxic effects ascribed to the "inactive" *R*-enantiomers [42]. It has also been suggested that as a prodrug the *R*-enantiomer may be a safer alternative to the available racemate [56].

Clinical data [57] have confirmed that *S*-ibuprofen inhibits the activity of COX 1 and COX 2 equally. *R*-Ibuprofen inhibits COX 1 less potently than *S*-ibuprofen and demonstrates no inhibition of COX 2. Conversely, *R*-ibuprofenyl-CoA thioester inhibition of COX 2 is greater than that of COX 1. These data suggest a contribution of *R*-ibuprofen to therapeutic effects not only *via* chiral inversion to *S*-ibuprofen but also *via* inhibition of COX 2 mediated by *R*-ibuprofenyl-CoA thioester. These findings are consistent with racemic ibuprofen being one of the safest NSAID drugs used clinically. The site of temperature reduction by ibuprofen is probably within the brain. During the inversion of the *R*-enantiomer, the lipophilic *R*-ibuprofenyl-CoA may pass through the blood-brain barrier more easily than the *S*-ibuprofen, and once in the brain, it may be converted into the active *S*-ibuprofen. The 2-arylpropionyl-CoA epimerase mRNA and protein have been localized into the brain in animal studies [8, 58]. In such a case, *R*-ibuprofen may contribute to temperature-reducing, analgesic effects of the administered racemic ibuprofen, even if it itself is "inactive".

S-Ibuprofen shows appreciably higher solubility, a lower melting temperature, higher dissolution rates and a different crystal structure compared with the racemic form. Therefore, a formulation of *S*-ibuprofen may have a more rapid absorption and physiological availability, which could produce a shorter period for pharmacological effects to occur [59]. *S*-Ibuprofen has become clinically available in Austria since 1994. A post-marketing surveillance study in 1400 patients demonstrated high effectiveness with a markedly low adverse event potential [48].

In addition to a pivotal role in the overall inversion process, the formation of CoA intermediate has led to incorporation of *R*-ibuprofen into adipose tissue triglycerides. Clofibric acid treatment induces a number of hepatic enzymes associated with fatty acid metabolism including the microsomal long chain CoA ligase and various acyl-CoA hydrolases and could modulate the chiral inversion of *R*-ibuprofen by virtue of either inducing both ligase and hydrolase activities and/or increasing synthesis of cofactor [47, 60]. Pretreatment of rats with clofibrate significantly increased the concentrations of ibuprofen in fat, lung, brain and liver tissue [61]. Clofibrate also altered the stereoselective disposition of ibuprofen in healthy volunteers by increased formation of *R*-ibuprofenoyl-coenzyme A rather than by an effect on oxidative metabolism of ibuprofen [62]. Gender and oral contraceptive steroids have little or no effect on chiral inversion of *R*-ibuprofen to the pharmacologically active *S*-enantiomer [63].

Many other profens undergo unidirectional chiral inversion. Fenoprofen undergoes *in vivo* unidirectional chiral inversion with the inactive *R*-isomer converted into its active antipode. The chiral inversion of fenoprofen has been studied in many species including humans. The results show large interspecies variations in the magnitude of inversion related to the expression of long-chain ACS. This inversion was studied in rat [16, 20, 64, 65], guinea pig [14, 65], cat [66], sheep and dog [65], horse [67], and humans [68, 69]. Fenoprofen is inverted presystemically as well [70]. The three-step mechanism was observed in brain subcellular compartments leading to a minor chiral inversion of fenoprofen compared with that in liver [17].

R-Benoxaprofen is stereospecifically inverted to the *S*-enantiomer by rats [71] and humans [72]. The rate of inversion is much higher in rats than in humans. Inversion in rats apparently does not occur in the liver, but can be brought about *in vitro* by an everted intestinal sac preparation, suggesting that the transformation takes place while passing through the gut wall [72]. Structurally very similar to benoxaprofen is flunoxaprofen, which has fluorine instead of chlorine atom in its molecule. Flunoxaprofen also undergoes unidirectional chiral inversion in rats [73], rabbits and humans [74].

Studies in mice revealed that pranoprofen acyl glucosides are formed *in vitro* and *in vivo*, and their formation is administrative, route and dose dependent as well as stereoselective [75]. While glucuronidation occurs mainly in the liver, the kidney was found to be preferential site of glucosidation favoring formation of the glucoside of *S*- as compared with *R*-pranoprofen. An appreciable enrichment in the *S*-enantiomer was observed after incubation of both racemate and *R*-indoprofen with rat liver enzymes [76].

The *rac*-2-[4-(2-oxocyclohexylidenemethyl) phenyl] propionic acid (CS-670), is a new pro-drug anti-inflammatory agent of the APA type. Stereoselective biotransformation of the pro-drug to pharmacologically active forms in humans and rats is related to reduction of the oxocyclohexylidene moiety [77]. The carboxyl group must be activated to the CoA thioester by acyl CoA ligase when compounds containing a carboxyl group are metabolized to the amino acid conjugate. As the CoA thioesters are obligate intermediates

for amino acid conjugation, the 2*S*-enantiomer of the *trans*-OH metabolite serves as a substrate for canine ACS, as well as the 2*R*-enantiomer, but only the CoA thioester with the 2*S*-configuration is a substrate for taurine *N*-acyl transferase. It is worth mentioning that these results are not consistent with the chiral inversion mechanism by which the 2*R*-enantiomers of profen NSAIDs are stereospecifically converted to CoA thioester intermediates [78].

1.5. Group II: Unidirectional or No Chiral Inversion

Inversion process is species and substrate dependent. Flurbiprofen does not appear to undergo enantiomeric inversion in humans [79,80]. Pharmacokinetics of this drug shows the stereoselectivity characteristic of a number of NSAIDs. However, flurbiprofen is extensively metabolized *via* hydroxylation and methylation products, all of which retain the chiral center. The major metabolites in humans are the hydroxy and methoxy derivatives. Stereoselectivity is exhibited at the level of protein binding and metabolite formation. Hence, the data obtained by using non-stereoselective assays may not be used to explain the pharmacokinetics of the individual enantiomers. Flurbiprofen is eliminated after it has undergone extensive biotransformation to acylglucuronides. The conjugates are excreted in urine, and approx. 20 % of flurbiprofen is eliminated unchanged [81].

In animal species, inversion of *R*-flurbiprofen to its optical antipode occurred to different extents in the dog and in the guinea pig, but only to a negligible degree in the rat [80, 82-84]. The inversion process takes place in a variety of cell types, including hepatocytes, mesangial cells, and glia cells [80].

R-Flurbiprofen, which is not an inhibitor of prostaglandin synthesis *in vitro*, had only marginal anti-inflammatory effects (as defined by the carrageenin edema of the rat paw) in contrast to the *S*-enantiomer. However, both enantiomers showed similar potency as antinociceptive drugs in the rat. Application of the pure enantiomers of flurbiprofen makes it possible to establish a more specific drug treatment: the *R*-enantiomer in occasional pain, the *S*-enantiomer in rheumatic disorders [80, 82].

In average, 7% of an administered dose of the *R*-isomer of suprofen was stereospecifically inverted to the *S*-isomer in humans [85], but no chiral inversion was found in the cat [86]. In previous studies carried out with fenoprofen, it was observed that the cat is capable of performing unidirectional *R* to *S* chiral inversion to a degree similar to that observed in the dog [66, 67]. Therefore, the lack of chiral inversion of suprofen is not due to a metabolic characteristic of this species. The results might suggest that suprofen is not a substrate of acyl CoA ligase in the cat, and the formation of the *R*-suprofenyl CoA intermediate thioester that leads to chiral inversion would not take place [86].

Wide species variation has been observed in the extent of inversion of naproxen. The enantioselective glucuronidation of naproxen was investigated *in vitro* with immobilized microsomal protein from human, rhesus monkey, and rabbit liver as the source of UDP-glucuronosyl-transferases. The glucuronidation of *S*-naproxen by human liver enzymes was inhibited in the presence of *R*-naproxen and vice versa [74]. The clearance of *S*-naproxen was similar to the value for *R*-naproxen in the rat. The *R* to *S* inversion ratio for naproxen was 0.02 [73]. Naproxen is marketed as the *S*-enantiomer [87].

1.6. Group III: Unidirectional, Bi-directional or No Chiral Inversion

The inversion has often been considered to be unidirectional, transforming the *R*-enantiomers to their *S*-antipodes. But reverse reaction might occur with some NSAIDs in some species. The rate of this inversion is very low, almost insignificant [43]. Ketoprofen is an example of both types of chiral inversion, and in humans this drug is not inversed.

Presystemic gastrointestinal and systemic inversions of *R*- to *S*-ketoprofen, extensive elimination of drug through bile, and extensive re-absorption following the biliary excretion were found in rats [88-92]. Unidirectional chiral inversion was further observed in dogs, sheep [93, 94], cattle [95, 96] and horses [97-101].

Differences in the chiral inversion of *R*-ketoprofen in cattle of different ages could be explained by different expressions of ACS, related to the lipid levels in the diets. Dietary factors and xenobiotics have the capacity to influence the formation of both endogenous and xenobiotic acyl-CoA [96].

The *S* to *R* chiral inversion, on the other hand, is rare and has been observed for ketoprofen enantiomers in CD-1 mice. After the administration of single doses of racemic ketoprofen or its optically pure enantiomers to male CD-1 mice, substantial chiral inversion in both directions was found [102].

R-Ketoprofen does not undergo a substantial metabolic inversion in humans [90, 103, 104].

1.7. Group IV: Bi-directional or No Chiral Inversion

There are two representatives in this group: tiaprofenic acid and 2-phenylpropionic acid.

After administration of *R*-tiaprofenic acid to rats it was found that inversion was bi-directional but in favor of inversion of *R*- to *S*-enantiomer. Bi-directional inversion was also observed when tiaprofenic acid enantiomers were incubated with rat liver homogenates. Chiral inversion of tiaprofenic acid may involve metabolic routes different from those associated with inversion of other APAs such as ibuprofen [105].

2-Phenylpropionic acid was isomerized slowly but significantly in rat liver slices. Kidney slices showed an about 3-fold higher isomerizing activity toward *R*-(–)-2-phenylpropionic acid than liver slices. The *S*-enantiomer was also slightly isomerized to *R*-enantiomer of 2-phenylpropionic acid in kidney slices. The liver and the kidney were considered to be the major organs for isomerization of 2-phenylpropionic acid in rat, since the isomerizing activity of the small intestine was low and other tissues examined were inactive [34, 36, 106]. Tanaka *et al.* [107] observed a significant extent of chiral inversion in both directions following administration of 2-phenylpropionic acid to dogs.

In contrast to the rat, and in contrast to most APA anti-inflammatory drugs, tiaprofenic acid enantiomers do not have different disposition kinetics, and the metabolic chiral inversion of one to the other does not occur in humans or it is so slow that the drug is eliminated before significant inversion takes place [36, 44]. A new direct stereospecific HPLC method was developed to assay tiaprofenic acid enantiomers in plasma. No chiral conversion was found when a stereochemically pure enantiomer of tiaprofenic acid was administered to 4 healthy volunteers [108].

Similarly, no inversion was detected, when 2-phenylpropionic acid was administered to mice [36].

1.8. Group V: No Chiral Inversion

In an *in vitro* experiment using everted rat jejunum, no chiral inversion of pirprofen was discernible. The drug was marketed as the racemate prior to its removal from the pharmaceutical market due to several reported cases of hepatotoxicity [109].

Carprofen showed no metabolic chiral inversion in rats [73] and dogs [93, 110]. Studies in humans suggest similar results [73, 111]. Chiral inversion did not occur in either direction.

It is generally known that there are large inter-species differences in chiral inversion of various NSAIDs. The existence of these differences gives rise to at least two important issues. The choice of animal species that can be used in the research of drugs designed for human therapeutics: the most pertinent animal species should demonstrate an enantiomeric plasma profile closest to that observed in man. For application in veterinary therapeutics, a careful balance must be established between the requirement of favorable bio-availability of the active S-(+)-enantiomer and the potential of any possible chiral inversion of R-(–)-enantiomer to generate hybrid molecules in meat and milk which in turn may lead to residues, the toxicity of which is still unknown to the human consumer.

1.9. Adverse Responses to NSAIDs

However, over the past 3 decades there has been a dramatic increase in the number of NSAIDs available for the treatment of postoperative pain but, on the other hand, they have numerous adverse effects as well. NSAIDs can damage gastrointestinal mucosa, impair renal function and may be associated with an increased risk of postoperative hemorrhage. They can also provoke acute asthma in susceptible patients. However, gastrointestinal adverse effects, such as dyspepsia with a tendency to gastric erosions and ulceration, are the main adverse effects of NSAIDs. Non-gastrointestinal adverse reactions to NSAIDs (i.e. renal, hepatic, allergic and hematological) were less common [112].

Some of NSAIDs were withdrawn from the pharmaceutical market due to several reported cases of hepatotoxicity and nephrotoxicity. Examples of withdrawals include benoxaprofen, indoprofen, suprofen, flunoxaprofen and pirprofen.

1.10. Structure-Inversion Relationship

The fungus *Verticillium lecanii* was shown [113, 114] to be capable of inverting the chirality of ibuprofen and 2-phenylpropionic acid from the *R*-enantiomer to the corresponding *S*-antipode. *V. lecanii* is capable of inverting the chirality of all the APAs investigated (ibuprofen, ketoprofen, indoprofen, suprofen, flurbiprofen and fenoprofen), together with the structurally related compounds (2-phenylbutyric acid, 2-phenoxypropionic acid, mandelic acid, atrolactic acid, etodolac and 2-methoxyphenylpropionic acid). All of them were inverted in the *R* to *S* direction with the exception of ketoprofen, where inversion was observed in both directions. The application of the structurally related compounds as substrates showed that the size of the alkyl substituent at the alpha-carbon at the methyl group, and the presence of the methyl group at the chiral center were critical [115].

2. CHIRAL INVERSION OF OTHER DRUGS

Until now, many drugs or potential drugs not belonging to APAs have been reported to undergo chiral inversion. The aim of this section is to collect data about the greatest possible number of these drugs and to show possible mechanisms of their chiral inversion.

The first information regarding the chiral inversion of mandelic acid enantiomers was published by Kenyon and Hegeman in 1970. This compound has been known to undergo chiral inversion in bacteria (from *S*-enantiomer to its *R*-antipode) [116], Fig. (**2**), and has also been observed in rodents [117]. The mechanism of this inversion appears to be unrelated to the inversion of the APA derivatives.

Unidirectional chiral inversion in rats *in vivo* was reported in the case of stiripentol, an antiepileptic agent [118]. Actually, the chiral inversion of stiripentol should have been reported there as bi-directional, despite the relatively large difference in the extent between the two directions. Zhang *et al.* [119] reported mechanistic studies on this chiral inversion in rats *in vivo*. They suggested that chiral inversion of stiripentol must involve cleavage of the C–O bond at the asymmetric center. On the basis of MS studies with radiolabeled stiripentol and its saturated

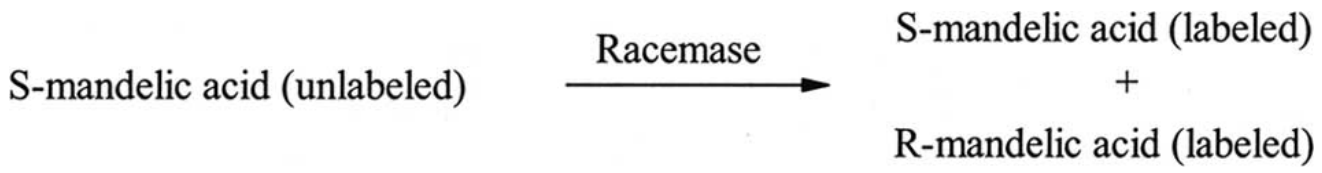

Fig. (2). Mandelic acid racemase in tritiated water converted unlabeled *S*-enantiomer to the tritium-labeled *S*-enantiomer and its tritium-labeled *R*-antipode.

analogs, they suggested a possible mechanism for the metabolic chiral inversion of *R*-stiripentol Fig. (**3**). Unfortunately, the authors' attempts at identifying the respective conjugate have not brought final results yet.

Unidirectional chiral inversion of anti-rheumatic drug KE-298 was observed in rats. Chiral inversion is reported for *R*-(–)-KE-298 or its active *S*-methyl metabolite *R*-(–)-KE-748 in rats after oral administration [120]. The same authors also found a relationship between the metabolic chiral inversion and chemical structure of derivatives of KE-298 in rats [121]. Finally, Yoshida *et al.* [122] proposed a scheme for the chiral inversion mechanism of KE-748, which differs in the localization and enzymes, involved from those in APAs Fig. (**4**).

Another example of drug with massive chiral inversion is NSAID flobufen. The chiral inversion of flobufen enantiomers to their antipodes was observed *in vitro* in rat hepatocytes after incubation of the suspension [123]. Similar *in vitro* inversion was observed later in primary cultures of hepatocytes in three species of ruminants [124], in guinea pigs [125], and also in male pigs [126]. Kral *et al.* [127] also reported chiral inversion in guinea pigs *in vivo*. Chiral inversion is not the same in all these cases, but individual species differed in direction and rate of this inversion. In more detail, the chiral inversion of flobufen was studied in primary culture of hepatocytes in man [128]. On the basis of this study, it was suggested that not only chiral inversion of flobufen enantiomers, but also chiral inversion of 4-dihydroflobufen stereoisomers takes place Fig. (**5**).

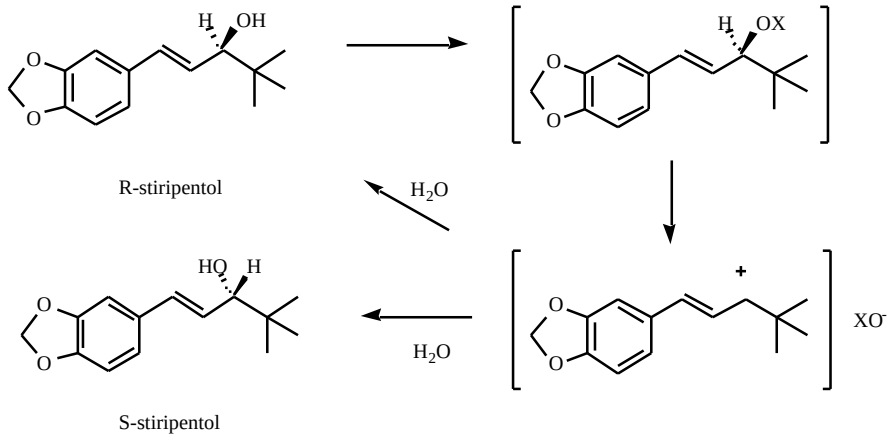

Fig. (3). A conjugation of stiripentol leads to an intermediate that collapses to a symmetrical resonance-stabilized carbocation, which, in turn, is trapped by water of the medium to generate a mixture of *R*- and *S*-enantiomers of the parent drug.

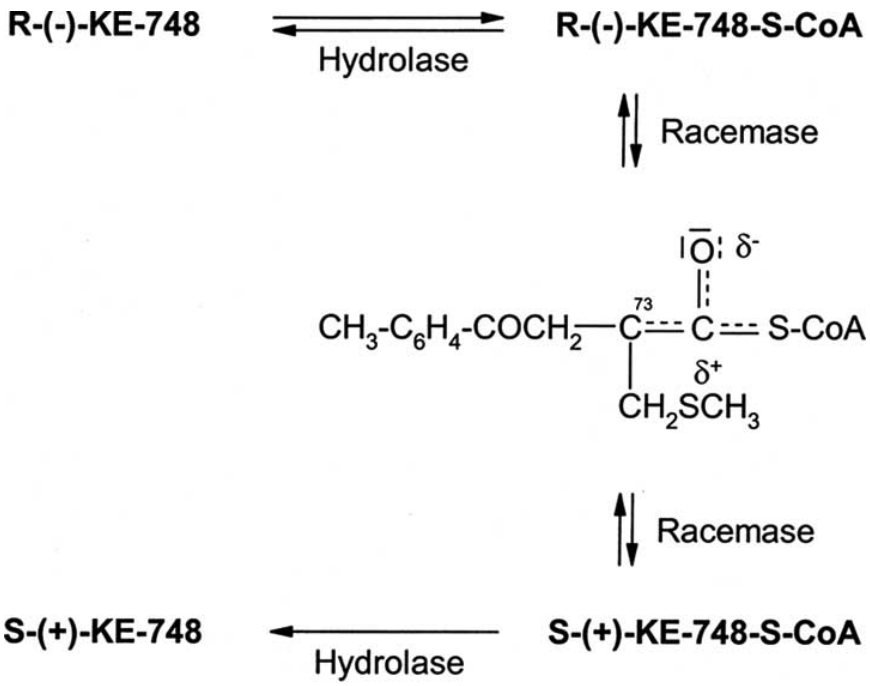

Fig. (4). The chiral inversion of R-(–)-KE-748 is catalyzed by the short- and medium-chain fatty acid CoA ligases in the mitochondrial matrix.

Kashiyama *et al*. [129] reported chiral inversion of flosequinan, a peripheral vasodilator with effect on both arterial and vascular beds, going by mechanisms mediated by intestinal bacteria in rats. These mechanisms involve initial reduction of one enantiomer of flosequinan to the corresponding achiral sulfide by the respective reductases with subsequent reoxidation of the sulfide by the respective mono-oxygenases Fig. (**6**).

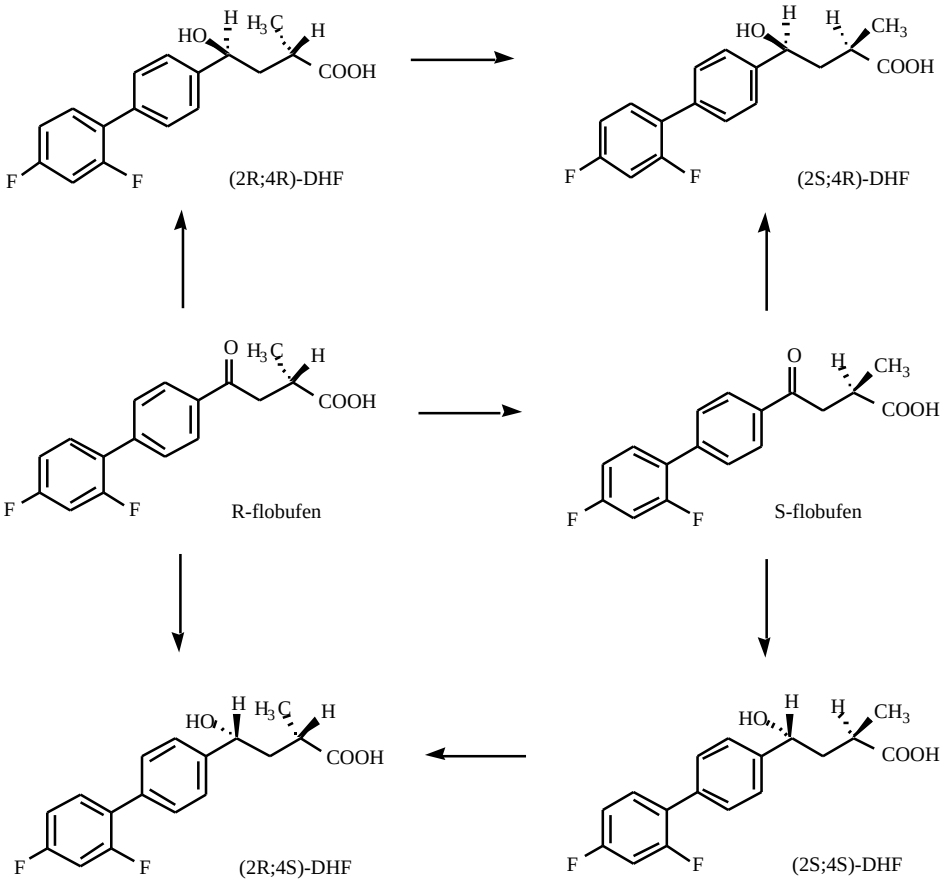

Fig. (5). The chiral inversion of flobufen enantiomers and 4-dihydroflobufen diastereoisomers (DHF) observed in primary culture of hepatocytes in man. The respective arrows indicate the main direction of inversion [128].

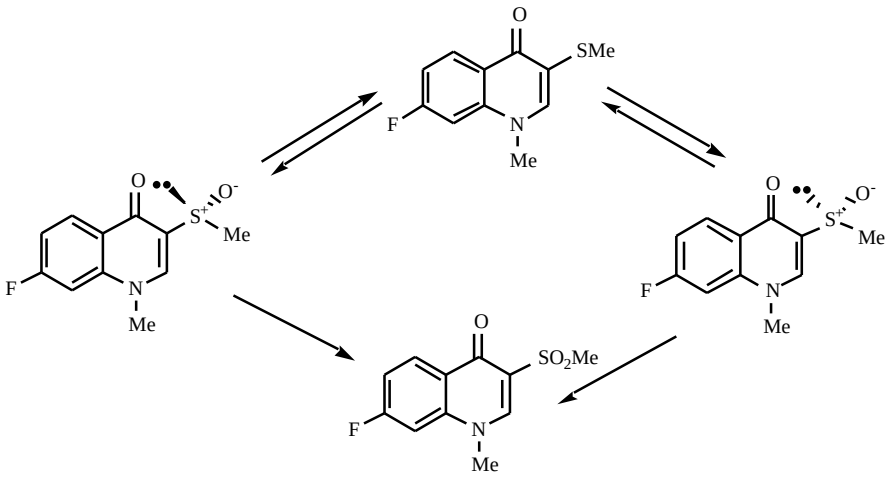

Fig. (6). The chiral inversion of flosequinan observed by Kashiyama *et al*. [129].

An interesting observation was reported in the context of the main metabolite of promising anticancer drug oracin. Prochiral oracin is metabolized by the respective reductases to its principal metabolite, 11-dihydrooracin (DHO), which is chiral. Subsequently, the DHO enantiomers are partially oxidized back to the parent oracin, but the extent of reoxidation is different from that of reduction Fig. (**7**) [130, 131]. This "futile cycle" could probably be a common feature of many drugs.

In principle, two main mechanisms of chiral inversion of drugs can be observed which can in addition differ in enzyme(s) involved in the process. One mechanism involves formation of conjugated intermediate of drug and subsequent chiral inversion of one enantiomer of conjugated intermediate to the opposite enantiomer followed by transformation of the opposite enantiomer of conjugated intermediate to the respective enantiomer. The other mechanism can be explained by the fact that many drugs use two opposing metabolic processes, such as oxidation and reduction. The two processes go by different mechanisms and are controlled by different enzymes; hence the net result can be chiral inversion of the drug.

Unfortunately, in most cases, the mechanism of chiral inversion has not been studied in detail, the authors just reporting that chiral inversion can be observed at certain conditions. Since no relationship was observed between chiral inversion and the structure of drug or affiliation to the therapeutic group, the following individual drugs are listed at random (Table **1**).

Some of the papers reported that no, or no substantial, chiral inversion could be observed. But it is just these results where the evidence for chiral inversion can be found. In many cases the only question is about the extent.

A relatively low extent of chiral inversion was found in ibutilide fumarate, a new drug for the treatment of cardiac arrhythmias. Pharmacokinetic studies in adult beagle dogs revealed up to 1% of chiral inversion after intravenous administration and even up to 5% after oral administration [132]. Contrary to that observation, Humphrey *at al.* [133] published data regarding cardiovascular effects of the *R*- and *S*-enantiomers of ibutilide in conscious beagle dogs, and they did not find any chiral inversion. In 1991 it was reported that both enantiomers of ibutilide undergo chiral inversion in a sterile solution [134]. This report also provides a clear illustration of how ignoring the physico-chemical properties of racemic drugs can potentially lead to a pharmaceutically useless or even dangerous drug product.

Another example describes the opposite observation. Chiral inversion was reported in the group of 1,4-benzodiazepines, in oxazepam and temazepam [135, 136], and 3-hydroxy metabolite of halazepam [136]. The racemization half-live was reported to depend on the structure of the respective drug. But, Aso *et al* did not observe chiral inversion of oxazepam acetate [137].

The reason why some drugs undergo chiral inversion is not clear but it is obvious that chemical structure of the drug itself plays an important role in this process. Undoubtedly, the presence of some functional groups or atoms and their spatial arrangement are important factors.

3. CHIRAL DRUGS WITHOUT REPORTED CHIRAL INVERSION

The previous section gave only drugs that undergo (at least partial) chiral inversion, but many papers have also been published which report no chiral inversion of drugs studied. Is it really true that none of those drugs undergoes any chiral inversion? Some of the results may be influenced by insufficient methodology for preparation and measurement of the drug samples, some of the papers concentrated on other aims and their failing to report any chiral inversion is just a "side product" lacking sufficient precision. Some papers claim that "chiral inversion is not considerable", but what are the limits justifying such claims? For someone, this limit is 1%, for someone 5%, and for someone perhaps even more. In other papers it is reported that a chiral impurity in the biological material measured (plasma, urine, feces, etc.) is similar to the chiral impurity of the individual enantiomer that was applied. However, should we expect the same

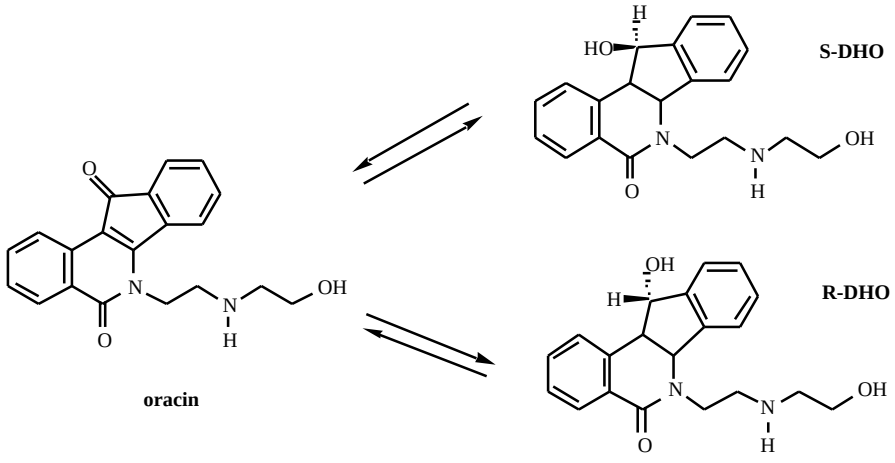

Fig. (7). Chiral inversion of DHO, the main metabolite of promising anticancer drug oracin.

Table 1. Further Drugs which Undergo Chiral Inversion

Drug	Therapeutic Group	Species	*Model System*	References
Carbenicillin	Antimicrobial agents	Man	*In vitro*	137
Ethiazide	Diuretics			
Etoposide	Cytostatics			
Ketamine	Central anaesthetics	Rat	*In vivo*	162
Thalidomide	Immunomodulating and antiangiogenic agents	Man	*In vivo*	138
Zopiclone	Hypnotics and sedatives	Rat	*In vivo*	156
BRL37344	Antidiabetic and antiobesity agents	Rat	*In vitro*	169
		Guinea-pig		
Pantoprazole	Proton pump inhibitors	Rat	*In vivo*	160
Clopidogrel	Antiagregate agents	Rat	*In vivo*	151
RS-8359	Monoamine oxidase inhibitors, antidepressants	Rat	*In vivo*	152
		Mouse		
		Dog		
		Monkey		
L-648,051	NSAIDs	Rat	*In vivo*	170
		Dog		
Ketorolac	NSAIDs	Rat	*In vivo*	140
		Man		
Albendazol-sulfoxide	Anthelmintics	Sheep	*In vitro*	157
		Cattle		
Lifibrol	Hypolipidemic agents	Dog	*In vivo*	161
5-Aryl-Thiazolidinedione	Antidiabetic agents	Dog	*In vitro*	149
		Man		
XK469	Cytostatics	Rat	*In vivo*	171

enantiomeric impurity? Pharmacokinetic fates of two enantiomers often differ, and their metabolisms differ qualitatively and quantitatively, too. Considering all these facts, one cannot reliably claim that the below-given drugs (Table **2**) really fail to undergo (at least partial) chiral inversion.

4. FACTORS AFFECTING CHIRAL INVERSION

4.1. Sample Handling and Interpretation of Results

In studies of metabolic chiral inversion, it is crucial to avoid spontaneous or chemical racemization of enantiomers. Racemization of enantiomers in any step of experiments could cause misleading results. Therefore, potential racemization of enantiomers should be carefully tested in all solutions and media used, in plasma, after extraction or analytical procedures.

The stability of enantiomers during experiments is strictly dependent on their structure [136]. Proper handling of solutions and biological samples is reported to be very important in experiments with thalidomide enantiomers [138], oxazepam, lorazepam, temazepam [139], halozepam [136], ketorolac [140] and many others. The most important factors influencing racemization of drugs include the temperature and pH [141-147], and the nature of the organic solvent [139, 141, 142, 144, 145, 148]. Various, more or less successful techniques have been described for handling solutions and samples [138]. Sometimes it is impossible to study pharmacokinetics or metabolism of individual enantiomers of a drug (e.g. 5-aryl-thiazolidinediones, new derivatives for treatment of diabetes) because of their rapid racemization in aqueous solvents and plasma [149]. Application of an unsuitable analytical procedure can also affect the study of possible chiral inversion. For example, the derivatization of enantiomers of tiaprofenic acid with L-leucinamide resulted in 7% racemization [108]. This racemization can have masked chiral inversion of these compounds in organism.

Table 2. Some Chiral Drugs which are Reported not to Undergo "Considerable" Chiral Inversion

Drug	Therapeutic Group	Species	Model System	References
R-albuterol	Bronchodilators	Dog	*In vivo*	172
Levocetirizine	Antihistamines	Man	*In vivo*	173, 174
Levobupivacaine	Local anesthetics	Man	*In vivo*	175, 176
Reboxetine	Antidepressants	Man	*In vivo*	177
Gacyclidine	Neuroprotective agents	Rat	*In vivo*	178
Levetiracetam	Antiepileptics	Dog	*In vivo*	179
Candoxatrilat	Antihypertensives	Mouse	*In vivo*	180
		Rat		
		Rabbit		
		Dog		
		Man		
SCH 66336	Cytostatics	Monkey	*In vivo*	150
		Rat		
SCH 56592	Antifungal agents	Rat	*In vivo*	181
		Dog		
		Monkey		
		Man		
SCH 39304	Antifungal agents	Rat	*In vivo*	182
		Monkey		
Amlodipine	Calcium channel antagonists	Man	*In vivo*	183
Remoxipride	Antipsychotic agents	Rat	*In vivo*	184
		Dog		
		Man		
		Man	*In vivo*	185
Losigamone	Antiepileptics	Man	*In vivo*	186
Nivaldipine	Calcium channel antagonists	Dog	*In ivo*	187
Nitrendipine	Calcium channel antagonists	Man	*In vivo*	188
Manidipine	Calcium channel antagonists	Dog	*In vivo*	189
E3810	Proton Pump Inhibitors	Dog	*In vivo*	190

In several cases, significant role of human serum albumin in drug racemization and epimerization was described [137]. Human serum albumin also retarded racemization of (–)-ethiazide to (+)-ethiazide. Attachment of the drugs to albumin may inhibit the attack by hydroxyl ion and/or water molecule and thus retard the racemization [137].

Also interpretation of results can seriously affect tests of chiral inversion. It is not clearly specified what extent of transformation of one enantiomer to its antipode in biological system is considered as chiral inversion. Ibutilide (substance for treatment of cardiac arrhythmias) was reported to exhibit no chiral inversion in the dog *in vivo* although "racemization" amounting nearly 5% was observed after oral administration [132]. Also in the case of a novel anti-tumor agent, SCH 66336, the detection of 2.5 – 3% of the respective antipode in rat and monkey plasma during *in vivo* experiment was interpreted as no chiral inversion [150].

The use of substrate with enantiomeric impurity is another complication in studies of chiral inversion. Enantiomeric impurity of 1.1-5.7% was reported in studies with

clopidogrel and its main metabolite [151]. In such cases, chiral inversion (occurring to a lower extent) can hardly be observed.

4.2. Species Differences

All factors determining expression, substrate affinity and activity of enzyme can affect the enzyme mediated chiral inversion. As the significant species differences in xenobiotic metabolism, including chiral inversion, are well known, the species of animals to be used play a crucial role in all experiments testing chiral inversion. Different species can differ in the rate at which chiral inversion occurs as well as in routes and mechanisms of inversion.

Many examples have been reported. Chiral inversion of *R*-fenoprofen in dogs *in vivo* reached 90% but only 38% of inversion was observed in horses [67]. The extent of bi-directional chiral inversion of RS-8359 (monoamine oxidase inhibitor, potential antidepressant) significantly differed in rats, mice, dogs and monkeys. The most extensive chiral inversion was found in rats, the least extensive one in dogs [152]. *R*-Ketoprofen is inverted in rats but is not inverted in humans; *R*-flurbiprofen is inverted in guinea pigs and dogs, but again not in humans [83, 153]. Several other examples of species differences in chiral inversion of profens have been demonstrated, e.g. [43].

With respect to all the above-mentioned examples, it must be taken into account which species was used for a particular test of chiral inversion, because extrapolation of these data from one species to another is impossible. Moreover, not only animal species but also the animal strain used in tests is important [152].

Besides humans, laboratory and farm animals, chiral inversion has also been studied in some non-mammalian species and in microorganisms.

Microorganisms are capable of carrying out a wide variety of biotransformations of xenobiotics. It was of interest to examine the microbial metabolism of ibuprofen to determine if microbial systems could also mediate the chiral inversion. An extensive screening study resulted in the identification of the mould *Verticillium lecanii*, which was found to invert ibuprofen and its hydroxymetabolite. The chiral inversion of a range of profens has been examined, using *V. lecanii*, and like in mammalian systems the extent of inversion was found to be substrate dependent [115]. Other studies have indicated that fungus *Cordyceps militaris* showed similar versatility as regards the substrate for inversion. Extensive metabolic inversion of mandelic acid mediated by mandelate racemase was observed in bacteria [154]. Intestinal bacteria are also able to contribute to inversion of one enantiomer to its antipode in the organism. This phenomenon and its significance are discussed in the following chapter.

4.3. Tissue Differences

The site(s) of inversion has/have been subject of many investigations, with pre-systemic intestinal metabolism and hepatic systemic metabolism receiving the greatest attention. As the investigation of site-specific metabolism in humans has obvious difficulties, the use of isolated organs (tissues) from animals served for providing the specific information.

Direct evidence for the contribution of the liver to the chiral inversion of profens has been the subject of many studies e.g. [16, 45, 155], which clearly show that inversion depends upon drug structure, species and the method of tissue preparation. On the other hand, chiral inversion of some drugs without participation of liver (hepatocytes) was observed. The short-acting hypnotic zopiclone undergoes chiral inversion of (–)-enantiomer to (+)-enantiomer *in vivo*, but hepatocytes are not involved in this inversion and spontaneous racemization was excluded [156]. Inversion was also observed in a pharmacokinetic study of anticoagulant agent clopidogrel but no chiral inversion was detected in hepatocytes [151].

Gastrointestinal tract is another organ (tissue) exhibiting important ability to invert enantiomers of drugs. Its contribution to chiral inversion of profens has been widely studied. Inversion of fenoprofen was tested *in vitro* [16]. Incubation of fenoprofen with everted intestinal tissues (duodenum, jejunum, ileum, colon) and non-everted stomach pouch revealed that chiral inversion occurs on both the serosal and mucosal sides of all everted intestinal tissues with the greatest extent detected in jejunum. No chiral inversion was detected in stomach. Similar experiments with ibuprofen showed that everted jejunum, ileum and colon from rats inverted enantiomer of this drug to the extent of 12.2%, 14.2% and 4.4%, respectively [53]. The epithelial cells were found to be the major site of inversion of ibuprofen in intestinal wall. Intestinal contents contributed only to a small extent to the process [70]. No chiral inversion in jejunum was found in studies with pirprofen in rats *in vitro* [109].

The potential contribution of intestinal bacteria to chiral inversion can be demonstrated on flosequinan. Chiral inversion at sulfoxide position of flosequinan enantiomers occurred in conventional rats but not in either germ-free rats or rats treated with antibiotics. Several strains of intestinal bacteria stereoselectively reduced flosequinan to give sulfoxide, followed by oxidation of the sulfoxide in the body to produce the antipode of flosequinan [129]. On the other hand, no differences between the *in vitro* chiral inversion of ibuprofen in parts of intestine from control and antibiotic-treated rats were reported [70].

Ruminal fluid from cattle and sheep is able to mediate bi-directional inversion of albendazole-sulfoxide enantiomers *in vitro*. This inversion does not occur in boiled ruminal fluid. The mechanism *via* reduction of one enantiomer of albendazole-sulfoxide into albendazole and following oxidation into sulfoxide antipode is suggested [157].

Hall *et al.* [158] have demonstrated the participation of the lungs in the chiral inversion of fenoprofen and ibuprofen. They have concluded that, due to anatomical location of the lungs, the pulmonary metabolism could account for the first pass effect after intravenous administration of these compounds.

Studies of Yamaguchi and Nakamura [159] involved kidney as a potential site of inversion of 2-phenylpropionic acid in rats.

Chiral inversion can probably occur also in brain. Fenoprofenoyl-CoA-ligase activity exists in rat cerebral microsomes. The subsequent steps of chiral inversion (epimerization and hydrolysis) were also observed in cerebral fractions [17].

It should be taken into account that the results concerning the relative amount of inversion seen in each isolated segment of tissue may not reflect the *in vivo* situation, because the extent of inversion depends greatly on the time the drug has been in contact with absorbing surface [16].

4.4. Effect of Route of Administration

If the gastrointestinal tract plays important role in chiral inversion of the drug, the route of its administration may affect the extent of inversion *in vivo.* Ibuprofen is subject to significant pre-systemic chiral inversion *via* gastrointestinal tract. Moreover, the extent of chiral inversion of ibuprofen in humans is related to the residence time in intestine; the greater extent of inversion was observed with slowly absorbed tablets but not with rapidly absorbed forms [52]. This phenomenon was also observed in rats *in vivo*. After administration of sustained release granules, the *S:R* AUC ratios were significantly (approx. 2 times) higher than after administration of suspension or solution. A significant positive linear correlation was found between the *S:R* AUC ratios and corresponding T_{max} for *R*-ibuprofen [53]. Stiripentol, an anticonvulsant agent and antiepileptic drug, undergoes chiral inversion only after *p.o.* administration, while no its inversion occurs after *i.v.* or *i.p.* administration. The *in vitro* study revealed that apparent metabolic chiral inversion of *R*-stiripentol results from a combination of at least two factors: partial acid-catalyzed racemization in gastric acid and enantioselectivity in absorption, first pass metabolism or biliary excretion [118]. In the case of pantoprazole, a higher chiral inversion was reported after *i.v.* administration than after *p.o.* administration [160].

On the other hand, there are many drugs whose extent of chiral inversion did not differ after different route of administration. Examples are lifibrol [161], fenoprofen [16], pirprofen [109], and ketoprofen [97].

Paradigm of i.v. administration affected chiral inversion of ketamine in rats. During wash-in infusion (long, constant rate), when circulation concentrations of ketamine enantiomers were high, the formation of R-norketamine (the main metabolite) predominated. In the following washout infusion (brief, only 5 min.), when circulating concentrations of ketamine enantiomers were low, inversion of R- to S-enantiomer appeared with subsequent metabolism to S-norketamine [162].

4.5. Inter-Individual Variability

It has been noted above that significant differences in chiral inversion are found between species. It is equally true, however, that such differences exist within species. Inter-individual variability has been documented mostly in chiral inversion of profens. It has been recognized for a long time that large variations in effect and response to profens occur in man. Later, it was suggested that variation in the extent of chiral inversion may contribute significantly to the variability in response to profens, namely ibuprofen [54, 163]. The results reported by Geisslinger *et al.* [164] do not support this hypothesis, as the inter-individual variation in pharmacokinetics of *S*-ibuprofen following administration of racemate was similar to that following the administration of the single isomer, which suggests that chiral inversion is not a major factor contributing to variability in disposition of this drug. On the other hand, a significant inter-individual variability in ratio of ibuprofen enantiomers plasma concentration in healthy volunteers has been clearly documented [54, 112]. Now it is no doubt that chiral inversion of profens varies in both rate and extent between people [50]. The origin of these variations is under study.

All factors affecting drug-metabolizing enzymes can affect enzyme-mediated chiral inversion. Genetic polymorphism and gender are considered to be the basic factors determining inter-individual variability in drug metabolism in humans. The influence of these factors on variability in inversion of profens was suggested by Cashman and McAnulty [112]. Clear information about genetic polymorphism in chiral inversion has not been reported. No differences between males and females were observed in pharmacokinetics of ibuprofen enantiomers [63], but these results cannot be extrapolated to other compounds. Thus, the impact of these factors remains largely unexplored.

During the life of individual person (animal), the enzymes participating in chiral inversion can be affected by many other factors, such as age, biological rhythm, pregnancy, diseases, stress, diet, environmental pollutants, and medications. The age of a person may markedly influence his or her ability to invert enantiomers of drugs. Igarza *et al.* [96] reported the significantly more extensive inversion of *R*-ketoprofen in calves than in cows. Influence of pregnancy on *R*-ketoprofen inversion was also found [96]. Landoni and Soraci [41] reported correlation between the severity of inflammatory reactions in horses and the reduction on the inversion rate of ketoprofen. The same is true of ibuprofen in humans as a consequence of stress [165]. An altered chiral inversion of ibuprofen was also found in patients with liver cirrhosis [166] and in children after a minor genito-urinary surgery [167]. On the other hand, no apparent effects of adjuvant-induced arthritis on the chiral inversion of *R* to *S*-ketoprofen were detected [91].

The influence of chemicals on enzyme-mediated chiral inversion is intimately connected with the above-mentioned intra-individual factors. Enzyme induction and enzyme inhibition, as possible consequences of the presence of chemicals in organism, are phenomena of great significance. Enzyme inducers increase the concentration and hence activity of enzymes, while inhibitors decrease the enzyme activity. Enhanced chiral inversion of ibuprofen was found in liver from rats treated with clofibric acid [47]. In healthy volunteers clofibrate pre-treatment also significantly increased chiral inversion of ibuprofen by increased formation of R-ibuprofenyl-CoA [61]. Two distinct mechanisms of clofibrate action are proposed: induction of acyl-CoA ligase and pantothenate kinase (after 3-days pre-treatment of rats with clofibric acid) and increase of intracellular pools of CoA during addition of clofibric acid into perfusate or medium [60]. Pre-treatment of guinea pigs with clofibrate

increased also the chiral inversion of R-fenoprofen [14]. In rats, phenobarbital significantly induced inversion of R- to S-indoprofen via an unknown mechanism [76]. Palmitic acid (substrate of long-chain ACSs) was described as a competitive inhibitor of R-ibuprofen inversion in rat hepatocytes [168].

CONCLUSIONS

With respect to all the above-mentioned facts it is possible to answer the questions we are interested in. Are enantiomers of profens the extraordinary compounds that enzymes are able to invert? No, there is no doubt that profens are not the only compounds that undergo the metabolic chiral inversion. Stereoisomers of many other drugs or their metabolites are inverted to the antipodes via enzyme catalysis, and chiral inversion is a relatively frequent metabolic pathway of chiral drugs. However, can all chiral drugs undergo the chiral inversion? No, many drugs can be considered as stereo-stable in organism. Why are some enantiomers of drugs inverted by enzymes and others are not attacked? The reason lies into the structure.

The proper arrangement of chiral center that can be attacked by isomerase or by biotransformation enzymes seems to be crucial for potential chiral inversion. There are several mechanisms by which metabolic processes can lead to chiral inversion. In rare cases (e.g. in mandelic acid), isomerase directly attacks the chiral center in compound and changes the configuration of substituents. Isomerization of drugs in conjugated form is a much more frequent mechanism of chiral inversion. Some compounds can be reversibly conjugated, leading to an isomerization of conjugated intermediates. This mechanism operates in chiral inversion of profens and probably also that of other structurally different drugs, such as KE-748 [122], stiripentol [119], etc.

The other type of chiral inversion is based on the metabolic processes that have opposite chemical consequences. Although most metabolic processes are not reversible in terms of the substrate and product inter-conversion, the opposite biotransformation reactions sometimes happen. This is the consequence of the wide pool of biotransformation enzymes that are ready to metabolize every xenobiotic. By this way, some metabolites, formed from a drug via catalysis by one enzyme, serve as substrates for another enzyme with an opposite effect, leading back to formation of the original drug. If this drug is a chiral compound and the metabolite is achiral, one of the net results can be the conversion of one enantiomer into the other.

Examples where chiral inversion takes place by the intermediacy of two opposing metabolic processes can be found among oxidation-reduction mechanisms, such as the alcohol-ketone inter-conversion. Oxidation of a chiral secondary alcohol leads to the corresponding achiral ketone. In the next step, this ketone is reduced to give the chiral secondary alcohol [3]. These two processes are controlled by different enzymes, hence the net result of chiral inversion depends on stereoselectivity and stereospecificity of these enzyme systems. Another group of compounds whose enantiomers can be inverted are chiral sulfoxides (e.g. albendazole-sulfoxide, [157]) and tertiary amine *N*-oxides. These compounds can be enzymatically reduced to the corresponding achiral sulfides or amines, which then undergo re-oxidation by mono-oxygenases to give enantiomers of the parent compounds. In all these cases, the extent of chiral inversion observed depends on stereoselectivity and stereospecificity of the enzymes involved in both reactions. If the achiral metabolite is stereoselectively formed only from one enantiomer of drug, and the same enantiomer of drug is formed stereospecifically from this achiral metabolite, then no chiral inversion is detected. But it is necessary to take into account that these "useless" futile biotransformation cycles always burden the organism.

Chiral inversions of drugs vary in extent, significance and mechanisms. In many cases, a chiral inversion alters the ratio of enantiomers. As the enantiomers of a drug, as well as those of its metabolites, generally show different biological activities, the chiral inversion alters the pharmacodynamic effect and pharmacokinetic behavior of drug. Moreover, chiral inversion may cause some other undesirable effects depending on its mechanism. The pharmacological and toxicological consequences of potential chiral inversion in individual chiral drugs should be evaluated. Without exception, chiral inversion must be excluded when the antipode of a drug that is used in pure enantiomeric form is toxic. Nevertheless, chiral inversion (in minor extent) should not serve as argument against the use of drug in pure enantiomeric form instead of the racemate. If chiral inversion occurs with one or both enantiomer(s) it almost certainly occurs in the racemate. Moreover, inversion (especially the bi-directional one) can hardly be detected if racemate is administered. From this point of view, chiral inversion should be tested also in all drugs administered as racemates.

When chiral inversion of drugs is tested, all factors affecting it should be taken into account. The correct evaluation depends strictly on samples handling and interpretation of results, on species, tissue and the administration route chosen. Probably, other factors may affect chiral inversion, too, and other mechanisms of chiral inversion can occur. New investigations should throw some more light on this field.

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