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Catechol-*O*-methyltransferase and Its Inhibitors in Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is a neurological disorder characterized by the degeneration of dopaminergic neurons, with consequent reduction in striatal dopamine levels leading to characteristic motor symptoms. The most effective treatment for this disease continues to be the dopamine replacement therapy with levodopa together with an inhibitor of aromatic amino acid decarboxylase (AADC). The efficacy of this therapy, however, decreases with time and most patients develop fluctuating responses and dyskinesias. The last decade showed that the use of catechol-*O*-methyltransferase inhibitors as adjuvants to the levodopa/AADC inhibitor therapy, significantly improves the clinical benefits of this therapy.

The purpose of this article is to review the current knowledge on the enzyme catechol-*O*-methyltransferase (COMT) and the role of COMT inhibitors in PD as a new therapeutic approach to PD involving conversion of levodopa to dopamine at the target region in the brain and facilitation of the continuous action of this amine at the receptor sites. A historical overview of the discovery and development of COMT inhibitors is presented with a special emphasis on nebicapone, presently under clinical development, as well as entacapone and tolcapone, which are already approved as adjuncts in the therapy of PD. This article reviews human pharmacokinetic and pharmacodynamic properties of these drugs as well as their clinical efficacy and safety.

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INTRODUCTION

Parkinson's disease (PD) is characterized by tremor, rigidity, bradykinesia, and postural instability. Other primary symptoms are slowness, stiffness, and the inability to initiate movements. The symptoms of PD become apparent after death of ca. 240,000 nigral cells (60% of the total). It is a chronic degenerative disease affecting just over 1 person per every 1000 with increasing incidence at an older age. The peak onset of PD is at 60 years of age; it progresses slowly over the next 10 to 20 years. In PD the loss of nigral cell is accelerated, as compared to normal healthy individuals; and is associated with the concurrent loss of dopamine in the striatum.

The discoveries by Carlsson et al. (1957) and by Ehringer and Hornykiewicz (1960) and Hornykiewicz (2006) provided evidence for the striatal dopamine deficiency in PD, and led to the symptomatic treatment with 3,4-dihydroxyphenylalanine (DOPA). Although initial trials led to inconsistent results, Cotzias et al. (1967) reported impressive antiparkinsonian effects of long-term oral therapy with DL-DOPA, establishing the basis for the modern pharmacotherapy of PD. The efficacy of the therapy was improved using a combination of L-DOPA (levodopa) with a peripheral decarboxylase inhibitor. Subsequently, the major adverse effects of this therapy were described (Cotzias et al. 1969).

Unlike dopamine, levodopa crosses the blood–brain barrier and is then decarboxylated to dopamine by aromatic amino acid decarboxylase (AADC) and released by presynaptic terminals in the striatum replenishing the dopaminergic deficiency. The amount of levodopa reaching the brain after oral administration is, nevertheless, very low, about 1% of the administered dose. Absorption, primarily in the duodenum and jejunum occurs through an amino acid carrier-mediated transport system and is dependent upon gastric emptying rate and protein intake among other factors. Levodopa is then rapidly metabolized, largely by decarboxylation, but also by *O*-methylation, transamination, and oxidation. The transport of levodopa through the blood–brain barrier is also subject to competition with other amino acids and eventually with its own metabolite 3-*O*-methyldopa (3-OMD).

The combination of levodopa with a peripheral AADC inhibitor, that is unable to enter the central nervous system, remains the most effective treatment for PD. However, even with this combination only 5 to 10% of administered levodopa reaches the brain. Levodopa is particularly useful when given with decarboxylase inhibitors, but its subsequent metabolism by catechol-*O*-methyltransferase (COMT) markedly limits its availability to the brain. COMT inhibitors avert the normal-*O*-methylation of levodopa to its metabolite 3-OMD and, by limiting this metabolic route, they increase the availability of levodopa for conversion to dopamine in the brain.

COMT

Physiological Role

Catechol-*O*-methyltransferase (COMT; EC 2.1.1.6), first described by Axelrod and Tomchick (1958), is a magnesium-dependent enzyme that catalyzes the methylation of catechol substrates using S-adenosyl-L-methionine (SAM) as a methyl donor and yielding, as reaction products, the *O*-methylated catechol and S-adenosyl-L-homocysteine (Guldberg and Marsden 1975). COMT substrates include a wide variety of catechols,

namely catecholamines, their hydroxylated metabolites, catecholestrogens, ascorbic acid, dietary phytochemicals, and medicinal compounds (Guldberg and Marsden 1975; Männistö et al. 1992a; Zhu et al. 2000). The major physiological role of COMT is the elimination of biologically active or toxic catechols. Of special importance is the methylation of levodopa to 3-OMD in levodopa/AADC inhibitor-treated PD patients (Männistö and Kaakkola 1990; Sharpless and McCann 1971). COMT has also been suggested to play a relevant role in modulating prefrontal dopamine neurotransmission (Akil et al. 2003; Bilder et al. 2004; Egan et al. 2002; Huotari et al. 2002).

Gene and Tissue Distribution

Queries at the National Center for Biotechnology Information databases (Wheeler et al. 2006) show that the COMT gene has been found and characterized in a variety of species, such as *Homo sapiens*, *Pan troglodytes*, *Canis familiaris*, *Rattus norvegicus*, *Mus musculus*, *Equus caballus, Gallus gallus*, and *Schizosaccharomyces pombe*, and is conserved in the fungi/metazoa group. COMT is present in prokaryotes and eukaryotes, namely bacteria (Dhar et al. 2000; Kim et al. 2004 Vilbois et al. 1994), yeast (Veser 1987; Veser et al. 1979), plants (Guldberg and Marsden 1975; Legrand et al. 1976) and animals (Guldberg and Marsden 1975), and invertebrates and vertebrates. In mammals COMT is present in two molecular forms: a soluble form (S-COMT) and in another form associated with membranes (MB-COMT) (Borchardt et al. 1974; Grossman et al. 1985; Jeffery and Roth 1984). Both forms are encoded by a single gene (Lundström et al. 1991; Salminen et al. 1990; Winqvist et al. 1992).

The overall genomic organization of the COMT gene is similar in both humans and rats; however, there are some significant transcriptional and translational differences (Tenhunen et al. 1994). In humans the COMT gene is located on chromosome 22 band q11.21 (Grossman et al. 1992; Winqvist et al. 1992) and is composed of six exons. The first two exons are noncoding and the translation initiation codons for the membrane bound and soluble isoforms are located on the third exon. Rat COMT gene, located on chromosome 11 band q23 (Yeung et al. 1996), is composed of five exons with the translational initiation codons for the two isoforms located in exon 2 (Tenhunen et al. 1993). In both species, two separate promoters direct the synthesis of two partially overlapped transcripts, one of 1.5 kb (human) or 1.9 kb (rat) that is constitutively expressed and another of 1.3 kb (human) or 1.6 kb (rat) that is subject to tissue-specific transcription regulation (Tenhunen et al. 1993, 1994). The short transcript translates S-COMT and the longer transcript translates MB-COMT, but also the soluble form by the leaky scanning mechanism of translational initiation (Kozak 1989).

The long transcript has been found in all tissues analyzed, with higher levels in human liver, brain, kidneys, adrenals, and lungs. The short transcript, on the other hand, is particularly abundant in liver, kidneys, and mammary glands (Tenhunen et al. 1994). In very small amounts it is found in the human brain (Hong et al. 1998; Tenhunen et al. 1994), but is completely absent in rat brain (Tenhunen et al. 1993; Tenhunen and Ulmanen 1993). As a consequence the soluble to membrane-bound isoform ratios differ among tissues; there is, however, no direct correlation between transcript and protein levels. In most human and rat tissues the levels of S-COMT greatly exceed the levels of MB-COMT, except for the human brain, where it represents about 30% of the total COMT (Karhunen et al. 1994;

Tenhunen et al. 1994). The reason for the discrepancy of the soluble to membrane-bound ratios between human and rat brain, where mainly the long transcript is found, is probably related to the fact that in humans, but not in rats, the MB-AUG initiation codon is located in a more favorable context than the S-AUG initiation codon. As a result, most of the translation is initiated from the MB-AUG site (Lundström et al. 1991; Tenhunen and Ulmanen 1993; Ulmanen and Lundström 1991). A higher membrane-bound to soluble ratio has also been reported in human and rat adrenals and pheochromocytomas (Eisenhofer et al. 1998; Ellingson et al. 1999).

The more detailed analysis of the COMT mRNA and the protein distribution in the mammalian brain revealed its presence both in neuronal and nonneuronal cells (Karhunen et al. 1994, 1995; Kastner et al. 1994) with COMT mRNA levels being significantly higher in neurons than in nonneuronal glial cells (Matsumoto et al. 2003). As for the subcellular location of COMT, the soluble protein is found mainly in the cytoplasm; however, its presence has been reported in the nucleus of transfected cells (Ulmanen et al. 1997) and also of mammary epithelial cells under certain circumstances, such as increased levels of catecholestrogens (Weisz et al. 1998, 2000). MB-COMT, on the other hand, although previously assigned to the outer mitochondrial membrane (Grossman et al. 1985), and plasma membrane (Aprille and Malamud 1975; Head et al. 1985; Lundström et al. 1992; Raxworthy et al. 1982), is actually localized in the rough endoplasmic reticulum (Ulmanen et al. 1997).

Enzyme Activity

Much work on the biochemical characterization of COMT was done with purified enzyme from rat tissues and human placenta. However, with the disclosure of mammalian COMT cDNAs and genes it was possible to express both rat and human S- and MB-COMT in heterologous systems, eukaryotic (Tilgmann et al. 1992; Ulmanen et al. 1997) and prokaryotic (Bonifácio et al. 2001; Lundström et al. 1992; Malherbe et al. 1992), allowing a more thorough characterization of the functional properties of the enzyme, its subcellular localization, and ultimately its three-dimensional structure.

Both human and rat S-COMT are nonglycosylated proteins containing 221 amino acid residues and having molecular weights of 24.3 kDa and 24.7 kDa, respectively (Lundström et al. 1991; Salminen et al. 1990). The MB-COMT has an additional peptide in its amino terminal of 50 (human) or 43 (rat) amino acid residues and a corresponding molecular weight of 30 kDa or 29.6 kDa, respectively. This extra peptide contains a stretch of 21 (human) or 17 (rat) hydrophobic amino acid residues that constitute the membrane anchor region (Bertocci et al. 1991; Lundström et al. 1991). MB-COMT is an integral membrane protein with the catalytic portion of the enzyme oriented toward the cytoplasmic side of the membrane (Ulmanen and Lundström 1991).

MB-COMT and S-COMT share similar affinities for SAM (Jeffery and Roth 1987; Lotta et al. 1995), similar magnesium dependency, inhibition by calcium, and a similar optimal pH for the activity (Männistö and Kaakkola 1999). However, they can have significantly different affinities for substrates as shown in Table 1. The affinity of MB-COMT for catecholamines is 10- to 100-fold higher than that of S-COMT, and this characteristic appears to be common to different species. The reasons for these differences remain to be determined. However, it can be hypothesized that additional interactions between the

TABLE 1. Affinities of S- and MB-COMT, from different sources, for several substrates

Enzyme source		Substrate	S-COMT K_m (μM)	MB-COMT K_m (μM)	References
Human	Brain	Dopamine	280	3.3	(Jeffery and Roth 1987)
	Recombinant *E.coli*	Catechol	108	10	(Malherbe et al. 1992)
	Recombinant Sf9 cells	Dopamine	207*	15*	(Lotta et al. 1995)
		Norepinephrine	369*	24*	(Lotta et al. 1995)
		DBA	39*	30*	(Lotta et al. 1995)
		Levodopa	613*	266*	(Lotta et al. 1995)
Rat	Liver & Brain	Epinephrine	168–345	0.9–3	(Bonifácio et al. 2000)
	Liver & Brain & Kidney	Norepinephrine	304–464	5.5–11.4	(Masuda et al. 2003)
Pig	Brain	R-Salsolinol	156	43	(Hotzl and Thomas 1997)
Mouse	Liver	Epinephrine	242	12	Data not published
Rabbit	Aorta	2-Hydroxyestradiol	0.27	0.15	(Reid et al. 1986)
		Isoproterenol	121	0.91	

*Values for the 3-*O*-methylation.

substrate and the extra N-terminal residues of MB-COMT (absent in S-COMT), or a change in the conformation of the active site, due to membrane interactions, could account for such differences (Bonifácio et al. 2000; Lotta et al. 1992).

Genetic Polymorphisms

COMT activity is ubiquitous in animal tissues. COMT levels vary, however, not only among different species, but also in individuals of the same species as well as in tissues from the same individuals. Within individuals, liver, followed by kidneys and gastrointestinal tract, has the highest enzymatic activity, while cardiac tissue has the lowest activity (Ellingson et al. 1999; Guldberg and Marsden 1975; Männistö and Kaakkola 1999). When comparing enzyme levels in different species, studies with erythrocytes revealed that humans were among the species with the lowest enzymatic activity. Monkeys had the highest levels followed by mouse, rat, dog, and guinea pig (Schultz et al. 1989; Zurcher et al. 1996).

Intra-species activity variations have been reported in two rat strains (Weinshilboum et al. 1979), monkeys (Zurcher et al. 1996), and humans. In humans the activity of COMT has trimodal distribution with high ($COMT^{H/H}$), intermediate ($COMT^{H/L}$), and low ($COMT^{L/L}$) activity groups. The difference in activity is correlated with a functional COMT polymorphism at codon 108/158 (S-COMT/MB-COMT) involving a Met→Val substitution in the polypeptide chain. The Met108/158 variant is associated with low enzymatic activity and decreased thermal stability, while the Val108/158 is associated with high activity (Lachman et al. 1996; Lotta et al. 1995). The low activity was associated with decreased protein levels (Doyle et al. 2004; Shield et al. 2004), rather than diminished transcript expression (Chen et al. 2004).

Although several other polymorphisms have been reported for COMT (Chen et al. 1996; Kinnear et al. 2001; Saito et al. 2001; Shield et al. 2004), none of them appeared to have any physiological significance. COMT genetic variation led to a multitude of linkage and genetic association studies searching for correlations between COMT polymorphisms and neuropsychiatric disorders. Most of these studies failed, however, to establish a significant correlation between COMT genotype and either obsessive-compulsive disorder (Azzam and Mathews 2003; Meira-Lima et al. 2004), bipolar disorder (Craddock et al. 1997, 2001), attention deficit hyperactivity disorder (Mills et al. 2004; Taerk et al. 2004; Turic et al. 2005), schizophrenia (Egan et al. 2001; Liou et al. 2001; Shifman et al. 2002; Strous et al. 2006; Tsai et al. 2006), or Parkinson's disease (PD) (Bialecka et al. 2005; Contin et al. 1996; Lee et al. 2001; Syvanen et al. 1997; Tai and Wu 2002). COMT genotype appears, however, to affect prefrontal cortex physiology (Akil et al. 2003; Egan et al. 2001; Malhotra et al. 2002; Rosa et al. 2004). With respect to the clinical efficacy of COMT inhibitors or the response to levodopa, available reports suggest that COMT genotype plays, at most, only a minor role (Contin et al. 2005; Lee et al. 2002; Rinne et al. 2003).

Enzyme Structure

The three-dimensional structure of rat recombinant S-COMT in complex with the co-substrate SAM, one magnesium ion and the competitive inhibitor 3,5-dinitrocatechol was resolved for the first time by Vidgren and collaborators (Vidgren et al. 1991, 1994). The detailed atomic arrangement of the active site was disclosed and encouraged a series of structural and theoretical studies of the catalytic and inhibition mechanisms of the enzyme. COMT is composed of one single domain with α/β-folded structure, containing eight α-helices arranged around a central mixed β-sheet (Fig. 1). This topology represents a variation of the Rossman fold and is characteristic of the catalytic domain of other SAM-dependent methyltransferases. Indeed, a high degree of structural homology is found in the SAM binding region between COMT and other known nucleotide binding proteins, namely DNA- and RNA-methyltransferases (O'Gara et al. 1995; Schluckebier et al. 1995), suggesting a common evolutionary origin. COMT active site consists of the actual catalytic site, which binds one magnesium ion and the catechol substrate, and the SAM-binding region (Fig. 2). While SAM is buried within the structure of the enzyme, the catalytic site is a shallow groove located on the outer surface of COMT. The analysis of the X-ray structure and of kinetics experiments made it possible to establish that the methylation reaction follows a sequentially ordered mechanism with SAM binding first, followed by Mg^{2+} and, finally, the catechol substrate (Lotta et al. 1995).

The Mg^{2+} has an octahedral coordination to two aspartic acid residues (Asp141 and Asp169), one asparagine residue (Asn170), and one crystallographic water molecule. The two free coordination sites are occupied by the two catechol hydroxyl groups of the substrate. Hence, the Mg^{2+} ion is responsible for "anchoring" the substrate (or inhibitor) molecules in a catalytic position. The two catechol hydroxyls form additional hydrogen bonds with the side chains of residues Glu199 and Lys144, and one of the hydroxyls is positioned in proximity to the activated methyl group of the co-substrate. In addition, the so-called "gatekeeper" residues (Trp38, Trp143, Pro174, and Leu198), which flank the hydrophobic entrance of the catalytic pocket, help to keep the planar catechol ring in the correct orientation. These residues are also responsible for the selectivity of COMT toward different substrates (Lautala et al. 2001) and for the observed regioselectivity

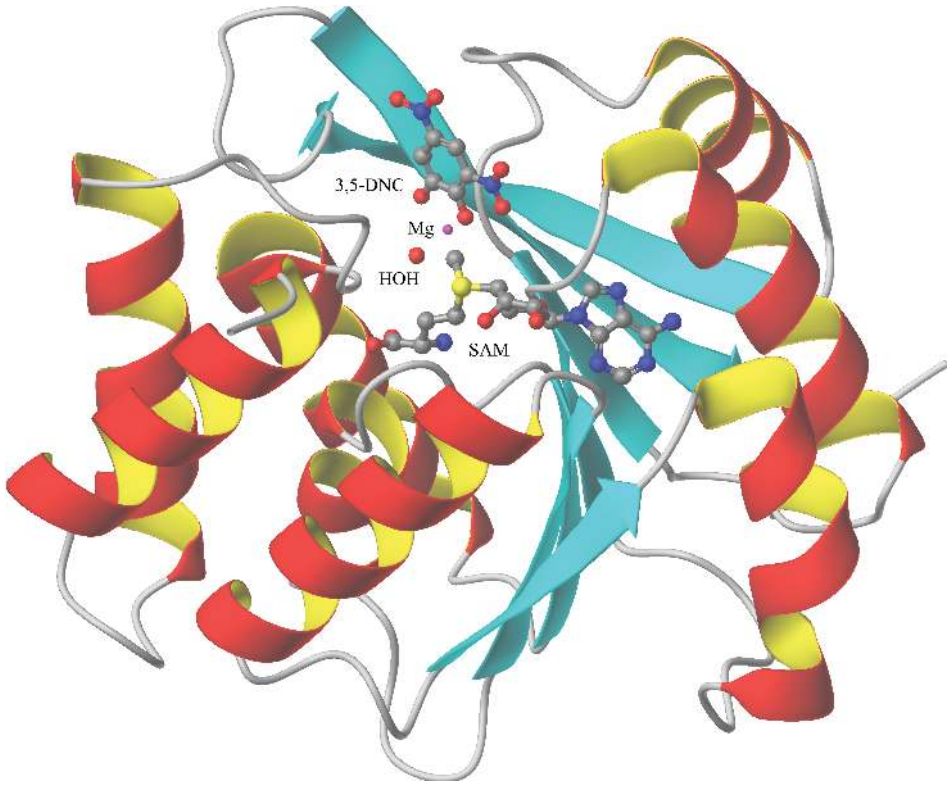

FIG. 1. Schematic representation of the three-dimensional structure of COMT. The S-adenosyl-L-methionine co-substrate (SAM), the inhibitor 3,5-dinitrocatechol (3,5-DNC), the magnesium ion, and coordinated water molecules are depicted.

of *O*-methylation of several substrates and inhibitors (Lotta et al. 1995; Palma et al. 2003, 2006). This atomic arrangement described has recently been confirmed by the X-ray structures of COMT in complex with three other inhibitors (Bonifácio et al. 2002; Lerner et al. 2001; Palma et al. 2006).

The catalytic and inhibition mechanisms of COMT have been extensively studied by structural and theoretical methods (Chen et al. 2005; Kahn and Bruice 2000; Kuhn and Kollman 2000; Lau and Bruice 1998; Lautala et al. 2001; Sipila and Taskinen 2004; Vidgren and Ovaska 1997; Zheng and Bruice 1997). In addition to the structural function, the positively charged Mg^{2+} ion also has an important catalytic role, by lowering the pK_a of Lys144 and of the catechol itself. At physiological pH, the ϵ-amino group of Lys144 is believed to be deprotonated and to act as a general catalytic base, attracting the proton from the nearby catechol hydroxyl group. The proton transfer is the initial step of the *O*-methylation catalytic process. Once ionized, the newly formed nucleophilic hydroxylate oxygen attacks the electron-deficient methylsulfonium group of SAM and the methyl transfer proceeds through a S_N2-type transition state (Woodard et al. 1980).

The factors affecting the substrate-binding affinity and turnover rate in the reaction catalyzed by S-COMT were analyzed by a QSAR study where a diverse set of 46 catecholic substrates was used (Lautala et al. 2001). It was concluded that the most prevalent factor,

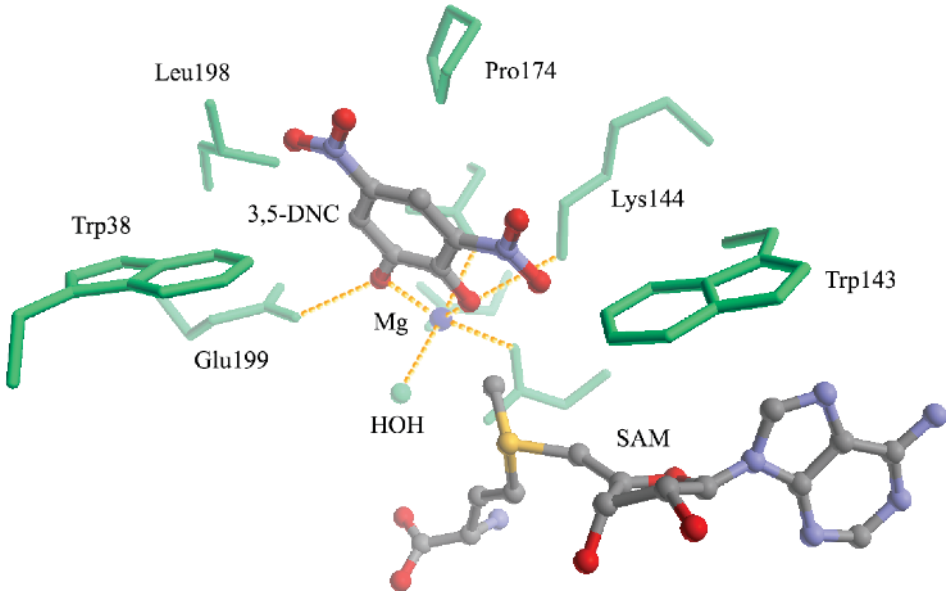

FIG. 2. Close-up view of the catalytic site of COMT complexed with SAM and 3,5-dinitrocatechol (3,5-DNC). The coordination bonds to the magnesium ion and hydrogen bonds to Lys144 and Glu199 are depicted in gold.

which may lower the rate of the *O*-methylation reaction (therefore increasing inhibition), was the electronic substituent effect on the reactive hydroxyl group. Introducing an electron-withdrawing substituent in the catechol ring has the effect of lowering the pK_a of the catechol hydroxyls and stabilizing the catechol anion, through charge delocalization within the enzyme–substrate complex. In this case, the negative charge of the catechol and the positive charge of the methylsulfonium are neutralized through the transition state. However, stabilization of the Michaelis complex rather than the transition state leads to an increase of the activation energy and, therefore, a decrease of the reaction rate. The electronic effects of substituents on the catechol hydroxyls have also been successfully utilized to explain the high affinity and lack of reactivity of the potent second-generation nitrocatechol COMT inhibitors (Lotta et al. 1992; Shinagawa 1992; Taskinen et al. 1989) (see next section). The effectiveness of COMT substrates was also found to depend on the steric fit, flexibility, and hydrophobicity of the side chain substituents (Lautala et al. 2001; Sipila and Taskinen 2004). To a certain degree, an increase of the steric bulkiness and hydrophobicity of the catechol substituents extending out of the catalytic pocket can decrease K_m and increase the turnover rate, as long as they do not conflict with the gatekeeper residues.

COMT INHIBITORS

First-Generation Inhibitors

Subsequent to the first purification and characterization of COMT, in the late 1950s (Axelrod and Tomchick 1958), several classes of COMT inhibitors were identified (for a review see Guldberg and Marsden 1975). Those compounds contain a catechol structure, or some related bioisosteric moiety, and are typically competitive substrates of COMT, in

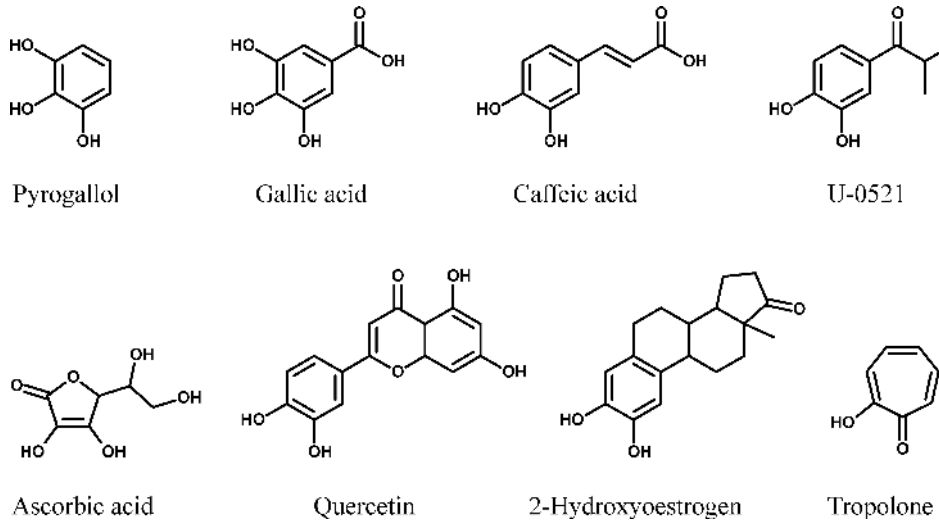

FIG. 3. Representative base structures of some "first-generation" COMT inhibitors.

the classical sense. They include derivatives of pyrogallol and catechols, such as gallic acid, caffeic acid, U-0521, 2-hydroxyoestrogens, or flavonoids like quercetin or rutin. Several other noncatecholic compounds such as ascorbic acid, tropolones, and derivatives of 8-hydroxyquinolines and 3-hydroxylated pyrones and pyridones were also identified as COMT inhibitors (Fig. 3).

The potencies of these compounds were only moderate, with dissociation constants typically within the micromolar range, but nevertheless, they proved to be useful as tools for the study of adrenergic mechanisms. Some of the early COMT inhibitors have been subjected to limited clinical tests, but disappointingly, they showed little value as pharmacological agents, owing to unfavorable pharmacokinetics, poor selectivity, or toxicity (Ericsson 1971; Guldberg and Marsden 1975; Reches and Fahn 1984). For instance, pyrogallol, *n*-butylgallate, and tropolone have low efficacy *in vivo* and are toxic. The toxicity mechanisms attributed to some of these compounds are possibly a direct consequence of their pharmacological action and not directly related to their structure. Large doses of these compounds are required for COMT inhibition *in vivo*, and by being themselves substrates of COMT they may depress tissue levels of the methyl donor component (Baldessarini and Chace 1972). Another COMT inhibitor, U-0521, was given orally to a single patient with Parkinson's disease, but no COMT inhibition in erythrocytes or drug accumulation in plasma was observed (Reches and Fahn 1984). In addition, this compound has shown a nonspecific depressant effect on the isotonic contraction of the rat vas deferens (Rice et al. 1997).

Second-Generation Inhibitors

The discovery of a new generation of potent and selective COMT inhibitors (Bäckström et al. 1989; Borgulya et al. 1989; Männistö and Kaakkola 1989) in the late 1980s, triggered a new interest in COMT as a therapeutic target. These "second-generation" inhibitors were developed simultaneously by two independent groups and constituted a new class of

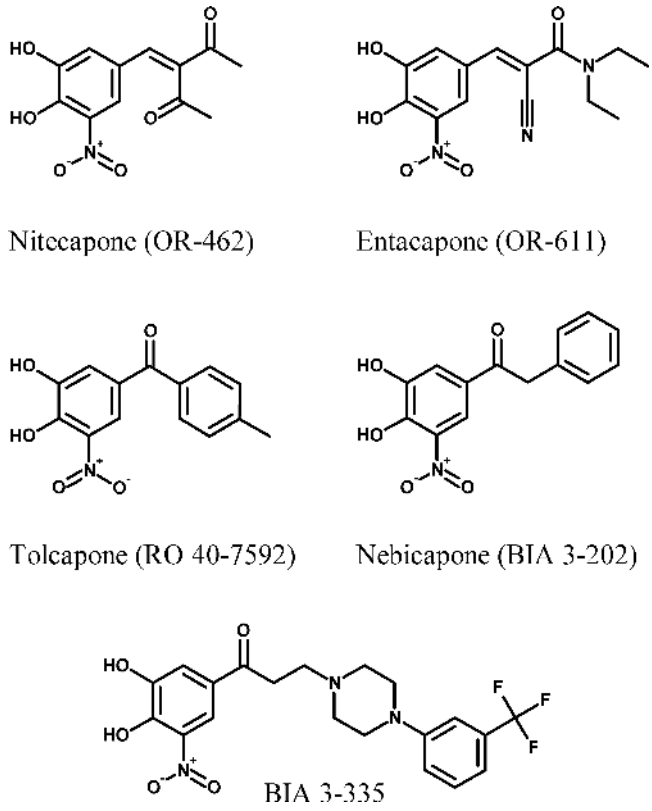

FIG. 4. Nitrocatechol-type second-generation COMT inhibitors.

di-substituted catechols. Structure–activity relationship analysis established that enhanced potency was obtained by substitution with electron-withdrawing groups at a position *ortho* to a hydroxyl group of the catechol moiety. The best results were obtained with the nitro group, hence giving rise to a new class of nitrocatecholic COMT inhibitors. One of the starting structures used by the two research groups was 3,5-dinitrocatechol, bearing two nitro groups in *ortho* and *para* positions to the same hydroxyl group. Although this compound was shown to be highly potent *in vitro*, it soon raised concerns of toxicity and of unfavorable pharmacokinetics, thus limiting its value as an effective drug. The same studies also evidenced that a second substituent, at *para* position to the same hydroxyl group, could be adjusted to modulate the potency, bioavailability, and toxicity of various derivatives. Substituents at this position containing a carbonyl group or carbon–carbon double bond, especially those bearing a multiple conjugated system throughout the whole molecule, were highly effective (Fig. 4) (Bäckström et al. 1989; Borgulya et al. 1989). The concentration of the most potent nitrocatechols necessary to inhibit 50% of COMT activity *in vitro* was typically in the low nanomolar range, which indicates a potency three orders of magnitude higher than that of typical first-generation COMT inhibitors.

Nitrocatechols are kinetically characterized as reversible tight-binding inhibitors of COMT (Borges et al. 1997; Lotta et al. 1995; Schultz and Nissinen 1989). Although poor substrates for the enzyme, they behave competitively with respect to the catechol

substrate and are uncompetitive with respect to the co-substrate SAM (Schultz and Nissinen 1989). Moreover, the new inhibitors showed increased selectivity for COMT over other enzymes involved in the metabolism of catecholamines, such as tyrosine hydroxylase, dopamine-β-hydroxylase, AADC, or monoamine oxidase (A and B forms) (Bäckström et al. 1989).

Among the various nitrocatechol inhibitors initially described, tolcapone, nitecapone, and entacapone were further studied and characterized (Fig. 4). They were shown to inhibit COMT with inhibition constant (K_i) values of 1.02, 0.30, and 0.27 nM for nitecapone, entacapone, and tolcapone, respectively (Lotta et al. 1995). There are some discrepancies among the reported K_i values for the three compounds (Lotta et al. 1995; Nissinen et al. 1992; Schultz and Nissinen 1989; Zurcher et al. 1990b), which are probably due to the different experimental conditions and mathematical models used in data fitting. The kinetic analyses of tight-binding inhibitors are complicated and the determination of inhibition parameters is highly dependent on the enzyme preparations and assay conditions.

Ex vivo, tight-binding inhibitors are orally active and reversibly inhibit COMT activity, to a significant level, in a variety of tissues in the rat. While nitecapone has its main site of action in the duodenum (Nissinen et al. 1988), the structurally related entacapone shows significant COMT inhibition in other peripheral tissues, such as the liver and erythrocytes. However, it is essentially a peripheral COMT inhibitor, with only limited and transient inhibition of brain COMT at the higher doses tested (Learmonth et al. 2002; Nissinen et al. 1988). Tolcapone, on the other hand, by oral administration to rats has a higher potency and a longer duration of action than entacapone (Learmonth et al. 2002; Zurcher et al. 1990b). Moreover, tolcapone can easily cross the blood–brain barrier, showing almost indiscriminate inhibition of both peripheral and brain COMT (Borgulya et al. 1991; Learmonth et al. 2002; Zurcher et al. 1990a, 1990b).

When levodopa plus an inhibitor of AADC is orally administered to rats, together with nitecapone (Nissinen et al. 1988), entacapone (Nissinen et al. 1992), or tolcapone (Borgulya et al. 1989; Zurcher et al. 1990a, 1990b), the bioavailability of plasma levodopa is increased and the 3-OMD levels are markedly reduced. These effects are directly dependent on the administered dose of the COMT inhibitor, and tolcapone shows a more pronounced and longer duration of action than nitecapone or entacapone. Moreover, the levels of levodopa that reach the brain are also augmented, as indicated by the increase in striatal levels of levodopa, dopamine, and DOPAC (dihydroxyphenylacetic acid) and the decrease in those of 3-OMD (Borgulya et al. 1989; Linden et al. 1988; Nissinen et al. 1992; Zurcher et al. 1990a, 1990b). It has been suggested that the response of striatal levels of HVA (homovanillic acid) and 3-MT (3-methoxytyramine), the *O*-methylation metabolites of DOPAC and dopamine, respectively, to oral levodopa/AADC inhibitor, reflects the level of central action of different COMT the inhibitors (Bonifati and Meco 1999; Männistö et al. 1992a). The levels of HVA and 3-MT in the striatum are markedly decreased by tolcapone (Zurcher et al. 1990a), indicating that this compound penetrates the blood–brain barrier and inhibits the brain COMT. On the other hand, the strictly peripheral COMT inhibitor nitecapone increases the striatal levels of HVA (Linden et al. 1988), which reflects an increased amount of levodopa reaching and being utilized in the brain, due to inhibition of COMT in the peripheral tissues, but not in the brain. Entacapone, a mainly peripheral inhibitor, but with detectable central COMT inhibition (Learmonth et al, 2002; Nissinen et al. 1988), does not induce any observable change in the striatal levels of HVA (Nissinen et al. 1992), despite the increase of its precursor, DOPAC.

It has been the subject of some debate whether a COMT inhibitor, to be used clinically as an adjunct in levodopa therapy of PD, should present broad tissue selectivity or, instead, should have a predominance of peripheral over central COMT inhibition, that is, peripheral selectivity. By blocking the activity of COMT, both peripherally and in the brain, centrally active COMT inhibitors such as tolcapone reduce the utilization and depletion of the methyl donor SAM in the striatum, observed after administration of levodopa plus benserazide (da Prada et al. 1994; Yassin et al. 1998). SAM has been claimed to have an antidepressant role (Zurcher et al. 1993) and, therefore, it was suggested that this SAM-sparing effect could be of some advantage in treating patients afflicted with PD, who often show symptoms of depression (Da Prada et al. 1994). On the other hand, inhibition of peripheral COMT is shown to be sufficient to increase the amount of levodopa reaching the brain, by protecting its *O*-methylation in the periphery (Linden et al. 1988; Nissinen et al. 1992; Parada and Soares-da-Silva 2003). Therefore, COMT inhibitors with limited access to the brain may serve as effective adjunct therapy of PD, while avoiding potential undesired central side effects.

Recently, as a result of various structure–activity relationship studies, two new nitrocatecholic COMT inhibitors were developed. Nebicapone (Learmonth et al. 2002) and BIA 3–335 (Learmonth et al. 2004) (Fig. 4) are potent and reversible competitive COMT inhibitors that possess the typical tight-binding properties of other nitrocatechols (Learmonth et al. 2002). These SAR studies demonstrated that, although the nitrocatechol moiety was mainly responsible for the "anchoring" of the inhibitor to the enzyme active site, variation of the side chain substituent exerts a profound influence on both the peripheral selectivity and duration of COMT inhibition. *In vivo*, the two inhibitors are endowed with a potent and long-acting inhibition of COMT, similar to that of tolcapone, yet they show limited access to the brain (Learmonth et al. 2004, 2005), resembling in that matter the peripherally selective, but short-acting entacapone. Nebicapone decreased the levodopa-induced rise in 3-OMD and increased the levels of levodopa in the plasma and striatum of rats treated with levodopa plus benserazide. The effects on the levels of striatal DOPAC and HVA, on the other hand, were dependent on the administered dose of the inhibitor. The rise of DOPAC and HVA observed in the striatum after levodopa plus benserazide was not significantly affected by a dose of 3 mg/kg of nebicapone. By contrast, with higher doses of the inhibitor (30 mg/kg), an increase of DOPAC and a decrease of HVA were observed, indicating that some level of COMT inhibition in the brain may occur at higher doses of the inhibitor (Parada et al. 2001; Parada and Soares-Da-Silva 2003). BIA 3-335 was found to selectively inhibit the peripheral COMT in the mouse, even at doses of 30 mg/kg, while maintaining a long duration of action (Learmonth et al. 2004).

Apart from the chemical nature of the side chain substituent, the alteration of its position of attachment within the nitrocatechol pharmacophore was also found to have a profound effect on the *in vitro* potency, *in vivo* selectivity, and duration of action of COMT inhibitors (Learmonth et al. 2005; Perez et al. 1992, 1993). Two of these late inhibitors were successfully co-crystallized with the rat-recombinant S-COMT. The crystallographic structure of COMT with BIA 3-335 (Bonifácio et al. 2002; Learmonth et al. 2004) (pdb code: 1h1d) was of particular interest, since it was the first complex co-crystallized with a clinically relevant COMT inhibitor. Moreover, this structure revealed the details of the interaction of a nitrocatecholic inhibitor containing a substituted carbonyl side chain, thus providing a structural model to better understand the interactions of other, related and clinically

relevant, COMT inhibitors, such as tolcapone or nebicapone, whose bound structures have not been determined. Recently, COMT was co-crystallized (Palma et al. 2006) with BIA 8-176 (Learmonth et al. 2005), a novel nitrocatecholic COMT inhibitor possessing the nitro group in *ortho* position relative to one of the catechol hydroxyls and to the side chain substituent. The authors discussed the implications of changing the nitro position from the "classical" *meta* position to the *ortho* position in relation to the side chain substituent, regarding the interaction of the inhibitor with the enzyme active site and its inactivation by *O*-methylation. The alteration of the position of the substituent has a profound effect on the *in vitro* regioselectivity of the *O*-methylation reaction (Palma et al. 2006).

Late Atypical Inhibitors

Alongside the discovery of the nitrocatecholic COMT inhibitors, a pyridine compound, CGP 28014 (Fig. 5), was found to increase DOPAC levels in the rat striatum and to reduce those of HVA after oral or intraperitoneal administration (Waldmeier et al. 1990a, 1990b). Like tropolone, this compound lacks the typical catechol structure and does not significantly inhibit COMT *in vitro.* While showing a weak *in vivo* inhibition of 3-OMD formation in the periphery (Männistö et al. 1992a) it mimics the effects of centrally active COMT inhibitors, by decreasing the levels of striatal HVA and 3-MT after clorgyline treatment and by reducing the formation of 3-OMD after administration of levodopa (Männistö et al. 1992a). Consequently, CGP 28014 behaves as a COMT inhibitor-like compound but, unlike other peripherally selective or broadly selective COMT inhibitors, it appears to act

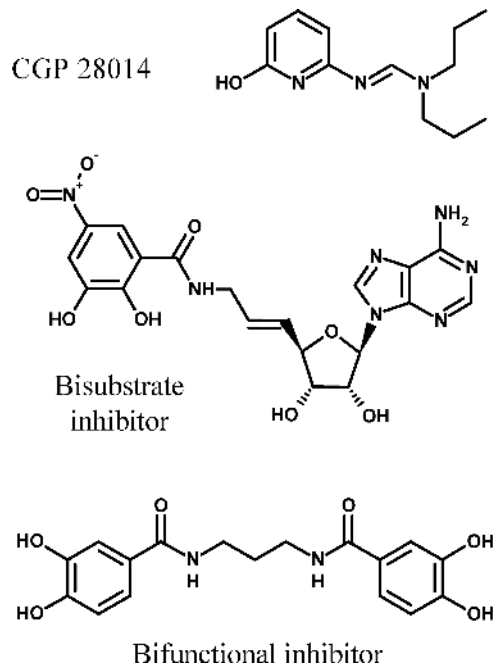

FIG. 5. Atypical late COMT inhibitors.

preferentially in the brain (Männistö et al. 1992b). The mechanism of action of CGP 28014 remains, however, to be further elucidated.

The design of bisubstrate inhibitors of COMT has long been attempted by targeting simultaneously the catechol and the SAM binding sites (Anderson et al. 1981). However, potent inhibitors with multisubstrate adducts were only recently obtained. A structure-based approach, taking advantage of the X-ray structure of the quaternary complex between COMT, 3,5-dinitrocatechol, SAM, and Mg^{2+}, led to the design of a new generation of bisubstrate inhibitors. The most potent of them had an IC_{50} of 9 nM (Lerner et al. 2001, 2003; Masjost et al. 2000). Structurally, it incorporates a catechol moiety covalently connected to the C(5′)-OH group of the adenosine fragment of SAM, through an appropriate spacer (Fig. 5). Structure–activity relationships showed that the particular size and rigidity of the spacer group were critical for the inhibitor's high affinity, and the binding mode was confirmed by X-ray crystallography (pdb code: 1jr4) (Lerner et al. 2001). However, no *in vivo* data have been published and, therefore, their *in vivo* efficacy is still to be demonstrated. Nevertheless, these bisubstrate inhibitors had the merit of demonstrating that potent COMT inhibitors can be designed by circumventing the alleged prerequisite for a nitro group (Paulini et al. 2004).

Another attempt to increase the potency of COMT inhibitors was made by including two catecholic pharmacophores in the same inhibitor molecule (Fig. 5). Depending on the nature of the catechol substituents and the size of the inter-catechol spacer, such bifunctional compounds were shown to have an increased *in vitro* potency over that of the corresponding monofunctional analogs (Bailey and Tan 2005; Brevitt and Tan 1997). However, the nature of the increased potency remained unclear and is complicated by the fact that the insertion of the second functionality often induced a change in the mechanism of enzyme inhibition.

COMT INHIBITION IN PARKINSON'S DISEASE

The COMT inhibitors currently approved as adjuncts to levodopa therapy in Parkinson's disease are tolcapone and entacapone. Tolcapone was first introduced in Europe, United States (USA), and Canada in 1997. Entacapone was introduced in Europe in 1998 and one year later in the USA and Canada. At present, entacapone is available in over 60 countries worldwide. Nebicapone is presently undergoing clinical development. Nitecapone and CGP-28014 were not developed for PD or any other clinical indication.

Pharmacokinetics of COMT Inhibitors

Following oral administration, tolcapone, entacapone, and nebicapone are rapidly absorbed, but due to significant first-pass metabolism their oral bioavailability is not complete. The oral bioavailability ranges between approximately 35% (Heikkinen et al. 2001) for entacapone and 60% for tolcapone (Jorga 1998). The maximum plasma concentration (C_{max}) is reached within 0.5–2 h; their elimination is rapid, with apparent elimination half-life ($t_{\frac{1}{2}}$) between 1.6 and 3.4 h. The main pharmacokinetic parameters following single-dose oral administration in healthy subjects are summarized in Table 2.

Area under the plasma-concentration time curve (AUC) and C_{max} values are dose-proportional after tolcapone (Dingemanse et al. 1995b), entacapone (Keranen et al. 1994),

TABLE 2. Main pharmacokinetic parameters following single oral doses of COMT inhibitors in healthy subjects

Compound and oral dose	C_{max} (μg/mL)	T_{max} (h)	AUC_∞ (μg.h/mL)	$t_{\frac{1}{2}\beta}$ (h)
Tolcapone (Dingemanse et al. 1995b)				
50 mg	2.4	1.2	5.7	1.7
100 mg	4.6	1.7	12.2	2.0
200 mg	6.3	1.8	18.5	2.1
Entacapone (Kaakkola et al. 1990)				
100 mg	1.1	0.6	0.7	1.6
200 mg	1.8	0.7	1.6	3.4
Nebicapone (Almeida and Soares-da-Silva 2003b)				
50 mg	2.7	0.5	4.0*	2.4
100 mg	4.1	1.5	7.4*	2.9
200 mg	5.9	2.0	16.0*	2.0

*AUC_{0-t} — Area under the plasma concentration-time curve (Saito et al. 2001) from time 0 to the last sampling time at which concentrations are equal or above the limit of quantification.
AUC_∞ — AUC from time 0 to infinity; $C_{\max}$ — maximum plasma concentration; $t_{\frac{1}{2}\beta}$ —plasma apparent terminal elimination half-life; $T_{\max}$ — time to reach $C_{\max}$.

and nebicapone oral administration (Almeida and Soares-da-Silva 2003b). The volume of distribution is relatively small and all nitrocathecols are highly bound to plasma proteins (>97%) (Männistö and Kaakkola 1999). The pharmacokinetics of COMT inhibitors is not significantly influenced by the presence of food.

During repeated administration at therapeutic doses, tolcapone, entacapone, and nebicapone did not accumulate in plasma (Almeida and Soares-da-Silva 2003a; Dingemanse et al. 1996; Keranen et al. 1994). Pharmacokinetic properties of the COMT inhibitors are similar following a single dose and following frequent multiple dosing. Pharmacokinetic properties of tolcapone and entacapone were shown to be independent of age. It appears that systemic availability of nebicapone is higher in elderly patients (Ferreira et al. 2006) when compared with young healthy subjects.

Tolcapone, entacapone, and nebicapone are extensively metabolized, mostly in liver. The major metabolic pathways of these compounds involve conjugative reactions. The 3 *O*-β,D-glucuronide conjugates of tolcapone and nebicapone are the major plasma metabolites. Tolcapone and nebicapone are also methylated (Jorga et al. 1999; Loureiro et al. 2006) and the respective metabolites, although present in minor concentrations in human plasma, have very long half-lives, which may suggest that accumulation could occur. However, during long-term administration only a relatively small accumulation of the methylated metabolite occurs, due to the suppression of its formation by the COMT inhibitor itself. Entacapone is not methylated in humans. Minor metabolic pathways in humans include oxidative or reductive reactions and further conjugation of the derived products for tolcapone (Jorga et al. 1999), and sulfate conjugation and nitro reduction followed by acetylation for nebicapone (Loureiro et al. 2006).

For entacapone, the only metabolite found in plasma is the Z-isomer, an active metabolite, but representing only about 5% of the total AUC of both isomers. The major fraction of entacapone urinary metabolites consists of the 3 *O*-β,D-glucuronide conjugates of entacapone (~70%) and its Z-isomer (~25%); the remaining metabolites are the

products of cleavage and reduction of entacapone's side chain and sulfate conjugates (Keranen et al. 1994). It is estimated that excretion in feces is responsible for the elimination of 80–90% of orally administered entacapone (Wikberg et al. 1993), whereas only about 40% of an oral dose of tolcapone is excreted in feces (Jorga et al. 1999). A dose reduction for COMT inhibitors is recommended in liver-impaired patients because of their increased bioavailability and reduced clearance (Männistö and Kaakkola 1999).

Pharmacodynamics of COMT Inhibitors

In clinical trials, the COMT inhibitory effect is usually evaluated by assaying the erythrocyte S-COMT activity. All COMT inhibitors decrease erythrocyte S-COMT activity in a dose-dependent and reversible fashion. The time to maximum inhibition is rapid (<2 h), but the level of inhibition and the time for enzyme activity recovery may differ with the inhibitor. The COMT inhibition profile is similar for tolcapone and nebicapone, and both these inhibitors cause a more profound and sustained inhibition than entacapone. Following oral doses of 100 mg and 200 mg, maximum COMT inhibition is, respectively, 72% and 80% for tolcapone (Dingemanse et al. 1995b) and, respectively, 69% and 80% for nebicapone (Silveira et al. 2003). COMT activity returns to the baseline at approximately 18 h following administration of a 200-mg dose of either tolcapone or nebicapone (Dingemanse et al. 1995b; Silveira et al. 2003). With a 200-mg dose of entacapone, maximum COMT inhibition is 65% and the enzyme recovers full activity within 8 h (Keranen et al 1994). In a comparative study in patients with Parkinson's disease, 75 mg of nebicapone and 200 mg of entacapone caused a similar peak COMT inhibition (approximately 60%), but the inhibitory effect of nebicapone, 75 mg, was more sustained than that observed with entacapone, 200 mg (Ferreira et al. 2006).

In single-dose studies in healthy young subjects in which nebicapone was used concomitantly with levodopa/carbidopa or levodopa/benserazide, nebicapone caused a rapid and reversible inhibition of COMT ranging from 56% (nebicapone 50 mg) to 86% (nebicapone 400 mg) (Almeida et al. 2004; Silveira et al. 2003). In a phase IIa study in Parkinson's disease patients, nebicapone caused a rapid and reversible inhibition of COMT activity, and at 75 or 150 mg its inhibitory effects were stronger and more sustained than with entacapone 200 mg (Ferreira et al. 2006). There is no indication of pharmacodynamic tolerance after repeated administration of tolcapone, entacapone, or nebicapone (Almeida and Soares-da-Silva 2003a; Dingemanse et al. 1996; Rouru et al. 1999).

Effect on Levodopa Pharmacokinetics

COMT inhibitors have a significant effect on the systemic availability and elimination of levodopa. In healthy volunteers, the combined administration of levodopa/AADC inhibitor with increasing doses of entacapone, tolcapone, or nebicapone, 50, 100, and 200 mg, leads to a dose-dependent increase in levodopa AUC without significant changes in levodopa C_{max} or T_{max}. Doses of COMT inhibitors above 200 mg may not increase (or may even decrease) levodopa systemic availability (Almeida and Soares-Da-Silva 2003b; Dingemanse et al. 1995b; Rouru et al. 1999). Explanations that have been considered include degradation of levodopa by other metabolic pathways, while inhibition of COMT becomes more marked (Sedek et al. 1997), or competition during absorption between the COMT inhibitors and

levodopa for the saturable transport of large neutral amino acids at the proximal small intestine level (Contin et al. 2005; Dingemanse et al. 1995a). This competition for absorption between the COMT inhibitors and levodopa may also explain the trend for an increase in mean levodopa T_{max} values when the dose of COMT inhibitor increases, as has been reported for all COMT inhibitors (Almeida et al. 2004; Keranen et al. 1993; Sedek et al. 1997).

In studies in which the COMT inhibitors were administered to healthy subjects concomitantly with standard release levodopa/AADC inhibitor, nebicapone, 200 mg, seemed to induce a higher increase than entacapone, 200 mg, and a lower increase than tolcapone 200 mg in levodopa AUC. For single doses of 100 mg or 200 mg to healthy subjects, the increase in levodopa AUC following concomitant administration with levodopa/AADC inhibitor was 50–88% for tolcapone (Sedek et al. 1997), 29–42% for entacapone (Keranen et al. 1993), and 50–60% with nebicapone (Almeida et al. 2004; Silveira et al. 2003). Studies in healthy subjects have shown that at 200 mg tolcapone did not change (Dingemanse et al. 1995a; Jorga et al. 1998) or increased the C_{max} of levodopa (Jorga et al. 1997a, 1997b; Muller et al. 2000; Sedek et al. 1997). Entacapone, 200 mg, decreased levodopa C_{max} by about 15% (Heikkinen et al. 2002; Keranen et al. 1993), while a decrease of 7–19% was reported for nebicapone, 200 mg (Almeida et al. 2004; Silveira et al. 2003). This flattening (decrease in C_{max}) and enlargement (increase in AUC) in the concentration-time curves for levodopa may have important clinical consequences, especially for safety. A decrease in C_{max} may lower the risk of dyskinesia at peak concentrations and could improve tolerability (Mouradian et al. 1989). More sustained plasma concentrations of levodopa may reduce the fluctuations in brain dopamine levels; the delivery of more continuous stimulation of dopaminergic receptors may reduce the risk for the development of motor complications (Chase 1998; Olanow and Obeso 2000; Olanow et al. 2000).

The formation of 3-OMD from levodopa is dependent on COMT activity, particularly at the intestinal level, the main site of *O*-methylation of levodopa. For all the COMT inhibitors, dose-dependent decreases of 3-OMD C_{max} and AUC values were reported. For a dose of 200 mg, the magnitude of 3-OMD decrease was 65% for tolcapone (Sedek et al. 1997), 45% for entacapone (Keranen et al. 1993), and 61% for nebicapone (Almeida et al. 2004).

Clinical Efficacy of COMT Inhibitors

Tolcapone, entacapone, and nebicapone increase levodopa bioavailability in PD patients, similar to what occurs in healthy volunteers, by significantly increasing levodopa AUC, leaving C_{max} and T_{max} without significant changes. A more sustained and less fluctuating levodopa level is obtained, which results in an improved therapeutic response and lower risk of developing dyskinesias (Chase 1998; Olanow and Obeso 2000; Olanow et al. 2000). Patients experience an increase in the daily ON time (prolonged motor response) and correspondent reduction of OFF time, with consequent improvement in the activities of daily living and general quality of life (Parkinson Study Group 1997; Larsen et al. 2003). In patients with nonfluctuating Parkinson's disease the administration of COMT inhibitors may also have beneficial effects in daily living activities and motor functions as was observed with tolcapone (Waters et al. 1997).

Head-to-head studies comparing different COMT inhibitors are missing and the evaluation of data obtained from noncomparative studies is difficult because of the different study

designs used. A comparison of two separate, simultaneous, long-term open-label studies, one for tolcapone and the other for entacapone, suggested that tolcapone has efficacy superior to that of entacapone with respect to motor symptoms, "OFF" time, and change in levodopa requirements (Factor et al. 2001). The only published head-to-head comparison performed according to a randomized, double-blind, and controlled design is a phase IIa study in PD patients with the wearing-OFF phenomenon (Ferreira et al. 2006). In this study a 4-way crossover design was adopted and nebicapone 75 mg and 150 mg, entacapone 200 mg, and placebo were administered concurrently with levodopa/carbidopa during four sequential treatment periods of 6 to 9 days each. The sequence of treatments occurred according to randomization and the number of daily doses ranged from 4 to 6. Overall, nebicapone 75 mg, improved motor function similar to entacapone, 200 mg; on the other hand, nebicapone, 150 mg had higher efficacy, with the efficacy results being consistent with the results reported for COMT inhibition and levodopa pharmacokinetics (Ferreira et al. 2006).

Safety and Toxicity of COMT Inhibitors

The adverse effects associated with the nitrocatecholic COMT inhibitors can be classified as those related to dopaminergic potentiation and those not related to dopamine. Within the first group, dyskinesia is by far the major adverse effect, followed by nausea and dizziness. Other common adverse effects include orthostatic hypotension, vomiting, anorexia, sleep disorders, somnolence, and hallucinations. The most common non-dopaminergic-related adverse event and whose mechanism is at present unknown, is diarrhea; it is usually more severe with tolcapone than with the other COMT inhibitors. Headache, abdominal pain, and urine discoloration are also observed with tolcapone or entacapone; the latter is due to the presence of the compounds and its metabolites in the urine.

In phase III clinical trials, alterations in liver enzyme levels were reported for 1–3% patients treated with tolcapone, leading to the necessity to closely monitor liver function. After marketing of the drug, cases of serious hepatotoxicity were reported, some of them in the form of fatal fulminant hepatitis (Olanow 2000). Cases of neuroleptic malignant syndrome have also been reported in patients receiving tolcapone (Keating and Lyseng-Williamson 2005). Tolcapone was withdrawn from the European Union and Canadian markets in 1998 and in the USA its use has been restricted. Recently, tolcapone was reintroduced in the European Union, although under stringent monitoring conditions. At present it is available in 27 European countries, most Latin America, Australia, Asia and Pacific countries. With respect to entacapone, no cases of hepatitis or other serious liver failure cases have been reported in phase III trials or after marketing authorization. Increases in liver enzyme levels are rare, and could not be definitely attributed to entacapone (Brooks et al. 2005), and thus no restrictions were applied to its commercialization. Regarding nebicapone no signs of concern arise from the existing data; however, clinical experience is still limited, which precludes the reliable judgment of its liver safety profile.

The mechanisms by which tolcapone triggers severe hepatotoxicity are still under discussion. Tolcapone was shown to be an uncoupler of the oxidative phosphorylation *in vitro* (Nissinen et al. 1997) and possibly also *in vivo* (Haasio et al. 2002a). Besides, acute toxicity does not appear to be due to any reactive or toxic metabolites but to the compound itself, supporting its capability to disrupt mitochondrial function (Borroni et al. 2001). However, formation of glutathione adducts from the amine and acetoamine metabolites was shown

to occur in liver microsomes (Smith et al. 2003). Furthermore, tolcapone was shown to impair energy production in neuroblastoma cells with or without a functional respiratory chain suggesting that its toxicity may also involve a mechanism independent of oxidative phosphorylation (Korlipara et al. 2004). Tolcapone toxicity has been observed mainly at drug levels much higher than those usually seen in the plasma of patients, indicating that variations in the metabolism of the compound, due to genetic factors, comedications, and comorbidities could lead to an increased risk of toxicity (Acuna et al. 2002; Borges 2005; Borroni et al. 2001).

The inhibition of COMT, could, in theory, result in an increase in the half-life of catecholamines or other drugs mainly metabolized by COMT, such as isoproterenol, epinephrine, dobutamine, and apomorphine. In fact, the use of COMT inhibitors is not recommended if patients are taking nonselective inhibitors of monoamine oxidase, the other major enzyme involved in the metabolism of catecholamines (Eisenhofer et al. 2004). As for other COMT metabolized drugs, they should be used cautiously in patients receiving COMT inhibitors.

CONCLUSIONS

The progression of PD and the long-term treatment with levodopa lead to motor complications which, although their pathophysiology is not yet fully understood, could be reduced and the patients could benefit from a more continuous, rather than pulsatile, dopaminergic stimulation. A more continuous dopaminergic stimulation is provided by the triple therapy with levodopa/AADC inhibitor/COMT inhibitor. The use of COMT inhibitors in levodopa/AADC inhibitor–treated patients with advanced and fluctuating Parkinson's disease increases the ON time, reduces the OFF time, and significantly improves the activities of daily living and general quality of life. The use of COMT inhibitors is, therefore, indicated in PD patients treated with levodopa/AADC inhibitor, who begin to experience the signs and symptoms of end-of-dose "wearing-off." Very few compounds—tolcapone, the use of which is restricted, and entacapone, a safer but less efficacious compound—are currently available. Nebicapone is in phase II trials; it appears to be more efficacious than entacapone and safer than tolcapone. This field still has the potential for the development of newer and different COMT inhibitors with a good and safe therapeutic profile.

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