Inhibition of catechol-O-methyltransferase in the cynomolgus monkey by opicapone after acute and repeated administration

Takashi Kitajima, Shintaro Mizote, Maria João Bonifácio, Takeo Umemura, Kazuhiro Yoneda, Paul Mose, Patrício Soares-da-Silva, Makoto Tanaka

* Research Headquarters, Ono Pharmaceutical Co., Ltd., 3-1-1 Sakurai, Shimamoto, Osaka, Japan
b Department of Research & Development, BIAL-Portela & Cª, S.A, 4745-457, São Mamede do Coronado, Portugal
MedInUP – Center for Drug Discovery and Innovative Medicines, University of Porto, Porto, Portugal

HIGHLIGHTS
- Confirmed strong and sustained COMT inhibition by opicapone in a dose dependent manner.
- Opicapone was rapidly absorbed and eliminated after oral administration in monkeys.
- No accumulation of plasma opicapone occurred after multiple dosing.
- Opicapone may fit the unmet need for a sustained COMT inhibitor.

ABSTRACT

Introduction: The aim of the study was to clarify the dose response for inhibition of catechol-O-methyltransferase (COMT) by opicapone, a third generation COMT inhibitor, after acute and repeated administration to the cynomolgus monkey with pharmacokinetic evaluation at the higher dose.

Methods: Three cynomolgus monkeys were used in the study. In the first experiment, COMT inhibition was evaluated over 24 h after the first and at 24 h after the last of 14 daily oral administrations of vehicle, 1, 10 and 100 mg/kg opicapone using a crossover design. In the second experiment, the effect of the maximally effective dose, 100 mg/kg, was retested under the same conditions with additional monitoring of plasma opicapone levels to explore the relationship between pharmacokinetics and pharmacodynamics.

Results: Opi cane dose-dependently inhibited COMT activity, significantly so at 10 and 100 mg/kg. Maximal inhibition was 13.1%, 76.4% and 93.2% at 1, 10 and 100 mg/kg respectively, and COMT remained significantly inhibited at 24 h after 10 and 100 mg/kg (42.6% and 60.2% respectively). Following repeated administration of opicapone residual COMT inhibition at 24 h was 15-25% greater at all doses. In contrast to its pharmacodynamic effect, opicapone was rapidly absorbed and eliminated, with no accumulation in plasma following repeated administration.

Conclusion: Opi cane showed sustained and dose-dependent COMT inhibition despite being rapidly eliminated from plasma and with no evidence for accumulation in plasma after 14 days administration. Opi cane fills the unmet need for a compound with sustained COMT inhibition which will improve levodopa bioavailability in patients with Parkinson’s disease.

1. Introduction

Parkinson’s disease (PD) is associated with the selective loss of neurons in the midbrain area referred to as the substantia nigra pars compacta (Shulman et al., 2011; Saiki et al., 2012). These neurons contain the neurotransmitter dopamine and project their axons to the striatum. Because these neurons control voluntary movements, PD is characterized by motor symptoms such as akinesia, muscle rigidity and tremor at rest. Levodopa (L-3,4-dihydroxyphenylalanine, L-DOPA) replacement therapy is still the most effective symptomatic treatment for PD (Olanow, 2015). However, L-DOPA undergoes rapid and extensive metabolism by a peripheral aromatic L-amino acid decarboxylase (also known as dopa decarboxylase) and catechol-O-methyltransferase (COMT), and only 1% of an oral dose of L-DOPA reaches the brain (Gordin et al., 2003; Nissinen, 2010). Therefore, L-DOPA is usually co-administered with a dopa decarboxylase inhibitor (DCI), which increases L-DOPA bioavailability. Nevertheless approximately 90% of the L-DOPA dose is still converted by COMT to 3-O-methyldopa, which competes with L-DOPA for transport at the blood-brain barrier. Thus, reducing L-DOPA metabolism in the periphery even further by using a
COMT inhibitor is an effective strategy to increase delivery of L-DOPA to the brain. Second generation COMT inhibitors demonstrate enhanced inhibitory potency as compared to the first generation, e.g. gallates and U-0521, by adding a strong electron withdrawing group at the ortho- position to one of the hydroxyl groups in catechols (Nissinen, 2010; Learmonth et al., 2010). Second generation COMT inhibitors include entacapone, a short-acting, peripherally selective COMT inhibitor (Keränen et al., 1994) and tolcapone, a more potent, longer acting compound but which inhibits both cerebral and peripheral COMT, and additionally has potential for hepatotoxicity (Assal et al., 1998).

Opicapone is a third generation COMT inhibitor, discovered by BIAL-Portela & C’, S.A (Kiss et al., 2010), with improved properties as compared to other COMT inhibitors such as tolcapone. It is a highly potent compound, leading to sustained COMT inhibition with no cell toxicity. Opicapone has a relatively short plasma half-life but produces long-lasting COMT inhibition in rats and humans (Bonifácio et al., 2014). This prolonged COMT inhibition is explained by the very high binding affinity of opicapone for COMT, resulting in a slow dissociation rate constant and consequent long duration of action in vivo (Palma et al., 2012; Walkup et al., 2015). Opicapone improved PD symptoms (especially increased motor fluctuations which cannot be stabilized with these combinations alone.

The cynomolgus monkey is a species that is physiologically and pathologically similar to humans and has been used to characterize the pharmacological properties of COMT inhibitors (Bonifácio et al., 2014). In addition, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated monkeys have been used to develop COMT inhibitors as this animal model reproduces symptoms that resemble PD and can be used to evaluate the therapeutic effects of L-DOPA (Filion et al., 1991; Doudet et al., 1997). Repeated oral treatment of 100 mg/kg opicapone reduced soluble COMT activity in erythrocytes and increased the L-DOPA level systemically and in the dorsal striatum, substantia nigra and prefrontal cortex in cynomolgus monkeys (Bonifácio et al., 2014). However, the effect of lower doses and a direct relationship between pharmacodynamic and pharmacokinetics has not yet been reported. Therefore, in this study, we investigated the pharmacodynamics of opicapone, assayed using erythrocyte S-COMT activity (Ferreira et al., 2008), after acute and repeated administration of increasing opicapone dose levels to cynomolgus monkeys and the pharmacodynamic/pharmacokinetic relationship of opicapone at the top dose of 100 mg/kg.

2. Materials and methods

2.1. Animal welfare and ethical statement

All experimental procedures performed on animals were approved by the Institutional Animal Care and Use Committee of Sekisui Medical Co., Ltd. (Ibaraki, Japan) that are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and were performed in accordance with the ethics criteria contained in the bylaws of the committee.

Three purpose-bred female cynomolgus monkeys aged 4–5 years and weighing 3.0–4.5 kg were used in the experiments. Monkeys were purchased from Hamri Co., Ltd. (Ibaraki, Japan). All monkeys were free of simian immunodeficiency virus, Salmonella bacteria, dysentery bacteria, and B virus. They were housed in individual bracket cages (W680 × D670 × H770 mm) under controlled environmental conditions (12 h light/dark cycle and room temperature 18–29 °C), allowed free access to water, and given 160 g food (PS-A, Oriental Yeast Co., Ltd. Tokyo, Japan) once a day at 13:00.

2.2. Animal treatments

During the repeated administration of opicapone, food was given 4 h after administration and removed 2 h after feeding. Opicapone and vehicle (0.2% hydroxypropylcellulose) were administered via the oral route using a sterile catheter with a syringe once a day for 14 days (10 mL/kg). Blood samples were collected at pre-dose, 1, 2, 4 and 24 h after the first opicapone administration, and 24 h after the final administration. The sampling times were chosen to provide sufficient data on COMT inhibition, but not for full pharmacokinetic analysis.

In the first experiment, each of the three monkeys received vehicle or 1, 10 and 100 mg/kg PO opicapone using a 4-way randomized crossover design (Fig. 1). Each treatment was administered once a day for 14 days during four separate periods with an intervening washout period of at least 4 weeks between each treatment period. The dose response of opicapone for COMT inhibition was evaluated over 24 h following the first administration. An additional measure of COMT inhibition was carried out at 24 h following the last of the 14 administrations.

In the second experiment, the three monkeys were first administered vehicle for 14 days and then, following a three-week wash-out period, administered the maximally effective dose established from experiment 1, 100 mg/kg PO opicapone, once a day for 14 days. The relationship between COMT inhibition and opicapone pharmacokinetics was evaluated over 24 h after the first administration. Additional
blood samples were taken at 24 h after the last of the 14 daily administrations of opicapone (100 mg/kg PO) to evaluate recovery of COMT activity and also to determine if there was an increase in residual plasma levels of opicapone after repeated administration. A further sample was taken 3 weeks after the final administration of opicapone to evaluate recovery of COMT activity.

2.3. Blood collection and processing

Whole blood samples (3 mL) were collected from the femoral vein using a sterile needle with a blood collection tube containing EDTA-2K, and were immediately placed on wet ice. Blood was centrifuged at 1500 × g for 10 min at 4 °C. After plasma removal (used for measuring opicapone levels as described in section 2.5 below), the upper buffy white coat was discarded and cold 0.9% NaCl was added at twice the volume of erythrocytes. After the sample was mixed gently, erythrocytes were centrifuged at 1500 × g for 10 min at 4 °C, and the supernatant discarded. Erythrocytes were washed twice under the same conditions, and then stored frozen at −80 °C until used for the COMT assay as described in section 2.4.

2.4. Determination of COMT activity

COMT activity was determined using S-adenosylmethionine (SAM) as a methyl donor and protocatechuic acid (PCA) as a methyl acceptor. COMT activity was measured by a modification of our coupled assay procedure (Nikodejovic et al., 1970). The reaction mixture (total volume of 100 μL) contained 20 μL erythrocytes, 8.85–10.18 μM [14C]-labeled SAM (S-adenosyl-L-(methyl-[14C])-methionine), 184 μM cold SAM, 160 μM PCA, 1 mM MgCl₂, and 5 mM Tris-HCl buffer pH 7.9. The reaction was carried out at 37 °C for 2 h and was stopped by addition of 1.0 mL of 1 M HCl. The sample was mixed with 5 mL ethyl acetate for a few minutes and centrifuged at 1600 × g for 5 min at 4 °C. The ethyl acetate phase (4 mL) was collected and radioactivity (dpm) was quantified using a liquid scintillation counter (model 2500/3100, PerkinElmer Life & Analytical Sciences, Boston, MA). The COMT activity was adjusted for the hemoglobin (Hb) concentration obtained using a hemoglobin assay kit (Wako Pure Chemical Industries, Osaka, Japan) and expressed as pmol/mg hemoglobin/h.

2.5. Bioanalysis of opicapone

Plasma concentrations of opicapone were determined with [13C₆]-opicapone as an internal standard by liquid chromatography-tandem mass spectrometry, after precipitation of plasma proteins with acetonitrile. An API 5000 mass spectrometer with a Turbo-Ionspray Interface (Applied Biosystems/MDS SCIEX, Foster City, CA, USA) operating in negative ion mode was used for mass spectrometric determination. The validated quantitation range was 1.00–1000 ng/mL. The assay precision was ±15% for the coefficient of variation (CV) and the accuracy was within 15% of the nominal values for opicapone.

2.6. Data analysis

All data are presented as mean ± SD. In the pharmacodynamic analysis, the mean COMT inhibition was calculated from individual inhibition from baseline of each treatment period. The maximum inhibition of COMT activity (Eₘₐₓ) and the corresponding time to achieve Eₘₐₓ (tₘₐₓ) were obtained from the experimental data. The area under the time-effect curve from time zero to 24 h (AUEC₀–2₄h) was calculated by the linear trapezoidal method. Statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA). Differences between groups were evaluated by Dunnett test for multiple comparisons and Student t-test for single comparison of two groups. Differences with P < 0.05 were considered significant.

For the pharmacokinetic analysis, the maximum peak concentration (Cₘₐₓ) in plasma, and the corresponding time to reach Cₘₐₓ (tₘₐₓ) were taken from the measured values. Although this was not a complete pharmacokinetic study, some parameters were estimated from this data to allow comparison with human data. The elimination half-life (tₜₐₜ), and the area under the concentration-time curve from time zero to time infinity (AUCₜₐₜ) of opicapone were obtained from individual plasma concentrations of opicapone on Day 1 and calculated using the non-compartmental method with WinNonlin v.6.3 (Pharsight Co, Mountain View, CA, USA). AUCₜₐₜ was calculated from AUCₜₐₜ = Cₜₐₜ/kₜₐₜ, where Cₜₐₜ is the quantifiable concentration at the time of the last measurable drug concentration and kₜₐₜ is the apparent plasma elimination rate constant calculated by log-linear regression of the terminal segment of the concentration-time profile.

3. Results

3.1. Baseline COMT activity

Baseline COMT activities just prior to each treatment period were consistent over the course of the first experiment (Table S1). COMT activity over time is depicted in Fig. 2, the dose-response results are shown in Fig. 3 and pharmacodynamic parameters are presented in Table 1. Like humans, monkeys showed high inter-individual differences in baseline COMT activity which varied from 2370 to 4175 pmol/mg Hb/h in the three monkeys as measured prior to the first treatment period. However, for each monkey the baseline COMT activity throughout the study remained relatively constant (CVs from 7.3% to 9.5%) indicating that the 4-week washout periods were effective in preventing any carryover effect of prolonged COMT inhibition by opicapone. The mean COMT activity during the vehicle administration
period was also relatively constant (3161–3424 pmol/mg Hb/h).

3.2. Experiment 1: Dose response for COMT inhibition after administration of opicapone (1–100 mg/kg)

The effect of acute administration of opicapone (1, 10 or 100 mg/kg, n = 3) on COMT activity was examined following the first of the 14 daily administrations. COMT activity was markedly inhibited by opicapone at 10 and 100 mg/kg, with a mean peak COMT inhibition of 76.4% and 93.2% from baseline, respectively, and a maximal effect at 2 h at both doses. The inhibition of COMT by opicapone, as measured over the 24 h period after the first administration, was dose-dependent (Fig. 4A). COMT activity slightly recovered at 24 h after the first administration, but the inhibition of COMT in the two groups was still 42.6% and 60.2% from baseline, respectively. Following 14-days repeated administration, COMT inhibition in the 10 and 100 mg/kg groups was slightly increased at 24 h post-administration compared to the same time-point after the first administration, to 63.6% and 75.0%, respectively (Fig. 4B). A similar increase was observed at 1 mg/kg opicapone with residual COMT inhibition at 24 h post-administration increasing from 6.6% on Day 1, to 29.5% at 24 h after 14 days.

3.3. Experiment 2: Relationship between pharmacokinetics and pharmacodynamics of opicapone at 100 mg/kg

The inhibition profile for 100 mg/kg opicapone against COMT was very similar in this experiment compared to the first, as shown in Fig. 5A. Maximal inhibition of COMT on day 1 was 95.2% compared to baseline at 1 h post-administration. COMT activity was still inhibited by 61.0% compared to baseline at 24 h post administration and this increased to 73.9% at 24 h after the last administration following repeated opicapone administration for 14 days. Recovery of COMT activity at 3 weeks after the final dose was still not complete, as enzyme activity remained 24.9% below basal values.

Fig. 5B shows the corresponding plasma concentration profile over time obtained for the acute administration of opicapone to monkeys on day 1 of the repeated administration phase (100 mg/kg QD, n = 3). The highest plasma concentrations were measured at 1 h after administration in 2 animals and at 2 h in the third (mean ± SD Cmax: 1810 ± 960 ng/mL) and there then followed a rapid decline in plasma concentrations such that at 4 h the mean plasma concentration was 446 ng/mL and at 24 h only 1.84 ng/mL. From this data the elimination half-life was calculated as 2.4 ± 0.6 h and the AUCinf as 4950 ± 1610 ng h/mL. The plasma opicapone level at 24 h after the last administration was below the lower limit of quantification (< 1.00 ng/mL) suggesting that no accumulation of plasma opicapone has occurred after repeated administration of this dose.

4. Discussion

This study was designed to first establish the maximally effective dose of opicapone for inhibition of COMT in cynomolgus monkeys and second to compare the temporal profile for COMT inhibition with plasma exposure of opicapone at the maximally effective dose. The first experiment established a dose-dependent inhibition of plasma COMT over the range 1–100 mg/kg and identified 100 mg/kg as a dose which almost completely inhibited COMT (Emax 93.2%). This inhibition was long-lasting and still significant at 24 h after the initial dose (60.2% inhibition). Following repeated administration, the inhibition of COMT at 24 h post-administration was higher, at 75.0%. This increase in residual COMT inhibition at 24 h was observed at all doses.

The second experiment confirmed the pharmacodynamic profile of opicapone at 100 mg/kg and demonstrated that this was independent of opicapone plasma concentrations. At this dose, COMT inhibition was nearly complete (95.2% inhibition at 1 h), and then gradually decreased, but was still 61.0% at 24 h after administration. In contrast, the

| Parameters | Dose (mg/kg/day) | 0 | 1 | 10 | 100 |
|------------|------------------|---|---|----|-----|
| \( E_{\text{max}} \) (%) | 4.5 ± 6.1 | 13.1 ± 5.3 | 76.4 ± 11.7 | 93.2 ± 3.3 |
| \( t_{\text{max}} \) (h) | 9.3 ± 12.9 | 3.3 ± 1.2 | 2.0 ± 1.7 | 2.0 ± 1.7 |
| AUEC\(_{0-24h}\) (%)h | 10.2 ± 223.5 | 190.9 ± 172.2 | 1415.6 ± 138.5 | 1822.2 ± 121.3 |

Data are shown as mean ± SD (n = 3).
plasma opicapone level was very low (1.29 ng/mL compared to 1810 ng/mL at Cmax) at trough, 24 h after administration. Thus, even though opicapone has a profile of high clearance and a short half-life of 2.4 h, COMT inhibition is sustained far beyond that expected based on the plasma opicapone level. This agrees with the data seen in humans (Almeida et al., 2013) and is probably due to the very high binding affinity of opicapone for COMT (Palma et al., 2012).

The dose level used in Experiment 2 gave exposure that is comparable to that obtained clinically in humans. Administration of 100 mg/kg opicapone gave only slightly higher exposure in monkeys (Cmax: 1.81 μg/mL, AUCinf: 4950 ng h/mL) compared to humans at 50 mg (Cmax: 1.54 μg/mL, AUCinf: 3756 ng h/mL in Caucasian subjects) (Falcão et al., 2016). The total inhibitory effect of 100 mg/kg opicapone on Day 1 in Experiment 2 in monkeys was ~75% less than baseline which is comparable to that seen in humans at 50 mg, of 61.7% less than controls. These results indicate that COMT inhibition and plasma opicapone exposure in monkeys at 100 mg/kg are similar to that seen in humans, suggesting that the relationship of COMT inhibition and pharmacokinetics in monkeys is similar to that in humans.

There was no accumulation of opicapone in plasma following repeated administration for 14 days (Fig. 5B), but COMT inhibition at trough was slightly increased by repeated administration of opicapone (Figs. 2 and 5A) with the dose-response curve for trough activity shifting leftward (Fig. 4B). These data indicate that repeated opicapone administration led to more sustained COMT inhibition without accumulation of opicapone in plasma. The sustained COMT inhibition is probably due to a long dissociation half-life and is dose-independent (Rocha et al., 2013).

Opicapone showed strong and prolonged COMT inhibition in monkeys, but the data for basal COMT activity prior to each treatment
session (Table S1) indicates that a 4-week washout period was sufficient to completely recover functional COMT enzyme from its opicapone-bound state although COMT was still inhibited by 24.9% 3 weeks after the final treatment in Experiment 2. This shows that recovery of monkey COMT enzyme from the opicapone-bound state has a long half-life of > 50 h, which is similar to that of 61.6–130 h in humans (Rocha et al., 2013; Svetel et al., 2018). A Basic Local Alignment Search Tool (BLAST) search showed that the amino acid sequence of human COMT has greater similarity to that of cynomolgus monkey (94%, Fig. S1). These data suggest that monkeys are a good species for translational research to evaluate COMT inhibitors such as opicapone.

There are several limitations to this study. Firstly, plasma opicapone exposure was only monitored with opicapone 100 mg/kg and at limited sampling points. The exposure to opicapone increases almost dose-proportionally with increasing doses at 100–1000 mg/kg (BIAL, unpublished data). We expect that plasma opicapone levels below 100 mg/kg in monkeys are also likely to show dose-proportionality. The pharmacokinetic parameters were obtained without data before 1 h or from the elimination phase (4 h–24 h). The possibility of an early plasma peak before 1 h is considered unlikely because the solubility of opicapone is not high and tmax of individual monkeys were either 1 h or 2 h. The t1/2 was considered reasonable to estimate because of an approximately straight exponential decline in the elimination phase has been observed in previous studies (BIAL, unpublished data). Second, opicapone is highly metabolized, but no metabolites were measured. An active metabolite BIA 9–1079 (amine N-oxide reduced form, IC50 = 429 nM for rat liver COMT vs that of opicapone = 224 nM) is responsible for some COMT inhibition in rats, but not in humans (Almeida et al., 2013; Falcão et al., 2015; Rocha et al., 2013; Gonçalves et al., 2017). In monkeys, the level of BIA 9–1079 in plasma was slightly lower than that of opicapone at tmax of around 1 h, and the metabolite was rapidly eliminated from plasma with low systemic accumulation in repeated administration (BIAL, unpublished data). This metabolite may contribute to COMT inhibition in monkeys.

In conclusion, opicapone was rapidly eliminated from plasma, but showed sustained erythrocyte COMT inhibition with significant inhibition at 24 h after administration. This was not due to drug accumulation as trough levels remained below the limit of quantification after 14 days administration. Finally, the similar time profile of COMT inhibition and plasma opicapone in clinical studies and in the present study suggests that the cynomolgus monkey is a suitable species with which to further explore the activity of opicapone. Overall, these data support the view that opicapone will support the view that opicapone will

Conflicts of interest statement

This study was sponsored by Ono Pharmaceutical Co., Ltd. All authors were involved in the design or conduct of the study; collection, management or analysis of the data; and preparation or review of the manuscript. At the time of the study, T.K., S.M., T.U., K.Y. and M.T. were employees of Ono Pharmaceutical Co., Ltd.; and M.J.B., P.M. and P.S.S. were employees of BIAL-Portela & Cª, S.A.

Chemical compounds

Oopicapone (PubChem CID: 76966913).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2018.10.001.

Abbreviations

AUC area under the curve
AUCinf area under the drug concentration-time curve from time zero to infinity
AUC0-∞ area under the drug concentration-time curve from time zero to the time of the last measurable concentration
BLAST Basic Local Alignment Search Tool
Cmax maximum concentration
COMT catechol-O-methyltransferase
CV coefficient of variation
DCI dopa decarboxylase inhibitor
EDTA ethylenediaminetetraacetic acid
Emax maximal effect
Hb hemoglobin
t-DOPA levodopa
MPTP 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PCA protocatechuic acid
PD Parkinson’s disease
SAM S-adenosylmethionine
S-COMT soluble COMT
tEmax time to Emax
tmax time to Cmax

References

Almeida, L., Rocha, J.F., Falcão, A., Palma, P.N., Loureiro, A.I., Pinto, R., Bonifácio, M.J., Wright, L.C., Nunes, T., Soares-da-Silva, P., 2013. Pharmacokinetics, pharmacodynamics and tolerability of opicapone, a novel catechol-O-methyltransferase inhibitor, in healthy subjects: prediction of slow enzyme-inhibitor complex dissociation of a short-living and very long-acting inhibitor. Clin. Pharmacokinet. 52, 139–151.
Aassl, F., Spahr, L., Hadengue, A., Rubbia-Brandt, L., Burkhard, P.R., 1998. Tolcapone and fulminant hepatitis. Lancet 352, 958.
Bonifácio, M.J., Sutcliffe, J.S., Torrão, L., Wright, L.C., Soares-da-Silva, P., 2014. Brain and peripheral pharmacokinetics of levodopa in the cynomolgus monkey following administration of opicapone, a third generation nitrocatechol COMT inhibitor. Neuropharmacology 77, 334–341.
Bonifácio, M.J., Torrão, L., Loureiro, A.I., Palma, P.N., Wright, L.C., Soares-da-Silva, P., 2015. Pharmacological profile of opicapone, a third-generation nitrocatechol catechol-O-methyl transferase inhibitor, in the rat. Br. J. Pharmacol. 172, 1739–1752.
Doudet, D.J., Chan, G.L., Holden, J.E., Morrison, K.S., Wyatt, R.J., Ruth, T.J., 1997. Effects of catechol-O-methyltransferase inhibition on the rates of uptake and reversibility of 6-fluoro-L-Dopa trapping in MPTP-induced parkinsonism in monkeys. Neuropharmacology 36, 363–371.
Falcão, A., Rocha, J.F., Santos, A., Nunes, T., Soares-da-Silva, P., 2016. Opicapone pharmacokinetics and pharmacodynamics comparison between healthy Japanese and matched white subjects. Clin Pharmacol Drug Dev 5, 150–161.
Ferreira, J.J., Almeida, L., Cunha, L., Tiemea, M., Rosa, M.M., Januário, C., Mitu, C.E., Coelho, M., Correia-Guedes, L., Morgadinho, A., Nunes, T., Wright, L.C., Falcão, A., Sampaio, C., Soares-da-Silva, P., 2008. Effects of nebicapone on levodopa pharmacokinetics, catechol-O-methyltransferase activity, and motor fluctuations in patients with Parkinson disease. Clin. Neuropharmacol. 31, 2–18.
Ferreira, J.J., Lees, A., Rocha, J.F., Poeve, W., Rasol, O., Soares-da-Silva, P., Bi-Park 1 investigators, 2016. Opicapone as an adjunct to levodopa in patients with Parkinson’s disease and end-of-dose motor fluctuations: a randomised, double-blind, controlled trial. Lancet Neurol. 15, 154–165.
Filion, M., Tremblay, L., Bédard, P.J., 1991. Effects of dopamine agonists on the spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res. 547, 152–161.
Gonçalves, D., Alves, G., Fortuna, A., Bonifácio, M.J., Soares-da-Silva, P., Falcão, A., 2017. A single- and multiple-dose study to investigate the pharmacokinetics and pharmacodynamics of opicapone, a novel COMT inhibitor, in rat. Neuropharmacology 125, 146–155.
Gordin, A., Kaakola, S., Teräväinen, H., 2003. Position of COMT inhibition in the treatment of Parkinson’s disease. Adv. Neurol. 91, 237–250.
Keränen, T., Gordin, A., Karlsson, M., Korpela, K., Pentikäinen, P.J., Rita, H., Schultz, E., Seppälä, L., Wikberg, T., 1994. Inhibition of soluble catechol-O-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone. Eur. J. Clin. Pharmacol. 46, 151–157.
Kiss, L.E., Ferreira, H.S., Torrão, L., Bonifácio, M.J., Palma, P.N., Soares-da-Silva, P., Learmonth, D.A., 2010. Discovery of a long-acting, peripherally selective inhibitor of catechol-O-methyltransferase. J. Med. Chem. 53, 3396–3411.
Learmonth, D.A., Kiss, L.E., Soares-da-Silva, P., 2010. The chemistry of catechol-O-methyltransferase inhibitors. Int. Rev. Neurobiol. 95, 119–162.
Lees, A.J., Ferreira, J., Rasol, O., Poeve, W., Rocha, J.F., McCray, M., Soares-da-Silva, P., BIPARK-2 Study Investigators, 2017. Opicapone as adjunct to levodopa therapy in patients with Parkinson disease and motor fluctuations: a randomized clinical trial. JAMA Neurol. 74, 197–206.
Nikodejevic, B., Senoh, S., Daly, J.W., Creveling, C.R., 1970. Catechol-O-methyltransferase. II. A new class of inhibitors of catechol-O-methyltransferase: 3,5-dihydroxy-4-methoxybenzoic acid and related compounds. J. Pharmacol. Exp. Therapeut. 174, 83–93.

Nissinen, E., 2010. Introductory remarks: catechol-O-methyltransferase inhibition - an innovative approach to enhance L-dopa therapy in Parkinson's disease with dual enzyme inhibition. Int. Rev. Neurobiol. 95, 1–5.

Olanow, C.W., 2015. Levodopa: effect on cell death and the natural history of Parkinson's disease. Mov. Disord. 30, 37–44.

Palma, P.N., Bonifácio, M.J., Loureiro, A.L., Soares-da-Silva, P., 2012. Computation of the binding affinities of catechol-O-methyltransferase inhibitors: multisubstrates relative free energy calculations. J. Comput. Chem. 33, 970–986.

Rocha, J.F., Almeida, I., Falcão, A., Palma, P.N., Loureiro, A.L., Pinto, R., Bonifácio, M.J., Wright, L.C., Nunes, T., Soares-da-Silva, P., 2013. Opicapone: a short lived and very long acting novel catechol-O-methyltransferase inhibitor following multiple dose administration in healthy subjects. Br. J. Clin. Pharmacol. 76, 763–775.

Saiki, S., Sato, S., Hattori, N., 2012. Molecular pathogenesis of Parkinson's disease: update. J. Neurol. Neurosurg. Psychiatry 83, 430–436.

Shulman, J.M., De Jager, P.L., Feany, M.B., 2011. Parkinson's disease: genetics and pathogenesis. Annu. Rev. Pathol. 6 193–222.

Svetel, M., Tomić, A., Kresojević, N., Kostić, V., 2018. Pharmacokinetic drug evaluation of opicapone for the treatment of Parkinson’s disease. Expet Opin. Drug Metabol. Toxicol. 24, 1–8.

Walkup, G.K., You, Z., Ross, P.L., Allen, E.K., Daryaei, F., Hale, M.R., O'Donnell, J., Elmann, D.E., Schuck, V.J., Buurman, E.T., Choy, A.L., Hajec, L., Murphy-Benenato, K., Marone, V., Patey, S.A., Grooser, L.A., Johnstone, M., Walker, S.G., Tonge, P.J., Fisher, S.L., 2015. Translating slow-binding inhibition kinetics into cellular and in vivo effects. Nat. Chem. Biol. 11, 416–423.