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PHARMACOLOGY REVIEW(S)

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TASMAR (tolcapone)
100 and 200 mg tablets
NDA 20-697

Hoffman-La Roche Inc
Nutley, NJ 07110-1199
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INTRODUCTION

Tolcapone (TASMAR), an inhibitor of catechol-O-methyltransferase (COMT) has been proposed for use in the treatment of Parkinson's disease as an adjunct to levodopa/carbidopa therapy. Administered concomitantly with levodopa and an aromatic amino acid decarboxylase (AADC) inhibitor it leads to more stable plasma levels of levodopa by reducing its metabolism to 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD). This may lead to an improvement in symptomatic response and may allow a reduction of the daily dose of levodopa. When TASMAR is administered together with levodopa/carbidopa, it increases the relative bioavailability (AUC) of levodopa by approximately two-fold. The average peak levodopa plasma concentration (C_{max}) and the time of its occurrence (T_{max}) were unaffected. Studies in healthy volunteers and Parkinson's disease patients suggest that the maximal effect occurs with 100 mg to 200 mg tolcapone. Plasma levels of 3-OMD were markedly and dose-dependently decreased by tolcapone when given with levodopa/carbidopa.

Tolcapone is a yellow, odorless, non-hygroscopic, crystalline compound with a relative molecular weight of 273. The chemical name of tolcapone is 3,4-dihydroxy-4'-methyl-5-nitrobenzophenone.

Tolcapone is rapidly absorbed with a T_{max} of approximately 2 hours. The absolute bioavailability following an oral administration is around 60% (200 mg dose). Tolcapone does not accumulate with t.i.d. dosing of 100 mg or 200 mg. At these doses, C_{max} is approximately 3 $\mu\text{g/mL}$ and 6 $\mu\text{g/mL}$, respectively. Food delays the absorption of tolcapone, but the relative bioavailability of a tolcapone dose taken with a meal is still 80% to 90% of that taken under fasting conditions. The steady-state volume of distribution (V_{ss}) of tolcapone is small (9 L). Tolcapone does not distribute widely into tissues due to its high plasma protein binding (>99.9%). In vitro experiments have shown that tolcapone binds mainly to serum albumin. Tolcapone is almost completely metabolized prior to excretion with only a very small amount (0.5% of the dose) found unchanged in urine. The main metabolic pathway of tolcapone appears to be conjugation leading to its inactive glucuronide. In addition, the compound is methylated by COMT to 3-O-methyl-tolcapone and metabolized in-vitro by cytochrome P450 3A4 and P450 2A6 to a primary alcohol

(hydroxylation of the methyl group) which is subsequently oxidized to the carboxylic acid. The reduction to a putative amine as well as the subsequent N-acetylation occur to a minor extent. After oral administration 60% of drug-related material is excreted into urine and 40% into feces. Tolcapone is a low-extraction-ratio drug (extraction ratio = 0.15) with a moderate systemic clearance of about 7 L/h. The elimination half-life of tolcapone is approximately 2-3 hours.

Studies in healthy volunteers have shown that tolcapone reversibly inhibits human erythrocyte COMT activity after oral administration. The inhibition is closely related to plasma tolcapone concentration. With an oral dose of 200 mg tolcapone, maximum inhibition of erythrocyte COMT activity is about 80% and at 200 mg t.i.d., erythrocyte COMT inhibition at trough is 30% to 45%.

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A total number of 51 studies were submitted of which 22 were reviewed. All 4 bioequivalence studies were reviewed. Of 30 pharmacokinetic studies, drug interaction, dissolution, formulation and in-vitro studies, 18 were reviewed. Sixteen analytical studies were condensed into 2 studies.

SUMMARY

Bioequivalence Study:

In a bioequivalence study, 25 subjects received either 100 mg or 200 mg tolcapone tablets to compare the formulations used in clinical trials and the formulations intended for marketing. The study indicated that in terms of AUC, 90% confidence interval is within the required range of 80% to 125% for all formulations but the C_{max} meets this criteria only for marketing formulations of 100 mg (F21) vs 200 mg (/029). The clinical trial tablets and the tablets intended for marketing were not bioequivalent in terms of C_{max} (study #2).

In a separate bioequivalence study conducted in the Japanese volunteers using formulations /010, /030 and /029, both C_{max} and AUC were within the confidence interval of 80 to 125% (study #2a).

In another bioequivalence study conducted in the Japanese volunteers using clinical formulations of 50 mg (/012), 100 mg (/021) and 200 mg (/010), both C_{max} and AUC were within the confidence interval of 80 to 125% (study #2b)

The 200 mg US marketing formulation (F20) is qualitatively and quantitatively similar to the 200 mg non-US marketing formulation (/029) with only a minor coloring difference in the film coat. Therefore, formulation /029 is representative of F20, the 200 mg tablet to be marketed in the US. Formulations /030 (100 mg) and /029 (200 mg) are the non-US marketing tablets. Like /029 and F20, the difference between /030 and F21 (100 mg tablet to be marketed in the USA) is in the coloring agent. Therefore, it can be concluded that qualitatively and quantitatively both 100 mg and 200 mg tablets to be marketed in the US are similar to the tablets to be marketed outside the US.

The in-vitro dissolution profiles of 200 mg tablet lot used in clinical trials (GMZ 0012 in caucasian) was slower than both the 100 mg and 200 mg to be marketed tablets (study #2). This slow dissolution may be responsible for the lower C_{max} for 200 mg clinical tablets. When 100 mg clinical tablet was tested with the 200 mg clinical tablet for the bioequivalence, the 90% confidence interval on the C_{max} was outside the 125% limit. It appears that the failure of C_{max} between the clinical tablets and to be marketed tablets to meet the 90% confidence interval may be an artifact and a difference of 2.5 $\mu\text{g/mL}$ between clinical and marketing tablets may not be of any clinical significance.

Therefore, based on the dissolution profiles, similarity between the US and non-US marketing formulations and the bioequivalence study in the Japanese volunteers it can be concluded that tolcapone tablets used in the clinical trials at 100 mg and 200 mg strengths are bioequivalent to the to be marketed tablets of the same strengths.

Absorption:

Absorption of tolcapone is rapid and peak concentrations (6 µg/mL) are reached within 2 h after 200 mg oral dosing. Following a 200 mg oral dose of tolcapone, absolute bioavailability of tolcapone was 60% (study #1).

Distribution:

The apparent volume of distribution at steady state (V_{ss}) following 50 mg IV infusion over 10 minutes was 8.6 ± 1.1 L (study #1).

Equilibrium dialysis experiments with tolcapone in human plasma showed that protein binding was high (>99.8%) over the concentration range of 0.32 to 210 µg/mL. The binding was attributed almost entirely to albumin (>99.9%), and not to α_1 -acid glycoprotein or low density lipoproteins. The blood to plasma ratio of radioactivity was 0.6 (study #5).

Metabolism:

In a mass balance study, healthy volunteers (n =6) received single oral doses of ^{14}C -labelled tolcapone. The radioactive material was administered in the form of a capsule (200 mg, 50 µCi). On average, 40% of the drug-related material was excreted within 24 h of administration, and it took 168 h to recover at least 90% of the radioactivity from all 6 individuals. The recovery of radioactivity was virtually complete (mean 96.7%, range 95.3 to 98.2%) within 9 days (up to 216 hours) (study #5).

Tolcapone is almost completely metabolized and tolcapone-related compounds are eliminated into urine (60%) and feces (40%). However, the majority of ^{14}C -labeled dose of tolcapone was unidentified in urine and feces (only 25% of the administered dose has been identified). In plasma, more than 80% of the drug-related material was identified. Only 0.5% unchanged tolcapone was found in urine. The major drug-related compounds in plasma were tolcapone (35%), 3-O-methyl tolcapone (Ro 40-7591, 26%) and a glucuronide conjugate (Ro 61-1448, 12%). The formation of 3-O-methyl tolcapone is dependent on COMT activity. Conjugation to the 3-O-glucuronide (Ro 61-1448) metabolite is the major elimination pathway of tolcapone. Oxidation of the 4'-methyl group (Ro 47-1868) and reduction of the 5'-nitro group (Ro 47-1669) represent minor pathways (study #5).

The metabolism of tolcapone was studied in vitro in liver microsomes from humans, rats and dogs. The primary metabolic product during incubations with liver microsomes was the 3-O-glucuronic acid conjugate of tolcapone (Ro 61-1448) which was formed by the action of UDP-glucuronyl transferase (UDPGT) enzymes. The highest therapeutic concentrations of tolcapone in vivo (about 32 µM) are lower than its K_m ($75 \pm$

9 μ M) suggesting that the UDPGT enzymes will not be saturated. Hydroxylation of the 4'-methyl group to a primary alcohol, Ro 47-1868, was mediated by cytochromes P450 3A4 (CYP3A4) and 2A6 (CYP2A6) in man with CYP3A4 being predominant. The affinity of tolcapone for CYP3A4 in human microsomes appeared to be higher than for glucuronyl transferase, but the V_{max} for the conjugation was about five times higher, consistent with the fact that glucuronidation appears to represent the main elimination pathway in humans in vivo. Therefore, inhibitors of CYP3A4 are expected to have little effect on the clearance of tolcapone. Metabolic reduction of tolcapone can occur but, compared to the oxidation to Ro 47-1868, this is likely to be a minor pathway (study #16).

Elimination:

Following IV administration, the mean total plasma clearance of tolcapone was 7.1 \pm 1.5 L/h and the elimination half-life of tolcapone was 1.3 \pm 0.3 hours (study #1).

Dose Proportionality:

Pharmacokinetics of tolcapone is linear from 50 to 400 mg dose. However, Ro 40-7591 (an inactive metabolite of tolcapone) was not linear over this dose range (Study #3).

Food Effect:

The effect of food (200 mg single dose given 45 minutes after breakfast) was assessed in healthy Japanese male volunteers (n =6). Food decreased C_{max} and AUC by 48% and 23%, respectively. Food increased the T_{max} almost by two fold. Food also decreased the Area Under the Effect Curve (AUE) of COMT inhibition by 25% (study # 4).

Based on these results no restrictions with respect to drug intake in relation to meals were applied in the therapeutic trials with tolcapone. It was therefore possible to evaluate the effect of food on the pharmacokinetics of tolcapone directly in the target population. A study using the population approach confirmed that the bioavailability of tolcapone taken with a meal (within 1 h before and up to 2 h after) was reduced to 80 - 90% (study # 14).

Multiple Dose Kinetics:

In a multiple ascending dose study, tolcapone (100-800 mg, t.i.d.) along with multiple fixed doses of Sinemet^R (25-100 mg) was administered to 55-75 years old volunteers for 7 days. Tolcapone did not accumulate in plasma during the multiple dosing of 100, 200 or 400 mg t.i.d. Only at 800 mg t.i.d. there was accumulation (almost 2 fold), as shown by increased C_{max} and AUC values from Day 1 to Day 7. Ro 40-7591-were

accumulated at all doses of tolcapone up to day 5, after which the morning levels remained relatively constant (study #9).

Pharmacodynamics of Tolcapone:

Erythrocyte COMT activity was assessed as a pharmacodynamic marker in several studies and it was found that tolcapone dose-dependently inhibits the enzyme activity. Tolcapone increased the general exposure to L-DOPA (AUC) and its elimination half-life, without affecting its peak plasma concentrations, leading to a more sustained supply of L-DOPA to the brain. The improved L-DOPA plasma concentration-time profile occurred after the first administration of tolcapone and was maintained throughout the duration of treatment. It was shown that tolcapone substantially reduced the formation of 3-OMD in all studies.

Following a single dose of tolcapone (5 to 800 mg), inhibition of COMT in erythrocytes was rapid following administration of all doses of tolcapone. The data could be described using a simple inhibitory E_{max} model. Time to minimum COMT activity in erythrocytes (T_{min}) being achieved within 2 hours for most subjects. Maximum % inhibition of COMT activity increased with increasing dose of Ro 40-7592, from 19.5 ± 4.6 at a dose of 5 mg to 72.4 ± 10.0 at a dose of 100 mg to $88.5 \pm 2.9\%$ at a dose of 800 mg. A rapid rise in maximum % COMT inhibition was observed with doses from 5 mg to 50 mg of Ro 40-7592 and this rise continued at a diminished rate up to a dose of 800 mg. The maximum drug effect as expressed by maximum % COMT inhibition was achieved at doses of 200 - 800 mg. The time to recovery of baseline COMT activity (T_{rec}) ranged from 4.5 hours at a 5 mg dose to 24 hours at a 800 mg dose (Study #3).

SPECIAL POPULATION:

Hepatic Impairment:

The pharmacokinetics of tolcapone was assessed in 8 healthy volunteers, 8 patients with cirrhotic liver disease (Child Pugh Classification 'B') and 8 patients with non-cirrhotic liver disease (alanine aminotransferase at least twice upper limit of normal, alkaline phosphatase at least 1.5 times upper limit of normal). The 3 groups of volunteers in this study were similar with respect to age, weight and gender. The volunteers received 50 mg tolcapone IV or a single oral dose of 200 mg tolcapone. There were no substantial effects of hepatic impairment on the systemic clearance or volume of distribution of tolcapone, based on total drug concentrations after i.v. dosing. The patients with moderate liver cirrhosis had reduced albumin levels because of their disease (study #6).

Following 200 mg oral dose of tolcapone, the C_{max} and AUC increased by 30% and 20%, respectively in the cirrhotic patients. The plasma concentration of the tolcapone glucuronide increased in patients with hepatic impairment, probably due to a combination of increased formation and decreased elimination. The C_{max} and AUC of tolcapone glucuronide increased by 4 fold and 7 fold, respectively in cirrhotic patients. There was almost a 2 fold increase in unchanged drug excreted in the urine of cirrhotic patients and 2 fold decrease in renal clearance. The pharmacokinetics of Ro 40-7591 (a tolcapone metabolite) and Ro-471669 (glucuronide conjugate) were not altered in patients with liver disease.

An almost 50% reduction in unbound clearance of tolcapone in patients with moderate liver cirrhosis was observed. Since the volume of distribution of unbound tolcapone decreased almost to the same extent as the unbound clearance, the elimination half-life was not changed. There was almost 10 fold increase in unchanged drug excreted in the urine of cirrhotic patients.

Based upon 50% reduction in unbound clearance of tolcapone, the usual recommended dose of tolcapone should be halved in Parkinsonian patients with moderate cirrhotic liver disease, to achieve the same unbound drug concentrations. An adjustment of the dosing interval is not warranted.

Renal Impairment:

The mass balance study showed that 60% of the drug-related material was excreted into the urine. However, very small amount of tolcapone was excreted unchanged by the kidneys (0.5%). Renal impairment should therefore not affect tolcapone concentrations, which was confirmed by the lack of a significant relationship between creatinine clearance and tolcapone clearance in Parkinsonian patients. It was calculated that a patient with a creatinine clearance of 50 mL/min would still have at least 70% of the typical clearance of tolcapone and adjustment of tolcapone dosage in this situation is considered unnecessary (study #13).

Although the main metabolite of tolcapone, 3-O-glucuronide (Ro 61-1448) is excreted into the urine, an alternative route exists into the bile. The accumulation of tolcapone glucuronide was evaluated in Parkinsonian patients at the end of a 6 week treatment period of 100 or 200 mg tolcapone t.i.d. In these patients the renal function showed a wide variability and the creatinine clearance estimates ranged from 42 to 146 mL/min. It was shown that even in the patients at the lower end of the creatinine clearance, tolcapone glucuronide trough levels were low and no relationship between glucuronide concentrations and creatinine clearance was observed. The variability in glucuronide

concentrations in plasma was mainly accounted for by the tolcapone concentrations in these patients. Accumulation of this inactive, stable conjugate, which does not regenerate parent drug, should not represent a risk. Given the very high protein binding of tolcapone, no significant removal of the drug by hemodialysis is expected. No extra dose at the end of a hemodialysis session would be necessary.

Age:

There were no substantial differences between the young (18-40 years) and the elderly (55-75 years) volunteers in the mean pharmacokinetic parameters at dose levels of 100-400 mg of tolcapone. At 800 mg dose, the AUC and half-life increased by 30% and 70%, respectively in the elderly as compared to young adults (studies # 3 & 9).

Gender:

No gender difference was observed in the pharmacokinetics of tolcapone at the dose levels of 100-800 mg (Study #9).

Race:

No ethnic differences in the pharmacokinetics of tolcapone were observed between Caucasians (n = 6) and Japanese (n = 6) or Caucasians (n = 18) and Blacks (n = 11). (study #9).

Drug-Interactions:

The effects of tolcapone on the elimination of other drugs could depend either on competition for elimination pathways with tolcapone itself or on the ability of tolcapone to inhibit the pathways of other drugs.

Effect of tolcapone on carbidopa:

Carbidopa has been one of the most frequent concomitant medications with tolcapone since it is contained in all Sinemet formulations. The effect of tolcapone on carbidopa pharmacokinetics (study #10) was investigated. The AUC of carbidopa at baseline before starting tolcapone was similar to the AUC of carbidopa 6 weeks later. Since the peak concentrations, time to peak and peak-to-trough fluctuations followed the same pattern as with or without tolcapone, it was concluded that tolcapone did not affect the pharmacokinetics of carbidopa. The pharmacokinetics of tolcapone was not altered due to carbidopa.

Effect of tolcapone on levodopa:

After administration of Sinemet^R 25/100 either with placebo or single ascending doses of tolcapone (5-800 mg), the most pronounced result observed regarding the effect of tolcapone was the dose-dependent decrease in 3-OMD plasma levels. At a dose of 10 mg tolcapone, a 33% decrease in the AUC of 3-OMD plasma levels was achieved. A nearly complete inhibition of the formation of this metabolite was obtained with the highest dose (800 mg), where 3-OMD levels remained close to baseline values. Because the formation of levodopa metabolite (3-OMD) was inhibited, a 2-fold increase in the AUC of L-DOPA was observed. The pharmacokinetics of tolcapone was not altered due to levodopa (study #7).

Tolcapone when given in single doses (10 - 800 mg) together with Madopar^R (25 mg benserazide and 100 mg L-Dopa) to healthy male volunteers, exhibited dose-dependent inhibition of COMT activity in erythrocytes and inhibition of formation of 3-O-methyldopa from L-Dopa was observed. This was associated with an increase in the bioavailability of L-Dopa (AUC increased by a factor of 2 at 200 mg). Madopar appears to have no effect on the pharmacokinetics of tolcapone (study #8).

Glucuronidation:

The tricyclic antidepressant, desipramine is a possible concomitant medication with tolcapone in Parkinson's disease patients and has glucuronidation as an important elimination pathway. A study was performed in 22 healthy volunteers to investigate the potential pharmacokinetic interaction between desipramine and tolcapone (Study # 11). It was confirmed that tolcapone did not influence the pharmacokinetics of desipramine and 2-hydroxy desipramine. The median increase in desipramine C_{max} and AUC was approximately 10% when Sinemet was added to desipramine, and tolcapone did not cause any additional change. This lack of pharmacokinetic interaction between desipramine and tolcapone was also predicted from in vitro experiments in human liver microsomes, which were shown to have a high capacity for glucuronidation (study # 14). The effect of desipramine on tolcapone pharmacokinetics was not evaluated.

Cytochrome P450:

Interactions of tolcapone with the metabolism of various drugs have been investigated in preparations of human liver microsomes (study #15). Although not oxidatively metabolized by CYP 2C9, tolcapone appears to inhibit this enzyme. It was found that tolcapone has a large enough affinity for CYP 2C9 to potentially inhibit metabolism of tolbutamide and diclofenac, while the oxidative demethylation of naproxen is considered to be less affected.

Tolbutamide was chosen as a model substrate of CYP 2C9, for an interaction study in humans (study #12). Co-administration of tolcapone did not change the pharmacokinetics of tolbutamide. The unchanged pharmacokinetic parameters for hydroxytolbutamide, the CYP450 2C9 dependent metabolite, confirmed the lack of effect of tolcapone on this metabolic pathway in vivo. The effect of tolbutamide on tolcapone pharmacokinetics was not evaluated.

S-warfarin is also a CYP 2C9 substrate and it is recommended to monitor coagulation parameters after initiation of combined warfarin and tolcapone treatment.

In vitro, tolcapone competed with coumarin for CYP 2A6-mediated metabolism (study #15). However, since the affinity of tolcapone for CYP 2A6 is considerably smaller than that of coumarin, no significant inhibition of coumarin metabolism in vivo is expected.

Tolcapone did compete in vitro with the metabolism of caffeine, even though it is not oxidatively metabolized by CYP 1A2 (study #14). While tolcapone is metabolized by CYP 3A, its affinity for the enzyme is smaller than those of midazolam, terfenadine and cyclosporine (study #15). No clinically significant metabolic interactions between tolcapone and these CYP 3A substrates are expected. No significant interference of tolcapone with CYP 2C19 and CYP 2D6 was observed (study #14).

Population Pharmacokinetics:

The total number of subjects who took part in population pharmacokinetic study was 412 (275 evaluable for tolcapone and 393 for L-DOPA). There were 262 males and 150 females (402 Caucasians, 1 Black, 3 Orientals, 6 Others) in the study. Their age ranged from 34-83 years and they weighed from 36-153 kg. One or more blood sample(s) was taken either prior to test drug intake, between 0.5 and 2 hours or between 3 and 4 hours after drug intake. All samples were assayed for tolcapone, L-DOPA and its 3-O-methylmetabolite (3-OMD). The data were analyzed using the mixed effect modelling software package NONMEM (study #13).

The pharmacokinetic characteristics derived for tolcapone in the target population of Parkinsonian patients were in general in good agreement with the results from the conventional pharmacokinetic studies in young and elderly volunteers. A two compartment open model with first-order absorption and potentially a lag-time was found to be appropriate to describe the concentration/time data of tolcapone.

Tolcapone was rapidly absorbed with an absorption rate constant of approximately $1-2 \text{ h}^{-1}$. The effect of food on the bioavailability of tolcapone was confirmed. It could be shown that tolcapone taken within 1 hour before to 2 hours after any meal resulted in a decrease of 10 to 20% in relative bioavailability. The total volume of distribution (V/F) of tolcapone estimated in Parkinsonian patients was 16 L, which is higher than the observed

value of approximately 4.5 L after intravenous administration of tolcapone in healthy young volunteers. Oral clearance of tolcapone was approximately 4.5 to 5 L/h. The inter-subject variability of tolcapone clearance was 30%.

Several factors which could influence tolcapone pharmacokinetics (age, sex, creatinine clearance, co-administration of dopamine agonists, serum protein and albumin levels, body weight and lean body weight) were investigated and even though some showed statistically significant effects, none of the effects were of a magnitude considered of clinical relevance. The covariate showing the biggest impact on clearance was lean body weight in one dataset, whereas it was creatinine clearance in other dataset. The estimated elimination half-life of tolcapone (5 - 8 hours after 200 mg) is longer than that observed in healthy volunteers (approximately 2 hours), which is due to the higher volume of distribution in Parkinsonian patients.

The pharmacokinetics of tolcapone were similar between 2 and 9 weeks of treatment. The effects of tolcapone on the pharmacokinetics of L-DOPA in Parkinsonian patients were also well in agreement with those observed in healthy volunteers. The decrease in L-DOPA clearance was generally compensated for by a reduction in L-DOPA dose and the resulting total exposure of the patient to L-DOPA was close to the exposure in absence of tolcapone.

Analytical Method:

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Review Outline

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A. PHARMACOLOGY

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A.1 Mechanism of Action

Tolcapone inhibits the activity of both central and peripheral catechol-O-methyl transferase, the enzyme that catalyzes the methylation of catechols at the 3-hydroxyl position. The 3-O-methylation of L-DOPA, yielding 3-O-methyl-DOPA (3-OMD) is a primary means of drug inactivation. Thus, tolcapone increases plasma concentrations and exposures to L-DOPA, resulting in an improved pharmacokinetic profile and antiparkinsonian activity of L-DOPA.

The COMT-inhibitory activity of tolcapone was demonstrated in several *in vitro*, *in vivo* and *ex vivo* experiments. In *in vitro* studies with rat brain and liver homogenates, or purified recombinant human COMT, the inhibitory potency of tolcapone was in the nanomolar range (Sponsor Table 1):

ENZYME PREPARATION	IC ₅₀ or Ki (nmoles/liter)
Rat liver homogenate	36 (IC ₅₀)
Rat brain homogenate	10 (IC ₅₀)
Purified human recombinant S-COMT	0.27 (Ki)
Purified human recombinant MB-COMT	0.29 (Ki)

IC₅₀, median inhibitory concentration; Ki, inhibition constant. From [2001, 2004].

In an *ex vivo* study of COMT inhibition by tolcapone in various organs, rats were administered tolcapone orally, sacrificed one hour later, and COMT activity was determined by a radioenzymatic method. The ED₅₀s for tolcapone inhibition of COMT in peripheral organs ranged from 0.4 (stomach) to 6.0 (vas deferens) mg/kg. The ED₅₀ in brain was lower (24 mg/kg) suggesting that peripheral COMT inhibition by tolcapone may be more critical to the drug's action than central COMT inhibition.

COMT inhibition was also assessed *ex vivo* by analyzing rat brain levels of 3-methoxy dopamine metabolites (3-MT and HVA), a dose of 10 mg/kg, p.o., tolcapone caused a 50% depletion of 3-MT. Doses as high as 100 mg/kg tolcapone do not significantly alter brain (or plasma) levels of catecholamines as alternative routes of metabolism (MAO) compensate for the inhibition of the COMT pathway.

In vivo microdialysis studies have also demonstrated central COMT inhibition by tolcapone. Oral administration of 30 mg/kg tolcapone to rats increased striatal DOPAC and decreased HVA and 3-MT (Sponsor Fig. 5):

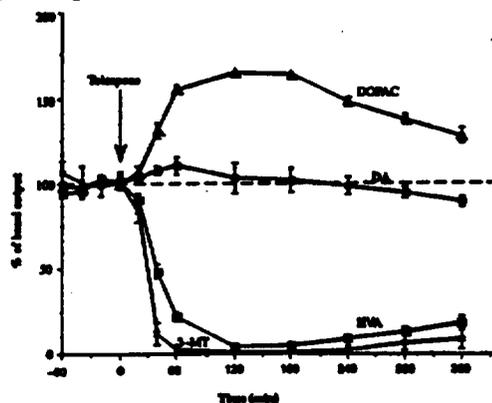


Figure 5. Tolcapone is a potent inhibitor of striatal COMT as shown by its effect on dopamine metabolism. Tolcapone (30 mg/kg p.o.) did not modify extracellular levels of DA (■), decreased the levels of HVA (●) and 3-MT (▲) and increased those of DOPAC (◆). Intracellular experiments done *in vivo*.

Relative to other COMT inhibitors, tolcapone appears to be approximately equivalent in potency to entacapone in central and peripheral tissue (Sponsor Table 1):

SEPPO KAAROLA *et al.*

Table 1. Effects of the new COMT inhibitors on COMT activity *in vitro* and *in vivo*

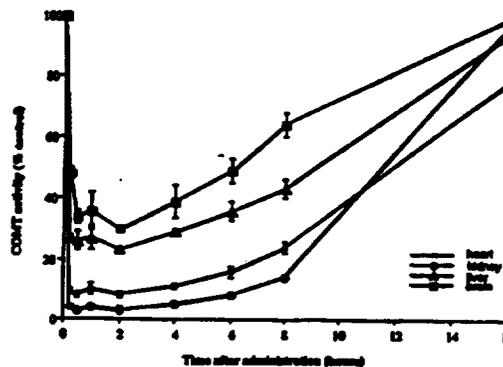
Reference	Entacapone	Tolcapone	Tolcapone	COP 2804
	a, b	a, d, e, f	a, g, h	i
IC_{50} (nM)				1 nM
Brain	10	12	16	—
Liver	100	207	36	—
K_i (nM liver, nM)	14	23	36	—
			—	—
ED_{50} (nM, mg/kg p.o.)				—
Dorsal	1.1	0.7	0.9	—
Liver	6.7	6.2	4.5-6.3	—
Hydroxy	3.4	>30	4.3	—
Brain	24.3	>30	26-38	—
COMT inhibition (mean, %)	25 (100 mg)	41 (100 mg)	60 (100 mg)	—
(human erythrocytes)	52 (200 mg)	—	90 (200 mg)	—

—Not available.

References: (a) Miettinen *et al.* (1992), (b) Kaarola *et al.* (1994), (c) Miettinen *et al.* (1990a), (d) Scheider and Miettinen (1989), (e) Zharov *et al.* (1990a), (f) Kaarola *et al.* (1990), (g) Borgegna *et al.* (1991), (h) Zharov *et al.* (1991), (i) Waldmeier *et al.* (1990a).

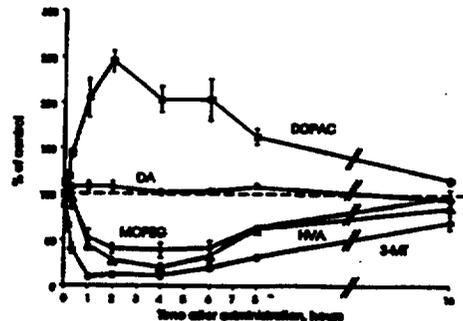
The reversibility of tolcapone as a COMT inhibitor was studied by administration of a high dose to rats (100 mg/kg, p.o.) and monitoring the time course COMT activity in several tissues. By 16 hrs post-treatment, only rat heart COMT appeared to be inhibited (Sponsor Fig. 6):

Figure 6. Tolcapone is a reversible COMT inhibitor as determined by the recovery of COMT activity in various rat organs after a single administration of tolcapone at a dose of 100 mg/kg p.o.



A similar time-course of recovery of COMT activity was observed in rat brain after 13 mg/kg using the tissue level method of analysis (Sponsor Fig. 7):

Figure 7. Tolcapone is a reversible inhibitor of central COMT as shown by the time-course of its effect on the level of dopamine, noradrenaline and their main metabolites O-MET, DOPAC, HVA and MHPG in rat brain. The levels of dopamine and noradrenaline metabolites returned to basal values 16 hours after administration of tolcapone.



After subchronic treatment with tolcapone (30mg/kg, p.o., b.i.d., 11 days), levels of dopamine metabolites returned to control levels by 16-24 hrs post-treatment.

A.2 Selectivity of Neurochemical Actions

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Enzyme Selectivity

The selectivity of tolcapone for COMT versus other enzymes important in neurotransmitter metabolism was evaluated *in vitro* and *ex vivo*. In *in vitro* studies, phenylsulfotransferase was inhibited to 31% of control by 0.1 mM tolcapone, and 5-hydroxyindole-O-methyl transferase was inhibited to 41% of control by 1 mM tolcapone. PNMT, AADC and AChE were not inhibited. Tolcapone (100 mg/kg) did not inhibit MAO A or B, PNMT, or AADC activities *ex vivo*.

Monoamine Uptake Inhibition

At concentrations of 100 μ M, tolcapone moderately inhibited the *in vitro* synaptosomal uptake of serotonin (48%) and norepinephrine (40%). Due to the high concentration required, this mechanism is probably not significantly affected by tolcapone at therapeutic concentrations.

The sponsor stated that dopamine uptake inhibition by tolcapone was evaluated in striatal synaptosomes, but this data could not be found in the application.

Receptor Binding

Tolcapone had no substantial affinity for 38 neurotransmitter binding sites at concentrations up to 10 μ M.

Subchronic administration of tolcapone to rats (10 mg/kg, i.p., b.i.d. for 3 weeks) did not significantly alter the density (B_{max}) or affinity (K_d) of brain D2, 5-HT1A, 5-HT2, or β receptors.

A.3 Effect of Tolcapone on L-DOPA pharmacokinetics

Plasma levels in Rats

A single oral administration of 30 mg/kg tolcapone caused an approximate four-fold increased in plasma L-DOPA exposure in rats that had received 20 mg/kg L-DOPA and 15 mg/kg benserazide (Sponsor Figure 1). Tolcapone-induced increases in L-DOPA exposures were lower when carbidopa was used as the AADC inhibitor (Sponsor Figure 2). Plasma levels of 3-O-methyl-DOPA were correspondingly reduced in tolcapone-treated rats.

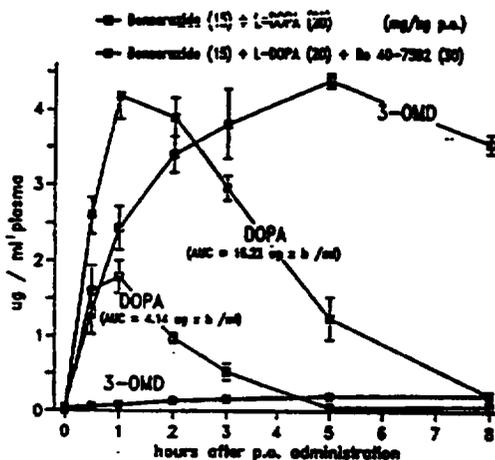


Figure 1

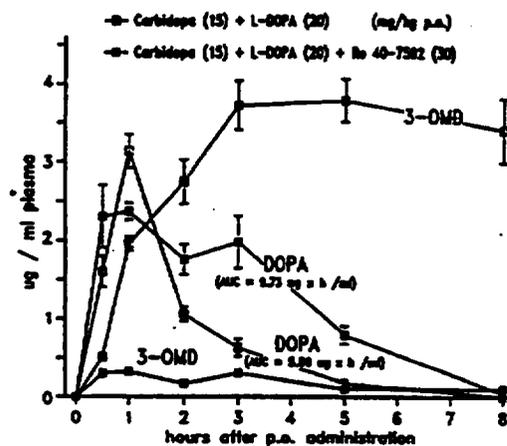


Figure 2

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Brain Levels in Rats

Using the same dosage regimen as that described in the plasma level study (30 mg/kg tolcapone, 20 mg/kg L-DOPA, 15 mg/kg benserazide), an approximate four-fold increase in striatal L-DOPA exposure, and a virtual abolition of striatal 3-O-methyl-DOPA formation, was observed (Sponsor Figure 1). Striatal dopamine levels were also increased by tolcapone.

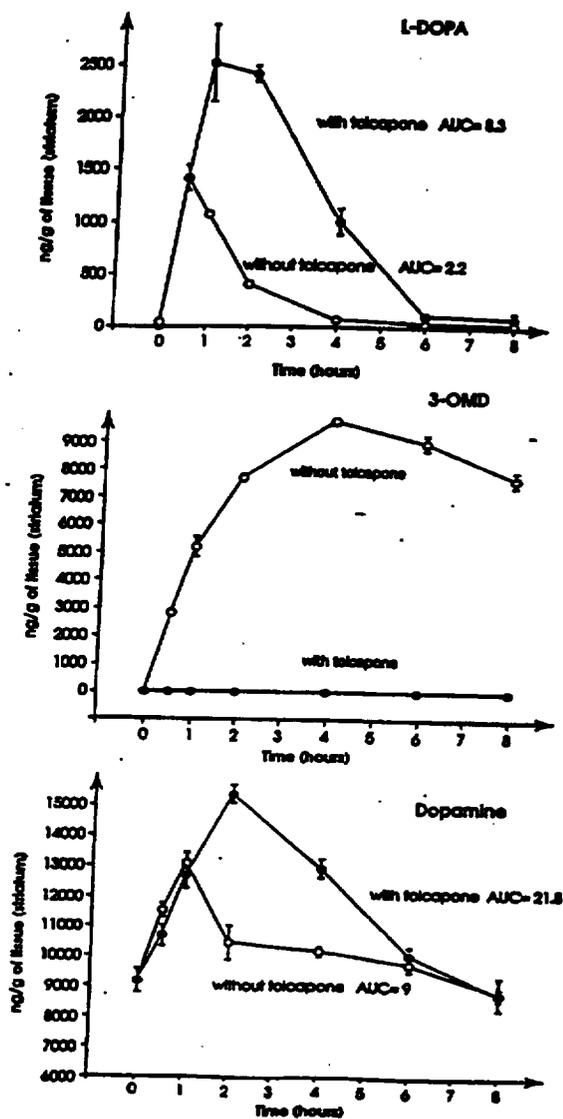


Figure 1

Plasma levels in Monkeys

Results of experiments by an independent investigator on L-DOPA pharmacokinetics following tolcapone in macaques were presented in abstract form. Baseline plasma levels of L-DOPA were obtained following administration of 10 mg/kg L-DOPA/10mg/kg benserazide, p.o. The effect of tolcapone (10 mg/kg, p.o.) was determined after administration of a lower dose of L-DOPA (5 mg/kg). Tolcapone still caused a 3-fold increase in the AUC for L-DOPA.

A.4 Efficacy in Animal PD Models

6-OHDA-lesioned rats

Albino rats (n = 8; 250-300g) were unilaterally-lesioned by injection of 8 µg 6-OHDA into the substantia nigra. After recovery, rotational behavior was tested following administration of L-DOPA (10mg/kg, i.p.) + benserazide (15 mg/kg, i.p.), alone or in combination with tolcapone (30 mg/kg, i.p.) (benserazide and tolcapone were administered 30 min before L-DOPA).

The effect of L-DOPA/benserazide on rotation was over by 2 hrs. Tolcapone prolonged the duration of effect to by 2-2.5 hr. Peak effects were similar in the presence and absence of tolcapone (Sponsor Figure 10).

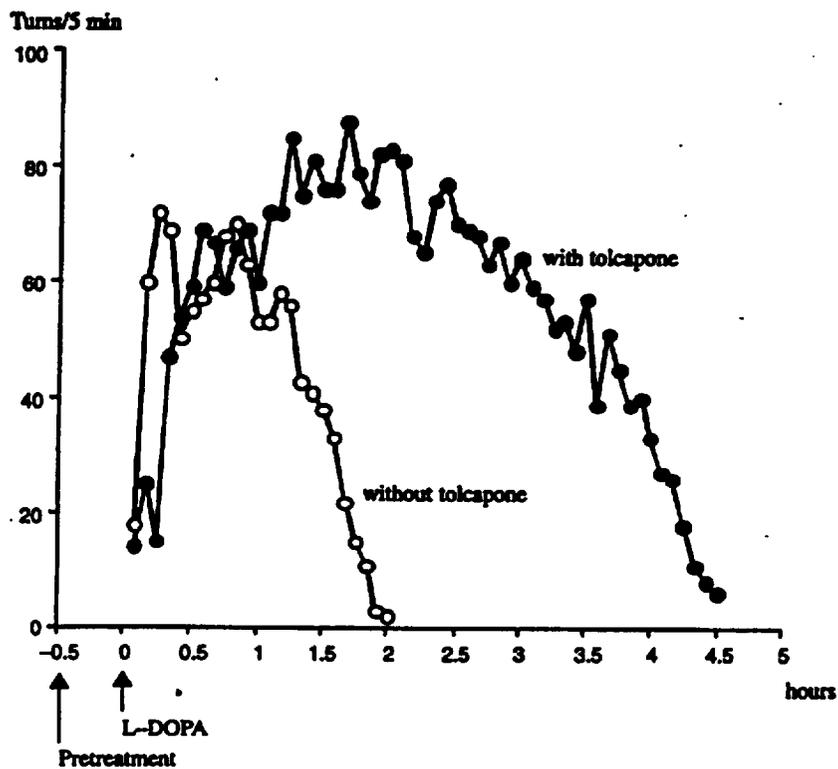


Figure 10. Tolcapone prolonged the response to L-DOPA in an animal model of Parkinson's disease, i.e., the turning behaviour induced by L-DOPA in rats carrying a unilateral 6-OHDA lesion. Rats were pre-treated (arrow) either with benserazide (15 mg/kg i.p.) or with benserazide plus tolcapone (30 mg/kg i.p.) and injected 30 minutes later with L-DOPA (10 mg/kg i.p.). Points are means of 8 animals.

This figure is from the sponsor's Integrated Summary, and is different from that shown in the original research report. Based on the raw data, the figure above is correct.

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MPTP Hemiparkinsonian Monkeys

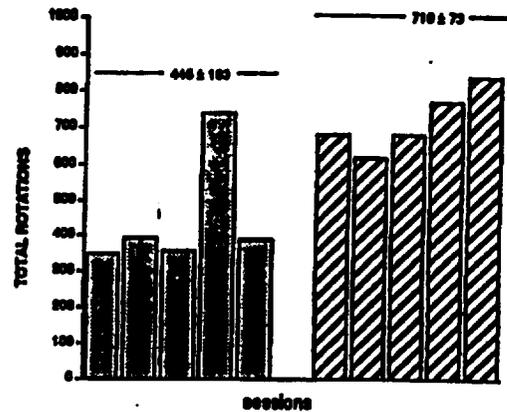
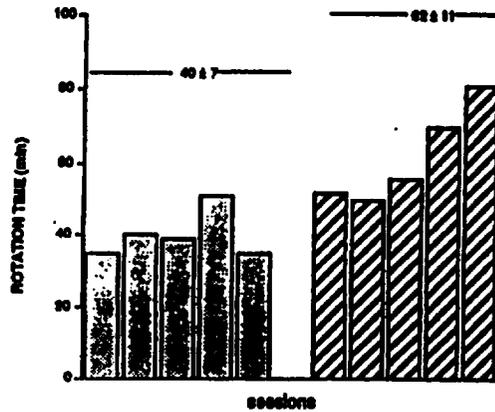
Two female rhesus monkeys were administered a single unilateral intracarotid infusion of MPTP (0.4 mg/kg), and monitored for the appearance of parkinsonian symptoms for 2-3 months.

Administration of tolcapone (10 mg/kg, i.p., or 30 mg/kg, p.o.) caused an approximate 50% increase in the number of rotations/session and the duration of action of L-DOPA (10 mg/kg, i.m.) alone, or L-DOPA (10 mg/kg, p.o.) + benserazide (3 mg/kg, p.o.). Data from the oral L-DOPA experiment in one animal is shown (Sponsor Figure 3).

Oral doses lower than 15 mg/kg tolcapone did not potentiate the effect of L-DOPA.

Figure 3.

Date	10/1	10/2	10/3	10/4	10/5	9/27	9/28	10/10	10/11	10/12
Ro 40-7592 (mg/kg) p.o.	-	-	-	-	-	15	15	15	15	15
Benserazide (mg/kg) p.o.	3	3	3	3	3	3	3	3	3	3
L-dopa (mg/kg) p.o.	10	10	10	10	10	10	10	10	10	10



	Ro 40-7592		Increase (%)
	-	+	
Rotation Time (min)	40 ± 7	82 ± 11	85
Total Rotations	445 ± 183	719 ± 73	62
Rotations / min	11.1	11.8	

■ Benserazide + L-dopa

▨ Ro 40-7592 + Benserazide + L-dopa

B. SAFETY PHARMACOLOGY

B.1. Central Nervous System Effects

Tolcapone was lethal at doses of 625 mg/kg, p.o. in mice, and 2500 mg/kg in rats. Death was preceded by hypomotility and respiratory depression.

Oral tolcapone was devoid of anti- and pro-convulsant effects in the D.B.A./2J mouse audiogenic seizure model at doses of 10-300 mg/kg, and analgesic activity in the mouse hot plate assay at doses of 10-100 mg/kg. Tolcapone did not affect body temperature in mice at doses of 10-100 mg/kg, p.o. Tolcapone (3-300 mg/kg, p.o.) alone did not modify the hypnotic effect of methylhexital in mice. MADOPAR (100 mg/kg, p.o.), alone and in combination with tolcapone, significantly prolonged methylhexital sleeping times.

In an EEG study in cats, abnormal morphologies did not observed after doses of 1-10 mg/kg, i.v. A significant decrease in peak frequencies in the dorsal hippocampus were evident, as were some minor changes in cortical δ - and β 1-waves.

The dependence and cross-dependence (codeine, diazepam) potential of tolcapone was evaluated in rats. In the direct dependence study, no signs of withdrawal were evident in rats that were escalated up to 200 or 600 mg/kg, p.o., tolcapone over a 4-week period. In the cross-dependence study, the same doses of tolcapone did not prevent withdrawal from codeine or diazepam.

Tolcapone (0.12, 0.6, 3.0 mg/kg/injection, i.v.) was not self-administered by cynomolgus monkeys that were trained to self-administer cocaine (0.05 mg/kg/injection).

B.2. Cardiovascular/Respiratory Effects

In conscious stump-tail macaques (n=4) monitored by continuous telemetry, oral doses of 10 and 30 mg/kg tolcapone did not alter mean arterial blood pressure or general behavior. A slight decrease (<10%) in heart rate occurred at 3-4 hr post-dose.

The hemodynamic effects of oral tolcapone (10, 30 mg/kg), Sinemet (5.5 mg/kg) and the combination of 30 mg/kg tolcapone with Sinemet (5.5 mg/kg) was evaluated in conscious dogs. No significant effects on mean arterial pressure were noted in any treatment group. Heart rate was significantly reduced in all reduced in all treatment groups. The greatest effects occurred in the HD tolcapone and HD tolcapone + Sinemet groups, but no additive effect of Sinemet was apparent.

The cardiovascular (pressure, rate, flow, ECG) and respiratory effects of intravenous tolcapone (1, 3 and 10 mg/kg) were studied in anesthetized beagles for up to 1 hr post-treatment. Dose-dependent decreases in systolic and diastolic pressure and femoral blood flow were evident ($p < 0.05$ at 10 mg/kg). Heart rate decreases were slight. At 10 mg/kg, a significant increase in respiration occurred between 0-30 min. The only notable ECG change was a slight shortening of the QRS complex.

In spontaneously hypertensive rats, oral doses of 10, 30 and 100 mg/kg tolcapone did not alter systolic pressure or heart rate at 1, 3 or 6 hours post-dose.

B.3. Gastrointestinal Effects

An oral dose of 300 mg/kg tolcapone caused a slight decrease in gastrointestinal motility in mice. In *in vitro* smooth muscle preparations, high concentrations of tolcapone inhibited the contractile responses to acetylcholine and histamine in guinea pig trachea (100 μ M), and the contractile responses to histamine in the guinea pig ileum (10-100 μ M). Tolcapone (100 μ M) decreased the spontaneous contractile amplitude of isolated uterine strips.

B.4. Renal Effects

The effect of 10 and 100 mg/kg, p.o., tolcapone on urine and electrolyte excretion was monitored in female rats from 0-5 hrs post-dose. At the high dose, urine output and sodium excretion were significantly reduced.

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C. TOXICOLOGY

C.1. Acute Toxicology

Tolcapone only studies conducted by: F. Hoffmann-LaRoche Ltd.
CH-4002 Basel, Switzerland

Combination studies conducted by: F. Hoffmann-LaRoche Ltd.
Nutley, NJ

Sponsor Volume: 1.18
These studies complied with GLP.

Summary

Acute toxicology studies were conducted in mice, rats and dogs. Animals were observed for up to 14 days following treatment. The lowest lethal oral doses in rats and mice 500 and 400 mg/kg, respectively, and high rates of lethality occurred at 1000 and 800 mg/kg (data from the tolcapone-only animals in the combination studies). By the intraperitoneal route, doses of 80 and 71 mg/kg caused high rates of lethality in rats and mice, respectively. Sinemet did not markedly enhance the lethality of tolcapone. Prominent signs of toxicity were ataxia, hypomotility, respiratory depression, and loss of righting reflex. In dogs, oral doses of 100 mg/kg or greater caused emesis, and diarrhea/mucoid feces occurred at higher doses. No fatalities resulted from doses up to 300 mg/kg. Reddening of the gastrointestinal mucosa was observed in both dogs at necropsy.

C.1.a. Acute Toxicology of Tolcapone

Acute Oral Toxicity in Mice

Doses: 1400, 1600, 1800 mg/kg n = 10 (5M, 5F)

Results:

Deaths: LD: 4/10; MD: 3/10; HD: 9/10

Signs: ataxia, hypomotility, decreased respiratory rate (seen only at 30 and 60 min); no gross path findings at necropsy (14 days)

Acute Intraperitoneal Toxicity in Mice

Doses: 45, 56, 71, 90 mg/kg n = 10 (5M, 5F)

Results:

Deaths: 71: 8/10; 90: 9/10

Signs: ataxia, hypomotility, decreased respiratory rate (seen only at 30 and 60 min); no gross path findings at necropsy (14 days)

Acute Oral Toxicity in Rats

Doses: 1600, 1800, 2000 mg/kg n = 10 (5M, 5F)

Results:

Deaths: LD: 3/10; MD: 2/10; HD: 1/10

Signs: ataxia, hypomotility, decreased respiratory rate (seen only at 30 and 60 min); no gross path findings at necropsy (14 days)

Acute Intraperitoneal Toxicity in Rats

Doses: 50, 63, 80, 100 mg/kg n = 10 (5M, 5F)

Results:

Deaths: 80: 9/10; 100: 9/10

Signs: no other signs besides death (rapid onset) were reported; no gross path findings at necropsy (14 days)

Acute Oral Toxicity in Beagles

Doses: escalating scheme from 10-300 mg/kg; n = 1/sex

Results: Signs: emesis at ≥ 100 ; diarrhea at ≥ 200

Autopsy: reddening of the gastrointestinal mucosa in both animals

C.1.b. Acute Toxicity Studies of Tolcapone in Combination with Sinemet

Acute Oral Toxicity of Tolcapone and Sinemet in Rats

Doses: Tolcapone - 500, 1000, 2000 mg/kg N= 10 (5 M/5 F)
Sinemet - 1100 mg/kg (1000 L-DOPA; 100 carbidopa)

Deaths

Dose TOL	TOL	TOL/SIN
0	-	0/10
500	1/10	3/10
1000	7/10	5/10
2000	9/10	5/10

The deaths in the tolcapone-only animals occurred within 6 hrs. Four deaths in the combination groups occurred after 7 hrs.

Signs:

Tolcapone alone: hypomotility, respiratory depression, loss of righting reflex

Tol + Sinemet: CNS depression, respiratory depression, gnawing; decrease food intake (day 1)

Sinemet alone: gnawing

Acute Oral Toxicity of Tolcapone and Sinemet in Mice

Doses: Tolcapone - 50-400; 25-800 mg/kg N= 12 (6 M/6 F)
 Sinemet - 1100 or 2200 mg/kg (10:1, L-DOPA:carbidopa)

Results: Study I - Sinemet = 2000
 # Deaths

Dose TOL	TOL	TOL/SIN
0	-	2/12
50	0	6/12
100	0	4/12
200	0	4/12
400	8/12	8/12

The deaths in the tolcapone-only animals occurred within 6 hrs, whereas all but 2 deaths in the combination groups occurred after 7 hrs.

Study II - Sinemet = 1000
 # Deaths

Dose TOL	TOL	TOL/SIN
0	-	0/12
25	0	0
50	0	0
100	0	1/12
200	0	1/12
400	4/12	3/12
800	8/10	8/10

The deaths in the tolcapone-only animals occurred within 6 hrs. Three deaths in the combination groups occurred after 7 hrs.

Signs:

Tolcapone alone: hypomotility, respiratory depression, loss of righting reflex
 Tol + Sinemet: CNS depression, respiratory depression, gnawing; decrease food intake (day 1)
 Sinemet alone: gnawing

C.2. Multiple-Dose Combination Toxicology Studies

(Note: In the combination studies, the following abbreviations are used for group designations:

LT: Low-dose Tolcapone; MT: Mid dose Tolcapone; HT: High dose Tolcapone
S: Sinemet (one fixed dose, usually a 4:1 ratio of L-DOPA:carbidopa)

C.2.a. 13-Week Oral Toxicity Study of Tolcapone and Sinemet in Rats

GLP Research Report #: J-146,441; Sponsor Volume: 1.34
Conducted by: Nippon Roche Research Center
Dept. Toxicology and Pathology
200, Kajiwara, Kamakura 247
Japan

Summary:

Tolcapone (20 and 200 mg/kg/day) was orally administered alone and in combination with Sinemet (100 mg/kg/day; 80 mg/kg L-DOPA/20 mg/kg carbidopa) to Sprague-Dawley rats for 13 weeks. Clinical signs of hypomotility, salivation, and lacrimation were observed in the groups that received Sinemet. Only slight effects on body weight were seen in HT, LT/S, and HT/S males. The most notable histopathological findings were in the forestomach epithelium (hyperkeratosis, thickening, and one case of necrosis) of HT and HT/S males, and a low incidence of changes in kidney (degeneration, pigment deposits, and vacuolation of the PTE). A nephroblastoma was diagnosed in one HT male. Relative kidney weights were increased in all combination groups and in Sinemet-only males. High incidences of acinar cell hypertrophy in submandibular gland were seen in the combination groups, but not in animals that received tolcapone alone. Isolated cases of hepatocyte necrosis were identified.

Toxicokinetic data demonstrated dose proportional increases in tolcapone, and that the drug does not accumulate. The relative increases in L-DOPA due to tolcapone coadministration were rather small (1.3-fold) at both dosage levels of tolcapone. The exposures (AUC_{0-24}) to tolcapone in HDM were 4.5 times higher than the expected exposures in humans receiving 200 mg, t.i.d. (80 μ g.hr/ml). Exposures to L-DOPA in rats were approximately 8-20 times higher than therapeutic human exposures.

Methods:

Dosage Groups

(Tolcapone Lot G PUL 606 090)

Group	tolcapone	Sinemet
CON	0	0
LT	20	0
HT	200	0
S	0	100
LT/S	20	100
HT/S	200	100

Route of Administration: orally (gavage) in an 0.5% sodium carboxymethylcellulose suspension
Species/Strain/Number: Sprague-Dawley Rat (males: 160-210 g; females: 110-150 g)
10/sex/dose for main study
5/sex/dose for TK study

Results:

Mortality: none

Clinical: ↑ spontaneous activity - S, LT/S, HT/S
salivation, lacrimation - S, LT/S, HT/S

Body Weight (Sponsor Figures 2-5):

males: slight decrease in HT, LT/S and HT/S groups
females: no effect

Food Intake:

males: slight decrease in HT group

Hematology: (at end of study included bone marrow count)

No treatment-related effects

Clinical Chemistry: (at end of study)

Several statistically significant mean variations occurred, but these are not considered toxicologically relevant.

Individual variations:

↑ SGOT	-	male:	1 Con, 2 LT, 1 HT
↑ SGPT	-	male:	1 LT
↑ alkaline phosphatase	-	female:	1 LT/S, 1 HT/S
↑ A/G	-	female:	2 LT/S

Urinalysis: (samples were collected before final dose by squeezing abdomen; urine volume and specific gravity were determined from 16 hr collections)

Round epithelial cells were detected in urine sediments from all HT/S males.

Slight decreases in urine volume in HT/S males, and specific gravity in high-dose tolcapone females were also noted.

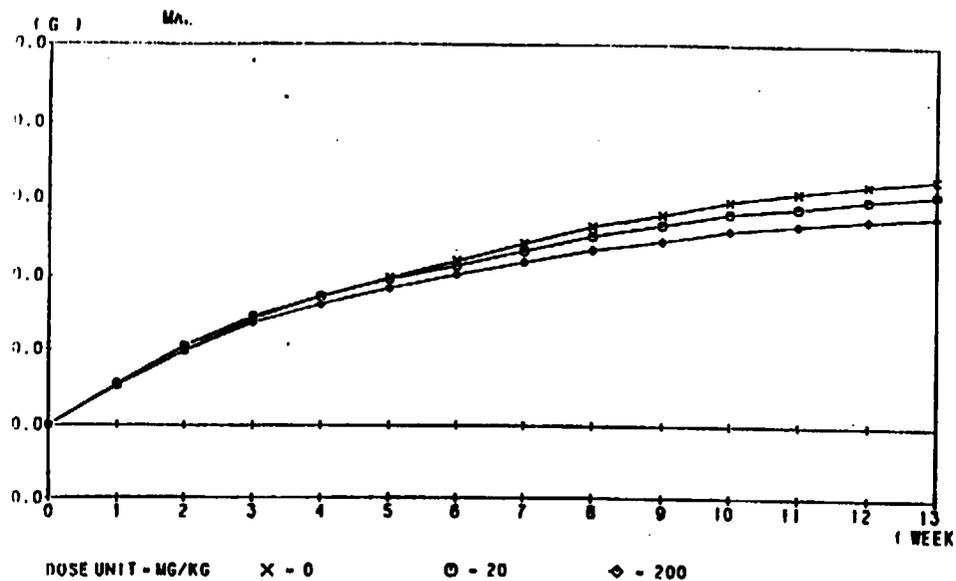


FIG. 2 : 13-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7592/001 AND SINEMET IN RATS (RO 40-7592/001 ALONE).
BODY WEIGHT GAIN

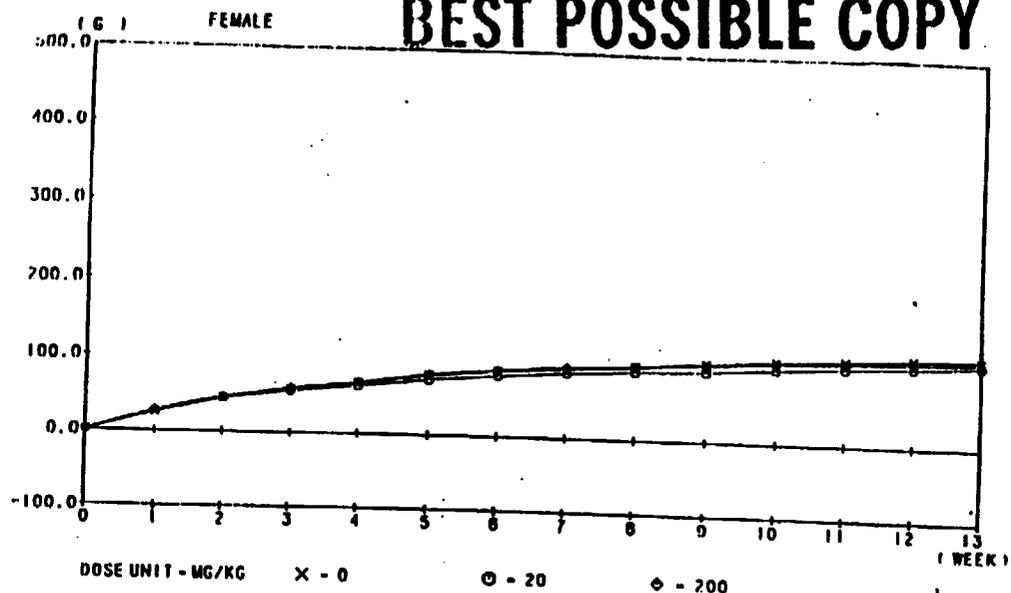


FIG. 3 : 13-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7592/001 AND SINEMET IN RATS (RO 40-7592/001 ALONE).
BODY WEIGHT GAIN

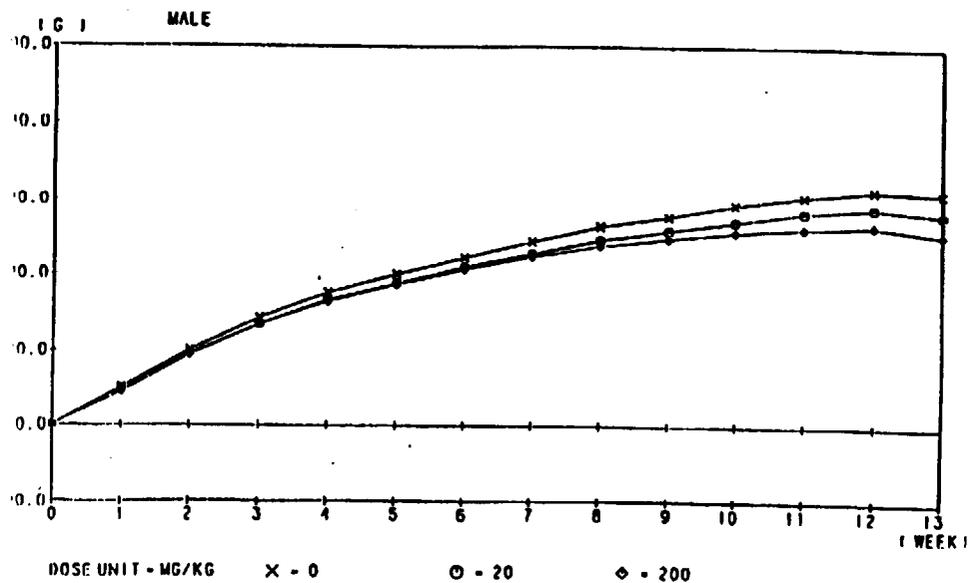


FIG. 4 : 13-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7592/001 AND SINEMET IN RATS (COMBINATION TREATMENT).
BODY WEIGHT GAIN

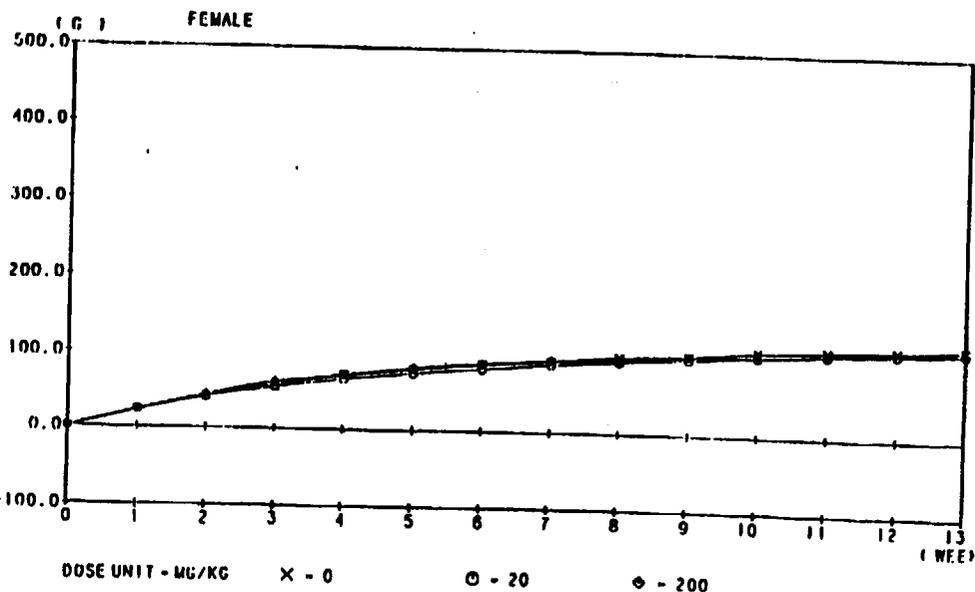


FIG. 5 : 13-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7592/001 AND SINEMET IN RATS (COMBINATION TREATMENT).
BODY WEIGHT GAIN

Ophthalmology: (after final dosing)

No treatment-related changes were noted.

Organ Weights:

Kidney weights were increased in Sinemet-only males (16%), in the male combination groups (22% with both low- and high-dose tolcapone), and in HT/S females (9.5%). Weights of salivary glands, testes, epididymides, and ovaries were increased in the combination groups.

Histopathology:

A low incidence of renal histopathological changes was observed. The prevalence and severity of the changes were far lower than those seen in the 1- and 2-year rat studies. However, since no renal changes were evident in the 6-month rat study (which was not reviewed), the findings in the present study suggest that the onset of renal toxicity may be earlier than expected (based on the absence of findings in the 6-month study). Because of the low incidence and distribution of renal findings in all treatment groups, it is not clear that Sinemet markedly enhanced the renal toxicity of tolcapone.

One nephroblastoma was found in a tolcapone-treated rat, although its presence cannot be conclusively linked to drug treatment.

forestomach epithelium,		
necrosis	-	1 HT(M)
hyperkeratosis	-	3 HT(M), 3 HT/S(M)
thickening	-	2 HT(M), 3 HT/S(M)
kidney,		
nephroblastoma	-	1 HT(M)
degeneration of PTE	-	1 HT/S(M)
pigment deposits in PTE	-	2 LT/S(F), 2 HT/S(F)
vacuolation of PTE	-	1 HT(M), 1 LT/S(M), 1 HT/S(M)
(grade 1)		1 Con(F), 1 HT(F), 1 HT/S(F)
hyaline droplets in PTE	-	1 HT/S(F)
liver,		
hepatocyte necrosis,		
single cell	-	1 S(M), 1 HT/S(M)
		1 LT/S(F), 1 HT/S(F)
focal	-	1 HT/S(M)
salivary glands,		
hypertrophy of acinar cells		
in submandibular gland	-	1 S(M), 5 LT/S(M), 5 HT/S(M)
		7 LT/S(F), 3 HT/S(F)

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Toxicokinetics: (1 rat/group/time point at 1, 2, 3, 5 and 24 hr post-dose on days 1, 30 and 72)

Increases in the concentrations of tolcapone were dose proportional. The drug did not accumulate, and there was no gender difference (only slightly higher in females) (Sponsor Table A). Levels of the 3-O-methyl metabolite were low (<0.4 µg/ml) under all conditions (Sponsor Table B). Sinemet did not influence the exposures to LD tolcapone, but slightly slowed absorption of higher doses. The low dose of tolcapone increased exposure to L-DOPA by 1.3 (Sponsor Table C) due to inhibition of 3-O-methyl-DOPA formation (Sponsor Table D). The high dose of tolcapone did not increase L-DOPA exposure any further.

Table A: Pharmacokinetic parameters of Ro 40-7992

Dose group	C _{max} (µg/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
B	5.789	9.792	8.500	6.260	9.679	12.13
C	40.69	54.11	50.23	74.95	65.33	140.0
E	3.623	3.240	4.477	4.994	4.526	4.978
F	30.24	35.99	34.67	39.69	23.84	29.40

tolcapone

Dose group	AUC _{0-24h} (h·µg/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
B	14.04	20.28	25.01	13.34	16.36	20.36
C	160.6	188.2	133.9 ^a	178.83	149.9	291.2
E	14.16	12.63	13.67	14.35	14.73	16.36
F	116.2	114.1	135.5 ^b	132.0	83.49	97.40

a: AUC_{0-24h} (h·µg/ml) 268.3

b: AUC_{0-24h} (h·µg/ml) 264.7

Table B: Pharmacokinetic parameters of Ro 40-7992

Dose group	C _{max} (ng/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
B	0.234	0.271	0.280	0.149	0.145	0.126
C	0.515	0.260	0.270	0.521	0.177	0.259
E	0.382	0.276	0.267	0.298	0.221	0.191
F	0.274	0.223	0.229	0.249	0.168	0.177

3-O-Methyl-Tolcapone

Dose group	AUC _{0-24h} (h·ng/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
B	0.900	0.910	1.00	0.30 ^a	0.306 ^a	0.260 ^a
C	1.23	1.0	0.930 ^b	1.18	0.76	0.825
E	1.29	1.12	1.05	0.680	0.780	0.710
F	1.25	1.04	0.940	0.880	0.740	0.760

a: AUC_{0-24h} (h·ng/ml)

b: AUC_{0-24h} (h·ng/ml) 1.87

Dose group B: 20 mg/kg/day Ro 40-7992

Dose group C: 200 mg/kg/day Ro 40-7992

Dose group E: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 20 mg/kg/day Ro 40-7992

Dose group F: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 200 mg/kg/day

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Table C: Pharmacokinetic parameters of Ro 05-6799 L-DOPA

Dosage group	C _{max} (ng/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
D	8.104	13.10	13.21	10.4	10.26	9.699
E	9.539	10.70	18.28	11.26	11.94	16.96
F	8.989	11.95	12.07	6.82	13.18	13.45

Dosage group	AUC ₀₋₈ (h·ng/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
D	24.25	40.98	44.29	24.10	37.56	34.15
E	33.88	45.44	32.78	33.72	44.62	58.99
F	29.04	40.69	48.58	28.68	43.24	57.25

Table D: Pharmacokinetic parameters of Ro 08-9609 3-OMe-DOPA

Dosage group	C _{max} values (ng/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
D	10.51	16.89	19.30	11.53	21.98	25.03
E	0.440	0.926	0.838	0.472	0.796	0.865
F	0.068	0.127	0.089	0.089	0.124	0.154

Dosage group	AUC values 0-24(h·ng/ml)					
	Male rats			Female rats		
	Day 0	Day 30	Day 72	Day 0	Day 30	Day 72
D	121.1	269.7	336.6	192.7	309.6	342.9
E	6.971	13.71	13.88	7.924	13.30	14.74
F	1.551	2.909	1.496	2.0	2.428	3.008

Dosage group D: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa

Dosage group E: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 20 mg/kg/day Ro 40-7992.

Dosage group F: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 200 mg/kg/day Ro 40-7992.

C.2.b. 13-Week Oral Toxicity Study of Tolcapone and Sinemet in Dogs

GLP Research Report #: J-146,426
Conducted by: Nippon Roche Research Center
Dept. Toxicology and Pathology
200, Kajiwara, Kamakura 247
Japan

Sponsor Volumes: 1.37-1.38

Summary:

Tolcapone (10 and 80 mg/kg/day) was orally administered alone and in combination with Sinemet (100 mg/kg/day; 80 mg/kg L-DOPA/20 mg/kg carbidopa) to beagle dogs for 13 weeks. The major clinical sign was emesis in the Sinemet only and combination group males and females, and in the HT males. The Sinemet dosage was reduced to 50 mg/kg on day 42 to reduce this problem. No remarkable changes in hematology, clinical chemistry, urinalysis, or ECG were observed. Notable histopathological findings were prostate atrophy/reduced spermatogenesis in males receiving the combinations and Sinemet only, and pigment deposits in submandibular glands of animals from the combination and Sinemet only groups. Thus, no remarkable changes were unique to the tolcapone-only groups. A similar profile was observed in the combination study with Madopar (not reviewed).

Toxicokinetic data were confounded by emesis. Thus, increases in L-DOPA exposures due to tolcapone were generally small and not clearly dose-related. A solid comparison to human exposures is not possible since the AUCs in dogs were calculated for 0-7 hrs. These values in HD dogs were 1-2.5 times higher than the expected therapeutic exposures (80 µg.hr/ml) in humans receiving 200 mg, t.i.d., tolcapone. L-DOPA exposures in dogs were 3-30 times higher than human therapeutic exposures (3 µg.hr/ml).

Methods:

Dosage Groups:

(Tolcapone Lot 307005)

Group	tolcapone	Sinemet*
CON	0	0
LT	10	0
HT	80	0
S	0	100
LT/S	10	100
HT/S	80	100

* Sinemet dose was reduced to 50 mg/kg/day at day 42 because of vomiting

Route of Administration: oral in gelatin capsule

Species/Strain/Number: beagle dogs (5-6 months old; males: 10-13 kg, females: 9-11 kg)
3/sex/dose

Results:

Mortality: none

Clinical: vomiting - S, LT/S, HT/S (both M&F); sporadic in HT group
salivation - S, LT/S, HT/S (both M&F)

Body Weight: Slight decrease in HT, LT/S and HT/S males & females until Sinemet dosage reduction at day 42 (Sponsor Figures 2-5).

Food Intake: No treatment-related effect

Hematology: (predose and weeks 5, 9 and 13)

No treatment-related effect

Clinical Chemistry: (predose and weeks 5, 9 and 13)

1 SGOT - 1 LT/S on day 91

Urinalysis: (at the end of dosing)

No treatment-related effect

Ophthalmology: (general exam 2X weekly; fundus exam after final dosing)

No treatment-related effect

EKG: (beginning and end of dosing; the time of measurement with respect to dosing was not indicated)

There were no notable differences in electrocardiogram parameters, although it is difficult to draw conclusions from the data since the timing of recordings with respect to dosing was not indicated.

Organ Weights:

↓ prostate weights - S, LT/S, HT/S

Gross Necropsy:

submandibular glands,
black spots - S, LT/S, HT/S (both sexes)

stomach,
hemorrhage in glandular mucosa - 1 HT/S female

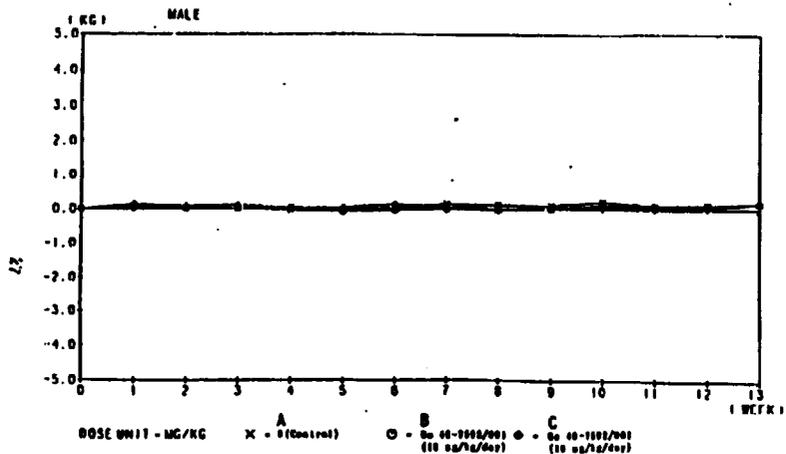


FIG. 2 : THIRTEEN-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7502/001 AND SINEMET IN DOGS BODY WEIGHT GAIN

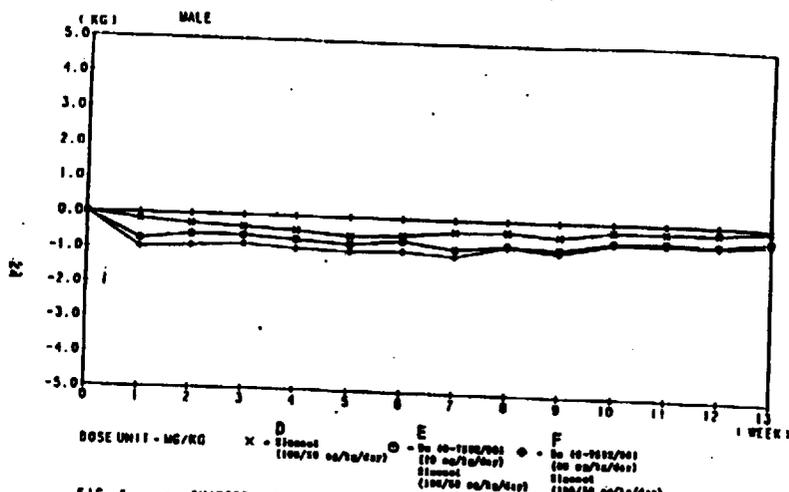


FIG. 3 : THIRTEEN-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7502/001 AND SINEMET IN DOGS BODY WEIGHT GAIN

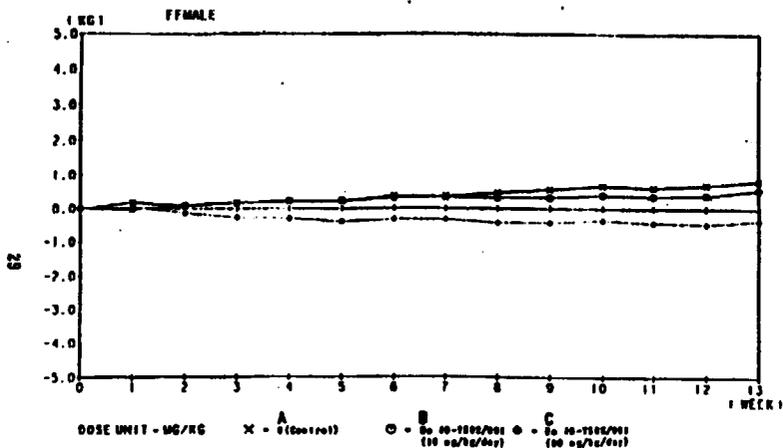


FIG. 4 : THIRTEEN-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7502/001 AND SINEMET IN DOGS BODY WEIGHT GAIN

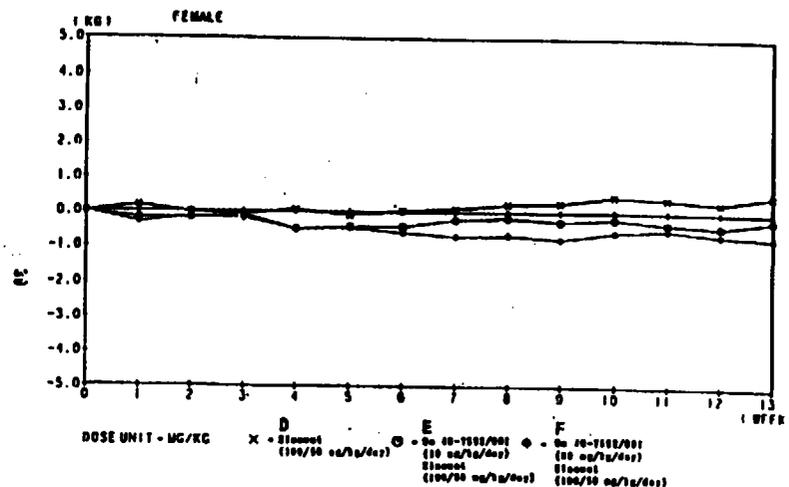


FIG. 5 : THIRTEEN-WEEK ORAL TOXICITY STUDY WITH CONCOMITANT ADMINISTRATION OF RO 40-7502/001 AND SINEMET IN DOGS BODY WEIGHT GAIN

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Histopathology:

↓ spermatogenesis	-	1 S 1 LT/S
prostate atrophy	-	1 S 2 LT/S 1 HT/S
submandibular glands, pigment deposits	-	2 S females 1 LT/S female 1 HT/S male and female

Toxicokinetics: (on day 0, 14 and 90 at predose and 1, 2, 3, 5, 7 and 24 hr post-dose; on days 28 and 56 at 5 and 24 hrs post-dose)

Emesis led to highly variable exposures within groups; it most obvious early in study (days 0 and 14) prior to Sinemet dosage reduction. Increases in tolcapone appeared less than dose proportional, probably because of emesis. Accumulation of tolcapone was not marked, although levels tended to be higher on day 90 then on day 14; this may have resulted from the reduction in L-DOPA dose. No consistent gender effects were noted (Sponsor Table 1). Exposures to the 3-O-methyl metabolite were low, and did not vary much among treatment groups (Sponsor Table 2).

Tolcapone did not produce the expected dose-related increase in L-DOPA exposure (again, likely because of emesis) (Sponsor Table 3), but reduced the amount of 3-O-methyl metabolite present (Sponsor Table 4).

Table 1: Summary table on mean pharmacokinetic parameters of Ro 40-7592

tolcapone

Dosage group	C _{max} (ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
B	8.47	9.60	13.2	10.0	6.03	11.6
C	23.0	32.0	35.3	42.1	46.6	54.4
E*	5.68	2.39**	10.3	3.94	4.74	6.34
F*	15.1	5.98	25.2	21.0	8.10	22.5

Dosage group	AUC 0-7 (h.ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
B	26.3	32.5	33.5	33.9	21.3	26.2
C	86.6	106	113	106	154	201
E*	9.88	6.62**	27.8	7.57	15.3	28.8
F*	31.9	16.5	94.8	49.1	34.4	76.8

*: dose reduced to 40 mg/kg/day L-Dopa and 10 mg/kg/day carbidopa from day 42 onward

** : mean from 2 values

- Dosage group B: 10 mg/kg/day Ro 40-7592
- Dosage group C: 80 mg/kg/day Ro 40-7592
- Dosage group E: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 10 mg/kg/day Ro 40-7592
- Dosage group F: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 80 mg/kg/day Ro 40-7592

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Table 2: Summary table on mean pharmacokinetic parameters of Ro 40-7591

3-O-Methyl-Tolcapone

Doseage group	C _{max} (ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
B	0.799	0.963	0.992	0.930	1.03	1.31
C	1.29	1.57	1.64	0.956	1.17	1.03
E*	0.676	0.802	1.32	0.701	0.816	1.16
F*	1.01	1.21	1.54	0.836	0.937	1.19

Doseage group	AUC 0-24 (h·ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
B	12.9	16.6	17.3	16.1	17.8	23.0
C	22.4	31.3	31.5	15.9	21.8	20.2
E*	9.89	13.3	24.8	12.1	15.2	24.5
F*	17.5	23.2	30.5	14.1	18.3	22.2

* : dose reduced to 40 mg/kg/day L-Dopa and 10 mg/kg/day carbidopa from day 42 onward

Table 3: Summary table on mean pharmacokinetic parameters of Ro 65-4759 (L-Dopa)

L-DOPA

Doseage group	C _{max} (ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
D	11.1	7.76	15.9	3.12	20.0	16.8
E*	4.12	14.0	15.8	4.41	7.09	18.9
F*	2.81	9.60	14.8	1.73	6.39	7.47

Doseage group	AUC 0-7 (h·ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
D*	86.0**	18.4	46.2	8.14***	40.6	66.1***
E*	10.8	37.1	64.5	8.90	24.3	90.4
F*	9.61	33.5	59.0	6.36	29.9	26.4

* : dose reduced to 40 mg/kg/day L-Dopa and 10 mg/kg/day carbidopa from day 42 onward.

** : single value ***: mean of 2 values

Table 4: Summary table on mean pharmacokinetic parameters of Ro 66-3609 (3-O-methyldopa)

3-O-Methyl/DOPA

Doseage group	C _{max} (ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
D*	16.1	32.1	40.0	6.17	31.2	33.9
E*	3.17	21.2	12.9	3.98	17.8	19.1
F*	1.80	13.2	6.58	0.902	8.31	6.16

Doseage group	AUC 0-24 (h·ng/ml)					
	Male dogs			Female dogs		
	Day 0	Day 14	Day 90	Day 0	Day 14	Day 90
D*	266	574	679	101	541	540
E*	51.9	361	233	68.5	321	378
F*	30.2	233	131	15.3	163	115

* : dose reduced to 40 mg/kg/day L-Dopa and 10 mg/kg/day carbidopa from day 42 onward.

Doseage group D: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa

Doseage group E: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 10 mg/kg/day Ro 40-7592

Doseage group F: 20 mg/kg/day carbidopa + 80 mg/kg/day L-Dopa + 10 mg/kg/day Ro 40-7592

Supplemental Study: Measurement of Testosterone and Prolactin in Male Dogs Treated with a Combination of Tolcapone and Sinemet or Madopar

As described above in the multiple-dose combination oral toxicity study of tolcapone and Sinemet, atrophy of the male sex organs (testis, epididymis, prostate) occurred in dogs that received the combination treatment. Similar changes were observed in dogs that received the combination of tolcapone and Madopar. To assess if inhibition of testosterone and prolactin by the combination treatment is a possible mechanism for these changes, the levels of these hormones were measured in sera from treated dogs.

The sera for hormonal determinations were collected at the end of the study (week 13) or from animals sacrificed moribund. Testosterone and prolactin were measured using standard RIA kits.

Results:

Mean testosterone levels in the Sinemet groups (alone or in combination with tolcapone) were significantly lower than those in the groups that did not receive Sinemet.

Only dogs that received Sinemet alone (1) or in combination with tolcapone (2 LD, 1 HD) had histopathological changes of male reproductive structures. As shown in Sponsor Table 2, 2 of the 4 animals had very low testosterone levels (#10003, 10005), and the other two had levels that were not abnormally low. These data are thus not entirely supportive of the hypothesis that lowering of testosterone levels is causally related to the male reproductive changes. The sponsor has not indicated the timing of sampling with respect to dosing. Knowledge of this relationship would aid in interpretation of this result.

The Madopar combination study in dogs was not reviewed since this compound is not approved for use in the U.S., and thus is not relevant for this NDA. Male reproductive changes were more prominent in dogs treated with Madopar alone and in combination with tolcapone (8 of 9 animals had some histopathological alteration). In all affected animals, testosterone levels were below or slightly above the limit of detection (0.2 ng/ml). Thus, these data provide a stronger case for the involvement of hormonal mechanisms in the male histopathological changes due to the combination treatment.

Prolactin was not detectable in any samples.

Table 1 Summary results of the combination study with Ro 40-7592/001 and Madopar in male dogs

Ro 40-7592 (mg/kg/day)	Madopar (mg/kg/day)	Animal No.	Testosterone (ng/ml) mean±SD	Prolactin (ng/ml) mean±SD	Histopathological Findings*** Testis Epididymis Prostate
0	0	55001	1.170	<1.5	- - -
		55002	<0.2	<1.5	- - -
		55003	1.092 ±0.634	<1.5 ±0.000	- - -
10	0	55004	1.099	<1.5	- - -
		55005	1.693 ±0.355	<1.5 ±0.000	- - -
80	0	55006	1.734	<1.5	- - -
		55007	0.909	<1.5	- - -
		55008	3.512 ±1.307	<1.5 ±0.000	- - -
0	100	56001	<0.2	<1.5	1 1 -
		56002	<0.2	<1.5	- - 1
		56003	<0.2 ±0.000	<1.5 ±0.000	- - 1
10	100	56004	<0.2	<1.5	2 1 3
		56005	<0.2	<1.5	- - 1
		56006	<0.2 ±0.000	<1.5 ±0.000	2 1 2
80	100→50°	56007**	0.229	<1.5	- - -
		56008	0.347	<1.5	1 1 2
		56009**	<0.2 ±0.176	<1.5 ±0.000	1 1 1

* : Dosage of Madopar was reduced from day 17 onward.
 ** : 56007 was dead on day 13, 56009 was sacrificed on day 16.
 Detection limits : Testosterone; 0.2 ng/ml, Prolactin; 1.5 ng/ml
 The data under the detection limits were calculated as 0.

--- : Histopathological findings (Grade)
 (Grade 1 ; Slight, Grade 2 ; Moderate, Grade 3 ; Severe change)
 Testis : Decrease of spermatogenesis
 Epididymis : Decrease of sperms in ducts
 Prostate : Atrophy of glands

<Statistical analysis of Testosterone values>

- Analysis of variance
 groups A, B, C v.s. groups D, E, F : Statistically significant (p<0.01)
- Student's t-test
 group C v.s. group D : Statistically significant (p<0.01)
 group C v.s. group E : Statistically significant (p<0.01)
 group C v.s. group F : Statistically significant (p<0.01)
 group A v.s. group C : Statistically significant (p<0.05)
 group B v.s. group D : Statistically significant (p<0.05)
 group B v.s. group E : Statistically significant (p<0.05)
 group B v.s. group F : Statistically significant (p<0.05)
 other combinations : not statistically significant

Table 2 Summary results of the combination study with Ro 40-7592/001 and Sinemet in male dogs

Ro 40-7592 (mg/kg/day)	Sinemet (mg/kg/day)	Animal No.	Testosterone (ng/ml) mean±SD	Prolactin (ng/ml) mean±SD	Histopathological Findings*** Testis Epididymis Prostate
0	0	09001	8.215	<1.5	- - -
		09002	1.729 ±3.823	<1.5 ±0.000	- - -
		09003	1.526	<1.5	- - -
10	0	09004	9.250	<1.5	- - -
		09005	1.937 ±5.384	<1.5 ±0.000	- - -
80	0	09006	4.835 ±3.672	<1.5 ±0.000	- - -
		09007	4.690	<1.5	- - -
0	100→50°	09008	3.757 ±3.266	<1.5 ±0.000	- - -
		09009	1.350 ±1.723	<1.5 ±0.000	- - -
		10001	1.397	<1.5	- - -
10	100→50°	10002	0.977	<1.5	- - -
		10003	<0.2 ±0.717	<1.5 ±0.000	1 1 2
		10004	4.476	<1.5	1 1 1
80	100→50°	10005	<0.2	<1.5	- - 1
		10006	0.575 ±2.435	<1.5 ±0.000	- - -
		10007	0.301	<1.5	- - -
0	100→50°	10008	1.154 ±0.928	<1.5 ±0.000	- - 1
		10009	1.306 ±0.542	<1.5 ±0.000	- - -

* : Day 39, 40 and 41; withdrawal, Dosage of Sinemet was reduced from day 42 onward.
 Detection limit : Testosterone; 0.2 ng/ml, Prolactin; 1.5 ng/ml
 The data under the detection limits were calculated as 0.

--- : Histopathological findings (Grade)
 (Grade 1 ; Slight, Grade 2 ; Moderate, Grade 3 ; Severe change)
 Testis : Decrease of spermatogenesis
 Epididymis : Decrease of sperms in ducts
 Prostate : Atrophy of glands

<Statistical analysis of Testosterone values>

- Analysis of variance
 groups O, H, I v.s. groups J, K, L : Statistically significant (p<0.05)
- Student's t-test
 group H v.s. group J : Statistically significant (p<0.05)
 other combinations : not statistically significant

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C.2.c. 4-Week Oral Toxicity Study of Tolcapone and Sinemet in Cynomolgus Monkeys

GLP

Research Report #:

Sponsor Volumes: 1.39-1.41

Conducted by:

Summary:

Tolcapone (10, 40 and 150 mg/kg/day) was orally administered alone and in combination with Sinemet (250 mg/kg/day; 4:1, L-DOPA:carbidopa) to cynomolgus monkeys for 13 weeks. Because of excessive hyperactivity in monkeys treated with Sinemet, the dose was reduced in steps to 100 mg/kg by day 9. One HT/S female was sacrificed on day 3 due to self-mutilation. Aside from the clinical signs, which also included occasional emesis, no remarkable toxicological findings were noted. Some LDH elevations (relative to control values) were noted.

Toxicokinetic data suggested that Sinemet may decrease Cmax for the high dose of tolcapone, and increase Cmax for the low dose of tolcapone. Tolcapone did not markedly increase exposures to L-DOPA, possibly because of decreased absorption of Sinemet (or an effect of emesis). At the highest dose of tolcapone, exposures were only slightly higher (1.5 times) than expected therapeutic exposures in humans receiving 200 mg, t.i.d. (80 µg.hr/ml). L-DOPA exposures in monkeys were 10-50 fold higher than those of humans receiving Sinemet and tolcapone (3 µg.hr/ml).

Methods:

Dosage Groups:

(Tolcapone Lot GPUL 606 090)

Group	tolcapone	Sinemet*
CON	0	0
LT	10	0
MT	40	0
HT	150	0
S	0	250-100
LT/S	10	250-100
HT/S	150	250-100

* Reduction in Sinemet dosing due to excessive hyperactivity:

Days 1,2	-	200 mg/kg/day L-DOPA + 50 mg/kg/day carbidopa
Day 3	-	none
Day 4	-	160 mg/kg/day L-DOPA + 40 mg/kg/day carbidopa
Day 8	-	120 mg/kg/day L-DOPA + 30 mg/kg/day carbidopa
Day 9-end	-	80 mg/kg/day L-DOPA + 20 mg/kg/day carbidopa

Route of Administration: oral (gavage); tolcapone in 0.2% CMC-Na; Sinemet in 0.1% Tween 80.

Species/Strain/Number: cynomolgus monkeys (males: 2.4 - 3.7 kg; females: 2.2 - 2.8 kg)
3/sex/dose

Results:

Mortality: one HT/S female exhibiting self-mutilation (biting of left foot) and hyperactivity was sacrificed for humane reasons on day 3

Clinical:

discolored urine	-	all treated groups
hyperactivity	-	observed on days 1-8 in all three groups that received Sinemet; peak effect was ca. 3 hr post-dose; dose was reduced to eliminate this effect (no hyperactivity observed after day 11)
emesis (sporadic)	-	HT: 3/3 males, 2/3 females LT/S: 2/3 females HT/S: 2/3 males, 1/2 females

Body Weight: No treatment-related effect (mild increase in all groups)

Hematology: (predose and day 25)

No clear treatment-related effect; 1 LT female was anemic on day 25 (↓ RBC, Hb, Hct, ↑ reticulocytes). Slightly decrease WBC counts were found in 1 MT male and 1 HT female, and 1 HT female had a slightly prolonged APTT.

Clinical Chemistry: (predose and day 25)

↑ ALT and Alk. P	-	1 HT male (JO3817)
↑ BUN	-	1 HT male (JO3816)
↑ LDH (>2X con)	-	1 LT male 1 HT male (JO3815) 1 S male 1 LT/S male 1 HT/S male 1 vehicle female

Prolactin: (day 14)

Generally lower prolactin levels (relative to pretreatment) were recorded in tolcapone-treated males and females, and combination treatment females on No consistent trend was observed in the combination treatment males.

Urinalysis: (at end of dosing)

No treatment-related effect; occult blood was detected in several animals, including controls.

Organ Weights, Gross Necropsy, Histopathology:

No treatment-related effects

Toxicokinetics: (days 1, 10 and during week 4 at predose and 1, 2, 3, 5, 8 and 12 hr post-dose)

Increases in tolcapone exposure were approximately (slightly greater than) dose proportional. Coadministration of Sinemet tended to decrease C_{max} for the high dose of tolcapone, and slightly increased C_{max} for the low dose of tolcapone. Tolcapone did not accumulate, and no gender difference was evident (only slightly higher in females) (Sponsor Table 1). Exposures to the 3-O-methyl metabolite were low (< 0.35 µg/ml), and were not influenced by L-DOPA.

Tolcapone reduced the amount of 3-O-methyl-DOPA, but did not increase exposures to L-DOPA (Sponsor Table 2), possibly because of decreased absorption of Sinemet (or an effect of emesis).

L-DOPA exposures were 4-10-fold higher than those of humans receiving Sinemet (25/100) and tolcapone.

Table 1
Mean (± S.D., n=6) pharmacokinetic parameters of Ro 40-7592 in cynomolgus monkeys during a 4-week interaction study with Ro 11-7618 (carbidopa 50 mg/kg/day / L-Dopa 200 mg/kg/day)

Dosage group	Day 1		Day 10 a)		Day 28 a)	
	C _{max} (µg/ml)	AUC (h·µg/ml)	C _{max} (µg/ml)	AUC (h·µg/ml)	C _{max} (µg/ml)	AUC (h·µg/ml)
Ro 40-7592 10 mg/kg/day	1.13 (0.44)	2.65 (0.53)	1.23 (0.32)	3.81 (1.43)	1.27 (0.30)	3.78 (0.71)
Ro 40-7592 40 mg/kg/day	7.56 (3.11)	22.6 (5.6)	5.29 (1.92)	19.4 (5.4)	5.86 (1.88)	22.0 (3.9)
Ro 40-7592 150 mg/kg/day	27.1 (11.9)	96.1 (25.0)	25.1 (8.30)	104 (30.0)	29.6 (12.8)	126 (34.2)
Ro 40-7592 10 mg/kg/day + Ro 11-7618	3.16 (1.88)	7.01 (3.59)	2.28 (1.01)	5.23 (1.97)	2.01 (0.67)	4.29 (1.72)
Ro 40-7592 b) 150 mg/kg/day + Ro 11-7618	17.9 (4.00)	90 (18.9)	18.2 (6.58)	92.3 (33.6)	12.5 (3.93)	92.1 (36.1)

a) carbidopa : 20 mg/kg/day
L-Dopa : 80 mg/kg/day

b) n=5

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Table 2

Mean (\pm S.D., n=6) pharmacokinetic parameters of Ro 05-4759 in cynomolgus monkeys during a 4-week interaction study with Ro 40-7592 and Ro 11-7618 (carbidopa 50 mg/kg/day / L-Dopa 200 mg/kg/day)

(L-DOPA)

Dosage group	Day 1		Day 10 a)		Day 28 a)	
	Cmax (μ g/ml)	AUC (h. μ g/ml)	Cmax (μ g/ml)	AUC (h. μ g/ml)	Cmax (μ g/ml)	AUC (h. μ g/ml)
Ro 11-7618	17.79 (5.02)	103 (26)	11.61 (4.97)	44 (14)	12.81 (1.75)	43 (4.4)
Ro 11-7618 + 10 mg/kg/day Ro 40-7592	31.81 (27.22)	98 (55)	10.32 (3.54)	35 (7.6)	11.09 (3.11)	43 (5.4)
Ro 11-7618 b) +150 mg/kg/day Ro 40-7592	19.98 (8.56)	158 (81)	7.31 (2.10)	42 (12)	7.63 (1.64)	45 (6.2)

a) carbidopa : 20 mg/kg/day
L-Dopa : 80 mg/kg/day

b) n=5

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Clinical: No marked treatment-related effects

Body Weight: HDM & F - sig. reduction week 5-52 (18% in males, 25% in females at termination, Sponsor Figures 2-5)

Food Intake: HDM & F - sig. reduction at most time points (ca. 10%)

Ophthalmology: (daily examination of anterior region; complete fundus exam and ERG on 3 rats/sex/dose after final dosing)

No treatment-related effects

Hematology: (termination)

Statistically significant mean changes were:

decrease RBC, Hct, Hb	-	HDM (6), HDF
decrease MCHC	-	LDF, HDF, HDM
decrease basophils	-	HDM
decrease neutrophils	-	MDF, HDF
decrease monocytes	-	HDF
increase lymphocytes	-	MDF, HDF

Six HDM (#61, 64, 69, 73, 75, 79) were anemic which accounted for the mean change. Three of the six had elevated reticulocyte counts (61, 64, 79), and 3 had elevated platelets (61, 73, 79). At necropsy, #64 was found to have a nephroblastoma and #75 had a renal adenocarcinoma.

The magnitude of the other mean variations were marginal.

Clinical Chemistry: (termination)

Statistically significant dose-related mean changes were:

Increases:	alkaline phosphatase	-	HDF
	cholinesterase	-	HDF
	A/G	-	HDM, HDF
	albumin	-	HDF
Decreases:	glucose	-	HDM, HDF
	triglycerides	-	LDF, HDF
	cholesterol	-	HDM
	creatinine	-	HDF
	calcium	-	HDM

The most notable individual variations occurred with alkaline phosphatase activity (greater than 2X control in 2 HDM, 2 MDF, 7 HDF), albumin (> 5.8 in 2 ConF, 2 LDF, 1 MDF, 7 HDF), and A/G ratio (> 3.0 in 6 ConF, 5 LDF, 2 MDF, 13 HDF).

Clinical: No marked treatment-related effects

Body Weight: HDM & F - sig. reduction week 5-52 (18% in males, 25% in females at termination) (Sponsor Figures 2,3)

Food Intake: HDM & F - sig. reduction at most time points (ca. 10%, Sponsor Figures 4,5)

Ophthalmology: (complete fundus exam and ERG on 3 rats/sex/dose after final dosing)

No treatment-related effects

Hematology: (termination)

Statistically significant mean changes were:

decrease RBC, Hct, Hb	-	HDM (6), HDF
decrease MCHC	-	LDF, HDF, HDM
decrease basophils	-	HDM
decrease neutrophils	-	MDF, HDF
decrease monocytes	-	HDF
increase lymphocytes	-	MDF, HDF

Six HDM (#61, 64, 69, 73, 75, 79) were anemic which accounted for the mean change. Three of the six had elevated reticulocyte counts (61, 64, 79), and 3 had elevated platelets (61, 73, 79). At necropsy, #64 was found to have a nephroblastoma and #75 had a renal adenocarcinoma.

The magnitude of the other mean variations were marginal.

Clinical Chemistry: (termination)

Statistically significant dose-related mean changes were:

Increases:	alkaline phosphatase	-	HDF
	cholinesterase	-	HDF
	A/G	-	HDM, HDF
	albumin	-	HDF
Decreases:	glucose	-	HDM, HDF
	triglycerides	-	LDF, HDF
	cholesterol	-	HDM
	creatinine	-	HDF
	calcium	-	HDM

The most notable individual variations occurred with alkaline phosphatase activity (greater than 2X control in 2 HDM, 2 MDF, 7 HDF), albumin (> 5.8 in 2 ConF, 2 LDF, 1 MDF, 7 HDF), and A/G ratio (> 3.0 in 6 ConF, 5 LDF, 2 MDF, 13 HDF).

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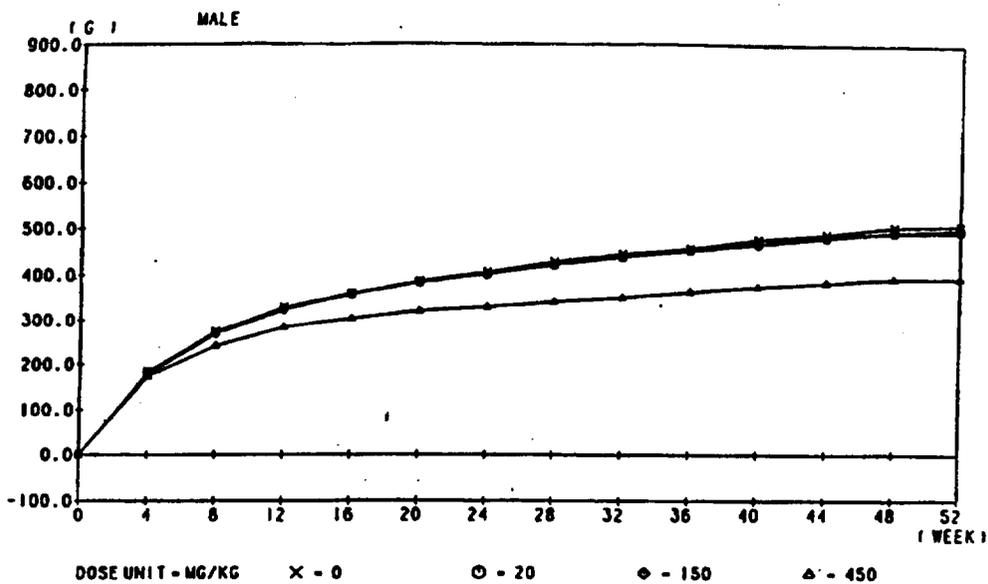


FIG. 2 : 52-WEEK ORAL TOXICITY STUDY OF RO 40-7592/001 IN RATS (FEED ADMIX)
BODY WEIGHT GAIN

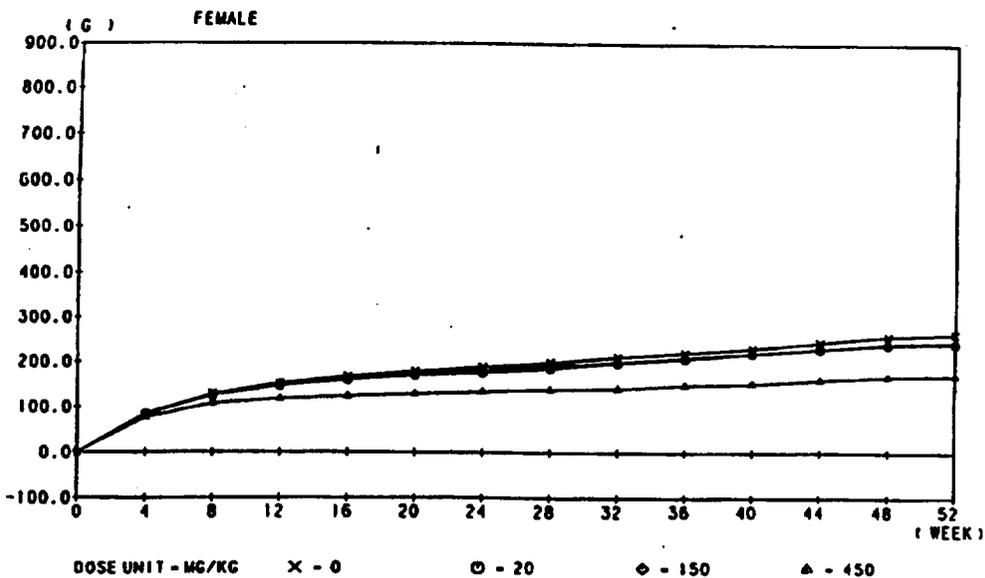


FIG. 3 : 52-WEEK ORAL TOXICITY STUDY OF RO 40-7592/001 IN RATS (FEED ADMIX)
BODY WEIGHT GAIN

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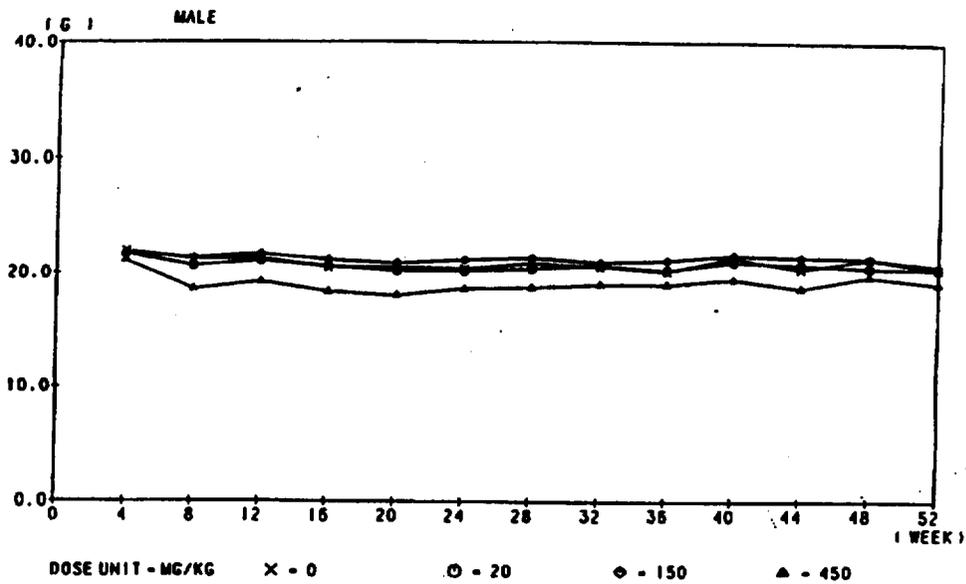


FIG. 4 : 52-WEEK ORAL TOXICITY STUDY OF RO 40-7592/001 IN RATS (FEED ADMIX)
FOOD CONSUMPTION

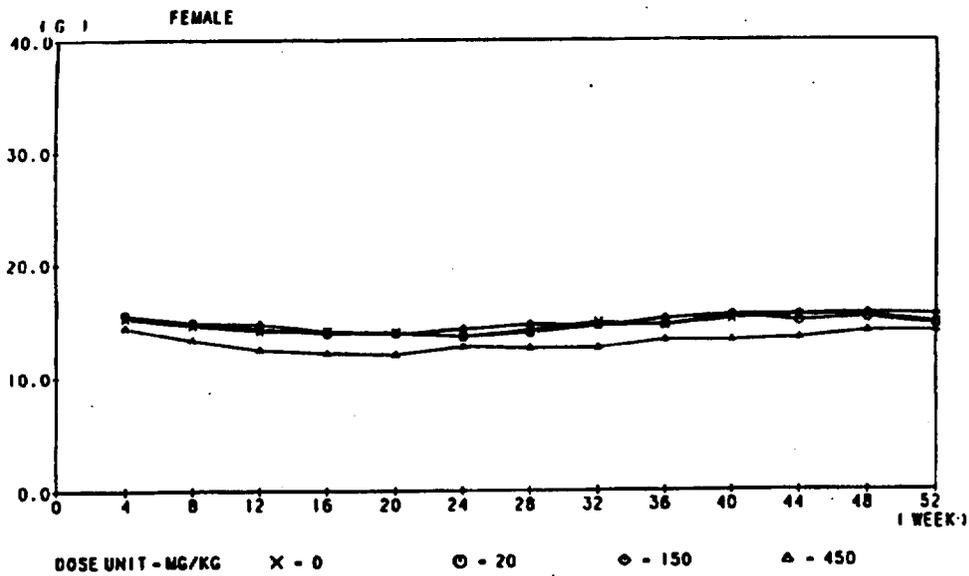


FIG. 5 : 52-WEEK ORAL TOXICITY STUDY OF RO 40-7592/001 IN RATS (FEED ADMIX)
FOOD CONSUMPTION

Urinalysis: (samples were collected before final dose by squeezing abdomen; urine volume and specific gravity were determined from 16 hr collections in 6 rats/sex/group)

The main finding was an increase in round epithelial cells (MDM, HDM, HDF) and white blood cells (HDM) in urine sediments.

Organ Weights:

Statistically significant increases in relative weights were:

kidney	-	MDM, HDM, HDF
brain	-	HDM, HDF
pituitary	-	HDM, HDF
salivary glands	-	HDM, HDF
heart	-	HDM, HDF
lung	-	HDM, HDF
liver	-	MDM, HDM, HDF
spleen	-	HDF
adrenal	-	HDF
male sex organs	-	HDM
uterus	-	HDF

Gross Pathology: Renal masses were observed in 2 HDM.

Histopathology: (complete examination only on control and HD rats; kidney and forestomach were identified as affected tissues, and were evaluated in the LD and MD groups)

		20	150	450
kidney	atypical nuclei in PTE	M		10
		F	10	20
	degeneration of PTE	F		6
	nephroblastoma	M		1
	adenocarcinoma	M		1
forestomach	epithelial hyperplasia	M	3	14
		F	1	8
adrenal	↑ lipid droplets	M		6
	(zona fasciculata)	F		
retina	atrophy	F	1	2
	degeneration	F		1

Plasma Concentrations: (days 4, 10 and 35, at 0900 and 1700, and on days 77, 161, 252 and 350 at 0600)

Increases in plasma concentrations were approximately dose-proportional. Levels tended to be higher in females than in males, although the gender effect was not as dramatic as observed in the 2-year carcinogenicity study. The sponsor has calculated a drug accumulation factor based on means that suggests some accumulation occurs; however, the individual data do not reveal consistent trends of drug accumulation (Sponsor Table 4).

Levels of the 3-O-methyl metabolite were not detectable in most samples (LOQ = 0.2 µg/ml).

Table 4 Mean concentrations (µg/ml) of Ro 40-7532 in plasma of rats during a 52-week toxicokinetic study with Ro 40-7532/001 dietary administration

Dose / sex (mg/kg)	Mean concentration on days 4-35				Mean concentration on days 77-350		Systemic Accumulation Factor
	At 9:00h (µg/ml)	Gender Differences	At 17:00h (µg/ml)	Gender Differences	At 6:00h (µg/ml)	Gender Differences	
20 / M	0.99±0.24	NA	0.90±0.43	NA	1.25±0.15	NA	1.3
20 / F	0.94±0.38	1.0	0.75±0.26	0.9	1.50±0.35	1.3	1.7
150 / M	6.20±1.03	NA	4.19±1.04	NA	9.40±1.30	NA	1.5
150 / F	6.83±2.23	1.1	4.90±1.21	1.2	19.10±2.39	1.4	1.9
450 / M	24.17±5.59	NA	12.19±1.67	NA	38.73±2.22	NA	1.6
450 / F	27.67±12.01	1.1	19.46±5.24	1.6	55.59±12.04	1.4	2.0

NA : Not Applicable
 Gender differences : Ratio of mean concentration in females to mean concentration in male animals
 Systemic accumulation factor : Ratio of mean concentration after repeated administration (days 77-350, at 6:00h) to the mean concentration on days 4-35 (9:00h).

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One-Year Rat Study Addendum: Immunohistopathological Assessment of Proximal Tubular Epithelium for Proliferating Cell Nuclear Antigen.

Purpose:

To determine if the atypical nuclei observed in the PTE of high dose rats is associated with high proliferative rates, five block specimens from control and HD rats were tested for the presence of proliferating cell nuclear antigen (PCNA), an immunohistochemical marker of highly proliferative cells.

The animal number of specimens as follows:

Dose level	Animal No.
Control	47081, 47083, 47085, 47086, 47087
450 mg/kg/day	47142, 47143, 47146, 47150, 47159

All specimens were from female rats. All treated animals had atypia. Three of the five treated animals had grade 1 degeneration of the proximal tubule epithelium.

Results:

Table 1 PCNA immunohistochemical findings of tubular epithelium in kidney

Item	Control	450 mg/kg/day
Number of cells per visual field	402 ± 21	462 ± 31 *
Number of atypical nuclei cells per visual field	0	135 ± 12
Number of PCNA positive cells per visual field	1.72 ± 0.58	7.52 ± 1.75 *
% of PCNA positive cells to all cells	0.42 ± 0.13	1.63 ± 0.39 *
% of PCNA positive cells in atypical nuclei cells	NANC	3.73 ± 0.78

NANC : No atypical nuclei cell

* : P < 0.01

PCNA : Proliferating cell nuclear antigen

The sponsor concludes that the number of PCNA-positive cells among cells with atypia did not clearly increase (Sponsor Table 1). This conclusion is difficult to support since there were no cases of atypia in control animals, and no statistical comparisons can be made. Thus, these data do not support the sponsor's contention that atypia is not necessarily indicative of the carcinogenic potential of tolcapone.

Tubular hyperplasia was not observed in this study.