$$\text{Transformed Score} = B_0 + \frac{Emax \times Ce^n}{Ce^n + EC_{50}{}^n}$$

where Bo is the baseline score, Emax is the maximum stimulatory effect, Ce is the drug concentration at the site of action, EC_{50} is the drug concentration that cause 50% of the maximum effect, n is the Hill's sigmoid coefficient Exponential error models were employed for the between-subject variability of EC_{50} and Bo, and additive error model was used for the between-subject variability of Emax (η_{ECS}, η_{BoS} and η_{EMX} as shown in the following equations)

$EC_{50} = TVECS \cdot \exp(\eta_{ECS})$

$Bo = TVBoS \cdot \exp(\eta_{BoS})$

$Emax = TVEMX + \eta_{EMX}$

where TVECS is the typical value of EC_{50}, TVBoS is the typical value of Bo and TVEMX is the typical value of Emax

Since pharmacodynamic data were obtained from 3 occasions in each patient, exponential error models were employed to account for between-occasion variability (BOV) in EC_{50}, Emax and Bo To avoid over-parameterization, all BOV parameters (variance) were fixed to 0 1 in the analysis

Results

Apomorphine Pharmacokinetics

Bayesian estimates of apomorphine PK parameters of individual subjects obtained were used to calculate the drug concentrations of each subject in PK/PD modeling

Summary of estimates of PK parameters for apomorphine in patients with Parkinson's disease are provided in the following Table

Estimates of Pharmacokinetic Parameters of Apomorphine

Parameters	Mean	SE (%)
Ka (1/h)	14 9	7 05
CL (L/h)	191	9 11
V (L)	153	7 25
BSV of Ka	141%	136 5
BSV of CL	30 2%	42 6
BSV of V	24 7%	40 2
Residual variance (proportional error)	13 8%	40 1
Residual variance (additive error, ng/mL)	1 0	57 7

Correlation of Motor Scores and Apomorphine concentrations

The mixed-effect PD parameter estimates are presented in the Table below The EC_{50} estimate of apomorphine based on motor score is 10 7 ng/rnL

Table Motor Score Pharmacodynamic Parameter Estimates

Parameter	Estimate	SE (%)
Keo (hr^{-1})	5 36	31 0
EC_{50} (ng/mL)	10 7	30 8
Bo	24 3	16 9
Emax	1 0 (fixed)	-
N	3 0 (fixed)	-
BSV of keo	0% (fixed)	-
BSV of EC50	63 7%	59 6
BSV of Bo	35 8%	57 8
Residual variance (proportional error)	30 7%	38 3
Residual variance (additive error)	1 0 (fixed)	-

Correlation of Step Second Scores and Apomorphine concentrations

Transformation of the modified Webster's step second score is performed prior to PK/PD modeling The PK/PD relationship is based on a stimulatory sigmoid Emax model

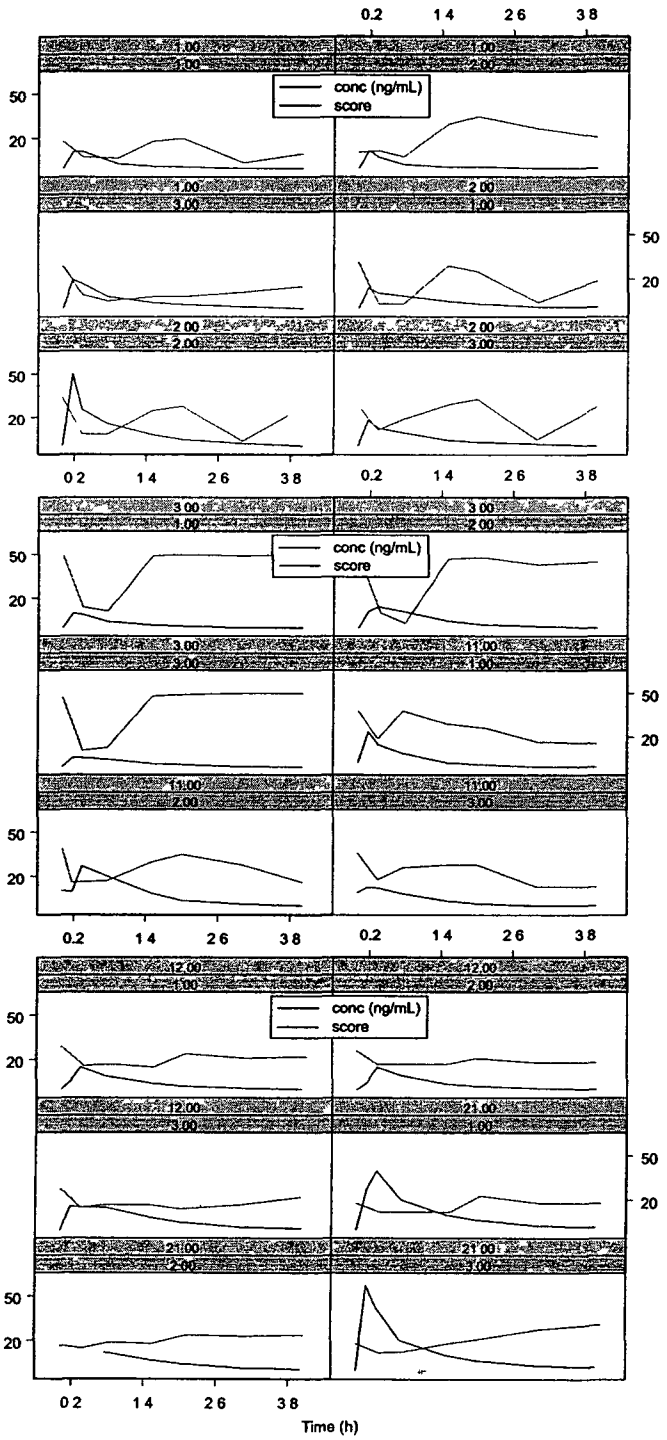
The mixed-effect PD parameter estimates are presented in the Table below The EC_{50} estimate of apomorphine based on step second scores is 5 3 ng/mL

Table Step Second Score Pharmacodynamic Parameter Estimates

Parameter	Estimate	SE (%)
Keo (hr^{-1})	6 08	16 6
EC_{50} (ng/mL)	5 3	24 5
Bo	0 70	125
Emax	7 9	36 7
N	3 0 (fixed)	-
BSV of keo	0 (fixed)	-
BSV of EC50	47 5%	56 6
BSV of Bo	155%	136
BSV of Emax	15 5%	72 2
Residual variance (additive error)	4 19	24 8

Comments

1 The applicant used 0 to 2 hour data although the data until 4 hour are available The reviewer plotted the data from 0 to 4 hours as shown in the following figure and found the concentrations after 2 hours were low and in most cases were 0 and the motor scores were flat in most cases However, it is noted that two troughs appear in subject 1 and 2 (especially in subject 2, two troughs appear in all three occasions) Since there were no controls in this



study, no obvious reasons could be found responsible for this phenomenon According to the protocol, co-medications were not allowed until 2 hours after apomorphine dose The co-medications after 2 hours could be a reason In addition, when the PK parameter estimates were based on the data until 4 hours, the results were similar to that using data until 2 hours as shown in the following table

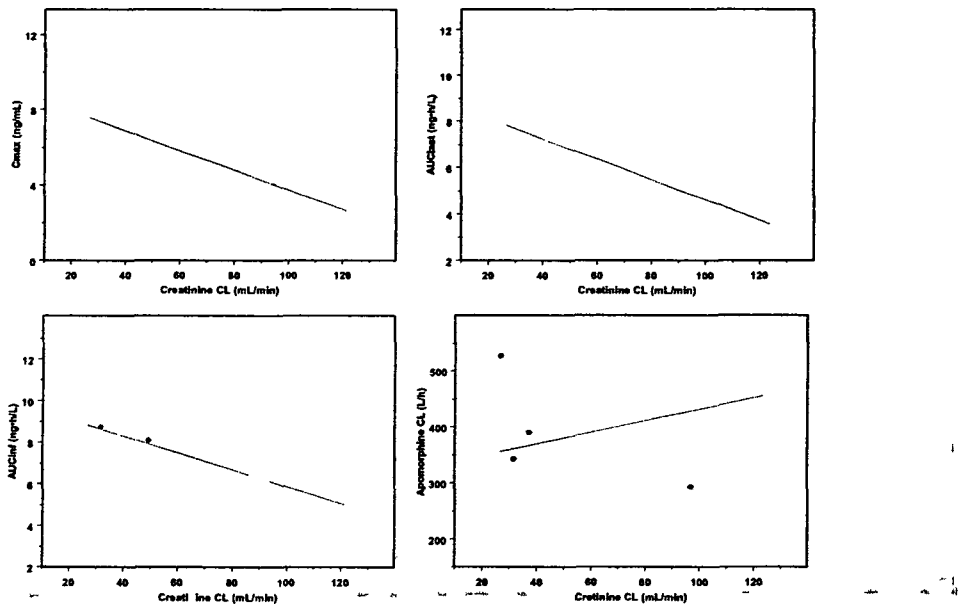
Parameters	Mean (se%) to 4 h	Mean (se%) to 2 h
Ka (1/h)	21 4 (21 0)	14 9 (7 05)
CL (L/h)	182 (8 3)	191 (7 25)
V (L)	158 (6 5)	153 (11 4)

2 Literatures suggested biphasic elimination for the pharmacokinetics of apomorphine However, the applicant used a one-compartment model The reviewer tried a two-compartment model and compared the objective function values between these two models The results are shown below

Model	Objective function values
One compartment	396 6
Two compartment	403 7

Based on the objective function values, the two-compartment model is not preferable over the one-compartment model The reason for this may be due to the sampling times in this study that did not well characterize the distribution phase

3 In the study comparing the pharmacokinetics in renally impaired subjects and normal subjects, the clearance in the renally impaired subjects reduced by 14% (422 vs 363 L/h) and the C_{max} increased 50% A regression analyses between different parameters and creatinine clearance is conducted The following figures show the regression results



Using the UPDRS score (primary clinical end point) versus concentration model, a simulation was done to investigate the impact of renal impairment on the effectiveness of apomorphine The following figure (on next page) shows the comparison of plasma concentration and effect between normal and renal impairment subjects As can be seen, the onsets of the effect are not considerably affected especially when the dose is higher (8 mg) Similarly, the magnitudes of the maximum effect are not different considerably However, the offsets of the profiles are separated resulting in the longer duration of the effects for the renal impairment subjects

To make a judgement for this modest increase of effect, we need to balance the benefit and risk In the literature (van Laar, et al Clin Neuropharm, 1998, 21(3) 152-158), a therapeutic window for apomorphine was described for ten Parkinson's disease patients by defining the minimum effective concentration (MEC) and the minimal toxic concentration (MTC) Of the five patients who demonstrated both a clinical benefit and an adverse event, the mean MEC was 3 9 ng/mL (1 4 to 5 3 ng/mL), and the mean MTC was 15 2 ng/mL (8 5 to 24 5 ng/mL) Substantial inter-individual variability in this window between efficacy and toxicity was observed Based on the simulation, the differences of effect (UPDRS score) between renal impairment and normal subjects are relatively small However, the reported therapeutic window and the adverse events (see below) should be considered Since both effectiveness and adverse events are related to concentrations, the starting dose of 2-mg for renally impaired patients would be equivalent to 3 mg (taking 50% increase of C_{max} in renally impaired patients into consideration)

Therefore, the starting dose for renally impaired patients is recommended to be reduced to 1 mg

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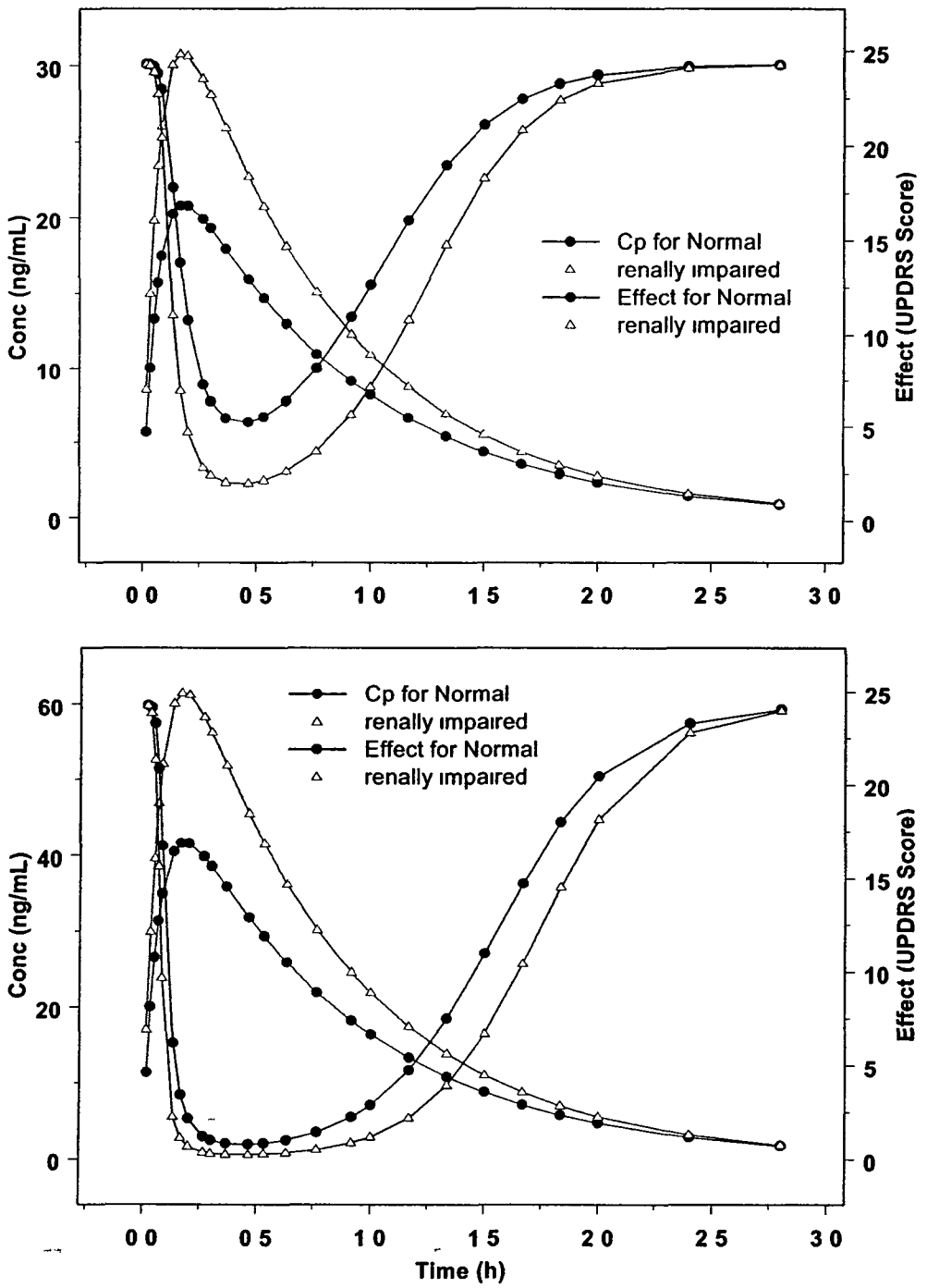
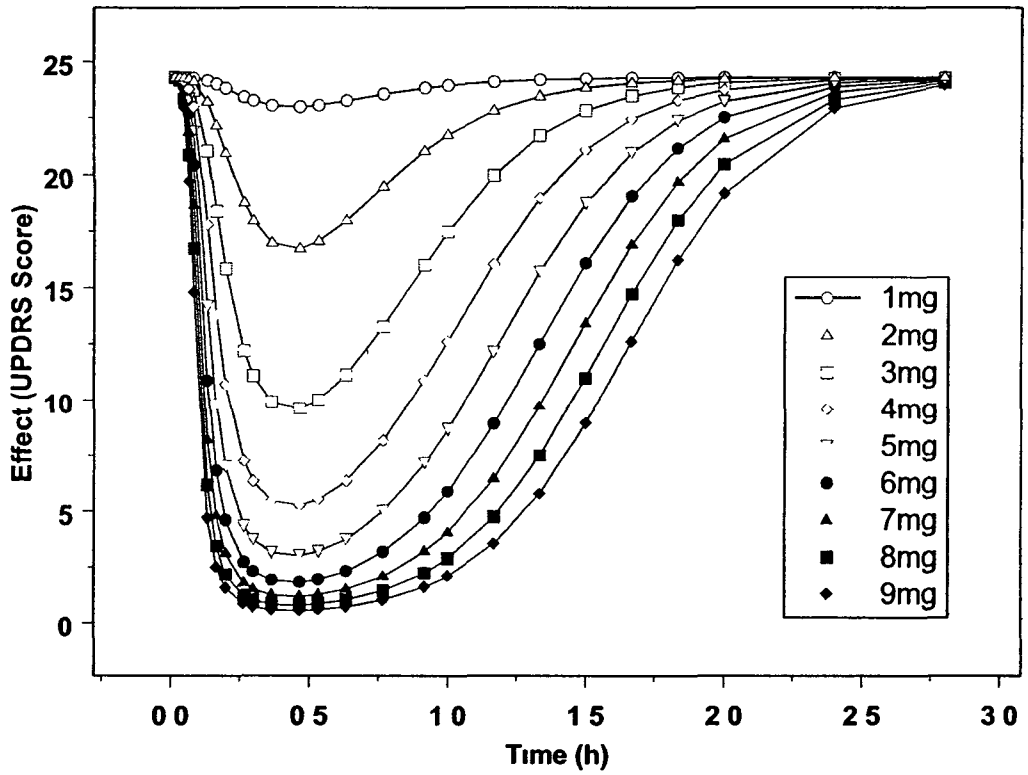
Figure. Comparison of plasma concentration and effect between normal and renal impairment subjects. The red lines represent plasma concentration (Cp) and the blue lines represent the effect (UPDRS-motor score) The round solid circles symbolize the profiles for normal subjects and the triangles symbolize the profiles for renal impairment subjects The upper panel is for dose 4 mg and lower panel for dose 8 mg (please note that the symbols are for the purpose of differentiation, and they do not stand for the actual data points)



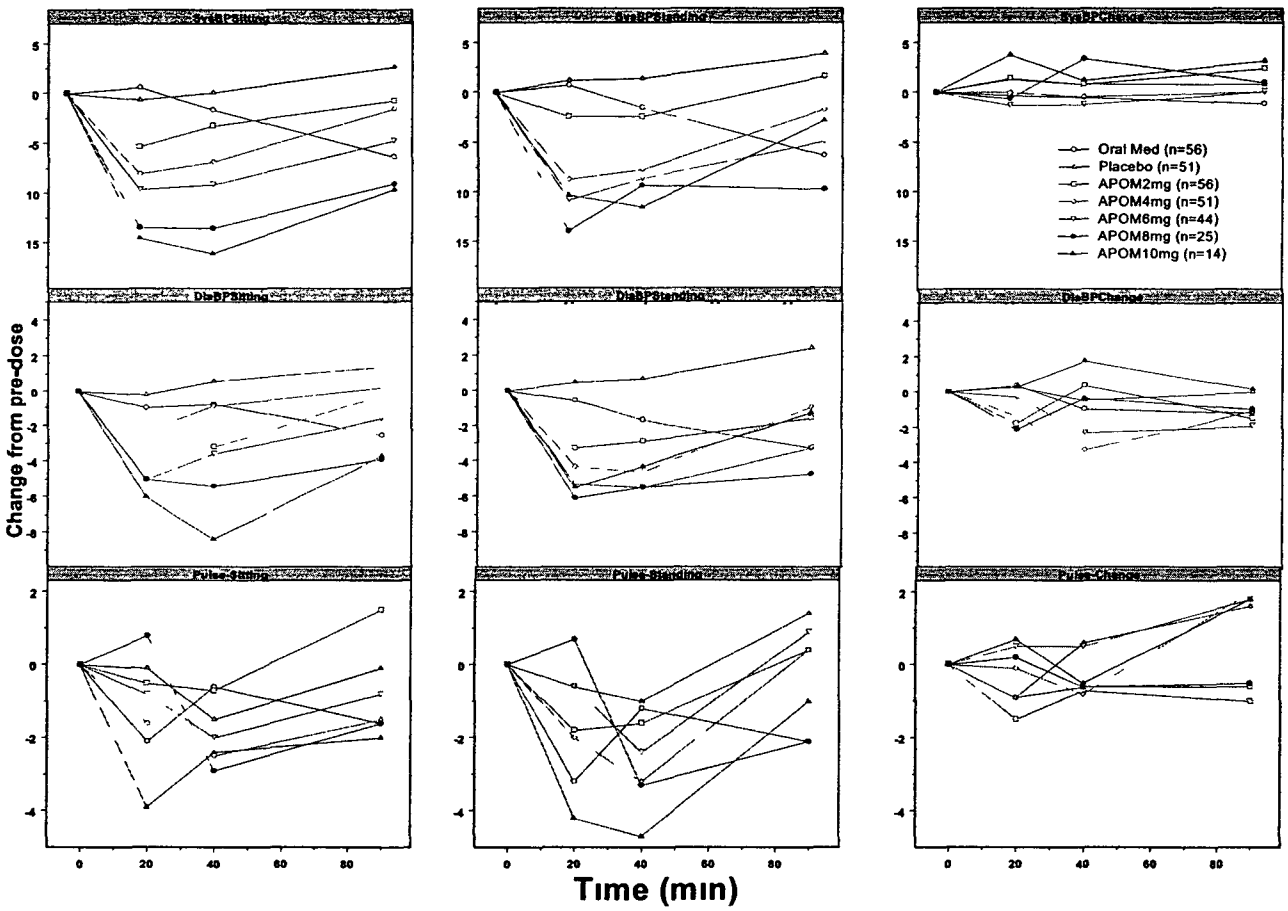
4 It can be seen from the above figure that when higher dose (8 mg) is given, the magnitudes of effect (decrease of UPDRS-motor score) do not increase dramatically, whereas the duration of the effect is prolonged In order to investigate the effect at different doses, another simulation was done As shown in the following figure (symbols are for differentiation purpose only and they do not stand for actual data points), dose increases from 1 mg to 5 mg, the effect increases considerably, either in magnitude or duration On the other hand, when dose is above 5 mg, the changes of magnitudes are smaller and the major changes are prolongation of the duration of the



effects (although it is smaller than the lower doses)

5 From modeling of the PK/PD relationship, it can be seen that doses higher than 6 mg may not produce considerable extra improvement although the duration of the effect may last longer Consequently, the relationship between dose and adverse events were examined to balance the benefit and risk The study APO303 was reviewed as suggested by the Medical Officer Changes in blood pressure that were qualified as orthostatic hypotension were dose related, occurred primarily at the 20- and 40-minute time points, tended to return toward baseline by 90 minutes, as shown in the following figures

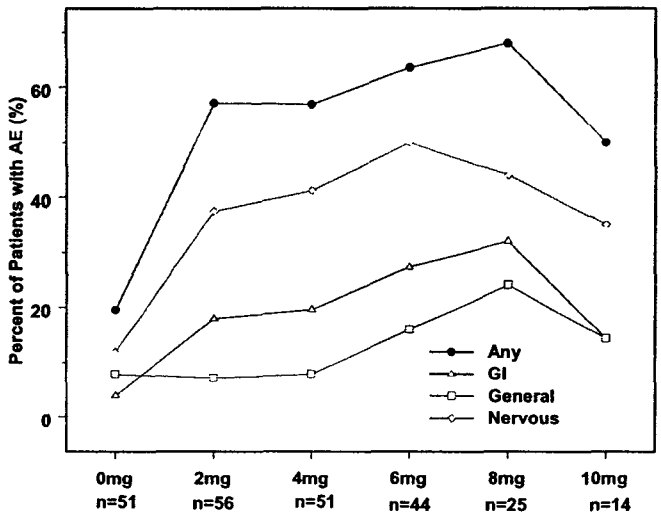
Fig The blood pressure and pulse changes from pre-dose at different time points for different treatment groups. The left column shows the values at sitting; the middle column shows the values at standing, and the right column shows the value changes from sitting to standing. The upper row shows the values of systolic blood pressures; the middle row shows the values of diastolic blood pressures, the lower row shows the values of pulse The y-axis is designated as change from pre-dose For systolic and diastolic blood pressure, the unit is mm Hg and for pulse, the unit is beats per minute.



As shown, the decreases of blood pressures (systolic and diastolic) and pulse are dose-dependent Higher doses may produce higher probability causing lower blood pressures and pulse

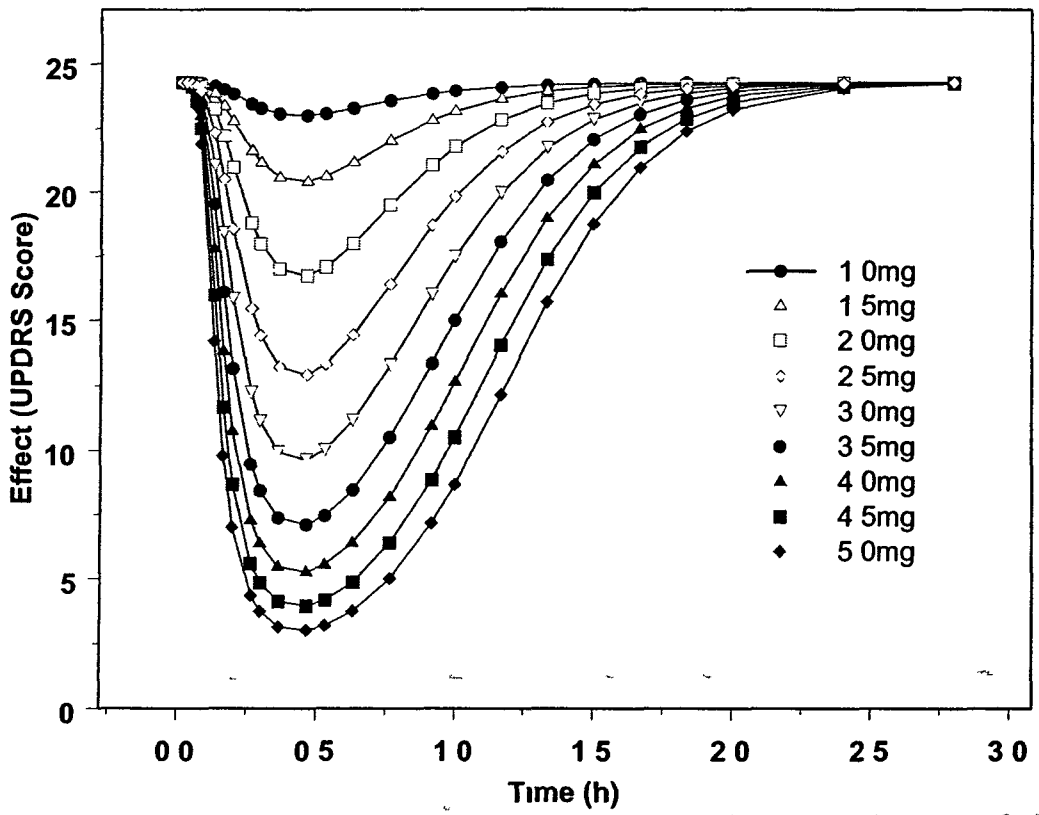
Other adverse events from this study may also have dose dependency as shown in the following figure All type adverse events (labeled as "Any"), GI system adverse events, and general adverse events increase with dose until 8 mg The 10 mg dose group have less patients (n=14) and may not reflect the real situation Further, these patients might be tolerated to the drug while other patients dropped out at lower doses (Note that there were 44 patients until 6 mg dose After that the number decreased to 25 patients at 8 mg and to 14 patients at 10 mg due to drop out)

Therefore, the dosing regimen should be selected by balancing the benefit and the risk It can be

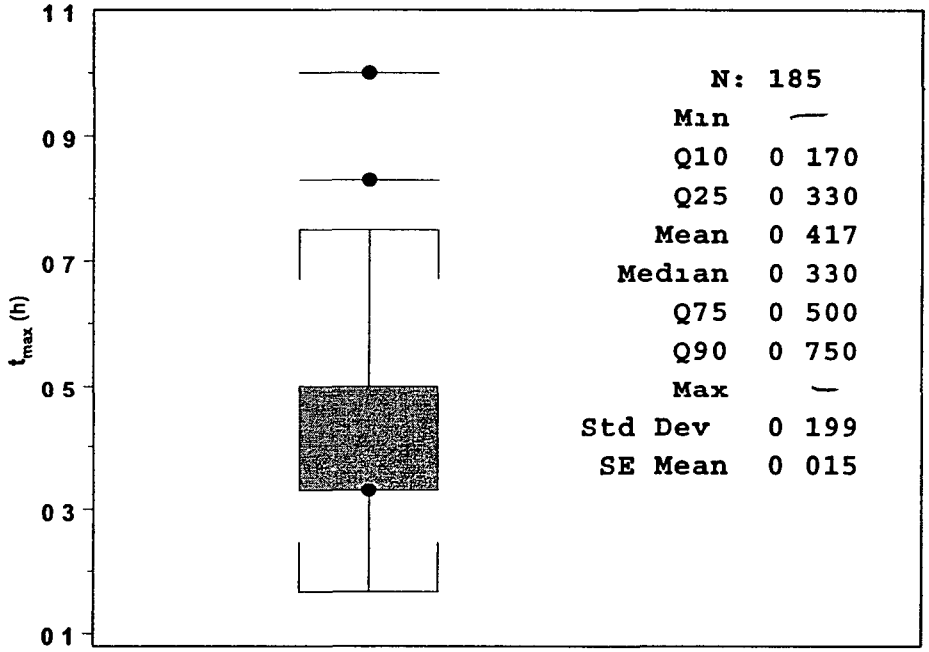


inferred that doses higher than 6 mg may not have considerable extra benefit whereas the probability of adverse reaction may increase Doses above 6 mg are not recommended

6 Due to the large increase of the effects when the dose increases during the lower dose range (from 1 mg to 4 mg), dose increment of 0 5 mg is recommended instead of 1 mg as proposed by the applicant The following figure shows the simulated effects by 0 5 mg increment



7 In the proposed labeling, the applicant did not indicate the interval of repeated dosing For selection of the repeated dosing regimen, we first consider the time to reach maximum plasma concentration (t_{max}), since it is shown that concentration and effect are correlated The t_{max} distribution in all the studies conducted by the sponsor including studies APOM-0053, APOM-0058, APOM-0073, APOM-0083, APOM-02115 and APOM-0222 is shown in the following figure In a total of 185 subjects, t_{max} is ranged from 0 167 hour to 1 hour The 25 percentile and 75 percentile are 0 33 hour and 0 5 hour, respectively



Next, we consider the delay between the plasma concentration and the effect A simulation is conducted to generate the concentration and effect (UPDRS scores) profiles for 1000 subjects From the simulation, the delay between plasma t_{max} and t_{Emax} is calculated The descriptive statistics show that the delay is ranged from — ? hour with a mean of 0 107 hour and a standard deviation of 0 095 hour as shown in the following table

Mean	Std	Min	Q25	Median	Q75	Max
0 107 h	0 095 h	—	0 003 h	0 163 h	0 163 h	—

Taking the ranges of delay and t_{max} into consideration, the time to reach the maximum effect would take at least 0 17 hour and could take as long as 1 58 hours If we take 75 percentile as a cutoff point for both t_{max} and delay, the time taken to reach the maximum effect would be 0 66 hour Before this time, the effect may not be fully observed and hence dosing adjustments should not be considered

On the other hand, at 20, 40 and 90 min after 6-mg dose administration, the decrease of systolic and diastolic blood pressures are as follows based on the data from study 303

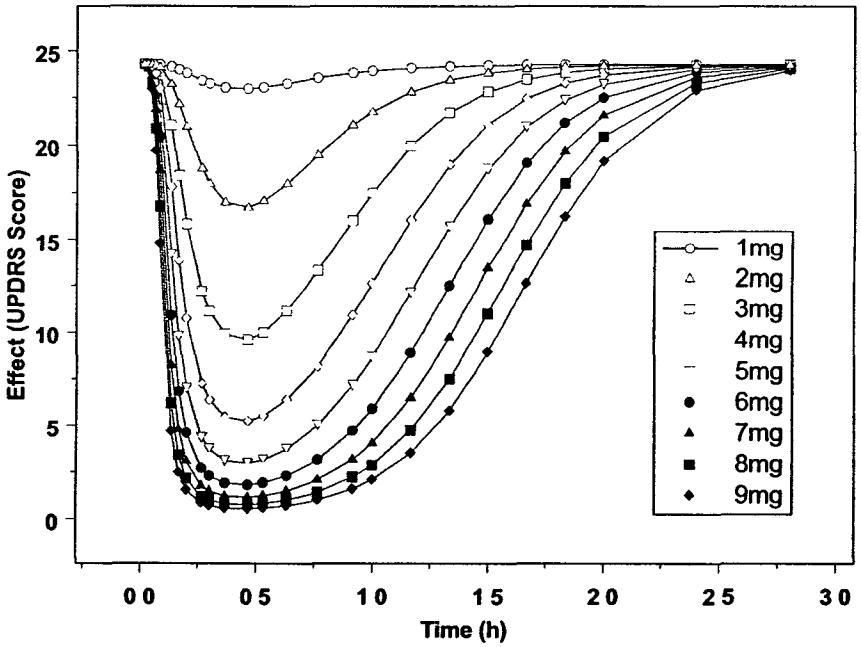
			Change from pre-dose, Mean (SE, N)	
Time (min)	Parameters		Placebo	Dose 6 mg
20	SysBP (mm Hg)	Sitting	-0 6 (1 6, 50)	-9 6 (1 9, 44)
		Standing	1 2 (1 0, 48)	-10 8 (2 8, 41)
	DiaBP (mm Hg)	Sitting	-0 2 (1 2, 50)	-5 (1 2, 44)
		Standing	0 5 (1 0, 48)	-5 3 (1 4, 41)
40	SysBP (mm Hg)	Sitting	0 1 (1 7, 50)	-9 1 (2 4, 44)
		Standing	1 4 (1 1, 48)	-8 6 (2 4, 42)
	DiaBP (mm Hg)	Sitting	0 6 (1 3, 50)	-3 6 (1 4, 44)
		Standing	0 7 (0 9, 48)	-5 5 1 4, 42)
90	SysBP (mm Hg)	Sitting	2 7 (2 0, 49)	-4 7 (1 5, 44)
		Standing	3 9 (1 5, 47)	-4 9 (2, 42)
	DiaBP (mm Hg)	Sitting	1 4 (1 7, 49)	-1 6 (1 1, 44)
		Standing	2 4 (1 1, 47)	-3 3 (1 3, 42)

The blood pressures (systolic and diastolic) at 20 and 40 minutes decrease the most At 90 min, although they tend to return toward baseline, the blood pressures are still lower than pre-dose levels Ideally, the repeated dose should not be administrated until the blood pressure comes back to baseline Therefore, the repeated dose interval should be more than 90 minutes from safety perspective

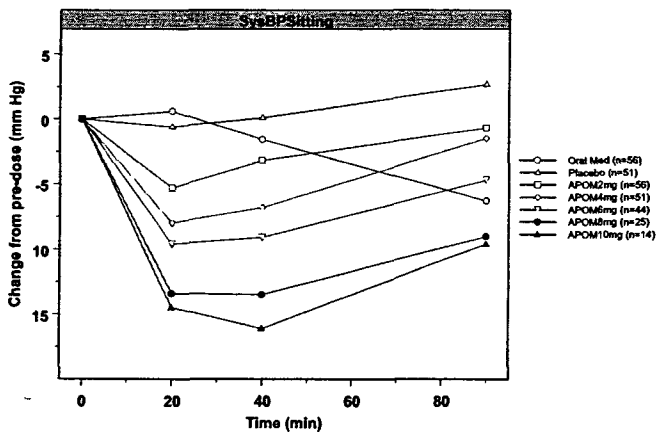
Conclusion

1 What is a reasonable maximum dose?

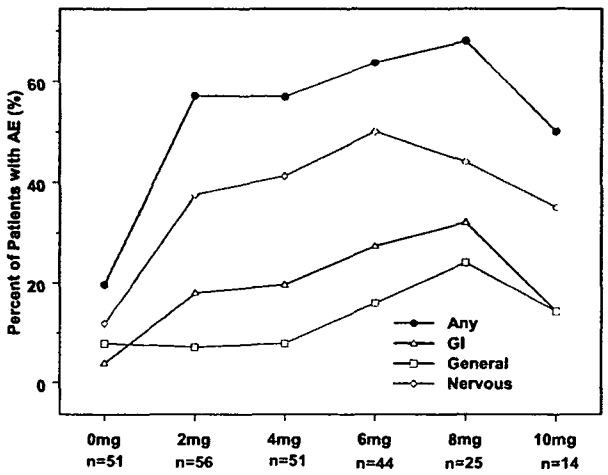
When higher dose is given, the magnitudes of effect (decrease of UPDRS-motor score) do not increase dramatically, whereas the duration of the effect is prolonged As shown in the following figure (symbols are for differentiation purpose only and they do not stand for actual data points), dose increases from 1 mg to 5 mg, the effect increases considerably, either in magnitude or duration However, when dose is above 5 mg, the changes of magnitudes are much smaller when the dose is increased At the same time, the prolongation of the duration is not as obvious as that at lower doses It can be seen that doses higher than 6 mg may not produce considerable extra improvement although the duration of the effect may last longer



On the other hand, decreases in blood pressures that were qualified as orthostatic hypotension were dose related, occurred primarily at the 20- and 40-minute time points, tended to return toward baseline by 90 minutes, shown in the following figure as an example



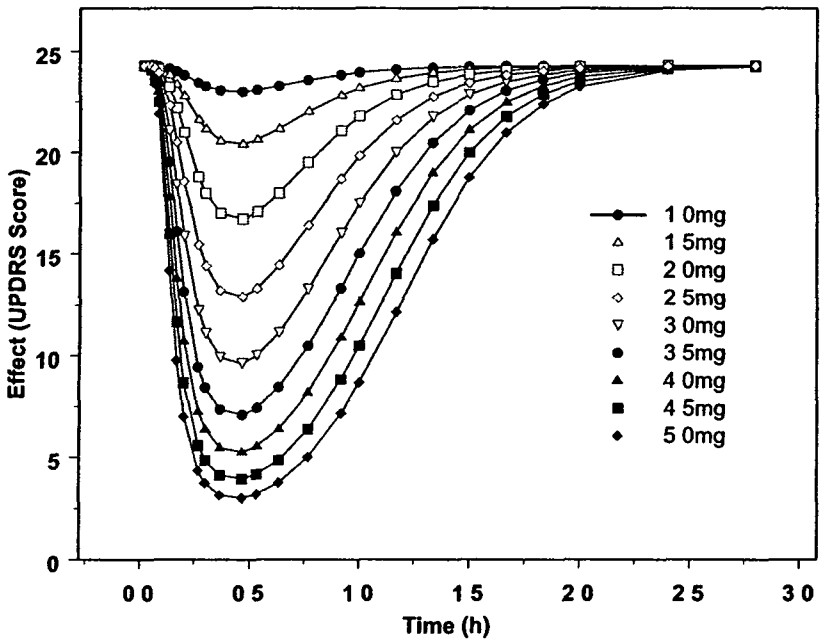
Other adverse events are also dose related as shown in the following figure



Therefore, the maximum dosing should be selected by balancing the benefit and the risk It can be inferred that doses higher than 6 mg may not have considerable extra benefit whereas the probability of adverse reaction may increase Doses above 6 mg are not recommended

2 What is a reasonable dose increment?

Within the lower dose range (from 1 mg to 4 mg), the increases of the effect (UPDRS scores) are considerable The following figure shows the simulated effects by 0 5-mg increment

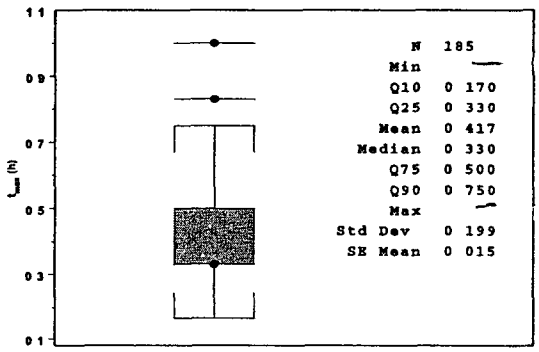


During dose titration, larger increment may miss the appropriate dose and go to an unnecessarily high dose Consequently, this may result in avoidable adverse events The Medical Officer found

that the rate of adverse events was 8-fold higher during titration stage than those during maintenance stage Therefore, dose increment of 0 5 mg is recommended instead of 1 mg as proposed by the applicant

3 What is a reasonable repeated dosing regimen?

In the proposed labeling, the applicant did not indicate the interval of repeated dosing For selection of the repeated dosing regimen, three factors are considered including the time to reach peak concentration (t_{max}, see the following figure), the delay between the plasma concentration and the effect, and the adverse event rate



In a total of 185 subjects, t_{max} is ranged from 0 167 hour to 1 hour The 25 percentile and 75 percentile are 0 33 hour and 0 5 hour, respectively From a simulation conducted in 1000 subjects, the calculated delay between plasma t_{max} and t_{Emax} is ranged from — hour with a mean of 0 107 hour and a standard deviation of 0 095 hour Taken the ranges of delay and t_{max} together, the time to reach the maximum effect would take at least 0 17 hour and could take as long as 1 58 hours The blood pressures (systolic and diastolic) at 20 and 40 minutes decrease the most as shown in the following figure At 90 min, although they tend to return toward baseline, the blood pressures are still lower than pre-dose levels Ideally, the repeated dose should not be administrated until the blood pressure comes back to baseline Therefore, the repeated dose interval should be at least 90 minutes

Time (min)	Parameters		Change from pre-dose	SE	N
20	SysBP (mm Hg)	Sitting	-9 6	1 9	44
		Standing	-10 8	2 8	41
	DiaBP (mm Hg)	Sitting	-5	1 2	44
		Standing	-5 3	1 4	41
40	SysBP (mm Hg)	Sitting	-9 1	2 4	44
		Standing	-8 6	2 4	42
	DiaBP (mm Hg)	Sitting	-3 6	1 4	44
		Standing	-5 5	1 4	42
90	SysBP (mm Hg)	Sitting	-4 7	1 5	44
		Standing	-4 9	2	42
	DiaBP (mm Hg)	Sitting	-1 6	1 1	44
		Standing	-3 3	1 3	42

4 Should renal impairment patients be dosed differently?

Since both effectiveness and adverse events are related to concentration, the starting dose of 2-mg for renally impaired patients would be equivalent to 3 mg (considering the 50% increase of C_{max} in renally impaired patients) To avoid adverse events, the starting dose for renally impaired patients is recommended to be 1 mg For the same reason, the starting dose for hepatically impaired patients is recommended to be 1 5 mg

Appendix III Individual study synopsis

1 Study 090059 (APOM-0003-DM) Volume P7 16

Study title: *In Vitro* Metabolism of Apomorphine by Human Liver Microsomes

Investigator: [redacted]

Study period not provided, Report date 10/10/2000

Study Objectives The objective was to determine the *in vitro* metabolism of apomorphine and identify the cytochrome P450 enzymes involved

Study Design Preliminary evaluations were conducted including assessment of the stability of apomorphine and incubations with human liver microsomes to observe metabolite formation prior to validation of the HPLC method After the HPLC method for metabolite detection was validated, apomorphine was incubated with a pool of human liver microsomes to assess proportionality of the metabolite formation with respect to incubation time and microsomal protein concentration These results were used to design experiments to determine the kinetic constants, Km and Vmax Additional experiments were then undertaken to identify the CYP450 enzymes involved in apomorphine metabolism Combinations of the following three experimental approaches were taken

1 Correlation analysis between the rate of apomorphine metabolite formation and the activity of the major cytochrome P450 enzymes in a panel of human liver microsomes

2 An evaluation of the effects of known chemical inhibitors or specific inhibitory antibodies against selected cytochrome P450 enzymes on the metabolism of apomorphine by human liver microsomes

3 Metabolism of apomorphine by individual recombinant human cytochrome P450 enzymes (cDNA-expressed human P450s)

Results

Assay performance

The HPLC method was used and the validation results are shown in the following table

LLQ	Linear range	Accuracy	Between day precision	QC accuracy	Freeze/thaw stability
[redacted]	[redacted] µM				[redacted]

The assay validation is acceptable

In vitro metabolism of apomorphine

In the presence of NADPH, apomorphine was metabolized *in vitro* by liver microsomes to a metabolite (M1) Based on the literature, this metabolite was suspected to be the N-demethylated metabolite, norapomorphine, however, the actual structure of M1 has not been confirmed Percent of M1 formed in incubations of apomorphine at 8 8, 26 4, and 88 0 μM were 0 8, 0 9, and 1 0%, respectively

The kinetic constants for M1 were determined as shown in the following table

Parameters	Value
Vmax (pmol/mg/min)	21±0 4
Km (μM)	89±4
Vmax/Km (μL/mg/min)	0 23

Since the Km value of M1 was rather high and the objective of the study was to study the metabolism of apomorphine under pharmacologically relevant conditions, all subsequent experiments for the determination of the CYP450 involved were carried out at the lowest substrate concentration at which metabolite formation could be quantified (26 4 μM) The following table shows that M1 formation correlated modestly with CYP2B6, CYP2C8 and CYP3A4/5

Enzyme	Marker activity	Correlation coefficient (r)
CYP1A2	7-Ethoxyresorufin *O*-dealkylation	-0 209
CYP2A6	Coumarin 7-hydroxylation	0 154
CYP2B6	*S*-Mephenytoin *N*-demethylation	**0.812**
CYP2C8	Paclitaxel 6α-hydroxylation	0 737
CYP2C9	Diclofenac 4′-hydroxylation	0 409
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	0 310
CYP2D6	Dextromethorphan *O*-demethylation	0 267
CYP2E1	Chlorzoxazone 6-hydroxylation	0 207
CYP3A4/5	Testosterone 6β-hydroxylation	0 733
CYP4A9/11	Lauric acid 12-hydroxylation	-0 094

Comments

- The confirmation of M1 to be norapomorphine should have been conducted
- NADPH-dependent metabolism of apomorphine by human liver microsomes appeared to be relatively minimal

2. Study – 990060. **Volume P7 16**

Study title *In Vitro* Evaluation of Apomorphine HCl as an Inducer of Cytochrome P450 and UDP-glucuronosyltransferase Expression in Human Hepatocytes

Investigator [redacted]

Study period not provided Report date 11/22/2000

Study Objectives The objective was to evaluate the effect of apomorphine HCl on the expression of liver microsomal cytochrome P450 and UDP-glucuronosyltransferase enzymes in primary cultures of human hepatocytes, with the aim of ascertaining the potential of apomorphine to cause drug interactions

Study Design Cultures of human hepatocytes were treated with 8 8, 44 or 88 µM apomorphine HCl twice daily, or various positive controls once daily for three days Approximately 24 hr after the final treatment, microsomes were prepared, and the expression of CYP was determined by measuring the marker activities as shown in the following table

CYP expression	Marker activities
CYP1A2	7-ethoxyresorufin O-dealkylation
CYP2B6	S-mephenytoin N-demethylation
CYP2C9	diclofenac 4'-hydroxylation
CYP2C19	S-mephenytoin diclofenac 4'-hydroxylation
CYP2E1	chlorzoxazone 6-hydroxylation
CYP3A4/5	testosterone 6β-hydroxylation
UGT1A6/2B8	4-methylumbelliferone glucuronosyltransferation

Results Under the conditions examined, treatment of cultured human hepatocytes twice daily for three days with apomorphine HCl produced the results shown in the following table

Treatment	Enzyme Activity (pmol/ mg protein/ min)						
	7-Ethoxyresorufin O-dealkylation (CYP1A2)	S-Mephenytoin N-demethylation (CYP2B6)	Diclofenac 4'-hydroxylation (CYP2C9)	S-Mephenytoin 4' hydroxylation (CYP2C19)	Chlorzoxazone 6-hydroxylation (CYP2E1)	Testosterone 6β-hydroxylation (CYP3A4)	4-MU glucuronidation (UGT 1A6 and 2B6) [b]
Metabisulfite Sodium	0.621 ± 0.200	7.99 ± 5 07	325 ± 169	7.66 ± 10.96	228 ± 115	1330 ± 770	36 1 ± 12 2
8.8 µM Apomorphine	1 81 ± 0 82	11.3 ± 8.8	422 ± 201	9.53 ± 15 66	310 ± 118 *	2320 ± 860 *	43 7 ± 12.8
	(2.9)	(1 4)	(1.3)	(1 2)	(1 4)	(1 7)	(1.2)
44 µM Apomorphine	2.02 ± 0.83	16 6 ± 7 7 *	313 ± 139	10.5 ± 18.6	301 ± 126 *	2290 ± 960 *	46.0 ± 15 8
	(3 3)	(2.1)	(0 96)	(1.4)	(1 3)	(1 7)	(1 3)
88 µM Apomorphine	4.02 ± 2 73 *	9.46 ± 7.21	312 ± 212	9.47 ± 16.61	257 ± 126	1430 ± 720	41.0 ± 12 0
	(6.5)	(1.2)	(0.96)	(1 2)	(1 1)	(1 1)	(1 1)
Dimethyl sulfoxide	1 61 ± 0 45	13 1 ± 11 1	484 ± 291	8.54 ± 14 82	644 ± 361	1890 ± 860	35.2 ± 7 9
β-Naphthoflavone	31.3 ± 15 3 *	14 0 ± 10.3	591 ± 113	13 8 ± 13.2	710 ± 330	775 ± 415	43 4 ± 13 7
	(19)	(1 1)	(1 2)	(1.6)	(1 1)	(0 41)	(1.2)
Phenobarbital [a]	3 04 ± 1.37	60 4 ± 42.8 *	689 ± 287	17 3 ± 18 8	930 ± 293 *	5690 ± 2040 *	41.0 ± 13 7
	(1.9)	(4 6)	(1 4)	(2.0)	(1 4)	(3.0)	(1.2)
Rifampin	4 77 ± 0.73	89.0 ± 19 8 *	1170 ± 280 *	68 8 ± 71.2 *	1130 ± 180 *	6700 ± 3510 *	46.5 ± 10.3
	(3 0)	(6.8)	(2.4)	(8 1)	(1 8)	(3.5)	(1 3)
Saline	1 05 ± 0.53	5 74 ± 3 81	301 ± 154	4 49 ± 6.78	187 ± 77	1270 ± 570	30 1 ± 7 2
Isoniazid	1.05 ± 0.34	3.20 ± 1.50	593 ± 660	2.44 ± 3 14	424 ± 182 *	906 ± 355 *	28.3 ± 7 3
	(1 0)	(0.56)	(2.0)	(0.54)	(2.3)	(0 71)	(0 94)

[a] Values are the mean ± standard deviation of five of human hepatocyte preparations: H226, H228, H230, H231 H232.
Values in parenthesis refer to the fold increase over control rounded to 2 significant figures.
[b] 4-MU: 4-Methylumbelliferone
Mean ± standard deviation are expressed in nmol/mg protein/min
* Significantly different from control according to Dunnett's test ($p < 0.05$).

It caused a modest increase in measuring 7-ethoxyresorufin O-dealkylase (6 5-fold), S-mephenytoin N-demethylase (2 1-fold), chlorzoxazone 6-hydroxylase (1 4-fold) and testosterone 6β-hydroxylase (1 7-fold) activities, which are markers for CYP1A2, CYP2B6, CYP2El and CYP3A4/5 activities, respectively

Comments

- The data suggest that apomorphine HCl may be a weak inducer of CYP1A2, CYP2B6, CYP2El, and CYP3A4/5 enzymes in human hepatocytes
- Apomorphine HCl concentrations used in this induction study (8 8 to 88 μM) were significantly greater than those at expected therapeutic plasma levels (approximately 0 04 μM)
- Although no concentrations close to therapeutic plasma levels (approximately 0 04 μM) were used, significant induction is not expected

3 Study — 990058 **Volume P7 16**

Study title Evaluation of Apomorphine as an Inhibitor of Human Cytochrome P450 Enzymes *In Vitro*
Investigator ———

Study period not provided Report date 12/19/2000

Study Objectives The objective was to evaluate the ability of apomorphine HCl to inhibit the major P450 enzymes in human liver microsomes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5), with the aim of ascertaining the potential of apomorphine to inhibit the metabolism of other drugs

Study Design Apomorphine HCl was evaluated as a direct-acting (metabolism "independent") reversible inhibitor of P450 activity Human liver microsomes from a pool of 9 individuals were incubated with marker substrates in the presence or absence of apomorphine HCl at concentrations ranging from 0 088 to 88 µM The concentrations of marker substrates were approximately equal to 1/2, 1, 2, and 4 times their Km with the exception of CYP3A4/5 at 1/3, 1/2, 1, and 2 times its Km due to solubility constraints The CYP enzymes evaluated are shown in the following table

CYP enzymes	Activities
CYP1A2	7-ethoxyresorufin O-dealkylation
CYP2C9	diclofenac 4'-hydroxylation
CYP2C19	S-mephenytoin diclofenac 4'-hydroxylation
CYP2D6	dextromethorphan O-demethylation
CYP2E1	chlorzoxazone 6-hydroxylation
CYP3A4/5	testosterone 6β-hydroxylation

In addition, apomorphine HCl was evaluated for its ability to function as a metabolism-dependent (mechanism-based) reversible or "irreversible" inhibitor, in which case, apomorphine HCl was pre-incubated with human liver microsomes and NADPH for 15 minutes to allow for the formation of metabolites that could inhibit cytochrome P450 Wherever possible, known metabolism-"independent" or metabolism-dependent inhibitors of P450 enzymes were included as positive controls

Results The summary of the results of this study is shown in the following table As a direct acting (metabolism-"independent") reversible inhibitor, 1) apomorphine appears to be a competitive inhibitor of CYP2C9 with a Ki value of 370 µM, 2) apomorphine appears to be a mixed inhibitor of CYP2C19, CYP2E1, CYP1A2 and CYP2D6 with Ki values of 440, 290, 55, and 50 µM, respectively, 3) apomorphine appears to be a non-competitive inhibitor of CYP3A4/5 with a Ki value of 33 µM The rank order of Ki values for the inhibition of the enzymes listed above is as follows CYP3A4/5 < CYP2D6 < CYPIA2 < CYP2El < CYP2C9 < CYP2CI9

		Metabolism-independent inhibition				Metabolism-dependent inhibition	
Enzyme	P450 Activity	Ki (μmol/L)	[I]/Ki (μmol/L)	I[b]	I unbound[d]	Reversible	Irreversible
CYP1A2	7 Ethoxyresorufin *O*-dealkylase	55 ± 12 [MI]	0 000645	0 0645%	0 000645%	Little or no inhibition	Little or no inhibition
CYP2C9	Diclofenac 4 -hydroxylase	370 ± 70 [CI]	0 000096	0 00959%	0 000096%	Little or no inhibition	Little or no inhibition
CYP2C19	*S*-Mephenytoin 4 -hydroxylase	440 ± 170 [MI]	0 000081	0.00807%	0 000081%	Little or no inhibition	Little or no inhibition
CYP2D6	Dextromethorphan *O*-demethylase	50 ± 9 [MI]	0 000710	0.0710%	0.000710%	Little or no inhibition	Little or no inhibition
CYP2E1	Chlorzoxazone 6-hydroxylase	290 ± 40 [MI]	0 000122	0 0122%	0 000122%	Little or no inhibition	Weak inhibition
CYP3A4/5	Testosterone 6β-hydroxylase	33 ± 1 [NC]	0 001076	0.108%	0 001076%	Little or no inhibition	Little or no inhibition

Inhibition constants (Ki values) were calculated with the software program GraFit (version 4 0 Erithacus Software Ltd., London U K.) with simple weighting. Values are shown ± standard errors, which were calculated by GraFit using a [illegible] method.

[b] I = estimated plasma concentration of apomorphine in humans 0 0355 μM.
I = predicted fractional inhibition *in vivo* based on the maximum human plasma concentration of 0 0355 μM

[d] $I_{unbound}$ = predicted fractional inhibition *in vivo* based on greater than 99% protein binding.

MI Mixed inhibition CI Competitive inhibition NC Noncompetitive inhibition
Metabolism-dependent inhibition
Little or no inhibition 0 – 10% Inhibition Weak inhibition 11 – 20% Inhibition
Moderate inhibition 21 – 40% Inhibition Marked inhibition > 40% Inhibition

With the possible exception of CYP2El, where apomorphine has a weak capacity to act as an irreversible metabolism-dependent inhibitor, apomorphine has little or no capacity to function as a reversible or "irreversible" metabolism-dependent inhibitor of any of the other P450 enzymes examined From these results and the estimated plasma concentration of apomorphine (10 8 ng/mL or 0 0355 μM) following the administration of a therapeutic dose (3 mg), the fractional inhibition of CYP3A4/5, the enzyme most potently inhibited by apomorphine, was calculated to be 0 108%

Comments

This study suggests that apomorphine would not be expected to cause drug interactions with drugs that are metabolized by the P450 enzymes evaluated in this study

4 Study APOM-0083 **Volume P7 1-P7 3**

Study title A Phase I, Open-Label, Sequential Dose Ranging Study of Apomorphine HCl in Healthy Volunteers

Investigator [redacted]

Study period Dosing dates were January 14, 2001, January 15, 2001, and January 20, 2001 for Group A, Group B, and Group C, respectively Samples were assayed in the Bioanalytical Department of Mylan Pharmaceuticals Inc , from the period of May 29, 2001 to June 13, 2001

Study Objectives The objective was to determine the pharmacokinetic profiles and tolerance for single doses of subcutaneous apomorphine HCl (1, 2, and 3 mg) injection in healthy adult male and female volunteers

Study Design This is an open-label, three-group, one period study Nine healthy, non-smoking, male and female subjects between the ages of 20 and 41 participated in this study The subjects were dosed in three groups, with three subjects in each Group (A B, and C) as shown in the following table

Group	N	Dose (mg)	Regimen	Comedication
A	3	1	Single SC	Tigan 250mg tid from 3 days before dosing to 6 h after dosing
B	3	2	Single SC	
C	3	3	Single SC	

Subjects were housed from the evening prior to dosing until 6 hours after dosing After a supervised overnight fast (at least 10 hours) each subject received a single subcutaneous injection of the assigned formulation according to the dosing schedule Subjects received a standard meal 4 hours post-dose, water was permitted at all times

Serial blood samples, 10 mL (1 x 10 mL), were collected before dosing, and at 0 17, 0 33, 0 50, 0 75, 1 0, 1 5, 2 0, 3 0, 4 0, and 6 0 hours post-dose Due to the sensitivity of apomorphine to photodegradation, blood samples were collected and processed under conditions minimizing light exposure

Results

Assay performance

The assay used [redacted]
Its performance is summarized in the following tables

Linearity	LOQ	Recovery %	Accuracy (%error)	precision (%CV)

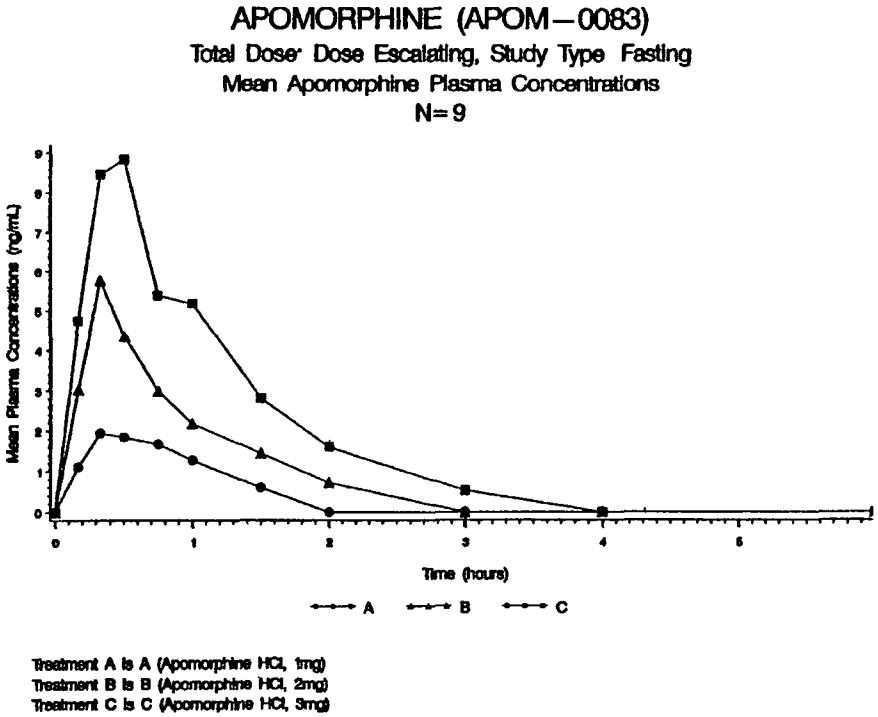
		Low	High	Internal standard	Intra-day	Inter-day	Intra-day	Inter-day

Stability				Stock solution stability			
Room temp	Freeze-thaw	-20°C	Post extraction	Apomorphine		Internal standard	
				4°C	Room	4°C	Room

The pre-study validation had an accuracy (%error) of –3 2 to –7 2% and precision (%CV) of 1 6 to 3 9% The in-study validation had an accuracy (%error) of –3 1 to –7 3% and precision (%CV) of 2 3 to 8 0% Based on current standard, the assay is acceptable

Pharmacokinetics

Pharmacokinetic parameters were derived from plasma apomorphine concentration time curves as shown in the following figure



The pharmacokinetic parameters for apomorphine were calculated using noncompartmental techniques Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC) Mean (% CV) single-dose pharmacokinetic parameters of apomorphine HCl are summarized in Table below

MEAN (%CV) APOMORPHINE HCL PHARMACOKINETIC PARAMETERS IN NINE HEALTHY MALE AND FEMALE SUBJECTS FOLLOWING A SINGLE, SUBCUTANEOUS INJECTION OF THE ASSIGNED TREATMENT UNDER FASTING CONDITIONS.

PROTOCOL NUMBER APOM-0083

Treatment	Parameter							
	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	CL (L/hr)	VD (L)
A 1 mg n=3	1 856 (27.51)	2.777 (20 01)	2.060 (13 62)	0 470 (51.59)	1 026 (23 14)	0 703 (25 80)	369.5 (19 17)	373 8 (29 99)
B: 2 mg n=3	4 746 (19.40)	6.046 (16.60)	5 778 (6.59)	0.330 (0 000)	0 861 (15 89)	0 820 (16 50)	337.5 (17.85)	393 4 (12.94)
C 3 mg n=3	10 06 (22.60)	10 90 (20 02)	10.35 (27 17)	0.443 (22.14)	1 039 (15 42)	0 680 (16.70)	282.3 (19.06)	276.3 (24 14)
Source: Study Report APOM-0083 Section 14 1 Attachment 1 part 3 and part 5								

Mean apomorphine CPEAK, AUCL and AUCI values increased with the ascending dose levels Mean apomorphine TPEAK values were similar among the increasing doses and ranged from 20 to 45 minutes There were no significant treatment differences in the non dose-normalized parameters for the between treatment comparisons for TPEAK, HALF , KEL, CL, and VD following a single subcutaneous injection of apomorphine HCl Additionally, there were no significant treatment differences between the 1 mg dose versus the 2 mg dose and the 2 mg dose versus 3 mg dose in the dose-normalized treatment comparisons of AUCLN, AUCIN, CPEAKN, and their log transformations However, statistically significant differences were detected between the 1 mg dose and 3 mg dose for AUCLN, CPEAKN and their log transformations The parameters, AUCIN and LNAUCIN, were similar between the 1 mg dose and the 3 mg dose

Adverse events

All nine subjects completed this study Sixteen adverse events were experienced in seven subjects during the study Adverse events included nausea (5), drowsiness (6), hot flashes (1), sweating (1), clamminess (1), facial twitching (1), and vomiting (1) One adverse event (clamminess) was listed as definitely related to drug administration and all the other adverse events were classified as probably related to drug administration

Comments

1 The pharmacokinetic parameters obtained from this study were similar to those in literature reports

2 No definitive conclusion in regards to linearity can be made from the study

5. Study APOM-0053 **Volume P7 4-7.7**

Study title A Phase I, Open-Label Study Comparing the Single-Dose Pharmacokinetics of Apomorphine HCl in Volunteers with Impaired Hepatic Function to Healthy Volunteers

Investigator [ ]

Study period Subjects were dosed between January 17,2001 and April 23, 2001 Samples were assayed in the Bioanalytical Department of Mylan Pharmaceuticals Inc , from the period of June 6, 2001 to June 19, 2001

Study Objectives The objective was to determine the effect of hepatic impairment on the single-dose pharmacokinetic parameters of apomorphine HCl

Study Design This is an open-label, single-dose, two-group, one-period study The effects of hepatic impairment were assessed by comparing the single-dose pharmacokinetic parameters of apomorphine in moderately impaired hepatic subjects to healthy individuals with comparable ages, weights, smoking habits, ethnic background and gender Sixteen volunteers participated in the study as shown in the following table

Group	N	Child-Pugh classification	Dosing regimen	Comedication
Healthy	8		3 mg single SC	Tigan 250mg tid from 3 days before dosing to 6 h after dosing
Hepatic impaired	7	B	3 mg single SC	
	1	C	3 mg single SC	

Subjects were housed from the evening three days prior to dosing until 9 hours after dosing Breakfast, lunch and dinner were provided at the appropriate times during the 72-hour confinement period prior to apomorphine dosing After a supervised overnight fast (at least 10 hours) each subject received a single 3-mg subcutaneous injection of apomorphine into the wall of the abdomen Subjects received a standard meal 4 hours post-dose, water was permitted at all times

Serial blood samples, 10 mL (1 x 10 mL), were collected in EDTA tubes before dosing, and at 0 17, 0 33, 0 50, 0 75, 1 0, 1 5, 2 0, 3 0, 4 0, 6 0 and 9 0 hours post-dose Due to the sensitivity of apomorphine to photodegradation, blood samples were collected and processed under conditions minimizing light exposure

Pharmacokinetic parameters for apomorphine were calculated using noncompartmental techniques Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC)

Results

Assay performance

The assay used

] Its performance is summarized in the following tables

Linearity (ng/mL)	LOQ (ng/mL)	Recovery %			Accuracy (%error)		precision (%CV)	
		Low	High	Internal standard	Intra-day	Inter-day	Intra-day	Inter-day

Stability				Stock solution stability			
Room temp	Freeze-thaw	-20°C	Post extraction	Apomorphine		Internal standard	
				4°C	Room	4°C	Room

Based on current standard, the assay is acceptable

Pharmacokinetics

Pharmacokinetic parameters were derived from plasma apomorphine concentration time curves Mean (% CV) single-dose pharmacokinetic parameters of apomorphine are summarized in the following Table

MEAN (%CV) APOMORPHINE HCL PHARMACOKINETIC PARAMETERS IN HEALTHY AND HEPATICALLY IMPAIRED SUBJECTS FOLLOWING A SINGLE, 3-MG SUBCUTANEOUS INJECTION OF APOMORPHINE HCL UNDER FASTING CONDITIONS.

PROTOCOL NUMBER APOM-0053

Treatment	Parameter							
	AUCL* (ng x hr/mL)	AUCI# (ng x hr/mL)	CPEAK* (ng/mL)	TPEAK* (hr)	KEL# (hr^{-1})	HALF# (hr)	CL*§ (L/hr)	VD*¶ (L)
A = Healthy	5 283 (29.20)	6.971 (16 74)	3.854 (44 92)	0 645 (42.04)	0 701 (19 95)	1 029 (23 09)	531 9 (50 11)	656 9 (29.50)
B = Hepatic Impaired	5.936 (38 64)	7 833 (28.48)	4 848 (44.50)	0 604 (52.59)	0 828 (45 28)	0.969 (36.75)	501.5 (55 39)	547 7 (33 88)
P value (hepatic vs. healthy)	0 672^	0.223^	0.441^	0 816	0.917	0.952	0 840	0 445

* n = 8 subjects for this parameter per treatment
\# n = 7 subjects for this parameter per treatment
^ Used Natural Log Transformed Parameter
§ Apparent clearance
¶ Apparent volume of distribution

Source: Study Report APOM-0053 Section 14 1 – Attachment 1 part 3, and Section 14.2 – Attachment 2 part 1

The severe and moderately hepatic impaired groups were treated as one group for all statistical analyses The applicant concluded that there was no statistically significant difference and therefore, dosing adjustments are not necessary for either mild or moderate hepatic impaired individuals prescribed apomorphine HCl for subcutaneous injection However, the reviewer calculated the 90% confidence interval of the ratios of C_{max} and AUC between healthy individuals and moderately hepatic impaired individuals following a subcutaneous injection of apomorphine The results indicated the ratios fall outside the 80-125% range as shown in the following table

Parameters	Ratio	90% CI

C_{max}	124 8	85 6-181 9
AUC	109 4	78 4-152 6
AUC	109 7	87 5-137 7

Adverse events

Sixteen subjects were entered into and completed this study Seven adverse events were experienced in four subjects during the study Adverse events included nausea (2), drowsiness (1), lightheadedness (1), bradycardia (1), and hypotension (2) All adverse events were listed as mild in severity, with the exception of two severe adverse events experienced by Subject 015 This healthy male volunteer experienced a transient, but clinically significant bradycardia and hypotension that required the administration of intravenous fluids and elevation of the subject's legs Both events began 30 minutes after receiving the study medication and resolved after several minutes All adverse events were classified as possibly or probably related to drug administration

Comments

1 The 90% confidence interval of the ratios of C_{max} and AUC between healthy individuals and moderately hepatically impaired individuals fell outside the 80-125% range

2 In order to make a label claim for severe hepatically impaired individuals, further studies would need to be performed

6 Study APOM-0058 Volume P7 8-7 11

Study title A Phase I, Open-Label Study Comparing the Single-Dose Pharmacokinetics of Apomorphine HCl in Volunteers with Impaired Renal Function to Healthy Volunteers

Investigator [ ]

Study period The first subject was screened on March 9, 2001 and received apomorphine on March 19, 2001 and the last subject was screened on October 19, 2001 and received apomorphine on October 26, 2001 Samples were assayed in the Bioanalytical Department of Mylan Pharmaceuticals Inc , from the period of December 17, 2001 to January 4, 2002

Study Objectives The objective was to determine the effect of renal impairment on the single-dose pharmacokinetic parameters of apomorphine HCl

Study Design This is an open-label, single-dose, two-group, one-period study The effects of renal impairment were assessed by comparing the single-dose pharmacokinetic parameters of apomorphine in moderately impaired renal subjects to healthy individuals with comparable ages, weights, ethnic background, smoking habits, and gender Sixteen volunteers participated in the study as shown in the following table

Group	N	Renal impairment	Dosing regimen	Comedication
Healthy	4	None	3 mg single SC	Tigan 250mg tid from 3 days before dosing to 6 h after dosing
	4		2 mg single SC	
Renal impaired	6	Moderate	3 mg single SC	
	1		2 mg single SC	
	1	Severe	3 mg single SC	

Renal impairment was assessed by an estimation of a subject's creatinine clearance using the Cockroft-Gault equation

Subjects were housed from the evening three days prior to dosing until 9 hours after dosing Breakfast, lunch and dinner were provided at the appropriate times during the 72-hour confinement period prior to apomorphine dosing Food was not allowed 2 hours prior to apomorphine dosing and until 1 hour following a single subcutaneous injection of apomorphine into the wall of the abdomen Subjects received a standard meal 4 hours post-apomorphine dosing, and water was allowed at all times throughout the study Initially, subjects received a 3 mg dose of apomorphine However, following a serious adverse event experienced by Subject 015, the dosing level of apomorphine was reduced to 2 mg for the remainder of the study Therefore, 7 renally impaired and 4 healthy matched volunteers received the 3 mg dose of apomorphine and 1 renally impaired and 4 healthy volunteers received the 2 mg dosing level of apomorphine

Serial blood samples, 10 mL (1 x 10 mL), were collected in EDTA tubes before dosing, and at 0 17, 0 33, 0 50, 0 75, 1 0, 1 5, 2 0, 3 0, 4 0, 6 0 and 9 0 hours post-dose Due to the sensitivity of

apomorphine to photodegradation, blood samples were collected and processed under conditions minimizing light exposure

Pharmacokinetic parameters for apomorphine were calculated using noncompartmental techniques Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC)

Results

Assay performance

The assay used

Its performance is summarized in the following tables

Linearity (ng/mL)	LOQ (ng/mL)	Recovery %			Accuracy (%error)		precision (%CV)	
		Low	High	Internal standard	Intra-day	Inter-day	Intra-day	Inter-day

Stability				Stock solution stability			
Room temp	Freeze-thaw	-15°C	Post extraction	Apomorphine		Internal standard	
				4°C	Room	4°C	Room

Based on current standard, the assay is acceptable

Pharmacokinetics

Pharmacokinetic parameters were derived from plasma apomorphine concentration time curves The dose normalized mean (%CV) single-dose pharmacokinetic parameters of apomorphine are summarized in the following Table

MEAN (%CV) APOMORPHINE HCL PHARMACOKINETIC PARAMETERS IN EIGHT (8) HEALTHY AND EIGHT (8) RENALLY IMPAIRED SUBJECTS FOLLOWING A SINGLE SUBCUTANEOUS INJECTION OF APOMORPHINE HCL UNDER FASTING CONDITIONS.

PROTOCOL NUMBER APOM-0058

Treatment	Parameter							
	nAUCL† (ng x hr/mL)	nAUCI† (ng x hr/mL)	nCPEAK† (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	CL§ (L/hr)	VD¶ (L)
A = Healthy	6.106 (23)	7 723 (26)	4.967 (38)	0.560 (32)	0 848 (36)	0 941 (46)	422.2 (36)	523 8 (28)
B = Renally Impaired	7 895 (32)	8.982 (31)	7 777 (45)	0 490 (58)	0 990 (45)	0 828 (40)	361 7 (29)	411 4 (40)
P value (renal vs. healthy)	0.137^	0 375^	0 119^	0.550	0 389	0.559	0.381	0 152

† This parameter has been normalized to 3 mg of apomorphine administered
^ Used Natural Log Transformed Parameter
§ Apparent clearance
¶ Apparent volume of distribution

Source: Study Report APOM-0058 Section 14 1 – Attachment 1 part 3, and Section 14.2 – Attachment 2, part 1

The severely impaired individual's pharmacokinetic data was pooled with the moderate impaired group's data for subsequent statistical analysis

The applicant concluded that there was no statistically significant difference and therefore, dosing adjustments are not necessary for either mild or moderate renal impaired individuals prescribed apomorphine HCl for subcutaneous injection However, the reviewer calculated the 90% confidence interval of the ratios of C_{max} and AUC between healthy individuals and moderately renal impaired individuals following a subcutaneous injection of apomorphine The results indicated the ratios fall outside the ranges suggested by the relevant Guidance as shown in the following table

Parameters	Ratio	90% CI
C_{max}	150 06	101 78-221 24
AUC_{last}	127 80	98 26-166 21
AUC_{inf}	115 88	88 53- 151 67

Adverse events

Sixteen subjects were entered and completed this study Twenty-two adverse events were experienced in eleven subjects during the study Five adverse events were classified as definitely not study drug related, two were possibly study drug related, nine were probably related to apomorphine administration, and six were definitely related to the administration of apomorphine There were fifteen mild adverse events, six of moderate severity, and one severe adverse event The severe event involved a fainting episode, which was probably a result of a vasovagal reflex As a direct result of this adverse event, the dosage level of apomorphine was reduced from 3-mg to 2-mg for subsequently enrolled subjects Subjects who had already completed the study at the 3-mg level were not re-dosed at the 2-mg level

Comments

1 The 90% confidence interval of the ratios of C_{max} and AUC between healthy individuals and moderately renally impaired individuals fall outside the ranges suggested by the relevant Guidance

2 In order to make a label claim for severe renally impaired individuals, further studies would need to be performed

7 Study APOM-0073 **Volume P7 12-P7 15**

Study title A Phase II, Open-Label Pharmacokinetic (PK) and Pharmacodynamic (PD) Study Following Subcutaneous Administration of Apomorphine HCl to Patients with Idiopathic Parkinson's disease

Investigator [ ]

Study period The first subject was enrolled into the study on September 14, 2001, and the last subject completed all aspects of the study on December 11, 2001 Samples were assayed in the Bioanalytical Department of Mylan Pharmaceuticals Inc , from the period of November 30, 2001 to January 7, 2002

Study Objectives The primary objective of this study was to evaluate the pharmacokinetics and dose proportionality of apomorphine HCl in patients with idiopathic Parkinson's disease An attempt to identify the pharmacokinetic and pharmacodynamic relationships in apomorphine activity was also made In addition, the effects of repeated apomorphine doses on cardiac parameters (heart rate and blood pressure) were investigated

Study Design This is an open-label study Six idiopathic Parkinson's disease patients participated in this study Patients were accepted into this study if they were enrolled in Mylan's clinical APO401 study, had received active apomorphine treatment, and had established a clinically stable maintenance dose under APO401 for at least one month

This study consisted of a baseline visit followed by four treatment day visits as shown in the following table

Visits	Apomorphine dose	Dose frequency	Co-medication	PK sample	UPDRS sample	Webster step-second
Baseline	No apom	-				
Day 1	Titrated dose	Single	Not allowed	0, 0 17, 0 33, 0 75, 1 5, 2, 3, and 4 hours	0, 0 33, 0 75, 1 5, 2, 3, and 4 hours	Each PK blood collection sample
Day 2	+2mg or – 2mg	Single	Not allowed			
Day 3	+2mg or – 2mg	Single	Not allowed			
Day 4	Titrated dose	3 doses 1 5 h apart	Allowed	0, 20, 90, 110, 180, 200, and 270 min	Follow each PK sample	

After midnight on the evenings prior to the scheduled study dosing of apomorphine, no apomorphine was to be administered to the patient For Treatment Days 1 and 4, the patient's currently stable maintenance dose of apomorphine (their "titrated dose") from the APO401 study was utilized For Treatment Days 2 and 3, the patients received their titrated dose plus or minus 2 mg according to the randomization scheme If the patient's titrated dose was less than 4 mg, then the lowest dose administered was 2 mg If the patient's titrated dose was greater than 8 mg, then the highest dose administered was 10 mg On Treatment Days 1, 2, and 3, only one dose of

apomorphine was administered for study purposes following the first "Off' state of the morning On these days, no additional apomorphine injections were given from midnight until four hours after study apomorphine administration Concomitant medication was not allowed to be administered from 1 5 hours prior to study apomorphine dosing until 2 hours after apomorphine administration On Treatment Day 4, patients received three doses of study apomorphine at 1 5 hour intervals beginning with the first "Off' of the morning Concomitant medications for Treatment Day 4 were allowed to be administered without regard to study apomorphine dosing or pharmacokinetic sampling times

On Treatment Days 1, 2, and 3, 5 mL (1 x 5 mL) serial blood samples were collected at the following times relative to dosing 0, 10, 20, and 45 minutes and 1 5, 2, 3, and 4 hours On Treatment Day 4, 5 mL (1 x 5 mL) serial blood samples were collected at the following times relative to the first apomorphine dose 0, 20, 90, 110, 180, 200, and 270 minutes The 0, 90, and 180 minute blood collections were obtained within 5 minutes of apomorphine-administration on Treatment Day 4 All serial blood samples were collected into tubes containing EDTA and immediately transferred to glass centrifuge tubes containing 20 to 30 mg of ascorbic acid to combat oxidative breakdown Due to the sensitivity of apomorphine to photodegradation, blood samples were collected and processed under conditions minimizing light exposure Plasma samples were stored in suitably labeled tubes at -20°C ± 5°C until analysis

The pharmacodynamic measurements taken during this study included a modified Webster Step-Seconds Test, the Unified Parkinson's Disease Rating Scale (UPDRS) assessment, dyskinesia assessment, and orthostatic "tilts" The Webster Step-Seconds Test was performed on each Treatment Day at each pharmacokinetic blood collection time period The UPDRS motor portion of the rating scale was performed prior to apomorphine dosing and at 20 and 45 minutes, and 1 5, 2, 3, and 4 hours post-dose on Treatment Days 1, 2, and 3 On Treatment Day 4, the UPDRS motor score evaluation occurred prior to study apomorphine dosing and following each pharmacokinetic blood collection The assessment of dyskinesia was performed at each blood collection time point on Treatment Days 1, 2, 3, and 4 In addition, orthostatic "tilts" (encompassing systolic and diastolic blood pressure measurements and pulse rate) and ECG measurements were performed prior to each dose of apomorphine HCl during the pharmacokinetic blood collection interval and following the collection of each 20 minute blood sample on Treatment Day 4

The pharmacokinetic parameters were derived from plasma apomorphine concentration time curves Single-dose pharmacokinetic parameters for each apomorphine dose the patient received were calculated using noncompartmental techniques Regression analyses were performed on AUCL, AUCI, and CPEAK versus apomorphine dose administered using the REG procedure of SAS Software (SAS Institute, Cary, NC) To assess for the presence of apomorphine accumulation following multiple apomorphine doses on Treatment Day 4, analysis of variance was performed using the GLM procedure of SAS Software The regression of the UPDRS motor score parameters on the administered dose of apomorphine and the regression of the modified Webster step second score parameters on apomorphine dose were also performed using the REG procedure of SAS software Blood pressure and pulse percent change relative to baseline values for both standing and sitting conditions along with the change from sitting to standing on

Treatment Day 4 also underwent regression analysis with correlation to the respective plasma concentration at 20 and 90 minutes post-apomorphine administration

Results

Assay performance·

The assay used [redacted]

Its performance is summarized in the following tables

Linearity (ng/mL)	LOQ (ng/mL)	Recovery %			Accuracy (%error)		precision (%CV)	
		Low	High	Internal standard	Intra-day	Inter-day	Intra-day	Inter-day

Stability				Stock solution stability			
Room temp	Freeze-thaw	-15°C	Post extraction	Apomorphine		Internal standard	
				4°C	Room	4°C	Room

Based on current standard, the assay is acceptable

Pharmacokinetics/pharmacodynamics

The pharmacokinetic parameters, the pharmacodynamic parameters for the UPDRS motor scores and the pharmacodynamic parameters for the modified Webster step-seconds test are shown in the following tables

SINGLE-DOSE APOMORPHINE HCL PHARMACOKINETIC PARAMETERS IN FIVE (5) IDIOPATHIC PARKINSON'S DISEASE PATIENTS TAKING THREE (3) DIFFERENT DOSES OF APOMORPHINE SUBCUTANEOUSLY (data deleted)[1]

PROTOCOL NUMBER APOM-0073

	Parameter							
	NAUCL[2]	NAUCI[2]	NCPEAK[3]	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	CL[4] (L/hr)	VD[5] (L)
Mean Parameter Value	4 643	4 960	4.867	0.227	0 988	0 720	225 0	230 4
Coefficient of Variation (%)	35 6	34 0	36 3	35 0	16 4	18 0	35 1	36.2
Range								

[1] Patient 21 s Treatment Day 2 deleted due to incomplete concentration profile Patient 11 s data deleted due to pre-dose apomorphine concentrations on Treatment Days 1 2, and 3

[2] Dose normalized parameter is expressed as (ng hr/mL)/ mg apomorphine

[3] Dose normalized parameter is expressed as (ng/mL)/ mg apomorphine

[4] Apparent clearance

[5] Apparent volume of distribution

Source: Study Report APOM-0073 Section 14.2 – Attachment 2, part 7

UPDRS-MOTOR SCORE PARAMETERS IN FIVE (5) IDIOPATHIC PARKINSON'S DISEASE PATIENTS ON TREATMENT DAYS 1, 2, AND 3 USING THREE (3) DIFFERENT DOSES OF APOMORPHINE HCl ADMINISTERED SUBCUTANEOUSLY[1]

PROTOCOL NUMBER APOM-0073

	Parameter			
	MBASE[2]	MAUCT[3]	MPEAK[4]	MTPEAK[5] (hour)
Mean Parameter Value	29	21.2	19	0.69
Coefficient of Variation (%)	42	68	74	72
Range				

[1] Patient 11 s data has been omitted from this summary
[2] MBASE = UPDRS – motor score baseline value.
[3] MAUCT = area under the UPDRS – motor score improvement curve from 0 to 2 hours post apomorphine administration.
[4] MPEAK = the largest improvement in UPDRS – motor score from baseline from 0 to 2 hours post apomorphine dosing. Calculated by subtracting the UPDRS – motor score at that time point from MBASE.
[5] MTPEAK = the time point at which MPEAK occurred relative to apomorphine administration

Source: Study Report APOM-0073 Section 14.2 – Attachment 2, part 10

MODIFIED WEBSTER STEP SECONDS SCORE PARAMETERS IN FIVE (5) IDIOPATHIC PARKINSON'S DISEASE PATIENTS ON TREATMENT DAYS 1 2, AND 3 USING THREE (3) DIFFERENT DOSES OF APOMORPHINE HCl ADMINISTERED SUBCUTANEOUSLY[1]

PROTOCOL NUMBER APOM-0073

	Parameter			
	SBASE[2]	SAUCT[3]	SPEAK[4]	STPEAK[5] (hour)
Mean Parameter Value	5048	5685	4929	0 82
Coefficient of Variation (%)	96	101	98	77
Range				

[1] Patient 11 s data has been omitted from this summary
[2] SBASE = modified Webster step-seconds baseline value.
[3] SAUCT = area under the modified Webster step-seconds improvement curve from 0 to 2 hours post apomorphine administration.
[4] SPEAK = the largest improvement in modified Webster step-seconds from baseline from 0 to 2 hours post apomorphine dosing. Calculated by subtracting the modified Webster step-seconds at that time point from SBASE.
[5] STPEAK = the time point at which SPEAK occurred relative to apomorphine administration.

Source: Study Report APOM-0073 Section 14.2 – Attachment 2, part 13.

The table below compares the pharmacokinetic parameters between this study and the literature report

Parameters	This study	Literature
AUCL (ng•hr/mL)	4 59	2 03-7 12
CPEAK (ng/mL/mg)	4 86	3 63-6 96
V (L)	218	203-276 6
CL (L/h)	223	228

The following table shows the results from AUCL, AUCI, and CPEAK parameter regression analyses

Dependent	Coefficient	R-square
AUCL	4 78	0 9161
AUCL	5 05	0 9181

CPEAK	5 17	0 8958

The results also indicate that apomorphine does not have a tendency to accumulate in patient's with idiopathic Parkinson's disease even after multiple 90-minute time interval administrations

In order to study the pharmacokinetic/pharmacodynamic relationship between plasma apomorphine concentrations and the UPDRS motor scores and modified Webster step-seconds scores, an inhibitory sigmoid Emax model and a stimulatory sigmoid Emax model were utilized, respectively Based upon the UPDRS motor scores and the modified Webster step second test scores the apomorphine plasma concentration that will elicit a 50% maximum pharmacodynamic improvement effect (EC50) in patients suffering from Parkinson's disease is 10 7 ng/mL and 5 3 ng/mL, respectively (See Pharmacometrics Review)

The percent change relative to baseline values for systolic and diastolic blood pressure along with pulse rates were poorly correlated to the plasma apomorphine concentrations at 20 and 90 minutes post dosing on Treatment Day 4 Even after multiple administrations of apomorphine within a 3-hour time frame, orthostasis was rarely observed

Adverse events

Seven (7) patients (2 females and 5 males) were enrolled into the study However, six patients were dosed and completed this study Thirty-seven adverse events were experienced in six patients during the study Thirty-three events (89%) were reported as mild in severity, while four events (11 %) were considered moderate in severity Twenty-eight (76%) of the reported or observed adverse events were considered to be drug-related (27 events deemed mild, with 1 event deemed moderate in severity by the investigator) The most common reported adverse events were yawning (9 of 37), somnolence (4 of 37), and flushing (4 of 37) There were three reports of dizziness, and two episodes each of nausea, dystonia, and hiccups

Comments

1 The study indicated that apomorphine possesses pharmacokinetic dose proportionality over the dosage interval studied (2 mg to 8 mg) in idiopathic Parkinson's disease patients

2 The pharmacokinetic values determined in this study were consistent with the previously reported literature values

3 This study indicated that apomorphine does not have a tendency to accumulate in patient's with idiopathic Parkinson's disease

4 The apparent clearance of 280 L/h, which is higher than hepatic blood flow, supports the existence of autooxidation of apomorphine as an elimination route However, the autooxidation could not account for the most apomorphine eliminated The major route of elimination is not obvious

8. Toxicology Study **Volume P2 2, P2 5-P2 6, P2 8-P2 10**

A study to evaluate potential toxicity of apomorphine when administered as combination of subcutaneous injections and oral administration of levodopa/cabidopa to rats for duration of 13 weeks was conducted The study demonstrated that apomorphine was absorbed and eliminated rapidly following the subcutaneous injection in rats There was no accumulation of apomorphine following a subcutaneous dose of 0 75 mg/kg apomorphine 4 times a day for 7 days when given as monotherapy or in combination of oral doses of L-dopa/carbidopa The concentrations at 15 minutes on day 7 were not statistically different between the treatment groups with or without the coadministration of levodopa/cabidopa

A similar study showed that a dosage level of 3 mg/kg/day apomorphine alone and dosage levels of 0 3, 1 and 3 mg/kg/day apomorphine in combination with levodopa/carbidopa produced known pharmacological (behavioral) effects in males and females In addition, a dosage level of 3 mg/kg/day apomorphine alone and in combination with levodopa/carbidopa produced significantly decreased body weights and food consumption in males only With the exception of predicted reaction, the no-observed-adverse-effect level (NOAEL) for rats in this study following qid administration for 13 weeks was considered to be 1 mg/kg/day apomorphine in combination with 40/10 mg/kg/day levodopa/cabidopa The 14 day study showed similar results

Note The pharmacologist Dr Paul Roney mentioned that the L-dopa doses used here are less than the therapeutic human doses

9 Study APOM-0222 **Volume P15 5-P15 8**

Study title

A Phase I, Open-label, Two-way Crossover Study in Healthy Volunteers to Compare the Pharmacokinetics of Apomorphine HCl Formulated with Benzyl Alcohol and Delivered from a Cartridge Using a Pen Device Versus Apomorphine HCl Formulated without Benzyl Alcohol, Delivered Using a Syringe Manually Filled from an Ampoule (MYLAN Protocol # APOM-0222)

Investigator

Clinical [ ]

Analytical Mylan Pharmaceuticals Inc , Bioanalytical Department 3711 Collins Ferry Road, Morgantown, WV 26505

Study period

Clinical Phases Phase 1 July 23, 2002- July 27, 2002 Phase 2 July 30,2002 -August 3,2002
Analytical Phase September 16, 2002 -September 26, 2002

Study Objectives

- To compare the pharmacokinetics (PK) of apomorphine HCl formulated with benzyl alcohol and delivered from a cartridge using a pen device to the PK of apomorphine HCl formulated without benzyl alcohol and delivered using a syringe manually filled from an ampoule

- To assess the instructions for use (IFU) for the apomorphine delivery device (pen/cartridge)

Study Design

This is a randomized, open-label, two-period crossover study Pharmacokinetic parameters were derived from plasma apomorphine concentration-time curves for each formulation Eleven (11) healthy, non-smoking, male and female subjects between the ages of 19 and 44 completed the study

Treatment	Contents	Device
A	Apomorphine HCl Injection containing 0 05% benzyl alcohol, 1 x 2 mg	pen/cartridge
B	no benzyl alcohol, 1 x 2 mg	syringe filled from an ampoule

Apomorphine treatments (A or B) were separated by seven (7) days To decrease the likelihood of nausea and vomiting associated with apomorphine, all volunteers received 250 mg (1 x 250 mg capsule) of the anti-emetic drug trimethobenzamide (Tigan) three to four times a day starting three days prior to dosing until six (6) hours after apomorphine HCl dose administration for each

dosing phase Subjects received a standard meal 4 hours post-dose, water was permitted at all times The Tigan dose (250 mg) was increased from three times/day to four times/day on Days 9 and 10, in order to reduce the incidence of emesis and nausea during the second dosing phase (Day 11)

Serial blood samples (1 x 10 mL) were collected prior to dosing (0 hr) and at 10, 20, 30, and 45 minutes, and 1, 1 5, 2, 3 and 4 hours after dose administration Single-dose pharmacokinetic parameters for apomorphine were calculated using non-compartmental techniques Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC)

Results

Assay performance

The assay used

Its performance is summarized in the following tables

Linearity (ng/mL)	LOQ (ng/mL)	Recovery %			Accuracy (%error)		precision (%CV)	
		Low	High	Internal standard	Intra-day	Inter-day	Intra-day	Inter-day

Stability				Stock solution stability			
Room temp	Freeze-thaw	-15°C	Post extraction	Apomorphine		Internal standard	
				4°C	Room	4°C	Room

Based on current standard, the assay is acceptable

Pharmacokinetics

Mean (%CV) single-dose pharmacokinetic parameters of apomorphine HCl are summarized in the following Table

The 90% confidence intervals for the statistical comparison fell outside the acceptable bioequivalent range of 80-125% for the log transformed parameters LNAUCL, LNAUCI and LNCPEAK for treatment comparisons

Mean apomorphine CPEAK, AUCL, AUCI and CL/F values for the pen/cartridge (Treatment A) were estimated at 5 9 ng/mL, 5 08 (ng•hr)/mL, 5 9 (ng•hr)/mL and 344 L/hr respectively These values were substantially lower than the mean values for CPEAK, AUCL, AUCI and CL/F estimated for subjects receiving an ampoule/syringe injection of apomorphine HCl (Treatment B, 8 1 ng/mL, 6 9 (ng•hr)/ml, 7 9 (ng•hr)/mL and 260 L/hr, respectively)

MEAN (%CV) PHARMACOKINETIC PARAMETERS FOR APOMORPHINE IN ELEVEN (11) HEALTHY SUBJECTS FOLLOWING A SINGLE DOSE OF APOMORPHINE HCl (2 mg) ADMINISTERED BY SUBCUTANEOUS INJECTION (0 2 mL) PROTOCOL NUMBER APOM-0222				
Parameter	**Arithmetical Mean (%CV) A. Cartridge and Pen (w/BA)**	**Arithmetical Mean (%CV) B Ampoule and Syringe (w/o BA)**	**LSMEANS Ratio (A/B)***	**90% Confidence Interval****
AUCL ((ng•hr)/mL)	5 08 (20%)	6 87 (19%)	0 74	65% - 84%
AUCI ((ng•hr)/mL)	5 93 (15%)	7 94 (18%)	0 75	68% - 83%
CPEAK (ng/mL)	5 908 (28%)	8 111 (54%)	0 77	63% - 94%
TPEAK (hr)	0 35 (33%)	0 41 (49%)	—	—
KEL (1/hr)	1 07 (28%)	1 04 (32%)	—	—
HALF (hr)	0 71 (34%)	0 74 (35%)	—	—
Cl/F* (L/hr)**	344 (15%)	260 (19%)	—	—
Vd/F* (L)**	352 (43%)	269 (29%)	—	—

*Ratio (A/B) = $e^{[\text{LSMEAN of LNA} \quad \text{LSMEAN of LNB}]}$

**Used Natural Log Transformed Parameter

***Where Cl/F=Cl_F and Vd/F=Vd_F on the PK data output (14 1 - Attachment 1) and statistics (14 2 - Attachment 2)

Abbreviations w/BA-Formulated with benzyl alcohol (0 05% w/v), w/o BA-Formulated without benzyl alcohol

Source Section 14 1-Attachment 1, part 3 and part 5

Differences between Treatments A and B show that the extent of absorption (F) was lower following Treatment A (delivery with pen/cartridge), as compared to Treatment B (delivery with syringe/ampoule) CPEAK, AUCI, and AUCL values suggest that lower than expected drug concentrations may have been caused by incomplete priming of the pen/cartridge device It has been noted that the bubbles present in each cartridge is not displaced by the recommended single small volume purge (1 x 0 02 mL) as stated in the instructions for use (IFU) utilized in this study Inadequate priming of the pen/cartridge delivery system (Treatment A) may have led to inaccurate drug delivery, a lower than expected dose, and the appearance of a lower extent of absorption (e g , decreased bioavailability, F)

Adverse Events

Clinical laboratory testing, vital signs and ECG monitoring indicated no safety risk associated with subcutaneous dosing of apomorphine HCl at a dose level of 2 mg Eleven subjects were enrolled and completed this study Seventeen instances of adverse effects were experienced by eight subjects during the study All adverse effects were classified as probably related to drug administration, except for one (loose stool), which was listed as possibly related to drug administration All adverse effects were listed as mild in severity with vomiting and nausea-related effects (lightheadedness, sweating, and dizziness) being the most common complaints, especially during the first dosing phase

Comments

1 This study did not show the bioequivalence between pen/cartridge with benzyl alcohol and syringe/ampoule without benzyl alcohol The reason might be the incomplete priming of the pen/cartridge device Therefore, the study needs to be repeated with a revised priming instruction

10 Study APOM-02115 **Volume P15 1-P15 4**

Study title A Phase I, Open-label, Three-way Crossover Study in Healthy Volunteers to Compare the Pharmacokinetics of Apomorphine HCl Formulated with Benzyl Alcohol Versus Apomorphine HCl Formulated without Benzyl Alcohol A comparison of Apomorphine HCl Delivery from a Cartridge Using a Pen Device to That from a Syringe Manually-filled from an Ampoule or Cartridge (MYLAN PROTOCOL # APOM-02115)

Investigator.

[ ]

Analytical Mylan Pharmaceuticals Inc , Bioanalytical Department 3711 Collins Ferry Road, Morgantown, WV 26505

Study period

Clinical Phases Group A November 13,2002 to December 1,2002, Group B November 22, 2002 to December 10, 2002, Group C December 5,2002 to December 23,2002
Analytical Phase January 7,2003 to January 20,2003

Study Objectives

The objectives of this study were to assess

- The overall effect of the apomorphine delivery device plus that of 0 05% (w/v) benzyl alcohol in the cartridge formulation (pen/cartridge vs syringe/ampoule)
- Potential effects of the apomorphine delivery device (pen/cartridge vs syringe/cartridge)
- Possible effects of benzyl alcohol (0 05% w/v) used in the cartridge formulation (syringe/cartridge vs syringe ampoule)
- The revised instructions for use (IFU, the priming instructions were revised so that a larger priming dose setting 0 06 mL instead of 0 02 mL is required) for the apomorphine delivery device (pen/cartridge)

Study Design

This is an open-label, randomized, three-period crossover study Pharmacokinetic parameters were derived from plasma apomorphine concentration-time curves for each formulation/delivery device Thirty-four (34) healthy, non-smoking, male and female subjects between the ages of 19 and 55 completed this study Subjects were dosed in three groups as a single subcutaneous injection into the subjects abdominal wall

Treatment	Contents	Device

A	Apomorphine HCl Injection containing 0 05%	pen/cartridge
B	benzyl alcohol, 1 x 2 mg	syringe filled from a cartridge
C	no benzyl alcohol, 1 x 2 mg	syringe filled from an ampoule

Apomorphine treatments (A, B, or C) were separated by seven (7) days To decrease the likelihood of nausea and vomiting associated with apomorphine, all volunteers received 250 mg (1 x 250 mg capsule) of the anti-emetic drug trimethobenzamide three to four times a day starting three days prior to dosing until six (6) hours after apomorphine HCl dose administration for each dosing phase Subjects received a standard meal 4 hours post-dose, water was permitted at all times

Serial blood samples, 10 mL (1 x 10 mL), were collected prior to dosing (0 hr) and at 10, 20, 30, 40 and 50 minutes, and 1, 1 5, 2, 2 5, 3 and 4 hours after dose administration Single-dose pharmacokinetic parameters for apomorphine were calculated using non-compartmental techniques Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC)

Results

Assay performance

The assay used

Its performance is summarized in the following tables

Linearity (ng/mL)	LOQ (ng/mL)	Recovery %			Accuracy (%error)		precision (%CV)	
		Low	High	Internal standard	Intra-day	Inter-day	Intra-day	Inter-day

Stability				Stock solution stability			
Room temp	Freeze-thaw	-15°C	Post extraction	Apomorphine		Internal standard	
				4°C	Room	4°C	Room

Based on current standard, the assay is acceptable

Pharmacokinetics

Mean (% CV) single-dose pharmacokinetic parameters of apomorphine HCl are summarized in the following Table and Figure

The 90% confidence intervals for the statistical comparison were within the acceptable bioequivalent range of 80-125% for the log transformed parameters LNAUCL, LNAUCI and LNCPEAK for all treatment comparisons

MEAN (%CV) PHARMACOKINETIC PARAMETERS FOR APOMORPHINE IN THIRTY-FIVE (35)[a] HEALTHY SUBJECTS FOLLOWING A SINGLE DOSE OF APOMORPHINE HCl (2 mg) ADMINISTERED BY SUBCUTANEOUS INJECTION (0.2 mL) PROTOCOL NUMBER APOM-02115									
Parameter	Mean (%CV) A Cartridge and Pen (w/BA)	Mean (%CV) B Cartridge and Syringe (w/BA)[a]	Mean (%CV) C Ampoule and Syringe (w/o BA)	LSMEANS Ratio[b]			90% Confidence Intervals[c]		
				(A/C)	(B/C)	(A/B)	(A/C)	(B/C)	(A/B)
AUCL ((ng•hr)/mL)	5 303 (24)	6 149 (23)	5 814 (19)	0 90	1 04	0 87	86% - 95%	99% - 109%	82% - 91%
AUCI ((ng•hr)/mL)	6 150 (22)	7 060 (22)	6 740 (18)	0 90	1 04	0 87	87% - 94%	99% - 108%	84% - 91%
CPEAK (ng/mL)	5 482 (30)	6 082 (34)	5 471 (27)	0 99	1 09	0 91	91% - 109%	99% - 120%	83% - 100%
TPEAK (hr)	0 400 (48)	0 406 (38)	0 404 (38)	–	–	–	–	–	–
KEL (1/hr)	0 997 (40)	0 928 (36)	0 922 (35)	–	–	–	–	–	–
HALF (hr)	0 775 (30)	0 830 (31)	0 835 (33)	–	–	–	–	–	–
Cl/F[d] (L/hr)	344 (27)	298 (24)	307 (19)	–	–	–	–	–	–
Vd/F[d] (L)	366 (26)	344 (29)	357 (26)	–	–	–	–	–	–

a For Treatment B, n = 34, because Subject 22 did not complete Dosing Phase 3

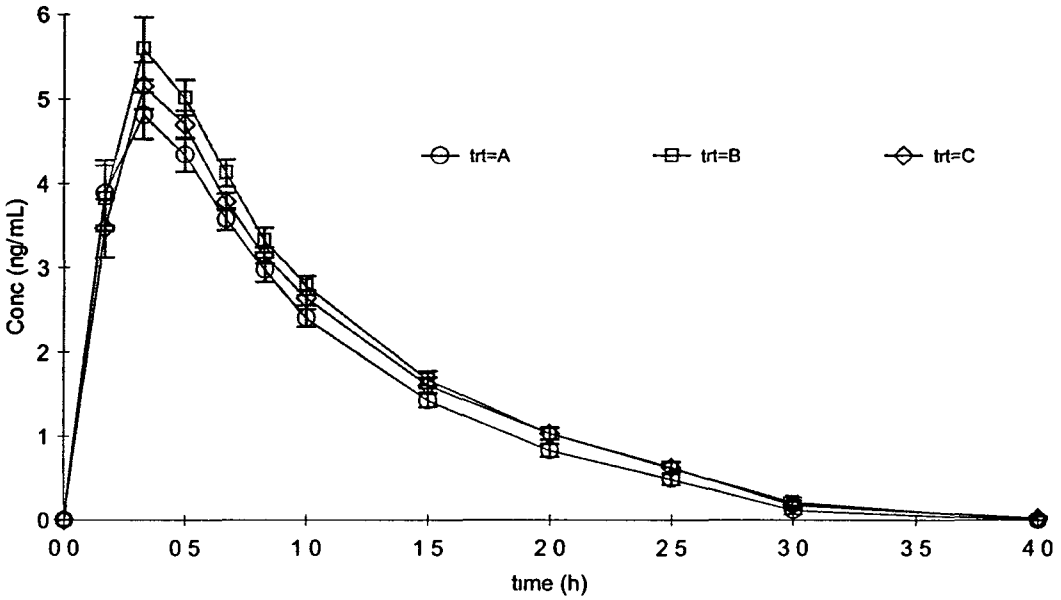
b Ratio (A/B) = $e^{[\text{LSMEAN of LN(NUMERATOR)} - \text{LSMEAN of LN(DENOMINATOR)}]}$

c Used Natural Log Transformed Parameter

d Where Cl/F=Cl_F and Vd/F=Vd_F on the PK data output (14 1 - Attachment 1) and statistics (14 2 - Attachment 2)

Abbreviations w/BA-Formulated with benzyl alcohol (0 05% w/v), w/o BA-Formulated without benzyl alcohol

Source Section 14 1-Attachment 1, part 3 and part 5



Adverse Events

Thirty-six (36) subjects were enrolled and thirty-four (34) completed this study One volunteer (Subject No 22) was discontinued after the second dosing phase due to an adverse effect that was unrelated to dose administration Clinical laboratory testing, vital signs and ECG monitoring indicated no safety risk associated with subcutaneous dosing of apomorphine HCl at a dose level of 2 mg Thirty-five instances of adverse events were experienced by 13 subjects during the study Most adverse events were thought to be possibly or probably related to drug administration Adverse events were generally mild in severity, with headache, nausea and vomiting being the most common complaints

Comments

1 The relative bioavailability was not significantly affected by the delivery device

2 Benzyl alcohol showed no significant effect on relative bioavailability

3 The revised instructions for use (IFU) provide directions for more precise operation of the Apomorphine HCl injection (10 mg/mL) delivery pen

4 Since the bioequivalence study was conducted at lower dose (2 mg), certain side effect of benzyl alcohol, such as irritation at injection site might not manifest Therefore, the safety of cartridge formulation should be monitored and reported

11 Literature Study **Volume P7 16**

Study title Pharmacokinetics, Enantiomer Interconversion, and Metabolism of R-Apomorphine in Patients with Idiopathic Parkinson's Disease

Authors van der Gees, T van Laar, P P Kruger, J M Gubbens-Stibbe, H E Bodde, R A C Roos, and M Danhof

Publication information Clinical Neuropharmacology 1998 Vol 21(3) 159-168

Study Objectives

The objectives of this study were to determine the pharmacokinetics and metabolism of apomorphine in patients with idiopathic Parkinson's disease after I V infusion

Study Design

Ten patients participated in this study (5 men and 5 women) Intravenous infusion apomorphine was given during 15 min at dose level of 30µg/kg Blood samples were collected at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 40, 45, 50, 60, 75, 90, 110, 130, 150, 170, 190, 210, 230, 250, 270, and 290 min after the start of infusion

The contribution of autooxidation to the overall pharmacokinetics of apomorphine was determined in the dark at 37°C Freshly obtained blood (40 mL) was incubated for 30 min Ten min after the incubator was saturated with oxygen, it was spiked with 125 µl of 15 7 µg/ml R-apomorphine Two-milliliter samples were taken at 0, 3, 6, 12, 18, 25, 35, 60, 90, 135, 175, and 235 min, respectively, after the addition of R-apomorphine The plasma concentration time profile was fitted to determine the degradation rate constant k

The plasma-to-blood ratio was determined by spiking a blood sample with a known concentration of R-apomorphine Plasma-to-blood ratio is then determined by dividing this value by the subsequently determined concentration in plasma The extent of protein binding (free fraction) also was determined in this experiment For that purpose, 1-ml plasma samples were spiked with 1257, 2107, and 3112 ng of R-apomorphine, respectively After a 1-h incubation at 37°C degrees, free R-apomorphine was separated from protein-bound R-apomorphine using an Amicon Micropartition System Free R-apomorphine was separated from plasma protein-bound R-apomorphine by ultrafiltration at 1090 g for 10 min at 37°C The concentration of free R-apomorphine was determined in 250 µL of ultrafiltrate From the R-apomorphine concentration in the ultrafiltrate and the concentration in plasma, the protein binding was calculated

Results

In most patients, the plasma concentration versus time profile was characterized by a biexponential function The values of relevant pharmacokinetic parameters were as follows

clearance 40 ± 15 mL/min/kg, volume of distribution at stead state 1 6 ± 0 5 L/kg and terminal half-life 41 ± 13 min

No measurable concentrations of S-apomorphine were detected in plasma, indicating that enantiomeric interconversion does not occur in vivo

No measurable concentrations of the methylated metabolites apocodeine and isoapocodeine could be detected in plasma The metabolism of apomorphine was characterized on basis of the excretion of unchanged R-apomorphine, S-apomorphine, apocodeine, isoapocodeine, and their respective sulfate and glucuronide conjugates in urine The total excretion of unconjugated S-apomorphine, apocodeine and isoapocodeine was less than 0 1% of the administered dose The total excretion of unchanged apomorphine, apomorphine sulfate, and apomorphine glucuronide amounted to 0 3 ± 0 4%, 3 8 ± 1 0 and 6 0 ± 2 2% of the administered dose, respectively The findings of this study show that on intravenous administration, S-apomorphine and the metabolites apocodeine and isoapocodeine are unlikely to interfere with the pharmacologic actions of R-apomorphine in patients with idiopathic Parkinson's disease Furthermore, no pharmacokinetic interaction between R-apomorphine and catechol-O-methyltransferase inhibitors is expected

The plasma-to-blood ratio was determined to be one No free apomorphine could be detected The protein binding was concluded to be more than 99 9%

The in vitro rate of autooxidation was influenced by multiple factors such as temperature, oxygen levels, multivalent cations, and pH After fitting, the half-life was determined to be 142 ± 35 min From this, the clearance from the blood as a result of autooxidation was calculated to be 0 035 L/min using a total blood volume of 5 L Further, a contribution of 1% of autooxidation to the overall clearance from the blood was calculated A higher contribution is obtained when it is assumed that the oxidation rates in the blood and in the peripheral tissues are equal By substituting the blood volume by the volume of distribution in the calculations, a value of 30% is obtained

Comments

1 This study used I V infusion The results may not be applicable to subcutaneous injection
2 The in vitro study may not reflect the in vivo situation
3 The concentration of apomorphine used in protein binding study was much higher than the plasma concentration (around 10 ng/mL)

Office of Clinical Pharmacology and Biopharmaceutics
New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-264	Brand Name	[redacted]
OCPB Division (I, II, III)	I	Generic Name	Apomorphine
Medical Division	HFD 120	Drug Class	Anti-Parkinson's
OCPB Reviewer	John Duan	Indication(s)	Treatment of "off episodes" associated with Parkinson s disease
OCPB Team Leader	Ramana Uppoor	Dosage Form	Subcutaneous injection
	Joga Gobburu	Dosing Regimen	2 mg starting dose with 1 mg increment
Date of Submission	1/2/03	Route of Administration	Subcutaneous
Estimated Due Date of OCPB Review	6/18/03	Sponsor	BERTEK Pharmaceuticals Inc
PDUFA Due Date	7/2/03	Priority Classification	P
Division Due Date	6/20/03		

Clin Pharm and Biopharm Information

	"X if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports tables data etc	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I Clinical Pharmacology				
Mass balance				
Isozyme characterization				
Blood/plasma ratio				
Plasma protein binding				
Pharmacokinetics (e g , Phase I) -				
Healthy Volunteers-				
Single dose	X	5		
Multiple dose				
Patients-				
Single dose	X	1		
Multiple dose				
Dose proportionality				
Fasting / non fasting single dose				
Fasting / non fasting multiple dose				
Drug-drug interaction studies -				
In-vivo effects on primary drug				
In-vivo effects of primary drug				
In vitro				
Subpopulation studies -				
ethnicity				
gender				
Pediatrics				
Geriatrics				
renal impairment.	X	1	1	
hepatic impairment	X	1	1	
PD				
Phase 2				
Phase 3				
PK/PD				
Phase 1 and/or 2 proof of concept.	X	1	1	

Phase 3 clinical trial				
Population Analyses -				
Data rich	X	1	1	
Data sparse				
II Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference				
alternate formulation as reference				
Bioequivalence studies -				
traditional design, single / multi dose	X	2	2	
replicate design single / multi dose				
Food-drug interaction studies				
Dissolution				
(IVIVC)				
Bio-wavier request based on BCS				
BCS class				
III Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	X	19	19	
Total Number of Studies				

Filability and QBR comments

	X" if yes	*Comments*
Application filable ?	X	Reasons if the application is not filable (or an attachment if applicable) For example is clinical formulation the same as the to be marketed one?
Comments sent to firm ?	X	Comments have been sent to firm (or attachment included) FDA letter date if applicable
QBR questions (key issues to be considered)	**What is the major elimination route What drug interaction study should be conducted What is a reasonable maximum dose What is a reasonable dose increment What is a reasonable repeated dose regimen**	
Other comments or information not included above		
Primary reviewer Signature and Date	**John Duan**	
Secondary reviewer Signature and Date	**Ramana Uppoor Joga Gobburu**	

CC NDA 21-264, HFD-850(Electronic Entry or Lee), HFD-120(CSO), HFD-860(RUppoor, JGobburu, MMehta, CSahajwalla), CDR

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature

/s/

John Duan
6/24/03 03 58 06 PM
BIOPHARMACEUTICS

Jogarao Gobburu
6/24/03 05 16 55 PM
BIOPHARMACEUTICS

Ramana S Uppoor
6/25/03 11 55 25 AM
BIOPHARMACEUTICS

COMPLETED JUL 2 4 2000

JUL 2 0 2000

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

Date of Document 4/6/2000

NDA	21-264
Name of Drug	—— (Apomorphine HCL) Intermittent Subcutaneous Injection
Sponsor	Mylan, Morgantown, WV
Indication of Drug	Parkinson's Disease
Type of Document	New NDA
Reviewer	Hong Zhao, Ph D

45 Day-Filing

Introduction
R-apomorphine hydrochloride is a potent, short-acting dopamine agonist The subcutaneous injection formulation of Apomorphine is currently approved in Europe for the management of refractory motor fluctuations in patients with Parkinson's disease This application is seeking approval of this same formulation in the U S for use via intermittent subcutaneous injection as abortive (rescue) therapy for acute refractory episodes of immobility or hypomobility ("off episodes") in patients with late stage Parkinson's disease

Human Pharmacokinetics Section
The sponsor has not conducted any apomorphine pharmacokinetics studies The rationale for not conducting any pharmacokinetics studies is that apomorphine is an "old" compound and the pharmacokinetics of which have been studied and published extensively The human pharmacokinetics section of this NDA consists of a description of apomorphine pharmacokinetics compiled from reports in the literature ranging from 1989 to 1999 (37 references)

Comments
PK as per Labeling dosage regimen Pharmacokinetics of R-apomorphine has not been characterized as per proposed labeling dosage regimen - the daily dose should not exceed 50 mg and each individual dose should not exceed 10 mg Dose proportionality was characterized only up to 4 mg Multiple dose study with total daily dose up to 50 mg and 10 mg/dose has not been conducted

PK in Special Populations The pharmacokinetics of R-apomorphine in special population, such as in patients with hepatic impairment or renal impairment, has not been conducted

Metabolic Pathways The metabolic pathways of R-apomorphine have not been characterized The sponsor submitted three proposed protocols as follows

Protocol APOM-0001-DM In vitro evaluation of apomorphine HCl as an inducer of cytochrome P450 expression in human hepatocytes — Protocol — 990060)

Protocol APOM-00021-DM Evaluation of apomorphine HCl as an inhibitor of cytochrome P450 enzymes in vitro — Protocol — 990058)

Protocol APOM-00021-DM In vitro metabolism of apomorphine by human liver microsomes — Protocol —990059)

Drug-Drug Interactions The only drug-drug interaction study was done with levodopa/benserazide at a single oral dose and the coadministration of the dopamine agonist apomorphine The effect of levodopa on pharmacokinetics and pharmacodynamics of apomorphine was not evaluated

Outcomes of 45-Day Filing Meeting on May 22, 2000
The decision of refuse to file for this NDA was made because it does not meet preclinical and clinical requirements for filing

Hong Zhao, Ph D /S/ 7/20/2000

RD/FT Initialed by Raman Baweja, Ph D /S/ 7/20/00

cc NDA 21-264 — Injection (Apomorphine HCL), HFD-120, HFD-860 (Zhao, Baweja, Mehta), Central Documents Room (CDR-Biopharm)