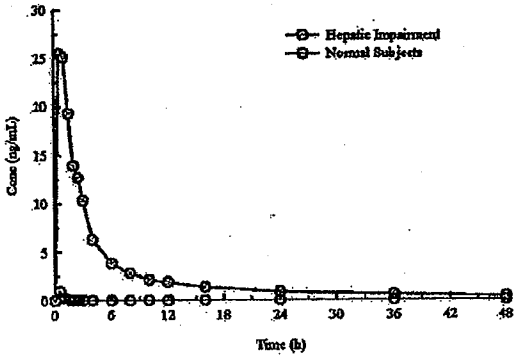
Figure 1: Mean plasma concentrations of tetrabenazine after oral administration of 25 mg of tetrabenazine (2×12.5 mg tablets) under fasting conditions to subjects with hepatic impairment and healthy volunteers — linear axes.



Pharmacokinetic parameters (arithmetic mean) for TBZ, α-HTBZ and β-HTBZ (Study TBZ 203,010)

	Normal (% CV) n=12	Hepatic Impairment (% CV) n=12
TBZ*		
t_{max} (h)[a]		1.0 (0.5-2.5)
C_{max} (ng/mL)		43.8 (81)
AUC $_{0-t}$ (ng*h/mL)		151 (72)
AUC $_{0-\infty}$ (ng*h/mL)		170.0 (79)
λz (hr^{-1})		0.1245 (200)
$t_{1/2}$ (h)		17.5 (44)
α-HTBZ		
t_{max} (h)[a]	1.0 (0.5-2.0)	1.75 (0.5-12.03)
C_{max} (ng/mL)	35 (32)	30.5 (49)
AUC $_{0-t}$ (ng*h/mL)	182 (53)	247 (46)
AUC $_{0-\infty}$ (ng*h/mL)	191 (51)	259 (52)
λz (hr^{-1})	0.1317 (41)	0.084 (44)
$t_{1/2}$ (h)	6.1 (39)	10.1 (55)
β-HTBZ		
t_{max} (h)[a]	1.0 (0.5-2.0)	1.75 (0.5-12.0)
C_{max} (ng/mL)	18.8 (52)	17.4 (74)
AUC $_{0-t}$ (ng*h/mL)	81.6 (87)	107.2 (48)
AUC $_{0-\infty}$ (ng*h/mL)	85.6 (85)	119.4 (53)
λz (hr^{-1})	0.213 (34)	0.1251 (66)
$t_{1/2}$ (h)	3.7 (39)	8.42 (72)

[a] median (range)

* For TBZ, plasma concentrations in normal subjects were < LOQ at most sampling times and no PK parameters were estimated. In impaired subjects, there were no concentrations ≥ LOQ for subject #13, 2 concentrations for subject ##22, and 1 concentration for subject #23, so for those subjects no PK parameters were calculated. PK parameters for TBZ in hepatic impairment reflect n=9.

As reviewed in the interim report, in most hepatic impairment patients, there were detectable concentrations of TBZ that were similar to or higher than exposures seen with α- or β-HTBZ. The highest TBZ concentrations in normal subjects ranged from 0.23-6.39 ng/ml when they were detectable. The mean TBZ Cmax concentration in hepatic subjects was approximately 7 to 190

fold higher than the detectable peak concentrations in healthy subjects in the present study. There were also increases in exposure to α- and β-HTBZ, in general, although to a lesser extent than the increase in exposure to TBZ. Half-life was also prolonged.

The PK parameters in each hepatic impairment subject are shown in the tables on page 110-11 in the Appendix. The patient with moderate impairment can be distinguished from the mildly impaired subjects. Generally, AUC and elimination half-life were longer for subjects with worse impairment (please refer to review of the study report, in the Appendix, for details on PK in individual subjects).

Without an understanding of the contribution of elevated levels of TBZ to safety, it is not possible to recommend a dosage adjustment in hepatic impairment. We recommend that TBZ be contraindicated in hepatic impairment, in agreement with the recommendation from the review of the original NDA submission.

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4 Clinical Pharmacology Individual Study Reviews

4.1 Analysis of Mass Balance Study

A PHASE I, OPEN-LABEL STUDY TO ASSESS THE ABSORPTION, METABOLISM AND ELIMINATION OF [^{14}C]-LABELLED TETRABENAZINE (ANALYTICAL PHASE)

Study Investigators and Site:

Protocol Number: CAM/O6

BACKGROUND:

Cam/06 identified the moieties found in human plasma and urine after administration of 25 mg [^{14}C]-tetrabenazine in 6 healthy male subjects after overnight fast. The clinical study (RD 204/24124) and preliminary analytical reports were reviewed by OCP with the original NDA. The following information is from the OCP review of that submission:

"After administration of TBZ, 64.97-81.39% of the dose (mean 75.4%) was excreted in urine in the 9 day collection period. The majority was recovered within 72 hours after dosing. Fecal recovery in the 9 days after dose accounted for 7.07-16.05% of the dose. The mean total recovery (urine + feces) was 87.49% (range 76.88-92.84%)".

At the time of that submission, identification of the major circulating component in humans (P16) had not been reported. In this submission, the characterization has been completed.

Only the updated results will be presented here.

RESULTS:

Identification of Metabolites

Chromatographic analysis of urine showed up to 19 distinct metabolites (U1-U19). None of these components exhibited co-chromatography with ^{14}C-TBZ. Analysis of urine using LC-MS/MS indicated the presence of the following compounds (as provided by the Sponsor):

Table 12 **Proportions of radioactive components in urine following administration of single oral doses of [^{14}C]-tetrabenazine to six male human subjects**

Component	Time (hours)					Total (0 – 48h)
	0 – 4	4 – 8	8 – 12	12 – 24	24 – 48	
U1	0.12 (0.71)	0.22 (1.93)	0.18 (1.77)	0.30 (2.00)	0.21 (1.52)	1.01
U2	0.18 (1.09)	0.27 (2.38)	0.25 (2.65)	0.39 (2.58)	0.38 (2.76)	1.47
U3	0.09 (0.55)	0.17 (1.48)	0.14 (1.48)	0.85 (5.66)	0.68 (4.91)	1.93
U4	0.65 (3.91)	0.79 (7.05)	0.68 (7.31)	0.62 (4.11)	0.60 (4.34)	3.34
U5	0.23 (1.36)	0.31 (2.81)	0.17 (1.79)	0.27 (1.78)	0.22 (1.58)	1.20
U6	0.58 (3.50)	0.66 (5.94)	0.47 (5.09)	1.18 (7.91)	1.13 (8.21)	4.02
U7	0.31 (1.86)	0.33 (2.93)	0.39 (4.18)	ND	ND	1.03
U8	0.92 (5.54)	0.58 (5.20)	0.44 (4.71)	0.38 (2.55)	0.28 (2.05)	2.60
U9	0.29 (1.75)	0.13 (1.12)	0.13 (1.38)	0.26 (1.74)	0.33 (2.40)	1.14
U10	1.02 (6.17)	0.65 (5.79)	0.50 (5.35)	0.92 (6.13)	0.61 (4.41)	3.70
U11	4.24 (25.61)	2.19 (19.56)	1.62 (17.43)	2.43 (16.23)	2.20 (15.97)	12.68
U12	0.71 (4.31)	0.64 (5.74)	0.57 (6.18)	0.62 (4.14)	0.57 (4.15)	3.11
U13	4.15 (25.07)	3.12 (27.91)	2.48 (26.63)	3.65 (24.40)	3.69 (26.78)	17.09
U14	0.27 (1.64)	0.16 (1.45)	0.13 (1.37)	0.62 (4.11)	0.15 (1.06)	1.33
U15	0.82 (4.94)	0.27 (2.44)	0.21 (2.26)	0.71 (4.77)	0.63 (4.60)	2.64
U16	0.15 (0.91)	0.05 (0.43)	0.06 (0.60)	0.12 (0.80)	0.15 (1.12)	0.53
U17	1.06 (6.41)	0.40 (3.56)	0.43 (4.63)	0.24 (1.61)	0.25 (1.80)	2.38
U18	0.19 (1.13)	0.04 (0.38)	0.03 (0.34)	0.11 (0.71)	0.06 (0.47)	0.43
U19	0.07 (0.45)	ND	ND	ND	ND	0.07
Other	0.51 (3.09)	0.21 (1.90)	0.45 (4.85)	1.31 (8.77)	1.64 (11.87)	4.12

Results expressed as % dose administered (Values in parentheses indicate % sample radioactivity
ND Component not detected
Components 1 – 9 were identified as mono-hydroxy-dihydro tetrabenazine(s) and glucuronides of O-dealkylated dihydro tetrabenazine(s)
Component 10 was identified as monohydroxy dihydrotetrabenazine
Components 11 and 13 were identified as sulphate conjugates of O-dealkylated dihydro tetrabenazine(s)
Components 15 and17 were tentatively identified as O-dealkylated dihydro tetrabenazine and/or β-dihydro tetrabenazine
Component 18 was identified as α-dihydro tetrabenazine

The following components and their proportions were found circulating in the plasma in pooled plasma samples. It can be seen that there were several metabolites (P11, P13, P 16) that were circulating to a greater extent than α – HTBZ (P 18). (P11 and P13 are sulfate conjugates of P16). In addition, although P17 was not circulating to a greater extent than P18, it represented more than 10% of components circulating in the plasma up to 1.5 hours after the dose.

Monohydroxy HTBZ (P10) that has been identified as a major metabolite *in vitro* (Studies CAM 19 and CAM20) was not detected in the plasma in the mass balance study. (It was detected as less than 4% of radioactivity in urine up to 48 hours).

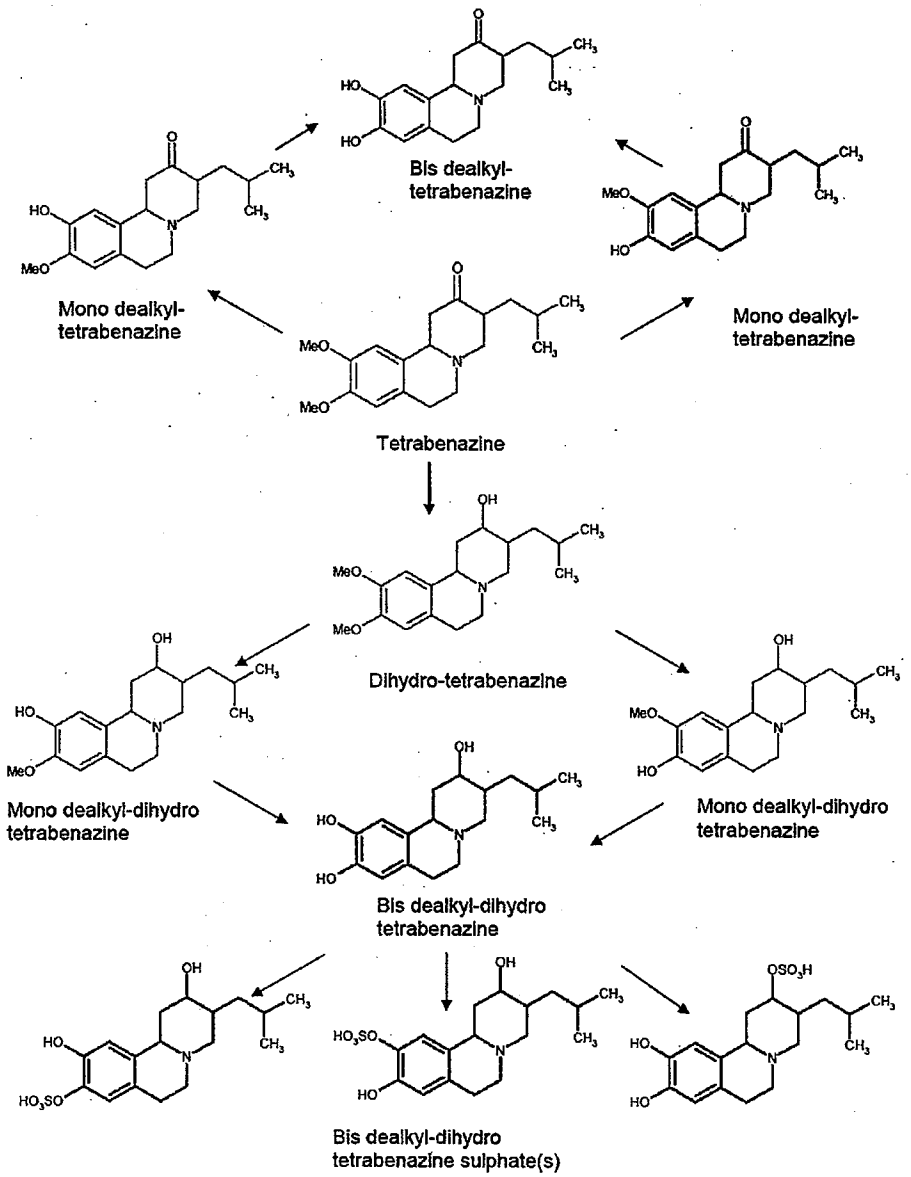
Table 15 **Proportions of radioactive components in plasma extracts following administration of single oral doses of [^{14}C]-tetrabenazine to six male human subjects**

Component	0.25 – 1.5 hours	2 – 3 hours	4 – 8 hours	Mean
P1	ND	ND	ND	NC
P2	ND	ND	ND	NC
P3	ND	ND	ND	NC
P4	ND	ND	ND	NC
P5	ND	ND	ND	NC
P6	ND	ND	4.10 (4.55)	NC
P7	ND	ND	ND	NC
P8	ND	ND	8.23 (9.14)	NC
P9	ND	ND	ND	NC
P10	ND	ND	ND	NC
P11	23.44 (16.67)	29.52 (20.41)	15.23 (16.90)	22.73 (17.99)
P12	ND	ND	ND	NC
P13	26.73 (19.01)	41.77 (28.88)	24.22 (26.88)	30.91 (24.92)
P14	ND	ND	ND	NC
P15	ND	ND	ND	NC
P16	49.54 (35.24)	50.11 (34.65)	19.77 (21.95)	39.81 (30.61)
P17	13.65 (9.71)	6.54 (4.52)	4.48 (4.97)	8.22 (6.40)
P18	25.73 (18.30)	16.70 (11.55)	14.05 (15.60)	18.83 (15.15)
Other	1.52 (1.08)	ND	ND	NC

Results expressed as ng equivalents/mL (Values in parentheses indicate % sample radioactivity)
ND Component not detected NC Not calculable
Components 1 – 9 were identified as mono-hydroxy-dihydro tetrabenazine(s) and glucuronides of O-dealkylated dihydro tetrabenazine(s)
Component 10 was identified as monohydroxy dihydrotetrabenazine
Components 11 and 13 were identified as sulphate conjugates of O-dealkylated dihydro tetrabenazine(s)
Component 16 was identified as O-dealkylated dihydro tetrabenazine
Components 15 and17 were tentatively identified as O-dealkylated dihydro tetrabenazine and/or β-dihydro tetrabenazine
Component 18 was identified as α-dihydro tetrabenazine

Proposed Metabolic Scheme

The Sponsor has proposed the following metabolic scheme. This has not changed since the original submission. It should be noted, however, that this scheme does not include the Phase 2 metabolites (sulfated or glucuronidated conjugates of HTBZ).



CONCLUSIONS:

The Sponsor has identified several Phase I and Phase II metabolites (glucuronidation and sulfation) circulating with significant exposures in the plasma. P16, the largest component circulating in the plasma, has now been identified as O-dealkylated HTBZ. The sulfated conjugates of O-dealkylated HTBZ (P11 and P13) are also major components of the circulating moieties in the plasma, and are the predominant metabolites in human urine.

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4.2 In Vitro TBZ Metabolism Study

Identification of the Major Drug Metabolizing Enzymes Involved in the Human Hepatic Metabolism of Tetrabenazine

Study Investigators and Site:

Protocol Number: CAM/16

OBJECTIVES:

To identify which enzyme or enzymes are responsible for metabolizing TBZ in human liver and in human whole blood.

METHODS:

Human Liver Microsomes

Human liver microsomes were supplied by and consisted of a pool from 50 individual human donors (mixed gender). Microsomes had been fully characterized by for total P450 content and enzyme activities for the major human P450s.

Mixtures containing ^{14}C-TBZ (final incubation concentrations of 1, 3, and 10uM), pooled human liver microsomes (1 mg microsomal protein/ml incubation mixture) and phosphate buffer were pre-incubated for 5 minutes and then reactions were initiated by addition of co-factor solution (2 mM NADPH final concentration). At 0, 30, 60, and 120 minutes, reactions were terminated by transferring 900 µL of incubation mixture into an Eppendorf tube containing ice-cold acetonitrile. Control incubations were run in the absence of microsomal protein and co-factor solution. All incubations were performed in duplicate at 37° C in a shaking water bath and tubes open to the atmosphere.

Following the preliminary experiments with pooled human liver microsomes only, further incubations were conducted in the presence of chemical inhibitors. Incubation mixtures contained ^{14}C-TBZ (final incubation concentrations of 1, 3 and 10 µM), pooled human liver microsomes (0.5 mg microsomal protein/mL incubation mixture), cofactor solution (2 mM NADPH final concentration), and phosphate buffer (100 mM, pH 7.4) in the presence or absence of chemical inhibitors. The following chemical inhibitors were used:

- Quercetin - final concentration of 100 µM (use as a chemical inhibitor of carbonyl reductase)
- Quinidine -final concentration of 5 µM (used as a selective chemical inhibitor of CYP2D6)

- SKF-525A - final concentration of 500 µM (used as a broad-spectrum chemical inhibitor of cytochrome P450)

Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of ^{14}C-TBZ. At 15 minutes following the initiation of the reaction, reactions were terminated by addition of ice-cold acetonitrile.

Further incubations were then performed to assess the involvement of carbonyl reductase and P450, using multiple concentrations of quercetin (final concentrations of 100, 500 and 1000 µM) or ketoconazole (final concentration of 150 µM) that the Sponsor used as a broad-spectrum chemical inhibitor of P450 at this concentration.

Expressed microsomes

^{14}C-TBZ was incubated with recombinant cytochrome P450 enzymes and the metabolic profiles obtained with each CYP450 enzyme compared to the hepatic microsomal profile of TBZ obtained with human liver microsomes. Recombinant cDNA expressed human cytochrome P450 enzymes [redacted]) were derived from heterologous bacterial expression (Bactosomes, *E coli*) and expressed a single cytochrome P450 enzyme as shown below:

CYP1A2 Batch C1A2R005, protein concentration of 11.9 mg/mL (13.3 nmol P450/mL)
CYP2C8 Batch C2C8R001, protein concentration of 9.0 mg/mL (10.9 nmol P450/mL)
CYP2C9 Batch C2C9R009A, protein concentration of 11.7 mg/mL (5.1 nmol P450/mL)
CYP2C19 Batch C2C19R005, protein concentration of 14.5 mg/mL (5.5 nmol P450/mL)
CYP2D6 Batch C2D6R017, protein concentration of 26.8 mg/mL (3.8 nmol P450/mL)
CYP2E1 Batch C2E1R014, protein concentration of 6.6 mg/mL (4.8 nmol P450/mL)
CYP3A4 Batch C3A4R018B, protein concentration of 9.4 mg/mL (3.9 nmol P450/mL)

Incubation mixtures contained ^{14}C-TBZ (final concentration of 3 µM), cDNA expressed CYP450 enzymes (125 pmol cytochrome P450/mL), cofactor solution (2 mM NADPH final concentration), phosphate buffer (100 mM, pH 7.4) and in the presence or absence of selective chemical inhibitors. The following chemical inhibitors were used:

- Quercetin (final concentration of 500 µM) - to assess the potential inhibition by quercetin on CYP450 enzymes (potential non-selective inhibition of carbonyl reductase
- Ketoconazole (final concentration of 150 µM) to confirm broad-spectrum chemical inhibition of cytochrome P450 at this concentration

Control incubations (conducted in parallel) were run in the absence of microsomal protein and co-factor solution.

Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of ^{14}C-TBZ. All incubations were performed individually at 37°C. Thirty minutes following the initiation of the reaction, reactions were terminated by the addition of ice-cold acetonitrile.

Human Whole Blood and Human Liver Cytosol

Incubations were conducted in human whole blood and in human liver cytosol to investigate the contribution to TBZ metabolism of carbonyl reductases that are present in these tissues. Pooled human liver microsomes were also used in these incubations to provide a reference sample for comparison.

Human liver cytosol was supplied by [redacted] and consisted of a pool from 50 individual human donors (mixed gender). The protein concentration was 10 mg/ml. Fresh control human whole blood was collected from 3 male healthy volunteers who had taken no medication in the previous 7 days or alcohol for 24 hours. Blood samples were pooled, hemolyzed, and stored until use.

For *cytosol or human liver microsomes*, incubation mixtures contained ^{14}C-TBZ (final incubation concentration of 3 μM), pooled human liver microsomes or human liver cytosol (0.5 mg microsomal protein/mL incubation mixture), cofactor solution (2 mM NADPH final concentration) and phosphate buffer (100 mM, pH 7.4) and were performed in the presence or absence of the following chemical inhibitors:

- Quercetin (final concentration of 500 μM) – a chemical inhibitor of carbonyl reductase.
- Furafylline (final concentration of 10 μM) – a potent and selective mechanism-based inhibitor of CYP1A2 (required pre-incubation (10 minutes) with cofactor solution (NADPH) and pooled human liver microsomes prior to initiation of reaction
- Ketoconazole (final concentration of 150 μM) - broad spectrum inhibitor of cytochrome P450 at this concentration.

All incubations were performed in duplicate at 37°C. Reactions were initiated by the addition of ^{14}C-TBZ, carried out for 15 minutes, and terminated as described above.

To assess the potency of CYP1A2 inhibition by furafylline, additional incubations containing ^{14}C-Phenacetin (20 μM) and pooled human liver microsomes in the presence and absence of furafylline (10 μM) were performed. Phenacetin undergoes O-deethylation to acetaminophen in human liver microsomes – a reaction that is selective for CYP1A2 catalysis.

For whole blood, incubation mixtures were similar to those described above, including the concentrations of chemical inhibitors. Following pre-incubation of 10 minutes, reactions were initiated by addition of ^{14}C-TBZ. At 2 hours following initiation, reactions were terminated as described above.

Assay

Samples were assayed by HPLC for ^{14}C -TBZ, by LC-MS/MS for metabolites and for TBZ, and by HPLC for analysis of Phenacetin samples. Radiochromatograms were evaluated for metabolite formation and substrate disappearance.

RESULTS:

Human Liver Microsomes

Nine radiolabeled metabolite fractions were detected as shown below:

Component	Approximate retention time (min)	Identity
MET 1	16.5	Bis-O-dealkylated-HTBZ
MET 2	17.5	O-dealkylated-HTBZ
MET 3	18.0	Bis-O-dealkylated-HTBZ
MET 4	19.0	Mono-hydroxy-TBZ
β-HTBZ	20.5	Confirmed as β-HTBZ
MET 5	22.0	O-dealkylated-HTBZ
α-HTBZ	23.5	Confirmed as α-HTBZ
MET 6	27.0	Impurity in Dose Solution
MET 7	28.0	O-dealkylated TBZ
TBZ	32.0	Confirmed as TBZ
MET 8	34.5	Isomeric impurity of TBZ

^{14}C-Tetrabenazine was rapidly metabolized by pooled human liver microsomes at all three substrate concentrations used (1, 3 and 10 μM) in a time- dependent manner and depending on the metabolite in a concentration-dependent manner, and that after a 30 minute incubation in the presence of NADPH, the majority of TBZ had been consumed (85.8% loss at 10 μM, and > 90% consumption at the lower concentrations).

α-HTBZ and β-HTBZ were the major metabolites, each metabolite comprising >20% of total sample radioactivity at 60 minutes. The O-dealkylated-HTBZ metabolite (MET 2) accounted for approximately 11-17% of total radioactivity at 60 minutes and approximately 20% at 120 minutes. The other metabolites each accounted for < 10%. Both α-HTBZ and β-HTBZ were further metabolized to secondary metabolites, as the levels decreased after the 60 minute time point, although this secondary metabolism was substantially reduced at the highest concentration of TBZ.

Table 1 Extent of ^{14}C-Tetrabenazine metabolism and metabolite formation by pooled human liver microsomes in the presence of cofactor (NADPH) (Experiment 1)

1 μM ^{14}C-Tetrabenazine

Time (min)	MET 1[1]	MET 2[1]	MET 3[1]	MET 4[1]	β-HTBZ[1]	MET 5[1]	α-HTBZ[1]	MET 6[1]	MET 7[1]	TBZ[2]	MET 8[1]
0	ND	ND	ND	ND	ND	ND	4.22	ND	ND	23.72	7.09
30	2.15	12.29	2.44	4.95	23.50	3.89	28.27	ND	ND	94.12	ND
60	4.13	17.41	3.11	4.17	23.25	4.39	26.80	1.11	2.36	97.97	ND
120	4.94	22.07	4.37	3.95	18.90	3.91	22.69	ND	ND	100.00	ND

3 μM ^{14}C-Tetrabenazine

Time (min)	MET 1[1]	MET 2[1]	MET 3[1]	MET 4[1]	β-HTBZ[1]	MET 5[1]	α-HTBZ[1]	MET 6[1]	MET 7[1]	TBZ[2]	MET 8[1]
0	ND	ND	ND	ND	ND	ND	3.22	ND	ND	21.87	7.04
30	2.00	10.95	1.88	5.18	20.64	4.14	26.57	1.04	6.44	91.28	ND
60	3.29	16.01	3.04	5.09	22.33	4.94	26.10	ND	3.53	97.73	ND
120	4.49	19.96	3.62	3.69	19.30	3.35	20.77	ND	1.41	100.00	ND

Data expressed as mean of duplicate incubations
ND – not detected or below limit of accurate quantification (deemed to be 1% of total eluted material)
[1] Individual metabolite data expressed as a percentage of total eluted material
[2] TBZ data expressed as percentage metabolism (i.e. 100% - amount TBZ expressed as a percentage of total eluted material)
See Section 4.2 for key to metabolite identities

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10 µM ^{14}C-Tetrabenazine

Time (min)	MET 1[1]	MET 2[1]	MET 3[1]	MET 4[1]	β-HTBZ[1]	MET 5[1]	α-HTBZ[1]	MET 6[1]	MET 7[1]	TBZ[2]	MET 8[1]
0	ND	ND	ND	ND	ND	ND	2.04	ND	ND	19.52	6.94
30	1.65	7.73	1.60	6.26	18.48	4.53	21.82	1.39	9.00	85.83	1.43
60	3.02	11.49	2.33	7.79	20.57	5.11	22.93	1.25	6.10	94.76	ND
120	5.29	15.54	3.60	7.24	20.48	4.72	21.60	ND	2.55	99.17	ND

Data expressed as mean of duplicate incubations
ND – not detected or below limit of accurate quantification (deemed to be 1% of total eluted material)
[1] Individual metabolite data expressed as a percentage of total eluted material
[2] TBZ data expressed as percentage metabolism (i.e. 100% - amount TBZ expressed as a percentage of total eluted material)
See Section 4.2 for key to metabolite identities

In the absence of cofactor solution (NADPH), conversion of TBZ to both α-HTBZ and β-HTBZ was still observed, although in a subsequent experiment very little metabolism of TBZ or formation of α-HTBZ or β-HTBZ was observed. (The relevance of this is unclear, since carbonyl reductase, responsible for the formation of α-HTBZ and β-HTBZ, is NADPH dependent). MET 8 was formed in the no-protein control, indicating non-enzymatic formation.

Inhibition experiments were performed using a lower microsomal protein concentration (0.5 mg/ml) compared to the initial studies, as the Sponsor was attempting to reduce the extent of TBZ metabolism. In the absence of chemical inhibitor, the extent of parent TBZ metabolism (consumption) under these conditions was 71, 63, and 52% at 1, 3, and 10 µM TBZ. Quercetin (100 µM) reduced TBZ consumption by approximately 23% at each concentration of TBZ. It also inhibited formation of MET 7, the O-dealkylated TBZ (quercetin inhibits human P450-mediated metabolism *in vitro* including CYP1A2, CYP2C9 CYP2D6 and CYP3A with IC50s of < 100 µM (Obach RS. JPET 2000; 294:88-95)). However, it did not reduce the formation of α-HTBZ or β-HTBZ. However, further study with quercetin at 100 µM, 500 µM and 1000 µM showed a concentration related inhibition of TBZ metabolism and of formation of α-HTBZ and β-HTBZ and MET 7. The CYP2D6 inhibitor quinidine (5 µM) had no effect on the formation of metabolites or consumption of TBZ in this assay. (The IC_{50} for quinidine mediated inhibition of CYP2D6 has been reported as 0.4 µM in the literature). However, the broad-spectrum p450 inhibitor SKF-525A (500 uM) reduced TBZ metabolism by approximately 15% (and had little effect on formation of α-HTBZ or β-HTBZ). SKF-525A inhibited formation of MET 7 by approximately 20-40%. Ketoconazole (150 uM), a broad spectrum P450 inhibitor at this concentration, caused an approximate 15% -20% inhibition of TBZ metabolism, and inhibited MET 7 formation by approximately 80%, with no effect on α-HTBZ or β-HTBZ formation. These results are shown in the tables below. The results support a P450-mediated mechanism for formation of MET 7 from TBZ, and a carbonyl reductase pathway for metabolism of TBZ to α-HTBZ or β-HTBZ.

Table 3 **Extent of ^{14}C-Tetrabenazine metabolism by pooled human liver microsomes in the presence and absence of quercetin, quinidine and SKF-525A (Experiment 2)**

1 µM ^{14}C-Tetrabenazine

Sample	MET 2[1]	β-HTBZ[1]	MET 5[1]	α-HTBZ[1]	MET 6[1]	MET 7[1]	TBZ[2]	MET 8[1]
No inhibitor (Solvent control)	4.05	16.64	2.66	20.80	2.35	10.38	70.98	2.76
Quercetin (100µM)	ND	15.50	ND	20.85	3.49	1.81	55.62	4.65
Quinidine (5µM)	3.69	18.76	2.84	22.29	1.85	8.96	70.79	3.22
SKF-525A (500 µM)	3.00	14.37	ND	21.41	4.14	8.42	63.28	3.52

3 µM ^{14}C-Tetrabenazine

Sample	MET 2[1]	β-HTBZ[1]	MET 5[1]	α-HTBZ[1]	MET 6[1]	MET 7[1]	TBZ[2]	MET 8[1]
No inhibitor (Solvent control)	2.95	15.00	1.97	16.79	2.30	11.42	63.32	3.79
Quercetin (100µM)	ND	12.95	1.06	16.79	3.03	1.98	48.62	5.12
Quinidine (5µM)	2.26	14.59	2.23	16.44	2.11	10.41	61.68	3.93
SKF-525A (500 µM)	1.93	11.18	ND	17.16	2.79	7.94	53.72	4.58

10 µM ^{14}C-Tetrabenazine

Sample	MET 2[1]	β-HTBZ[1]	MET 5[1]	α-HTBZ[1]	MET 6[1]	MET 7[1]	TBZ[2]	MET 8[1]
No inhibitor (Solvent control)	1.50	7.80	ND	10.63	1.93	11.33	51.79	4.64
Quercetin (100µM)	ND	7.75	ND	11.75	3.16	2.50	39.29	5.83
Quinidine (5µM)	1.46	8.16	ND	11.77	1.98	10.76	51.71	4.36
SKF-525A (500 µM)	1.02	7.79	ND	12.46	3.31	6.85	44.72	5.27

Data expressed as mean of duplicate incubations
ND – not detected or below limit of accurate quantification (deemed to be 1% of total eluted material)
[1] Individual metabolite data expressed as a percentage of total eluted material
[2] TBZ data expressed as percentage metabolism (i.e. 100% - amount TBZ expressed as a percentage of total eluted material)
See Section 4.2 for key to metabolite identities

Note: the table below is for 10 µM TBZ only. Similar results were seen with 1 µM and 3 µM TBZ.

10 µM ^{14}C-Tetrabenazine

Component	Solvent control (NaOH)	Quercetin 100 µM	Quercetin 500 µM	Quercetin 1000 µM	No cofactor (NaOH)	Solvent control (Methanol)	Ketoconazole 150 µM	No cofactor (Methanol)
β-HTBZ[1]	102	91.3	72.7	55.6	0.00	89.9	104	0.00
α-HTBZ[1]	162	132	109	104	0.00	144	229	0.00
MET 7[1]	125	51.1	0.00	0.00	0.00	135	28.9	0.00
TBZ[2]	691	559	468	424	283	707	597	297

Data expressed is from individual incubations
[1] Individual metabolite data expressed as pmol/min/mg protein
[2] TBZ data expressed as rate of TBZ metabolised pmol/min/mg protein
See Section 4.2 for key to metabolite identities

Table 5 **Percentage inhibition of ^{14}C-Tetrabenazine metabolism and metabolite formation by pooled human liver microsomes in the presence of quercetin and ketoconazole (Experiment 3)**

1 µM ^{14}C-Tetrabenazine

Component	Quercetin 100 µM	Quercetin 500 µM	Quercetin 1000 µM	Ketoconazole 150 µM
β-HTBZ	4.04	33.8	47.0	7.18
α-HTBZ	0.386	21.6	33.2	0
MET 7	68.1	92.7	100	82.3
TBZ	19.6	38.6	45.7	19.0

3 µM ^{14}C-Tetrabenazine

Component	Quercetin 100 µM	Quercetin 500 µM	Quercetin 1000 µM	Ketoconazole 150 µM
β-HTBZ	0	36.2	49.4	1.88
α-HTBZ	0	30.5	44.2	0
MET 7	61.0	100.0	100	83.5
TBZ	17.2	41.4	45.2	19.2

10 µM ^{14}C-Tetrabenazine

Component	Quercetin 100 µM	Quercetin 500 µM	Quercetin 1000 µM	Ketoconazole 150 µM
β-HTBZ	10.5	28.7	45.5	0
α-HTBZ	18.5	32.7	35.8	0
MET 7	59.1	100	100	78.6
TBZ	19.1	32.6	38.6	15.6

See Section 4.2 for key to metabolite identities

Quercetin at these concentrations is reported in the literature to be a carbonyl reductase inhibitor. Because the quercetin concentration is in excess relative to its Ki for the P450s evaluated, specificity of the P450 inhibition cannot be determined, and a role in specific P450-mediated metabolism of TBZ is not concluded from this experiment. However, data in the following sections further support the role of carbonyl reductase and the lack of P450-mediated formation of α and β-HTBZ.

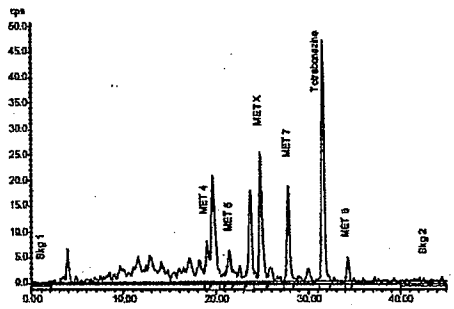
Expressed human P450s
Quantitative results from these experiments were not presented.

α-HTBZ and β-HTBZ were not produced by any expressed P450 enzyme.

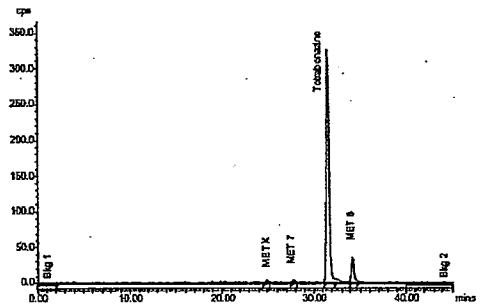
CYP1A2 produced MET 4 (mono-hydroxy-TBZ) and MET X (bis-O-dealkylated-TBZ) and MET 7 as major metabolites. Other oxidative metabolites were also formed, but their identities were not confirmed. Quercetin and ketoconazole completely inhibited metabolism of TBZ by CYP1A2, as shown in the figure below.

CYP1A2, 3 µM Tetrabenazine

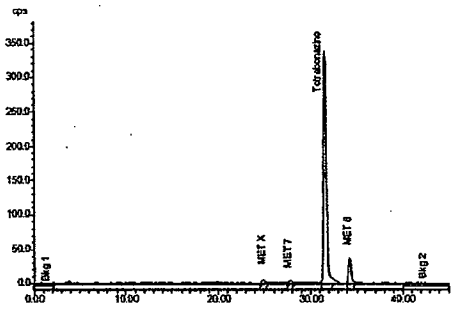
30 m



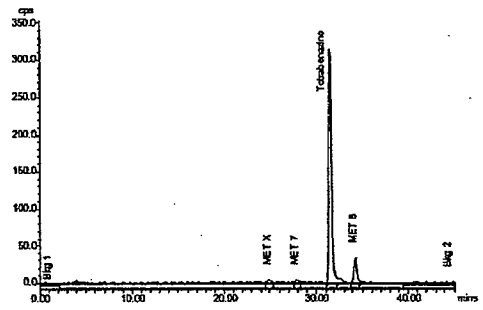
30 m, 500 µM Quercetin



30 m, no cofactor



30 m, 150 µM Ketoconazole



CYP2C9 produced MET 7 and this was partially inhibited by quercetin and completely inhibited by ketoconazole (broad spectrum concentration).

CYP2C19 produced MET 1 (bis-O-dealkylated-HTBZ), MET 4, and MET 7. Production of MET 1 and 4 was partially inhibited by quercetin, but production of MET 7 increased. Ketoconazole reduced the production of all 3 metabolites.

CYP2D6 produced MET 4 and MET 7. Quercetin had little effect on CYP2D6-mediated metabolism, but ketoconazole reduced production of both metabolites.

CYP3A4 produced MET 4 and this was inhibited in the presence of quercetin. The Sponsor states that data from ketoconazole were not available.

CYP2C8 and CYP2E1 did not metabolize TBZ in this assay.

The results are summarized in the table below where "X" indicates formation of the specific metabolite. Of the isozymes evaluated, MET 1 was formed only by CYP2C19 and MET X (Bis-O-dealkylated-TBZ) was formed only by CYP1A2. MET 4 and MET 7 were formed by multiple P450s.

	MET 1 (Bis-O-dealkylated HTBZ)	MET 4 (mono-hydroxy-TBZ)	MET 7 (O-dealkylated TBZ)	MET X (Bis-O-dealkylated-TBZ)
CYP1A2		X	X	X
CYP2A6	Isozyme not studied			
CYP2B6	Isozyme not studied			
CYP2C8				
CYP2C9			X	
CYP2C19	X	X	X	
CYP2D6		X	X	
CYP2E1				
CYP3A		X		

Human Whole Blood and Human Liver Cytosol, and Human Liver Microsomes

The rate of metabolism in human liver microsomes was 240 pmol/min/mg microsomal protein, in cytosol was 96 pmol/min/mg protein, and in human whole blood (hemolyzed) was 5 pmol/min.

In cytosol, TBZ was metabolized to α-HTBZ and β-HTBZ and MET 7. In human whole blood only α-HTBZ was formed (and this was only about 5% of total sample radioactivity).

Results of formation and inhibition studies are shown in the table below and summarized in the text below.

Table 6 **Rate of ^{14}C-Tetrabenazine (3 µM) metabolism and metabolite formation by pooled human liver microsomes, cytosol and whole human blood in the presence and absence of quercetin, furafylline and ketoconazole (Experiment 5)**

Human liver microsomes

Component	Solvent control (NaOH)	Quercetin 500 µM	Solvent control (Methanol)	Furafylline 10 µM	Ketoconazole 150 µM
β-HTBZ[1]	40.3	34.0	42.0	44.7	46.6
α-HTBZ[1]	56.7	46.2	61.9	56.4	85.6
MET 7[1]	42.5	3.00	46.4	15.8	6.98
TBZ[2]	232	152	240	198	203

Human liver cytosol

Component	Solvent control (NaOH)	Quercetin 500 µM	Solvent control (Methanol)	Furafylline 10 µM	Ketoconazole 150 µM
β-HTBZ[1]	8.74	6.14	8.94	9.58	4.10
α-HTBZ[1]	11.9	6.46	11.9	12.3	7.88
MET 7[1]	4.98	0.00	5.12	5.04	0.00
TBZ[2]	90.3	78.6	96.4	96.1	82.1

Whole human blood

Component	Solvent control (NaOH)	Quercetin 500 µM	Solvent control (Methanol)	Furafylline 10 µM	Ketoconazole 150 µM
α-HTBZ[3]	*	0.940	1.02	0.930	1.04
TBZ[4]	*	4.49	4.82	4.52	4.53

Data expressed as mean of duplicate incubations, * solvent control data (NaOH) not available for blood incubations
[1] Individual metabolite data expressed as pmol/min/mg protein, [2] TBZ data expressed as rate of TBZ metabolised pmol/min/mg protein, [3] Individual metabolite data from whole human blood expressed as pmol/min
[4] TBZ data from whole human blood expressed as rate of TBZ metabolised pmol/min
See Section 4.2 for key to metabolite identities

Inhibitors:

In human liver microsomes, quercetin 500 uM inhibited TBZ about 35% and formation of α-HTBZ and β-HTBZ about 16-19%. MET 7 formation was inhibited by approximately 93%.

In cytosol, quercetin inhibited TBZ metabolism by approximate 13%, and inhibited formation of α-HTBZ and β-HTBZ by approximately 46% and 30%, respectively. MET 7 formation was inhibited approximately 100%.

Formation of α-HTBZ was inhibited by quercetin < 10%.

In human liver microsomes, furafylline at 10 uM (approximately 13x > the reported Ki for CYP1A2 inhibition) had a mild inhibitory effect on TBZ metabolism (about 18%). MET 7 formation was inhibited by approximately 66%, and α-HTBZ and β-HTBZ were inhibited < 10%. (Metabolism of phenacetin to acetaminophen, used as a positive control reaction, was completely inhibited by furafylline)

In human liver cytosol, TBZ metabolism and metabolite formation were unaffected by furafylline.

Formation of α-HTBZ in whole blood was inhibited by furafylline < 10%.

Ketoconazole (150 uM) had a mild inhibitor effect on TBZ metabolism (15%) in human liver microsomes. MET 7 formation was inhibited by approximately 85%, and α-HTBZ and β-HTBZ formation were not inhibited.

In cytosol, ketoconazole had a mild inhibitory effect on TBZ metabolism (15%). Formation of α-HTBZ and β-HTBZ was inhibited by approximately 34% and 54%, respectively. MET 7 formation was inhibited by 100%.

Formation of α-HTBZ in whole blood was not inhibited by ketoconazole.

CONCLUSIONS and COMMENTS:

These experiments used1) individual recombinant human P450s and 2) chemical inhibitors in human liver microsomes and cytosol to characterize the pathways for metabolism of TBZ. (It should be noted that while recombinant P450s may indicate pathways involved in metabolism, they do not characterize the relative importance of each pathway. In addition, since a primary pathway of metabolism of TBZ is via non-P450-mediated reduction to α-HTBZ and β-HTBZ, the present evaluation may not be relevant as it emphasizes P450-mediated metabolism of TBZ).

TBZ is metabolized by microsomal and cytosolic carbonyl reductases to α-HTBZ and β-HTBZ, the major metabolites.

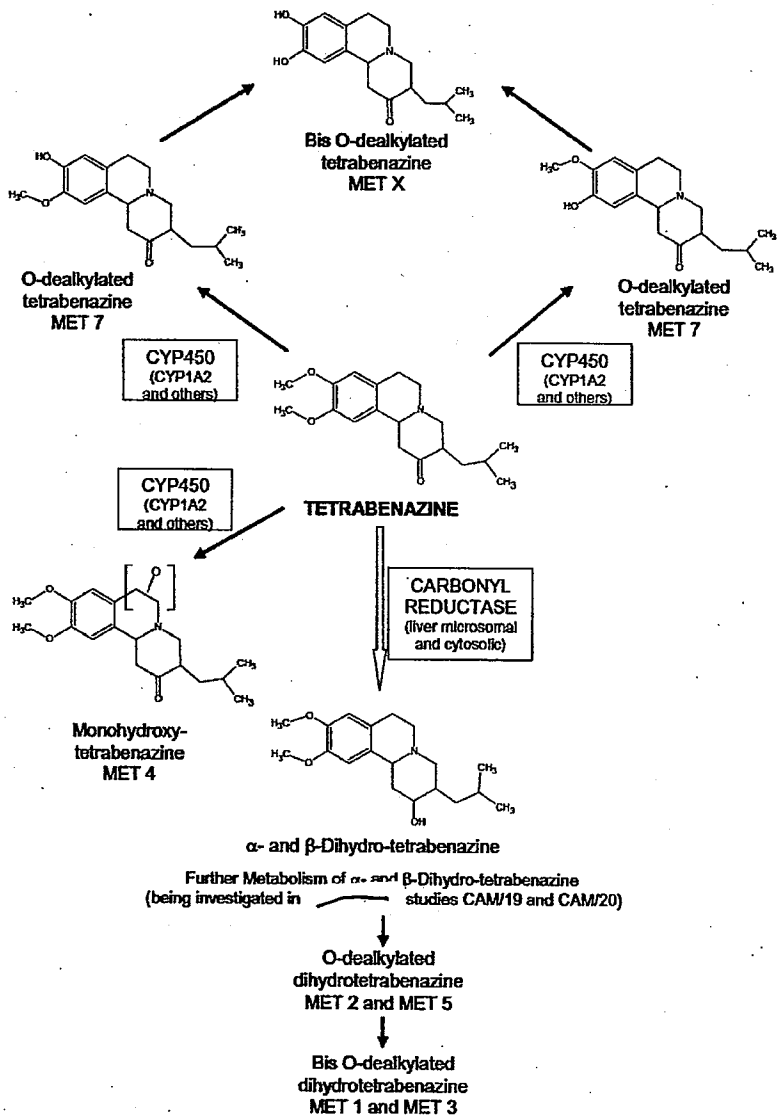
TBZ is metabolized by microsomal P450 to hydroxylated (MET 4) and O-dealkylated (MET 7) metabolites.

- MET 4 and MET 7 appeared to be minor metabolites, each accounting for < 10% of total radioactivity in these *in vitro* studies in human liver microsomes.
- Although CYP2D6 resulted in formation of MET 4 and MET 7 in expressed enzymes, quinidine did not show inhibition of TBZ metabolism in human liver microsomes.
- MET 7 formation was inhibited (approximately 66%) by the CYP1A2 inhibitor furafylline in human liver microsomes.

- MET X (bis-O-dealkylated TBZ) was identified as a metabolite formed by P450s but the Sponsor does not provide details on this metabolite, other than formation by CYP1A2.
- The Sponsor has incompletely characterized the pathways involved in formation of MET 7, with still approximately 40% of the P450 metabolism not accounted for by furafylline-mediated inhibition of CYP1A2. Expressed enzymes suggest a role for CYP2D6, 2C9, and 2C19. Specific chemical inhibitors have not been used to characterize the contribution of these pathways.

The results support the Sponsor's proposed biotransformation pathway shown below.

Figure 52 Proposed biotransformation pathway for ^{14}C-tetrabenazine in human liver microsomes



4.3 In Vitro TBZ Metabolism Study in Several Species

Comparative in vitro metabolism studies of ^{14}C – tetrabenazine (TBZ) with mouse, rat, dog, Cynomolgous monkey and human liver microsomes

Study Investigators and Site:

Protocol Number: CAM/26

OBJECTIVES:

To assess microsomal metabolism of TBZ in commonly encountered laboratory species and to compare these data with the human microsomal metabolism of TBZ.

(*Note:* only the human liver microsome data will be reviewed here).

METHODS:

Human Liver Microsomes

Pooled human liver microsomes were purchased from ——— and had been fully characterized by ——— for total P450 content and enzyme activities for the major human P450s (including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5).

^{14}C-TBZ (5 uM) was incubated with human liver microsomes (0.5 mg microsomal protein/ml incubation mixture) and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of co-factor solution (2 mM NADPH final concentration). At pre-determined time intervals following the initiation of the reaction (0, 5, 10, 20, 30, and 60 minutes), reactions were terminated by the addition of an aliquot to organic solvent (acetonitrile). Samples were analyzed by HPLC directly after centrifugation. Control incubations (conducted in parallel) were run in the absence of microsomal protein or co-factor solution to assess:
i) the relative non-metabolic stability of ^{14}C-TBZ in the incubation matrix.
ii) the extent of any non NADPH-dependant metabolism. All incubations were performed in singlicate at 37°C.

Assay

Samples were assayed by HPLC for TBZ and authentic reference standards ———

For metabolite identification, samples were analyzed by LC/MS/MS. Radiochromatograms were evaluated for metabolite formation and metabolism (disappearance of parent ^{14}C-TBZ). The amount of each metabolite formed and amount of parent ^{14}C-TBZ remaining were expressed as a percentage of total eluted sample radioactivity for each chromatogram. Metabolic rate was

determined based on a time point where the rates of metabolism/formation were considered to be linear.

RESULTS:

Composition of the metabolites, confirmed by LC/MS/MS is shown below, as provided by the Sponsor:

Component	Approximate retention time (min)*	Identity
MET 1	16.2	Multi-component constisting: Mono-hydroxy O-dealkylated tetrabenazine O-dealkylated dihydrotetrabenazine Bis-O-dealkylated dihydrotetrabenazine
MET 2	17.9	Mono-hydroxy tetrabenazine
MET 3 (β-HTBZ**)	18.8	Multi-component constisting: β-HTBZ Second isomer of mono-hydroxy tetrabenazine O-dealkylated dihydrotetrabenazine (First regioisomer)
α-HTBZ	22.1	Confirmed as α-HTBZ
MET 4	25.1	[redacted]
MET 5	27.9	Multi-component consisting: [redacted] O-dealkylated tetrabenazine
MET 6 [redacted]	34.3	[redacted]

* Retention times taken from LC-MS/MS analysis
** Major component

The major metabolites detected were MET 3, MET 5, and α-HTBZ that represented 11.3%, 10.21%, and 9.81% of total eluted sample radioactivity. These metabolites were formed as a result of O-dealkylation, hydroxylation, or reduction.

In comparing mouse, rat, and dog with human liver microsomes, there were no species-specific metabolites identified (although the Bis-O-dealkylated dihydrotetrabenazine was only seen in human and mouse).

All metabolites except MET 6 (stereoisomer that was formed non-enzymatically in the no protein control) were formed only in the presence of NADPH cofactor

CONCLUSIONS and COMMENTS:

TBZ undergoes NADPH-dependent Phase I metabolism in human liver microsomes, primarily mediated by O-dealkylation, hydroxylation, or reduction to metabolites alpha- and beta-dihydrotetrabenazine that undergo further oxidative metabolism.

4.4 In Vitro α-HTBZ Metabolism Study

Identification of the Major Drug Metabolizing Enzymes Involved in the Human Hepatic Metabolism of α –Dihydrotetrabenazine-^{14}C

Study Investigators and Site:

Protocol Number: CAM/19

OBJECTIVES:

To identify which enzyme or enzymes are responsible for metabolizing α-HTBZ in human liver microsomes.

METHODS:

Human Liver Microsomes

Both pooled and individual donor microsomes (16 individual donors) were used in this study. Microsomes prepared from a pool of n=50 donors were also used for preliminary experiments. Human liver microsomes were supplied by ——— Microsomes had been fully characterized by ——— for total P450 content and enzyme activities for the major human P450s (including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5).

Preliminary experiments were performed to establish the microsomal metabolism of α-HTBZ -^{14}C (1, 5, and 10 μM) and the linear reaction rates of metabolism with respect to microsomal protein (0.1, 0.5, 1, and 2 mg protein/ml incubation mixture) and incubation time (0, 10, 20, 30, and 60 minutes). Incubations were performed at 37 °C.

Correlation analysis was performed using individual donor microsomes. HTBZ -^{14}C was incubated with individual donor microsomes that had been previously characterized. Incubation conditions had been derived from the preliminary experiments to ensure linear metabolite reaction rates.

Incubation mixtures contained α-HTBZ-^{14}C (5 μM), pooled human liver microsomes (0.5 mg microsomal protein/ml incubation mixture) and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of co-factor solution. At pre-determined time intervals following the initiation of the reaction (30 minutes), reactions were terminated by the addition of organic solvent (acetonitrile). Samples were analyzed by HPLC directly after centrifugation. Control incubations (conducted in parallel) were run in the absence of microsomal protein or co-factor solution to assess:
i) the relative non-metabolic stability of α-HTBZ-^{14}C in the incubation matrix.
ii) the extent of any non NADPH-dependant metabolism.
All incubations were performed in duplicate at 37°C.

Chemical inhibitors were used to identify individual P450s responsible for metabolism. α-HTBZ-^{14}C was incubated with pooled human liver microsomes, phosphate buffer (100 mM, pH 7.4) and performed in the presence or absence of CYP450-selective chemical inhibitors as follows:

CYP1A2 activity Furafylline (final concentration of 10 μM)
CYP2C8 activity Quercetin (final concentration of 10 μM)
CYP2C9 activity Sulphaphenazole (final concentration of 30 μM)
CYP2C19 activity Ticlopidine (final concentration of 10 μM)
CYP2D6 activity Quinidine (final concentration of 5 μM)
CYP2E1 activity Diethyldithiocarbamate (final concentration of 50 μM)
CYP3A4 activity Ketoconazole (final concentration of 2 μM)

These concentrations are approximately 10x the Ki values of the specific inhibitors for the specific enzymes.

For the quercetin, sulphaphenazole, quinidine and ketoconazole incubations, incubation mixtures contained α-HTBZ-14C (final concentration of 5 μM), pooled human liver microsomes (0.5 mg/mL), chemical inhibitor and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of cofactor solution.

Furafylline, ticlopidine and diethyldithiocarbamate are mechanism-based inhibitors and therefore required pre-incubation with cofactor solution in order to generate the inhibitory species. Therefore, these incubations contained chemical inhibitor, pooled human liver microsomes (0.5 mg/mL), cofactor solution and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 20 minutes microsomal reactions were initiated by the addition of α-HTBZ-^{14}C (final concentration of 5 μM).

Control incubations (conducted in parallel) were run in the absence of microsomal protein, co-factor solution and chemical inhibitor (solvent blanks)

All incubations were performed in duplicate at 37°C. At pre-determined time intervals following the initiation of the reaction (30 minutes), reactions were terminated by the addition of ice-cold acetonitrile.

Expressed P450s were used to further characterize the isozymes involved in the metabolism of α-HTBZ. Recombinant cDNA expressed human cytochrome P450 enzymes [redacted] were derived from heterologous bacterial expression (Bactosomes, *E coli*) and expressed a single cytochrome P450 enzyme as shown below:

CYP1A2 Batch C1A2R005, protein concentration of 11.9 mg/mL (13.3 nmol P450/mL)
CYP2C8 Batch C2C8R002/A, protein concentration of 6.0 mg/mL (9.1 nmol P450/mL)
CYP2C9 Batch C2C9R009A, protein concentration of 11.7 mg/mL (5.1 nmol P450/mL)
CYP2C19 Batch C2C19R005, protein concentration of 14.5 mg/mL (5.5 nmol P450/mL)
CYP2D6 Batch C2D6R017, protein concentration of 26.8 mg/mL (3.8 nmol P450/mL)

CYP2E1 Batch C2E1R014, protein concentration of 6.6 mg/mL (4.8 nmol P450/mL)
CYP3A4 Batch C3A4R018B, protein concentration of 9.4 mg/mL (3.9 nmol P450/mL)

Incubation mixtures contained α-HTBZ-^{14}C (final concentration of 5 µM), cDNA expressed CYP450 microsomes (125 pmoles cytochrome P450/mL) and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of cofactor solution. Control incubations (conducted in parallel) were run in the absence of co-factor solution to assess the extent of any non NADPH-dependant metabolism. All incubations were performed in duplicate at 37°C.
Reactions were terminated as described above.

Assay
Samples were assayed by HPLC for ^{14}C –α-HTBZ and by LC-MS/MS for metabolites. Radiochromatograms were evaluated for metabolite formation and substrate disappearance.

RESULTS:

Preliminary Experiments

The radiolabeled components were identified as follows:

Component	Approximate retention time* (min)	Identity
MET 1	14.5	Mono-hydroxy- α-HTBZ**
MET 2	16.2	Mono-hydroxy- α-HTBZ**
MET 3	20.2	O-dealkylated- α-HTBZ
MET 4	20.7	Mono-hydroxy- α-HTBZ**

* Retention times taken from LC-MS/MS analysis
** Isomers of mono-hydroxylated-HTBZ (position/configuration of hydroxylation unknown)

Of these metabolites, the major metabolites detected were the mono-hydroxy-α-HTBZ (MET 2) and the O-dealkylated-α-HTBZ (MET 3) based on the chromatograms. No metabolite formation was evident in the no-cofactor control samples indicating that that the formation of MET 1 - 4 is catalyzed by cytochrome P450 enzymes. The no protein control samples indicated that α-HTBZ-^{14}C was chemically stable in the incubation matrix.

Metabolism of α-HTBZ-^{14}C and formation of metabolites MET 2 and MET 3 was linear up to a protein concentration of approximately 0.5 mg/ml. Therefore, this was the protein concentration chosen for subsequent experiments. Formation of MET 2 and MET 3 increased with increase in incubation time and was generally linear up to 60 minutes. The 30 minute incubation time selected therefore is under linear conditions.

Correlation Analysis
Radiochromatograms were evaluated for loss of parent and formation of MET 1-3. Formation of MET 4 was too low to accurately quantify for correlation analysis, according to the study report. Results were expressed as % of total eluted radioactivity present, and this was converted into

rates of metabolism of parent of formation of metabolite. An r^2 value of ≥ 0.7 was considered to be positive.

Inter-individual differences in the rate of parent metabolism and metabolite formation were identified in the donor data set, and the Sponsor refers to extensive, intermediate, and poor metabolizers of α-HTBZ-^{14}C being observed. However, neither α-HTBZ-^{14}C metabolism nor MET 1 formation correlated with any of the P450 selective activities. For MET 1 formation the best correlation was with CYP2D6 (r^2= 0.6491, significance (F =0.0002).

Formation of MET 2 (mono-hydroxy- α-HTBZ) correlated with testosterone-6B-hydroxylation (r^2= 0.9309), a marker for CYP3A4/5

Table 11 Correlation of MET 2 formation by individual donor microsomes with CYP450-isoform-selective activities

CYP450 isoform	Correlation coefficient (R^2)	Significance (F)	Significant/ Not significant
CYP1A2	0.0148	0.6535	NS
CYP2A6	0.2531	0.0470	NS
CYP2B6	0.3923	0.0094	NS
CYP2C8	0.1613	0.1231	NS
CYP2C9	0.0465	0.4224	NS
CYP2C19	0.4728	0.0032	NS
CYP2D6	0.1020	0.2279	NS
CYP2E1	0.0885	0.2631	NS
CYP3A4/5	**0.9309**	**0.0000**	**S**
CYP4A11	0.0559	0.3781	NS

0.0000 = <0.00005
R^2 = 0.7 to 1 possible correlation
S = significant (F < 0.01, 99% certainty in R^2)
NS = not significant
Data calculated using Microsoft Excel statistical software.

Formation of MET 3 was correlated with dextromethorphan O-demethylation, a marker for CYP2D6.

Table 12 Correlation of MET 3 formation by individual donor microsomes with CYP450-isoform-selective activities

CYP450 isoform	Correlation coefficient (R^2)	Significance (F)	Significant/ Not significant
CYP1A2	0.0849	0.2734	NS
CYP2A6	0.0390	0.4635	NS
CYP2B6	0.0027	0.8474	NS
CYP2C8	0.0768	0.2987	NS
CYP2C9	0.2757	0.0367	NS
CYP2C19	0.0532	0.3902	NS
CYP2D6	**0.8128**	**0.0000**	**S**
CYP2E1	0.2673	0.0403	NS
CYP3A4/5	0.0251	0.5575	NS
CYP4A11	0.2065	0.0770	NS

0.0000 = <0.00005
R^2 = 0.7 to 1 possible correlation
S = significant (F < 0.01, 99% certainty in R^2)
NS = not significant
Data calculated using Microsoft Excel statistical software.

Therefore, correlation analysis suggests the involvement of CYP2D6 and CYP3A in the metabolism of α-HTBZ and formation of its metabolites MET 2 and MET 3.

Chemical Inhibitors

Results from chemical inhibition studies in pooled human liver microsomes are shown in the tables below. The report does not discuss any results with either MET 1 or MET 4.

Table 13 **Metabolism of α-HTBZ-^{14}C in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% Inhibition [1]
+ furafylline (10 µM)	23.5	19.7	16.4
+ quercetin (10 µM)	23.5	23.4	0.40
+ sulphaphenazole (30 µM)	23.5	24.0	0.00
+ ticlopidine (10 µM)	23.5	14.1	40.0
+ quinidine (5 µM)	23.5	12.5	47.0
+ diethyldithiocarbamate (50 µM)	23.5	17.8	24.2
+ ketoconazole (2 µM)	23.5	12.0	48.9

Data expressed as mean (n = 2) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of α-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of α-HTBZ-^{14}C metabolism in the absence of inhibitor (negative values reported as 0.00)

Table 14 **Formation of MET 2 in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% Inhibition [1]
+ furafylline (10 µM)	9.95	9.46	4.90
+ quercetin (10 µM)	9.95	9.95	0.00
+ sulphaphenazole (30 µM)	9.95	8.89	10.6
+ ticlopidine (10 µM)	9.95	8.03	19.3
+ quinidine (5 µM)	9.95	9.44	5.10
+ diethyldithiocarbamate (50 µM)	9.95	8.33	16.3
+ ketoconazole (2 µM)	9.95	0.00	100

Data expressed as mean (n = 2) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of α-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of α-HTBZ-^{14}C metabolism in the absence of inhibitor

Table 15 **Formation of MET 3 in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% Inhibition [1]
+ furafylline (10 µM)	13.6	10.2	25.1
+ quercetin (10 µM)	13.6	13.5	0.00
+ sulphaphenazole (30 µM)	13.6	15.1	0.00
+ ticlopidine (10 µM)	13.6	6.08	55.3
+ quinidine (5 µM)	13.6	2.98	78.1
+ diethyldithiocarbamate (50 µM)	13.6	9.49	30.2
+ ketoconazole (2 µM)	13.6	12.0	11.7

Data expressed as mean (n = 2) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of α-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of α-HTBZ-^{14}C metabolism in the absence of inhibitor

These chemical inhibition results suggest involvement of the following enzymes (>25% inhibition):

	P450s involved (% inhibition)	**Comments**
Metabolism of α-HTBZ	CYP2D6 (47%), CYP3A (48.9%) (CYP2C19 (40%))*	*Ticlopidine was used as the inhibitor of CYP2C19. It inhibited α-HTBZ metabolism, but it is not selective for 2C19; according to the literature (also inhibits 2D6 and 2B6)
Formation of MET 2	CYP3A (100%)	
Formation of MET 3	CYP1A2 (25.1%) CYP2C19 (55.3%)* CYP2D6 (78.1%) CYP2E1 (30.2%)**	*Ticlopidine was used as the inhibitor of CYP2C19. It inhibited MET 3 formation, but it is not selective for 2C19; according to the literature (also inhibits 2D6 and 2B6) **The Sponsor states that diethyldithiocarbamate has limited selectivity for CYP2E1; Draper et al in Archives of Biochemistry and biophysics (1997; 341:47-61) reports a Ki for inhibition of CYP2A6 that is approximately the same as the concentration used here.

Expressed P450s

Quantitative results were not provided. The results of incubations with expressed P450 are as follows.

CYP1A2 catalyzed the formation of MET3.
CYP2D6 catalyzed formation of MET 3; smaller amounts of MET 1 and MET 4 were formed.
CYP3A4 primarily catalyzed the formation of MET 2.

CYP2C8, CYP2C9, CYP2C19, and CYP2E1 did not metabolize α-HTBZ appreciably.

CYP2B6 was not evaluated.

CONCLUSIONS and COMMENTS:

- α-HTBZ metabolism mediated primarily by CYP2D6 (47%) and CYP3A (48.9%) in chemical inhibition studies
  - Cannot rule out CYP2C19
- MET 2 (monohydroxy α-HTBZ) and MET 3 (O-dealklyated α-HTBZ) were the primary metabolites formed from α-HTBZ in human liver microsomes *in vitro*.
  - MET 1 was also formed
  - MET 4 formation was too low to quantify
- Formation of MET 2 is mediated by CYP3A (100% in chemical inhibition studies)
- Formation of MET 3 is mediated primarily by CYP2D6 (shown in correlation studies and >78% in chemical inhibition studies) and by CYP1A2 (>25% in chemical inhibition studies)
  - Cannot rule out CYP2C19 or CYP2E1
- CYP 2C8 and CYP2C9 not appreciably involved in metabolism of α-HTBZ.
- The role of CYP2B6 has not been evaluated.

RECOMMENDATION

- Since monohydroxy HTBZ (formed by CYP3A) was not detected in the plasma in the mass balance study and accounted for < 1% and < 5% in fecal and urine extracts, respectively (as % of dose administered), in vivo drug interaction studies with CYP3A inhibitors need not be pursued.

- Need to conduct *in vivo* studies with strong inhibitor of CYP2D6 and 1A2 to determine impact on O-dealkylated α-HTBZ since O-dealkylated HTBZ has been detected as the major circulating component in plasma.

- The role of CYP2B6 in the metabolism of α-HTBZ should be evaluated *in vitro*.

4.5 In Vitro β-HTBZ Metabolism Study

Identification of the Major Drug Metabolizing Enzymes Involved in the Human Hepatic Metabolism of β –Dihydrotetrabenazine-^{14}C

Study Investigators and Site:

Protocol Number: CAM/20

OBJECTIVES:

To identify which enzyme or enzymes are responsible for metabolizing β -HTBZ in human liver microsomes.

METHODS:

Human Liver Microsomes

Both pooled and individual donor microsomes (16 individual donors) were used in this study. Microsomes prepared from a pool of n=50 donors were also used for preliminary experiments. Human liver microsomes were supplied by [redacted]. Microsomes had been fully characterized by [redacted] for total P450 content and enzyme activities for the major human P450s (including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5).

Preliminary experiments were performed to establish the microsomal metabolism of β -HTBZ -^{14}C (1, 5, and 10 μM) in pooled human liver microsomes (0.1-2 mg/ml) to optimize the incubation conditions (linear metabolite formation) for subsequent incubation with liver microsomes from individual donors (correlation analysis). Microsomal protein concentrations were 0.1, 0.5, 1, and 2 mg protein/ml incubation mixture and incubation times were 0, 10, 20, 30, and 60 minutes. Incubations were performed at 37 °C.

Correlation analysis was performed using individual donor microsomes. HTBZ -^{14}C was incubated with individual donor microsomes that had been previously characterized. Incubation conditions had been derived from the preliminary experiments to ensure linear metabolite reaction rates.

Incubation mixtures contained β -HTBZ-^{14}C (5 μM), pooled human liver microsomes (0.5 mg microsomal protein/ml incubation mixture) and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of co-factor solution. At pre-determined time intervals following the initiation of the reaction (30 minutes), reactions were terminated by the addition of organic solvent (acetonitrile). Samples were analyzed by HPLC directly after centrifugation. Control incubations (conducted in parallel) were run in the absence of microsomal protein or co-factor solution to assess:
i) the relative non-metabolic stability of β-HTBZ-^{14}C in the incubation matrix.
ii) the extent of any non NADPH-dependant metabolism.

All incubations were performed in duplicate at 37°C.

Chemical inhibitors were used to identify individual P450s responsible for metabolism. β -HTBZ-^{14}C was incubated with pooled human liver microsomes, phosphate buffer (100 mM, pH 7.4) and performed in the presence or absence of CYP450-selective chemical inhibitors as follows:

CYP1A2 activity	Furafylline (final concentration of 10 μM)
CYP2C8 activity	Quercetin (final concentration of 10 μM)
CYP2C9 activity	Sulphaphenazole (final concentration of 30 μM)
CYP2C19 activity	Ticlopidine (final concentration of 10 μM)
CYP2D6 activity	Quinidine (final concentration of 5 μM)
CYP2E1 activity	Diethyldithiocarbamate (final concentration of 50 μM)
CYP3A4 activity	Ketoconazole (final concentration of 2 μM)

These concentrations are approximately 10x the Ki values of the specific inhibitors for the specific enzymes.

For the quercetin, sulphaphenazole, quinidine and ketoconazole incubations, incubation mixtures contained β -HTBZ-^{14}C (final concentration of 5 μM), pooled human liver microsomes (0.5 mg/mL), chemical inhibitor and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of cofactor solution.

Furafylline, ticlopidine and diethyldithiocarbamate are mechanism-based inhibitors and therefore required pre-incubation with cofactor solution in order to generate the inhibitory species. Therefore, these incubations contained chemical inhibitor, pooled human liver microsomes (0.5 mg/mL), cofactor solution and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 20 minutes microsomal reactions were initiated by the addition of β -HTBZ-^{14}C (final concentration of 5 μM).

Control incubations (conducted in parallel) were run in the absence of microsomal protein, co-factor solution and chemical inhibitor (solvent blanks).

All incubations were performed in duplicate at 37°C. At pre-determined time intervals following the initiation of the reaction (30 minutes), reactions were terminated by the addition of ice-cold acetonitrile.

Expressed P450s were used to further characterize the isozymes involved in the metabolism of β-HTBZ. Recombinant cDNA expressed human cytochrome P450 enzymes were derived from heterologous bacterial expression (Bactosomes, *E coli*) and expressed a single cytochrome P450 enzyme as shown below:

CYP1A2 Batch C1A2R005, protein concentration of 11.9 mg/mL (13.3 nmol P450/mL)
CYP2C8 Batch C2C8R002/A, protein concentration of 6.0 mg/mL (9.1 nmol P450/mL)
CYP2C9 Batch C2C9R009A, protein concentration of 11.7 mg/mL (5.1 nmol P450/mL)
CYP2C19 Batch C2C19R005, protein concentration of 14.5 mg/mL (5.5 nmol P450/mL)

CYP2D6 Batch C2D6R017, protein concentration of 26.8 mg/mL (3.8 nmol P450/mL)
CYP2E1 Batch C2E1R014, protein concentration of 6.6 mg/mL (4.8 nmol P450/mL)
CYP3A4 Batch C3A4R018B, protein concentration of 9.4 mg/mL (3.9 nmol P450/mL)

Incubation mixtures contained β-HTBZ-^{14}C (final concentration of 5 μM), cDNA expressed CYP450 microsomes (125 pmoles cytochrome P450/mL) and phosphate buffer (100 mM, pH 7.4). Following a pre-incubation period of 5 minutes, microsomal reactions were initiated by the addition of cofactor solution. Control incubations (conducted in parallel) were run in the absence of co-factor solution to assess the extent of any non NADPH-dependant metabolism. All incubations were performed in duplicate at 37°C.
Reactions were terminated as described above.

Assay
Samples were assayed by HPLC for ^{14}C –β-HTBZ and by LC-MS/MS for metabolites. Radiochromatograms were evaluated for metabolite formation and substrate disappearance.

RESULTS:

Preliminary Experiments
The radiolabeled components were identified as follows:

Component	Approximate retention time (min)*	Identity
MET 1	12.7	Mono-hydroxy-β-HTBZ**
MET 2	16.8	O-dealkylated-β-HTBZ
MET 3	17.4	Multi-component comprising of: • An isomer of mono-hydroxy-β-HTBZ** • A second regioisomer of O-dealkylated-β-HTBZ

* Retention times taken from LC-MS/MS analysis
** isomer of mono-hydroxylated-HTBZ (position/configuration of hydroxylation unknown)

Of these metabolites, the major metabolite detected was MET 2 (the O-dealkylated-β-HTBZ). (A quantitative analysis of this experiment is not provided). No metabolite formation was evident in the no-cofactor control samples indicating that the formation of MET 1 - 3 is catalyzed by cytochrome P450 enzymes. The no protein control samples indicated that β -HTBZ-^{14}C was chemically stable in the incubation matrix.

Metabolism of β -HTBZ-^{14}C/ metabolism formation was linear up to a protein concentration of approximately 0.5 mg/ml. Therefore, this was the protein concentration chosen for subsequent experiments. Formation of metabolites increased with increase in incubation time and was generally linear up to 60 minutes. The 30 minute incubation time selected therefore is under linear conditions.

Correlation Analysis
Radiochromatograms were evaluated for loss of parent and formation of MET 1-3. Formation of MET 4 was too low to accurately quantify for correlation analysis, according to the study report.

Results were expressed as % of total eluted radioactivity present, and this was converted into rates of metabolism of parent of formation of metabolite. An r^2 value of ≥ 0.7 for correlation with P450-selective activity was considered to be positive.

Inter-individual differences in the rate of parent metabolism and metabolite formation were identified in the donor data set, and the Sponsor refers to extensive, intermediate, and poor metabolizers of β-HTBZ-^{14}C being observed.

The results of the correlation studies are summarized in the tables below. For β-HTBZ-^{14}C metabolism and for formation of MET 2 (O-dealkylated metabolite) and MET 3 there was a significant correlation with CYP2D6 activity. For MET 1 (mono-hydroxy metabolite) formation there was a significant correlation with testosterone -6β hydroxylation that is indicative of CYP3A4/5 metabolism.

Table 9 Correlation of β-HTBZ-^{14}C metabolism by individual donor microsomes with CYP450-selective activities

CYP450 enzyme	Correlation coefficient (R^2)	Significance (F)	Significant/ Not significant
CYP1A2	0.1067	0.2169	NS
CYP2A6	0.0372	0.4739	NS
CYP2B6	0.0344	0.4917	NS
CYP2C8	0.1446	0.1463	NS
CYP2C9	0.3483	0.0161	NS
CYP2C19	0.0287	0.5307	NS
CYP2D6	**0.7985**	**0.0000**	**S**
CYP2E1	0.2519	0.0476	NS
CYP3A4/5	0.0015	0.8878	NS
CYP4A11	0.2142	0.0711	NS

0.0000 = <0.00005
R^2 = 0.7 to 1 possible correlation
S = significant (F < 0.01, 99% certainty in R^2)
NS = not significant
Data calculated using Microsoft Excel statistical software.

Table 10 Correlation of MET 1 formation by individual donor microsomes with CYP450-selective activities

CYP450 enzyme	Correlation coefficient (R^2)	Significance (F)	Significant/ Not significant
CYP1A2	0.0000	0.9750	NS
CYP2A6	0.1819	0.0995	NS
CYP2B6	0.4484	0.0045	NS
CYP2C8	0.3132	0.0242	NS
CYP2C9	0.1683	0.1145	NS
CYP2C19	0.3888	0.0098	NS
CYP2D6	0.0098	0.7147	NS
CYP2E1	0.0007	0.9224	NS
CYP3A4/5	**0.7215**	**0.0000**	**S**
CYP4A11	0.0030	0.8408	NS

Table 11 Correlation of MET 2 formation by individual donor microsomes with CYP450-selective activities

CYP450 enzyme	Correlation coefficient (R^2)	Significance (F)	Significant/ Not significant
CYP1A2	0.1157	0.1974	NS
CYP2A6	0.0774	0.2968	NS
CYP2B6	0.0059	0.7781	NS
CYP2C8	0.0913	0.2565	NS
CYP2C9	0.2814	0.0345	NS
CYP2C19	0.0800	0.2886	NS
CYP2D6	**0.8629**	**0.0000**	**S**
CYP2E1	0.2949	0.0297	NS
CYP3A4/5	0.0431	0.4402	NS
CYP4A11	0.2465	0.0505	NS

0.0000 = <0.00005
R^2 = 0.7 to 1 possible correlation
S = significant (F < 0.01, 99% certainty in R^2)
NS = not significant
Data calculated using Microsoft Excel statistical software.

Table 12 Correlation of MET 3 formation by individual donor microsomes with CYP450-selective activities

CYP450 enzyme	Correlation coefficient (R^2)	Significance (F)	Significant/ Not significant
CYP1A2	0.1126	0.2038	NS
CYP2A6	0.0744	0.3066	NS
CYP2B6	0.0053	0.7889	NS
CYP2C8	0.0839	0.2766	NS
CYP2C9	0.3497	0.0158	NS
CYP2C19	0.0917	0.2541	NS
CYP2D6	**0.7613**	**0.0000**	**S**
CYP2E1	0.2326	0.0585	NS
CYP3A4/5	0.0176	0.6244	NS
CYP4A11	0.2282	0.0613	NS

0.0000 = <0.00005
R^2 = 0.7 to 1 possible correlation
S = significant (F < 0.01, 99% certainty in R^2)
NS = not significant
Data calculated using Microsoft Excel statistical software.

Therefore, correlation analysis suggests that the metabolism of and formation of its metabolites is associated with CYP2D6 (for β-HTBZ, MET 2, and MET 3) and CYP3A (MET 1).

Chemical Inhibitors

Results from chemical inhibition studies in pooled human liver microsomes are shown in the tables below. Ticlopidine was used as a 2C19 inhibitor and had a mild inhibitory effect on β-

HTBZ metabolism and formation of MET 2 and MET 3; however it has also been shown to inhibit CYP2D6 and therefore these results cannot be definitive. CYP2D6 inhibition by quinidine inhibited β-HTBZ metabolism, and inhibition of MET 2 and MET 3 formation (100%) and inhibited formation of MET 1 by approximately 45%. Diethyldithiocarbamate (CYP2E1 inhibitor) had a mild inhibitory effect on β-HTBZ metabolism and formation of MET1, MET 2, and MET 3. Ketoconazole, a CYP3A inhibitor, had a mild inhibitor effect on β-HTBZ metabolism and formation of MET 2 and MET 3, and inhibited MET 1 formation approximately 59%.

Table 13 **Metabolism of β-HTBZ-^{14}C in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% Inhibition [1]
+ furafylline (10 µM)	33.8	29.0	14.2
+ quercetin (10 µM)	33.8	34.3	0.00
+ sulphaphenazole (30 µM)	33.8	35.8	0.00
+ ticlopidine (10 µM)	33.8	22.2	34.3
+ quinidine (5 µM)	33.8	3.63	89.3
+ diethyldithiocarbamate (50 µM)	33.8	27.0	20.1
+ ketoconazole (2 µM)	33.8	25.1	25.7

Data expressed as mean ($n = 2$) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of β-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of β-HTBZ-^{14}C metabolism in the absence of inhibitor (negative values reported as 0.00)

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Table 14 **Formation of MET 1 in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% Inhibition [1]
+ furafylline (10 µM)	6.61	5.75	13.1
+ quercetin (10 µM)	6.61	6.49	1.90
+ sulphaphenazole (30 µM)	6.61	6.89	0.00
+ ticlopidine (10 µM)	6.61	5.28	20.1
+ quinidine (5 µM)	6.61	3.63	45.1
+ diethyldithiocarbamate (50 µM)	6.61	5.83	11.9
+ ketoconazole (2 µM)	6.61	2.70	59.2

Data expressed as mean (n = 2) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of β-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of β-HTBZ-^{14}C metabolism in the absence of inhibitor (negative values reported as 0.00)

Table 15 **Formation of MET 2 in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% Inhibition [1]
+ furafylline (10 µM)	20.8	18.2	12.7
+ quercetin (10 µM)	20.8	21.7	0.00
+ sulphaphenazole (30 µM)	20.8	22.8	0.00
+ ticlopidine (10 µM)	20.8	13.2	36.5
+ quinidine (5 µM)	20.8	0.00	100
+ diethyldithiocarbamate (50 µM)	20.8	16.5	20.9
+ ketoconazole (2 µM)	20.8	17.8	14.4

Data expressed as mean (n = 2) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of β-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of β-HTBZ-^{14}C metabolism in the absence of inhibitor (negative values reported as 0.00)

Table 16 **Formation of MET 3 in the presence/absence of CYP450-selective chemical inhibitors**

Sample type	Rate of metabolism without inhibitor	Rate of metabolism in presence of inhibitor	% inhibition [1]
+ furafylline (10 µM)	6.28	5.06	19.4
+ quercetin (10 µM)	6.28	6.14	2.30
+ sulphaphenazole (30 µM)	6.28	6.08	3.50
+ ticlopidine (10 µM)	6.28	3.75	40.4
+ quinidine (5 µM)	6.28	0.00	100
+ diethyldithiocarbamate (50 µM)	6.28	4.75	24.4
+ ketoconazole (2 µM)	6.28	4.58	27.1

Data expressed as mean (n = 2) rate of metabolism (pmoles/min/mg protein)
[1] based upon rate of β-HTBZ-^{14}C metabolism in the presence of inhibitor expressed as a % of the rate of β-HTBZ-^{14}C metabolism in the absence of inhibitor (negative values reported as 0.00)

These chemical inhibition results suggest involvement of the following enzymes (>25% inhibition):

	P450s involved (% inhibition)	Comments
Metabolism of β-HTBZ	CYP2D6 (89%), CYP3A (25.7%) (CYP2C19 (34.3%))*	*Ticlopidine was used as the inhibitor of CYP2C19. It inhibited β -HTBZ metabolism, but it is not selective for 2C19; according to the literature (also inhibits 2D6 and 2B6)
Formation of MET 1	CYP2D6 (45.1%) CYP3A (59.2%)	
Formation of MET 2	CYP2D6 (100%) (CYP2C19 (36.5%))*	*Ticlopidine was used as the inhibitor of CYP2C19. It inhibited β -HTBZ metabolism, but it is not selective for 2C19; according to the literature (also inhibits 2D6 and 2B6)
Formation of MET 3	CYP2D6 (100%) CYP3A (27.1%) CYP2C19 (40.4%)*	*Ticlopidine was used as the inhibitor of CYP2C19. It inhibited MET 3 formation, but it is not selective for 2C19; according to the literature (also inhibits 2D6 and 2B6)

Expressed P450s
Quantitative results were not provided. The results of incubations with expressed P450 are as follows.
CYP1A2 catalyzed the formation of minor amounts of M2.
CYP2C19 produced minor amounts of MET 2 and MET 1.
CYP2D6 extensively metabolized β-HTBZ and produced MET 2 and MET3 as major metabolites and minor amounts of MET 1.
CYP3A4 catalyzed the formation of MET 1 as a major metabolite.

CYP2C8, CYP2C9, and CYP2E1 did not metabolize β-HTBZ appreciably.

CYP2B6 was not evaluated.

CONCLUSIONS and COMMENTS:

- β-HTBZ metabolism is mediated primarily by CYP2D6 and CYP3A
  - Cannot rule out CYP2C19
- MET 2 (O-dealkylated β-HTBZ) was the major metabolite and MET 1 (mono-hydroxy β-HTBZ) and MET 3 (multicomponent comprised of isomers of MET 1 and MET 2 that have not been resolved) were minor metabolites formed from β-HTBZ in human liver microsomes *in vitro*.
- Formation of MET 2 is mediated primarily by CYP2D6 (in chemical inhibition studies, correlation studies, and expressed P450s)
  - CYP1A2 and CYP2C19 cannot be ruled out
- Formation of MET 1 is mediated primarily by CYP3A
  - CYP2D6 plays a role
  - Cannot rule out CYP2C19 or CYP2E1
- Formation of MET 3 is mediated primarily by CYP2D6
  - CYP3A plays a lesser role
  - CYP2C19 cannot be ruled out
- The role of CYP2B6 has not been extensively evaluated, although correlation studies do not suggest a role.

RECOMMENDATION

- Since monohydroxy HTBZ was not detected in the plasma in the mass balance study and accounted for < 1% and < 5% in fecal and urine extracts, respectively (as % of dose administered), *in vivo* drug interaction studies with β-monohydroxy HTBZ need not be pursued.
- Need to conduct *in vivo* studies with strong inhibitor of CYP2D6 to determine impact on metabolism of HTBZ since its metabolite O-dealkylated HTBZ has been detected as the major circulating component in plasma.

4.6 In Vitro P450 Induction Study

***In Vitro* Evaluation of Tetrabenazine, α-Dihydrotetrabenazine and β-Dihydrotetrabenazine as Inducers of Cytochrome P450 Expression in Cultured human Hepatocytes**

Study Investigators and Site:

Protocol Number: 063015

OBJECTIVES:

To investigate the effect of tetrabenazine (TBZ), α-dihydrotetrabenazine (α-OH-TBZ), and β-dihydrotetrabenazine (β -OH-TBZ) on the expression of Cytochrome P450 enzymes in primary cultures of human hepatocytes.

METHODS:

Hepatocytes were isolated and cultured from 3 individual donor livers. Cultures were allowed to adapt to the culture environment for 3 days, replacing media daily. After this period, hepatocytes were examined under a light microscope and determined to be morphologically normal.

Hepatocytes were treated daily for 3 consecutive days in the presence of supplemented MCM media containing 0.1% DMSO (vehicle, negative control), one of three concentrations of tetrabenazine, α-OH-TBZ , or β-OH-TBZ (0.1, 1.0, or 10 μM) or one of three known human P450 inducers (positive controls) that were omeprazole (100 μM), Phenobarbital (750 μM), or rifampin (10 μM). The plasma concentrations of tetrabenazine that would be expected are << .1μM and would be up to 0.4 μM in hepatic impairment; the concentrations of metabolites used cover up to 10x the expected plasma concentrations of those metabolites in humans, based on previous OCP review of the original NDA. Therefore these concentrations are acceptable. and its metabolites cover the therapeutic range. The concentrations of omeprazole and rifampin are acceptable for CYP1A2 and for CYP3A4, respectively with 14-24x induction and 4-31X induction expected for each of those enzymes, respectively. Phenobarbital at those concentrations would be expected to induce CYP2B6, 2C8, 2C9, and 3A4.

After approximately 24 hours following the final treatment, cultures were visualized to document morphological integrity.

Microsomal samples were prepared to use for evaluation of enzyme activity. Microsomal incubations were carried out at 37° C with the following probe substrates at the concentrations indicated. Recommended protein concentrations for microsomal studies are typically < 1 mg/ml and therefore these conditions are acceptable. The probe substrates are those recommended in

the draft guidance, with measurement of the metabolite for each substrate also as recommended in the guidance. The substrate concentrations are higher than the Km values reported in the draft Guidance for CYP3A (Km for testosterone 52-94 μM) and other substrates except for paclitaxel for CYP2C8 and phenacetin for CYP1A2. The Investigators state in the study report that the experimental conductions exhibited acceptable linearity for both incubation time and protein concentration.

Assay Conditions Table

Enzyme	Substrate	Substrate concentration (μM)	Quantity of protein (mg)	Final volume (mL)	Time (min)
CYP1A2	Phenacetin	80	0.016	0.4	10
CYP2B6	Bupropion	500	0.032	0.4	10
CYP2C8	Paclitaxel	10	0.025	0.4	10
CYP2C9	Diclofenac	100	0.008	0.4	10
CYP2C19	*S*-Mephenytoin	400	0.032	0.4	10
CYP3A4/5	Testosterone	250	0.008	0.4	10

CYP Enzyme	Measurement
CYP1A2	Phenacetin *O*-dealkylation
CYP2B6	Bupropion hydroxylation
CYP2C8	Paclitaxel 6α-hydroxylation
CYP2C9	Diclofenac 4′-hydroxylation
CYP2C19	*S*-Mephenytoin 4′-hydroxylation
CYP3A4/5	Testosterone 6β-hydroxylation

RESULTS:

Results of the effects of control inducers or study drug on enzyme activity are shown in the Tables below, as provided by the Sponsor.

Table 3: Effects of treating cultured human hepatocytes with tetrabenazine, α-dihydrotetrabenazine or β-dihydrotetrabenazine or prototypical inducers on the expression of cytochrome P450 enzymes

Treatment	Concentration	Enzymatic activity (pmol/mg protein/min)*					
		Phenacetin *O*-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Paclitaxel 6α-hydroxylation (CYP2C8)	Diclofenac 4′-hydroxylation (CYP2C9)	*S*-Mephenytoin 4′-hydroxylation (CYP2C19)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl Sulfoxide	0.1%	43.6 ± 11.0	25.1 ± 4.6	9.63 ± 6.83	972 ± 434	10.2 ± 10.7	1880 ± 370
Terrabenazine	0.1 μm	43.8 ± 10.9	26.3 ± 4.9	10.6 ± 7.3	1030 ± 520	10.3 ± 10.4	2150 ± 470
Tetrabenazine	1.0 μm	42.3 ± 11.7	27.0 ± 4.4	9.45 ± 6.21	920 ± 456	9.37 ± 8.67	2100 ± 430
Tetrabenazine	10 μm	44.5 ± 12.2	29.3 ± 5.2	10.4 ± 7.5	902 ± 507	7.16 ± 5.98	2140 ± 460
α-Dihydrotetrabenazine	0.1 μm	38.6 ± 9.3	24.5 ± 5.4	8.43 ± 5.79	828 ± 434	13.3 ± 15.7	1900 ± 210
α-Dihydrotetrabenazine	1.0 μm	41.5 ± 8.5	24.4 ± 5.6	8.21 ± 4.47	956 ± 534	10.0 ± 10.1	2110 ± 470
α-Dihydrotetrabenazine	10 μm	44.2 ± 12.6	31.1 ± 7.9	10.2 ± 6.0	958 ± 555	9.53 ± 9.20	2510 ± 490
β-Dihydrotetrabenazine	0.1 μm	43.7 ± 17.0	27.2 ± 9.3	9.60 ± 7.83	861 ± 497	8.87 ± 8.49	1950 ± 180
β-Dihydrotetrabenazine	1.0 μm	43.4 ± 15.7	28.7 ± 11.7	9.58 ± 7.28	922 ± 531	4.15 (n=2)	2010 ± 290
β-Dihydrotetrabenazine	10 μm	41.8 ± 15.7	28.1 ± 10.8	9.33 ± 6.71	804 ± 426	6.28 ± 5.16	2080 ± 120
Omeprazole	100 μm	1450 ± 560	227 ± 163	26.1 ± 20.0	1620 ± 760	9.91 ± 4.39	5150 ± 1250
Phenobarbital	750 μm	80.9 ± 18.1	382 ± 276	54.3 ± 29.7	1810 ± 960	23.3 ± 14.3	11400 ± 1200
Rifampin	10 μm	69.6 ± 10.1	283 ± 180	57.9 ± 24.8	2130 ± 1140	63.2 ± 43.5	11100 ± 1100

Rates are rounded to three significant figures and standard deviation is rounded to the same degree of precision.
* Values are the mean ± standard deviation of three of human hepatocyte preparations: H666, H672 and H681, unless otherwise indicated (*i.e.*, n=2).

Table 4: Effects of treating cultured human hepatocytes with tetrabenazine, α-dihydrotetrabenazine or β-dihydrotetrabenazine or prototypical inducers on the fold induction of cytochrome P450 enzymes

Treatment	Concentration	Fold Induction [a]					
		Phenacetin O-dealkylation § (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Paclitaxel 6α-hydroxylation § (CYP2C8)	Diclofenac 4'-hydroxylation (CYP2C9)	S-Mephenytoin 4'-hydroxylation * (CYP2C19)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl Sulfoxide	0.1%	1.00 ± 0.25 [b]	1.00 ± 0.18 [b]	1.00 ± 0.71 [b]	1.00 ± 0.45 [b]	1.00 ± 1.05 [b]	1.00 ± 0.20 [b]
Tetrabenazine	0.1 μm	1.01 ± 0.08	1.05 ± 0.14	1.09 ± 0.18	1.03 ± 0.11	1.04 ± 0.11	1.14 ± 0.07
Tetrabenazine	1.0 μm	0.966 ± 0.062	1.08 ± 0.04	0.998 ± 0.100	0.926 ± 0.106	1.01 ± 0.25	1.12 ± 0.04
Tetrabenazine	10 μm	1.02 ± 0.08	1.17 ± 0.01	1.07 ± 0.10	0.875 ± 0.180	0.822 ± 0.173	1.15 ± 0.13
α-Dihydrotetrabenazine	0.1 μm	0.890 ± 0.088	0.971 ± 0.045	0.884 ± 0.021	0.821 ± 0.121	1.16 ± 0.24	1.02 ± 0.09
α-Dihydrotetrabenazine	1.0 μm	0.965 ± 0.132	0.982 ± 0.202	0.912 ± 0.156	0.945 ± 0.215	1.01 ± 0.21	1.12 ± 0.07
α-Dihydrotetrabenazine	10 μm	1.02 ± 0.18	1.23 ± 0.15	1.11 ± 0.21	0.937 ± 0.240	0.998 ± 0.199	1.34 ± 0.11
β-Dihydrotetrabenazine	0.1 μm	0.976 ± 0.174	1.06 ± 0.19	0.937 ± 0.174	0.844 ± 0.213	0.934 ± 0.160	1.06 ± 0.23
β-Dihydrotetrabenazine	1.0 μm	0.974 ± 0.130	1.11 ± 0.28	0.956 ± 0.235	0.890 ± 0.211	1.03 (n=2)	1.10 ± 0.27
β-Dihydrotetrabenazine	10 μm	0.935 ± 0.157	1.09 ± 0.25	0.955 ± 0.131	0.793 ± 0.117	0.729 ± 0.168	1.14 ± 0.23
Omeprazole	100 μm	32.6 ± 8.3	9.02 ± 5.57	2.60 ± 0.55	1.66 ± 0.18 †	2.02 ± 1.77	2.92 ± 1.31
Phenobarbital	750 μm	1.87 ± 0.15	15.3 ± 9.8 †	6.01 ± 1.35	1.81 ± 0.26 †	3.16 ± 1.65	6.20 ± 1.15 †
Rifampin	10 μm	1.64 ± 0.28	11.5 ± 6.8 †	6.78 ± 1.74	2.15 ± 0.37 †	8.15 ± 3.92	6.03 ± 0.71 †

Fold inductions are rounded to three significant figures and standard deviation is rounded to the same degree of precision.

[a] Values are the mean ± standard deviation of three human hepatocyte preparations: H666, H672 and H681, unless otherwise indicated (*i.e.*, n=2).

[b] For Vehicle Controls, CV, Coefficient of Variance (Rate Standard Deviation ÷ Mean Rate), is calculated instead of Fold Increase Standard Deviation to give a more realistic representation of variance between control samples"

§ Significance found among treatment groups (where 0.1% DMSO is the vehicle control) according to Kruskal-Wallis One Way Analysis on Ranks ($p < 0.05$) but unable to specify the groups that statistically differ from the other groups according to Dunnett's Method with positive controls.

† Statistically significant compared to control (0.1% DMSO) according to Dunnett's Test ($p < 0.05$) with positive controls.

* Significance found among treatment groups (where 0.1% DMSO is the vehicle control) according to Kruskal-Wallis One Way Analysis on Ranks ($p < 0.05$) but unable to specify the groups that statistically differ from the other groups according to Dunn's Method with positive controls.

Omeprazole and rifampin produced the expected induction of CYP1A2 and CYP3A4, respectively. In contrast, there was no significant induction of CYP1A2 by tetrabenazine or its metabolites α-OH-TBZ or β-OH-TBZ. Calculated by the reviewer as recommended in the draft Guidance where % positive control = 100*(activity of test drug treated cells-activity of negative control)/(activity of positive control-activity of negative control), tetrabenazine, α-OH-TBZ, and β-OH-TBZ caused a change that was < 10% of the positive control, whereas a change greater than 40% of the positive control would suggest induction.

Since there is no induction of CYP1A2 and CYP3A it can be concluded that the test drug is also not an inducer of CYP2C8, CYP2C9, or CYP2C19. This has been confirmed by the induction seen in activity of CYP2C8, and CYP3A in the presence of phenobarbital, whereas the change in the presence of test drug or its metabolites was < 10% of that of Phenobarbital as the positive control. Similarly, for CYP2B6, the drug-induced change was < 10% of that of Phenobarbital as the positive control.

CONCLUSIONS and COMMENTS:

- The study was adequately conducted to evaluate in vitro induction of P450s.
- The positive controls resulted in the expected induction of CYP1A2, CYP3A4, and CYP2B6.
- Neither tetrabenazine nor its metabolites α-OH-TBZ or β-OH-TBZ induced CYP1A2, CYP3A4, or CYP2B6 in vitro; it can be concluded that these are also not inducers of CYP2C8, CYP2C9, or CYP2C19.
- No further study of induction is required.

4.7 In Vitro P450 Inhibition Study

In Vitro Evaluation of Tetrabenazine and its Two Major Metabolites as Inhibitors of Human Cytochrome P450 Enzymes

Study Investigators and Site:

Protocol Number: [redacted]J65005

OBJECTIVES:

To evaluate the ability of tetrabenazine, α-dihydrotetrabenazine, and β-dihydrotetrabenazine to inhibit the major CYP enzymes in human liver microsomes.

TEST COMPOUNDS:

Tetrabenazine (lot number 105481)
α-dihydrotetrabenazine (batch number RUS 0406)
β-dihydrotetrabenazine (batch number IC12 130802)

METHODS:

Human liver microsomes from donated livers were prepared and characterized by
A pool of 16 individual, mixed gender human liver microsomal samples was used. The Km and Vmax used to select marker substrate concentrations and incubation conditions had been previously determined.

The incubation conditions used for tetrabenazine assays are shown in the table below, as provided by the Sponsor. These substrates are those recommended in the draft Guidance and are used at concentrations that are approximately their respective K_ms (except for 2-Mephenytoin 4-hydroxylation that has a Km in the guidance of 13-35 μM).

Table 1: Summary of experimental conditions for enzyme assays: Direct and metabolism-dependent inhibition of CYP enzymes by tetrabenazine (IC50 determinations)

Enzyme	CYP Reaction	SOP followed	Substrate concentration (μM)	Incubation volume (μL)	Protein[a] (μg/mL)	Incubation time (min)	Pre-incubation time (min)	Tetrabenazine	
								Target concentrations (μM)	Solvent volume[b] (μL)
CYP1A2	Phenacetin *O*-deethylation	L3250.04	60	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP2C8	Paclitaxel 6α-hydroxylation	L3250.04	10	400	50	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP2C9	Diclofenac 4'-hydroxylation	L3250.04	7.5	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	L3250.04	40	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP2D6	Dextromethorphan *O*-demethylation	L3250.04	7.5	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP2E1	Chlorzoxazone 6-hydroxylation	L3250.04	30	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP3A4/5	Testosterone 6β-hydroxylation	L3250.04	100	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP3A4/5	Midazolam 1'-hydroxylation	L3250.04	5.0	400	50	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4
CYP3A4/5	Nifedipine oxidation	L3250.04	7.5	400	100	5	30	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100	4

[a] The human liver microsomal sample used for these experiments was a pool of sixteen individuals (samples 16, 17, 27, 34, 79, 113, 116, 140, 152, 155, 171, 175, 177, 209, 223, and 233).

[b] Acetonitrile was the vehicle used to dissolve the test article (final incubation concentration: 1% (v/v)).

CYP enzyme	Positive control	Vehicle	Concentration studied
CYP1A2	α-Naphthoflavone	Methanol	0.5 µM
CYP2C8	Quercetin	Methanol	20 µM
CYP2C9	Sulfaphenazole	Methanol	2.0 µM
CYP2C19	Modafinil	DMSO	250 µM
CYP2D6	Quinidine	High purity water	0.5 µM
CYP2E1	4-Methylpyrazole	High purity water	15 µM
CYP3A4/5	Ketoconazole	Methanol	0.15[a] / 0.075[b] µM

[a] Testosterone 6β-hydroxylation and nifedipine oxidation
[b] Midazolam 1´-hydroxylation

Assay conditions for α -dihydrotetrabenazine, and β-dihydrotetrabenazine were the same as for tetrabenazine, except that acetonitrile concentrations in the β-dihydrotetrabenazine were 1.5% due to solubility of that compound. The assay conditions were in agreement with the recommendations in the draft Guidance such that microsomal protein concentrations are < 1 mg/ml, and the Sponsor states that the assay method allowed for the rate of reaction to be measured under initial rate conditions so that the percent metabolism of the marker substrate did not exceed 20%. Enzyme activity was expressed as percentage of the solvent control.

The reaction mixture contained buffer and substrate and were initiated by addition of an aliquot of an NADPH-generating system. Assays were carried out in duplicate. Reactions were terminated at 5 minutes by addition of the appropriate internal standard and stop reagent (acetonitrile). Precipitated protein was removed by centrifugation. Enzyme activity was expressed as percentage of the solvent control.

To examine mechanism-based inhibition (metabolism-based inhibition), test drugs were pre-incubated with human liver microsomes and an NADPH-generating system for approximately 30 minutes to allow for generation of intermediates that could inhibit P450s. After the pre-incubation period, the marker substrate was added and the incubation continued for 5 minutes and terminated as above. Incubations that contained vehicle only and incubations that contained test drug but were not pre-incubated served as negative controls. Enzyme activity was expressed as percentage of the solvent control.

Analyses were performed using validated HPLC/MS/MS methods according to SOPs.

Additional controls
For every experiment, incubations were also conducted at half and twice the normal protein concentration and for half and twice the normal incubation period to ascertain whether metabolite formation was linearly related to concentration and incubation time.

Additional incubations were carried out in the presence of marker substrate and inhibitors used as positive controls as shown in the table below. In all cases, the positive control inhibited the enzyme activity. For CYP1A2, CYP2C8, CYP2C9, CYP2D6, and CYP3A4 the substrates are all preferred or acceptable inhibitors for *in vitro* studies, according to the draft Guidance, and are used at concentrations > the published K_i values. Modafinil has been shown in the literature to inhibit CYP2C19 reversibly, with a K_i of approximately 39 µM (Robertson P, Decory H, Madan A, Parkinson A. Drug Metab Disp 2000; 28:664-71). In the present assay, modafinil inhibited CYP2C19 activity by approximately 71%. 4-Methylpyrazole is considered in the literature to be

an inhibitor of CYP2E1, and resulted in about a 73% inhibition of CYP2E1 activity in the present study when used as a positive control.

Positive controls were also evaluated for mechanism-based inhibition as shown in the table below as provided by the Sponsor. In all cases, the positive controls inhibited the enzyme activity in a time-dependent manner. The inclusion of a positive control is optional according to the draft Guidance, and the draft guidance only identifies furafylline (1A2) and methoxsalen (2A6) as positive controls for mechanism based inhibition. The results from this evaluation of positive controls supports the adequacy of the assay conditions.

Additional zero-minute and 30-minute pre-incubations were conducted (in the presence of the following metabolism-dependent inhibitors) with the normal pre-incubation time and microsomal protein concentration. The incubations were continued as described in section 3.2.2.

CYP enzyme	Positive control	Vehicle	Concentration studied
CYP1A2	Furafylline	DMSO	1.0 µM
CYP2C8	1-Aminobenzotriazole	DMSO	1000 µM
CYP2C9	Tienilic acid	Tris base (0.002 mg/mL)	0.25 µM
CYP2C19	Ticlopidine	High purity water	0.75 µM
CYP2D6	Metoclopramide	High purity water	20 µM
CYP2E1	3-Amino-1,2,4-triazole	High purity water	10,000 µM
CYP3A4/5	Troleandomycin	Acetonitrile	25[a] / 7.5[b] / 20[c] µM

[a] Testosterone 6β-hydroxylation
[b] Midazolam 1´-hydroxylation
[c] Nifedipine oxidation

RESULTS:

Tetrabenazine

The results for tetrabenazine are shown below, both for direct inhibition and for mechanism-based inhibition.

Table 5: Summary of results: *In vitro* evaluation of tetrabenazine as an inhibitor of human CYP enzymes

		Direct inhibition		Metabolism-dependent inhibition (MDI)		
		Zero-minute pre-incubation		30-minute pre-incubation		
Enzyme	CYP Reaction	IC50 (µM)	Maximum inhibition at 100 µM (%)[a]	IC50 (µM)	Maximum inhibition at 100 µM (%)[a]	MDI Potential[b]
CYP1A2	Phenacetin *O*-deethylation	99 ± 13	50	67 ± 10	55	Possible
CYP2C8	Paclitaxel 6α-hydroxylation	>100	21	>100	19	Little or no
CYP2C9	Diclofenac 4´-hydroxylation	34 ± 4	71	51 ± 8	70	Little or no
CYP2C19	*S*-Mephenytoin 4´-hydroxylation	35 ± 3	81	45 ± 4	82	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	>100	26	>100	33	Possible
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	0	>100	0	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	9.1	>100	18	Possible
CYP3A4/5	Midazolam 1´-hydroxylation	>100	0	>100	9.1	Possible
CYP3A4/5	Nifedipine Oxidation	>100	0	>100	15	Possible

Notes: Values were calculated using the average data obtained from duplicates for each incubation condition. The IC50 values were calculated using XLfit.

[a] Maximum inhibition (%) is calculated using the following formula and data for the highest concentration of test article for which usable data were collected from the IC50 determinations (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control (Appendix 6)

[b] Metabolism-dependent inhibition was determined by comparison of IC50 values with and without pre-incubation, increase in % inhibition upon pre-incubation at all concentrations, and by visual inspection of the IC50 plot.

In the clinical studies reviewed in the original NDA submission, plasma concentrations of tetrabenazine were up to 0.002 μM in the absence of hepatic impairment and up to 0.4 μM in the presence of hepatic impairment. Therefore, in the worst case of hepatic impairment, and considering that substrate concentrations were around their respective K_m values (and assuming competitive inhibition so that $K_i = IC_{50}/2$), the I/K_i values for CYP2C9 and CYP2C19 that had the greatest inhibition would not exceed 0.02. Therefore the likelihood of P450 inhibition of these enzymes is remote, if a direct inhibition is considered.

For the mechanism-based inhibition studies, the IC_{50} for inhibition of CYP1A2 was reduced from approximately 99 μM to approximately 67 μM, suggesting time-dependent inhibition. Similarly, this is suggested by the results of CYP2D6 and CYP3A. However, due to the very low circulating concentrations of tetrabenazine (usually undetectable in healthy volunteers or present at concentrations of < 0.002 μM in the absence of hepatic impairment), this is not likely to be clinically significant. Therefore no further evaluation is necessary.

α –Dihydrotetrabenazine

The results for α –dihydrotetrabenazine are shown below, both for direct inhibition and for mechanism-based inhibition.

Table 6: Summary of results: *In vitro* evaluation of α-dihydrotetrabenazine as an inhibitor of human CYP enzymes

		Direct inhibition		Metabolism-dependent inhibition (MDI)		
		Zero-minute pre-incubation		30-minute pre-incubation		
Enzyme	CYP Reaction	IC50 (μM)	Maximum inhibition at 100 μM (%)[a]	IC50 (μM)	Maximum inhibition at 100 μM (%)[a]	MDI Potential[b]
CYP1A2	Phenacetin *O*-deethylation	>100	0	>100	0	Little or no
CYP2C8	Paclitaxel 6α-hydroxylation	>100	7.9	>100	12	Little or no
CYP2C9	Diclofenac 4'-hydroxylation	>100	0	>100	0	Little or no
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	>100	6.6	>100	3.3	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	100 ± 20	49	>100	31	Little or no
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	0	>100	0	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	0	>100	5.8	Little or no
CYP3A4/5	Midazolam 1'-hydroxylation	>100	0	>100	0	Little or no
CYP3A4/5	Nifedipine Oxidation	>100	7.6	>100	8.7	Little or no

Notes
Values were calculated using the average data obtained from duplicates for each incubation condition. The IC50 values were calculated using XLfit.
a Maximum inhibition (%) is calculated using the following formula and data for the highest concentration of test article for which usable data were collected from the IC50 determinations (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control (Appendix 7)
b Metabolism-dependent inhibition was determined by comparison of IC50 values with and without pre-incubation, increase in % inhibition upon pre-incubation at all concentrations, and by visual inspection of the IC50 plot.

No inhibition by α –dihydrotetrabenazine was seen at concentrations of > 100 μM except for CYP2D6 that had an IC_{50} of 100 μM after direct inhibition experiments. With observed concentrations in the original NDA submission of up to 0.54 μM (according to the Sponsor Cmax values can be up to 0.83 μM) and considering that substrate concentrations were around their respective K_m values (and assuming competitive inhibition so that $K_i = IC_{50}/2$) the I/K_i would be approximately 0.01-0.02. Therefore the likelihood of inhibition of CYP2D6 (or other P450s evaluated) is remote, if a direct inhibition is considered.

Time-dependent inhibition was not observed in the pre-incubation studies, suggesting that α – dihydrotetrabenazine is not a mechanism-based inhibitor.

β –Dihydrotetrabenazine

The results for β –dihydrotetrabenazine are shown below, both for direct inhibition and for mechanism-based inhibition.

Table 7: Summary of results: *In vitro* evaluation of β-dihydrotetrabenazine as an inhibitor of human CYP enzymes

		Direct inhibition		Metabolism-dependent inhibition (MDI)		
		Zero-minute pre-incubation		30-minute pre-incubation		
Enzyme	CYP Reaction	IC50 (μM)	Maximum inhibition at 100 μM (%)[a]	IC50 (μM)	Maximum inhibition at 100 μM (%)[a]	MDI Potential[b]
CYP1A2	Phenacetin *O*-deethylation	>100	0	>100	0	Little or no
CYP2C8	Paclitaxel 6α-hydroxylation	>100	0	>100	0	Little or no
CYP2C9	Diclofenac 4'-hydroxylation	>100	0	>100	0	Little or no
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	>100	0	>100	0	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	46 ± 9	65	48 ± 9	60	Little or no
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	0	>100	0	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	0	>100	8.2	Little or no
CYP3A4/5	Midazolam 1'-hydroxylation	>100	0	>100	3.5	Little or no
CYP3A4/5	Nifedipine Oxidation	>100	10	>100	25	Possible

Notes
[a] Values were calculated using the average data obtained from duplicates for each incubation condition. The IC50 values were calculated using XLfit.
Maximum inhibition (%) is calculated using the following formula and data for the highest concentration of test article for which usable data were collected from the IC50 determinations (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control (Appendix 8)
[b] Metabolism-dependent inhibition was determined by comparison of IC50 values with and without pre-incubation, increase in % inhibition upon pre-incubation at all concentrations, and by visual inspection of the IC50 plot.

No β –dihydrotetrabenazine-mediated inhibition of the P450s evaluated was observed except for CYP2D6 for which the IC_{50} was approximately 46 μM. With observed concentrations in the original NDA submission of up to 0.55 μM and considering that substrate concentrations were around their respective K_m values (and assuming competitive inhibition so that $K_i = IC_{50}/2$) the I/K_i would be approximately 0.02-0.03. Therefore the likelihood of inhibition of CYP2D6 (or other P450s evaluated) is remote, if a direct inhibition is considered.

For the mechanism-based inhibition studies, a small time dependent inhibition of CYP3A can be observed, although a shift in the IC_{50} at relevant concentrations (in the expected therapeutic range) or to IC_{50} of < 100 μM is not observed.

CONCLUSIONS and COMMENTS:

- The study was adequately conducted to evaluate *in vitro* inhibition of P450s.
- Neither tetrabenazine nor its metabolites α-OH-TBZ or β-OH-TBZ resulted in *in vitro* inhibition that is likely to be clinically relevant when considered separately.
- Although both metabolites resulted in CYP2D6 inhibition, the likelihood of a significant in vivo inhibition due to the presence of both metabolites is remote even when the I/Ki values are summed.
- Potential for inhibition of CYP2B6 has not been evaluated.

4.8 In Vitro Pgp Study

The Assessment of Tetrabenazine (TBZ), α-OH-Tetrabenazine (α-OH-TBZ), and β- OH-Tetrabenazine (β -OH-TBZ) as Potential Substrates and Inhibitors of Human Pgp *In Vitro*

Study Investigators and Site:

Protocol Number: 6PRESP1

OBJECTIVES:

To assess the potential of tetrabenazine (TBZ), α-OH-tetrabenazine (α-OH-TBZ), and β- OH-tetrabenazine (β -OH-TBZ) to be substrates or inhibitors of human P-Glycoprotein (Pgp) *in vitro*.

METHODS:

Permeability Testing

General Procedure

Transfected MDR1-MDCK cells (with MDCK cells used as negative controls) were used in the bidirectional permeability assay (the preferred functional assay) to determine whether TBZ and its metabolites are substrates for Pgp. Caco-2 cells were used to determine whether TBZ and its metabolites are inhibitors of Pgp.

Prior to permeability experiments, suitability of testing conditions was evaluated by confirming that when compounds were dosed in the transport buffer (HBSSg supplemented with 0.2% DMSO) there was no significant loss of test compounds to nonspecific binding to the plastic of the Transwell apparatus.

Cell monolayers were certified after growing to confluence on collagen-coated polycarbonate microporous membrane filters. For MDCK and MDR1-MDCK cells the results are as follows. The transepithelial electrical resistance (TEER) of the polarized cells was determined and found to meet the Investigators' acceptance criteria of > 1400 for MDCK and MDR1-MDCK. Permeability of control compounds propranolol, atenolol, and digoxin (10 μM) was also assayed by incubation at 37 C with 5% CO2 in a humidified incubator and all treatments were performed in triplicate. Only the digoxin results will be evaluated as this is the probe substrate recommended in the guidance. For digoxin (considered in the draft Guidance) to be an acceptable substrate at the concentration used, the net flux ratio (ratio of (Permeability $_{app, B-A}$/Permeability $_{app, A-B})_{MDR}$ to (Permeability $_{app, B-A}$/Permeability $_{app, A-B})_{wild\ type}$ was 95.66/18.5 = 5 for the batches used for substrate assessment. A minimum net flux ratio of > 2 is recommended

in the guidance and the results for the substrate assessment met this criterion. Following completion of all test treatments, Lucifer yellow apparent permeability was measured on each cell monolayer to assess integrity. Its permeability was < 0.4 in the certification studies and in the permeability studies and this is acceptable. For Caco-2 cell monolayers, the certification results are as follows. TEER values were between 100-800 Ω cm^2, as recommended in the draft Guidance. Digoxin permeability (B-A)/A-B) ratio was >10. Lucifer yellow permeability was < 0.4.

A tolerability assessment was performed to see if the highest concentration (50 µM) of TBZ, α -OH-TBZ, and β -OH-TBZ used in the study would impair MDR1-MDCK, MDCK, and Caco-2 cell monolayer integrity. The pre-exposure and post-exposure change in TEER was similar between the blank treatment and treatments with the test compounds, and post-experiment lucifer yellow permeability values in monolayers exposed to test compounds were similar to values obtained in the blank treatment (< 0.8 x 10^{-6} cm/s).

Pgp substrate testing was performed as described above in MDR1-MDCK and MDCK cells in the absence and in the presence of Pgp inhibitors cyclosporine A and ketoconazole. The three concentrations of TBZ, α -OH-TBZ, and β -OH-TBZ used were 5 µM, 10 µM and 50 µM when dosed alone, or 5 µM in the presence of 10 µM cyclosporine or 10 µM ketoconazole. (The cyclosporine concentration is the K_i value reported in MDCK-MDR1 cells and the ketoconazole concentration is above the IC_{50} values reported for Caco-2 and above the K_i value reported for LLC-PK1 MDR1). Digoxin (10 µM) was also tested as a positive control in the absence and presence of these inhibitors. Cells were first pre-incubated with blank HBSSg buffer or HBSSg buffer containing inhibitor for 30 minutes. Then cells were dosed either with test compound alone or test compound with inhibitor. Sampling was performed at time 0 and at 30, 60, and 120 minutes from the receiver chamber and at 120 minutes from the donor chamber. The assay was performed in the apical to basolateral (A-B) and basolateral to apical (B-A) direction. Three replicate assays were performed.

Pgp inhibitor testing was performed to determine potential of the test compound to inhibit human Pgp-mediated transport in Caco-2 cell monolayers. The initial step evaluated the potential of TBZ (50 µM), α -OH-TBZ (50 µM), and β -OH-TBZ (50 µM) to inhibit Pgp. This was assayed by testing the bidirectional permeability of the marker Pgp substrate digoxin (10 µM) in the absence and presence of the test compounds. In addition, on parallel wells, the bidirectional assessment of digoxin transport was tested in the presence of known Pgp inhibitors cyclosporine A (10 µM) and ketoconazole (10 µM) as control treatments. Prior to onset of digoxin permeability testing, cells were pre-incubated with blank transport buffer or buffer containing CSA, ketoconazole, or test compound for 30 minutes. Cells were then dosed with either digoxin alone or digoxin with inhibitors or test compound. Digoxin permeation was tested in the A-B direction or the B-A direction for 120 minutes. Dosing solutions were sampled at 0 minutes, the donor chamber was sampled at 120 minutes, and the receiver chamber was sampled at 60 and 120 minutes and analyzed for digoxin concentration by LC-MS/MS.

If the test compounds inhibited efflux of digoxin more than 50% in the first step, then the effect at multiple concentrations (0.1, 1, 10, 20, 30, 40, 50, and 60 µM) on the bidirectional permeability of digoxin (10 µM) was tested to establish an IC_{50} value. As control treatments, on

parallel wells the bidirectional assessment of digoxin transport was assayed in the presence of cyclosporine A and ketoconazole at multiple concentrations. Testing and sampling was described as outlined above.

Analytical Assays:

A summary of the validation data for TBZ, α-OH-TBZ and β-OH-TBZ determination by LC-MS (validation and assay by ——— is shown below as provided by the Sponsor. The assay showed no interference from digoxin, ketoconazole or cyclosporine A at 10 μM concentrations.

	Tetrabenazine	α-dihydrotetrabenazine	β-dihydrotetrabenazine
Validated Range	0.1 – 62.5 μM	0.1 – 62.5 μM	0.1 – 62.5 μM
Calibration Model	Linear weighted $1/x^2$	Linear weighted $1/x^2$	Linear weighted $1/x^2$
Precision (%CV) Intra-assay	≤±10.1%	≤±8.8%	≤19.0%
Precision (%CV) Inter-assay	≤±9.0%	≤±10.5%	≤±14.4%
Accuracy (%RE) Intra-assay	≤±9.7%	≤±13.5%	≤±9.9%
Accuracy (%RE) Inter-assay	≤±8.1%	≤±2.1%	≤±9.3%
Accuracy (%RE) and precision (%CV) following 1/10 dilution	≤±3.8% RE ≤±5.3% CV	≤±9.9% RE ≤±5.7% CV	≤±5.7% RE ≤±6.3% CV
Stability in Hank's buffer	At least 4 hours at room temperature and 23 days at -70 °C	At least 4 hours at room temperature and 23 days at -70 °C	At least 4 hours at room temperature and 23 days at -70 °C
Freeze-Thaw Stability	At least 3 cycles at -70 °C	At least 3 cycles at -70 °C	At least 3 cycles at -70 °C
Processed Extract Stability	At least 4 days at room temperature At least 3 days at 4°C	At least 4 days at room temperature At least 3 days at 4°C	At least 4 days at room temperature At least 3 days at 4°C
Equipment			
———	———		

Digoxin was determined using a validated LC-MS/MS assay at ——— For the standard curve precision was < 5% for all concentrations at or above the LLQ. Accuracy was acceptable. Similarly both accuracy and precision were < 10% for the QC samples. Neither TBZ nor its metabolites α-OH-TBZ and β-OH-TBZ nor ketoconazole or cyclosporine A caused interference with the assay.

RESULTS:

Results of the Pgp substrate assessment are shown in the table below. The net efflux ratio is the ratio of Efflux $Ratio_{MDR1\text{-}MDCK}$/Efflux $Ratio_{MDCK}$. The results suggest that β-OH-TBZ is a substrate for Pgp.

Net Efflux Ratio

Treatment	B-A P_{app}/A-B P_{app} Efflux Ratio		Compound Net Efflux Ratio
	Cell Line		
	MDR1-MDCK	MDCK	Digoxin
Digoxin (10 µM)	73.4	30.6	2.4
Digoxin (10 µM) + CSA (10 µM)	0.9	1.0	0.9
Digoxin (10 µM) + Ketoconazole (10 µM)	8.2	2.3	3.6
			TBZ
TBZ (5 µM)	1.1	1.3	0.9
TBZ (5 µM) + CSA (10 µM)	1.0	1.3	0.8
TBZ (5 µM) + Ketoconazole (10 µM)	1.2	1.1	1.0
TBZ (10 µM)	1.1	1.3	0.9
TBZ (50 µM)	1.1	1.1	1.0
			α-OH-TBZ
α-OH-TBZ (5 µM)	1.6	1.3	1.2
α-OH-TBZ (5 µM) + CSA (10 µM)	1.0	1.2	0.9
α-OH-TBZ (5 µM) + Ketoconazole (10 µM)	1.0	1.1	0.9
α-OH-TBZ (10 µM)	1.3	1.2	1.1
α-OH-TBZ (50 µM)	1.0	1.1	0.9
			β-OH-TBZ
β-OH-TBZ (5 µM)	2.0	0.9	2.2
β-OH-TBZ (5 µM) + CSA (10 µM)	1.2	1.1	1.0
β-OH-TBZ (5 µM) + Ketoconazole (10 µM)	1.0	0.8	1.3
β-OH-TBZ (10 µM)	1.8	1.1	1.6
β-OH-TBZ (50 µM)	1.7	1.2	1.5

Digoxin, the control substrate, had a high efflux ratio in the MDR1-MDCK cell line and in the MDCK cell line, with a net efflux ratio of 2.4. Digoxin efflux was substantially decreased by the presence of cyclosporine (CSA) or ketoconazole in either cell line, with net efflux in the presence of cyclosporine decreased by > 50% reducing the net efflux ratio to near unity. These results show that the cell system is acceptable.

TBZ and α-OH-TBZ had efflux ratios of < 2 in both cells lines and net efflux ratios of < 2, indicating that these are not Pgp substrates. In contrast, 5 µM β-OH-TBZ, but not the higher concentrations, had an efflux ratio of 2.0 in MDR1-MDCK cells and a net efflux ratio of 2.2. This was inhibited by cyclosporine and by ketoconazole by approximately 50%, reducing the efflux ratio to near unity. These results suggest that β-OH-TBZ is a substrate for Pgp.

Results of the Pgp inhibitor studies in Caco-2 cells are as follows. Digoxin efflux was inhibited by 93.93% and by 92.33% by cyclosporine A and by ketoconazole, respectively. Digoxin efflux was inhibited by approximately 83.96% by TBZ but only by 38.72% and by 45.81% by α-OH-TBZ and by β-OH-TBZ, respectively. Therefore, the effect of TBZ on digoxin efflux was further investigated to establish the IC_{50} value. (The IC_{50} values of α-OH-TBZ and β-OH-TBZ are estimated to be higher than 50 µM). The IC_{50} for inhibition of digoxin efflux was estimated to be 0.32 µM, and the IC_{50} for inhibition of digoxin efflux in the presence of ketoconazole was calculated to be 0.42 µM. These results are generally in agreement with the IC_{50} values reported in the draft Guidance (1.3 and 1.2 µM, respectively). Results of the assessment of TBZ as an

inhibitor of digoxin efflux at concentrations of 0.1 μM-60 μM TBZ are shown below. At lower concentrations, TBZ appeared to have a stimulatory effect. The IC_{50} for inhibition was estimated to be 24 μM.

Inhibition of Digoxin Efflux by TBZ

Treatment	Average $P_{app,B-A} / P_{app,A-B}$ Digoxin Efflux Ratio	% Inhibition	IC_{50}
Digoxin Control	10.04	0	24 μM
Digoxin + 0.1 μM TBZ	13.06	-30.08	
Digoxin + 1 μM TBZ	16.30	-62.35	
Digoxin + 10 μM TBZ	7.15	28.78	
Digoxin + 20 μM TBZ	5.94	40.84	
Digoxin + 30 μM TBZ	3.80	62.15	
Digoxin + 40 μM TBZ	3.63	63.84	
Digoxin + 50 μM TBZ	2.26	77.49	
Digoxin + 60 μM TBZ	2.51	80.78	

In the absence of hepatic impairment, plasma concentrations of tetrabenazine are < 1 ng/ml (0.002 μM) and in the presence of hepatic impairment TBZ concentrations are up to 126 ng/ml (0.4 μM) based on the OCP review of the original NDA. In the case of hepatic impairment, I/IC_{50} would be 0.017, making TBZ a weak inhibitor. Similarly, clinically relevant plasma concentrations of α-OH-TBZ and β-OH-TBZ are up to
$5.4x10^{-7}$ M, and I/IC_{50} would be approximately 0.01 (if the IC_{50} were 50 μM). Therefore, at clinically relevant exposures, neither TBZ nor its α- and β-HTBZ metabolites are considered Pgp inhibitors. However, for inhibition in the GI tract, a worst case scenario would use a projected GI concentration of dose/250 ml as the inhibitor concentration for TBZ. In that case, [I] for a 37.5 mg dose would be approximately 472 μM, and this would result in I/Ki of approximately 19. In this case, further in vivo interaction study with digoxin is also recommended. However, as it would be difficult to achieve a worst case scenario in the setting of an in vivo evaluation, further in vivo study to investigate the potential for this drug interaction is not recommended, and the potential can be described in labeling.

CONCLUSIONS and COMMENTS:

1. The cell system was acceptable for use.
2. TBZ and α-OH-TBZ do not appear to be Pgp substrates.
3. β-OH-TBZ is a substrate for Pgp. However, due to its extensive metabolism in vivo that is not primarily mediated by CYP3A, no further evaluation for potential drug interaction with a Pgp inhibitor is necessary.
4. Neither TBZ nor its metabolites α-OH-TBZ and β-OH-TBZ are potent inhibitors of Pgp, as they have I/IC_{50} values of < 1 at the concentrations at which they will be circulating after clinical use.

4.9 Paroxetine Interaction Study

AN INTERACTION STUDY OF TETRABENAZINE AND REPEATED DOSES OF PAROXETINE

Study Investigators and Site:

Protocol Number: TBZ 107,018

Note: QT evaluation was included in the Sponsor's analysis but is not reviewed in detail here. A thorough QT study (104,015) has been referred to the IRT as a consult and exposure response for QT is being reviewed by Pharmacometrics.

OBJECTIVES:

The objective was to evaluate the effect of repeated doses of paroxetine, a potent CYP2D6 inhibitor, on the pharmacokinetics and safety of a single dose of tetrabenazine.

FORMULATION:

Table 1. Product used in TBZ 107,018

	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 25 mg tablet	L027/8B	2/2010 (5/9/07-6/21/07)
Paroxetine 20 mg tablets	2105B11	11/2008 (5/9/07-6/21/07)

STUDY DESIGN:

This study was a single-center, open-label sequential drug interaction study in CYP2D6 extensive metabolizers. Subjects checked into the unit on Day -1 and remained in-house until Day 12. On Study day 1 subjects received a single dose of tetrabenazine (50 mg) followed by a 2-day washout period. Subjects received 9 days of 20 mg paroxetine daily (Treatment Days 3-11), and on Treatment Day 10 another single dose of TBZ 50 mg was co-administered with paroxetine. Study drug was administered in the fasted state with 240 ml water.

Serial blood samples for measurement of plasma concentrations of tetrabenazine and α- and β-HTBZ were performed on Treatment Days 1, 2, 3, 10, and 11 and on the discharge day (Day 12). Serial tetrabenazine PK samples were collected on Treatment Day 1 (pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 16 hours post-dose), Treatment Day 2 (24 and 36 hours pose-dose), Treatment Day 3 (48 hours post-dose), Treatment Day 10 (pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 16 hours post-dose), Treatment Day 11 (24, 30, and 36 hours pose-dose), and Discharge Day 12 (48 hours post-dose). Pre-dose plasma concentrations of paroxetine were obtained on Treatment Days 8, 9, 10, and 11 and 24 hours after the dose on Treatment Day 11 (Discharge, Day 12).

Digital ECGs were collected on Treatment Days 1, 9, and 10 at pre-dose and 1, 2, 3, 4, and 5 hours post-dose to assess the effect of treatment on cardiac repolarization and to add an assessment at T_{max} for tetrabenazine (Treatment Days 1 and 10).

Safety was assessed by periodic measures of vital signs, physical examinations, laboratory parameters, paper and digital 12-lead electrocardiograms (ECGs) and indirect questioning regarding AEs. Continuous cardiac telemetry was performed for 24 hours beginning in the morning of Treatment Day 10.

Inclusion criteria included healthy males or females, between the ages of 18 and 50 years of age (inclusive). Subjects were to be non-smokers (refrained from any tobacco usage, including smokeless tobacco, nicotine patches, etc., for 3 months prior to administration of the study medication) and no significant history of smoking (i.e., less than 3 pack- years). Females had to be surgically sterile, at least 2 years postmenopausal, or practice an acceptable method of birth control that could include hormonal contraceptives. Exclusion criteria included CYP2D6 poor metabolizers (PMs) based on genotyping results; abnormal cardiac finding, based on a mean of three Screening 12-lead ECGs separated by 1 minute, as follows: PR > 210 msec, QRS >120 msec and QTc > 440 msec or clinically significant minor ST-T wave changes on the screening ECG; abnormal heart rate or blood pressure measurements at the pre-study visit as follows: heart rate <50 or >90 bpm; systolic blood pressure <90 or >140 mmHg; diastolic blood pressure <50 or 90 mmHg after subject had been resting in sitting position for 5 minutes; treatment with any known enzyme-altering drugs such as barbiturates, phenothiazines, cimetidine, carbamazepine, etc., or herbal products such as St. John's Wort within 30 days prior to or during the study; used any prescription medication within 30 days prior to Baseline (Day - 1); used any non-prescription medications within 7 days prior to first dose; excessive consumption of caffeinated coffee, tea, or chocolate – more than 6 cups/day on average, or xanthine-containing drinks (more than 6 glasses/day).

ASSAY:

Plasma concentrations of TBZ, α- HTBZ, and β-HTBZ were measured using a validated LC/MS/MS method (— 1266/1). 25x dilution integrity was previously demonstrated. According to the analytical study report, [redacted]

Table 2. Performance of Analytical Method for TBZ 203-009

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.990	0.2	0.5	11.5	6.1
					25.0	5.5	14.1
					100.0	7.8	6.8
α- HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.992	0.5	1.0	10.4	-13.2
					25.0	6.5	15.7
					100.0	9.2	5.7
β-HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.992	0.5	1.0	11.2	5.4
					25.0	5.9	15.5
					100.0	9.3	6.4

For TBZ, and α- and β-HTBZ, samples were stored at -70° C before shipping to ⁓ for analysis. Calibration curves with duplicate standards (9 calibration standards for α- and β-HTBZ; 11 for TBZ) and duplicate QC samples were analyzed with each batch of study samples. Samples were analyzed within the period for which the samples are stable at –80° C (321 days). Specificity with respect to interference from paroxetine was evaluated and the Sponsor states that it did not affect precision or accuracy of this assay. The performance of the assays for all analytes is considered acceptable.

Plasma was assayed for paroxetine. Paroxetine samples were stored at -20° C before shipping to ⁓ for analysis and upon arrival they were stored at -70° C . Sample analysis was performed using and LC/MS/MS method following extraction by ⁓ ⁓ for which a summary of the validation has been provided as follows:

QC Concentrations	0.2, 0.6, 4, 16, 40, and 80 ng/mL [a]
Standard Curve Concentrations	0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 ng/mL [a]
Lower Limit Of Quantitation (ng/mL)	0.2 ng/mL
Upper Limit Of Quantitation (ng/mL)	100 ng/mL [a]
Average Recovery of Drug (%)	101.6 %
Average Recovery of Int. Std (%)	94.6 %
QC Intraday Precision Range (%CV)	1.1 to 8.9 % [a]
QC Intraday Accuracy Range (%Diff)	-2.7 to 7.4 % [a]
QC Interday Precision Range (%CV)	4.1 to 10.4 %
QC Interday Accuracy Range (%Diff)	-7.7 to -3.5 %
Autosampler Stability in Reconstitution Solvent	456 Hours at 4°C [b]
Benchtop Stability in Plasma	7 Hours at Room Temperature [b]
Freeze/thaw Stability in Plasma	6 Cycles at -20°C [b]
Long-term Storage Stability in Plasma	78 Days at -20°C [b]
Long-term Storage Stability in Plasma	To be determined at -70°C
Dilution Integrity	200 ng/mL diluted 10-times [a]
Selectivity	≤ 20% LLOQ for analyte; ≤ 5% for IS

The performance of the assay in study 107-018 is as follows:

Table 3. Performance of Analytical Method for Paroxetine in Plasma in Study TBZ 107-018

Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
LC/MS/MS	0.2-100	>0.98	0.20	0.6	6.5	-5.7
				4.0	6.8	-5.5
				16.0	3.8	-6.3
				40.0	11.4	0.0
				80.0	4.8	3.9

Samples were stored at -70° C for 3 days instead of at -20° C. According to the study report, long-term frozen stability for at least 3 days will be established to cover the samples at -70° C, but these data have not yet been provided. Duplicate calibration curves (9 concentrations) and duplicate QC samples were analyzed with each batch of study samples for paroxetine plasma analysis. Samples were analyzed within 7 days of beginning the clinical study; this is within the period for which the samples are stable at -20° C, and long-term stability is being evaluated at -70° C. Specificity with respect to interference from TBZ, α-HTBZ, and β-HTBZ has been evaluated and the Sponsor states that there was no interference. The performance of the assays for all analytes is acceptable.

RESULTS:

Demographics

Thirty subjects entered the study (20 males and 10 females). Two subjects withdrew after dosing on Day 1 with TBZ – one due to adverse events (restlessness, nausea) and 1 for personal reasons. Twenty-eight subjects were dosed with paroxetine (19 males, 9 females), and one of those subjects withdrew on Day 5 due to a rash. Twenty-seven subjects (19 males, 9 females) completed the study. The three subjects who withdrew were not included in the PK analysis. In addition, 4 subjects experienced emesis after administration of TBZ. For 2 of the subjects, emesis occurred within 2x the median Tmax; these 2 subjects were excluded from PK analysis. Demographics of the 25 subjects completing the PK portion of the study are shown in the table below.

Table 4. Demographics of Subjects Completing Study 107-018

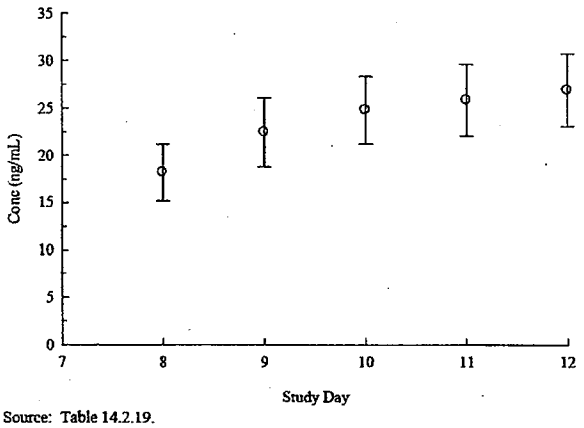
Mean Age (Range)	Gender	Weight (mean ± SD)	Race
31 (19-49)	18 males 7 females	76 ± 13 kg (n=25) 78 ± 12 kg (male) 63 ± 6 kg (female)	Caucasian 11 Black/African American 5 Hispanic 8 Other 1 (non-Asian, non-Native American)

Two subjects used hormonal contraceptives. There were no other concomitant medications in the PK population.

Pharmacokinetics

The pre-dose plasma concentrations for paroxetine are shown in the figure below, as provided by the Sponsor. Steady state is approached by Day 10.

Figure 2. Mean ± Standard Error Pre-dose Plasma Concentrations of Paroxetine after Oral Administration of 20 mg QD on Treatment Days 3 through 11



Source: Table 14.2.19.

Tetrabenazine and its metabolites

The plasma concentration time course curves for α-HTBZ and β-HTBZ are shown below, as provided by the Sponsor.

Figure 3. Mean Plasma Concentrations of α-HTBZ after After Single Dose Administration of Tetrabenazine 50 mg Under Fasting Conditions Before and After 8 Days of Dosing with Paroxetine 20 mg QD to Healthy Volunteers — Linear Axes

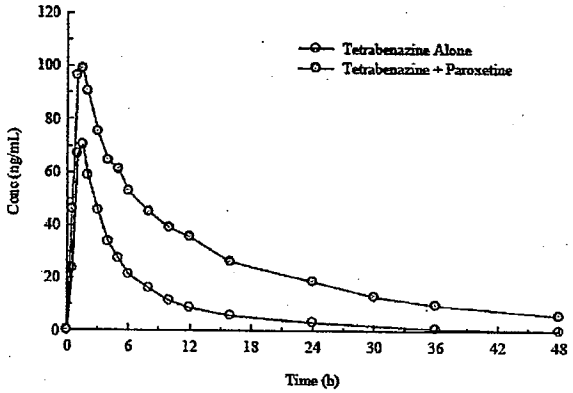
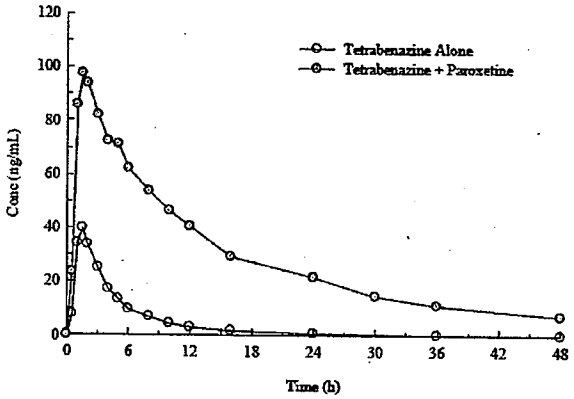


Figure 5. Mean Plasma Concentrations of β-HTBZ After Single Dose Administration of Tetrabenazine 50 mg Under Fasting Conditions Before and After 8 Days of Dosing with Paroxetine 20 mg QD to Healthy Volunteers — Linear Axes



Plasma concentrations for TBZ that were ≥ LLOQ ranged from 0.2 to 7.105 ng/ml, and after the two hour time point were generally below the limit of quantification. The highest Cmax on Day 1 as 7.1 and the highest Cmax on Day 10 was 3.3. The mean Cmax on Day 1 was 1.26 ng/ml and on Day 10 was 1.01 ng/ml. No formal analysis of TBZ PK has been performed.

PK parameters for α-HTBZ and β-HTBZ are shown in the table below. The parameters calculated by the Sponsor are generally in agreement with the parameters calculated by the reviewer for α-HTBZ and β-HTBZ.

Table 5. Pharmacokinetic parameters (arithmetic mean, %CV) for Study TBZ 107,018

		Day 1	Day 10
α-HTBZ	t_{max} (h)[a]	1.00 (1.0-3.1)	1.5 (1.0-6.0)
	C_{max} (ng/mL)	77.3 (34)	107 (25)
	AUC_{0-t} (ng*h/mL)	419 (52)	1235 (39)
	AUC_{0-inf} (ng*h/mL)	422 (53)	1365 (53)
	$t_{1/2}$ (h)	7.0 (29)	13.8 (32)
β -HTBZ	t_{max} (h)[a]	1.5 (1.0-3.1)	1.5 (1.0-4.0)
	C_{max} (ng/mL)	42.9 (56)	105 (31)
	AUC_{0-t} (ng*h/mL)	183 (94)	1368 (54)
	AUC_{0-inf} (ng*h/mL)	184 (95)	1638 (58)
	$t_{1/2}$ (h)	4.5 (57)	13.5 (40)

[a] median (range)

The highest reported Cmax for α-HTBZ was 156.5 ng/ml and the highest for β-HTBZ was 183.2 ng/ml on Day 10.

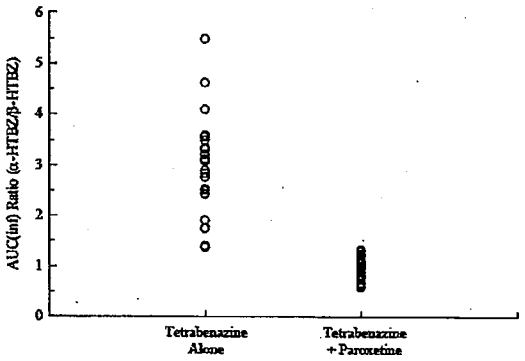
For α-HTBZ there was an approximate 1.3x increase in Cmax and an approximate 3.2x increase in AUCinf after administration of repeated doses of paroxetine. In addition, there was an approximate 2x increase in the elimination half-life in the presence of a strong CYP2D6 inhibitor. For β-HTBZ the Cmax was approximately 2.4x greater and the AUCinf was approximately 9x greater after administration of paroxetine compared to no CYP2D6 inhibitor. The β-HTBZ elimination half-life was approximately 3x greater after CYP2D6 inhibition than when TBZ was given alone. The BE assessment is shown below, as provided by the Sponsor.

Table 6. Bioequivalence Assessment for α- and β-HTBZ in Study TBZ 107,018

		Geometric Means			90% Confidence Interval	
Assay*	Parameter	Tetrabenazine + Paroxetine	Tetrabenazine Alone	Geometric Mean Ratio	Lower Limit	Upper Limit
AHT	CMAX	105.32	72.57	145.12	127.10	165.69
	AUC(0-t)	1,187.69	388.46	305.74	268.18	348.58
	AUC(inf)	1,302.38	378.19	344.37	305.95	387.62
BHT	CMAX	103.24	37.65	274.20	225.79	332.99
	AUC(0-t)	1,232.26	146.39	841.80	687.19	1031.2
	AUC(inf)	1,342.51	140.28	956.99	794.85	1152.2

In the absence of CYP2D6 inhibition, exposure to α-HTBZ is generally greater than to β-HTBZ (median ratio of 3). Following CYP2D6 inhibition with paroxetine, the median ratio is 1. These results are shown in the figure below. This, along with the PK data above, suggests the importance of CYP2D6, especially in the metabolism of β-HTBZ.

Figure 7. Individual Subject Ratios of α-HTBZ to β-HTBZ $AUC_{(0-t)}$ After Single Dose Administration of Tetrabenazine 50 mg Under Fasting Conditions Before and After 8 Days of Dosing with Paroxetine 20 mg QD to Healthy Volunteers



The Sponsor has also included the PK report produced by the PK consultant that provides information on the analysis of the desmethyl HTBZ metabolites from a limited number of subjects. According to that report, there are four (4) potential desmethyl metabolites of HTBZ, two (2) derived from α-HTBZ and two (2) derived from β-HTBZ. Throughout the report, these are referred to as 1-, 2-, 3-, and 4-desmethyl HTBZ where the number refers to the *order of elution* from the HPLC, *not to a position on the molecule*. The 1- and 2-desmethyl HTBZs are derived from β-HTBZ and the 3- and 4-desmethyl HTBZs are derived from α-HTBZ. Plasma concentrations for 2- and 4-demethyl HTBZ were < LOQ (5 ng/ml). Details of the analysis from the analytical report have not been provided.

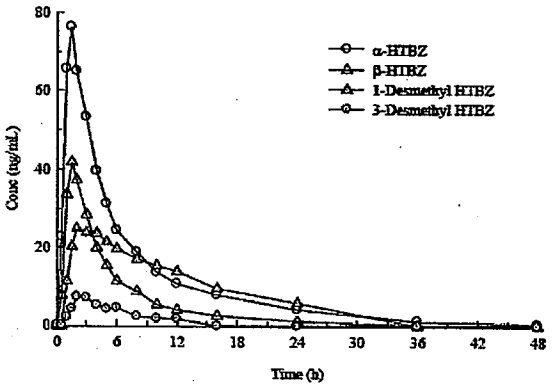
Table 1: Summary of pharmacokinetic parameters for α-HTBZ, β-HTBZ, 1-desmethyl HTBZ, and 3-desmethyl HTBZ after oral administration of a single 50 mg dose of tetrabenazine to healthy volunteers.

Parameter[1]	α-HTBZ	β-HTBZ	1-Desmethyl[2] HTBZ	3-Desmethyl[2] HTBZ
Cmax (ng/mL)	87.3 ± 22.7 (6)	46.8 ± 30.6 (6)	26.9 ± 8.41 (6)	9.53 ± 3.28 (5)
Tmax (h)	1.25 (6)	1.50 (6)	2.00 (6)	2.00 (5)
AUC(0-t) (h·ng/mL)	491 ± 269 (6)	219 ± 251 (6)	313 ± 113 (6)	59.3 ± 19.7 (4)
AUC(inf) (h·ng/mL)	506 ± 276 (6)	224 ± 252 (6)	466 ± 161 (6)	73.8 (1)
λz (h^{-1})	0.1017 ± 0.0340 (6)	0.1710 ± 0.0592 (6)	0.0631 ± 0.0209 (6)	0.1260 (1)
t½ (h)	7.33 ± 1.94 (6)	4.58 ± 1.86 (6)	12.2 ± 4.65 (6)	5.50 (1)

[1]Mean ± standard deviation (N) except for Tmax for which the median (N) is reported.
[2]The numbers correspond to the order of elution.

A comparison of the plasma concentration time course for each of these metabolites after a single 50 mg dose of TBZ is shown in the figure below, as provided by the Sponsor.

Figure 1: Mean plasma concentrations of α-HTBZ, β-HTBZ, 1-desmethyl HTBZ, and 3-desmethyl HTBZ after oral administration of a single 50 mg dose of tetrabenazine to healthy volunteers — linear axes.



Pharmacodynamics

Note: The Sponsor has included the results of ECG evaluation in a consideration of pharmacodynamics. These results will not be reviewed in detail here, as this study has been sent as a consult for QT review by the IRT.

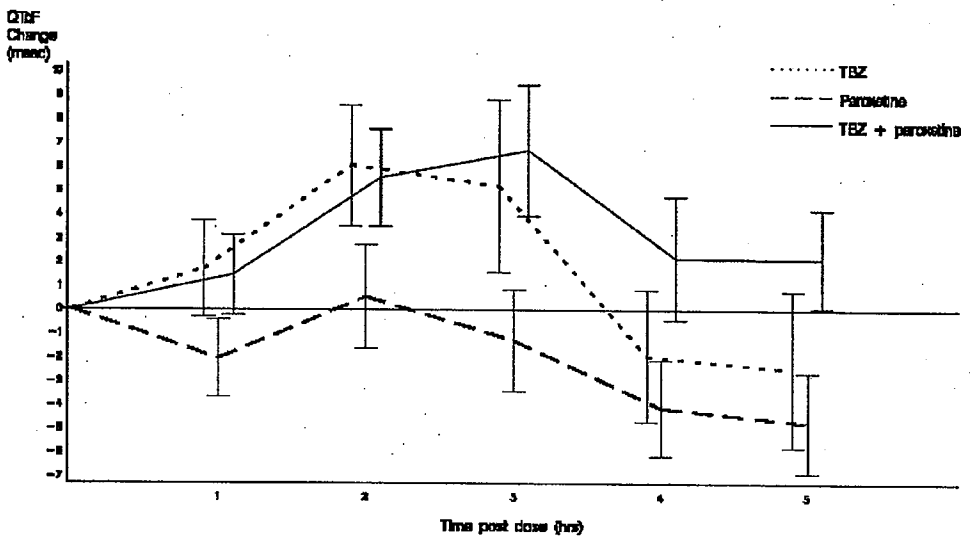
The following data has been provided by the Sponsor for change in QTcF:

Table 14. Maximum Change from Pre-dose in QTcF by Treatment

Treatment	N	Time Post-dose of Maximum Change (h post dose)	Maximum Mean Change (msec)	90% CI
Tetrabenazine 50 mg	30	2	6.0	3.5 to 8.6
Tetrabenazine 50 mg + Paroxetine 20 mg	27	3	6.7	3.9 to 9.4
Paroxetine 20 mg	27	2	0.5	-1.7 to 2.7

Source: Table 14.2.7.

Figure 8. Mean (SD) Change From Pre-Dose in QTcF Over Time by Treatment



In the outlier analyses, there were 2 subjects taking a single dose of TBZ 50 mg and 1 subject taking TBZ with paroxetine who had a change in QTcF $\geq$ 30 and < 60 msec. There were no subjects with change in QTcF $\geq$ 60 msec. There were no subjects with QTcF > 450 msec.

Safety

Adverse events will not be reviewed in detail here. There were no deaths or treatment emergent SAEs reported. Treatment emergent AEs reported by more than 1 subject and at a higher rate during the period of TBZ + paroxetine compared to when either drug was administered alone included (incidence presented for TBZ+paroxetine): dizziness (14.8%), fatigue (14.8%), nausea (14.8%), restlessness (14.8%), feeling hot (7.4%), and pallor (7.3%). AEs leading to premature discontinuation of study drug included rash for one subject on Treatment Day 4 after having receiving TBZ 50 mg on Day 1 and Paroxetine 20 mg on Days 3 and 4 and relentlessness and nausea for another subject after taking TBZ 50 mg on Treatment Day 1.

CONCLUSIONS and RECOMMENDATIONS:

- Administration of a single dose of TBZ 50 mg after CYP2D6 inhibition with the strong CYP2D6 inhibitor paroxetine resulted in an increase in Cmax and AUCinf of α-HTBZ of approximately 1.3-fold and 3.2-fold respectively. The Cmax and AUCinf of β-HTBZ increased approximately 2.4-fold and 9-fold, respectively. The elimination half-lives of both metabolites increased 2-fold and 3-fold for α-HTBZ and for β-HTBZ, respectively, resulting in elimination half-lives for these metabolites of approximately 13 hours.

- The ratio of α-HTBZ: β-HTBZ changed from approximately 3:1 to approximately 1:1 when TBZ was given after CYP2D6 inhibition.

- When TBZ is added to the regimen of a patient taking a strong CYP2D6 inhibitor, consideration should be given to using a lower daily TBZ dose and to [redacted]. The initial dose should be titrated up only as clinically necessary.

- When a strong CYP2D6 inhibitor is added to the regimen of a patient taking TBZ, consideration should be given to reducing the dose of TBZ and [redacted]

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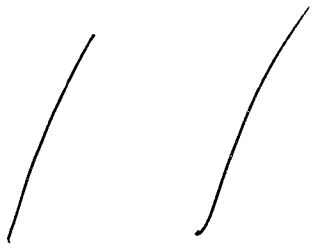
4.10 Hepatic Impairment Study

A COMPARISON OF THE PHARMACOKINETIC CHARACTERISTICS OF A SINGLE DOSE OF TETRABENAZINE IN LIVER IMPAIRED AND IN HEALTHY SUBJECTS

Study Investigators and Site:



Protocol Number: TBZ 203,010

Note: an interim report (dated February 28, 2005) was reviewed in the original NDA review (6 healthy subjects, 6 impaired subjects). The present review evaluates the final study report, completed in August 2007.

OBJECTIVES:

The primary objective was to compare the PK characteristics of TBZ and α- and β-dihydrotetrabenazine (HTBZ) in subjects with mild or moderate liver impairment to those of age-matched (+/- 5 years) healthy subjects.

The secondary objective was to evaluate safety and tolerability of TBZ in liver impaired subjects.

FORMULATIONS:

Table 1. Product used in TBZ 203,010

	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 12.5 mg tablet	6573804	6/8/05 (9/7/04 – 12/19/05)

Note: in a response from 11/27/07 the Sponsor states that the results of the stability testing shoed that the expiration date could be extended to 12/8/05 and that the last date that study drug was dispensed and administered was 12/7/05.

STUDY DESIGN:

This was a multi-center, single-dose, open-label study of the PK characteristics of TBZ, α-HTBZ, and β-HTBZ in liver-impaired and healthy subjects.

Inclusion criteria included male subjects 18-65 years of age, inclusive, with a body mass index ranging from 19-30 kg/m^2. Liver impaired subjects must have mild or moderate liver impairment (Child-Pugh classification 5 to 9) and stable chronic impairment of hepatic parenchymal function due to causes other than heart failure. Exclusion criteria included past history of depression or current depressive episode, subjects who consume more then 40 g of alcohol per day, subjects who currently smoke more than 10 cigarettes per day, subjects who drink more than 6 cups per day of caffeinated coffee, tea, or chocolate, or more than 6 glasses per day of cola/caffeine containing drinks. Also excluded were subjects with intake of any medication within less than 5 times its elimination half-life prior to study drug intake (with the exception of acetaminophen), and for healthy subjects, existence of any surgical or medical condition which might interfere with the absorption, distribution, metabolism, or excretion of study drug. Subjects with liver impairment must not suffer from CHF, hepatic encephalopathy that would impair ability to provide informed consent, or abnormal renal function.

Eligible subjects were admitted to the clinic on the day before treatment (Day -1). On Day 1, subjects who continue to be eligible received a 25 mg oral dose of TBZ with 200 ml of water at 8AM after a 10 hour fast. Subjects continued to fast for 4 hours after drug administration. Blood samples were drawn at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 36, and 48 hours after the dose of TBZ. Plasma was to be stored at -20 C or lower.

Safety monitoring included vital signs (supine and standing) 12-lead ECG, laboratory panel, and physical examination. Tolerability was evaluated on adverse events within each group (liver impaired and age-matched healthy subject).

ASSAY:

Plasma concentrations were measured using a validated LC/MS/MS method —→ 1266/1).

Table 3. Performance of Analytical Method for TBZ 203,010

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.990	0.2	0.5	5.3	2.1
					25.0	4.4	4.2
					100.0	5.7	-2.6
α- HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.990	0.5	1.0	5.8	4.4
					25.0	6.4	6.0
					100.0	5.6	-4.6
β-HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.984	0.5	1.0	7.2	0.3
					25.0	6.8	2.8
					100.0	5.5	-0.3

One calibration curve and duplicate QC samples were analyzed with each batch of study samples. Study samples were to be stored at –70° C according to the —→ analysis plan.

Samples were analyzed within the period for which the samples are stable at –70° C (321 days). The performance of the assays for all analytes is considered acceptable.

RESULTS:

Demographics

Twenty-four male subjects completed the study (12 healthy, 12 liver impaired). Two liver-impaired subjects exceeded 10 cigarettes/day. The control group is similar to the hepatic impaired group with respect to age, weight, and gender. Although the control group is not from the intended target population of Huntington's disease, the age range in the present study is within the age range enrolled in the pivotal efficacy study 103,004.

Table 4. Demographics of Subjects Completing the Study

Mean Age (Range)	Weight (mean ± SD)	Race *
54 (36-66) (n=24)	75 ± 11 kg (n=24)	Not available
53 (36-63) (healthy)	75 ± 8 kg (healthy)	
55 (39-66) (hepatic)	74 ± 14 kg (hepatic)	

*This study was conducted in France. French law prohibits recording of race in clinical studies.

The Child-Pugh classification for the hepatic impaired subjects is as follows where 5-6 is mild and 7-9 is moderate:

Subject	Classification
13	5
14	5
15	6
16	9
17	6
18	5
19	6
20	6
21	9
22	5
23	5
24	7

Pharmacokinetics

Note: PK data are generally in agreement with data from interim report.

Pharmacokinetic parameters were determined using noncompartmental analysis. The plasma concentration time course (provided by the sponsor) and the pertinent pharmacokinetic parameters for TBZ and for α-HTBZ and β-HTBZ are shown in Figure 1 and Table 6, below.

TBZ concentrations were less than the LOQ for the majority of sampling times in normal subjects.

Figures 1 and 2. Mean Plasma concentrations of TBZ after administration of TBZ to normal and hepatic impaired subjects.

Figure 1: Mean plasma concentrations of tetrabenazine after oral administration of 25 mg of tetrabenazine (2×12.5 mg tablets) under fasting conditions to subjects with hepatic impairment and healthy volunteers — linear axes.

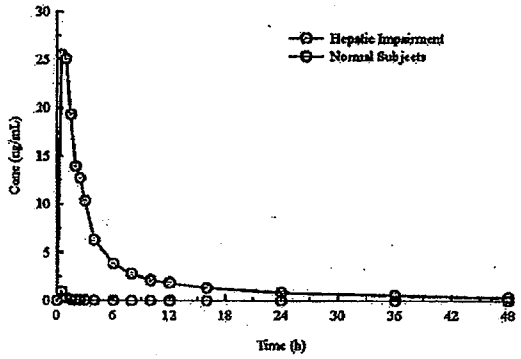
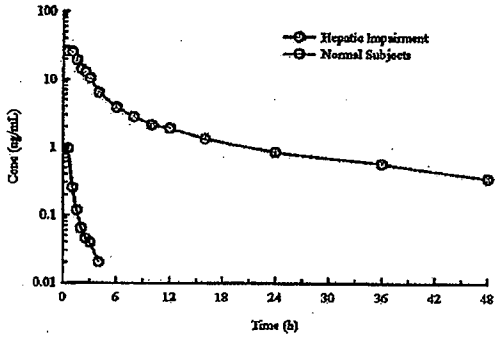


Figure 2: Mean plasma concentrations of tetrabenazine after oral administration of 25 mg of tetrabenazine (2×12.5 mg tablets) under fasting conditions to subjects with hepatic impairment and healthy volunteers — semi-logarithmic axes.



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Figures 3 and 4. Mean Plasma Concentration Time Course for α-HTBZ and for β-HTBZ after administration of TBZ to normal and hepatic impaired subjects.

Figure 7: Mean plasma concentrations of α-HTBZ after oral administration of 25 mg of tetrabenazine (2×12.5 mg tablets) under fasting conditions to subjects with hepatic impairment and healthy volunteers — semi-logarithmic axes.

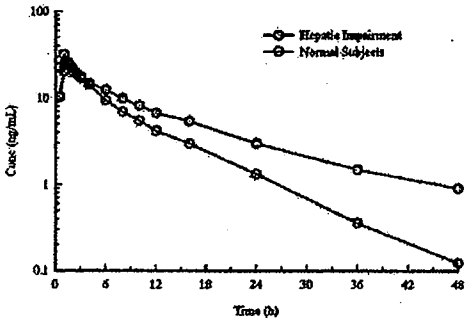

Figure 12: Mean plasma concentrations of β-HTBZ after oral administration of 25 mg of tetrabenazine (2×12.5 mg tablets) under fasting conditions to subjects with hepatic impairment and healthy volunteers — semi-logarithmic axes.

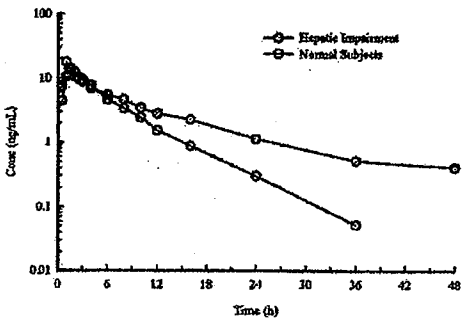

Table 5. Pharmacokinetic parameters (arithmetic mean) for α-HTBZ and β-HTBZ (Study TBZ 203,010)

	Normal (% CV) n=12	Hepatic Impairment (% CV) n=12
TBZ*		
t_{max} (h)[a]		1.0 (0.5-2.5)
C_{max} (ng/mL)		43.8 (81)
AUC $_{0-t}$ (ng*h/mL)		151 (72)
AUC $_{0-\infty}$ (ng*h/mL)		170.0 (79)
λz (hr^{-1})		0.1245 (200)
$t_{1/2}$ (h)		17.5 (44)
α-HTBZ		
t_{max} (h)[a]	1.0 (0.5-2.0)	1.75 (0.5-12.03)
C_{max} (ng/mL)	35 (32)	30.5 (49)
AUC $_{0-t}$ (ng*h/mL)	182 (53)	247 (46)
AUC $_{0-\infty}$ (ng*h/mL)	191 (51)	259 (52)
λz (hr^{-1})	0.1317 (41)	0.084 (44)
$t_{1/2}$ (h)	6.1 (39)	10.1 (55)
β-HTBZ		
t_{max} (h)[a]	1.0 (0.5-2.0)	1.75 (0.5-12.0)
C_{max} (ng/mL)	18.8 (52)	17.4 (74)
AUC $_{0-t}$ (ng*h/mL)	81.6 (87)	107.2 (48)
AUC $_{0-\infty}$ (ng*h/mL)	85.6 (85)	119.4 (53)
λz (hr^{-1})	0.213 (34)	0.1251 (66)
$t_{1/2}$ (h)	3.7 (39)	8.42 (72)

[a] median (range)

* For TBZ, plasma concentrations in normal subjects were < LOQ at most sampling times and no PK parameters were estimated. In impaired subjects, there were no concentrations ≥ LOQ for subject #13, 2 concentrations for subject ##22, and 1 concentration for subject #23, so for those subjects no PK parameters were calculated. PK parameters for TBZ in hepatic impairment reflect n=9.

As reviewed in the interim report, in most hepatic impairment patients, there were detectable concentrations of TBZ that were similar to or higher than exposures seen with α- or β-HTBZ. The highest TBZ concentrations in normal subjects ranged from 0.23-6.39 ng/ml when they were detectable. The mean TBZ Cmax concentration in hepatic subjects was approximately 7 to 190 fold higher than the detectable peak concentrations in healthy subjects in the present study.

In addition, there were increases in exposure to α- and β-HTBZ, in general, although to a lesser extent than the increase in exposure to TBZ. The AUC_{0-t} was approximately 31-35% higher in hepatic impairment for either α- or β-HTBZ than in normal subjects. The $AUC_{0-\infty}$ for α- HTBZ was approximately 35% greater and the $AUC_{0-\infty}$ for β-HTBZ was approximately 39% greater in hepatic impaired patients than in normal subjects. The half-life was also prolonged.

The tables below show the individual PK parameters for TBZ, α-HTBZ, and β–HTBZ, respectively for each liver impaired subject. Generally, AUC and elimination half-life were longer for subjects with worse impairment.

TBZ PK parameters by hepatic impairment subject

Subject Number	Child-Pugh Classification	Cmax (ng/ml)	AUC0-t (ng*hr/ml)	$t_{1/2}$ (h)
13	5	No conc ≥ LOQ	No conc ≥ LOQ	No conc ≥ LOQ
14	5	42.5	155.9	*
18	5	41.0	74.5	14.6
22	5	*	*	*
23	5	*	*	*
15	6	5.6	8.4	0.9
17	6	66.6	145.0	16.3
19	6	37.5	85.2	15.5
20	6	29.7	132.2	20.2
24	7	25.2	177.7	23.4
21	9	20	179.2	23.0
16	9	126.3	400.4	25.7

*PK parameters not calculated due to few detectable concentrations

α-HTBZ PK parameters by hepatic impairment subject

Subject Number	Child-Pugh Classification	Cmax (ng/ml)	AUC0-t (ng*hr/ml)	$t_{1/2}$ (h)
13	5	39.8	170.4	7.0
14	5	31.3	252.3	9.1
18	5	25.7	137	9.8
22	5	37.9	180.8	6.4
23	5	33.7	79.5	4.1
15	6	62.1	320.6	8.5
17	6	33.1	320.9	9.0
19	6	44.7	315.7	6.9
20	6	11.0	141.1	9.4
24	7	9.4	209.6	22.9
21	9	20.7	474.4	18.3
16	9	17	361.8	Not be estimated due to prolonged elimination phase

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β-HTBZ PK parameters by hepatic impairment subject

Subject Number	Child-Pugh Classification	Cmax (ng/ml)	AUC0-t (ng*hr/ml)	$t_{1/2}$ (h)
13	5	29	84.5	Could not be estimated*
14	5	14.8	111.9	Could not be estimated*
18	5	16.4	76.6	6.7
22	5	23.8	83.2	3.5
23	5	10.8	21.2	2.4
15	6	50.6	142.9	5.3
17	6	16.6	127.6	7.3
19	6	19.9	114.3	6.3
20	6	4.6	54.1	Could not be estimated*
24	7	3.9	84.7	18
21	9	9.6	202.3	17.8
16	9	9.0	183.5	Could not be estimated*

*prolonged elimination

Safety

The safety evaluation has not been reviewed in detail by OCP. The Sponsor reports that there were no deaths or SAEs reported during the course of the study. Four AEs reported occurred in 4 subjects, each subject having 1 AE. These AEs were :

- asthenia that was mild in intensity that was thought to be probably related to TBZ in a liver-impaired subject (mild impairment)
- rigors of mild intensity thought possibly related to TBZ in a liver impaired patient (mild impairment)
- venipuncture site hemorrhage in a healthy subject thought unrelated to TBZ
- Vasovagal syncope after blood draw (healthy volunteer) considered unrelated to TBZ

CONCLUSIONS:

1. Hepatic impairment reduces the metabolism of TBZ resulting in substantial exposures relative to normal subjects in whom minimal exposure to TBZ occurs (approximately up to more than 190 times higher exposure when TBZ was detectable in normal subjects). This was true for both mild and moderate hepatic impairment.

2. Exposure (AUC) to α- and β-HTBZ was 31-39% greater in the hepatically impaired subjects than in healthy controls.

3. The increase in TBZ exposure is not entirely accounted for by changes in exposure to α- and β-HTBZ.
4. The impact on safety of the increased exposure to TBZ in hepatic impairment is unknown and dose adjustment is not possible due to the magnitude of the increase in exposure. The label should contraindicate TBZ in hepatic impairment.

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Ramana S. Uppoor
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Mehul Mehta
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Pharmacometrics/Clinical Pharmacology Review

NDA :	**21,894**
Compound:	**Tetrabenazine tablets**
Submission Date:	**10/23/2005**
Consult Date:	**08/03/2006**
Sponsor:	**Prestwick Pharmaceuticals, Inc.,**
Reviewer:	**Joga Gobburu**

Tetrabenazine is being proposed for the treatment of Huntington's disease. Currently, there is no approved treatment for this disease and the prevalence rate is 4 to 10 per 100,000. Tetrabenazine is approved in other countries for this indication. The sponsor has conducted 2 double-blind, controlled clinical trials – TBZ 103,004 (study 004) and TBZ 103,005 (study 005). Study 004 won on the primary endpoint statistically. However, Study 005 failed on the primary endpoint. The sponsor also conducted 2 additional open-label un-controlled studies, Studies 007 and 006. These are extensions of studies 004 and 005, respectively. There is another open-labeled study 103,011 which includes an analysis of data collected from an open-labeled study.

With one of the two controlled trials not meeting the primary endpoint, the pharmacometrics group was consulted to gain insights into the dose-response characteristics. The purpose of this review is to investigate if the dose-response information can provide important support evidence towards the effectiveness of tetrabenazine for the treatment of Huntington's disease. The primary variable of interest, pertaining to effectiveness, is the total Chorea score. Higher scores reflect worse disease. The goal is to lower these scores.

Specifically, I will address two issues in this review, they are:

1. Is there adequate evidence for the effectiveness of Tetrabenazine on Chorea scores?
2. Is there adequate evidence for a sustained effect of Tetrabenazine on Chorea scores?

Recommendations

The Office of Clinical Pharmacology performed a detailed analysis of 3 clinical trials (TBZ 103,004, 103,005, 103,007). We find that Tetrabenazine demonstrates significant lowering of Chorea scores and that these effects are sustained over at least 13 weeks, the maximum maintenance period studied. The following presents the summary of the *'Analysis of Clinical Trial Data'* discussed subsequently in this review.

Is there adequate evidence for the effectiveness of Tetrabenazine on Chorea scores?

Yes, there is adequate evidence for the effectiveness of Tetrabenazine, as measured by Chorea scores, and this is internally consistent across clinical trials. Specifically, the evidence arises from the following:

1. Study TBZ 103,004, a placebo-controlled, 12 week study in 84 patients was successfully demonstrated that Tetrabenazine group significantly ($p<0.001$) lowered total Chorea scores, relative to placebo.
2. Study TBZ 103,004 also clearly demonstrated that upon withdrawal to Tetrabenazine treatment on week 12, the patients' total Chorea scores reached baseline by week 13, which is statistically significant ($p<0.001$).
3. Study TBZ 103,005, a placebo-controlled 5 day study clearly demonstrated that in patients in whom Tetrabenazine was withdrawn on Day 1 (N=12) or Day 3 (N=12), the total Chorea scores increased by Day 5, which is statistically significant ($p<0.001$). This increase in Chorea scores is consistent with the effect size observed in TBZ 103,004.
4. Study TBZ 103,004 data demonstrated a significant ($p<0.001$) dose-response. Data across all visits (titration and maintenance phases), for placebo and drug groups, were employed to test if there was a significant dose-response.
5. Study TBZ 103,007, an open-label study over 24 weeks in patients who completed TBZ 103, 004, demonstrated identical changes in Chorea scores between the two studies.
6. Study TBZ 103,007 data demonstrated a significant ($p<0.001$) dose-response. Data across all visits (titration and maintenance phases) were employed to test if there was a significant dose-response. This relationship is consistent with that found for TBZ 103,004.
7. Study TBZ 103,007 also clearly demonstrated that upon withdrawal to Tetrabenazine treatment on week 24, the patients' (N=10) total Chorea scores reached baseline by week 25.
8. The mean Chorea score changes in Study 006 are similar to those reported in Study 004, 005 and 007.

Is there adequate evidence for sustained effect of Tetrabenazine on Chorea scores?

Yes, there is adequate evidence for the sustained effectiveness of Tetrabenazine, as measured by Chorea scores, and this is internally consistent across clinical trials. Specifically, the evidence arises from the following:

1. TBZ 103,004 demonstrates that Tetrabenazine treatment over 5 weeks of maintenance offers superior lowering of Chorea scores, relative to placebo.
2. TBZ, 103, 004 also clearly shows a dose-response and an unequivocal effect upon withdrawal.
3. TBZ 103,007 demonstrates that Tetrabenazine effects are consistent with those observed in TBZ 103,004 and are sustained between week 11 and week 24. The fact that the same patients from TBZ 103,004 upon wash-out and re-titration gained similar effects in Chorea scores supports that the effect is reproducible too.
4. TBZ 103,006, which is an extension of TBZ 103,005, demonstrated that Tetrabenazine treatment led to significant lowering of Chorea scores. Increases in dosing from week 12 to 24 led to further decreases in Chorea scores. This observation supports the existence of a dose-response relationship. The need for further increase from their previously established 'best dose' after week 12 is not clear. But this could be due to differences between investigators.

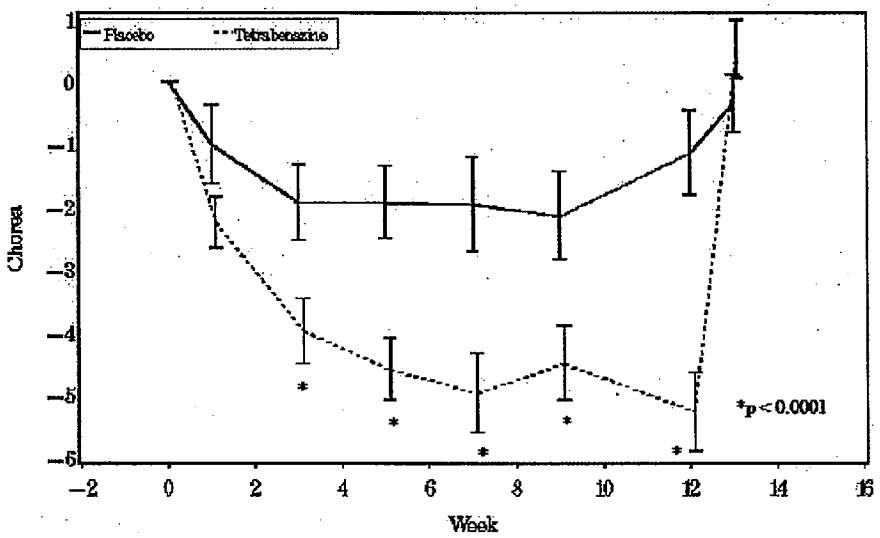
Analyses of Clinical Trial Data

Study TBZ 103,004

Patients (total N=84) were randomized to placebo (N=30) or tetrabenazine (N=54). Weekly dose titration was allowed until week 7 and doses maintained beyond that time for 5 weeks. The lowest tetrabenazine dose available was 12.5 mg and the highest allowed was 100 mg. Chorea scores were collected at weeks 1, 3, 5, 7, 9 and 12 on treatment.

The primarily analysis considered scores at week 12. During the treatment period, chorea scores for participants in the drug group declined by an estimated 5.0 units, as shown in Figure 1, while those in the placebo group declined by an estimated 1.5 units. The treatment effect of 3.5 units is highly significant ($p<0.0001$). **In study 103,004, the tetrabenazine group beat placebo according to the pre-specified analysis.**

Figure 1. Mean (± s.e.m.) Change in Total Chorea Score Over Time in 84 HD Participants Randomized to Tetrabenazine (N=54) or Placebo (N=30) - Protocol TBZ 103,004.

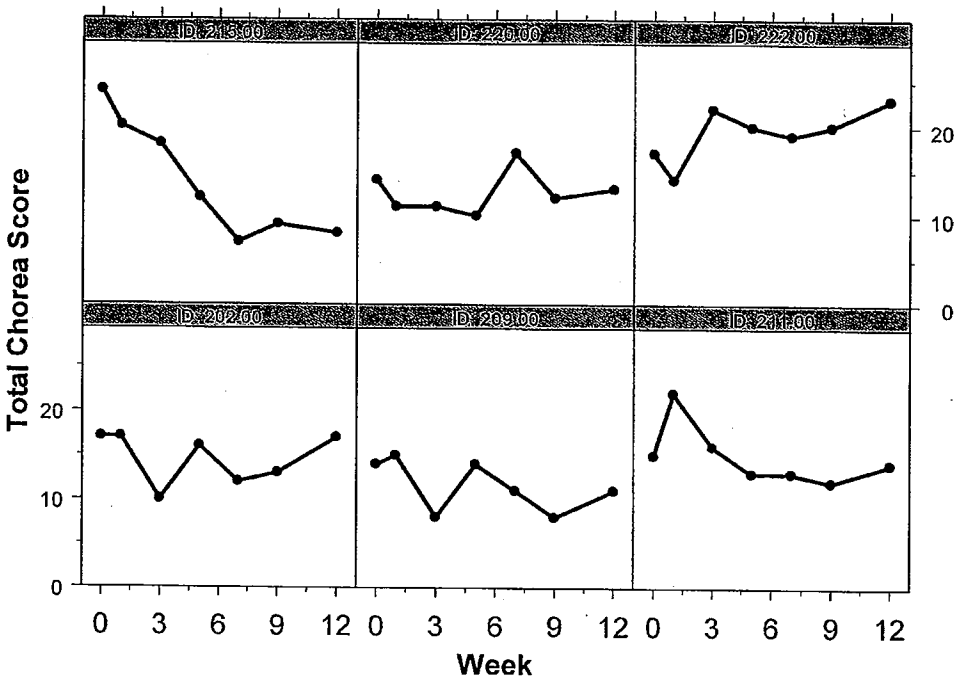


In addition to the primary analysis, I conducted a dose-response analysis by considering the doses (closest to each visit) and Chorea scores. There were a total of 574 observations in 84 subjects. The first challenge in investigating a dose-response relationship when the doses are titrated is to ensure that dose and time are not confounded. That is to say, we should not mis-attribute a time effect as a dose-effect. First, the half-life of tetrabenazine is about 5 hrs. The PK are at steady-state by the end of each day. **Hence, the PK will not confound the dose-response.**

The placebo data across 12 weeks were employed to further ensure that the Chorea scores do not change over time. Figure 1 above suggests that Chorea scores decline slightly till week 3 and by week 9 they start increasing back to baseline. However, a closer look at the individual time profiles of placebo patients together with statistical analysis suggests that Chorea scores remain reasonably unchanged over time in most patients.

Figure 2. Total Chorea score over 12 weeks in 30 HD patients randomized to placebo - Protocol TBZ 103,004. The graphs are presented as 5 sets with 6 plots per set. The patient ID is shown at the top of each plot. The ID number was

truncated to the last 3 digits, but the original ID can be restored by adding 447,000 to all ID numbers - Protocol TBZ 103,004.



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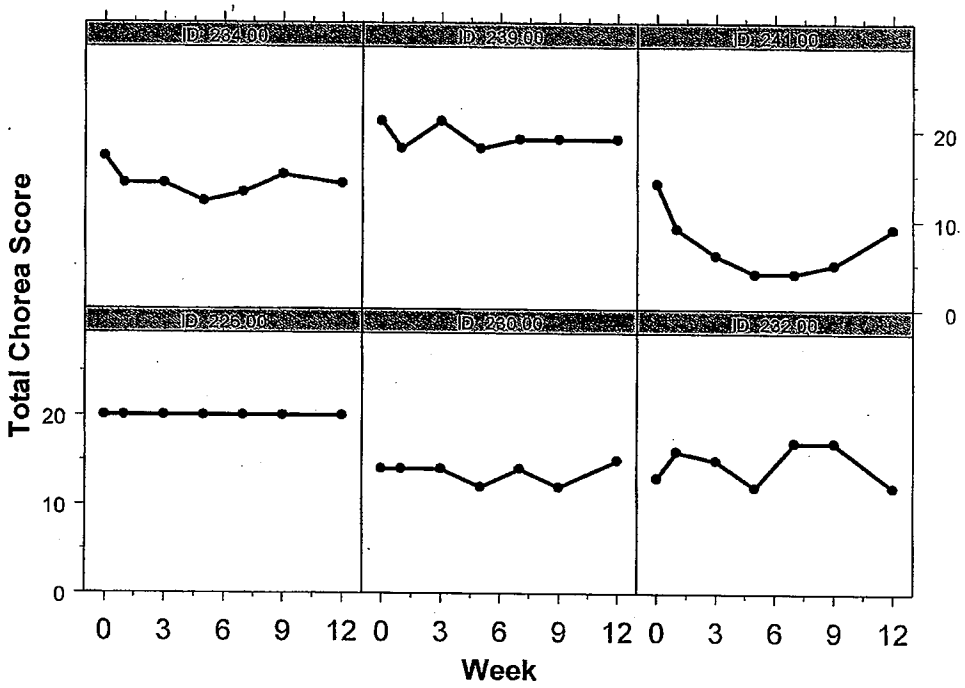

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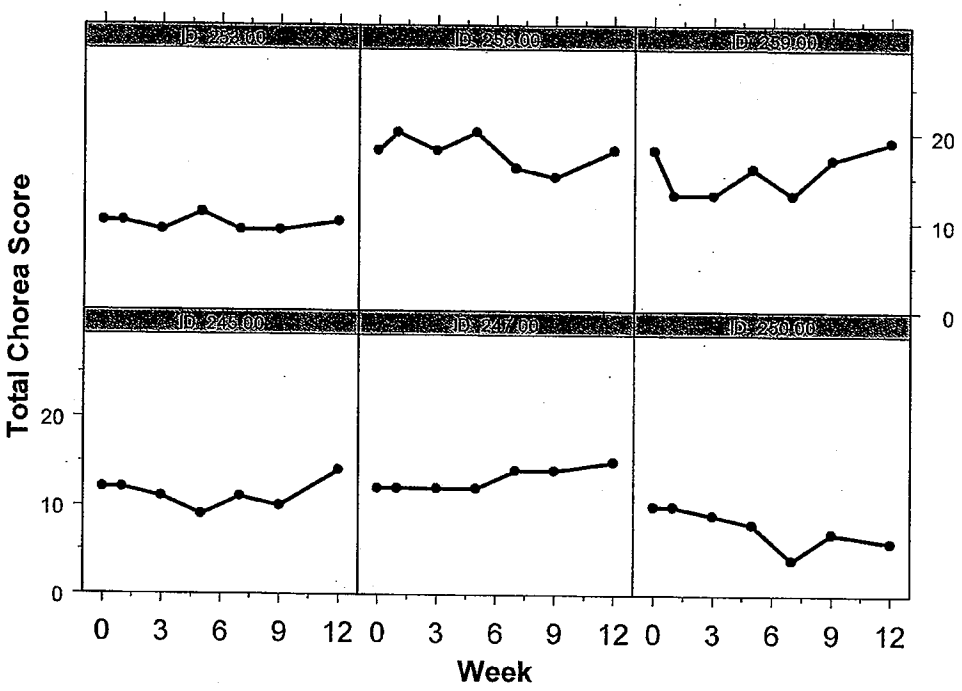

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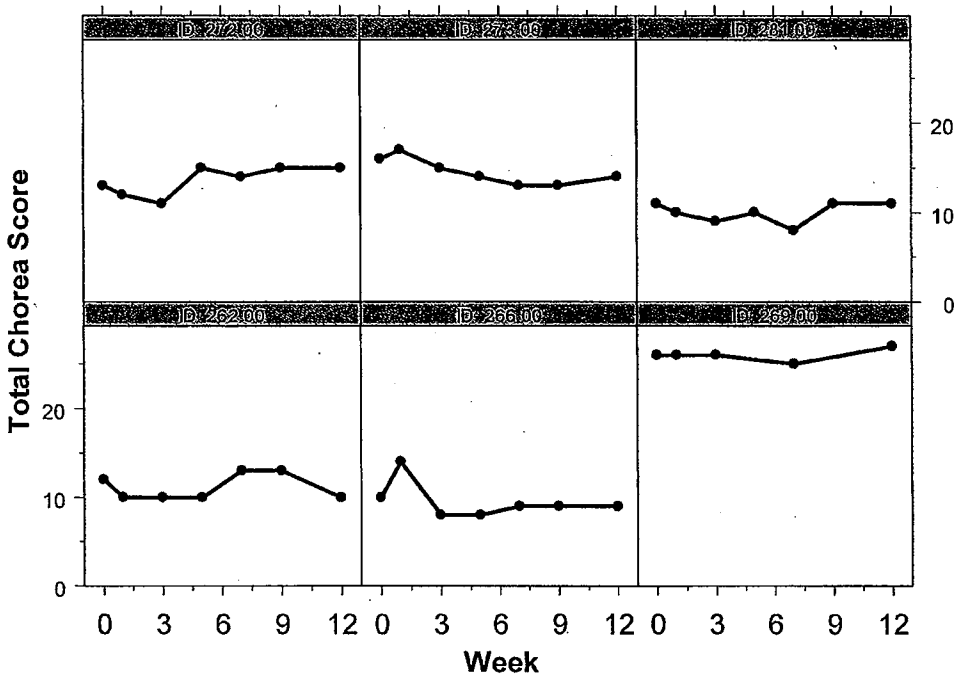

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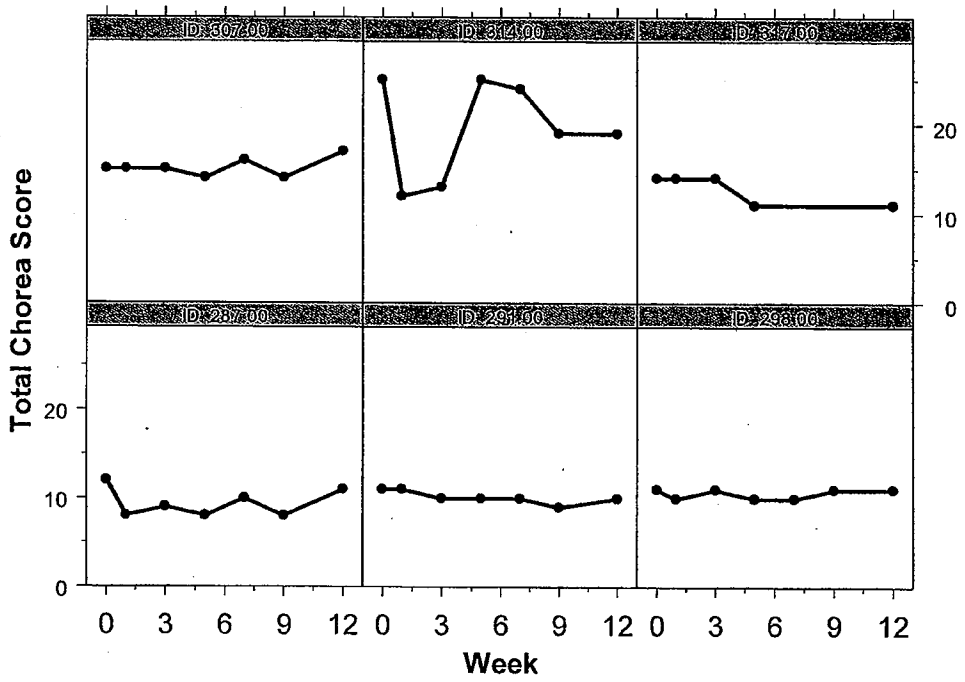


As shown in Figure 2 above, only patients 215, 211, 241 and 314 (of the 30 patients) show a trend towards declining Chorea scores over time. Even these trends are not consistent over time. For example, patient 314 shows a sudden drop by week 1, but then the scores increase back up in the next two visits.

To obtain more certainty on this issue, I conducted an analysis to test two hypothesis: 1) Is there a statistically significant change from baseline in the placebo group?; 2) Is there a statistically significant 'systematic' change over time in the placebo group? The first hypothesis was tested by comparing the baseline versus the subsequent visit data for significant difference (see Table 1). The second hypothesis included testing if the change in Chorea scores over time (slope) offers superiority over the first hypothesis. The expectation is that if there is a systematic change in Chorea scores over time then the second hypothesis should beat the first one.

Table 1. The statistical hypotheses and inferences to investigate whether there is a significant time course to **placebo** effects that could confound dose-response analysis - Protocol TBZ 103,004.

Hypothesis	Inference
Is there a statistically significant 'systematic' change over time (slope) in the placebo group?	There is no significant ($p=0.25$) systematic change in Chorea over 12 weeks, other than the constant placebo effect (above). Further, the maximal change of Chorea score was estimated to occur within one day (0.8 days). This signifies the rather flat placebo time course.

Based on each patient's Chorea time profile and the statistical analysis I conclude that there is no systematic change in the Chorea scores over time that could confound the dose-response relationship in the tetrabenazine group.

The data from placebo and tetrabenazine groups across 12 weeks were simultaneously analyzed to describe the dose-response. It is perhaps clear to most of us that mixed effects modeling is the best way to analyze data that arise from a dose titration study, if time is not a confounding factor (which it is not). The reason for this is that folks who are poor responders (low sensitivity to the drug) need the highest dose. This could lead to an apparent inverted U-shaped curve. Taking into account individual dose-response will circumvent this problem. Table 2 presents the 3 hypothesis tested and inferences drawn, regarding dose-response.

Table 2. The statistical hypotheses and inferences to investigate whether there is a significant dose-response relationship - Protocol TBZ 103,004.

Hypothesis	Inference
Is the change in Chorea on an average over 12 weeks in tetrabenazine group superior to that in placebo?	There is a significant drug effect on an average ($p<0.001$).
Is the tetrabenazine dose-response slope different from zero, after adjustment for placebo effect?	There is a significant (linear) dose-response relationship ($p<0.001$).

Figure 3 below supports that the linear model predicts the observed Chorea scores for both placebo and tetrabenazine groups reasonably well. The observed values are distributed around the line of identity. The dose-response model parameter estimates are provided in Table 3. The mean and variance estimates are well estimated as reflected by the tight confidence intervals.

Figure 3. A linear dose-response model describes the total Chorea scores for the placebo and tetrabenazine groups well. The predictions are distributed around the line of identity. Ideally, if the model were perfect the symbols and the line should be superimposed - Protocol TBZ 103,004.

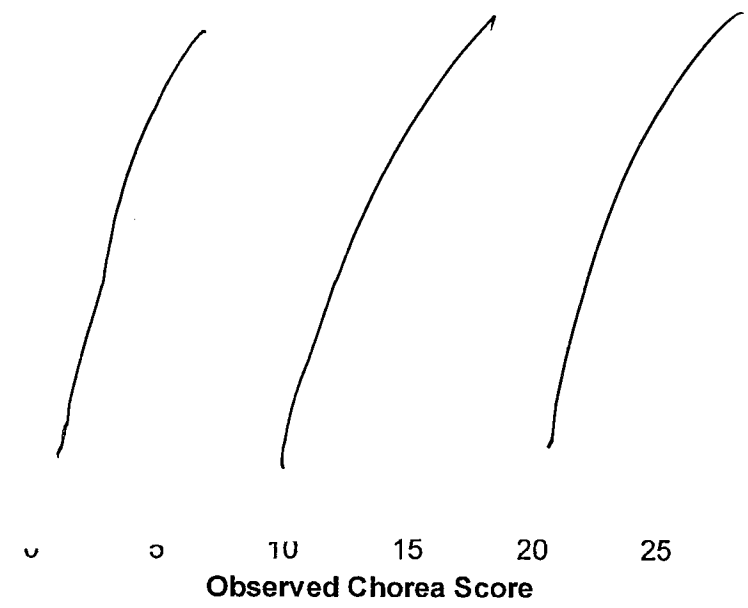
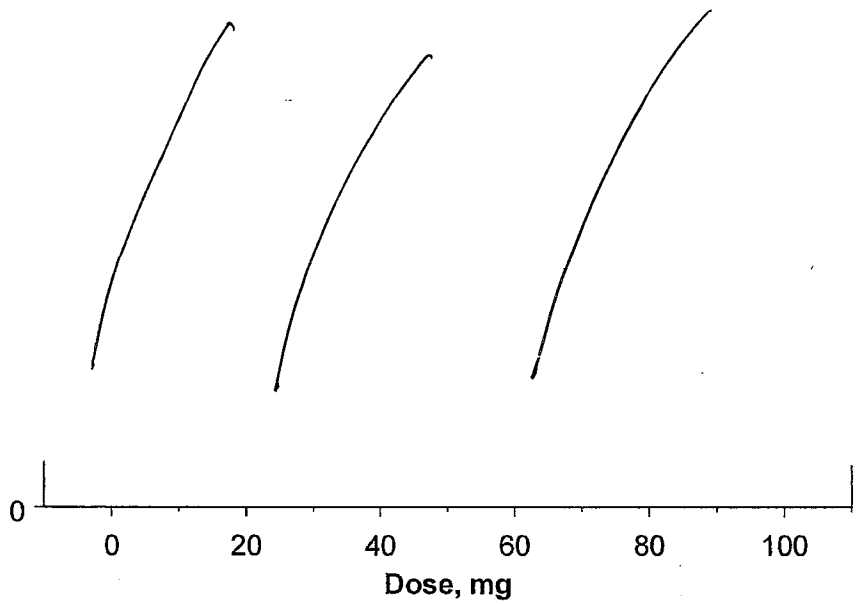


Table 3. Dose-response model parameter (mean and between-subject variability (BSV)) estimates and the 90% confidence intervals- Protocol TBZ 103,004. The slope of the dose-response is expressed as % change relative to baseline per mg of tetrabenazine dose. For instance, a dose of 100 mg will result in a net drug effect (placebo corrected) of 40% (=0.0042x100). In other words, 100 mg will reduce a baseline score of 14 to 8.4 (placebo corrected).

Parameter	Mean (CI)	BSV (CI)
Baseline score	13.4 (12.9, 13.9)	25% (21%, 29%)
Placebo effect, score	-1.2 (-0.7, -1.7)	Not estimable
Slope of dose-response, % per mg	0.0042 (0.0037, 0.0046)	58% (42%, 74%)
Residual Variability	24% (20%, 28%)	

Figure 4 depicts the individual (dotted line) and typical (solid line) dose-response curves. As can be seen in Figure 4, patients show varying degrees of dose-response relationship – some are shallow, several others are relatively steeper. On an average, higher doses lead to larger reductions in the Chorea score.

Figure 4. The individual and typical dose-response relationships describes the total Chorea scores for the placebo and tetrabenazine groups well. The predictions are distributed around the line of identity. Ideally, if the model were perfect the symbols and the line should be superimposed - Protocol TBZ 103,004.



Study TBZ 103, 004 Conclusions

1. **The tetrabenazine group beat placebo according to the pre-specified analysis ($p<0.001$).**
2. **There is a significant dose-response relationship, which provides a strong supportive evidence for the effectiveness of tetrabenazine.**
3. **Chorea scores significantly increase, in fact reach baseline, within a week upon cessation of tetrabenazine. This result also supports the effectiveness of tetrabenazine.**

Study TBZ 103,005

This was a randomized placebo controlled study that recruited 30 patients who were stabilized on tetrabenazine for at least 2 months. Tetrabenazine in 12 patients was stopped on day 1; on day 3 in another 12 patients; and on day 5 in the remaining 6 patients. Until stopped patients received their 'best dose'. The sponsor claims that the investigators instructed their patients to stop taking tetrabenazine on the previous evening. That is, if patients were supposed to receive their last dose on day 3, in reality the patients received their last dose on the evening of day 2. So, at least 12 hours have elapsed since the last dose. The primary analysis was Group 1 (tetrabenazine withdrawn on Day 1) versus Groups 2 (tetrabenazine withdrawn on Day 3) and 3 (tetrabenazine withdrawn on Day 5). This endpoint was not met. The sponsor attributed this failure to their belief that in all the groups tetrabenazine treatment was 'completely' washed out.

However, there is one useful piece of information from this trial that supports the effectiveness of tetrabenazine. Table 4 shows the mean total Chorea scores in the all the 3 groups and Table 5 presents the comparison between the different groups. Clearly, in Group 1 in which patients withdrew from tetrabenazine on Day 1, the scores increased significantly ($p<0.001$). The mean increase is 5.3, which is consistent with the effect size seen in Study 103, 004. This is further supported by the sustained increase on Day 5. The results are similar for Group 2 also.

Table 4. Mean (± SD) Total Chorea Scores throughout the Study by Withdrawal Group and by Study Day -- Protocol TBZ 103,005.

Withdrawal Group	Day 1	Day 3		Day 5
	On Tetrabenazine	Off Tetrabenazine	On Tetrabenazine	Off Tetrabenazine
Group 1 (N=12)	9.4 ± 4.9	14.8 ± 5.4		14.8 ± 7.1
Group 2 (N=12)	9.1 ± 6.2	12.7 ± 5.3		14.6 ± 5.4
Group 3 (N=6)	11.2 ± 4.4		12.8 ± 6.0	15.2 ± 6.0

Table 5. Mean (± SD) Change Scores with p-Value (By T-Test) of Total Chorea Scores By Treatment Group from Day 5 to Day 3 and Day 3 to Day 1 for 30 HD Participants - Protocol TBZ 103,005.

Treatment Assignment Group	Study Day	
	Day 3 to Day 1	Day 5 to Day 1
Group 1 (N=12)	5.3 ± 3.5; p-value = 0.000245*	5.3 ± 3.8; p-value = 0.000499†
Group 2 (N=12)	3.6 ± 2.8; p-value = 0.000951*	5.5 ± 3.4; p-value = 0.000159†
Group 3 (N=6)	1.7 ± 4.7; p-value = 0.426*	4.0 ± 3.0; p-value = 0.02†

Study TBZ 103, 005 Conclusions

1. **The fact that the total Chorea scores have increased significantly upon withdrawal of tetrabenazine is a strong evidence of its effectiveness. The increase in total Chorea scores is about 5.3 in patients who stopped drug intake either on Days 1 or 3. This effect size is consistent with that reported in TBZ 103, 004.**

Study TBZ 103,007

Patients who completed TBZ 103, 004 (placebo controlled study, which was positive), were included in the study TBZ 103,007. This was an open-label study with titration allowed for 11 weeks and the total Chorea score data up to 36 weeks were available. Total Chorea scores were measured in all patients at baseline (post wash-out period of Study TBZ 103, 004) and subsequently at weeks 2, 6, 12, 24, 25 and 36.

Figure 5 clearly shows that the mean tetrabenazine effects observed in Study 004 and 007 are identical. Of particular interest is the sustained effect of tetrabenazine over 24 weeks on maintenance dose. A concern with open-label studies is their vulnerability to influence the investigator assessments and patient response. However, this is not a concern in this case for several reasons. First, there are two well-controlled studies (Study 004 and 005) which unequivocally showed that tetrabenazine lowers Chorea scores. Second, the patients who participated in Study 004 exhibited identical effects (on an average) in this extension study (007). Third, in 10 patients in whom tetrabenazine was withdrawn at week 24, the Chorea scores increased and reached baseline by week 25. This observation is in congruence with the results from Study 005. Fourth, patients who received placebo in Study 004 had lower Chorea scores when they received tetrabenazine in Study 007.

Dose-response analysis for Study 007 indicated that the estimate of the slope was 0.0038 % per mg, which is in close proximity to 0.0042 % per mg for the Study 004 data alone. The ideal approach would be to establish dose-response for the combined data set. Table 6 presents the estimates of the linear dose-response model for the combined Study 004 and 007 data. The results are consistent internally. Further, in 10 patients who withdrew from Tetrabenazine treatment (planned) on week 24, the total Chorea scores reached baseline by week 25 (mean change at week 24 was 6 versus zero at week 25).

Figure 5. Mean total Chorea scores in Studies 004 and 007 for placebo and tetrabenazine groups. Effects of tetrabenazine in study 004 and 007 are identical. Titration schemes in both the studies were similar. Study 007 supports the durability of response over 36 weeks, specifically for about 24 weeks on maintenance.

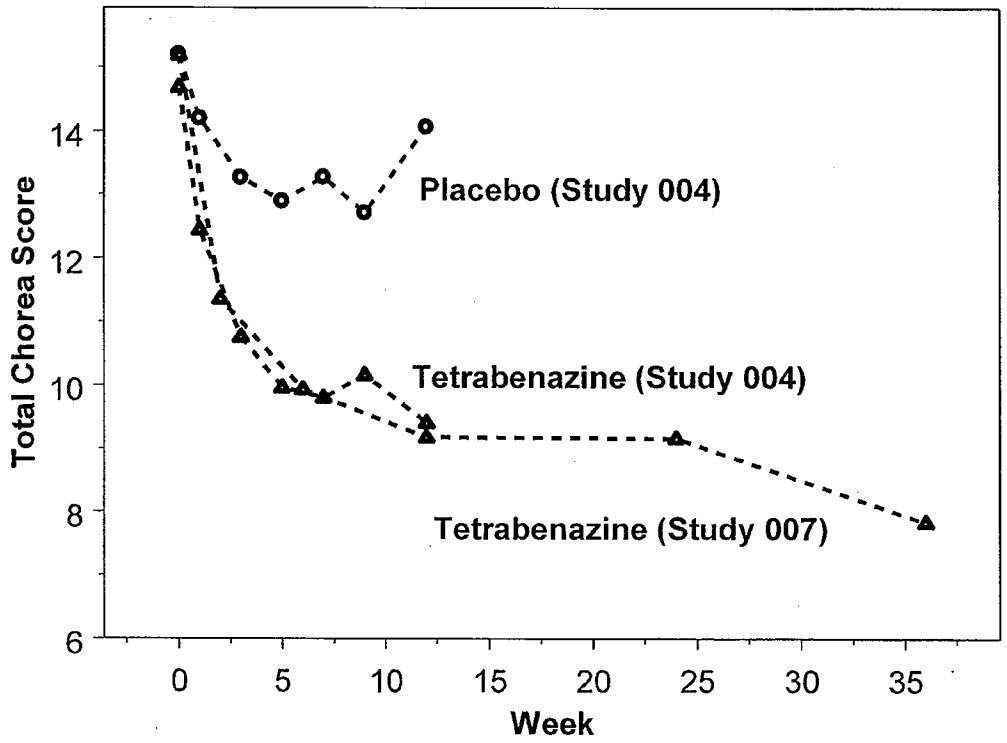


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Figure 6. Mean total Chorea scores of patients who received placebo in study 004 and then received tetrabenazine in study 007. This can be treated as a cross-over trial, for practical purposes. The total chorea scores are lower in patients when they received TBZ.

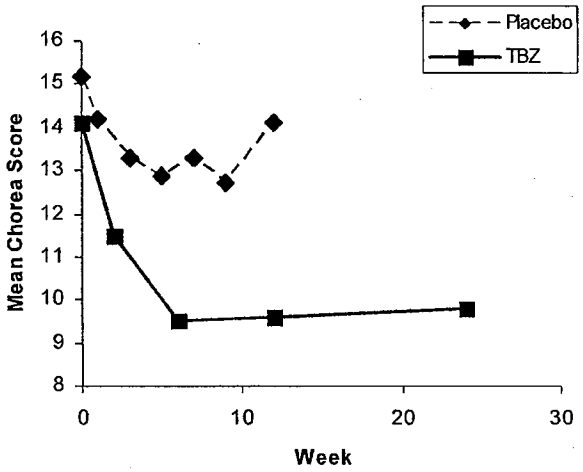


Table 6. Dose-response model parameter (mean and between-subject variability (BSV)) estimates and the 90% confidence intervals- Protocols TBZ 103,004 and TBZ 103,007 combined. The slope of the dose-response is expressed as % change relative to baseline per mg of tetrabenazine dose. These estimates are consistent with those reported in Table 5 for TBZ 103, 004 alone.

Parameter	Mean (CI)	BSV (CI)
Baseline score	13.4 (12.9, 13.9)	25% (21%, 29%)
Placebo effect, score	-0.8 (-0.5, -1.2)	Not estimable
Slope of dose-response, % per mg	0.0043 (0.0037, 0.0046)	56% (46%, 66%)
Residual Variability	26% (23%, 29%)	

Study TBZ 103, 007 Conclusions

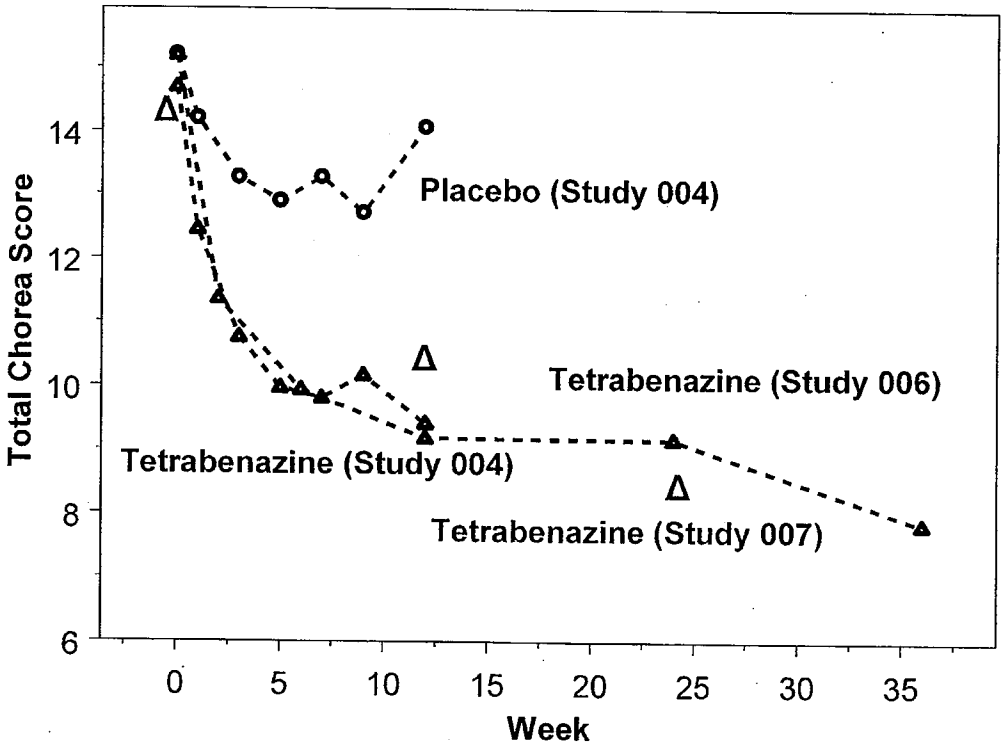
1. **The changes in Chorea scores from this study and TBZ 103,004 are super imposable.**
2. **There is a significant dose-response relationship, which provides a strong supportive evidence for the effectiveness of tetrabenazine. This relationship is consistent with that observed in TBZ 103,004.**

3. **Chorea scores significantly increase, in fact reach baseline, within a week upon cessation of tetrabenazine. This result also supports the effectiveness of tetrabenazine.**
4. **The lowering of Chorea scores is shown to be sustained over 24 weeks, but more importantly between week 11 and week 24 (maintenance phase).**

Study TBZ 103,006

This study enrolled patients who completed TBZ 103,005. In TBZ 103,005 thirty patients were off Tetrabenazine by Day 5. These patients were previously stabilized on 'best dose' of Tetrabenazine. In TBZ 103,006, these patients were resumed on the 'best dose' instead of upward titration again. Chorea measurements were performed on weeks 12 and 24. The mean total Chorea scores for 3 studies are shown in Figure 7.

Figure 7. Mean total Chorea scores in Studies 004, 007 and 006 for placebo and tetrabenazine groups. Effects of tetrabenazine in all studies are similar. Titration schemes in both 004 and 007 were similar. Study 006 did not employ titration for the first 12 weeks, but dose was increased in 53% (9 of 17) patients after week 12. Study 007 and 006 supports the durability of response over 36 weeks, specifically for about 24 weeks on maintenance.



As shown in Figure 6, the Chorea score changes for Study 006 are reasonably consistent with the Study 004 and 007. The mean change in Chorea score at week 12 was in this study was -3.7. For the same patients, at the so-called same best dose in Study 005, the change was -5.3. It is not clear if this is a serious discrepancy. More measurements between week 0 and 12 would have allowed appreciation of the time course of effect better. But the claim is that the maximal changes in Chorea scores occur shortly after giving the 'best dose'. Also, there is a further decrease in Chorea scores upon increasing doses after week 12. It is not again clear why patients needed higher doses than their previously established 'best dose'. The differences, if any, between the investigator assessment and that of the patients' previous physician could lead to the need for further titration. Nevertheless, there is a decrease in Chorea scores by week 12 and further decrease upon upward titration at week 24. The sponsor's proposal to resume patients at their previous best dose is reasonable.

Study TBZ 103, 006 Conclusions

1. **The mean Chorea score changes in Study 006 are similar to those reported in Study 004, 005 and 007.**
2. **Patients resuming their 'best dose' had a lower Chorea scores at week 12. This period can be treated as maintenance period as the doses were not changed (unless an AE happens).**
3. **The need for further increase from their previously established 'best dose' after week 12 is not clear. But this could be due to differences between investigators.**

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John Robert Powell
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Mehul Mehta
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NDA 21,894
Tetrabenazine

Clinical Pharmacology and Biopharmaceutics Review

NDA:	21-894
Brand Name:	Xenazine
Generic Name:	Tetrabenazine
Type of Dosage Form:	Tablet
Strengths:	12.5 mg, 50 mg
Indications:	Chorea of Huntington's Disease
Type of Submission:	New/Priority
Sponsor:	Prestwick Pharmaceuticals, Inc.
Submission Date:	September 23, 2005 October 18, 2005 December 9, 2005 December 14, 2005 December 15, 2005 December 19, 2005 December 23, 2005 January 18, 2006 February 21, 2006
OCPB Division:	DCP-I
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1 Executive Summary

1.1 Recommendations

The clinical pharmacology and biopharmaceutics information submitted to NDA 210894 is acceptable provided that the following are addressed prior to approval:

1) The Sponsor has been asked to clarify the rotation speed at which the dissolution method was generated (request was sent on 1/2/06 and as of 2/14/06 there has been no response). If the Sponsor has data to support the proposed rotation speed and agreement is reached between the Sponsor and the Agency regarding dissolution specifications, the method and agreed upon specifications can be accepted as interim method and specifications. The recommended dissolution method and specifications are as follows:

   Apparatus: USP Apparatus 2 (Paddles)
   Medium: 0.1 M HCl
   Volume: 900 ml
   Rotation Speed: 50 rpm
   Specification: ≥ — (Q) in 30 minutes

2) Since the 25 mg tablet is scored, the Sponsor should demonstrate dissolution similarity (with f2 testing and using the interim dissolution method above) between 2 half-tablets and 1 whole 25 mg tablet.
3) The P16 component, identified as the largest circulating component, in the mass balance study should be characterized. In addition, the extent to which the mono- and bis-dealkyl tetrabenazine metabolites (and other individual metabolites) are circulating should be clarified.
4) Adequately performed *in vitro* metabolism studies be submitted to address the potential for inhibition or induction of P450s by TBZ and its metabolites. The Sponsor should also

characterize the *in vitro* metabolism of TBZ and its metabolites as well as the role for PgP in TBZ disposition. Finally, the Sponsor should adequately address the role for TBZ as a PgP inhibitor *in vitro*. There is currently insufficient information to allow for adequate labeling regarding the potential for drug interactions.

5) Tetrabenazine should be contraindicated in patients with hepatic impairment since the exposure to TBZ in hepatic impairment is > 70-fold greater than the exposure in healthy subjects. Without information on changes in efficacy and safety, it is not possible to recommend a dosage adjustment.
6) Agreement is reached between the Sponsor and the Agency regarding labeling (Please refer to **Section 3** of this review).

Comments to Sponsor

The following additional comments should be sent to the Sponsor for guidance on performing the in vitro drug metabolism studies (and were generally communicated to the Sponsor in an email of 12/21/05):

1. The Sponsor has not taken a step-wise approach to understanding the metabolism of TBZ or its metabolites. The preferred first approach would be to directly identify metabolites after incubation with hepatocytes or liver slices. Subsequent studies can also eliminate non CYP oxidative pathways.
2. The studies to evaluate CYP pathways of TBZ and HTBZ metabolism are methodologically deficient. It is recommended that recombinant enzymes not be used alone, but in combination with other methods (such as use of inhibitors) for identifying drug metabolizing P450 isozymes. In addition, the probes used as controls in the submitted studies are not classical, preferred probes, and the Sponsor has not provided justification, so it is difficult to understand the acceptability of the reactions.
3. Studies characterizing the metabolism of TBZ *in vitro* should include measurement of the formation of metabolites (including the oxidative metabolites of TBZ and the oxidative metabolites of HTBZ) to identify the pathways by which they are formed.
4. The Sponsor should follow-up with the results of the submitted studies with *in vitro* inhibition studies that use well accepted methodology and preferred substrates to confirm lack of involvement of TBZ and its metabolites in inhibition of P450s.
5. The *in vitro* study of TBZ inhibition of PgP provided from the literature was not conducted with methods that are in agreement with current Agency thinking. The *in vivo* TBZ-digoxin interaction study was performed with a low dose of TBZ, and does not allow for conclusions about higher doses that will be used clinically. The Sponsor should perform an adequate *in vitro* inhibition study using preferred methodology to determine the need for further *in vivo* study.
6. The results of adequate *in vitro* drug metabolism studies will guide the need for further *in vivo* drug interaction studies.
7. Since CYP2D6 appears to be involved in the metabolism of TBZ and HTBZ, we recommend genotyping for CYP2D6 in future TBZ clinical trials.
8. The thorough QT study did not assess exposure to TBZ or metabolites outside of the ranges that might be normally observed after administration. The results of the in vitro drug metabolism studies may help guide decisions regarding the need and approach for further metabolically-based evaluation of QT.

1.2 Phase 4 Commitments

The Sponsor should address the following issues as Phase IV commitments:

1) *In vivo* study of the effect of CYP2D6 inhibition on TBZ disposition using a strong CYP2D6 inhibitor since CYP2D6 inhibition may increase the exposure to the inactive β-HTBZ relative to the active moiety α-HTBZ (based on evaluation of plasma concentrations in Phase III studies).
2) Evaluate the clinical relevance of CYP2D6 inhibition after administration of TBZ *in vivo* using a sensitive CYP2D6 substrate (such as desipramine) since *in vitro* studies suggest involvement of CYP2D6.
3) Other *in vivo* drug interaction studies should be guided by the results of the *in vitro* drug metabolism studies, in agreement with the Agency.
4) The discriminatory ability of the interim dissolution method should be determined in order to determine the final dissolution specifications.

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1.3 Summary of Clinical Pharmacology and Biopharmaceutics Findings

The present NDA (21-894) has been submitted to support the approval of XENAZINE (tetrabenazine) for the treatment of chorea associated with Huntington's disease. The strengths of tetrabenazine (TBZ) are 12.5 mg and 25 mg. The proposed starting dose is 25 mg daily (12.5 mg in the morning and 12.5 mg in the evening) with upward titration as follows. One week later the dose should be increased to 37.5 mg per day (12.5 mg in the morning, 12.5 mg at noon, and 12.5 mg in the evening) and then continue to be increased weekly by 12.5 mg increments until satisfactory control of chorea is achieved or until side effects occur or until a maximal dose of 100 mg per day (given in divided doses, generally tid) is reached. (Of note, the highest single dose in the pivotal efficacy studies was a 37.5 mg dose).

The following clinical pharmacology studies have been submitted and reviewed:

• Food Effect Study	TBZ103,003
• Bioequivalence of 12.5 mg and 25 mg	TBZ 104,012
• Effect on Prolactin	TBZ 202,001
• Effect on Prolactin and Other Hormones	TBZ 203,008
• Effect on Digoxin	TBZ 203,009
• Single and Multiple Dose PK	1700114
• Hepatic Impairment Study	TBZ 203,010
• QT Study 104,015	
• Mass Balance Study	RD 204/24124
• Protein Binding	AG-04001
• In Vitro P450 Studies	AG-A04002, AG-A05001, AG-A03001

In addition, the following double-blind, placebo-controlled Phase III studies had either PK data or dose-response data:

• Tetra HD	TBZ 103,004
• Tetra Withdrawal	TBZ 103,005

The key findings with respect to the Clinical Pharmacology and Biopharmaceutics of TBZ are as follows:

Pharmacokinetics

TBZ is rapidly metabolized after oral administration to several metabolites including the circulating metabolites α- and β-dihydrotetrabenazine (HTBZ). TBZ and α-HTBZ inhibit the human brain synaptic vesicular monoamine transporter (VMAT), resulting in depletion of monoamine stores, particularly with respect to dopamine. TBZ normally does not circulate to a significant extent (in healthy volunteers it is usually not detectable or is seen at concentrations approximately 5-fold lower than α-HTBZ, with an estimated half-life of approximately 0.4 hrs). Therefore α-HTBZ has been considered the active circulating moiety. On average, exposure to

α-HTBZ is greater than exposure to β-HTBZ, although the ratio ranges from approximately 0.4 to 6.8.

After oral administration, the extent of absorption is at least 75.4%, based on results of a mass balance study. Food does not alter exposure to α- or β-HTBZ. Protein binding is approximately 60% for α- and β-HTBZ and estimated to be approximately 80% for TBZ. Pharmacokinetic (PK) parameters have been characterized after single doses of 12.5 mg, 25 mg, and 50 mg and after repeated doses of 25 mg in healthy subjects. Across studies, Cmax and AUC for both α- and β-HTBZ increase slightly more than dose proportionately (approximately 2.3-2.6-fold with a 2-fold increase in dose) following single doses. Tmax occurs at approximately 1-1.5 hours for either enantiomer. The elimination half-life is approximately 4-8 hours for α- HTBZ and approximately 2.4-5.2 hours for β-HTBZ. Based on the half-life, an accumulation factor of 1.2-1.5 would be predicted for a dose given every 12 hours, although a slightly greater than expected increase in exposure (approximately 1.7 fold for β-HTBZ) is observed after repeated dosing. There is extensive variability in exposure (CV for Cmax and AUC 40-60% for α-HTBZ and 60-166% for β-HTBZ) in healthy volunteers. The PK has not been well characterized in patients, although based on the available data it seems be similar to that described in healthy subjects with respect to exposure, variability in PK, and relationship of α- to β-HTBZ.

TBZ is extensively metabolized, and metabolites are primarily renally eliminated (a mean of 75.4% of radioactivity in the mass balance study was excreted in the urine). More than 19 metabolites are reported to be found in the urine. TBZ is not found in the urine. The mass balance study identified circulating metabolites in addition to α- and β-HTBZ that may be circulating to a similar extent. The PK and pharmacologic activity of other metabolites have not been characterized. *In vitro* studies suggest a role for CYP2D6 in the metabolism of TBZ and α- and β-HTBZ, although deficiencies in the evaluation do not allow for ruling out the role of other P450s.

Exposure response relationships for efficacy have not been determined. Dose is titrated to "best dose" based on tolerability and efficacy, and in the PK data set from the efficacy studies there is extensive variability in exposure. With respect to safety, there is a positive correlation between exposure to α- HTBZ and prolactin secretion, as expected based on its mechanism of action, and between β-HTBZ and prolactin secretion that may be unrelated. In a study evaluating the effect of TBZ on QTc prolongation after a single dose of up to 50 mg there was an approximate 7 msec increase in QTcF or QTcI, and a 10 msec increase could not be ruled out. There was a slight correlation between QTc increase and β-HTBZ exposure.

The effect of renal impairment on TBZ PK or PD has not been evaluated. In mild to moderate hepatic impairment, the elimination half lives of α- and β-HTBZ were prolonged to approximately 10 hours. However, in hepatic impairment (interim report from an ongoing study), exposure to TBZ was similar to or higher than exposures seen with either α- or β-HTBZ, and exposures were approximately 70-fold higher for Cmax and 100 to 200-fold higher for AUC than the mean in healthy subjects. Without information on changes in efficacy and safety, it is not possible to recommend a dosage adjustment in hepatic impairment. Therefore, from an Office of Clinical Pharmacology (OCP) perspective, TBZ should be contraindicated in hepatic

impairment. The roles of age and race have not been sufficiently characterized to impact labeling. No significant gender effect is observed.

The role of pharmacogenetics in disposition to TBZ or its metabolites has not been well characterized, nor has the role of CYP2D6 inhibition. In Phase III clinical studies in which plasma concentrations of α- and β-HTBZ were determined, it appears that subjects taking concomitant CYP2D6 inhibitors had a ratio of α:β-HTBZ (< 1.6) that is lower than that observed in patients not identified as taking CYP2D6 inhibitors. In addition, in the 2 CYP2D6 PMs that were included in the QT study, the ratio of α-HTBZ: β –HTBZ was ≤ 1 (range 0.29-1.0) for Cmax and AUC and the elimination half life for those metabolites was 10-20 hours. Thus CYP2D6 may play a role in stereoselective elimination of HTBZ. An altered ratio of α-HTBZ: β –HTBZ in a patient already taking a stable dose of TBZ could result in change in efficacy due to a decrease in exposure to α-HTBZ, and the effects of an alteration in this ratio on efficacy and safety are unknown.

The effect of 12.5 mg TBZ on Pgp was evaluated after administration of digoxin, a PgP substrate. No effect was observed, although clinically relevant doses were not evaluated.

As described above the role of various P450s in metabolism of TBZ or its metabolites has not been adequately characterized *in vitro* nor has the effect of TBZ or its metabolites on P450s . *In vivo* studies have not been performed. There is insufficient information to describe the potential for P450-mediated drug interactions in the labeling.

Biopharmaceutics

The Sponsor has not provided adequate information to determine whether the dissolution method and specifications are appropriate. Information clarifying the methodology has been requested (1/24/06) as has been information showing discriminatory ability of the proposed method. As of 3/2/06 there has been no response.

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NDA 21,894
Tetrabenazine

Clinical Pharmacology Required *Office Level* Briefing:
March 1,2006
Attendees: Sally Yasuda, Mehul Mehta, Ramana Uppoor, Peter Hinderling, Phil Colangelo, Venkat Jaragula, Andrea Powell, Lois Freed, Elizabeth McNeil, Carole Davis, John Feeney, Shiew Mei Huang, Srikanth Nallani, Arzu Selen.

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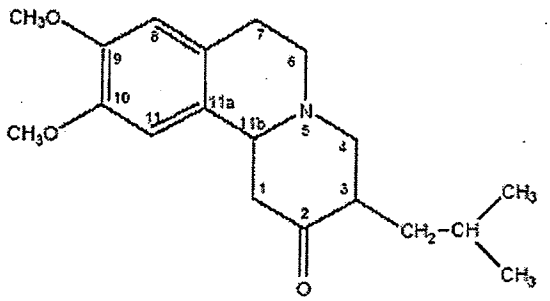
2 Question-Based Review

2.1 General Attributes

Tetrabenazine has been developed for treatment of chorea associated with Huntington's disease (HD). It was discovered in the 1950s-1960s and the Sponsor states that TBZ was first introduced for treatment of chorea in the United Kingdom in 1971. It is currently registered in Europe, Canada, Australia, and New Zealand where it is available as 25 mg immediate release tablets.

2.3.1 What are the highlights of the chemistry and physical-chemical properties of XENAZINE, and the formulation of the drug product?

Tetrabenazine (cis rac-1,3,4,6,7,11b-hexahydro-9,10,dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolizin-2-one) as a molecular formula of $C_{19}H_{27}NO_3$ and a molecular weight of 317.4. Its structural formula is shown below, as provided by the Sponsor.



Tetrabenazine (TBZ) has two chiral centers, C3 and C11b, and 4 isomers (RS, SR, SS, and RR) are possible.

The log P partition coefficient is 3.187 and the pKa is 6.51. The Sponsor states that TBZ is sparingly soluble in water, soluble in ethanol. Solubility

The composition of tetrabenazine tablets, 12.5 mg and 25 mg, as provided by Sponsor, is shown in the table below.

Component	Function	Amount per 12.5 mg Tablet (mg)	Amount per 25 mg Tablet (mg)
Tetrabenazine	Active Ingredient	12.5	25.0
Lactose Maize Starch Talc Magnesium Stearate			
Iron Oxide, Yellow	Coloring Agent	-	
Total		125	125

In Section 2.3.P.2. of the submission, the Sponsor states that the formulation and manufacturing process used to produce the product marketed in other countries was used to manufacture the clinical batch and the registration batches.

2.3.2 What is the proposed mechanism of drug action and what is the proposed therapeutic indication?

Tetrabenazine and its active metabolite HTBZ inhibit the brain synaptic vesicular monoamine transporter (VMAT) that is responsible for transporting monoamines (DA, NE, histamine, and 5HT) from the cytoplasm into the synaptic vesicle. This results in degradation of monoamines by monoamine oxidases in the cytoplasm and depletion of monoamine stores. In animal studies DA was the most affected and 5–HT was least affected by either tetrabenazine or HTBZ. Brain concentrations of monoamines return to control values 12 hours post-administration. Sigmoidal relationships were observed between monoamine levels and corresponding log brain or serum concentrations of tetrabenazine or HTBZ, according to the Sponsor's review of literature. There are two VMAT isoforms, VMAT1 (peripheral) and VMAT2 (brain). TBZ exhibits a 10,000 fold higher affinity for hVMAT 2 than for hVMAT 1, and according to the Sponsor, HTBZ binds exclusively to hVMAT2 (h refers to the human isoforms). According to the Sponsor, binding of HTBZ to VMAT is stereoselective, with only the α-isomer binding to hVMAT2. Selectivity for VMAT2 should result in selective CNS effects and low incidence of peripheral side effects such as hypotension and diarrhea.

The proposed therapeutic indication is for chorea associated with Huntington's disease (HD). HD is a progressive neurodegenerative disorder characterized by chorea, psychiatric manifestations, and cognitive impairment. The onset is generally in midlife with gradual worsening over approximately 10-20 years, until death. Huntington's disease is an autosomal dominant disease. The mutant gene that causes the disease contains an excessive number of CAG repeats (≥ 37) resulting in an abnormal huntingtin protein. Huntington's disease is characterized by debilitating chorea, personality disorder, and dementia. Drug treatment with

tetrabenazine is aimed at blocking DA transmission that is thought to be associated with the chorea.

2.3.3 What is the proposed dosage and route of administration?

The proposed starting dose is 25 mg/day (12.5 mg in the morning and 12.5 mg in the evening) given orally. The dose should be titrated up slowly at weekly intervals by 12.5 mg (e.g. after 1 week the dose should be increased to 37.5 mg/day given as 12.5 mg in the morning, 12.5 mg at noon, and 12.5 mg in the evening). The dose should continue to be increased by 12.5 mg increments until satisfactory control of chorea is achieved or until side effects occur or until a maximal dose of 100 mg/day is reached.

2.4 General Clinical Pharmacology

2.4.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Efficacy Study TBZ 103,004: Multicenter, randomized, double blind, placebo controlled, parallel group study to evaluate Placebo (n=30) vs TBZ (n=54) titrated to "best dose" starting at 12.5 mg daily with the highest daily dose of 100 mg.

Efficacy Study TBZ 103,005: Single center, randomized, placebo controlled staggered withdrawal of TBZ in patients suffering from HD and treated with TBZ (at "best dose").

Inclusion criteria for both studies included a confirmatory CAG repeat ($n \geq 37$).

2.4.2 What is the basis for selecting the response endpoints, i.e., clinical or surrogate endpoints, or biomarkers (collectively called pharmacodynamics, PD) and how are they measured in clinical pharmacology and clinical studies?

Efficacy in the pivotal efficacy studies (103,004 and 103,005) was evaluated based on the Total Chorea Score of the UHDRD motor portion since chorea is a debilitating symptom of the disease.

2.4.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The active moiety in plasma has been considered to be α- HTBZ. Both α- and β-HTBZ have been considered to be the major circulating moieties and these have been appropriately measured using a chiral assay (please refer to Section 2.6) except for Study 1700114 that used an achiral assay. However, results from the mass balance study (performed in June 2005 and submitted in December 2005) suggest that there may be other metabolites circulating to a significant extent, the pharmacology and clinical pharmacology (including PK) of which have not been determined. If these metabolites contribute substantially to efficacy or safety they should be determined in plasma at least to characterize an exposure response relationship.