Development of Ebselen, a Glutathione Peroxidase Mimic, for the Prevention and Treatment of Noise Induced Hearing Loss

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ABSTRACT

The development of therapeutics for the prevention and treatment of sensorineural hearing loss remains an illusive goal for auditory scientists and clinicians worldwide. While much research has focused on the histopathology associated with exposure to intense noise or ototoxins (i.e., loss of hair cells), the biochemical and genetic mechanisms that evoke or mediate hair cell death and dysfunction are still under investigation and debate. Many have observed an early oxidative burst in the cochlea that leads to an increase in lipoperoxidation and the activation of cell death pathways, ultimately resulting in apoptosis. In support of this hypothesis, many have protected the cochlea and preserved auditory function by injecting high doses of antioxidants or inhibitors of cell death activation prior to intense noise or ototoxin exposure. Here we discuss a compound currently in Phase II clinical testing for the prevention and treatment of noise-induced hearing loss. This paper will review the historic background, and pertinent preclinical and clinical data available for ebselen, a novel drug that mimics the activity of glutathione peroxidase, a catalytic antioxidant enzyme that is essential for the peripheral auditory system.

1.0 BACKGROUND

Exposure to intense noise or ototoxins results in loss of cochlear hair cells through a common pathway. An early oxidative burst in the cochlea leads to an increase in lipoperoxidation and activation of cell death pathways, ultimately resulting in apoptosis. In support of this observation, researchers have protected the cochlea and preserved auditory function by injecting high doses of antioxidants or inhibitors of cell death activation prior to intense noise or ototoxin exposure. The most common toxic metabolites are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS can destroy lipid membranes and mitochondria resulting in dysfunction or loss of function. Interestingly, several antioxidant enzyme activities have been shown to decrease during this time. Most notably glutathione peroxidase (GPx), the dominant catalytic antioxidant enzyme in the cochlea, decreases in activity resulting in an increase in ROS and RNS. This imbalance may cause irreparable damage to auditory hair cells and as a consequence a loss of hearing sensitivity.

The development of drugs to prevent and treat noise induced hearing loss (NIHL) is the next logical step for compounds that have shown efficacy in multiple pre-clinical models of NIHL. Here, we present a review of the published preclinical results for ebselen including the path towards the clinical evaluation of ebselen, in both FDA approved or allowed Phase I and Phase II clinical studies.

In 1984, ebselen, (2-phenyl-1,2-benzoisosenelazol-3(2H)-one, was first described as a catalytic antioxidant mimic in the laboratories of Dr. Albrecht Wendel[1] and Dr. Helmut Sies[2]. Wendel et al., characterized the glutathione peroxidase (GPx) activity of ebselen and demonstrated that it was associated with the selenium (Se) moiety present in ebselen, whereas a sulfur analog that did not

contain Se, had no GPx activity. Importantly, Wendel et al., showed that oral and injected ebselen that was radiolabel traced for selenium (synthesized with the selenium 75 isotope) did not increase the intracellular Se level or pool and therefore, was not bioavailable for incorporation into selenoenzymes such as GPx. Dr. Sies' group confirmed the GPx activity of ebselen and provided insights into its ability to reduce malondialdehyde formation in a rat liver microsomal assay. Malondialdehyde is the end-product of lipid peroxidation, which is a process where ROS and RNS degrade lipids. This compound is a reactive nitrogen species that cause toxic stress in cells. Malondialdehyde is used as a biomarker to measure the level of oxidative stress in an organism.

Ebselen has been evaluated preclinically in a broad range of animal models having the underlying theme of oxidative injury and stress ranging from diabetes mellitus, to ischemic stroke, to noise and ototoxin induced hearing loss. In the case of acute ischemic stroke, a series of clinical trials performed by Daiichi Pharmaceuticals, indicated that ebselen given orally for 14 days starting within 24 hours of the onset of stroke, had a positive benefit in patient outcome; $p = 0.038$ at 1 month and $p = 0.049$ at 3 months post stroke using a Wilcoxon Rank Sum with an Intent To Treat analysis[3]. However, if ebselen treatment was started between 24 and 48 hours after stroke onset, there were no significant differences between groups using the same methods of analysis. A third pivotal Phase III study proved non-significant and Daiichi did not continue the clinical and commercial development of ebselen for the treatment of stroke.

Recently, several publications showed that oral administration of ebselen was excellent in protecting the cochlea from intense noise exposure[4, 5]. These data are consistent with the observation that Reactive Oxygen and Nitrogen Species (ROS/RNS) are increased in the cochlea after noise exposure[6] and that deletion of the GPx1 gene in mice confers increased susceptibility to noise

induced hearing loss[7]. In our preclinical studies, we find that ebselen has maximum benefit if provided one day prior to noise exposure and if ebselen is continued for 14 days vs. 3 or 7 days. In a Guinea pig study designed to evaluate the induction of temporary threshold shift (TTS), a single ebselen dose (10 mg/kg) prior to noise exposure, prevented the TTS, where the mechanism of protection appeared to be a significant decrease in afferent dendrite swelling at the base of inner hair cells[8]. Similar otoprotective results have been reported in rats where ebselen (10mg/kg) given once or twice prior to noise, significantly reduced TTS. Here, ebselen suppressed inducible nitric oxide synthase, an enzyme that potentiates oxidative stress through increased ROS/RNS activity, in primarily non-sensory structures of the cochlea including the stria vascularis and spiral ligament (Park et al., ARO Mid-Winter Meeting 2006). More recently, we have shown that ebselen stimulates the GPx1 gene and protein expression *in vitro* and *in vivo*[9]. The ability of ebselen to induce catalytic antioxidant gene expression may explain why low oral doses of ebselen work so quickly to prevent a temporary loss of auditory sensitivity across a range of frequencies following intense noise exposure.

2.0 CURRENT DEVELOPMENT OF EBSELEN FOR THE PREVENTION AND TREATMENT OF NIHL

In an effort to establish a safety and pharmacokinetic profile for ebselen, we have performed an FDA approved Phase I trial in normal volunteers with oral doses of 200, 400, 800, and 1600 mg. The primary objective of this study was to evaluate the safety and tolerability of SPI-1005 (ebselen) in healthy subjects. Secondary objectives were to evaluate the single dose pharmacokinetic (PK) profile of SPI-1005 in healthy subjects and to determine the correlation between plasma selenium levels and plasma ebselen levels. This study had a randomized, double-blind, placebo-controlled, single

ascending dose design. All subjects were in-patient for 72 hours post-dose at MDS Pharma Services' 176 bed, clinical study facility in Lincoln, Nebraska. A total of 32 subjects were enrolled in the study, and 32 subjects completed the study. All 32 subjects were included in the safety and PK analyses. Subjects enrolled in this study were judged by the Investigator to be normal, healthy volunteers who met all inclusion and none of the exclusion criteria or were approved by the Investigator at check-in. The test product was single SPI-1005 capsules (active ingredient: ebselen 200 mg) administered as 200 mg, 400 mg, 800 mg, or 1600 mg doses with 240 mL of water following a minimum 10-hour overnight fast. The reference product was SPI-1000 matching placebo capsules administered with 240 mL of water following a minimum 10-hour overnight fast.

2.1 Safety

Physical examinations, vital signs, 12-lead electrocardiograms (ECG's), adverse events (AEs), hematology, clinical chemistry, and urinalysis were assessed for safety and tolerability. Vital sign measurements were collected with subjects in semi-recumbent and standing positions for the assessment of orthostatic changes in blood pressure and heart rate.

2.2 Pharmacokinetics

The PK parameters of ebselen and its three metabolites [2-methyl selenobenzanilide, 2-glucuronyl selenobenzanilide, and N-(4-hydroxyphenyl)-2-methylselenobenzamide)] were calculated from plasma concentration-time data (using actual blood draw times) using WinNonlin® Professional (Version 5.0). Plasma PK parameters (AUC_{0-t}, AUC_{0-inf}, AUC_{0-t}/AUC_{0-inf}, K_{el}, $t_{½}$, C_{max}, T_{max}, CL/F, and V_d/F) and urinary PK parameters (C_{urine}, Am, % dose excreted, and CL_r) were tabulated by treatment. PK parameters were also calculated for plasma selenium at

the same blood draw times. The area under the curve (AUC) is an estimation of plasma drug exposure over time. Cmax is the maximum peak plasma concentration (Cmax) determined after ingesting a drug. Tmax is the time by which the drug takes to reach a Cmax. The half life or $t_{½}$ is the time for 50% of the plasma concentration to decrease after some point of elimination. Typically the $t_{½}$ is the beta elimination of a drug from the plasma. These parameters are important in determining plasma exposure profiles and times between consecutive dosing in an individual patient which will be important in establishing exposure versus response relationships in future efficacy studies.

2.3 Statistical Methods

2.3.1 Pharmacokinetics

Summary statistics (including sample size [N], arithmetic mean, standard deviation [SD], minimum, maximum, median, and coefficient of variation [CV]) were calculated by treatment. Mean and individual concentration-time plots (both linear and log-linear) were presented. In addition, the geometric mean and geometric CV were calculated for Cmax and AUC values. Dose proportionality was assessed following a single dose by plotting ln-transformed (natural log-transformed) PK parameters Cmax, AUC0-t, and AUC0-inf against the ln-transformed dose proportionality was established if a linear relationship was demonstrated and if the 95% confidence intervals (CI) for the slope of the ln-transformed parameters included the value of 1 for dose-dependant parameters.Combined plasma ebselen and metabolites concentrations corresponding to selenium plasma concentrations were determined to assess the utility of tracking plasma selenium as a reliable surrogate of plasma ebselen. Combined plasma ebselen and metabolites concentrations were plotted against selenium plasma concentrations and a simple linear regression was performed. The resulting R-squared (R^2) value was used to assess the correlation between the two analytes.

2.3.2 Safety

All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA®) (Version 9.0) and were summarized by treatment for number of subjects reporting the AE and the number of AEs reported. Laboratory evaluations and change from baseline results were summarized by treatment and time point. Measurements for 12-lead ECGs and change from baseline results were summarized by treatment and time point. Vital sign assessments and change from baseline results were summarized by position, treatment, and time point. Orthostatic vital signs and change from semi-recumbent to standing were also summarized by

treatment and time point. Descriptive statistics were calculated for quantitative safety data.

2.4 Pharmacokinetic Results

The maximum peak plasma concentrations (C_{max}) and total exposure (AUC_{0-t}) increased with increasing doses of ebselen. The arithmetic means and SD of plasma ebselen PK parameters are presented in Table 1.

Table 1: Summary of the pharmacokinetic parameters for plasma ebselen for treatments A through D. Treatment A was 1 x 200 mg Ebselen. Treatment B was 2 x 200 mg Ebselen. Treatment C was 4 x 200 mg Ebselen, and Treatment D was 8 x 200 mg Ebselen.

Tmax is presented as Median (Minimum, Maximum).

	Treatment A	**Treatment B**	**Treatment C**	**Treatment D**
Pharmacokinetic Parameters	**Mean ± SD (N)**	**Mean ± SD (N)**	**Mean ± SD (N)**	**Mean ± SD (N)**
C_{max} (ng/mL)	30.3 ± 28.4 (6)	67.5 ± 31.9 (6)	70.3 ± 33.3 (6)	83.4 ± 27.0 (6)
T_{max} (hr)	1.53 (1.04, 3.00) (6)	2.25 (1.02, 5.00) (6)	2.00 (1.00, 6.02) (6)	1.50 (0.999, 4.02) (6)
AUC_{0-t} (ng*hr/mL)	117.37 ± 169.59 (5)	371.68 ± 305.68 (6)	690.06 ± 751.19 (6)	880.62 ± 494.10 (6)
AUC_{0-inf} (ng*hr/mL)	546.31 (1)	883.15 ± 497.96 (2)	710.10 ± 297.17 (2)	992.64 ± 736.47 (4)
AUCR	0.764 (1)	0.760 ± 0.0228 (2)	0.656 ± 0.152 (2)	0.694 ± 0.113 (4)
$t_{1/2}$ (hr)	6.42 (1)	12.1 ± 8.48 (2)	14.4 ± 11.7 (2)	16.7 ± 12.4 (4)
K_{el} (1/hr)	0.108 (1)	0.0762 ± 0.0535 (2)	0.0714 ± 0.0578 (2)	0.0575 ± 0.0323 (4)
CL/F (L/hr)	7397.42 ± 6352.62 (6)	1641.96 ± 995.851 (6)	2068.10 ± 1437.31 (6)	2664.13 ± 2051.30 (6)
Vd/F (L)	3391.57 (1)	7524.14 ± 1297.42 (2)	21358.8 ± 10057.1 (2)	40967.5 ± 16254.8 (4)
$\ln(C_{max})$	3.186 ± 0.6406 (6)	4.092 ± 0.5696 (6)	4.153 ± 0.5030 (6)	4.375 ± 0.3525 (6)
$\ln(AUC_{0-t})$	4.067 ± 1.245 (5)	5.685 ± 0.7219 (6)	6.195 ± 0.8342 (6)	6.610 ± 0.6889 (6)
$\ln(AUC_{0-inf})$	6.303 (1)	6.697 ± 0.5969 (2)	6.520 ± 0.4314 (2)	6.721 ± 0.6661 (4)
Cum. Am (mg)	0.09 ± 0.06 (6)	0.17 ± 0.07 (6)	0.23 ± 0.06 (6)	0.42 ± 0.20 (6)
Cum. %dose (%)	0.04 ± 0.03 (6)	0.04 ± 0.02 (6)	0.03 ± 0.01 (6)	0.03 ± 0.01 (6)
CLr (mL/hr)	2122.56 ± 1437.27 (6)	596.51 ± 278.52 (6)	570.23 ± 420.09 (6)	589.16 ± 286.80 (6)

The dose proportionality assessment of plasma ebselen following the administration of 200 mg, 400 mg, 800 mg, and 1600 mg ebselen is presented in Table 2.

Table 2: Summary of the dose proportionality assessment for plasma ebselen

Pharmacokinetic Parameter	Intercept	Slope	95% CI for Slope	R-square	p-value for Slope = 1
AUC_{0-t}	-2.6224	1.2927	(0.7631, 1.8222)	0.5381	0.2640
AUC_{0-inf}	5.7729	0.1274	(-0.4447, 0.6994)	0.0381	0.0087
C_{max}	0.6353	0.5232	(0.2248, 0.8216)	0.3754	0.0032

The statistical analyses were performed using the SAS Regression Procedure. A linear regression model with In-transformed parameter and In-transformed dose was used. Dose proportionality was not rejected if the 95% CI for the slope included the value of 1.

As the ebselen dose administered increased by 1:2:4:8, the corresponding ebselen AUC_{0-t} changed 1:3.2:5.9:7.5, indicating that ebselen exposure did not change in a dose proportional manner. The PK profile of ebselen was established from a timed series of plasma samples collected prior to and following dosing. Data was generated using a validated Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry (LC-MS/MS) method to quantify the concentrations of ebselen and each of its major and minor metabolites (Figure 1). The total amount of selenium in plasma was also quantified for each patient using a validated Inductively Coupled Plasma Mass Spectrometry (ICPMS) method and results were compiled to generate a

PK curve (Figure 2). The calculated amount of selenium available in the plasma contributed by plasma ebselen and metabolites concentrations was correlated to the observed amount of selenium in the plasma as determined by ICPMS across all dose groups and a regression analysis was performed (Figure 3). The combined plasma ebselen and metabolites concentrations demonstrated a strong positive correlation with selenium plasma concentrations across all dose groups ($R^2 = 0.8120$). The R^2 values for combined plasma ebselen and metabolites concentrations versus selenium plasma concentrations per dose group following the administrations of 200 mg, 400 mg, 800 mg, and 1600 mg ebselen were 0.9317, 0.8540, 0.7809, and 0.7648, respectively. These data indicate a strong relationship between plasma selenium and plasma ebselen/metabolite levels in the plasma of patients receiving 200 and 400 mg doses of ebselen.

2.5 Adverse Events Results

AEs were monitored for all subjects during the course of the Phase I trial and throughout the follow up period. AE results are summarized in Table 3.

Table 3. Adverse event reporting (AE)

Group	Rx	Subjects with AEs possibly related to Rx	Subjects with no AEs or AEs unrelated to Rx
1	**200 mg**	67%	33%
	Placebo	50%	50%
2	**400 mg**	0%	100%
	Placebo	50%	50%
3	**800 mg**	33%	67%
	Placebo	50%	50%
4	**1600 mg**	50%	50%
	Placebo	0%	100%
All SPI-1005 Subjects		**38%**	**63%**
All Placebo Subjects		**38%**	**63%**

There were no treatment- or dose-related trends in AE incidence. No serious AEs occurred and none of the subjects were discontinued from the study treatment due to an AE. All AEs in this study were mild or moderate in severity and resolved without sequelae. Although 14 (44%) of the 32 subjects exhibited insufficient orthostatic compensation during at least one time point in the study, there were no treatment- or dose-related trends. Additionally, 4 (13%) of the 32 subjects in

this study reported the AE of dizziness or postural dizziness: 2 subjects after 800 mg SPI-1005 administration, 1 subject after 200 mg SPI-1005 administration, and 1 subject after placebo administration. In addition, there were no treatment- or dose-related trends in clinical laboratory, ECG, or physical examination findings. The most common AE experienced was head ache, which is not unusual in a Phase I setting where all participants are restricted from accessing caffeinated beverages while participating in the study.

2.6 Conclusions from the Phase I study of ebselen

1. SPI-1005 (ebselen) capsules administered in a single oral dose up to 1600 mg appeared to be safe and well tolerated by the healthy male and female subjects in this study, compared to placebo.

2. Following the administration of escalating doses of ebselen (200 to 1600 mg) in the SPI-1005 formulation, the mean ebselen C_{max} values ranged from 30.3 ng/mL to 83.4 ng/mL; the mean ebselen AUC_{0-t} values ranged from 117.4 ng*hr/mL to 880.6 ng*hr/mL; the median ebselen T_{max} values ranged from 1.5 hours to 2.3 hours; and the mean ebselen $t_{1/2}$ values ranged from 6.4 hours to 16.7 hours.

3. 2-glucuronyl selenobenzanilide was the predominate metabolite of ebselen in plasma and urine.

4. Less than 11% of the administered dose of ebselen was excreted in urine as ebselen and its metabolites.

5. Combined plasma ebselen and metabolite concentrations demonstrated a strong positive correlation with plasma selenium concentrations across all dose groups ($R^2 = 0.8120$).

Following the successful completion of this Phase I trial, Sound Pharmaceuticals, Inc. is proceeding with the clinical development of ebselen in a Phase II trial designed to evaluate the safety and efficacy of ebselen in an at risk clinical population of noise exposed military personnel. The clinical trial design includes evaluating active Navy personnel exposed to intense levels of noise during live fire training exercises. Efficacy will be evaluated using a randomized double blinded placebo controlled trial design with the dosing of ebselen in 0, 200, 400, and 600 mg oral capsules taken twice daily starting one day prior to noise exposure and continuing for 14 days. Noise exposure will be from 5 days of live fire exercises with hearing protective devices for all trial participants. Hearing thresholds will be established using pure tone audiometry at baseline (prior to noise exposure), 3-6 hours after noise exposure, and at 2 and 4 weeks after noise exposure. Participants will complete a Tinnitus Handicap Inventory (THI) questionnaire at every audiometric assessment. Efficacy in this study will be evaluated based on TTS, PTS, and changes in the THI raw score. Post-noise pure tone thresholds will be compared with baseline testing to determine the threshold shift for multiple frequencies (0.5-8kHz) including those the military use to calculate significant threshold shift (STS). The primary efficacy endpoint of this study is a significant reduction in the incidence of hearing loss using STS criteria at either TTS or PTS intervals. A secondary efficacy endpoint is a significant reduction in the severity of hearing loss, determined by measuring at least a 10 dB reduced hearing threshold shift at one of the tested frequencies, occurring at either the TTS or PTS intervals. Raw THI scores will be determined for each treatment or placebo group, averaged per group, and compared for a significant reduction in mean score.

The basis of this novel clinical trial design is the years of epidemiology within the armed forces suggesting that brief exposures to intense noise, even with hearing protective devices such as foam

earplugs, lead to both TTS and PTS. Whether ebselen can exert an otoprotective effect at TTS and PTS in these human populations remains to be seen. The safety, pharmacokinetic profile and oral bioavailability of ebselen make it a worthy drug candidate for advancement into Phase II trials for the prevention and treatment of noise induced hearing loss. In addition, ebselen may prove to be valuable in preventing and treating drug induced hearing loss and age-related hearing loss since they are also influenced by Glutathione Peroxidase activity.

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Figure 1. Plasma ebselen concentrations over time as determined by LC-MS/MS.

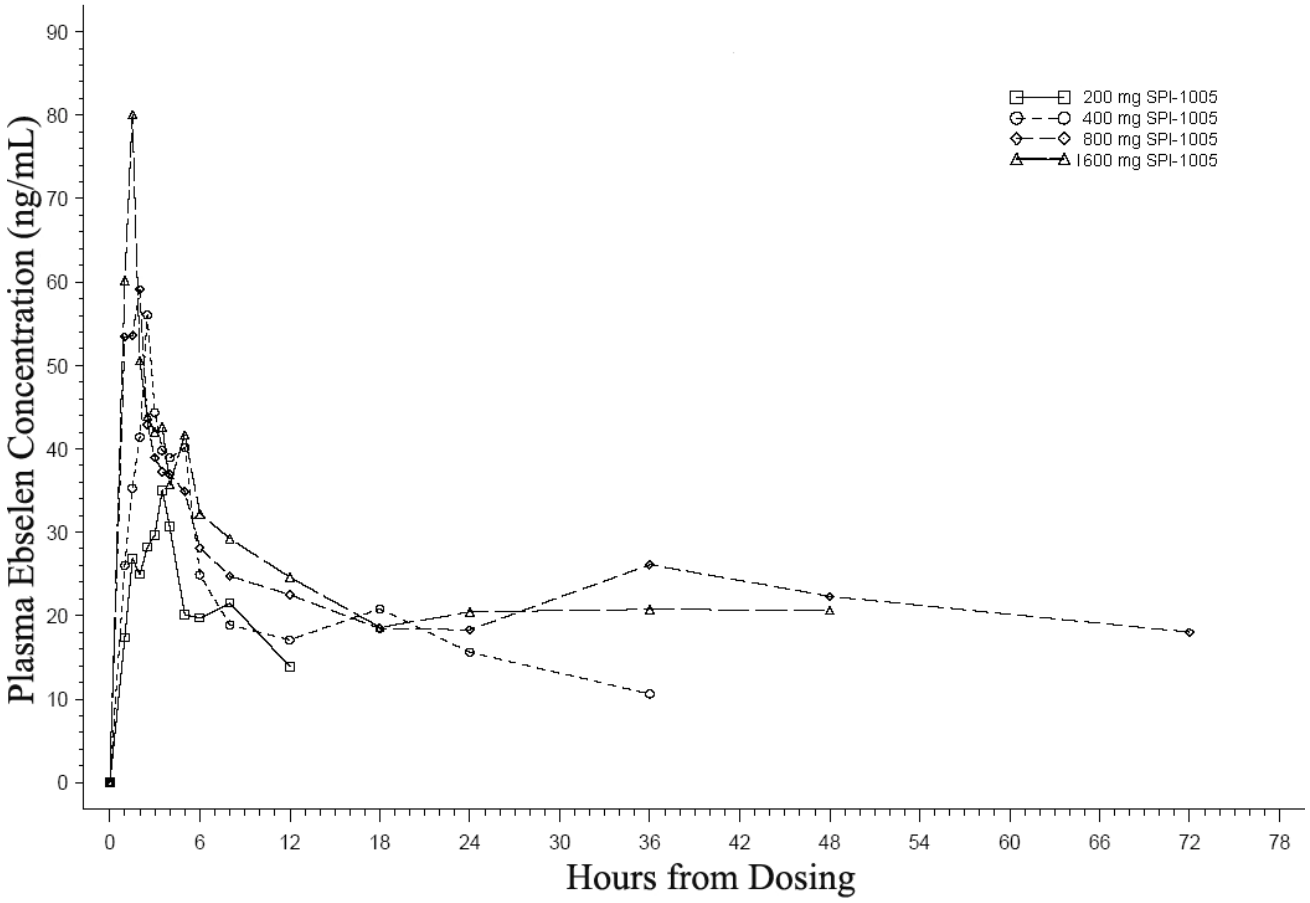

Figure 2. Plasma selenium concentrations over time as determined by ICPMS.

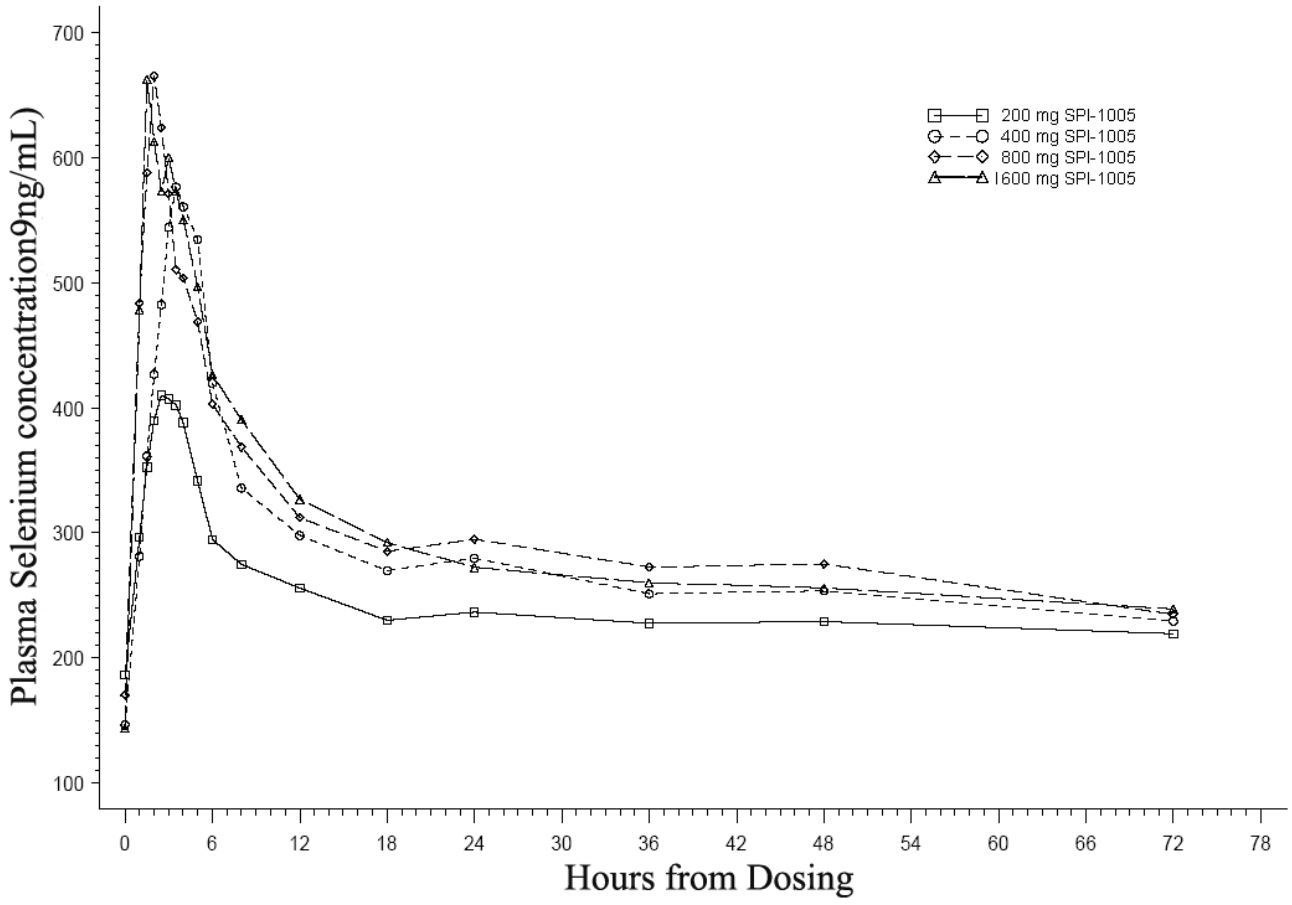

Figure 3. Plasma selenium concentrations versus combined plasma ebselen and metabolites concentrations following all dose groups.