THE EFFECTS OF LEVOTOFISOPAM, A NOVEL HOMOPHTHALAZINE, IN A PHASE 1B STUDY DESIGNED TO DEMONSTRATE EARLY SIGNAL DETECTION IN TREATMENT OF THE SYMPTOMS OF MENOPAUSE

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Background

Levotofisopam is the S-enantiomer of racemic tofisopam, a homophthalazine prescribed outside the United States to treat anxiety and disorders related to stress and autonomic dysfunction, such as functional gastrointestinal disorders and the symptoms of menopause.[1,2] Although the precise mechanism of action of levotofisopam is not yet clear, available data suggest that levotofisopam may modulate autonomic tone via interaction with subcortical 2,3-benzodiazepine receptors. Differing structurally from typical benzodiazepines, with ring nitrogen atoms in the 2,3 position rather than the 1,4 or 1,5 position (Figure 1), levotofisopam has shown little affinity for most receptors, enzymes, and ion channels, including the classical benzodiazepine binding site; however, it does bind to the 2,3-benzodiazepine receptor (Table 1). Unlike the classical benzodiazepine receptor, which is primarily cortical, the 2,3-benzodiazepine receptor is localized in subcortical brain regions, including the hypothalamus.[3]

Until recently, senescence of the ovary was considered the primary cause of menopause, but current evidence suggests that alterations in the hypothalamic-pituitary axis precede the loss of regular cyclicity,[4-6] resulting in extensive autonomic and neuroendocrine dysfunction. The hypothalamus also serves as the thermoregulatory center of the body. Manipulation of the hypothalamic-noradrenergic signaling pathway has been shown to alter the response to heating and cooling of the body.[7]

The most common symptom of menopause, affecting 75% of postmenopausal women,[8] is the hot flash, which presents as a heat-dissipation response, with peripheral vasodilation and perspiration on the head, neck, and chest. A variety of treatments have emerged for hot flashes and other symptoms associated with the menopause, most prominently hormone therapy (HT). However, a number of complications have been associated with the use of HT in the treatment of the menopause, including increased risk of deep venous thromboembolism,[9] gallstones or cholecystectomy,[10] breast cancer,[11,12] and coronary heart disease.[11] These findings have led to a surge of interest in nonhormonal treatments. Several compounds, including the α_2-adrenergic agonist clonidine, the γ-aminobutyric acid analogue gabapentin, selective serotonin-reuptake inhibitors (SSRIs—e.g., paroxetine), serotonin-norepinephrine reuptake inhibitors (SNRIs—e.g., venlafaxine), selective estrogen-receptor modulators (SERMs), vitamin E, and soy-based products or other phytoestrogens, have proven modestly effective and in many cases have been associated with unacceptable side-effects. Clearly, there is a need for alternative therapies for the treatment of the vasomotor symptoms of menopause.

With this need in mind, VelaPharm sponsored a single- and multiple-dose Phase 1B trial of levotofisopam in healthy male and postmenopausal female volunteers. The study was conducted at Pharma BioResearch, Zuidlaren, The Netherlands.

Objectives

The present study was conducted to evaluate the safety, tolerability, pharmacokinetics, and potential clinical utility of levotofisopam in a Phase 1B setting in women with symptoms of menopause. Safety, tolerability, and pharmacokinetics were also assessed in healthy young men.

Design

This was a randomized, double-blind, placebo-controlled trial utilizing ascending doses tested in sequential groups. Following a screening period, subjects received placebo (n = 7 women, 2 men) or a single 50 mg dose of levotofisopam (n = 18 women, 6 men) on Day 1 and 50 mg TID or placebo on Days 2-7. In the subsequent treatment period, a new cohort of patients was given placebo (n = 5 women, 2 men) or levotofisopam 150 mg (n = 17 women, 6 men) as a single dose and then BID. In female subjects, physiological (objective) and subjective hot flash frequency were recorded via skin conductance monitors (UFI, Morro Bay, CA). Self-rated menopausal symptoms were also collected using the Greene Climacteric Scale.

Results

After 7 days of treatment, both objective and subjective measures of hot flash frequency were reduced by levotofisopam. For the modified intent-to-treat (MITT) population, comprising all women who received at least one dose of study drug and had a baseline hot flash count of ≥5/day, median objective change in hot flash frequency was -33% for the 50 mg TID cohort (p = .002), -45% for the 150 mg BID cohort (p = .028), and -36% for the two cohorts combined (p = .0002; Table 2). Median subjective change from baseline in hot flash frequency for this population was -23% for the 50 mg TID cohort (p = .0001), -11% for the 150 mg BID cohort (NS), and -20% for the two cohorts combined (p = .003; Table 3). In addition, the "all subjects" analysis of Greene Climacteric Scale data showed significant changes from baseline in sleep quality and vasomotor symptoms (including night sweats) for the 50 mg TID group and in vasomotor symptoms for the 50 mg TID and 150 mg BID treatment groups combined. Placebo, in contrast, had little effect.

Multiple oral doses of levotofisopam 50 mg TID and 150 mg BID were generally well tolerated in these healthy men and postmenopausal women, with incidences of treatment-emergent adverse events (TEAEs) similar to placebo. The most frequently reported TEAEs are shown in Table 4. One serious adverse event (SAE) was reported, acute renal insufficiency in a male subject following a single 150 mg dose of levotofisopam. The subject was withdrawn from the study due to this event, which was considered probably related to study drug. There were no other premature discontinuations. Except for a transient increase in mean creatinine in men receiving 150 mg BID levotofisopam, due primarily to the SAE described above, there were no out-of-range mean values or clinically significant trends in laboratory, vital signs, ECG, or physical examination results.

Levotofisopam exhibited a more than dose-proportional increase of mean C_{max} and AUC values (Table 5). The time to reach C_{max} and the apparent terminal elimination half-life were similar for both dose levels (Figures 2 and 3). The extent of accumulation of levotofisopam was similar at both dose levels and in both males and females. Increased $AUC_{0-\tau}$ on Day 7 as compared to AUC_{0-inf} on Day 1 suggested time-dependent pharmacokinetics in all treatment groups, which also contributed to the relatively high exposure on Day 7.

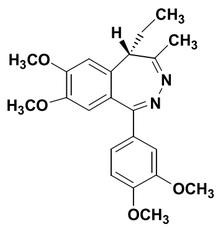

Figure 1. Chemical structure of levotofisopam [(S)-5-ethyl-7,8-dimethoxy-4-methyl-1-(3,4-dimethoxyphenyl)-5H-2,3-benzodiazepine].

Table 1. Selected Binding Activity of Levotofisopam

Assay	Radioligand	Activity[a]
GABA-A (GABA agonist site)	[^{3}H]GABA	None
GABA-A (typical benzodiazepine site)	[^{3}H]Flunitrazepam	None
GABA-B	[^{3}H]CGP 54626A	None
Serotonin (nonselective)	[^{3}H]LSD	None
Estrogen	[^{125}I]3,17B-Estradiol, 16a	None
Prostaglandin (leukotriene B_4)	[^{3}H]LTB_4	None
Histamine (H_2)	[^{125}I]Aminopotentidine	None
Dopamine (nonselective)	[^{3}H]Spiperone	None
Adrenergic (alpha 1)	[^{3}H]7-MeO-Prazosin	None
Adrenergic (alpha 2)	[^{3}H]RX 821002	None
Adrenergic (beta)	[^{3}H]DHA	None
Muscarinic (nonselective)	[^{3}H]QNB	None
Nicotinic	[^{3}H]Epibatidine	None
Opiate	[^{3}H]Naloxone	None
Dopamine transporter	[^{3}H]WIN 35428	None
Norepinephrine transporter	[^{3}H]Nisoxetine	None
Serotonin transporter	[^{3}H]Citalopram, N-Methyl	None
2,3-Benzodiazepine	[^{3}H]Girisopam	> 50% inhibition

[a] Activity is defined as >50% inhibition of specific binding of radioligand with 10 μM levotofisopam.

Table 2. Objective Skin Conductance Data for the MITT Population

Parameter/ Treatment Group[a]	N	Baseline	Day 6	Change from Baseline	P-Value	% Change from Baseline	P-Value
***Median* Objective Hot Flash Frequency**							
50 mg TID	11	10.0	5.0	- 3.0	**.002**	-33%	**.002**
Placebo	4	11.0	9.5	- 4.5	.25	-34%	.44
150 mg BID	16	14.5	8.5	- 5.0	**.009**	- 45%	**.028**
Placebo	3	8.0	8.0	+ 2.0	.50	+ 25%	.50
Combined groups	27	10.0	7.0	- 4.0	**.0001**	- 36%	**.0002**
Placebo	7	8.0	8.0	- 3.0	.17	- 19%	.41
***Mean* Objective Hot Flash Frequency**							
50 mg TID	11	9.4	5.7	- 3.6	**.005**	- 39%	**.003**
Placebo	4	11.0	8.5	- 2.5	.23	- 11%	.40
150 mg BID	16	15.5	9.0	- 6.5	**.010**	- 30%	**.036**
Placebo	3	8.0	6.3	- 1.7	.35	- 11%	.41
Combined groups	27	13.0	7.7	- 5.3	**.001**	- 34%	**.001**
Placebo	7	9.7	7.6	- 2.1	.17	- 11%	.34

Note: Statistically significant P-values are indicated in boldface type. The "combined groups" are the 50 mg TID and 150 mg BID levotofisopam groups.

Table 3. Subjective Hot Flashes (Event Marks), MITT Population

Parameter/ Treatment Group[a]	N	Baseline	Day 6	Change from Baseline	P-Value	% Change from Baseline	P-Value
***Median* Subjective Hot Flash Frequency**							
50 mg TID	14	8.5	5.5	- 3.0	**.0001**	- 23%	**.0001**
Placebo	4	7.5	7.0[a]	- 4.0	.50	- 36%	.50
150 mg BID	11	9.0	10.0	- 1.0	.20	- 11%	.34
Placebo	5	7.0	7.0	0.0	.50	0.0	.50
Combined groups	25	9.0	7.0	- 2.0	**.001**	- 20%	**.003**
Placebo	9	7.0	7.0[b]	0.0	.31	0.0	.42
***Mean* Subjective Hot Flash Frequency**							
50 mg TID	14	9.6	7.0	- 2.64	**.0001**	- 29%	**.0001**
Placebo	4	8.3	7.3[a]	- 1.33	.36	- 9%	.42
150 mg BID	11	10.8	9.9	- 0.91	.21	- 5%	.33
Placebo	5	9.2	8.4	- 0.80	.32	+ 1%	.55
Combined groups	25	10.2	8.3	- 1.88	**.001**	- 18%	**.002**
Placebo	9	8.8	8.0[b]	- 1.00	.25	- 2%	.44

Note: Statistically significant P-values are indicated in boldface type. The "combined groups" are the 50 mg TID and 150 mg BID levotofisopam groups.
[a] n = 3; [b] n = 8

Table 4. Most Frequently Reported TEAEs,[a] by Number (%) of Subjects

Adverse Event, Preferred Term	Placebo		Levotofisopam 50 mg TID		Levotofisopam 150 mg BID	
	Men (n=4)	Women (n=12)	Men (n=6)	Women (n=18)	Men (n=6)	Women (n=17)
Abdominal pain		3 (25%)		1 (6%)	1 (17%)	2 (12%)
Diarrhoea		1 (8%)		1 (6%)		7 (41%)
Disturbance in attention						4 (24%)
Dizziness		5 (42%)		7 (39%)	1 (17%)	3 (18%)
Feeling cold		2 (17%)				7 (41%)
Flatulence		2 (17%)		3 (17%)	1 (17%)	10 (59%)
Headache	2 (50%)	6 (50%)		7 (39%)	4 (67%)	10 (59%)
Myalgia	1 (25%)	2 (17%)		5 (28%)	1 (17%)	8 (47%)
Nausea		4 (33%)		3 (17%)	2 (33%)	5 (29%)
Paraesthesia		2 (17%)		1 (6%)	2 (33%)	1 (6%)
Peripheral coldness						5 (29%)
Pharyngolaryngeal pain	1 (25%)	1 (8%)		3 (17%)		1 (6%)
Pollakiuria	1 (25%)	1 (8%)	2 (33%)	2 (11%)		
Somnolence	1 (25%)	5 (42%)		6 (33%)	1 (17%)	1 (6%)

[a] TEAEs occurring in more than one subject and more than 20% of subjects within at least one treatment group

Table 5. Mean Pharmacokinetic Data, by Dosage, Gender, and Study Day

Cohort/ Day	C_{max} (ng/mL) [a]	t_{max} (hr) [a]	$t_{1/2}$ (hr) [a]	$AUC_{0-\tau}$ [b] (ng•hr/ mL) [a]	AUC_{0-inf} (ng•hr/mL) [a]	CL/F (L/hr) [a]	RA [a,c]
Levotofisopam 50 mg TID, Women (n = 18)							
Day 1	95.9 (65.7)	0.66 (0.24)	5.88 (2.44)	93 (59)	108 (69)	815 (877)	—
Day 7	137.9 (87.3)	0.67 (0.21)	6.75 (1.55)	176 (112)	—	418 (280)	2.1 (1.0)
Levotofisopam 50 mg TID, Men (n = 6)							
Day 1	52.3 (21.8)	0.82 (0.18)	4.47 (3.07)	69 (23)	75 (24)	754 (334)	—
Day 7	62.5 (34.3)	0.96 (0.33)	6.68 (4.40)	126 (80)	—	534 (269)	2.4 (2.8)
Levotofisopam 150 mg BID, Women (n = 17)							
Day 1	461 (257)	0.63 (0.16)	7.20 (1.90)	586 (307)	637 (332)	304 (153)	—
Day 7	639 (356)	0.73 (0.24)	6.78 (1.28)	1079 (608)	—	175 (81)	2.0 (0.7)
Levotofisopam 150 mg BID, Men (n = 6 for Day 1, n = 5 for Day 7)							
Day 1	442 (327)	0.83 (0.43)	7.02 (6.18)	650 (528)	696 (542)	306 (168)	—
Day 7	600 (443)	1.50 (0.92)	4.90 (2.25)	1043 (373)	—	163 (72)	2.1 (1.6)

[a] Arithmetic mean (SD)
[b] τ = 7 hours for TID treatment, 14 hours for BID treatment
[c] RA = Observed accumulation index = $AUC_{0-\tau,\ Day\ 7}/AUC_{0-\tau,\ Day\ 1}$

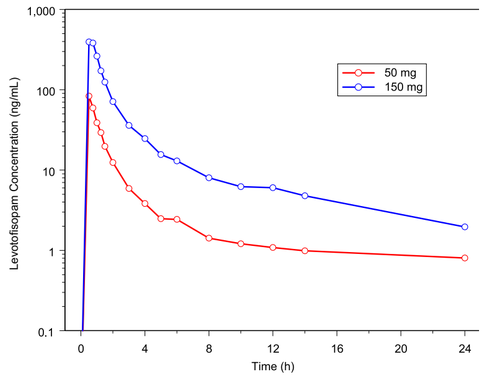

Figure 2. Logarithmic plot of plasma concentrations following initial dose of levotofisopam 50 mg or 150 mg (women only, Day 1).

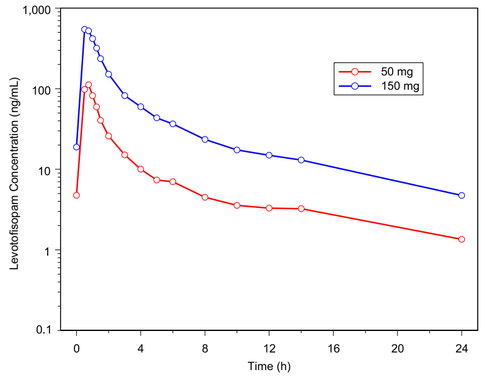

Figure 3. Logarithmic plot of plasma concentrations following final dose of levotofisopam 50 mg TID or 150 mg BID (women only, Day 7).

Table 6. Comparative Effect of Levotofisopam and Common Treatments for Menopausal Vasomotor Symptoms

Treatment	Mechanism of Action	Treatment Dose and Duration	Estimated Reduction, Hot Flash Frequency
Clonidine[13]	α_2-adrenergic agonist	2 weeks	Approx. 28-46% (mean)
Gabapentin[14]	γ-aminobutyric acid analogue	12 weeks	45% (mean)
Paroxetine[15]	SSRI	6 weeks	46-50% (mean)
Venlafaxine[16]	SNRI	4 weeks	30-58% (median)
Levotofisopam	Homophthalazine	7 days	33-45% (median)

Conclusions

Levotofisopam significantly reduced both objective and subjective measures of hot flash frequency in postmenopausal women within 7 days of treatment. These reductions compare favorably to those reported for commonly prescribed nonhormonal treatments for vasomotor symptoms of menopause (Table 6). Further, multiple oral doses of levotofisopam 50 mg TID and 150 mg BID were well tolerated in these postmenopausal women, with little difference from placebo.

In conclusion, data from the present Phase 1B trial suggest the potential utility of levotofisopam in the treatment of hot flashes in menopausal women.

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