UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549

FORM 8-K

CURRENT REPORT
Pursuant to Section 13 or 15(d) of
the Securities Exchange Act of 1934

Date of Report (Date of earliest event reported): May 20, 2009

VANDA PHARMACEUTICALS INC.

(Exact name of Registrant as specified in its charter)

Delaware
(State or other jurisdiction of incorporation)

000-51863	**03-0491827**
(Commission File No.)	(IRS Employer Identification No.)

9605 Medical Center Drive
Suite 300
Rockville, Maryland 20850
(Address of principal executive offices and zip code)

Registrant's telephone number, including area code: (**240) 599-4500**

Not Applicable
(Former Name or Former Address, if Changed Since Last Report)

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions:

o Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)

o Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)

o Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))

o Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Item 8.01. Other Events

Vanda Pharmaceuticals Inc. (the "Company" or "Vanda") made presentations regarding the Company's products, Fanapt™ (iloperidone) and tasimelteon, to medical professionals, analysts, investors and others at the Annual Meeting of the American Psychiatric Association (the "APA Meeting") on May 20, 2009. The posters that were be used for such presentations are furnished as Exhibit 99.1 to this Form 8-K. In addition, the posters will be posted on the Company's Web site http://www.vandapharma.com.

Various statements to be made in the presentations, including statements in the posters furnished as Exhibit 99.1 to this Form 8-K, are "forward-looking statements" under the securities laws. Words such as, but not limited to, "believe," "expect," "anticipate," "estimate," "intend," "plan," "targets," "likely," "will," "would," and "could," and similar expressions or words, identify forward-looking statements. Forward-looking statements are based upon current expectations that involve risks, changes in circumstances, assumptions and uncertainties. Vanda is at an early stage of development and may not ever have any products that generate significant revenue. Important factors that could cause actual results to differ materially from those reflected in the Company's forward-looking statements include, among others: delays in the completion of Vanda's clinical trials; a failure of Vanda's products to be demonstrably safe and effective; Vanda's failure to obtain regulatory approval for its products or to comply with ongoing regulatory requirements for its products; a lack of acceptance of Vanda's products in the marketplace, or a failure to become or remain profitable; Vanda's expectations regarding trends with respect to its costs and expenses; Vanda's inability to obtain the capital necessary to fund its commercial and research and development activities; Vanda's failure to identify or obtain rights to new products; Vanda's failure to develop or obtain sales, marketing and distribution resources and expertise or to otherwise manage its growth; a loss of any of Vanda's key scientists or management personnel; losses incurred from product liability claims made against Vanda; a loss of rights to develop and commercialize Vanda's products under its license and sublicense agreements and other factors that are described in the "Risk Factors" section (Part II, Item 1A) of Vanda's quarterly report on Form 10-Q for the fiscal quarter ended March 31, 2009 (File No. 001-34186). In addition to the risks described above and in Part II, Item 1A of Vanda's quarterly report on Form 10-Q, other unknown or unpredictable factors also could affect Vanda's results. There can be no assurance that the actual results or developments anticipated by Vanda will be realized or, even if substantially realized, that they will have the expected consequences to, or effects on, Vanda. Therefore, no assurance can be given that the outcomes stated in such forward-looking statements and estimates will be achieved.

All written and verbal forward-looking statements attributable to Vanda or any person acting on its behalf are expressly qualified in their entirety by the cautionary statements contained or referred to herein. Vanda cautions investors not to rely too heavily on the forward-looking statements Vanda makes or that are made on its behalf. The information in this release is provided only as of the date of this release, and Vanda undertakes no obligation, and specifically declines any obligation, to update or revise publicly any forward-looking statements, whether as a result of new information, future events or otherwise.

The information in the posters attached as Exhibit 99.1 to this Form 8-K will be provided only as of the date on which such posters are presented, and the Company undertakes no obligation to update any forward-looking statements contained in such posters from and after the date of such presentations whether as a result of new information, future events or otherwise.

The information in Item 8.01 of this Form 8-K and the posters attached as Exhibit 99.1 to this Form 8-K shall not be deemed "filed" for purposes of Section 18 of the Securities Exchange Act of 1934 (the "Exchange Act") or otherwise subject to the liabilities of that section, nor shall it be deemed incorporated by reference in any filing under the Securities Act of 1933, as amended, or the Exchange Act, except as expressly set forth by specific reference in such a filing.

Item 9.01. Financial Statements and Exhibits.

(d) Exhibits

Exhibit No.	Description
99.1	Presentation posters.

SIGNATURES

Pursuant to the requirements of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned hereunto duly authorized.

VANDA PHARMACEUTICALS INC.

By: /s/ STEPHANIE R. IRISH
Name: Stephanie R. Irish
Title: Acting Chief Financial Officer and Treasurer

Dated: May 20, 2009

NR7-100

Effect of Melatonin Agonist Tasimelteon on Sleep Parameters and Architecture in a Phase Advance Model of Transient Insomnia

Russell Rosenberg, PhD[1]; Gunther Birznieks, MS[2]; Christin H. Scott, MS[2]; Paolo Baroldi, MD, PhD[2]; Mihael H. Polymeropoulos, MD[2]; Thomas Roth, PhD.[3]

[1]NeuroTrials Research, Inc., Atlanta, GA; [2]Vanda Pharmaceuticals Inc., Rockville, MD; [3]Henry Ford Hospital Sleep Center, Detroit, MI

Abstract

Objective: Tasimelteon is an investigational dual MT1/MT2 receptor melatonin agonist. The clinical efficacy and safety of tasimelteon using a model of transient insomnia induced by both "First Night Effect" and phase advance was studied in this Phase III trial.

Methods: A randomized, double-blind, placebo-controlled, multi-center study of 412 healthy adults was conducted. Transient insomnia was induced via a combination of stress inducement (first night in a sleep laboratory) and circadian rhythm disruption (5 hour bedtime advance). Subjects received 20mg, 50mg, 100mg tasimelteon or placebo 30 minutes prior to bedtime, and sleep measures were assessed using polysomnography (PSG) with post-sleep questionnaires to measure subjective sleep onset and sleep time.

Results: The primary outcome measure, LPS, significantly improved for all tasimelteon doses (21.5 min., p<0.001; 26.3 min., p<0.001 and 22.8 min., p<0.001) and improvements in WASO were observed (24.2 min., p<0.02; 33.7 min., p<0.001 and 17.5 min., p<0.081) at 20, 50, and 100mg respectively compared with placebo. The sleep increase in tasimelteon groups was primarily observed in NREM sleep. Significant improvements in subjective assessments of sleep onset and sleep time were also demonstrated.

Conclusion: Tasimelteon demonstrated sleep onset and maintenance effects both objectively as measured by PSG and subjectively by self-assessment. Given the combined first night effect and circadian challenge, efficacy may reflect the combined soporific and circadian effects of tasimelteon. Tasimelteon was safe, well tolerated, and no next-day residual effects were observed.

Vanda Pharmaceuticals sponsored this study.

Introduction

- Circadian Rhythm Sleep Disorders (CRSD) are a group of dysomnias that result when the timing of an individual's circadian pacemaker is misaligned with the imposed sleep time. In patients suffering from CRSD, insomnia occurs because patients try to sleep at a time when a strong drive for wakefulness is emanating from the circadian pacemaker
- Because pharmacotherapies for CRSD do not currently exist, most people that suffer from these dysomnias are either untreated or are inadequately treated, usually with sedative-hypnotics
- Tasimelteon is a specific and potent agonist of the human MT1 and MT2 receptors which mediate melatonin's effect on circadian rhythm. Because of melatonin's direct association with sleep and its involvement in the control of circadian rhythm, tasimelteon is being developed for the treatment of CRSDs
- A previous Phase 2 study demonstrated the ability of tasimelteon to shift the body clock, as measured by the body's own production of melatonin, in a 'phase advance' direction and simultaneously improve sleep onset and maintenance parameters compared to placebo[1]
- This poster represents a follow up Phase 3 study to confirm the sleep benefits observed with tasimelteon in a phase-shifted population

Methods

- Phase III, randomized, double-blind, placebo-controlled trial conducted at 19 centers in the US
- Transient insomnia induced in healthy subjects using a combination of:
  - Stress inducement via first night in a sleep laboratory[2]
  - Circadian sleep-wake timing challenge via a 5-hour phase advance
- Participants randomized to one of four treatment arms: tasimelteon (20 mg), tasimelteon (50 mg), tasimelteon (100 mg), and placebo. Study drug administered 30 minutes prior to bedtime

Supported by funding from Vanda Pharmaceuticals Inc.

- Primary endpoint was Latency to Persistent Sleep (LPS), as measured by PSG on Night 1, and defined as the length of time elapsed between lights out and onset of persistent sleep (10 minutes of continuous sleep)
- Secondary endpoints included:
  - Wake after sleep onset (WASO) during Night 1, determined by PSG
  - Subjective post-sleep assessments of sleep, as reported by questionnaire on Day 2
  - Safety and tolerability

Core Criteria for Entry into the Study

- Men and women aged between 21 and 50 years
- Considered in good health
  - Determined by no clinically significant deviations from normal in medical history, clinical laboratory results, electrocardiogram (ECG) readings, and physical examinations conducted during the screening visit
  - No current medical, psychiatric, or sleep disorders (including history or evidence of the following: restless leg syndrome, periodic limb movement disorder, excessive daytime sleepiness, sleep apnea, sleep deprivation, or insomnia)
- Meet sleep history and sleep-wake schedule requirements
  - Between 7 and 9 hours of sleep per night with consistent bedtime and wake time for 1 month prior to enrollment
  - Compliant with a a sleep schedule during screening that required staying in bed and trying to sleep for approximately 8 to 9 hours per night (monitored via actigraphy)
  - No previous sleep in a sleep clinic
- No history of recent drug or alcohol abuse and willing to comply with drug (including over-the-counter and prescription drugs), alcohol, smoking, and caffeine consumption restrictions

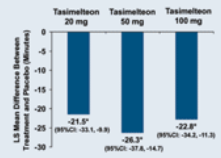
Statistics

- Included 411 randomized patients who received a dose of study drug and had PSG data
- Used analysis of covariance (ANCOVA), with treatment group and center being the covariates, for efficacy endpoint analysis. A hierarchical approach was used to test for a statistically significant difference between tasimelteon and placebo (proceeding from highest tasimelteon dose to lowest dose). The a priori analysis included a transformation of the data if assumptions of normality and equal variances were violated. Data from post-hoc analysis of untransformed data presented here for clinical interpretability. Results of transformed data were comparable
- TST, REM, and SWS results are from a post-hoc analysis

Table 1. Baseline Demographics

	Placebo (n = 103)	Tasimelteon 20 mg (n = 100)	Tasimelteon 50 mg (n = 102)	Tasimelteon 100 mg (n = 106)
Mean age, years (± SD)	30.9 (7.3)	30.8 (8.4)	31.0 (8.5)	31.2 (8.2)
Female gender, n (%)	68 (66.0)	62 (62.0)	58 (56.9)	73 (68.9)
Median weekly bedtime (range)	22:30 (21:00-01:00)	23:00 (21:00-01:00)	22:30 (21:00-00:30)	23:00 (21:00-01:00)
Median weekly wake time (range)	07:00 (05:00-09:30)	07:00 (05:00-09:30)	06:52 (04:30-09:00)	07:00 (05:00-08:30)
Sleep per night, mean hours (± SD)	8.3 (0.4)	8.3 (0.5)	8.2 (0.6)	8.2 (0.5)
Time taken to fall asleep, mean min. (± SD)	12.3 (6.8)	12.3 (7.3)	11.2 (5.9)	12.7 (6.7)

Figure 1. Latency to Persistent Sleep



*P < 0.001 for all treatment groups vs placebo

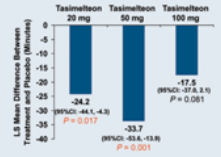
Results

- No significant differences in baseline characteristics were detected between the 4 treatment groups (Table 1)

Efficacy

- Significant improvement in LPS (p<0.001) was observed at all tasimelteon dose levels. The average improvement that was observed in LPS compared to placebo was 21.5 minutes, 26.3 minutes, and 22.8 minutes, at 20, 50, and 100 mg tasimelteon, respectively (Figure 1)
- Data from subjective self-reports on this night were consistent with this finding. Significant reduction in subjective measures of sleep onset were also observed at tasimelteon 50-mg (p<0.001) and 100-mg doses (p=0.004), compared with placebo (Table 2)

Figure 2. Wake After Sleep Onset



P values correspond to treatment vs placebo, red highlight designates $p < 0.05$

- Significant improvement in WASO was observed with the 20 and 50 mg tasimelteon groups compared with placebo (p=0.017 and p=0.001, respectively). The average improvement compared with placebo that we observed in WASO was 24.2 minutes, 33.7 minutes, and 17.5 minutes (p=0.081) at 20, 50, and 100 mg tasimelteon, respectively (Figure 2)

Table 2. Additional Sleep Parameters – Least-Square Mean Difference Between Treatment and Placebo

	TST (min)	sSL (min)	sTST (min)
Tasimelteon 20 mg	33.7 (95%CI: 13.0, 54.5) P=0.002	-10.3 (95%CI: -21.7, 1.0) p=0.075	22.7 (95%CI: -2.5, 47.8) p=0.077
Tasimelteon 50 mg	47.9 (95%CI: 27.2, 68.6) p<0.001	-21.1 (95%CI: -32.4, -9.7) p<0.001	33.9 (95%CI: 8.7, 59.1) p=0.009
Tasimelteon 100 mg	29.6 (95%CI: 9.1, 50.0) p=0.005	-16.5 (95%CI: -27.7, -5.3) p=0.004	13.8 (95%CI: -10.9, 38.6) p=0.273

- Significant improvements were observed in TST of 33.7 (p=0.002), 47.9 (p<0.001), and 29.6 (p=0.005) minutes at 20, 50, and 100 mg tasimelteon, respectively, compared with placebo (Table 2)

Table 3. REM and SWS Sleep- Least-Square Mean Difference Between Treatment and Placebo

	REM vs. Placebo (min)	SWS vs. Placebo (min)
Tasimelteon 20 mg	0.25 (95%CI: -6.7, 7.2) p=0.943	-0.35 (95%CI: -6.4, 5.7) p=0.908
Tasimelteon 50 mg	5.86 (95%CI: -1.1, 12.8) p=0.100	-4.69 (95%CI: -10.7, 1.3) p=0.128
Tasimelteon 100 mg	0.70 (95%CI: -6.2, 7.6) p=0.842	-2.49 (95%CI: -8.4, 3.5) p=0.4120

- Significant improvement in subjective measures of sleep duration was observed at tasimelteon 50 mg (p=0.009) compared with placebo (Table 2)
- The amount of REM (rapid eye movement) sleep and SWS (slow wave sleep, stage 3 and 4 of NREM) in tasimelteon-treated subjects was comparable to values observed in placebo-treated subjects (Table 3) indicating that tasimelteon was not suppressing SWS or REM sleep
- Although the study was not powered to detect differences between tasimelteon doses, the numerical superiority of both objective and subjective measures of sleep parameters on the 50mg dose suggests that 50 mg tasimelteon appears to be the optimal therapeutic dose. The 50 mg dose of tasimelteon being the optimal dose over the higher 100 mg dose supports the hypothesis that higher doses will not necessarily be more efficacious as observed with melatonin[3]

Safety

- No TEAEs met the criteria for most common, defined as a percentage incidence in any tasimelteon treatment group of at least twice that of the placebo group and a percentage incidence of 1% or more overall (across all 4 treatment groups)
- The most frequently reported TEAEs in all 4 groups combined for this study were nausea (2.9%) and headache (1.2%). The incidence of nausea and headache was similar between tasimelteon-treated subjects and placebo-treated subjects
- No next-day residual effects were seen after placebo or tasimelteon treatment based on assessments of cognitive performance (measured by DSST) and mood (measured by VAS)

Conclusion

- Tasimelteon was able to reduce sleep latency, improve sleep maintenance, and increase sleep duration in transient insomnia induced by both an abrupt shift in the sleep-wake cycle and first night effect stress
- Tasimelteon was able to increase the total sleep time without having an impact on the amount of REM sleep or SWS
- Tasimelteon was safe, well tolerated, and no next-day residual effects were observed
- Tasimelteon demonstrates an efficacy profile which may suggest therapeutic potential to treat the symptoms of insomnia associated with a misalignment between the timing of the sleep-wake cycle and the circadian sleep propensity rhythm

Acknowledgements

We thank the following investigators and their staff for their contributions during the study conduct: R. Bogan, M. Cohn, B. Corser, H. Emsellem, N. Feldman, J. Flescher, P. Haberman, B. Harris, J. Hudson, S. Hull, A. Jamieson, G. Pegram, M. Perlis, K. Rice, R. Rosenberg, M. Rosenthal, H. Schwartz, D. Seiden, and S. Thein.

References

1. Rajaratnam S.M.W., Polymeropoulos M.H., Fisher D.M., Roth T., Scott C., Birznieks G. and Klerman E.B. *Lancet.* 2009; 373:439-41
2. Rosenberg R., Caron J., Roth T., Amato D. *Sleep Med.* 2005; 56:15-22
3. Gerdin, M.J., Masana, M.I., Ren, D., Miller, R.J., and Dubocovich, M.L. *J Pharmacol Exp Ther.* 2003; 304:931-9

Presented at the 162nd Annual Meeting of the American Psychiatric Association May 19-21, 2009, San Francisco, CA

NR7-098 Effect of a Period 3 (*PER3*) Polymorphism on Sleep Architecture in Phase Advanced Transient Insomnia

Shruti Mitkus, PhD; Gunther Birznieks, MS; Charles A. Czeisler, PhD, MD; Andrew Thompson, BS; Christian Lavedan, PhD.
Vanda Pharmaceuticals Inc., Rockville, MD

Abstract

Objective: Insomnia is the most common sleep disorder and is also a symptom of circadian rhythm sleep disorders and other medical and psychiatric conditions. A 4-5 repeat polymorphism in a clock gene, Period 3 (*PER3*), plays a role in regulating the sleep-wake cycle. Although this polymorphism has been associated with delayed sleep phase syndrome, its role in transient insomnia is unknown. The effect of this polymorphism on polysomnographic sleep parameters was analyzed in individuals subjected to phase-advanced transient insomnia.

Method: Transient insomnia was induced in healthy subjects, through a 5-hour phase advance protocol and a "first night effect". Individuals (N=76) were genotyped by standard methods. Several sleep parameters were evaluated by polysomnography including sleep efficiency, total sleep time, latency to persistent sleep, wake after sleep onset, rapid eye movement (REM), non-REM, and slow wave sleep (SWS). Statistical analysis was performed using a generalized linear model for analysis of variance with pooled center as a covariate.

Results: *PER3* 5/5 individuals had significantly greater sleep efficiency over an 8-hour sleep episode as compared to the non-5/5 individuals (6.2 vs. 5.3 hours, p=0.023). Rate of REM accumulation was faster in 5/5 than non-5/5 individuals (5.8 vs. 3.7 hours to accumulate 30 minutes of REM, p=0.000055), but non-REM and SWS accumulation rate did not differ between genotypes (p>0.05).

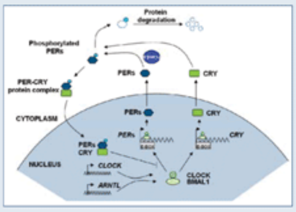
Conclusions: *PER3* 5/5 individuals were significantly less disrupted by phase advance induced-transient insomnia than the non-5/5 individuals, suggesting that PER3 plays a role in regulating circadian rhythm. These results also suggest that genetic variations in *PER3* may contribute to susceptibility to transient insomnia. This finding may have important implications for understanding the potential selective advantage of the *PER3* 5/5 genotype and its role in the pathophysiology of transient insomnia. Vanda Pharmaceuticals sponsored this study.

Introduction

- Insomnia is the most common sleep disorder estimated to affect millions worldwide, and is also a symptom of circadian rhythm sleep disorders and other medical and psychiatric conditions, such as depression[1,2]
- It is characterized by difficulty initiating and maintaining sleep or experiencing non-refreshing sleep[1,2]
- Transient insomnia is associated with daytime sleepiness and impairment in psychomotor performance[2]
- Heritability estimates for insomnia are at about 57%[3]
- Understanding the role of genetic factors in sleep disorders and in the modulation of the circadian rhythm may be valuable in the diagnosis and personalized treatment of these conditions
- Several genes have been implicated in the regulation of sleep and circadian rhythm (Figure 1) including *PER3, CRY1/2, CLOCK, ARNTL, CSNK1E, HOMER1*[4-6]
- *PER3* is a member of the Period family of genes, which are expressed in a circadian pattern in the suprachiasmatic nucleus
- A *PER3* Variable Number Tandem Repeats (VNTR) polymorphism, with 4 or 5 18-amino acid repeats, has been implicated in diurnal preference, delayed-sleep phase syndrome (DSPS), cognitive performance and neurobehavioral function during sleep deprivation at an adverse circadian phase[7-10]
- The aim of this study was to determine the effect of this *PER3* 4-5 polymorphism on sleep parameters in healthy individuals subjected to transient insomnia induced by a phase advance

Supported by funding from Vanda Pharmaceuticals Inc.

Figure 1. Schematic Representation of the Molecular Pathway Involved in Regulation of Sleep and Circadian Rhythm



PERs (period homolog 2,3), *CRYs* (cryptochrome 1, 2), *CLOCK* (circadian locomoter output cycles kaput), *ARNTL* (aryl hydrocarbon receptor nuclear translocator-like), *CSNK1E* (casein kinase 1 epsilon). Adapted from Nick Foulkes Max Planck Institute for Developmental Biology www.eb.tuebingen.mpg.de/research-groups/nick-foulkes.

Methods

Clinical Trial

- This study was part of a randomized double-blind placebo-controlled clinical trial in healthy individuals designed to evaluate a novel treatment for insomnia
- Transient insomnia was induced for one night in healthy individuals (N=288) using both the first night effect (new laboratory setting) and a 5-hour phase advance (subjects asked to go to sleep 5 hours before habitual bedtime)
- Of the 288 subjects, 212 received study medication and 76 received placebo
- Subjects had no history or evidence of periodic limb movement disorder, sleep apnea, primary insomnia or any other sleep disorder
- Sleep assessments evaluated by polysomnography (PSG) included:
  - latency to persistent sleep (LPS)
  - wake after sleep onset (WASO)
  - sleep efficiency (SE)
  - total sleep time (TST)
  - rapid eye movement sleep (REM)
  - non-REM sleep (NREM)
  - slow wave sleep (SWS)

Pharmacogenetic Study

- Retrospective study of healthy individuals who received placebo (N=76) and therefore were not affected by the study medication
- Genotyping was performed as previously described[8]

Statistical Analysis

- General Linear Model (GLM) of analysis of variance with pooled center as a covariate was performed between *PER3* 5/5 and non-5/5 genotype individuals
- LPS values were log transformed to fit a normal distribution and WASO transformation was performed using the Box-Cox procedure for normalization

Results

- Allele and genotype frequencies were similar to what has been reported in most populations[7-9,11] (Table 1)
- The 5-repeat allele was more common among Blacks than in Whites (Table 1), but the difference in genotype distribution was not statistically significant (chi^2 p=0.3)
- We detected similar frequencies in an independent population of 50 African American and Caucasian North American individuals (data not shown)

Table 1. *PER3* VNTR Genotype and Allele Frequencies (%)

	Overall Population (N=288)	Black (N=47)	White (N=224)	Others (N=17)	Placebo Group (N=76)
4/4	49.6	42.55	52.7	29.4	40.8
4/5	39.6	42.55	37.5	58.8	47.4
5/5	10.8	14.9	9.8	11.8	11.8
5-repeat allele	30.6	36.2	28.6	41.2	34.9

- Individuals with the *PER3* 5/5 genotype had significantly greater sleep efficiency for the during the phase advanced 8-hour sleep episode (78% vs. 66%, p=0.023) (Table 2 and Figure 2), particularly in the first third of the sleep episode, when 5/5 homozygotes experienced 75% sleep efficiency (2 hours) versus 60% (1.6 hours) for non-5/5 individuals (p=0.047)
- Individuals with the *PER3* 5/5 genotype showed a numeric trend for:
  - Accumulating more total NREM sleep (TNREM: 4 hrs 50 min vs 4 hrs 25 min)
  - Taking 45% less time to fall asleep (LPS: 29 vs. 53 min)
  - Spending 25% less time awake after falling asleep (WASO: 1 hr 39 min vs. 2 hrs 12 min)

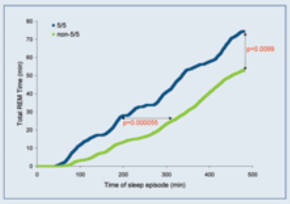
Table 2. Summary of PSG Sleep Parameters by *PER3* 4-5 Genotype

Genotype (N)	SE (%)	TST (min)	LPS (min)	WASO (min)	TREM (min)	TNREM (min)	TSWS (min)
5/5 (9)	78 ± 16	372 ± 76	29 ± 44	99 ± 74	74 ± 25	290 ± 54	22 ± 13
4/4 (31)	67 ± 18	323 ± 85	42 ± 78	140 ± 85	55 ± 24	269 ± 69	42 ± 27
4/5 (36)	66 ± 19	315 ± 86	63 ± 77	126 ± 74	50 ± 21	262 ± 71	35 ± 20
non-5/5 (67)	66 ± 18	319 ± 85	53 ± 78	132 ± 79	52 ± 23	265 ± 69	38 ± 23
p-value*	0.023	0.023	0.13	0.15	0.0099	0.061	0.11

*GLM analysis between PER3 5/5 and non-5/5 individuals. Mean ± SD
TREM: total REM sleep time TNREM - total nonREM sleep time TSWS - total slow wave sleep

- Analysis of the timing of REM sleep propensity revealed that *PER3* 5/5 individuals accumulated REM sleep much faster than non-5/5
- The total REM sleep episode, irrespective of *PER3* genotype, was ~60 minutes. It took non-5/5 individuals about 2 more hours than the 5/5 individuals to accumulate 30 minutes of REM sleep (5.8 vs. 3.7 hours, p=0.000055; Figure 2)
- At the end of the sleep episode, 5/5 individuals had accumulated 42% more REM sleep (1.2 hours) than non-5/5 individuals (52.5 minutes, p=0.0099; Table 2 and Figure 2)

Figure 2. Effect of *PER3* Genotype on REM Sleep



Discussion

- Individuals with the *PER3* 5/5 genotype who were subjected to phase-advanced transient insomnia had greater sleep efficiency over the 8 hour sleep episode than individuals with the non-5/5 genotype
- This effect was particularly prominent between hours 1 through 5 and is consistent with a hypothesis that the evening circadian wake maintenance zone occurs at a greater interval of time before habitual bedtime in 5/5 individuals than in non-5/5 individuals
- Such a widening of the phase angle of entrainment, i.e. the time between the onset of melatonin secretion and habitual bedtime (lights off), would be anticipated if the intrinsic circadian period was shorter in 5/5 individuals than in non-5/5 individuals[12]
- Analysis of the timing of REM sleep propensity, which is known to be under tight circadian regulation[13] revealed that individuals with the *PER3* 5/5 genotype accumulate REM sleep significantly faster than non-5/5 individuals
- This observation suggests that the endogenous REM propensity rhythm, which in healthy young subjects is coupled to the sleep propensity rhythm[14], is phase advanced in 5/5 individuals relative to non-5/5 individuals
- This also suggests the existence of a circadian component in the protection from phase advance-induced insomnia for *PER3* 5/5 individuals
- While we cannot exclude the possibility that this difference in the rate of REM sleep accumulation in 5/5 subjects is unrelated to the circadian system, we hypothesize that a wider phase angle of entrainment due to a shorter intrinsic circadian period in 5/5 individuals is the most parsimonious explanation for our observations

Mechanistic Hypothesis

The effect of the *PER3* VNTR on sleep may be mediated by the functional consequence of one additional or missing 18 amino acid motif

- It is believed that PER3 nuclear translocation and protein stability are regulated by casein kinase 1ε (CSK1ε)[15]
- Each PER3 repeat contains potential phosphorylation motifs for CSK1ε[8]
- The 4-repeat allele provides five fewer serine/threonine residues available for phosphorylation than the 5-repeat allele
- A reduced phosphorylation of the 4-repeat allele compared to the 5-repeat allele might lead to a lower rate of elimination of the PER3 protein from the nucleus
- This increased nuclear stability of PER3 and the subsequent delay in its translocation out of the nucleus in response to light may result in lengthening of the circadian period similar to that observed for the double-time (dbt) allele in Drosophila[16-18]

Conclusion

- *PER3* 5/5 individuals, appear to be protected against induced transient insomnia (greater sleep efficiency and total sleep time than non-5/5 individuals)
- Genotype differences in sleep efficiency in the first third of the night suggest that *PER3* 5/5 individuals may be phase advanced compared to non-5/5 individuals
- REM sleep accumulation, which is under tight circadian regulation[13], is faster in *PER3* 5/5 individuals suggesting the existence of a circadian component in the protection against transient insomnia for those individuals
- *PER3* non-5/5 individuals with transient insomnia may benefit from better sleep hygiene or therapies targeted towards advancing their circadian clock

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NR7-009

Common Effect of Antipsychotics on Biosynthesis and Regulation of Fatty Acids and Cholesterol Supports a Role of Lipid Homeostasis in Schizophrenia

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Vanda Pharmaceuticals Inc., Rockville, MD

Abstract

Objective: For decades, the dopamine hypothesis has gained the most attention in an attempt to explain the origin and the symptoms of schizophrenia. While this hypothesis offers an explanation for the relationship between psychotic symptoms and dopamine kinetics, it does not provide a direct explanation of the etiology of schizophrenia which remains poorly understood. Consequently, current antipsychotics that target neurotransmitter receptors, have limited and inconsistent efficacy. To gain insights into the mechanism of action of these drugs, we studied the effect of antipsychotics on the expression of the human genome, and compared it to that of an extensive library of drugs used in a variety of disorders.

Methods: The expression profile of 12,490 genes in a cell line treated with 11 typical and 7 atypical antipsychotics was analyzed with a Weighted Influence Model, Rank of Ranks method. The "antipsychotic signature" was compared to that of a library of 448 other compounds.

Results: Nineteen of the first 20 ranked probe sets in the antipsychotic group profile correspond to 13 genes involved in fatty acids and cholesterol biosynthesis, or in phospholipid metabolism. Typical and atypical antipsychotics had a similar effect on lipid homeostasis, regardless of their metabolic or lipid-related adverse event profile. It was observed that antipsychotics not only activate genes involved in lipid homeostasis but do this preferentially from all other genes.

Conclusions: We propose that the activation by antipsychotics of genes associated with lipid homeostasis is not just a common off target effect of these drugs but rather a common mechanism by which they achieve their antipsychotic activity. These results also support convergent clinical evidence for a lipid hypothesis of schizophrenia, and may help research aimed at the development of novel treatments for this devastating disease. Vanda Pharmaceuticals sponsored this study.

Introduction

- The underlying molecular etiology of schizophrenia remains poorly understood although the dopamine hypothesis has been the most influential for decades
- A number of antipsychotics are used for management of the disease symptoms. They share the ability to modulate neurotransmitters through the binding of an array of receptors. Their efficacy is limited and the mechanisms leading to induced adverse events are not well understood
- In an effort to discover molecular signatures of pharmaceutical agents, including antipsychotics, we have screened 466 compounds that belong to 14 different therapeutic classes (Figure 1) in a human cell line, and studied the resulting gene expression changes across 12,490 genes
- We describe here the discovery of an "antipsychotic signature" which gives insights into the therapeutic effect of these drugs, but also possibly into the etiology of schizophrenia

Methods

Cell Culture and Drug Treatment

- Retinal pigment epithelia cell line, ARPE-19/HPV-16
  - human origin
  - normal karyotype
  - easily grown as monolayer in 96-well plates (~2x10e5 cells/well)
  - expresses the dopamine receptor D2, the serotonin receptors 1A, 2A, and 2C, the muscarinic receptor M3, and the histamine receptor H1
- Cell line from glioblastoma origin (H4) used only for independent replication
- 24 hrs incubation before treatment with 10µM drug or vehicle

Gene Expression Profiles

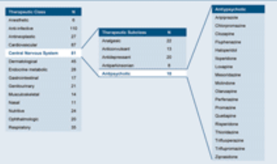
- RNA extracted after 24 hrs treatment
- 708 U133A2.0 Affymetrix microarrays analyzed (22,238 probe sets of 12,490 genes)
  - 74 for the 18 antipsychotics
  - 499 for the other 448 compounds
  - 135 for vehicle controls

Supported by funding from Vanda Pharmaceuticals Inc.

Data Analysis

- For each drug treatment-vehicle pair all probe sets were ranked by amplitude (A)
  A= (t-v)/[(t+v)/2] ; t = treatment instance; v = vehicle instance
- Drug group profiles created with the Weighted Influence Model, Rank of Ranks (WIMRR) method
- A gene-set enrichment metric based on the Kolmogorov-Smirnov (KS) statistic was used where the KS score gives a measure of how up (positive) or down (negative) the set of probes occurs within the profile of another treatment instance

Figure 1. Compounds Profiled Across Therapeutic Classes and Sub-classes


Therapeutic Class
Central Nervous System
Therapeutic Subclass
Antipsychotic
Aripiprazole
Chlorpromazine
Clozapine
Fluphenazine
Haloperidol
Loxapine
Molindone
Olanzapine
Perfenazine
Promazine
Quetiapine
Risperidone
Thioridazine
Trifluoperazine
Triflupromazine
Ziprasidone

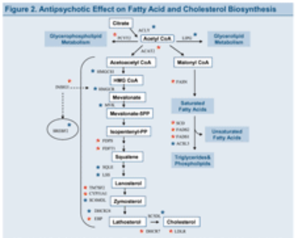
Results

- Nineteen of the first 20 (95%) ranked probe sets in the antipsychotic group profile correspond to 13 genes involved in fatty acids and cholesterol biosynthesis, or in phospholipid metabolism (Table 1 and Figure 2)
- This lipid homeostasis signature appears even stronger upon a comprehensive analysis beyond the top 20 probe sets, which revealed the up-regulation of more genes from the same biological pathways (Figure 2)

Table 1. Top Probe Sets of Antipsychotic Group Profile in ARPE-19 Cell Line

Rank	Probe set	Gene	Description
1	201627_s_at	*INSIG1*	insulin induced gene 1
2	200831_s_at	*SCD*	stearoyl-CoA desaturase (delta-9-desaturase)
3	200832_s_at	*SCD*	stearoyl-CoA desaturase (delta-9-desaturase)
4	211162_x_at	*SCD*	stearoyl-CoA desaturase (delta-9-desaturase)
5	202218_s_at	*FADS2*	fatty acid desaturase 2
6	202067_s_at	*LDLR*	low density lipoprotein receptor
7	208964_s_at	*FADS1*	fatty acid desaturase 1
8	201275_at	*FDPS*	farnesyl diphosphate synthase
9	201625_s_at	*INSIG1*	insulin induced gene 1
10	202068_s_at	*LDLR*	low density lipoprotein receptor
11	209608_s_at	*ACAT2*	acetyl-Coenzyme A acetyltransferase 2
12	210950_s_at	*FDFT1*	farnesyl-diphosphate farnesyltransferase 1
13	216607_s_at	*CYP51A1*	cytochrome P450, family 51, subfamily A, polypeptide 1
14	212218_s_at	*FASN*	fatty acid synthase
15	201790_s_at	*DHCR7*	7-dehydrocholesterol reductase
16	219562_at	*RAB26*	RAB26, member RAS oncogene family
17	210130_s_at	*TMTSF2*	transmembrane 7 superfamily member 2
18	208962_s_at	*FADS1*	fatty acid desaturase 1
19	209577_at	*PCYT2*	phosphate cytidylyltransferase 2, ethanolamine
20	202735_at	*EBP*	emopamil binding protein (sterol isomerase)

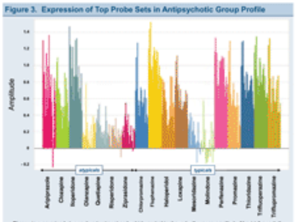
Figure 2. Antipsychotic Effect on Fatty Acid and Cholesterol Biosynthesis


Citrate
Acetyl CoA
Glycerophospholipid Metabolism
Glycerolipid Metabolism
Acetoacetyl CoA
Malonyl CoA
HMG CoA
Mevalonate
Mevalonate-5PP
Isopentenyl-PP
Saturated Fatty Acids
Unsaturated Fatty Acids
Triglycerides& Phospholipids
Squalene
Lanosterol
Zymosterol
Lathosterol
Cholesterol

Pathways of Fatty Acid and Cholesterol Biosynthesis are shown with major metabolite intermediates and enzymatic reactions (arrows). Genes coding for relevant enzymes, regulators, or transporters are shown with an asterisk: red or blue when the corresponding probe set(s) was (were) in the top 20 or top 100, respectively, of the antipsychotic profile.

- Typical and atypical antipsychotics had a similar effect (Figure 3)
- The effect on lipid homeostasis was confirmed in the H4 cell line
- Gene ontology (GO) analysis showed statistically significance
  - "lipid biosynthetic process" was the term most significantly associated with the top 20 and top 100 probes sets (p=5.23E-16 and 6.20E-33, respectively, after Bonferroni correction)
  - The genes most down-regulated were from various biological processes, with no clear common significance. The term most significantly associated with the bottom 20 probes sets was "mitosis" (p=1.06E-03 after correction)

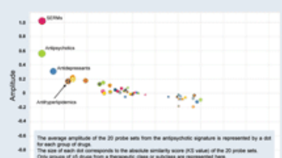
Figure 3. Expression of Top Probe Sets in Antipsychotic Group Profile



Change in expression between drug treatment and vehicle control is shown by the mean amplitude (Y axis) for each the top 20 ranked probe sets (left to right on the X axis, ordered by rank) for each antipsychotic tested. The mean amplitude graphed is the average amplitude across replicates.

- Selective estrogen receptor modulators (SERMs), affected lipid homeostasis in a manner similar to antipsychotics (KS scores of 0.997 and 0.806 for the top 20 and 100 probe sets, respectively) (Figure 4). See poster NR1-092 for details
- The antipsychotic signature appears unique among all other therapeutic classes and sub-classes of more than 400 compounds studied, with only a partial overlap with antidepressants and antihyperlipidemic agents (Figure 4)

Figure 4. Visualization of the Antipsychotic Gene Expression Signature Across a Library of Compounds.



The average amplitude of the 20 probe sets from the antipsychotic signature is represented by a dot for each group of drugs.
The size of each dot corresponds to the absolute similarity score (KS value) of the 20 probe sets.
Only groups of ≥5 drugs from a therapeutic class or subclass are represented here.

Discussion

Results are in agreement with independent observations

- In yeast, haloperidol, chlorpromazine, and trifluoperazine strongly affect genes involved in fatty acid biosynthesis[1]
- In a glioma cell line, clozapine, olanzapine, haloperidol, chlorpromazine, risperidone, ziprasidone activate *SCD, FASN, DHCR7, LDLR, FDPS, ACAT2, SC5DL*, and genes for 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (*HMGCR*), and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (*HMGCS1*)[2]
- *SCD* and *FASN* are up-regulated in blood cells from patients with psychotic disorders treated with olanzapine[3]
- Drug-free schizophrenia patients have significantly lower levels of plasma phosphoethanolamines (PE) than control subjects. Increase in PE, phosphatidylcholine (PC), and triacylglycerols (TG) was observed with olanzapine. Increase in PE, PC, and lysophosphatidylcholine (LY) was seen with risperidone[4]

Antipsychotic modulation of the ratio of polyunsaturated to saturated fatty acids and cholesterol content may alter the fluidity of cell membranes of neurons and supporting cells, resulting in changes in neuronal connectivity. This may lead to improvement of symptoms and not necessarily just to side effects

- The up-regulation of *SCD, FADS1* and *FADS2* points to a central role of the regulation of the composition of mono- and poly-unsaturated fats
- Reconstitution of the lipid membrane composition to achieve improvement of schizophrenia symptoms could explain the time lag of the therapeutic effect seen with these agents
- Several antipsychotics which share the gene signature do not have the same type of metabolic or lipid-related adverse event profile
- The antipsychotic signature is shared by SERMs which do not have the same type of metabolic-related adverse events as antipsychotics

Connection with CNTF

- A ciliary neurotrophic factor (CNTF) peptidomimetic (axokine) significantly decreases the expression of *SCD* in adipocytes, a function shared by leptin[5]
- *SCD* was one of the genes most consistently up-regulated by antipsychotics
- We reported that patients with a *CNTF* null mutation have a high placebo response[6]
- These patients may have a higher constitutive expression of *SCD* contributing to their ability to better overcome an acute exacerbation, at least in the short term, than patients with the normal CNTF protein

We propose that an aberration of lipid homeostasis may represent a key deficit in the etiology of this disease and possibly other behavioral disorders, in agreement with multiple clinical observations

- Disease onset is usually in late adolescence or early adulthood[7], at a period of significant changes in fatty acid composition in the cerebral cortex[8]
- Increase of intra-abdominal fat, impaired fasting glucose tolerance, and more insulin resistance in drug-naive schizophrenia patients than in healthy individuals[9-10]
- Decrease of phospholipids and polyunsaturated fatty acids in postmortem brain as well as in peripheral membranes[11-14]
- Abnormalities in phospholipase A2 activity which plays an essential role in the breakdown of phospholipids, and in the metabolism of prostaglandins[15-17]
- Reduction of essential polyunsaturated fatty acids in the red blood cell membrane of antipsychotic-naive patients[18]
- Nutritional deficiencies including ω-3 fatty acids in mental disorder patients, low incidence rate and better prognoses due to consumption of ω-3 fatty acids[19-22]
- Positive therapeutic effects of ω-3 fatty acids in patients with schizophrenia[23-28]
- Deficits of ω-3 fatty acids in the orbitofrontal region of brains of patients with schizophrenia; partially restored in patients treated with antipsychotics[29]

Conclusion

- We suggest that an array of defects in the fatty acids and cholesterol biosynthesis pathways may be involved in the pathogenesis of schizophrenia, and that alteration of lipid homeostasis may be a common mechanism by which antipsychotics achieve their therapeutic effect
- Further research in the lipid hypothesis of schizophrenia may provide fundamental knowledge of the gene-environment interactions in the causation and course of the disease
- It is our hope that the finding of a common effect of current antipsychotics on genes involved in lipid will spark research aimed at the development of new treatments for this devastating disease

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