PH80 Nasal Spray Effects on In Vitro Receptor Binding, Reproductive Organs in Mice, and Pharmacokinetic Profile in Humans: A Novel, Investigational, Rapid-Onset, Non-Hormonal Treatment for Vasomotor Symptoms Due to Menopause

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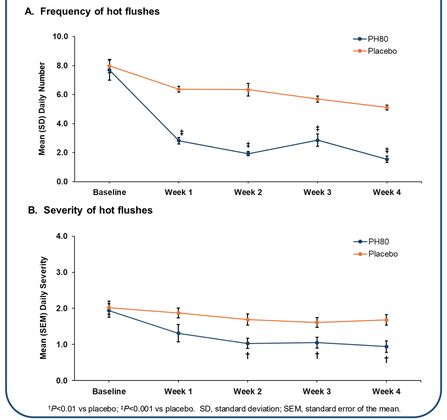
P-124

Introduction

- Vasomotor symptoms (VMS) due to menopause, including hot flashes and night sweats,[1,2] can have a negative impact on women's overall quality of life and mental health[3-6]
- Pherines are a novel class of odorless, tasteless, non-hormonal, nasal chemosensory receptor agonists that are under investigation by Vistagen as intranasal drug candidates for multiple psychiatric and women's health indications[7]
  - Pherines rapidly and selectively bind to receptors in human nasal chemosensory neurons (NCNs), activating olfactory bulb-to-brain neurocircuits
  - Activating receptors in NCNs avoids systemic absorption and brain uptake to achieve therapeutic effects with a favorable safety profile
- PH80 is a pherine with a proposed mechanism of action to treat VMS via its agonist activity on NCN receptors, activating microcircuits (glomeruli) in mood and thermoregulatory neurocircuits[7]
- In an exploratory, phase 2a study, PH80 nasal spray reduced the frequency and severity of hot flashes (**Figure 1AB**), as well as hot flash bother and sweating, for up to 4 weeks in menopausal women experiencing moderate to severe hot flashes.[8] The tolerability profile of PH80 did not differ from that of placebo.[8]

Figure 1. Phase 2a study: PH80 nasal spray reduced frequency and severity of hot flashes in menopausal women[8]



†P<0.01 vs placebo; ‡P<0.001 vs placebo. SD, standard deviation; SEM, standard error of the mean.

Objectives

To characterize PH80's

- In vitro binding profile on steroid hormone and neurotransmitter receptors
- In vivo effects on the uterus and seminal vesicle in animal studies
- Plasma levels after multiple intranasal doses in healthy volunteers

Methods

In vitro receptor binding

- Standard receptor binding assays were used to measure the binding of PH80 (doses of 1.0E-9, 1.0E-7, and 1.0E-5) to various steroid hormone and neurotransmitter receptors

In vivo effects

- Female mice (n=30) were given the 3 doses of PH80 (10 mg/kg) to evaluate potential estrogenic agonism and/or antagonism as measured by uterine weight
  - Agonist control: Estradiol benzoate (1 µg/kg)
  - Antagonist control: Tamoxifen (100 µg/kg)
- PH80 (10 mg/kg PO) given for 5 days was evaluated in male rats (n=30) for potential androgenic agonism and/or antagonism as measured by seminal vesicle weight
  - Agonist control: Testosterone propionate (3 mg/kg)
  - Antagonist control: Cyproterone (3 mg/kg)

Pharmacokinetic study

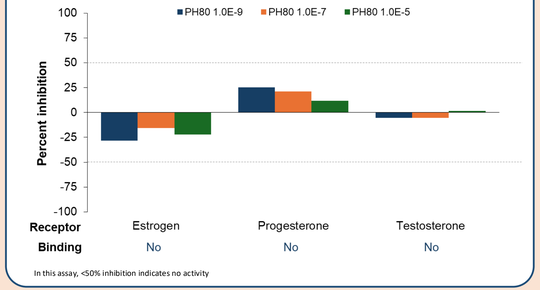
- A prospective, one-sequence, one-period, one-treatment, single-blind study
- Six clinically healthy, female volunteers (age 18-55 years) were administered PH80
  - 1.6 µg of PH80 intranasally (0.8 µg/100 µL in each nostril)
  - 7 additional doses were administered at 4, 5, 6, 8, 10, 12, and 14 hours after the initial dose
  - Each volunteer received a total of 12.8 µg of PH80
- Blood samples were drawn at pre-dose (control [0.00]), and at 0.084, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 12.00, and 24.00 hours post dosing; and stored at -70ºC
- PH80 was measured at each timepoint by liquid chromatography-tandem mass spectrometry; the lower limit of quantification was 0.2 ng/mL
- Adverse events were recorded

Results

In vitro receptor binding and activity

- PH80 lacks binding affinity and has no activity on steroid hormone receptors, including those for estrogen, progesterone, and testosterone (**Figure 2**)
- PH80 does not bind or have activity at multiple neurotransmitter receptors, including those for $GABA_A$, dopamine, serotonin, glutamate, and opiate (**Figure 3**)

Figure 2. PH80 activity and binding to steroid receptors



In this assay, <50% inhibition indicates no activity

In vivo effects

- No agonist or antagonist effects on estrogen receptors with PH80 were observed in female mice. PH80 had no effect on uterine weight, while estradiol benzoate increased and tamoxifen decreased uterine weight (**Figure 4**)
- No androgenic agonism or antagonism with PH80 was observed in male rats. PH80 had no effect on seminal vesicle weight, while testosterone propionate increased and cyproterone decreased seminal vesicle weight (**Figure 4**)

Figure 4. Lack of PH80 agonist and antagonist activity

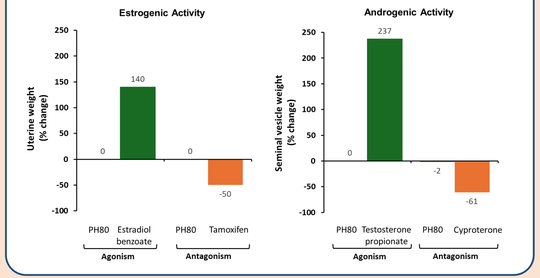
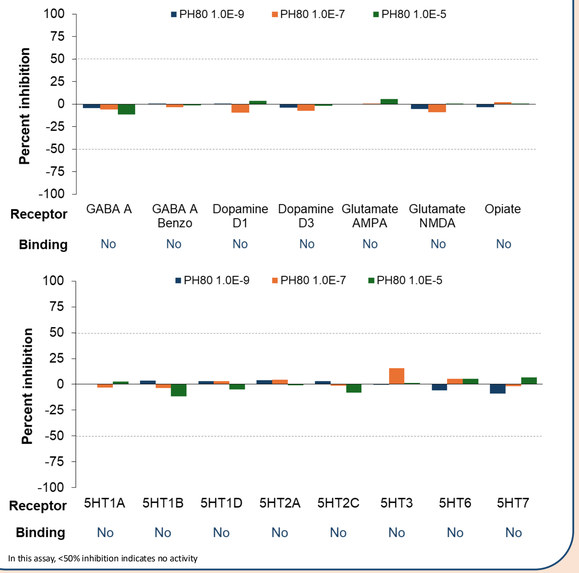


Figure 3. PH80 activity on neurotransmitter receptors



In this assay, <50% inhibition indicates no activity

Plasma levels and safety

- The women had a mean age of 27.0±5.6 years with a mean BMI of 22.8±2.2 kg/m^2
- PH80 was not detected (was below the limit of quantification) in any plasma sample at any time point (**Table 1**)
- Given that intranasally administered PH80 was not detected in the plasma, pharmacokinetic parameters could not be calculated
- No adverse events or serious adverse events were reported with PH80

Table. Mean plasma concentrations of PH80 over time

Subject	Time (hours)									
	0.00	0.84	0.25	0.50	1.00	2.00	4.00	8.00	12.0	24.0
Mean	0.00	NC	NC	NC	NC	NC	NC	NC	NC	NC
SD	0.00	NC	NC	NC	NC	NC	NC	NC	NC	NC
CV%	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

NC, concentration below the limit of quantification

Key Takeaways

- PH80 does not have binding activity on steroid hormone receptors, nor does it bind to neurotransmitter receptors, including receptors linked to abuse liability
- Intranasally administered PH80 was not detected systemically
- These in vivo and in vitro data in conjunction with early-phase efficacy and safety data[8] warrant further examination of PH80 for treating perimenopausal VMS in a phase 3 study

Conclusions

- The lack of in vitro binding affinity with steroid hormone receptors and in vivo agonist activity of PH80 support a non-hormonal mechanism of action
- PH80's lack of in vitro binding to abuse-related neurotransmitter receptors suggests that it is unlikely to have any abuse liability or withdrawal symptoms
- Undetected plasma levels and lack of adverse events with PH80 found here are consistent with its placebo-like safety profile reported from the phase 2a study[8]

References

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Disclosures

- LM and RAB are employees of and own stock or stock options in Vistagen Therapeutics, Inc (Vistagen). RH is a consultant to Vistagen.
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See also Poster #P-13 on the autonomic effects of PH80