Brain and peripheral tissue distribution of intranasal radio-labeled ituruvone (PH10) in laboratory rats

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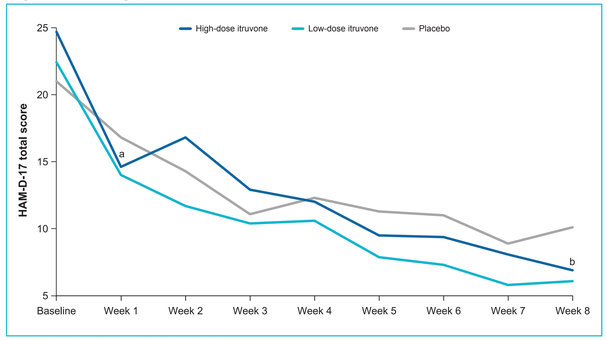
BACKGROUND

- Major depressive disorder (MDD) is a serious and often chronic disease with a lifetime prevalence estimate of 2% to 21% worldwide[1]
- Past meta-analyses of tricyclic, selective serotonin reuptake inhibitor, and serotonin norepinephrine reuptake inhibitor antidepressants indicate that only 38% to 49% of patients with moderate to severe MDD achieve remission[2,3]
  - Remission rates are likely even lower in the real-world treatment of patients outside of clinical trials[4]
  - Adverse events often negatively impact antidepressant adherence, further compromising treatment to remission[5]
- Ituruvone (PH10, pregn-4-en-20-yne-3-one) is an odorless and tasteless investigational synthetic neuroactive steroid formulated as a nasal spray[6]
- Ituruvone binds to and activates chemosensory neurons in the nasal epithelium that impact olfactory-amygdala neural circuits believed to increase limbic-hypothalamic sympathetic autonomic nervous system activity, increasing catecholamine release from the midbrain[6]
- In a randomized, double-blind, placebo-controlled phase 2A study in MDD (n=30),[6] high-dose ituruvone monotherapy (daily dose of 6.4 µg) provided a significant reduction in Hamilton Depression Rating Scale-17 (HAM-D-17) total scores vs placebo at week 1 (*P*=0.03) and at endpoint, week 8 (*P*=0.022; effect size: 0.95) (**Figure 1**)

Figure 1. Change from Baseline in HAM-D-17 Total Score[6]



[a]*P*=0.03 at week 1 and [b]*P*=0.022 at week 8 for the high-dose ituruvone group.

OBJECTIVE

- To assess the absorption, distribution, and excretion of radio-labeled [^{14}C]-ituruvone after single intranasal (IN) administration to rats

METHODS

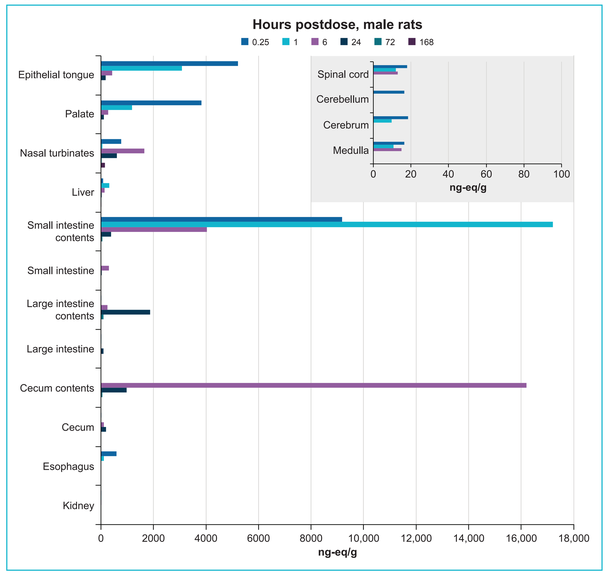
- After acclimation to study conditions, 6 male and 6 female Long Evans (LE) rats and 3 male and 3 female Sprague Dawley (SD) rats were selected via computer-generated random numbers based on body weight
  - Rats were 10 to 13 weeks old and weighed 217 to 312 g at selection
- Each rat was administered a single IN dose of [^{14}C]-ituruvone at a target dose of 92.9 µg/animal
- In LE rats, blood (approximately 1 mL) was collected from a jugular vein, transferred into K_2EDTA tubes, placed on wet ice until aliquoted for radioanalysis, and centrifuged to obtain plasma within 1 hour of collection
- After blood collection, 1 animal/sex/time point was sacrificed (isoflurane anesthesia) at 0.25, 1, 6, 24, 72, and 168 hours postdose and carcasses were prepared for quantitative whole-body autoradiography (QWBA)
  - 40 µm thickness whole-body sagittal sections were exposed to phosphor imaging screens, along with fortified blood standards for subsequent calibration, and scanned
  - Autoradiographic standard imaging data were sampled to create a calibrated standard curve; [^{14}C]-ituruvone tissue concentrations were interpolated from each standard curve as nanocuries/g and then converted to ng equivalents/g
- In SD rats, urine was collected at predose, 0 to 6 hours postdose, 6 to 24 hours postdose, and 24-hour intervals through 168 hours postdose; the weight of each sample was recorded
  - Feces were collected at predose and 24-hour intervals through 168 hours postdose; the weight of each sample was recorded. Feces were collected in plastic containers surrounded by dry ice; the weight of each sample was recorded
  - After each 24-hour excreta collection through 144 hours postdose, cages were rinsed with water, samples were collected, and weight was recorded
- Statistical analyses were descriptive, with calculations for mean and standard deviation

RESULTS

Absorption and Distribution of Radioactivity

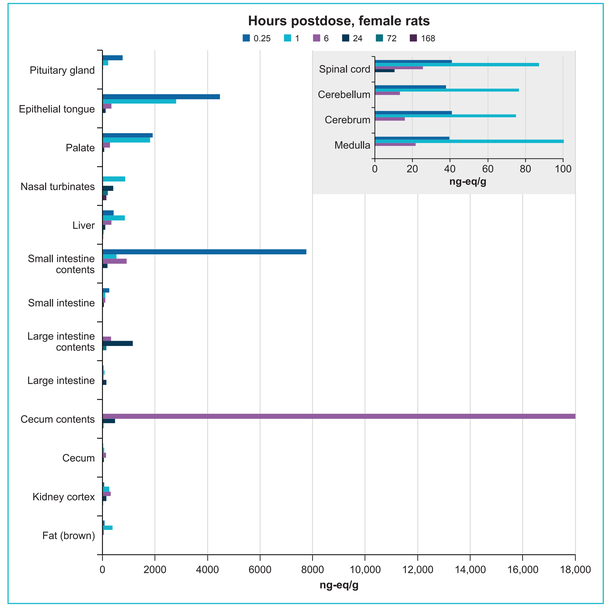
- After IN administration of [^{14}C]-ituruvone, radioactivity distributed to all tissues
  - In male LE rats, blood and plasma radioactivity concentrations peaked at 15 minutes at 12.3 and 21.2 ng-eq/[^{14}C]-ituruvone/g, respectively, declining 4.02 and 6.63 ng-eq/[^{14}C]-ituruvone/g by 6 hours postdose, respectively, and were below the limit of quantification (BLQ; defined as <9.54 ng-eq/[^{14}C]-ituruvone/g) thereafter through 168 hours postdose
  - In female LE rats, blood and plasma radioactivity concentrations peaked at 1 hour at 88.4 and 145 ng-eq/[^{14}C]-ituruvone/g, respectively, declining to 3.25 and 5.88 ng-eq/[^{14}C]-ituruvone/g by 24 hours postdose, respectively, and were BLQ thereafter through 168 hours postdose
  - Maximum radioactivity concentration (C_{max}) in most other tissues was achieved by 0.25 hours postdose in LE males (**Figure 2**) and by 1 hour postdose in LE females (**Figure 3**)
- Minimal levels of radioactivity were detected in the brain
  - In LE males, brain and spinal cord radioactivity levels were ≤18.5 ng-eq [^{14}C]-ituruvone/g from 15 minutes to 6 hours and were BLQ thereafter (**Figure 2 inset**)
  - In LE females, brain radioactivity levels were ≤102 at 1 hour and ≤21.5 ng-eq [^{14}C]-ituruvone/g at 6 hours and BLQ thereafter; in spinal cord, they were ≤87.0 ng-eq [^{14}C]-ituruvone/g through 24 hours and BLQ thereafter (**Figure 3 inset**)

Figure 2. Select Individual QWBA [^{14}C]-Ituruvone Radioactivity Concentrations (ng equivalents/g) in Male Long Evans Rats (92.9 µg/animal)



- The tissues exhibiting the highest maximum concentrations of radioactivity (>290 ng-eq/g) in LE males, excluding gastrointestinal (GI) contents, included the epithelial tongue, palate, nasal turbinates, esophagus, liver, and small intestine, with values of 5220, 3830, 1650, 591, 309, and 297 ng-eq/g, respectively

Figure 3. Select Individual QWBA [^{14}C]-Ituruvone Radioactivity Concentrations (ng equivalents/g) in Female Long Evans Rats (92.9 µg/animal)

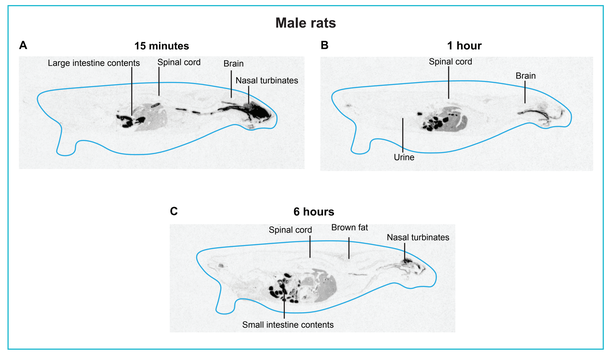


- The tissues exhibiting the highest C_{max} values of radioactivity (>300 ng-eq/g) in LE females, excluding GI contents, included the epithelial tongue, palate, liver, nasal turbinates, pituitary gland, fat (brown), and kidney cortex, with values of 4470, 1910, 847, 860, 775, 384, and 314 ng-eq/g, respectively

QWBA

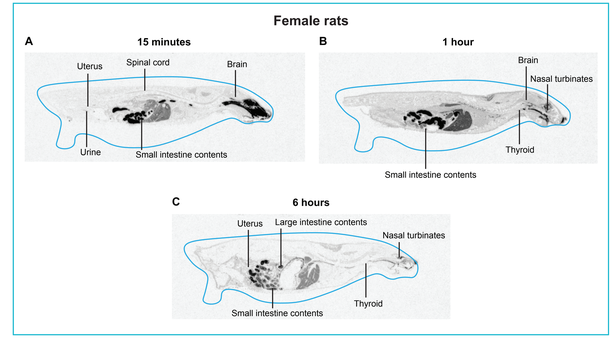
- QWBA images at 15 minutes, 1 hour, and 6 hours after IN administration of [^{14}C]-ituruvone in male and female LE rats are presented in **Figures 4 and 5**
  - The distributions of radioactivity in LE male and female rats were similar, and were largely confined to the nasal passages and digestive system
- In male LE rats, the highest calculated doses of absorbed radiation after IN administration of [^{14}C]-ituruvone were in the liver, small intestine, large intestine, stomach, and spinal cord (**Figure 4**)

Figure 4. QWBA of Male Rats After A) 15 Minutes, B) 1 Hour, and C) 6 Hours of [^{14}C]-Ituruvone Exposure



- In female LE rats, the highest calculated doses of absorbed radiation after IN administration of [^{14}C]-ituruvone were in the liver, kidney, small intestine, ovary, and spinal cord (**Figure 5**)

Figure 5. QWBA of Female Rats After A) 15 Minutes, B) 1 Hour, and C) 6 Hours of [^{14}C]-Ituruvone Exposure



Excretion and Mass Balance

- After IN administration of [^{14}C]-ituruvone to male SD rats, a mean of 69.1% and 1.73% of the dose was excreted in feces and urine, respectively
- After IN administration of [^{14}C]-ituruvone to female SD rats, a mean of 66.7% and 5.17% of the dose was excreted in feces and urine, respectively

CONCLUSIONS

- Overall, these data lend further support to the proposed mechanism of action whereby ituruvone binds to receptors in nasal chemosensory neurons, rather than neuronal receptors in the brain, minimizing potential blood-brain barrier penetration and systemic exposure
- Concentrations of radioactivity in GI tissues, liver, small intestine, large intestine, and stomach are consistent with the demonstrated fecal excretion
- Combined with previous preclinical findings,[6] the evidence suggests that ituruvone has the potential to achieve antidepressant effects while avoiding benzodiazepine- or antidepressant-associated side effects, such as weight gain, sexual dysfunction, and sedation, or psychotomimetic side effects and safety concerns potentially associated with intravenous or IN ketamine therapy
- The efficacy, safety, and tolerability of ituruvone monotherapy in the treatment of MDD will be further evaluated in a planned phase 2B study

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Acknowledgments

This study was sponsored by Vistagen Therapeutics, Inc., South San Francisco, CA, USA. Medical writing and editorial support for this poster were provided by John H. Simmons, MD, of Peloton Advantage, LLC, an OPEN Health company, and funded by Vistagen Therapeutics.

Disclosures

Jo Cato, Ross A. Baker, and Louis Monti: Employees and owners of stock or stock options in Vistagen Therapeutics, Inc.