Vistagen

Poster # 186

Metabolism of the Anxiolytic Neuroactive Steroid Fasedienol (PH94B) by Human Nasal Epithelial Cells

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ABSTRACT

Background
Many xenobiotic-metabolizing enzymes, such as the cytochrome P450 (CYP) enzymes, are expressed in the mammalian olfactory mucosa. These enzymes catalyze the biotransformation of exogenous compounds, including steroidal molecules active on olfactory receptors, to facilitate their elimination and avoid receptor saturation.
Fasedienol (PH94B or 3β-androsta-4,16-dien-3-ol) is a synthetic neuroactive steroid in development for treatment of anxiety disorders. It induces its rapid pharmacological and behavioral effects by- binding to receptors in the membrane of peripheral nasal chemosensory neurons. These, in turn, activate subsets of olfactory bulb neurons that project directly to the limbic amygdala which regulates fear and anxiety circuits. Intranasal spray administration of fasedienol to human volunteers significantly increases the local electrogram response recorded from the surface chemosensory mucosa in a dose dependent manner (ED_{50}= 1.0 microgram). Additionally, it rapidly lowers heart rate, respiratory rate, and electrodermal activity, consistent with sympatholytic activity and an anxiolytic effect.
Radiolabeled ^{14}C-fasedienol (10 µCu) administered intranasally to laboratory rats is recovered in the nasal passages and gut but not in the CNS and, in human PK studies fasedienol administered intranasally, could not be detected in plasma samples collected at 1 hour intervals during 24 consecutive hours after dosing. The objective of the present study was to investigate whether fasedienol is metabolized by enzymes in epithelial cells recovered from the human nasal passages to better understand the mechanism of clearance of fasedienol.
Methods
In this IRB-approved study (E&I IRB #2-IRB0007807), cells were extracted from the epithelial lining of the dorsal nasal septum of each nasal passage in healthy adult human volunteers. Extractions were dissociated for 5 minutes and then washed, pelleted, and resuspended in DMEM containing antibiotics. Samples were pooled, centrifuged, resuspended in 2 µM fasedienol in DMEM/0.1% DMSO, and immediately divided into four equal aliquots. Other than the zero timepoint, samples were incubated at 36°C in 5% CO_2 to facilitate the metabolism until collection. At four timepoints, reactions were stopped by addition of acetonitrile, centrifuged, and supernatants were frozen. Negative controls containing fasedienol without cells were similarly collected and frozen. The time course of depletion of fasedienol was measured using LC-MS (Oakland Analytics, Berkeley).
Results
Our preliminary results from incubation of fasedienol with human nasal epithelial cells show progressive depletion from the zero-time point of addition of the neuroactive steroid. Depletion reached 50-60% at 1-hour incubation and was 90-100% at 2 hours of incubation. No depletion of fasedienol was observed in no-cell controls.
Conclusions
We report that enzymes in nasal epithelial cells, including chemosensory cells, can metabolize fasedienol. This supports both its local nasal clearance and its absence from systemic circulation after intranasal administration.
Cytochrome P450 CYP enzymes in the human nasal mucosa play a significant role as biotransformation enzymes in the metabolism of airborne chemicals, including therapeutic agents. Our findings suggest that fasedienol may be metabolized by multiple CYP enzymes, including CYP2A6 and CYP1A1. This provides the basis for further investigation of the metabolic capacity of specific human nasal CYP enzymes in the catabolism of fasedienol and other neuroactive steroids.

BACKGROUND

The xenobiotic metabolic activity in the nasal epithelium has been investigated in several species including humans. The Phase I, cytochrome P-450 enzymes have been studied extensively for their toxicological significance since these enzymes (Table 1) metabolize drugs to facilitate their clearance from the nasal mucosa. The cytochrome P-450 activity in the nasal chemosensory epithelium is higher even than in the liver, mainly because of a three- to four-fold higher NADPH-cytochrome P-450 reductase content.
Fasedienol (PH94B or 3β-androsta-4,16-dien-3-ol) is a synthetic neuroactive steroid in development for treatment of anxiety disorders. It induces its rapid pharmacological and behavioral effects engaging receptors in the membrane of peripheral nasal chemosensory cells, which activate subsets of olfactory bulb neurons that project directly to the limbic amygdala.
Intranasal spray administration of fasedienol to human volunteers produces a local electrogram response in the surface chemosensory mucosa (ED_{50}= 1.0 microgram) followed by decreased heart rate, respiratory rate, and frequency of skin conductance events, consistent with sympatholytic activity and an anxiolytic effect (Monti et al, 2023 submitted to Human Psychopharmacology; Monti and Liebowitz, 2020 CNS Spectrums; Liebowitz et al 2016 Anxiety and Depression; Liebowitz et al 2014, American J. of Psychiatry). Radiolabeled ^{14}C-fasedienol (10 µCu) administered intranasally to laboratory animals is recovered in the nasal passages and gut but not in the CNS, and in human PK studies fasedienol administered intranasally could not be detected in plasma samples collected at 1-hour intervals during 24 consecutive hours after dosing.
The objective of the present study was to investigate whether fasedienol is metabolized by enzymes in epithelial cells from the human nasal passages, to understand the mechanism of clearance of fasedienol.

Table 1. Phase I and Phase II xenobiotic-metabolizing enzymes expressed in the human nasal mucosa.

PHASE I ENZYMES		PHASE II ENZYMES
CYP1A1, CYP1A2	CYP2F1	ALDH6, ALDH7
CYP1B1	CYP2J2	FMO1
CYP2A6, CYP2A13	CYP3A	GSTA, GSTP1
CYP2B6	CYP4B1	UGT2A1
CYP2C	NADPH-cytochrome P450 reductase	FMO1
CYP2E1		ALDH6, ALDH7
		GSTA, GSTP1
		Microsomal epoxide hydrolase
		Microsomal epoxide hydrolase

METHODS

Human Nasal Cell Studies
In order to test whether fasedienol is cleared by cells in the nose, human nasal cells were first extracted from the epithelial lining of the dorsal nasal septum of each nasal passage from 7 healthy adult volunteers. Extractions were dissociated for 5 minutes in DPBS without calcium or magnesium and then washed, pelleted, and resuspended in DMEM containing gentamicin, penicillin, and streptomycin. Samples were pooled, centrifuged, resuspended in 2 µM Fasedienol in DMEM/0.1% DMSO, and immediately divided into four equal aliquots, one for each time point. Other than the zero timepoint, samples were incubated at 36°C in 5% CO_2 to facilitate the metabolism until collection. At 0, 30, 60, and 120 minutes, reactions were stopped by addition of acetonitrile and then they were centrifuged and supernatants were frozen. Negative controls containing Fasedienol without cells were similarly collected and frozen. Additionally, 10 µM methoxsalen, a known CYP2A6 inhibitor, was added to some samples starting at time zero. The time course of depletion of Fasedienol was measured using LC-MS (Oakland Analytics, Berkeley), and the percent Fasedienol remaining at each time point relative to the zero time point was plotted. This study was IRB-approved (E &I IRB #2-IRB0007807).
Bactosome Studies
Bactosomes expressing specific, single CYP P450 enzymes (BioIVT/Xenotech) were used in reaction phenotyping assays to begin to determine which enzymes may be responsible for nasal clearance of Fasedienol. Reactions containing Tris buffer, NADPH, 10 µM Fasedienol, and 20 pmole bactosomes were incubated in a 37°C water bath to facilitate metabolization. At time zero and every 10 minutes thereafter for an hour, as well as an additional 5-minute time point, 100 µl samples were removed from the reaction and immediately stopped by addition of acetonitrile. The samples were then centrifuged to remove the bactosomes, and supernatants were frozen prior to LC-MS analysis. The percent Fasedienol remaining at each time point relative to the zero time point was plotted.

RESULTS

Volunteer	Sex	Age	Ethnicity	COVID
1	F	39	Asian	Negative
2	M	41	Caucasian	Negative
3	M	59	Caucasian	Negative
4	M	61	Asian	Negative
5	M	47	Caucasian	Negative
6	M	44	African American	Negative
7	F	54	Asian	Negative

Table 2. Demographics of the clinically healthy volunteers that participated in the study.

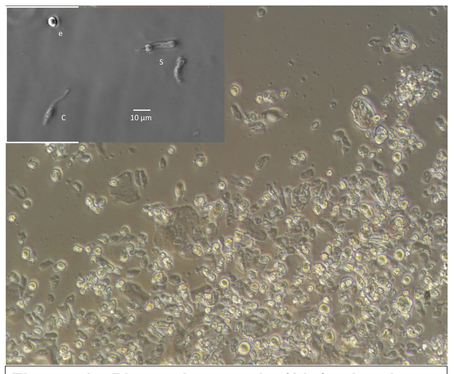

Figure 1. Photomicrograph (20x) showing a representative sample of the epithelial cells extracted from the nasal mucosa of human volunteer. The upper left inset shows different cell types producing xenobiotic enzymes; (C) chemo-sensory cell, (S) sustentacular cell, (e) erythrocyte.

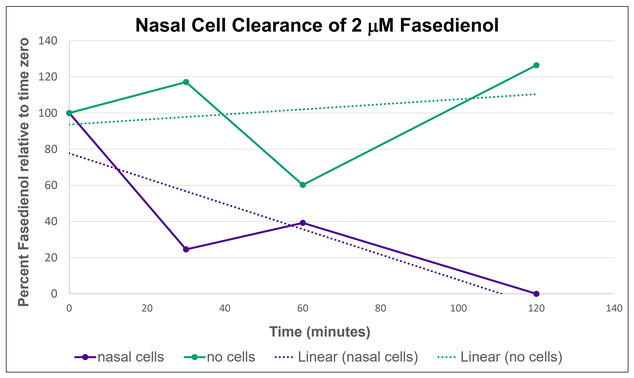

Figure 2. 2 µM Fasedienol is depleted within two hours in the presence of human nasal epithelial cells. To test whether nasal cells can clear Fasedienol, cells pooled from the dorsal nasal septum of 7 healthy adult volunteers were incubated at 36°C with 2µM Fasedienol to facilitate metabolism. At time 0, 30, 60, and 120 minutes, samples were collected and metabolism was immediately stopped by addition of acetonitrile. LC-MS analysis was then used to determine the percent Fasedienol remaining at each time point. As shown above, human nasal cells were able to completely deplete Fasedienol under these conditions within two hours. Negative controls without cells showed no Fasedienol depletion. As this was a pilot experiment from one set of pooled cells, regression trendlines were added to compensate for process variability.

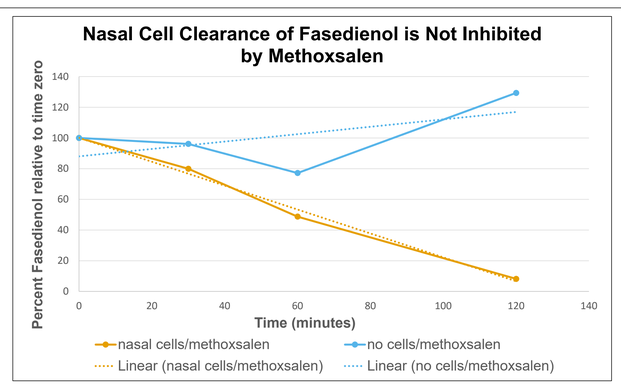

Figure 3. Methoxsalen, a known CYP2A6 inhibitor, does not inhibit nasal cell clearance of 2 µM fasedienol. Since we have some evidence that CYP2A6 may be able to metabolize fasedienol, we repeated the same experiment as in Figure 1 with the same cell pool but with the addition of 10 µM methoxsalen to block CYP2A6 activity. Methoxsalen did not inhibit nasal cell metabolization of fasedienol, as fasedienol was depleted by 90% within 2 hours. This is suggestive that fasedienol may be metabolized by multiple enzymes.

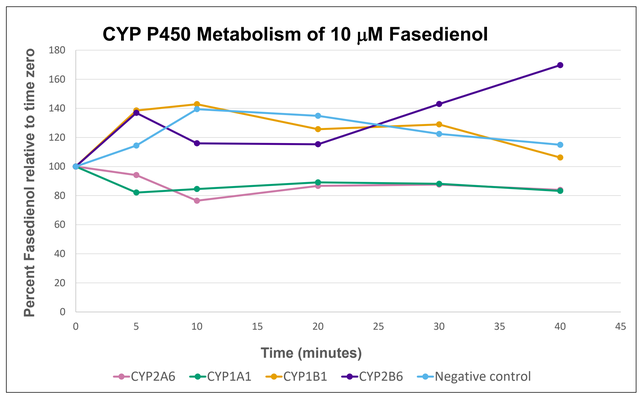

Figure 4. Reaction phenotyping suggests that Fasedienol is metabolized by more than one CYP P450 enzyme, including CYP2A6 and CYP1A1. To test whether CYP P450 enzymes may be able to metabolize Fasedienol, 10 µM Fasedienol was incubated with bactosomes that express single CYPs, and samples were collected throughout a 40 minute time course. In the presence of either CYP2A6 or CYP1A1, about 20% of Fasedienol was depleted, indicating that these CYPs can metabolize Fasedienol. On the contrary, CYP1B1 and CYP2B6 did not show evidence of metabolism of Fasedienol. These experiments will be repeated with a lower concentration of Fasedienol and an expanded panel of CYP enzymes.

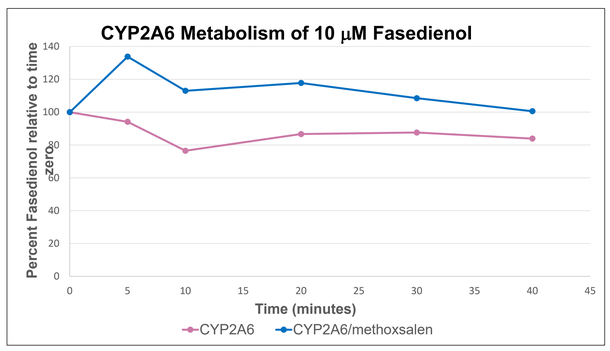

Figure 5. Metabolism of Fasedienol by CYP2A6-expressing bactosomes is inhibited by methoxsalen. CYP2A6 metabolism of Fasedienol was blocked in the presence of 10 µM methosalen, a known CYP2A6 inhibitor, confirming that CYP2A6 was responsible for the 20% depletion of Fasedienol seen in the absence of the inhibitor.

SUMMARY and CONCLUSIONS

- Fasedienol is a synthetic neuroactive steroid in development for treatment of anxiety disorders that is administered as a nasal spray.
- In these pilot studies, we show that human nasal epithelial cells are capable of completely clearing Fasedienol.
- Reaction phenotyping studies revealed that Fasedienol can be metabolized by CYP2A6 and CYP1A1.
- Many CYP P450 enzymes are expressed in the human nasal mucosa and play a role in metabolization of airborne chemicals. Our preliminary pilot studies suggest that Fasedienol is cleared from the nasal passages by multiple CYP P450s.
- This has important implications for understanding the mechanism of clearance of Fasedienol, as fasedienol is a fast-acting nasal spray that has not been detected in either blood plasma or the CNS.
- This work will be further expanded to investigate a larger panel of CYP P450 enzymes for catabolism of fasedienol and other neuroactive steroids.