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Lipidomics Evaluation of the Impact of Fentanyl Treatments on Neural Stem Cells

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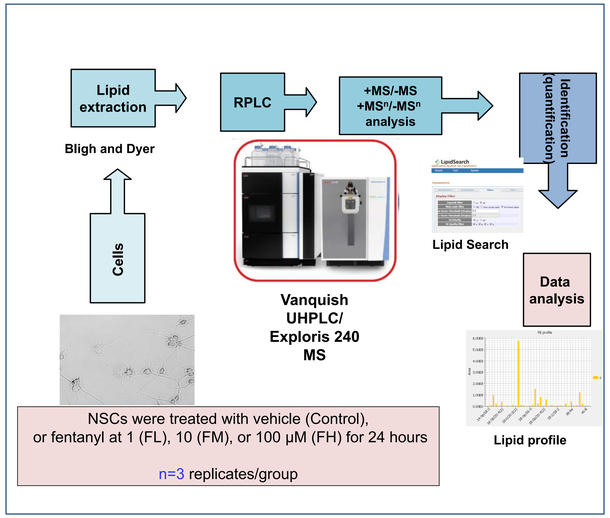
Abstract

Fentanyl is a potent and short-acting opioid medication that is often given to pediatric patients during surgery to relieve pain and as an adjunct to anesthesia. Because it is difficult to assess the adverse effects on human infants and children, the utilization of human-derived neural stem cell models, might be a good tool to evaluate the vulnerability of the developing nervous system to fentanyl exposure. Since neural cells contain a wide variety of lipid classes and lipid species, lipidomics analysis using ultra-high-performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (HRMS) was conducted to investigate the impacts of different doses of fentanyl on neural stem cells (NSCs) and neural cells differentiated/derived from NSCs. The neural cells were treated with vehicle (control), or fentanyl at 1, 10, or 100 μM for 24 hours. Although 24-hour fentanyl exposure of NSCs resulted in a dose-related increase (not significant) in the release of lactate dehydrogenase into the cell culture medium (indicator of cell death/damage), no significant reduction in the mitochondrial health marker (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) was observed vs control. Lipidomics analysis detected 1830 lipid species from 20 lipid classes. Consistent with MTT data, palmitoylcarnitine (indicating mitochondrial functioning) did not significantly accumulate after fentanyl exposure. Among the 20 lipid classes detected, the total abundance of cholesterol ester and sphingosine classes significantly decreased while ceramide and hexosylceramide classes significantly increased (>2 folds increases) in the high-dose group vs the control. This preliminary data indicated that the ceramide pathway might be disturbed by fentanyl treatments, which might provide the underlying mechanisms of fentanyl-induced neurotoxicity on developing neural cells.

Experimental Design



Materials and Methods

Lipid Extraction
LC/MS grade water (1 mL) was added to the Eppendorf tube containing neural stem cells (~1M counts), followed by vortexing for 40s.

Lipid extraction was achieved by using a modified version of the Bligh and Dyer extraction protocol, whereby 0.9 mL of H_2O, 2 mL methanol, and 0.9 mL dichloromethane (DCM) was added and mixed gently, but thoroughly for 5 s. Aliquots of stable internal standard mixtures-SPLASH® Lipidomix® Mass Spec Standard. Following two rounds of extraction, the bottom layers were combined and dried under nitrogen flow and reconstituted in 1 mL of ethanol and centrifuged just prior to analysis.

Un-targeted Lipidomics
The extracted lipids were separated by a Thermo Vanquish Ultimate 3000 UPLC (Thermo Scientific, Milford, MA, USA) equipped with a Thermo Accucore C30 column . The metabolomics data was collected with a Thermo Orbitrap Exploris 240 mass spectrometer (Thermo Scientific, Waltham, MA) operated in positive and negative ionization electrospray modes. Data were acquired in full-scan mode (*m/z* 70 to 1000) at a resolution of 120,000 for all samples.

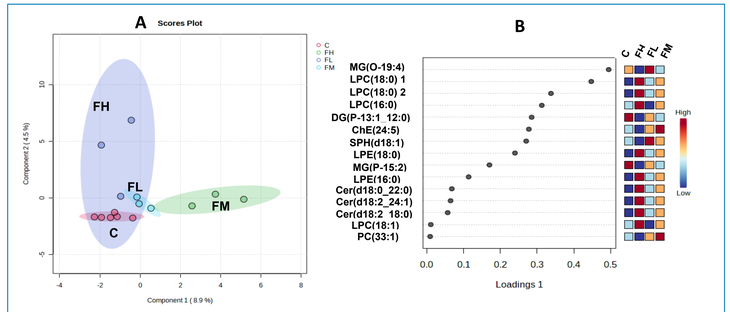


Figure 1. Principal component analysis (PCA) score plots (**A**), the top 15 variable importance in projection (VIP) plot (**B**) of lipidomics data from NSCs of control (Ctr), treated with 1 (FL), 10 (FM) or 100 μM (FH) fentanyl for 24 hours..

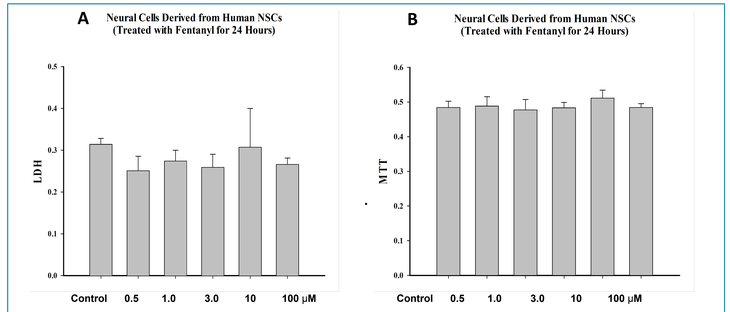


Figure 2. Markers of cell death/damage, LDH, and mitochondrial health, MTT, were used/monitored to determine the dose response effects of fentanyl on NSC viability. No significant changes were detected in LDH release and MTT uptake when NSCs were exposed (24 hours) to fentanyl at 0.5, 1, 3, 10 or 100 μM. Each treatment condition was assessed at least in triplicate, and experiments were repeated three times independently. .

Results

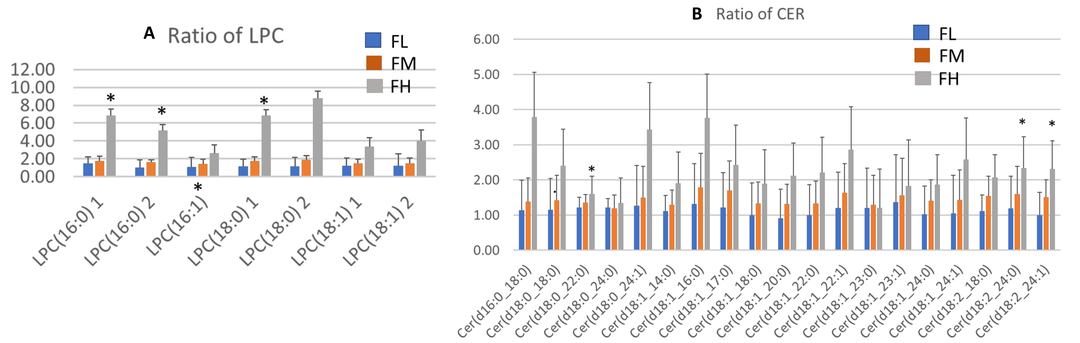


Figure 3. Bar graphs of the ratios of LPC and CER (treated/Ctr). Ratios of LPC and CER had a dose-dependent increase. * indicated ratios are significant changes vs control.

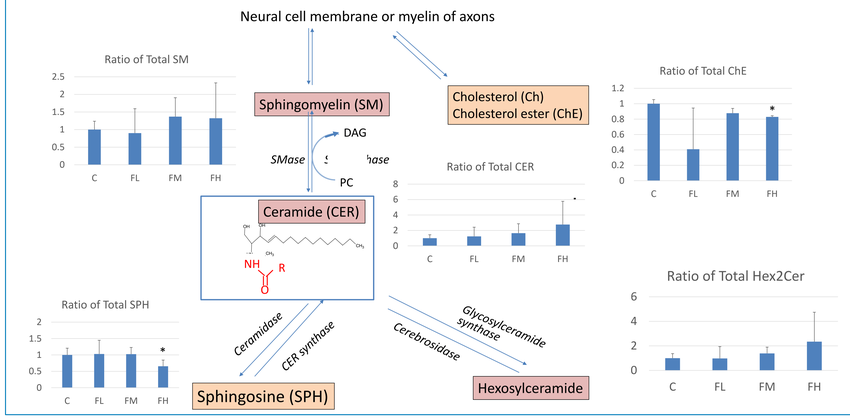


Figure 4. Summary illustration of the identified lipids involved in the ceramide pathways after 100 uM fentanyl treatment for 24 hours. The scheme of the pathway is simplified and shows only relevant lipids. The bar graph show the ratio of total lipid class level (Sum(treated)/Sum(Ctr)). The green (down-regulation) and pink (up-regulation) represent the changes in the total lipid class levels following fentanyl treatment. * indicated ratios are significant changes vs Ctr.

Conclusion

- Untargeted lipidomics analysis was conducted to assess the vulnerability of neural stem cells (NSC) exposed to fentanyl, a potent opioid analgesic used for medical pain management. To examine the impacts of different dosages of fentanyl on neural cells, particularly focusing on lipidome changes.
- Lipidomics data show that fentanyl exposure might cause ceramide pathway disturbance, potentially due to the adverse effects of fentanyl treatments.
- The study's preliminary data provided insights into the underlying mechanisms of fentanyl-induced neurotoxicity on developing neural cells.