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Metabolomics of Opiate-Induced Changes in Murine Brain by GC/Q-TOF

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Overview

Untargeted metabolomics was employed to characterize a murine model of opiate dependence. Metabolomics profiles of morphine-sensitive and resistant murine strains were evaluated with and without exposure to morphine. The study was performed using accurate mass high resolution GC/Q-TOF that took advantage of both EI and CI in full acquisition as well as MS/MS modes for compound confirmation and identification. Metabolomics changes revealed in this study offer an exciting new analytical approach towards a better understanding for the mechanisms of opiate dependence.

Introduction

A large body of evidence indicates that chronic opioid therapy often results in opioid dependence and addiction. Understanding the mechanisms of opiate addiction is important for developing novel therapeutic strategies. Metabolomics is a powerful approach for investigating the biochemistry of morphine addiction, since it provides the most direct information about a system's physiological state. There is currently very little information regarding metabolomics changes that mediate neurobehavioral responses. Similar to the different levels of susceptibility to narcotic addiction in humans, various murine strains also differ markedly in their sensitivity to these drugs. Therefore, an untargeted metabolomics approach was developed with the goal of characterizing two inbred murine strains displaying drastically different levels of physical dependency to morphine, the main natural opiate.

Methods

Sample Preparation

C57BL/6 and 129Sv1 murine strains were administered subcutaneously either with morphine for four consecutive days, or with saline control over the same period of time. Brainstem tissue was collected from 8 week old male C57BL/6 (morphine-sensitive) and 129Sv1 (morphine-resistant) mice in 7 - 8 biological replicates. Metabolites were extracted by the Folch method (Folch et al., *J Biol Chem*, 1957, 226, 497). The aqueous fraction was collected, dried under vacuum, and consecutively derivatized by methoximation using a saturated solution of hydroxylamine HCl in pyridine and by silylation with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and 1 % trimethylchlorosilane (TMCS), respectively.

Methods

Analytical Conditions

This study was performed using an Agilent 7890 GC coupled to an Agilent 7200 series Quadrupole-Time-of-Flight (Figure 1). GC and MS conditions are described in Table 1.

Figure 1. 7200 series GC/Q-TOF system.

GC and MS Conditions:	
Column	DB-5 MS UI, 30 meter, 0.25 mm ID, 0.25 µm film
Injection volume	1 µL
Split ratio	10:1
Split/Splitless inlet temperature	250 °C
Oven temperature program	60 °C for 1 min 10 °C/min to 325 °C, 3.5 min hold
Carrier gas	Helium at 1 mL/min constant flow
Transfer line temperature	290 °C
Ionization mode	EI (MS1 and MS/MS), positive CI (20 % methane flow)
Source temperature	230°C
Quadrupole temperature	150°C
Mass range	50 to 600 m/z
Spectral acquisition rate	5 Hz, collecting both in centroid and profile modes

Table 1. GC-MS conditions used in the study.

Data Processing

The data were processed by chromatographic peak deconvolution using the Unknowns Analysis tool of MassHunter Quantitative Analysis software followed by compound identification by comparison with the Agilent-Fiehn GC/MS Metabolomics Retention Time Locked (RTL) Library. Molecular Structure Correlator (MSC) software was used to further validate the structures of tentatively identified compounds. Statistical evaluation of the data was performed by Mass Profiler Professional (MPP), a multivariate statistical analysis package.

Results and Discussion

cholesterol and the neurotransmitter N-acetylaspartylglutamic acid among others.

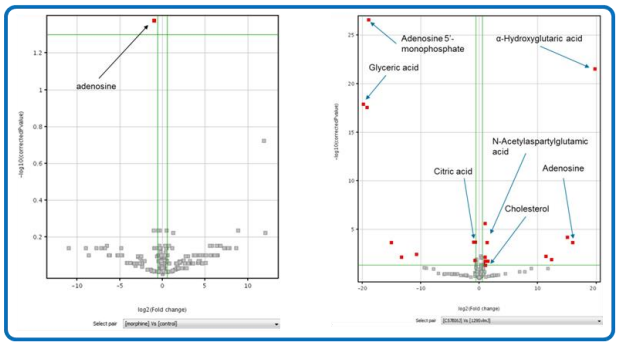

Figure 6. Volcano plots of log of fold change *vs.* log of p-Value (probability) for morphine-treated *vs.* control morphine-sensitive mice (left), as well as for morphine-sensitive (C57BL/6) *vs.* morphine-resistant (129Sv1) mice (right).

Identification of the Empirical Formulas of the Unknowns and Validation of the Tentative Hits

MS/MS was used to help identification of the molecular ion by finding contaminating ions in a peak of interest eluting at 10.34 minutes. The two most abundant ions in the unknown of interest (m/z 72.0808 and m/z 129.1022) are clearly not derived from ion m/z 228.0665, possibly making the latter a contaminant (Figure 7). This hypothesis was confirmed by tracking the changes in the abundance of these ions when comparing morphine-treated *vs.* control conditions.

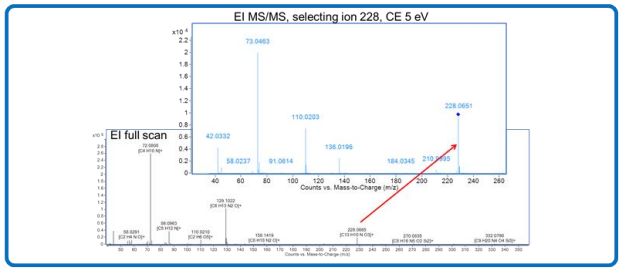

Figure 7. EI MS (lower) and MS/MS (upper) spectra of the unknown peak.

Further, methane PCI spectral data on the unknown confirmed that the molecular ion is m/z 158.1419 (Figure 8). After performing Molecular Formula Generation (MFG) with Fragment Formula Annotation, the spectrum shows typical PCI methane adducts of m/z 158.1419: 159.1496 (M+H)+, 187.1797 (M+C2H5)+, and 199.1801 (M+C3H5)+.

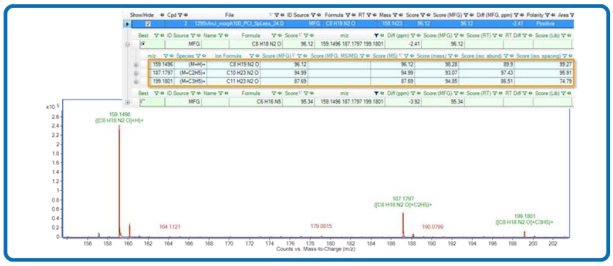

Figure 8. PCI adducts of the unknown.

Validation of the structure of a tentative hit (α-hydroxyglutaric acid), accumulated in morphine-sensitive strain C57BL/6 was performed using Molecular Structure Correlator (MSC, Figure 9). α-Hydroxyglutaric acid had a top compatibility score of 92.88. Although this type of confirmation is not completely unambiguous, it provides additional validation for a tentatively identified compound.

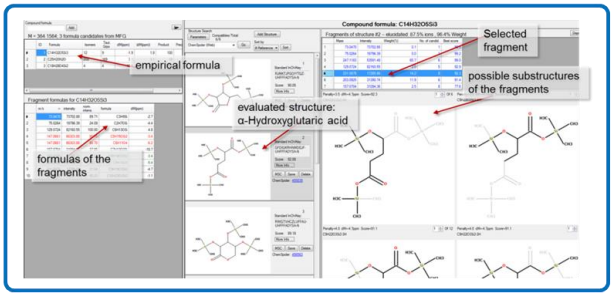

Figure 9. Structure validation results for the compound tentatively identified as α-hydroxyglutaric acid using MSC. ChemSpider database was searched to find all possible structural isomers.

Conclusions

- Untargeted metabolomics of the murine model of opiate dependency has been performed using a GC/Q-TOF.
- Accurate mass information, high sensitivity in full spectrum mode, both EI and CI modes as well as MS/MS capability enabled the identification and confirmation of about 70 metabolites.
- Differences in metabolomics profiles observed in this study could be correlated to phenotypic difference between morphine-dependent and morphine-resistant male mouse strains.

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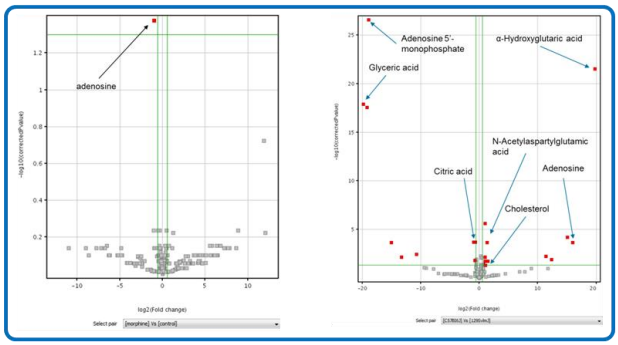

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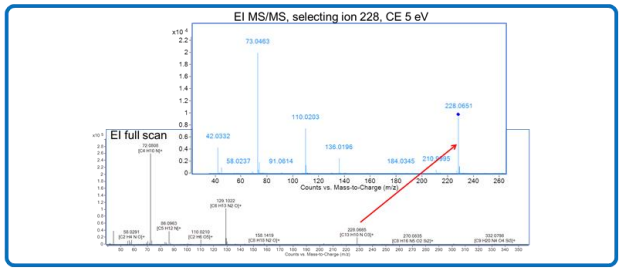

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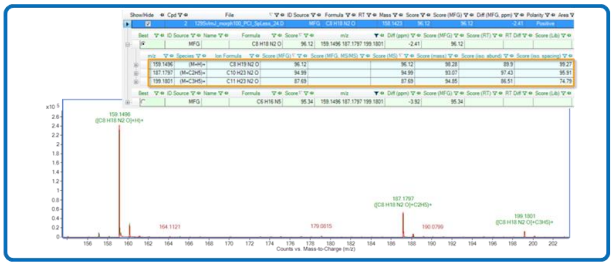

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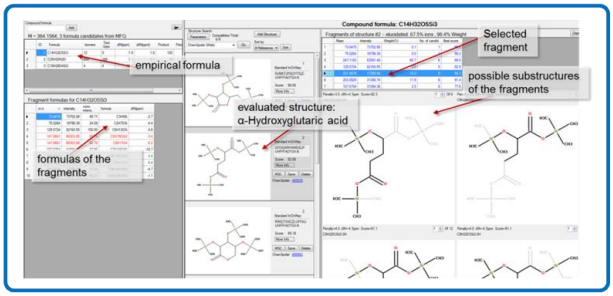

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