Metabolic Changes in Lung Tissue of Tuberculosis-Infected Mice Using GC/Q-TOF with Low Energy EI

Application Brief

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Introduction

The global burden of tuberculosis (TB) is vast, with an estimated 9.6 million new TB cases and 1.5 million deaths due to the disease in 2014 alone [1]. Using metabolomics, TB biomarkers can be identified to make progress in our understanding of the disease. This study used a mouse model of *Mycobacterium tuberculosis* (Mtb) infection to determine the metabolic profile of uninfected and infected lung tissues.

To identify new pathophysiological pathways involved in infection as well as biomarkers of TB, an untargeted metabolomics study was performed using uninfected and infected lung tissue at 9 weeks following infection. After initial compound annotation, low-energy EI data were used to confirm the molecular ions and identify molecular formulas of putatively identified compounds and unknowns, respectively.

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Experimental

Mice were infected with 5 × 10⁴ CFU of *Mycobacterium tuberculosis* (Mtb) H37Rv through the intratracheal route. The dried extracts of lung tissue were derivatized by O-methoximation followed by trimethylsilylation. GC/MS analysis was performed using an Agilent 7890B GC system coupled to a novel high-resolution Agilent 7250 GC/Q-TOF equipped with an EI source, allowing low-energy ionization (Figure 1). In addition to the new low-energy EI source, the 7250 system is capable of high resolving power (25,000 at *m/z* 272), improved mass accuracy, and a wide dynamic range. Table 1 shows the instrument parameters.

A retention time locked (RTL) Fiehn method was used to facilitate compound identification when using Fiehn.L RI library for initial compound identification. In addition, the National Institute of Standards and Technology Library (NIST.L) as well as an accurate mass Metabolomics PCDL were used to identify additional hits. Feature detection was performed using SureMass signal processing in Agilent MassHunter Unknowns Analysis B.08.00. Statistical analysis was performed in Agilent Mass Profiler Professional (MPP) version 13.0. Pathway Architect, an extenstion tool for MPP, was used to identify biochemical pathways associated with TB infection.

Figure 1.   Agilent 7250 GC/Q-TOF.

Table 1.   Agilent 7250 GC/Q-TOF Conditions

Parameter	Value
Column	Agilent DB-5MS, 30 m × 0.25 mm, 0.25 µm, DuraGuard, 10 m
Injection volume	1 µL
Split ratio	10:1
Split/Splitless inlet temperature	250 °C
Oven temperature program	60 °C for 1 minute 10 °C/min to 325 °C 9.5 minutes hold
Carrier gas	Helium at 1 mL/min constant flow
Transfer line temperature	290 °C
Ionization mode	Standard EI at 70 eV Low electron energy EI at 17 eV, 15 eV, and 12 eV
Source temperature	200 °C
Quadrupole temperature	150 °C
Mass range	50 to 950 *m/z*
Spectral acquisition rate	5 Hz

Results and Discussion

Experimental setup and feature detection

To identify new pathophysiological pathways involved in infection as well as biomarkers of TB, an untargeted metabolomics study was performed using uninfected and infected lung tissue extracts at 9 weeks following infection.

Following feature detection and a library search performed in Unknowns Analysis (Figure 2), the results were exported as .CEF files for further processing in MPP.

Differential analysis

In MPP, principal component analysis (PCA) was used to evaluate clustering of the data. Distinct clusters that represent clear separation between the uninfected control and infected tissues were formed (Figure 3).
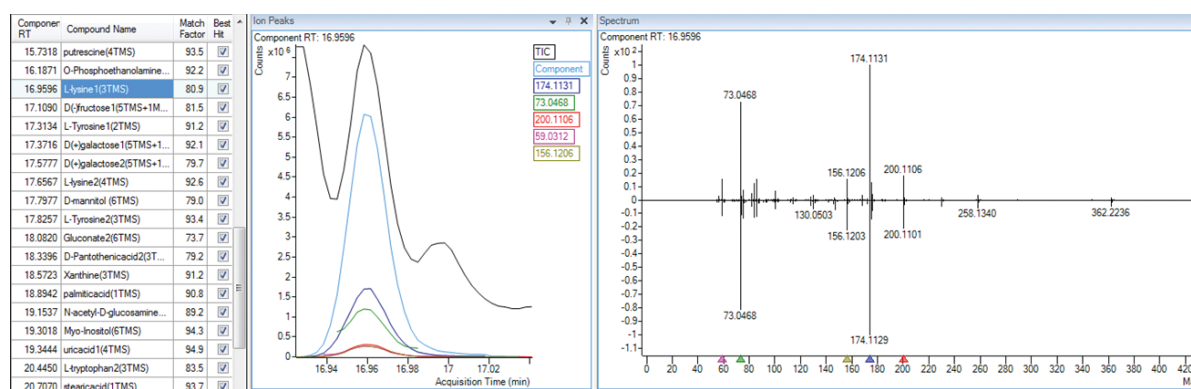

Figure 2.   Feature detection and library search performed in Agilent MassHunter Unknowns Analysis (using PCDL as an example).
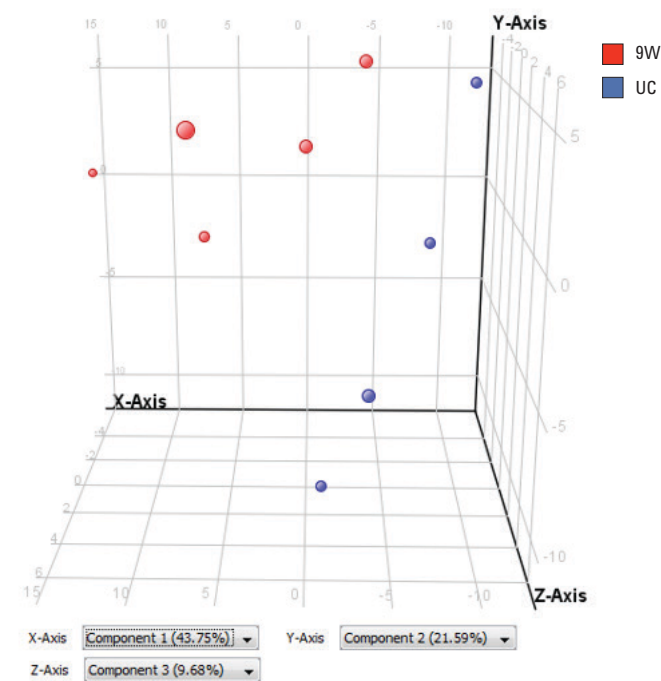

Figure 3.   PCA plot. Distinct clusters from uninfected control (UC, blue circles) and 9 weeks following the infection (9W, red circles) lung tissues were observed.

Differential analysis

Significant changes in the metabolome of lung tissue between infected and uninfected mice were further evaluated in MPP using fold change analysis (Figure 4) as well as a heatmap (Figure 5). Alteration in profiles of many metabolites, in particular amino acids and nucleobases, were observed.

In addition, a change in the profiles of itaconic acid and kynurenine, have also been detected.

Pathway Architect was also used to identify the biochemical pathways potentially associated with TB infection. Pathways of purine and pyrimidine metabolism as well as NAD biosynthesis II were among the most significant, Figure 6 shows one of the examples.
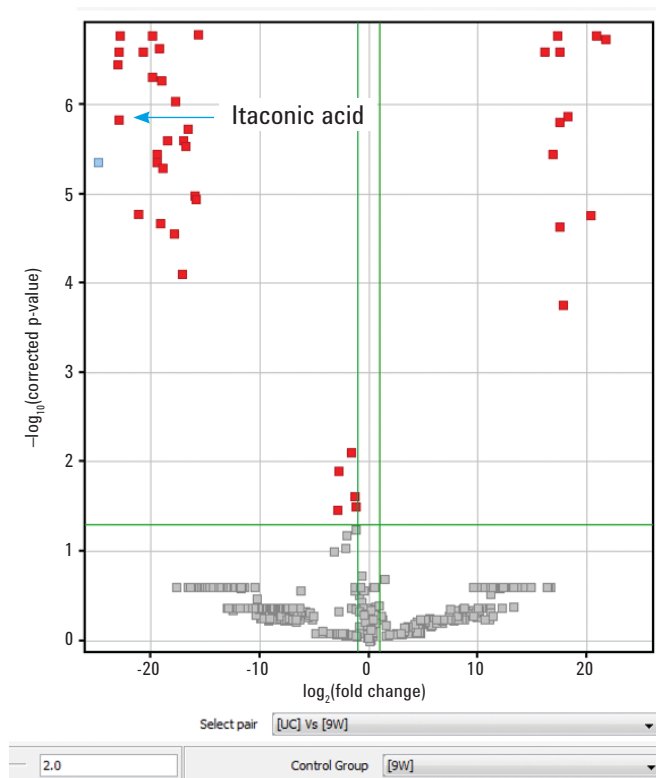

Figure 4.   Volcano plot visualizing of log of fold change versus log of p-Value for uninfected lung tissue versus 9 weeks following TB infection.
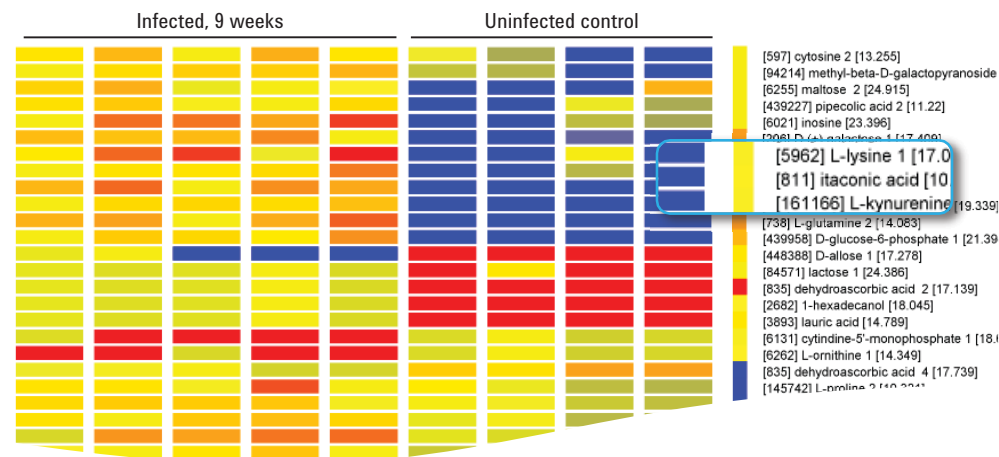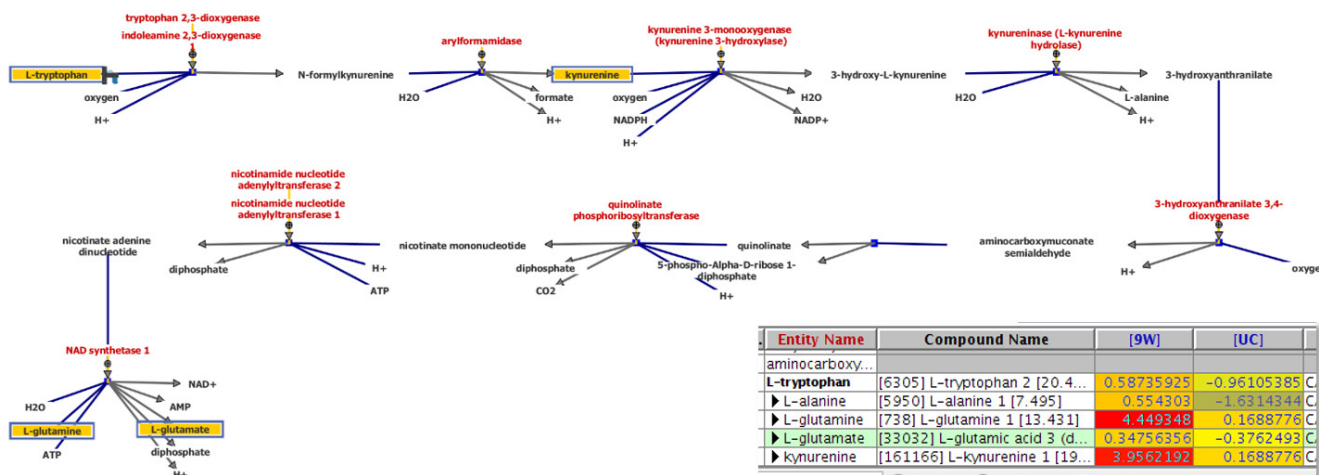

Figure 5.   Heatmap highlighting differentially regulated metabolites between uninfected and infected lung tissues.

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Figure 6.   Example of Pathway Analysis results: NAD Biosynthesis II.

Entity Name	Compound Name	[9W]	[UC]	
aminocarboxy...				
L–tryptophan	[6305] L–tryptophan 2 [20.4...	0.58735925	-0.96105385	C.
▶ L–alanine	[5950] L–alanine 1 [7.495]	0.554303	-1.6314344	C.
▶ L–glutamine	[738] L–glutamine 1 [13.431]	4.449348	0.1688776	C.
▶ L–glutamate	[33032] L–glutamic acid 3 (d...	0.34756356	-0.3762493	C.
▶ kynurenine	[161166] L–kynurenine 1 [19...	3.9562192	0.1688776	C.

Unknowns identification and confirmation of tentative hits

After initial compound annotation and differential analysis in MPP, low-energy EI spectra were used to confirm the molecular ions and identify molecular formulas of putatively identified differential compounds and unknowns, respectively (Figure 7).

The first step in an attempt to elucidate the structures of unknowns was to use low electron energy to help identify molecular ions (M+). MS/MS was further obtained at optimal electron energy to maximize the absolute abundance of M+ use as a precursor. Unknown compound MS/MS spectra were then extracted using the Find by Targeted MS/MS algorithm in Qual. The results were evaluated in Molecular Structure Correlator (MSC) (Figure 8).
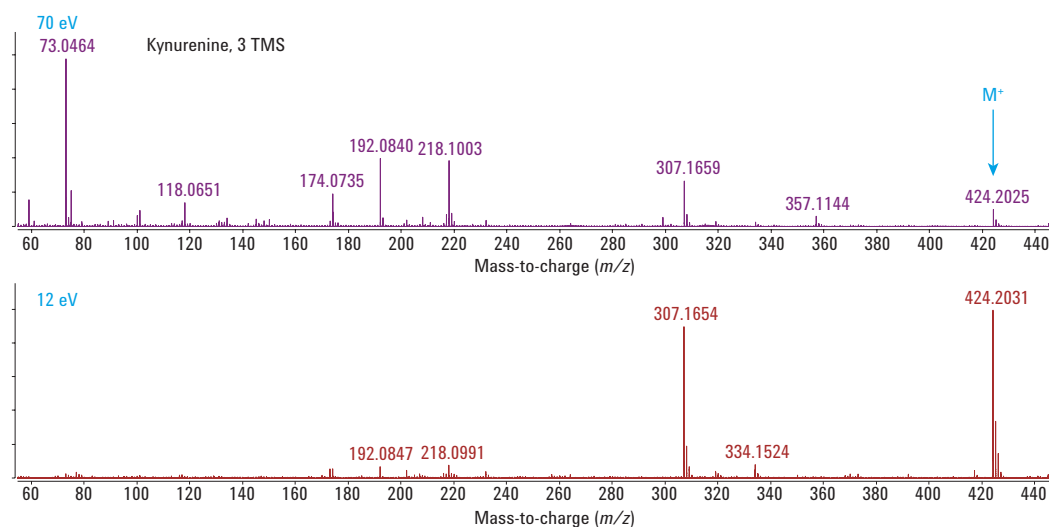

Figure 7.   Confirmation of the molecular ion (M+) using low electron energy.

5

70 eV

$[C_{10}H_{10}NO]^+$
160.0760

$[C_{14}H_{22}NO_2Si]^+$
264.1422

$[C_5H_5]^+$
65.0387

$[C_7H_7]^+$
91.0544

$[C_9H_7]^+$
115.0544

$[C_9H_9O]^+$
133.0652

$[C_{11}H_{14}NO_2]^+$
192.0994

$[C_{12}H_{16}NOSi]^+$
218.1002

$[C_{13}H_{18}NO_2Si]^+$
248.1105

12 eV

$[C_{14}H_{22}NO_2Si]^+$
264.1428

$[C_3H_9Si]^+$
73.0471

$[C_7H_7]^+$
91.0549

$[C_6H_{15}OSi]^+$
131.0890

$[C_{10}H_{10}NO]^+$
160.0763

$[C_{11}H_{14}NO_2]^+$
192.1026

$[C_{13}H_{18}NO_2Si]^+$
248.1110

$[C_{15}H_{25}NO_2Si]^+$
279.1660

17 eV, MS/MS at CE = 20 V

264.1423

73.0470

160.0759

91.0544   119.0495   190.1041   248.1468

60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290
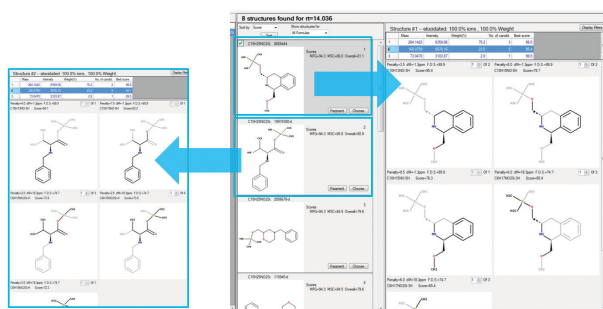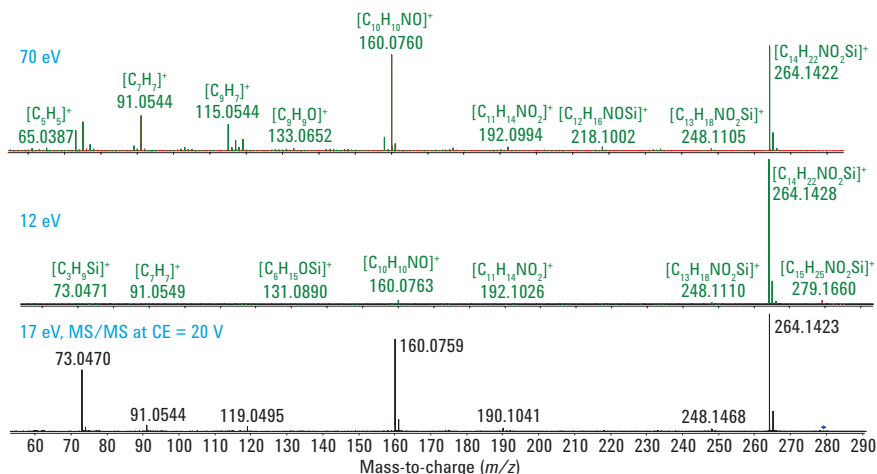Mass-to-charge (*m/z*)

Figure 8.   Structure elucidation of unknown compounds using low electron energy and MS/MS with Molecular Structure Correlator (MSC). Shown are possible structures of an unknown compound.

Conclusions

The untargeted metabolomics study demonstrated an alteration in amino acids profile, as well as a change in kynurenine and itaconic acid profiles. Interestingly, itaconic acid is not generally classified as a mammalian metabolite, however, it has recently been shown to likely play a role in macrophage-based immune response [2].

References

1. World Health Organization (http://www.who.int/ mediacentre/factsheets/fs104/en/)

2. Cheryl L. Strelko, *et al. J. Am. Chem. Soc.* **133(41)**, 16386–16389 (2011).

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