Lipid Mapping In Human Dystrophic Muscle By Cluster-Time-Of-Flight Secondary Ion Mass Spectrometry Imaging

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Abstract

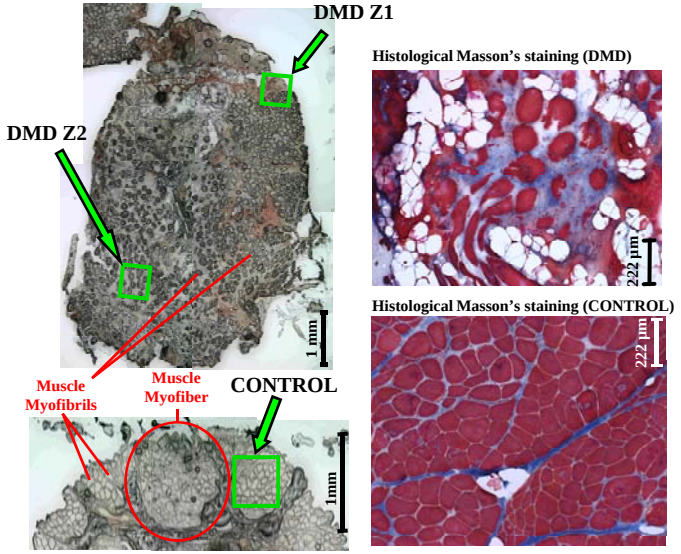
Human paravertebral striated muscle samples, from male DMD-affected and control 14 years old patients, have been subjected to cluster-time-of-flight secondary ion mass spectrometry (cluster-ToF-SIMS) imaging using a 25 keV Bi_3^+ primary ion liquid gun. Characteristic distributions of various lipids have been observed. Phosphocholines have been detected in necrotic areas, together with sphingomyelin and its fragment. Fatty acid chains composition varied depending on the region.

INTRODUCTION

Duchenne muscular dystrophy (DMD), a yet incurable severe X-linked pathology (1 in 3500 boys[1]) and induced by truncation or absence of dystrophin (involved in a transmembrane complex), results in a rapid muscular degeneration due to muscle cell membranes malfunctioning (permeability, ion drift disorder and oxidation stress). The most spread research model is the *mdx* (X-linked muscular dystrophy) mouse, developing an unusually high concentration of creatin and pyruvate kinases[2], a model of low cost, high availability and relatively short lifetime. Cluster-ToF-SIMS is powering its way in lipidomics and drugs/biomarkers mapping on biological tissues at a µm scale[3,4]. Phospholipids are key components of muscle cell walls and glycerophosphatidylcholines (PCs), a major component of membranes phospholipids. Fatty acid chains composition determine membrane flexibility, structure and resistance. In order to validate the coupling of the ToF-SIMS imaging methodology together with the murin model[5,6] as a suitable approach for research on the human pathology, we here report on our preliminary results obtained on human paravertebral striated muscle sections and compare them to our first outcomes on the *mdx* mouse.

1. **Blake DJ, Weir A, Newey SE, KE D.** *Physiol Rev.* **2002**;**82**(2):291-329.
2. **Bulfield G, Siller WG, Wight PA, Moore KJ.** *Proc Natl Acad Sci U S A.* **1984**;**81**(4):1189-1192.
3. **Touboul D, Halgand F, Brunelle A, Kersting R, Tallarek E, Hagenhoff B, Laprevote O.** *Anal Chem* **2004**;**76**(6):1550-1559.
4. **Brunelle A, Touboul D, Laprevote O.** *J Mass Spectrom* **2005**;**40**(8):985-999.
5. **Touboul D, Piednoel H, Voisin V, De La Porte S, Brunelle A, Halgand F, Laprevote O.** *Eur J Mass Spectrom* **2004**;**10**(5):657-664.
6. **Touboul D, Brunelle A, Halgand F, De La Porte S, Laprevote O.** *J Lipid Res* **2005**;**46**(7):1388-1395.

Optical Images of DMD-affected and Control Human Paravertebral Striated Muscle Sections



Histological Masson's staining (DMD)

Histological Masson's staining (CONTROL)

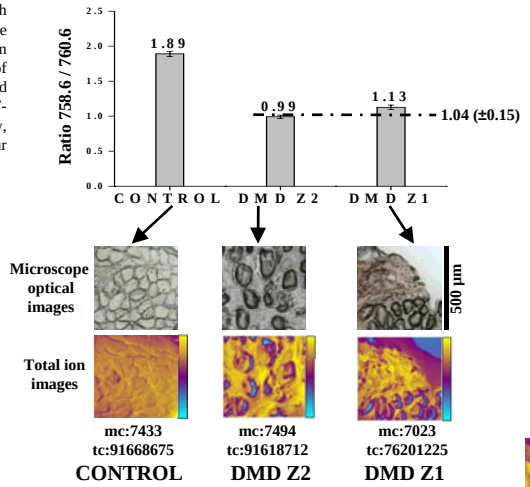
Experimental

We thank the **Banque de Tissus pour la Recherche** (BTR, AFM) for providing human muscle biopsies from DMD-affected and control patients. The BTR is a partner of the **EuroBioBank** network funded by the EC under the **Fifth Framework Programme** (QLRI-CT-2002-02769). 20 µm thick sections were cut at -20°C, in a **CM3050-S cryostat** (Leica Mycrosystèmes SA, Rueil-Malmaison, France) and stored at -80°C. Images were taken with an **Olympus BX51 microscope** (Rungis, France). Experiments were performed, without any prior treatment on a **ToF-SIMS IV mass spectrometer** (ION-TOF GmbH, Munster, Germany) with a bismuth cluster **(Bi_3^+) ion source** at a 45° incidence angle and an effective ion flight path of ~2m. A $2x10^{11}$ ions/cm^2 primary ion dose density, 0.27 pA current and a 150 µs cycle time. A low-energy electron flood gun neutralized the sample surface during analysis. The secondary ions were extracted with a 2 keV kinetic energy and post-accelerated to 10 keV. Due to the very low initial kinetic energy of the secondary ions, the relationship between the time of flight and the square root of *m/z* is linear over the whole mass range. Secondary ion **images field of view** was **500x500 µm^2** (256x256 pixels), compressed during data processing (128x128 pixels) to increase contrast for a final **lateral resolution of 4 µm**. **Masson's trichrome staining** (Sigma kit No. HT15) was applied to visualise connective tissue, muscle fibres and collagen (with aniline blue). Images were taken with a **Leica DMRXA2 microscope**.

RESULTS

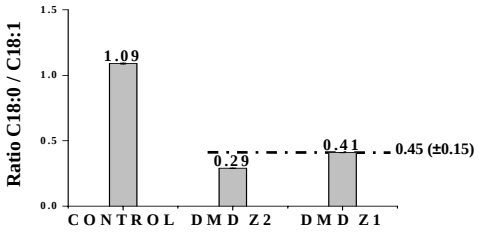
Same trend as in *mdx* model[5]:
- In each ROI, DMD cells exhibit a similar behavior to *mdx* mouse cells in healthy and control zones,
- PCs accumulate mainly in necrosed or intercellular area, similarly to *mdx* mouse destructured zone ⇒ intensive regenerating activity.

Global Intensity Ratio of 758 / 760

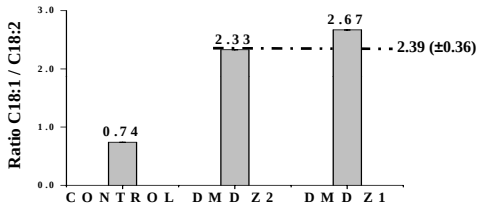


Global Intensity Ratio of C18:0 / C18:1 and C18:1 / C18:2

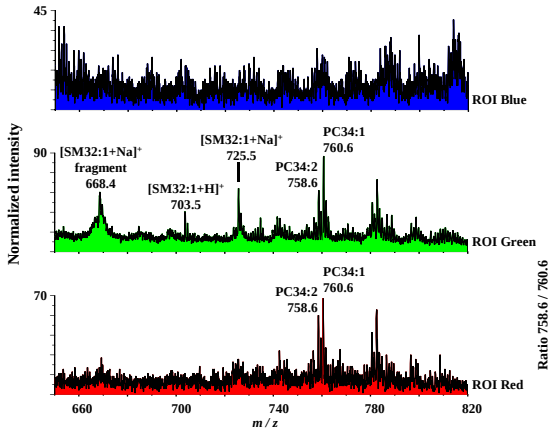
- high regeneration for control and degeneration for DMDZ2,
- inflammation in DMD: C18:1 & C18:0 accumulation in DMD necrosed or intercellular regions .



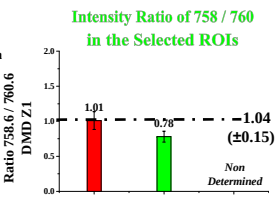
mdx mouse spectrum exhibited a high intensity signal for C18:2[7], comparable with human control spectrum but not with dystrophic ones, most probably due to the exceptionally high regeneration aptitude of mouse muscle even when developing Duchenne myopathy, while DMD-affected human muscle regeneration rate is far lower.



ToF-SIMS Positive Ion Mode Spectra of the Selected ROIs



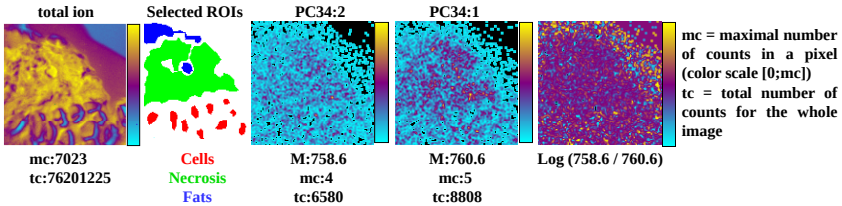
Intensity Ratio of 758 / 760 in the Selected ROIs



Sphingomyelin SM is present in DMD necrosed (green ROI) or intercellular regions, but not in the control ⇒ disorder in regulation of membrane sphingolipid composition (*i.e.* structure) and/or dysfunctionning of ceramide-mediated apoptosis pathway[7].

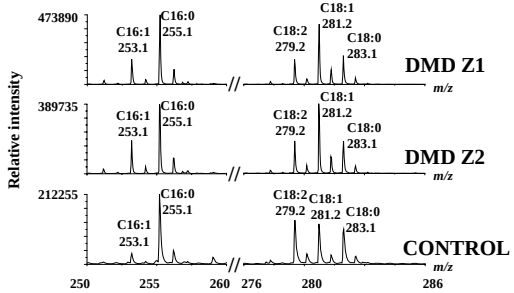
7. **Kolesnick RN, Kronke M.** Regulation of ceramide production and apoptosis. *Annu Rev Physiol.* **1998**;**60**:643-665.

ToF-SIMS Positive Ion Mode Images of the Ions 758 and 760 in spot DMDZ1



mc = maximal number of counts in a pixel (color scale [0;mc])
tc = total number of counts for the whole image

Global ToF-SIMS Negative Ion Mode Spectra of DMD and Control Zones



CONCLUSIONS

- Complete preservation of sample molecular and structural integrity: no prior sample treatment,
- Direct and relatively rapid analysis and chemical imaging of intact biological tissues and organ sections,
- Possibility of simultaneous but separate analysis of different muscle cells at µm scale,
- High molecular specificity and sensitivity, and no molecular delocalization at µm scale,
- Specific localization of different compounds in positive ion mode images and confirmation in negative mode,
- Association of spectra with images provides molecular information even with too low image signal and spatial information even with insufficient spectrum signal-to-noise ratio,
- Confirmation in human of similar trends observed previously in the *mdx* mouse model.

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