Analytical Biochemistry 488 (2015) 51–58

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Visualization of acetaminophen-induced liver injury by time-of-flight secondary ion mass spectrometry

Yohei Murayama [a, *], Shuya Satoh [a], Akinori Hashiguchi [b], Ken Yamazaki [b], Hiroyuki Hashimoto [a], Michiie Sakamoto [b]

[a] Frontier Research Center, Canon, Ohta-ku, Tokyo 146-8501, Japan
[b] Department of Pathology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160-8582, Japan

ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form
24 June 2015
Accepted 9 July 2015
Available online 21 July 2015

Keywords:
Time-of-flight secondary ion MS
Imaging MS
Tissue imaging
Drug-induced liver injury
Acetaminophen

ABSTRACT

Time-of-flight secondary ion mass spectrometry (MS) provides secondary ion images that reflect distributions of substances with sub-micrometer spatial resolution. To evaluate the use of time-of-flight secondary ion MS to capture subcellular chemical changes in a tissue specimen, we visualized cellular damage showing a three-zone distribution in mouse liver tissue injured by acetaminophen overdose. First, we selected two types of ion peaks related to the hepatocyte nucleus and cytoplasm using control mouse liver. Acetaminophen-overdosed mouse liver was then classified into three areas using the time-of-flight secondary ion MS image of the two types of peaks, which roughly corresponded to established histopathological features. The ion peaks related to the cytoplasm decreased as the injury became more severe, and their origin was assumed to be mostly glycogen based on comparison with periodic acid–Schiff staining images and reference compound spectra. This indicated that the time-of-flight secondary ion MS image of the acetaminophen-overdosed mouse liver represented the chemical changes mainly corresponding to glycogen depletion on a subcellular scale. In addition, this technique also provided information on lipid species related to the injury. These results suggest that time-of-flight secondary ion MS has potential utility in histopathological applications.

To investigate and diagnose diseases, various staining methods have been developed to visualize the morphological and chemical information in tissue specimens. Hematoxylin and eosin (H&E)[1] staining provides useful morphological information and is the "gold standard" for pathological diagnosis. Special staining and immunohistochemical techniques offer additional information on the chemical and molecular features of specimens and can be used to extend morphological observation. During recent decades, the development of analytical techniques has enabled large amounts of chemical information to be used for this purpose. There are many promising mass spectrometry (MS) techniques, including advanced proteomics and genomics using liquid chromatograph–MS [1–3], and metabolomics using gas chromatography–MS, which is now in practical use for newborn screening [4]. Nevertheless, the difficulty in connecting chemical information and morphological information has limited the utility of MS techniques in histopathological applications. It is widely accepted that subcellular chemical changes and the subsequent morphological changes are important for evaluating disease. The proteomics, genomics, and metabolomics techniques use purified extracts of specimens that provide useful chemical information but do not provide any morphological information on their origin. For this reason, imaging mass spectrometry (IMS) [5–7] has received considerable attention. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS), nanoscale secondary ion mass spectrometry (nano–SIMS), and time-of-flight secondary ion mass spectrometry (TOF–SIMS) are typical IMS techniques. These techniques are classified as in situ analysis techniques that can visualize chemical information as distributions of multiple substances. MALDI–MS is considered to be suitable for the detection of large intact molecules, such as proteins and peptides, although the spatial resolution is limited to several micrometers. Of the IMS techniques, nano-SIMS has the highest spatial resolution, approaching 50 nm, although the chemical information is limited to

* Corresponding author.
E-mail address: murayama.yohei@canon.co.jp (Y. Murayama).

[1] *Abbreviations used*: H&E, hematoxylin and eosin; MS, mass spectrometry; IMS, imaging mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; nano–SIMS, nanoscale secondary ion mass spectrometry; TOF, time-of-flight; PAS, periodic acid–Schiff; ROI, region of interest.

http://dx.doi.org/10.1016/j.ab.2015.07.005
0003-2697/

elemental analysis. TOF–SIMS produces secondary ion images that reflect the distribution of substances with a maximum mass of approximately 1000 at sub-micrometer spatial resolution and, therefore, has great potential for visualizing cellular and subcellular chemical information in biological samples.

Many excellent studies of single-cell imaging of cultured cells [8–18] and many approaches for visualizing the molecular distribution of tissue sections using TOF–SIMS have been reported, including for distributions of lipids in the brain [19], cholesterol ester in atherosclerotic aortic sinuses of mice [20], and specific lipids in nonalcoholic fatty liver [21]. TOF–SIMS has also been reported for subcellular visualization of tissue sections such as for visualization of cell nuclei, cell bodies and intercellular regions in mouse muscle tissue [22], and cholesterol localized in cell nuclei in rat kidney tissue [23] and brain tissue [24,25]. Furthermore, subcellular chemical changes of increased cholesterol in cell nuclei in mouse intestine tissue following cholera toxin treatment have also been visualized [19]. However, there have been few reports focused on subcellular chemical changes in individual cells in damaged tissue sections using TOF–SIMS, particularly as compared with histopathological changes.

In this study, we evaluated the use of TOF–SIMS for capturing subcellular chemical changes in damaged tissue sections. We selected mouse liver, which is a well-characterized metabolic organ, and prepared a typical acute liver injury model induced by acetaminophen overdose [26,27]. The changes on a subcellular scale were evaluated with conventional histopathological staining images. The acetaminophen-overdosed mouse liver tissue shows the following zonal features: necrotic hepatocytes in the centrilobular area, degeneration of hepatocytes in the transitional area, and nonaffected hepatocytes in the periportal area [28]. We visualized these zonal features with TOF–SIMS measurements and also evaluated the subcellular chemical changes in hepatocytes.

1. Materials and methods

1.1. Reagents

Acetaminophen, glycogen, and glucose were purchased from Sigma–Aldrich (St. Louis, MO, USA). Optimum cutting temperature compound was purchased from Sakura Fine Tek (Tokyo, Japan). Paraformaldehyde was obtained from Merck (Darmstadt, Germany). Other reagents were obtained from commercial sources.

1.2. Animals

Acetaminophen-induced liver injury model mice and control mice were prepared. All conditions were described in a previous report [13]. All animal studies were conducted in accordance with the animal experimentation guidelines of the Keio University School of Medicine.

1.3. Sample preparation

Livers harvested from mice were divided into three pieces. One piece was snap-frozen in liquid nitrogen and stored at −80 °C. The remaining pieces were fixed in 10% neutral buffered formalin solution. A formalin-fixed paraffin-embedded block was prepared from one of the fixed specimens and stained with H&E and periodic acid–Schiff (PAS) stain for standard histological evaluation. The other frozen specimen was embedded in optimum cutting temperature compound and cryosectioned into 6-μm-thick sections with a cryostat (Tissue-Tek Cryo$_3$, Sakura Fine Tek) at −20 °C. These sections were then transferred onto an indium tin oxide-coated glass slide and dried in air at room temperature for TOF–SIMS measurements. Glycogen and glucose solutions (1 mg/ml) were made with deionized water. Each solution (1 μl) was deposited onto a glass slide and dried in air at room temperature and then used to obtain the TOF–SIMS spectra of glycogen and glucose as reference compounds.

1.4. Instrumentation

TOF–SIMS spectra and images were obtained with a TOF–SIMS V instrument (ION–TOF, Münster, Germany) equipped with a Bi_n^+ cluster primary ion gun. Positive and negative ion spectra and images were recorded using Bi_3^+ cluster primary ions at 25 keV and electron flooding for charge neutralization. High-spatial resolution images were recorded using primary ions with a target current of 0.06 pA and a pulse width of 100 ns (high-spatial resolution mode). These images were recorded in an area covering the centrilobular zone of each section with a pixel size of approximately 1 × 1 μm. The high-mass resolution spectra were recorded using primary ions with a target current of 1.0 pA and a pulse width of 18 ns (high-mass resolution mode) for assigning and identifying the origin of the peaks. In high-mass resolution mode, the mass resolution ($m/\Delta m$, full width at half-maximum) was approximately 5000 at m/z 27. Internal mass calibration was performed using H_2^+, CH^+, CH_3^+, cholesterol ion ($C_{27}H_{45}^+$) [29], and phospholipid ion ($C_8H_{19}PNO_4^+$) [30] for positive ion spectra. For negative ion spectra, CH^-, C_2^-, C_2H^-, CHO^-, CH_3O^-, O_2^-, C_3H^-, C_4H^-, $C_3H_5O^-$, and fatty acid ion ($C_{16}H_{31}O_2^-$) [21] were used for precise mass calibration. Peak assignment was carried out using ion peak position, mass resolution, and mass tolerance (~±100 ppm; typical ion peaks are listed in Tables S1 and S2 of the online supplementary material) and also took into account the biological relevance obtained by pathological evaluation. In addition, many mass assignments were confirmed by comparison with reference spectra and/or the literature [21,29–35]. The images in the high-mass resolution mode were recorded with a pixel size of approximately 2 × 2 or 4 × 4 μm. All data were recorded with a primary ion dose density of less than 10^{13} ions cm^{-2}, which is the static SIMS limit. The data acquisition and processing were performed within Surface Lab 6 software (ION–TOF). A color scale bar, with amplitude as the number of counts, is shown on the right side of each image. The intensities of pixels were normalized to the intensity of the brightest pixel in each image. The spectra shown in each figure are the sum of all the pixels in each recorded field.

2. Results and discussion

2.1. Histology of acetaminophen-induced liver injury

Acetaminophen-overdosed mice livers and control livers were evaluated histologically by H&E and PAS staining, and centrilobular necrosis was confirmed in the acetaminophen-overdosed livers (Fig. 1). We classified the parenchyma of injured liver into the following three areas: a necrotic area devoid of glycogen, where it was difficult to distinguish individual cell borders and nuclei; a transitional area, which was distinguished by narrow bands of glycogen-depleted hepatocytes surrounding the necrotic area; and a nonaffected area. Separate parenchymal and periportal hepatocytes in nonaffected areas contained normal amounts of glycogen even in the injured liver.

2.2. Identification of nucleus and cytoplasm

First, we identified nucleus-related and cytoplasm-related ions using the TOF–SIMS spectrum and images obtained from the control mouse liver tissue. The nucleus-related ions were identified