BL-4A/2017G692, 2018G644

Evaluation of Copper Accumulation in The Liver of Chronic Bile Stasis by Synchrotron Radiation X-ray

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1 Introduction

Copper and iron are essential nutrients for all organisms. Both metals in excess are toxic with strong redox-activity. Humans as well as higher vertebrates developed the ingenious strategy to manage these double–edged swords; the effective absorption and excretion system for copper, and the strictly regulated absorption and no specific excretion system for iron. However, medical interests of copper in the beginning had been focused on the toxic effect, acute poisoning or chronic exposure by mine pollution. Since the discovery of mammalian copper transporters, ATP7A and ATP7B in 1993, molecular mechanisms of copper homeostasis and trafficking pathways in physiological and pathological conditions have been widely explored. We would like to introduce about what copper toxicosis is, namely Wilson disease (WD), and several problems encountered in clinics and laboratories. In this report, we show results of copper detection in copper storage conditions by synchrotron-based technique.

2 Experiment

Tissue sections were prepared from tissue blocks as standard pathological laboratory technique. The section to be measured was mounted on the folder. The synchrotron-radiation X-ray beam was irradiated and the fluorescence X-ray was measured based on energy dispersive spectrometry. Each sample was measured at least two times to standardize variation. Two dimentional image was created with Analysis software.

3 Results and Discussion

There are several examples of cooper accumulation I the liver including physiological and pathological conditions.

3.1 Chronic bile stasis

The bile duct obstruction including bile stone, cholecystitis, bile duct obstruction due to tumor can cause chronic bile stasis. Because the bile contains a large amount of copper, the bile static condition can induce copper retention in the liver. An extreme example is biliary atresia, congenital bile obstruction with unknown reason. Therefore, we examined the liver explant at the living liver transplantation due to advanced biliary atresia, in which chronic bile stasis caused liver cirrhosis. As shown in Figure 1, macroscopic H&E staining view did not show clear cirrhotic nodules. In contrast, copper signals clearly demonstrated cirrhotic nodules. Of interest, the copper signals were much stronger in the border of nodule (parenchyma) and surrounding portion (stroma, including fibrous tissues, bile ducts and vessels). To understand precise localization of copper signals, further study of other cases with high-resolution microbeam X-ray analysis is undergoing.

3.2 Neonatal livers

It has been noticed that human neonates presented physiologic newborn jaundice 2 to 4 days after birth and disappeared at 1 to 2 weeks. The incidence is about 98% in Japanese and about 60% in Caucasians. It could be occurred in the breast-feeding babies (breastfeeding jaundice). If the blood type of mother and baby was not appropriate (mother-infant blood type mismatch) such as Rh and ABO types, infants developed pathological jaundice due to over degradation of red blood cells followed by hyperbilirubinemia [1, 2]. We examined newborn livers by X-ray fluorescence (XRF) analysis. The copper signals were stronger than control adult liver and the copper signals were diffusely distributed throughout the newborn liver.

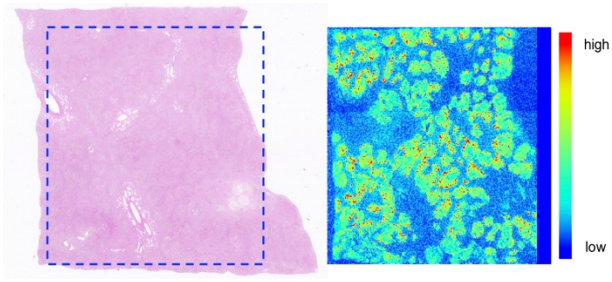

Figure 1. Copper localization in chronic bile stasis (BA). Hematoxylin and eosin stain (left) and Copper imaging (right)

4. Conclusion

Synchrotron X-ray fluorescence analysis (SXRF) is useful to evaluate the copper accumulation and distribution in various copper storage conditions [3, 4]. A unique distribution pattern found in patients with biliary atresia implied that copper storage mechanism is different from Wilson disease. To conduct more high-resolution analysis, the technical improvement is important [5]. We hope that scientists in the field of X-ray optics would continue hard works to bring biomedical researchers to the next step, beyond “precision medicine”.

Acknowledgement

We thank BL-4A user's group members (especially, Drs. Takahashi and Iida) for continuous help of the maintenance of BL-4A.

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