DIRECT VISUALIZATION OF MICROVASCULAR NO-REFLOW DURING HEPATIC ISCHEMIA-REPERFUSION WITH INTRAVITAL AUTOFLUORESCENCE MICROSCOPY

Ian Liau, Hsueh-Han Lu, Yao-Ming Wu

1Department of Applied Chemistry, National Chiao Tung University
1001 University Road, Hsinchu 300, Taiwan
2National Taiwan University Hospital
7 Chung-Shan South Road, Taipei 100, Taiwan
E-mail: ianliau@mail.nctu.edu.tw

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Ischemia-reperfusion injury, referring to an increased damage that occurs to a tissue during reperfusion after a period of ischemia, has long been recognized as a major complication of surgeries involving transplantation and resection of organs. The ability to evaluate timely and specifically various pathogenic mechanisms that underlie the injury is essential to improve our understanding of the pathogenesis and to help develop effective therapies targeting specific pathogenic mechanisms. By far, histopathological examination post reperfusion remains a commonly employed means to assess ischemia-reperfusion injury and is thus lacking kinetic information. Herein we report the employment of intravital autofluorescence microscopy to monitor kinetically hepatic ischemia-reperfusion of rats in vivo. Global analysis of the time-lapse autofluorescence images shows that the autofluorescence intensity of the liver decreases rapidly after ischemia, but recovers gradually during reperfusion, with a rate depending strongly on the duration of ischemia. The kinetic variation of autofluorescence is rationalized with the conversion of mitochondrial flavoproteins between the non-fluorescent reduced state and fluorescent oxidized state during ischemia and reperfusion, an interpretation supported with results of complimentary hypoxia-reoxygenation experiments and inhibitory assays performed on cultured hepatocytes. In particular, our approach enables direct visualization of microvascular occlusion (commonly termed “no-reflow”), a pathogenic mechanism that delays the reoxygenation of cells during reperfusion and inevitably causes more severe tissue damage, further demonstrating the unique capability of intravital autofluorescence microscopy to assess the progressive pathological change of organs in real time without employing any stains.

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Figure 1: Time-lapse autofluorescence images of rat liver during ischemia and reperfusion