Confocal laser endomicroscopy in dynamic evaluation of hepatic apoptosis in vivo

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The recent advance in confocal laser endomicroscopy (CLE) has led to an exciting renaissance for in vivo histology in human liver. CLE is a novel imaging modality providing in vivo histology through very-high-resolution images of human and animal liver (3, 6, 8). CLE is based on tissue illumination with a low-power laser with subsequent detection of the fluorescence light reflected from the tissue through a pinhole (11). The laser light is focused at a selected depth in the tissue of interest and reflected light is then refocused onto the detection system by the same lens. The light reflected and scattered at other geometric angles from the illuminated object or refocused out of plane with the pinhole is excluded from detection (7). This dramatically increases the spatial resolution of CLE, thus providing live histological examination of the superficial and internal layer of the liver (1, 5, 12). Scientists have successfully used CLE to visualize the human liver in vivo at greater imaging depths and at higher resolutions than was previously possible (9).

Fluorescein is the principal dye that has been used for CLE because of its fluorescence properties and safety profile (10). This has allowed some of the key histological properties of the liver to be observed in real time and has led to the development of novel functional tests of liver physiology. Initial studies showed that the diagnostic yield of confocal endomicroscopic images of human liver (from surface to 250 μm) were generated in real time after fluorescein injection, permitting visualization of hepatocytes, bile ducts, sinusoids, and collagen fibers in vivo (6). More recent studies have tried to go beyond this by showing that CLE is as effective a diagnostic tool as histological evaluation of liver biopsies. Findings from CLE compared favorably with histological results from liver biopsy samples and could correctly predict the presence of steatosis and fibrosis in 81 and 90% of cases, respectively (5, 9). Another study has demonstrated that CLE is an effective instrument for detecting cell death occurring in liver tumors (2). Currently there are multicenter trials testing whether the diagnostic yield is increased when confocal endomicroscopy is used in procedures such as screening liver tissue for dysplasia (HCC) or looking for cholangiocarcinoma in indeterminate biliary strictures.

Apoptosis, the process of programmed cell death, is fundamental to many biological and pathological events, including human liver development and liver injury. A precise balance of hepatic cell proliferation and cell death is required for liver tissue homeostasis, in which old or damaged hepatic cells are deleted and replaced by new cells. Several approaches to monitor hepatic apoptosis noninvasively have been developed, with varying degrees of sensitivity and specificity. Some rely on the structural changes occurring in the apoptotic liver tissue, which can be detected by ultrasound or diffusion-weighted MRI. Other methods include reagents developed for monitoring activation of caspase proteases by fluorescence, bioluminescence, or radiolabeled probes (13). However, much of the knowledge of the morphology of hepatic apoptosis has been gained ex vivo on fixed samples, subject to processing artifacts and yielding static information rather than continuous imaging, or from cell culture, which can only partially reflect the biological complexity of apoptosis in the intact, multicellular organism. Intravital imaging emerged as an indispensable tool in hepatobiliary research, and a variety of imaging techniques have been developed to noninvasively monitor liver tissues in vivo. Nevertheless, most of the current techniques have been limited by the lack of an appropriate reporter and the resolution to study events at the single-cell level.

In this issue of American Journal of Physiology Gastrointestinal and Liver Physiology, Goetz and colleagues (4) took advantage of CLE technology to make several important observations by continuously following distinct morphological, functional and molecular features of apoptosis in intact liver in vivo and at high resolution as follows: 1) The time course of apoptosis was visualized in vivo while liver perfusion and tissue integrity were maintained. 2) In contrast to most ex vivo studies, initial cell swelling was observed that coincided with early defects in barrier function of sinusoids and hepatocytes. 3) Cytoplasmic vesicle formation, nuclear condensation, cellular disintegration and macrophage infiltration were captured sequentially. 4) Labeling of caspases allowed molecular imaging of activated caspases targeted with the green probe FLIVO. Goetz and colleagues provide strong evidence that in vivo confocal microscopy can be used to continuously follow apoptosis with subcellular resolution in intact liver of live animals for up to 4 h. Instead of combining multiple snapshots of different cells from culture or biopsies, the investigators continuously followed individual cells within a multicellular context. Associated mediator profiles were preserved in their study, reflecting the natural environment for apoptosis. The lack of necrosis in control animals during CLE suggests that liver perfusion and tissue integrity were maintained throughout the examination. Various staining protocols allowed evaluation of morphological, functional, and molecular events in vivo at high resolution and enabled them to set a time frame for apoptosis based on continuous observation of individual hepa-
tocytos. Such in vivo microscopy allows dynamic identification of distinct features of apoptosis that are not uniformly described or detected in ex vivo studies and may represent a promising tool for future studies and clinical trials of apoptosis and its pharmacological manipulation in animals and humans.

Since CLE research in intact liver in vivo at high resolution is still in its infancy, the work of Goetz now published in American Journal of Physiology Gastrointestinal and Liver Physiology (4) is pioneering. Meanwhile, and without lessening the merit of the current investigation, caution is advised when interpreting the significance and broader applicability of their results. Because this investigation, similar to the majority of studies in the field, has been conducted in a limited field of view in relatively superficial areas of the liver, and in limited numbers of animals, the results will have to be replicated in multiple, independent animal or patient populations before being translated into clinical practice. Thus a major challenge still lies ahead to determine the true biological and clinical importance of CLE detection of pathological alterations in intact liver in human hepatic disorders.

REFERENCES