

Real-Time Bio-Imaging of Hyaluronic Acid Derivatives in Cirrhotic Mice Using Quantum Dots

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Statement of Purpose: Liver cirrhosis is one of the representative liver diseases and a major cause of morbidity and mortality worldwide.[1] For the treatment of liver fibrosis, a lot of anti-fibrotic compounds like interferon- α 2a have been investigated to check their *in vitro* and *in vivo* therapeutic effects. To improve the stability of anti-fibrotic compounds in the body, they were conjugated with synthetic polymer like poly(ethylene glycol) [PEG]. However, PEGylation is not intended for target delivery to the liver but for passing through the liver and long circulation in the body. In this respect, PEGylation might not be a good strategy for the development of liver disease therapeutic delivery systems. Accordingly, to assess hyaluronic acid (HA) derivatives as alternative target specific drug delivery carriers for the treatment of liver diseases, we carried out real-time bio-imaging of HA derivatives in cirrhotic mice using quantum dots (QDot).

Methods:

Synthesis of HA-QDot Conjugates: HA-QDot conjugate was synthesized as previously reported except for a slight modification in the reaction buffer and EDC deactivation method [2].

In vitro bio-imaging: Normal hepatocytes (FL83B), and liver disease cells of hepatic stellate cells (HSC-T6) and hepatoma cells (HepG2) were incubated with HA-QDot conjugates at a final concentration of 5 nM for 90 min [3]. The cells were washed, fixed, and observed with a confocal scanning microscope.

Cirrhotic mouse preparation: Hepatic fibrosis was induced by the intraperitoneal administration of CCl₄ dissolved in olive oil twice a week for 8 weeks.

In vivo bio-imaging: HA-QDot conjugates were administered to CCl₄-induced liver cirrhotic mice at an average age of 8 weeks by tail-vein injections. The fluorescence of injected HA-QDot conjugates was captured with a luminescent image analyzer in 30 min, 1 day, 3 days, 5 days and 8 days.

Results: Figure 1 shows confocal microscopic images of three liver cells (HepG2, HSC-T6, and FL83B) after incubation with QDots and HA-QDot conjugates. HA-QDot conjugates with ADH content of 22 mol% was thought not to be affected for the binding with HA receptors on the basis of the fact that three carboxyl groups (hexasaccharide) in the HA molecule are related with its binding to HA receptors [4]. Interestingly, more amount of HA-QDot conjugates were taken up to HepG2 and HSC-T6 than FL83B. The results supported that liver-injury had some physiological effect on the receptor mediated endocytosis of HA derivatives.

On the basis of *in vitro* bio-imaging study, we carried out *in vivo* real time bio-imaging of HA-QDot conjugates

in cirrhotic model mice. Figure 2 shows the fluorescence images of HA-QDot conjugates after tail vein injections to fibrotic balb/c mice. HA-QDot conjugates were accumulated mainly in the liver. The clearance of HA-QDot conjugates was slower in cirrhotic mice than normal mice remaining in 8 days. The results may be explained by the assumption that fibrotic liver becomes lethargic and unable to remove HA-QDot conjugates efficiently.

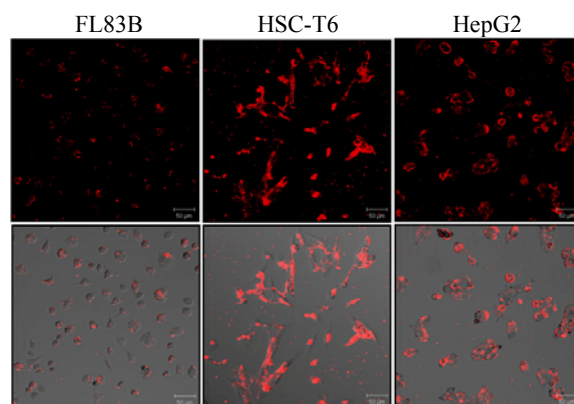


Figure 1. Confocal microscopic images of FL83B, HSC-T6, and HepG2 incubated for 2 hrs with HA-QDot conjugates with 22 mol % HA modification. Scale bar corresponds to 50 μ m.

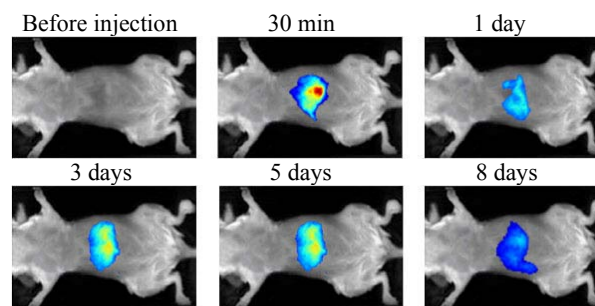


Figure 2. Fluorescent images of the livers in cirrhotic mice with intensity-weighted colors up to 8 days after tail-vein injections of HA-QDot conjugates.

Conclusions: Real-time bio-imaging of HA derivatives in cirrhotic mice using QDots demonstrated the feasibility of HA derivatives as novel target specific and long acting drug delivery carriers for the treatment of various liver diseases including hepatitis, liver cirrhosis and liver cancer.

References:

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