**Self-immolative boronated retinoic acid prodrug nanoassemblies for treatment of hepatic ischemia/reperfusion injury**

Nanhee Song, Eunkyong Jung, Manseok Yang, Soonyoung Kwon, Dongwon Lee
Department of Bionanotechnology and Bioconvergence Engineering
Chonbuk National University, Republic of Korea

**Statement of Purpose:** Retinoic acid (atRA) is an active metabolite of retinol, (vitamin A) and plays important roles in cell growth and proliferation. atRA is also known to exert anticancer and antioxidant activity. However, the clinical use of atRA has been limited by its low solubility, short half-life and insufficient therapeutic efficacy. Nanomedicine based on stimulus-responsive self-assembling prodrugs has emerged as a novel paradigm in controlled drug delivery because of high drug loading content. To enhance the therapeutic efficacy and expand clinical application of atRA, we developed boronated atRA prodrug nanoassemblies by exploiting the benefits of stimulus-responsive prodrug nanoassemblies. The therapeutic potential of atRA nanoassemblies was evaluated using a mouse hepatic ischemia/reperfusion (IR) model.

**Methods:** Boronated retinoic acid prodrug (RABA) was synthesized by conjugating boronic acid to atRA. RABA nanoassemblies were prepared under aqueous conditions by simple reprecipitation method. The size and morphology were investigated by dynamic light scattering and scanning electron microscope. Anticancer, antioxidant and anti-inflammatory activity of RABA nanoassemblies were evaluated by flow cytometric analysis using various cells. Hepatic IR injury was induced by ligating the portal vein for 10 min, followed by reperfusion. atRA nanoassemblies were intravenously injected 30 min before reperfusion. Liver and blood were collected to evaluate the therapeutic effects.

**Results:** RABA was designed as a self-assembling H$_2$O$_2$-activatable hybrid prodrug possessing boronic acid that endows hydrophilic nature and H$_2$O$_2$-triggered self-immolation (Figure 1). Upon the addition of H$_2$O$_2$, RABA underwent H$_2$O$_2$-triggered degradation of boronic acid, leading to the release of atRA and hydroxybenzyl alcohol (HBA) that exerts anti-inflammatory activity. RABA self-assembled under the aqueous condition to form stable RABA nanoassemblies. RABA nanoassemblies were spherical nanoparticles with smooth surface and a mean diameter of ~230 nm, verified by dynamic light scattering and SEM. RABA nanoassemblies scavenged H$_2$O$_2$ because of H$_2$O$_2$-triggered degradation of boronic acid. In H$_2$O$_2$-activated cells, RABA nanoassemblies exerted highly potent antioxidant and anti-inflammatory activity by suppressing the expression of TNF-α, IL-β, IL6, and (high mobility group box-1 (HMGB-1). In the mouse model of hepatic IR injury, RABA nanoassemblies accumulated in liver due mainly to the natural propensity of nanoparticles to target liver. A single injection of RABA nanoassemblies effectively protected liver from IR injury by scavenging the overproduced H$_2$O$_2$ and suppressing TNF-α, IL-β, IL6, and HMGB-1 (Figure 2). RABA nanoassemblies did not cause apparent toxicity, evidenced by histological examination of major organs and blood chemistry tests.

![Figure 1. Schematic illustration of RABA nanoassemblies as a targeted therapeutic agent for hepatic IR injury.](image)

![Figure 2. Protective effects of RABA nanoassemblies against hepatic IR injury. The serum level of (a) ALT and (b) AST. (c) Histological examination of liver tissues. Top: Gross photographs of liver excised 24 h after the treatment. The dotted lines indicate the boundary of damaged sites. Bottom: H&E-stained liver tissues.](image)

**Conclusions:** RABA was developed as a rationally designed hybrid prodrug of atRA and HBA. RABA self-assembled to form stable nanoassemblies that exert H$_2$O$_2$-activatable therapeutic actions. A single dose of RABA nanoassemblies exhibited significantly higher therapeutic actions than the combination of atRA and HBA in the mouse model of hepatic IR injury. RABA nanoassemblies have great potential as a pure nanodrug for hepatic IR injury because of their H$_2$O$_2$-responsiveness, self-assembling and self-immolating behaviors.

**References:**
E. Jung, *et al.* Biomaterials, 284, (2022), 121515