Nanobubbles, ultrasound deliver drugs that can treat infective endocarditis

A novel drug delivery method—a combination of nano-sized bubbles and ultrasound—can treat infective endocarditis by disrupting the biofilm that typically shields infected cardiac tissue from antibiotics, according to new research presented Sunday at Scientific Sessions.

A proof of concept experiment shows that the technique can successfully inject foreign material through the biofilm and into infected aortic valve tissue, researchers said.

"Endocarditis is refractory to treatment due to a biofilm which protects the bacterial vegetation from antibiotics and other external materials. In humans, endocarditis is a lethal disease," said study researcher Satoshi Nakatani, MD, PhD, professor in the department of functional diagnostics at Osaka University Graduate School of Medicine in Japan. "We want antibiotics to penetrate into the bacterial vegetation more easily in order to reduce the time and difficulty of treatment. That is why we thought of ultrasound."

Ultrasound already is used to facilitate delivery of drugs and genes through cellular membranes through sonoporation. Acoustic energy delivered by ultrasound causes the formation of microbubbles at the cell membrane surface that caviitate and open microscopic pores in cell membranes and force outside material through the membrane into the cell. The technique often is used to allow the uptake of DNA or other large molecules.

Adding microscopic bubbles to the delivery system can enhance the effects of sonoporation both in vitro and in vivo, Nakatani said, but the added microbubbles can dramatically increase sonoporation and cause unacceptable tissue damage.

Nakatani directed a research group that used nano-scale bubbles with an average diameter of less than 3 nanometers in an attempt to minimize the tissue damage seen with larger microbubbles. Results of the study were presented Sunday by lead author Kasumi Masuda, PhD, from the Department of Functional Diagnostics at Osaka University Graduate School of Medicine.

The research team induced aortic regurgitation in 43 rats using mechanical injury created by a catheter inserted into the left ventricle via the carotid artery. Three days after catheterization, the rats were infected with Enterococcus faecalis injected intravenously. Two days after infection, the rats were killed and the aortic valves were excised. Thirty of the excised valves showed signs of aortic regurgitation, and bacterial vegetation was found on 15 valves.

The infected valves were fixed in 400 mL saline solution containing 4 mL of India ink as a marker and assigned to one of three groups. A control group received neither ultrasound nor nanobubbles, a second group had ultrasound and no nanobubbles, and a third group had both ultrasound and nanobubbles.

The nanobubbles were injected in the fixing solution at the surface of the biofilm. Ultrasound was delivered at a frequency of 1.0 MHz at 1.0 W/cm2 using a one-second-ultrasound/four-second-stop pattern for 10 minutes.

After ultrasound exposure, all 15 samples were examined microscopically for evidence of India ink penetration. There was no penetration in the control group. The penetration score for the ultrasound-only group was 0.56 compared with 1.65 for the ultrasound plus nanobubble group.

Enterococcus faecalis infection was confirmed by gram stain and microscopic examination.

"Aquatic pressure from the ultrasound disrupts the nanobubbles and causes caviation," Nakatani explained. "Cavitation forms microjets which makes a port in the surface of the biofilm and allows the India ink to penetrate. We believe the same thing would happen if we used an antibiotic instead of India ink."

The next step is to confirm the in vitro activity of ultrasound plus nanobubbles in live rats using an inert marker, Nakatani said. If the mechanism is confirmed in live animals, the group plans to test the delivery system using antibiotics on active infections and compare survival rates to usual treatment.