Side effects of 'gene-silencing' treatment more wide-ranging than previously thought

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The side effects of an experimental "gene-silencing" treatment that is currently being investigated for a variety of diseases are even more wide-ranging than previously discovered, according to a study by a University of Kentucky researcher.

Following up on groundbreaking research published last year in the journal *Nature*, Dr. Jayakrishna Ambati, a UK ophthalmologist , and his colleagues found that the new drug modality, siRNA (21-nucleotide small-interfering RNA), is toxic not only to blood endothelial cells, which line blood vessels, but also to the cells lining the lymphatic channels.

These findings reinforce the note of caution sounded by Ambati's previous *Nature* study. But these side effects could themselves find useful application, for example, in cornea transplantation, where growth of new blood and lymph vessels is believed to be a major cause of graft failure.

The new findings are published in this week's online issue of Proceedings of the National Academy of Sciences, the official journal of the U.S. National Academy of Sciences.

In the earlier study, the Ambati laboratory discovered previously unrecognized immune side effects of siRNA, which is currently in FDA trials for numerous diseases including age-related macular degeneration and life-threatening viral infections.

Specifically, they showed that in two different established animal models of new blood vessel growth, siRNA killed these cells by activating an immune receptor called toll-like receptor 3 (TLR3). This was a critical finding, as immune and blood vessel toxicities were not believed to occur with this pharmacologic technique. As a result, siRNA is now recognized as a new class of anti-vascular drugs that could potentially be used to treat some of the 10 percent of the world's population suffering from neovascular diseases. However, this first study did not address other forms of specialized endothelial cells that exist in the human body, including those that line the lymphatic system, a critical component of immune responses.

The new study found that siRNAs block not only blood vessels but also lymphatic vessels. In the cornea, the clear part of the eye, injury often leads to the formation of both blood and lymphatic vessels. In fact, the formation of lymphatic vessels after corneal transplantation is purported to be a major mechanism through which transplant rejection occurs. Ambati's lab found that corneal injections of siRNA suppressed both blood and lymphatic vessel growth via endothelial cell toxicity.

Won Gil Cho, post-doctoral fellow, Dr. Romulo Albuquerque, and Dr. Mark Kleinman, researchers in the Ambati laboratory, also showed that siRNA directly activates TLR3, the first time this has been demonstrated in the literature. Addditionally, they showed, using time-lapse studies, that siRNA does not enter cells without a cell-permeating moiety such as cholesterol. This is important, because siRNA must enter cells in order to function as intended by specifically degrading intracellular messenger RNA bound for protein-forming machinery. Furthermore, this finding strengthens their finding that TLR3 positioned on the cell surface is responsible for mediating the toxic side-effects of siRNA. In concert with Sandro De Falco and Arturo Brunetti, researchers in Naples, Italy, they also found that siRNAs generically block blood and lymphatic vessel growth in muscle tissue as well. These findings illustrate this side

effect of siRNA can occur in many parts of the body.

Ambati's lab also reported last year in the *New England Journal of Medicine* that siRNA is deleterious to other cell types, such as the retinal pigmented epithelium, which is involved in age-related macular degeneration.

"This may be a broadly imprinted response in the mammalian immune system that is activated by siRNA," Ambati said. "In terms of benefit, siRNA may be utilized in the treatment of diseases of the lymphatic system, including lymphangiomas for which there is currently no effective targeted pharmacologic intervention."

Ambati is a Doris Duke Charitable Foundation Distinguished Clinical Scientist and a Burroughs Wellcome Fund Clinical Scientist in Translational Research. His laboratory is also supported by the National Eye Institute of the NIH, Research to Prevent Blindness, and American Health Assistance Foundation.

Source: University of Kentucky