Newly discovered cell mechanism uses amplified nitric oxide to fight C. diff

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Groundbreaking research encompassing Case Western Reserve University School of Medicine and University Hospitals Case Medical Center, has uncovered a natural defense mechanism that is capable of inactivating the toxin that spreads *Clostridium difficile*, or *C. diff*, an increasingly common bacterial infection in hospitals and long-term care settings. The research has immediate implications for developing a new form of treatment for antibiotic-resistant bacteria.

The newly discovered mechanism involves a nitric oxide (NO)-based molecule, S-nitrosoglutathione (GSNO), which binds to the toxins secreted by *C. diff* bacteria to deactivate them and prevent them from penetrating and damaging cells. The mechanism encompasses S-nitrosylation (SNO), a protein modification that attaches NO to cysteine residues in enzymes and other proteins.

"We've discovered a natural defense against *C. diff* that is based on nitric oxide, a ubiquitous molecule that is often produced by immune cells to kill pathogens," says Jonathan Stamler, MD, director of the Institute for Transformative Molecular Medicine and the Robert S. and Sylvia K. Reitman Family Foundation Distinguished Chair in Cardiovascular Innovation at the Case Western Reserve University Cardiovascular Center and University Hospitals Harrington-McLaughlin Heart & Vascular Institute. "Understanding how this mechanism deactivates toxins provides a basis for developing new therapies that can target toxins directly and thereby keep bacterial infections, like *C. diff*, from spreading," he says.

Dr. Stamler discovered the molecule GSNO, as well as the nitrosylation mechanism for control of protein function, in his previous research. He is one of the senior investigators studying how the protein modification inhibits the virulence of *C. diff* toxins. The resulting research findings appear in the Aug. 21 online issue of *Nature Medicine*.

In addition to Dr. Stamler, investigators from the University of Texas in Galveston, the University of California, Tufts University and the Commonwealth Medical College collaborated on the research. The University of Texas researchers first determined that NO helped protect cells against *C. diff* and approached Dr. Stamler to determine if SNO was also involved.

C. diff is the most common cause of hospital-acquired infectious diarrhea and life-threatening inflammation of the colon. It originates when normal, competing bacteria in the gastrointestinal tract are wiped out by the use of antibiotics. This allows *C. diff* bacteria to grow out of control.

The *C. diff* bacteria secrete a toxin that cleaves or cuts itself to generate a fragment that can penetrate cells, damaging them and resulting in a hemorrhagic injury to the gastrointestinal tract. The toxin is activated when inositolhexakisphosphate (InsP6), a substance prevalent in leafy vegetables and the gastrointestinal tract, binds to it, enabling the toxin to change shape and cleave itself.

The research shows that upon activation, GSNO, a NO donor molecule, binds to the toxin and nitrosylates it. This can only occur when InsP6 binds to the toxin.

The change in shape that results when InsP6 binds to the toxin is what enables the GSNO to target and inactivate the toxin by directly binding to the active site. There, the GSNO can nitrosylate (SNO) the cysteine to inactivate the toxin. These findings are especially significant as they suggest that GSNO has evolved to recognize shape changes in the toxins it targets.

Prior to this, researchers knew GSNO could produce SNO in many classes of proteins but there was little to no precedent for it binding to toxins or explaining how this SNO modification protects against infectious agents, Dr. Stamler says.

"The new research suggests GSNO, and S-nitrosylation, more generally, may have a universal function in protecting cells against microbial proteins, many of which have a design that is conducive to being s-nitrosylated by GSNO," Dr. Stamler says. "In this regard, GSNO-like molecules may represent a new class of antibiotics that can be developed, exploiting the shape changes in numerous bacterial proteins."

In their work, researchers also noted that increased levels of GSNO in the gut of *C. diff*-infected animals and increased levels of SNO-toxin in stools of patients, directly correlated with deactivation of the toxin, further confirming that the natural mechanism works to reduce disease activity in people. This provides a basis for measuring how much nitric oxide, a key molecule in cell immune activity, has bound to toxins to make SNO and limit the spread of bacteria.

The current treatment of *C. diff* is difficult and the infection often recurs. Resistance to antibiotics is also a serious worry. The researchers are currently developing a new class of anti-toxin treatment based on these findings. One advantage of such antitoxins, says Dr. Stamler, is that resistance won't occur. The researchers hope that the new treatment can enter clinical trials very rapidly.

Provided by Case Western Reserve University