Researchers detail how aging undermines bone healing

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Researchers have unraveled crucial details of how aging causes broken bones to heal slowly, or not at all, according to study results published today in the *Journal of Bone and Mineral Research*. The research team also successfully conducted preclinical tests on a potential new class of treatments designed to "rescue" healing capability lost to aging.

In the worst cases, an age-related delay in healing keeps the two sides of a fractured bone from ever rejoining (non-union), leaving many confined to wheelchairs, unable to walk or to live independently. Of the estimated 5.6 million fractures in the United States each year, between five and ten percent (up to 560,000) will heal slowly or incompletely. Researchers have known for 30 years that aging interferes with fracture healing, and have been filling in the details since on the complex web of biochemicals, stem cells and genes that bring about healing. The field is now reaching the point where precision designed drugs are in different stages of animal and human trials.

The current study is focused on cyclooxygenase 2 (COX-2), an enzyme known from past studies to drive stem cells to differentiate into cartilage, which then matures into bone. Researchers at the University of Rochester Medical Center 20 years ago discovered the gene in humans that is responsible for producing the COX-2 enzyme and revealed the enzyme's role in causing inflammation, the reason drugs like the painkiller Vioxx were developed to shut down its action. Then about seven years ago another research team here determined that COX-2 also plays an essential role in bone formation during skeletal repair.

The current study shows for the first time that COX-2 levels drop dramatically with age, and that the drop most explains why stem cells no longer turn into cartilage as efficiently, an early step in the chain reaction of healing. While a role for COX-2 in bone repair had been detailed prior to the current study, the cell populations responsible for the supply of COX-2 to the fracture callus, the layer of pre-cartilage cells (cartilage progenitors) that form first around a fracture to guide bone building, had not. The team also confirmed for the first time that healing ability lost with age can be rescued by manipulating the COX-2 pathway with existing, experimental drugs. The study was in mice, but is especially relevant to human medicine because of the similarity between human and mouse COX-2 gene, and because the study mice were engineered by the National Institute on Aging, and have inspired several major insights into aging that have been validated in humans.

"The skeleton loses the ability to repair itself as we age," said Regis J. O'Keefe, M.D., Ph.D., chairman of the Department of Orthopaedics at the University of Rochester Medical Center and corresponding author of the article. "Our results position the COX-2 pathway as one of several under exploration with the common goal of accelerating healing in aging humans, and with the potential to come together in future combination therapies."

Turning Back the Clock

In the current study, healing rates were compared between a group of young mice (7-9 weeks old) and a group of old mice (52-56 weeks of age), with healing evaluated by imaging and gene expression studies. Specifically, the current study found that the older mice experienced delayed fracture healing, decreased bone formation and decreased resupply of blood vessels to the healing site in aging mice. Expression of the gene that codes for production of the COX-2 was reduced by 75 percent in fractures between aged mice and young mice during the early

healing phase five days after a fracture. COX-2 expression in young mice peaked at the exact time that stem cells were changing into cartilage within the fracture callus of young mice, and was reduced during that period in older mice.

In addition, experiments confirmed that COX-2 is expressed primarily in early stem cell precursors of cartilage that also express collagen, type II, alpha 1 (col2a1), the gene that codes for production of a key part of type II collagen in mice and humans, the fibrous, structural protein that lends strength to bone. Researchers observed in aged mice a dramatic decrease as well in the expression of other genes known to contribute to bone formation as well (e.g. osteocalcin and type X collagen). Altogether the results suggest that in aging animals gene expression is altered early in fracture repair with consequences for the entire healing cascade.

Researchers found further proof that COX-2 is responsible for loss of bone healing ability with age when they were able to reverse the process with a drug known to encourage the COX-2 signaling effect. COX-2 catalyzes the conversion of a fatty acid to prostaglandin E2 (PGE2), a hormone with many functions in the animal body depending on the type of cell they interact with, from blood vessel dilation to embryo implantation in the womb to bone healing. PGE2 is known to have it effect on cells by reacting with one of four receptor proteins (EP1-EP4) on the surface of cells, including the surfaces of bone marrow stem cells, cartilage cells and bone-producing cells (osteoblasts). Human cells send and receive signals that switch on life processes through workhorse proteins called receptors that enable messages to penetrate cells.

Prior work in other labs had established that the ability of PGE2 to create new bone growth occurs in particular through its interaction with the EP4 receptor. In the current study, the team showed that delayed fracture healing observed in aged mice could be rescued with local delivery of an experimental drug, CP-734432, which directly activates

the EP4 receptor in place of the missing COX-2 (an EP4 agonist). The drug, provided to the team by Pfizer Inc., was also recently used to prevent osteoporosis in early animal studies. Local injection of an EP4 agonist to the fracture site of aged mice compensated for the reduced fracture repair observed with aging, with a significant reduction in immature cartilage seen and more efficient formation of mature bone.

Source: University of Rochester