Pantoprazole, a proton pump inhibitor, delays bone remodeling during fracture healing in mice

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INTRODUCTION:
Acid-suppression medications such as proton pump-inhibitors (PPIs) are among the most widely prescribed medications worldwide. PPIs are commonly used drugs for the therapy of reflux esophagitis, peptic ulcers and other acid-related gastrointestinal disorders. In addition, PPIs are used in combination with an analgetic therapy, first of all with nonsteroidal anti-inflammatory drugs (NSAIDs), to avoid stress ulcers by inhibition of gastric proton-pump (H+/K+-ATPase) activity in the final step of gastric acid secretion in the parietal cells. Recent studies have now reported potential adverse consequences of chronic acid suppression, including an increased risk for bone fractures. In vitro and human data suggest that PPIs decrease bone resorption by inhibiting osteoclastic vacuolar H+-ATPase. Vacular H+-ATPases are present in high concentration on the ruffled border of the resorbing osteoclasts, and this proton pump extrudes protons during the bone resorption process. Of interest, administration of a selective inhibitor of the osteoclastic vacuolar H+-ATPase prevents bone loss in ovariectomized rats. These findings raise the possibility that PPIs can prevent osteoporosis and fractures.

Although there may be substantial interest how these actions of PPIs influence bone regeneration, there is complete lack of information on whether PPIs can affect the process of fracture healing. Therefore, the aim of the present study was to analyze the effects of pantoprazole on fracture healing in a standardized femur fracture model in mice. We hypothesized that pantoprazole impairs bone remodeling during fracture repair through inhibition of bone resorption.

METHODS:
All animal procedures were performed according to the National Institute of Health guidelines for the use of experimental animals and were approved by the German legislation on the protection of animals. For the present study a total of forty-eight 12 to 14 week old CD-1 mice were used. Twenty-four mice were treated daily with 100mg/kg body weight (BW) pantoprazole i.p. Twenty-four vehicle (saline)-treated mice served as controls. Bone healing was studied in a murine closed femur fracture model using radiological, biomechanical, histomorphometric and protein biochemical analysis at 2 and 5 weeks after fracture. All data are given as means±SEM. After proving the assumption for normal distribution (Kolmogorov-Smirnov test) and equal variance (F-test), comparison between the two experimental groups was performed by Student’s t-test.

RESULTS:
Radiological analyses 2 (n=8) and 5 weeks (n=8) after fracture could not demonstrate significant differences between pantoprazole-treated animals and controls (p>0.05). However, biomechanical analysis showed in pantoprazole-treated animals compared to controls a slightly lower bending stiffness at 2 weeks (n=8) after fracture and a significantly lower bending stiffness (p<0.05) at 5 weeks (n=8) after fracture. This indicates a decreased quality of the newly formed bone at 5 weeks after pantoprazole treatment.

Histological analysis of the total callus area in relation to the femur diameter showed neither after 2 weeks (n=8) nor after 5 weeks (n=8) a significant difference between the two groups. However, after 5 weeks the amount of bone tissue was reduced and the amount of cartilage tissue was increased after pantoprazole treatment.

After 2 weeks (n=4) of fracture healing Western blot analysis of the callus tissue revealed that pantoprazole reduced the expression of bone formation markers BMP-4, Collagen I and CYR61. In addition, expression of PCNA, indicating cell proliferation, was also significantly reduced after pantoprazole treatment. Of interest, the expression of OPG, an inhibitor of osteoclastogenesis, was found slightly increased in the callus of pantoprazole-treated animals, while the expression of RANKL, a stimulator of osteoclastogenesis, was significantly reduced after pantoprazole treatment.

DISCUSSION:
We herein demonstrate for the first time that pantoprazole induces a delay at the late phase of fracture repair, as indicated by a significantly lower bending stiffness after 5 weeks when compared to non-treated controls. The action of pantoprazole may involve a reduction of osteoclast activity, as indicated by the down-regulation of RANKL. This inhibits bone resorption and delays bone remodeling. However, pantoprazole may also affect cell proliferation and bone formation, as indicated by the significantly reduced expression of PCNA and BMP-4 after pantoprazole treatment.

By investigating specific cellular activities in the bone resorptive process in vitro Zaidi [1] found inhibited osteoclastic bone resorption after treatment with a proton pump inhibitor. A recent study could also demonstrate that gastrointestinal proton pump inhibitors regulate osteoclast-mediated resorption of calcium phosphate cements in vivo [2]. In line with the results of these studies, the data of the present study demonstrate that the PPI pantoprazole significantly reduces RANKL expression. However, since remodeling during fracture healing requires osteoclast-mediated bone resorption, the reduced RANKL expression by pantoprazole may be the cause for the delay in remodeling, as indicated by the significantly lower callus stiffness during the late phase of fracture healing.

Furthermore, the callus size of pantoprazole-treated animals was not noteworthy larger compared to controls. This can be a compensatory consequence of the pantoprazole-induced reduction of RANKL expression, achieved by an inhibition of bone formation markers. Apart from reduction of RANKL expression, the present study indicates that pantoprazole also suppresses the expression of some bone formation markers such as BMP-4, collagen I and CYR61 during the process of fracture healing. This indicates that pantoprazole affects bone metabolism not only through the control of the RANKL/RANK/OPG system, but also through the regulation of bone formation. The decreased bending stiffness might indeed be caused by an impaired mineralization of the soft callus tissue due to the reduced expression of the bone formation markers. In conclusion, pantoprazole treatment delays the time course of bone repair most probably by affecting the process of remodeling through down-regulation of the expression of both RANKL and the bone formation markers BMP-4 and CYR61.

Significance:
There was complete lack of information on whether PPIs can affect the process of fracture healing. Now, our study demonstrates that pantoprazole treatment delays the time course of bone repair. This may indicate that the treatment of acid-related gastrointestinal disorders should be changed in patients incurring a fracture.


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