Angiotensin-converting enzyme inhibition stimulates fracture healing and periosteal callus formation in mice

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INTRODUCTION:

The renin angiotensin system (RAS) is classically known as circulating endocrine system, regulating blood pressure and electrolyte homeostasis. The main effector peptide in this system is Angiotensin II (Ang II), which is hydrolyzed from angiotensin I (Ang I) by the angiotensin converting enzyme (ACE), a key molecule in this system. Angiotensin I, however, is cleaved from angiotensinogen by renin. Ang II exerts its biological effects through binding with different specific angiotensin receptors, mainly the angiotensin type 1 and 2 receptors (AT1-receptor and AT2-receptor). The ACE furthermore interferes with the kallikrein-kinin system by degradation of bradykinin, which is responsible for prostaglandine (PG) and nitric oxide (NO) production through binding with specific receptors (B1 and B2).

In contrast to the classical (systemic) RAS, in the recent years a local RAS has been described in various tissues, as the heart, brain and the kidney, regulating different tissue functions as cell regeneration, cell proliferation, cell apoptosis, vascularization or inflammation. Almost nothing is known about a local RAS in bone. In vitro studies have shown that different components of the RAS can be synthesized in osteogenic cells. Additionally, clinical studies have shown a correlation between the angiotensin converting enzyme (ACE), a key molecule in the RAS, and bone mineral density in different populations. Nothing is known about the influence of the RAS on the process of fracture healing.

METHODS:

Right femora of 96 CD-1 mice were prestabilized using a pin-clip technique, as described previously. Briefly, an intramedullar pin was implanted retrograde in the right femur. Then, the femur was exposed through a lateral approach and a metallic clip was implanted ventro-dorsally through the medullary cavity, passing the intramedullar pin laterally. The operative procedure was performed using an operating microscope. Afterwards an osteotomy with a gap size of 0.25mm was created with a giggly wire saw in the middle of the femur, under the metallic clip.

48 CD-1 mice were treated with the ACE inhibitor perindopril (3mg/kg bw per day) in the drinking water and 48 mice served as a control. Animals were killed after 2, 5 and 10 weeks and fracture healing was analyzed by radiology, biomechanics, histomorphometry, immunohistochemistry and Western Blot. Additional animals (n=7 each group) were killed after 10 weeks for micro-CT analysis. All experiments were performed in adherence 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.

RESULTS:

Immunohistological analysis of the femora after 2 weeks showed that the ACE was expressed in hypertrophic chondrocytes and osteoblasts in the periosteal callus area. Western-Blot analysis confirmed expression of the ACE in the periosteal callus after 2 weeks, and further showed expression of the angiotensin type 1 receptor (AT1-receptor). Quantitative expression of the ACE was significantly lower in the perindopril treated group compared to controls after 2 weeks, whereas there was no difference in the expression of the AT1-receptor. Western Blot analysis further showed that apoptosis was significantly lower in the perindopril treated group, as indicated by reduced expression of cleaved Caspase-3 in the periosteal callus. We observed no difference in the expression of PCNA in both groups, indicating no difference in proliferation between both groups.

Radiological analysis showed a greater callus diameter after 2, 5 and 10 weeks, although the difference was not statistical significant. Of interest, whereas the relative callus size increased steadily in the control group from 2 to 10 weeks, we observed a significantly decrease of the relative callus size in the perindopril treated group between 5 and 10 weeks.

Histomorphometric analysis after 2 and 5 weeks showed a significantly greater periosteal callus formation in animals treated with perindopril. This was shown by a significantly greater relative callus diameter after 2 weeks (211.3±16.1% vs. 151.1±17.3%; p=0.02) and 5 weeks (164.1±9.5% vs. 127.8±8.6%; p=0.01) and also a significantly greater relative periosteal callus area after 2 weeks (5.2±0.6 mm² vs. 2.1±0.8 mm²; p=0.007) and after 5 weeks (3.2±0.5 mm² vs. 1.0±0.4mm²; p=0.003). At 5 weeks 7/8 animals in the perindopril treated group showed complete histomorphometric bone bridging of the osteotomy, compared to 3/8 animals in the control group (p=0.05; χ²-Test).

Biomechanical analysis showed a significantly greater max. torque at failure (0.3±0.04% vs.0.1±0.03%; p=0.0003) and a trend towards a greater torsional stiffness (0.5±0.2% vs.0.2±1.1%; p=0.12) in the perindopril treated group after 2 weeks. After 5 weeks max. torque (97.2±18.5% vs. 65.0±2.5%; p=0.18) and torsional stiffness (148.1±35.1% vs. 74.5±13.0%; p=0.07) still were greater in the perindopril treated group, however, the difference was not statistical significant.

Micro-CT analysis after 10 weeks showed that the bone volume in the gap region was smaller in the perindopril treated group compared to controls (3.9±0.2 vs. 4.7±0.3; p=0.055), although the difference did not prove to be statistical significant.

DISCUSSION:

We showed for the first time expression of components of the RAS during fracture healing in vivo. Expression of the ACE was shown in hypertrophic chondrocytes and osteoblasts in the periosteal callus. We further showed expression of the AT1-receptor in the periosteal callus. Inhibition of the ACE resulted in a reduced expression of the ACE in the healing callus after 2 weeks, which is in line with studies in other tissues.

Inhibition of the ACE resulted in an accelerated fracture healing, by stimulating periosteal callus formation. This was most probably due to a reduced apoptosis as indicated by reduced expression of cleaved Caspase-3 in the periosteal callus area after 2 weeks. Whether these effects are due to an interaction between angiotensin II and its receptors, or due to an interference with the kallikrein-kinin system cannot be concluded from the current data. Further studies will have to show whether there is also a local RAS in bone and how this local RAS probably influences fracture healing.

REFERENCES:


