Unfocused Extracorporeal Shock Waves Induce Anabolic Effects in Bone of Healthy Rats
+1Van der Jagt, O.P. , 1Piscaer, ... -34 

Introduction
Alternatively to drug therapy for the treatment of osteoporosis a treatment with biophysical stimuli might be useful. It has been shown that extracorporeal shock waves (ESW) stimulate the recruitment and the differentiation of mesenchymal stem cells towards osteoprogenitors and that they can induce the expression of osteogenic related growth hormones such as VEGF and BMPs. Therefore a treatment with ESW might affect bone turnover, bone architecture and the mechanical properties of treated bone. To examine this and subsequently evaluate if ESW might be a potential treatment for osteoporosis we investigated the effects of unfocused shock waves using in vivo SPECT scanning, in vivo microCT scanning, histological analyzes and mechanical testing.

Materials and Methods
6 Male Wistar rats 14 weeks of age were obtained. At 0 days 1000 electro-hydraulic unfocused shock waves with an energy flux density of 0.3 mJ/mm² were applied to the antero-lateral side of the right tibia. The large focal zone of 3.8 cm allowed treatment of the whole tibia. The contra-lateral left tibia was not treated and served as a control.

To analyze the bone turnover multipinhole-SPECT scans were made at 2, 7, 21 and 49 days. Using i.v. injected radioactive technetium labeled methylene diphosphonate (99mTc-mdp) a scan with a spatial resolution less than 1 mm was made under gas anesthesia. Differences between the right, treated leg and the left untreated control leg were evaluated in two regions of interest; one at the proximal metaphysis and one at the mid-diaphysis.

At 0 and 49 days in vivo micro CT scans were made under gas anesthesia. 3D reconstructions with a voxelsize of 18 µm were made. Similar to the SPECT-scans two region of interest were made, one at the proximal metaphysis and one at the mid-diaphysis. Bone was discriminated using a local threshold algorithm resulting in binary datasets. Mechanical properties of were determined at day 49 using three-point bending with a displacement rate of 0.01 mm/s. At the end of the experiment (day 49) histological analysis of 6 µm sections of methylmethacrylate embedded tibia were performed using thionine and Perls' stainings. For the statistical analysis of the SPECT data Wilcoxon matched pairs test was used, other outcome parameters were tested using paired t-tests (treated right vs. untreated left controls as pair).

Results
Shock waves induced an increased bone turnover in the treated tibia compared to the untreated control tibia (fig.1). The increase in the metaphysis at 7 days and in the diaphysis at day 7 and 21 days was more than twofold. In the diaphysis the bone turnover in the treated tibia was still increased 49 days after ESW were applied with 63% compared to the untreated control. In the metaphysis this was only 20%.

Further examination of the micro-CT scans showed the presence of trabecular structures and mineralizations in the bone marrow (fig.3a). On histology these mineralizations were present around fibrotic tissue and had a bony morphology with osteocytes (fig.3c). Active osteoblasts and osteoid could be observed around these ossifications. Perls’ staining showed the deposition of hemosiderin throughout the bone marrow of ESW treated legs only, suggesting that ESW induced (minor) bleedings (fig.3b). Furthermore, it was found on histology that in the marrow of treated legs there were much more adipocytes than in the untreated control legs.

Three-point bending tests showed that the stiffness of the ESW treated tibias was significantly higher than the untreated tibias (p=0.03). A non-significant trend indicated that the maximal force before failure was higher in the treated legs compared with the control legs (p=0.07).

Discussion
This study shows that unfocused shock waves affect bone turnover, micro-architecture and the mechanical properties of ESW treated bone. When ESW with a lower energy flux density were used no effects on bone turnover could be found (data not shown). Micro-CT analysis showed that both cancellous and cortical bone volume increased suggesting that ESW positively influenced the balance of the bone turnover. The longitudinal analysis of the local bone turnover showed that ESW affected the metaphysis and the diaphysis differently. Histological analysis showed an active process of intramembranous bone formation in the marrow, which can explain this different time course. The finding of depositions of hemosiderin, which has also been shown when bone was treated with focused shock waves, suggests that ESW induced minor bleedings. We hypothesize that these bleedings were accompanied by the formation of some fibrosis, which could subsequently be ossified. The increased number of adipocytes might be caused by ESW induced hypoxia. Whether these potential side effects would also occur in the human setting in which the ratio between the whole bone and the treated area is very different is not known. In conclusion we have shown that a single treatment with unfocused ESW can induce anabolic effects on bone, but ESW also induced bleedings in the marrow and affected the adipose tissue there. The large treatment area of unfocused shock waves allows the treatment of large skeletal sites in patients and since ESW induces anabolic effects on both the cancellous and the cortical bone it might be useful in the treatment of osteoporosis.