Introduction: The efficacy of Extracorporeal Shock Wave Therapy (ESWT) for delayed union and non-union of fractures has been described by several authors since 1991 (1,2). In their reports, however, conditions of ESWT, such as the number of irradiation, its frequency, focus area and energy level were not consistent, and a standard protocol of ESWT has not been established. Previous reports have shown that shockwave could induce osteogenesis in animal experiments (3,4,5), although the process of shockwave-induced osteogenesis has not been clarified. In this study, we irradiated the rat long bones by shockwaves, and evaluated the osteogenesis by measuring their bone mineral contents (BMC). In addition, we analyzed the molecular features of the process of osteogenesis by means of in situ hybridization (ISH). Based on the results, we discuss the details of the effects of shock waves on long bones. The final purpose of our experiments is to establish the best protocol of ESWT for delayed union and non-union of fractures.

Methods: Animal experiment: The present study was conducted in accordance with the Guide for Animal Experimentation, Inohana Campus, Chiba University, Chiba, Japan. Twenty-one male Sprague-Dawley rats aged 10 weeks were used in this study, and an Epos (Dornier MedTech Co., Germany) was used to produce shockwaves. Each rat was placed on a cage in prone position under general anesthesia, and shockwaves were applied to the right femoral-shaft from ventral side. Ultrasonic waves were used for focusing the irradiation. The focus was set on the center of the femoral diaphysis, where the energy flux density, the frequency and the number of shockwaves were set to be 0.5 mJ/mm², 4 Hz and 3000 times, respectively. The left femur was analyzed as an unexposed control. Determination of BMC: Twelve of 21 animals were used for this examination, and they were divided into Groups I and II. The animals were monitored for 21 days in Group I, and for 42 days in Group II. The irradiated rats were euthanized and their femurs were removed. Soft X-ray photos of the longitudinal and the lateral views of both bilateral femurs were harvested. The specimens were fixed with 4% paraformaldehyde, decalcified with 20% formic acid and embedded in paraaffin. Midsagittal sections were stained with hematoxylin-eosin and toluidine blue pH4.1. To examine mRNA expression for extracellular matrix proteins, sections were hybridized with digoxigenin-labeled cRNA antisense probes for type I procollagen (COL1A1), type II procollagen (COL2A1), type X procollagen (COL10A1), osteocalcin (OC) and osteopontin (OPN).

Results: After shockwave exposures, no fracture was occurred in the rats. BMC: Fig.2 shows the results of the determination of BMC. In the exposed femurs on day 21, BMC in PS, DS and D areas were significantly higher by 7.46%, 10.2% and 3.45% than those of the unexposed femurs, respectively. On day 42, BMC in P and PS areas of the exposed femurs were significantly higher by 4.02% and 10.2% than those of the unexposed femurs. On days 21 and 42, BMC in total areas of the exposed femurs were significantly higher by 5.05% and 5.71% than those of the unexposed femurs. ISH: On day 4, periosteal new bone formation occurred on the ventral cortex of the femurs, which was directly exposed with the shockwaves. Similar periosteal woven bone was also found on the dorsal cortex of the femurs, the other side of the exposures. At the endosteum, however, no bone formation was seen. In the newly-formed woven bone, COL1A1, OC and OPN mRNAs were expressed in osteoblastic cells under the periosteum. On day 7, periosteal bone formation progressed and trabeculae were formed. COL1A1 and OC mRNAs were strongly expressed in matured osteoblasts lining on the trabeculae (Fig.3). OPN mRNA was weakly expressed in immature osteoblastic cells and osteocysts. (Fig.3). On day 14, bone remodeling began within the periosteal bone. Expression of COL1A1, OC, and OPN mRNAs still occurred at this stage, but the signals were weak. Throughout the periosteal osteogenesis, COL2A1 and COL10A1 mRNAs were not detected in the newly-formed bone.

Discussion: In the experimental conditions employed in this study, osteogenesis was induced by the irradiation of shockwaves. In addition, the increased bone volume was maintained more than one month after the irradiation. Therefore, we suggest that the present experiment model in rats is adequate for investigating the molecular mechanisms of osteogenesis induced by shockwaves. The expression patterns of mRNAs for extracellular matrix proteins demonstrate that the shockwave-induced osteogenesis have the processes of intramembranous ossification, but not of endochondral ossification. Furthermore, the expression patterns were extremely similar to those found in the periosteal hard callus formation in the rat femoral-shaft fracture model (6). In conclusion, shockwaves can dramatically activate the cells in normal long bones, and drive the cells to express genes for osteogenesis. We suggest that clinical applications of shockwaves might regenerate the processes of fracture healing at sites of delayed union and non-union.


Fig1. Definition of the exposed area
Fig2. BMC of the exposed and unexposed femurs *p<0.05
Fig3. Expression of COL1A1, OC, and OPN mRNAs at the newly-formed bone on day 7 after irradiation. PO: periosteum, CB: cortical
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