Workshop II-A
2W-01
Bone formation and ECM remodeling cease within a limited period regardless of completion of bone healing in the rat calvarial defect

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Healing of bone defects depends on a size of the defect, i.e. a bone defect larger than a certain size (a critical size) does not heal completely. There have been few reports on healing of bone defects, whereas numerous studies have investigated that of bone fracture. It has not been known how and why bone formation ceases in the course of healing of the large bone defect. Bone formation during development involves extensive remodeling of extracellular matrices (ECM), which is achieved by both production and degradation of ECM. Our previous study suggested that osteoblasts and osteocytes secrete matrix metalloproteinases (MMPs) 2, 8 and 13 and play a role in ECM degradation as well as ECM production during bone development. The present study was designed to investigate the process of bone healing in the critical size defect focusing on the bone healing rate and the cellular activity of ECM production and degradation using the standardized rat calvarial bone defect model.

Twelve-week-old male Wistar rats were used. A full-thickness standardized trephine defect, 8.8 mm in diameter, was made in the rat parietal bone under anesthesia. The rats were fixed by perfusion through the aorta in days 1, 3 and weeks 1, 2, 3, 5, 8, 10, 12, 18, 24 and 36. The resected calvaria were radiographed for morphometric analysis of bone matrix apposition per week and then processed for in situ hybridization for type I collagen, osteocalcin and MMPs 2, 8 and 13. Alternatively, RNA was extracted from tissue that filled the original bone defect at the same time points and processed for quantitative analysis of expression of these bone matrix ECM proteins and MMPs using real-time PCR.

The bone healing rate (i.e. the rate of bone matrix apposition per week) was the largest in the fourth week and decreased thereafter. Little bone was apposed in the 36th week with leaving the defect unrepaird. The expression of type I collagen and osteocalcin as well as MMPs 2 and 13 increased towards weeks 2 and 3 and decreased thereafter. In contrast, the expression of MMP 8 was the highest in day 1 and decreased. The mRNA transcripts of type I collagen and osteocalcin were localized in osteoblasts and osteocytes. Some of those cells expressed MMPs 2, 8 and 13. Expression of the bone matrix ECM proteins and MMPs was no longer identified in week 24.

The results indicated that osteoblasts and osteocytes cease bone formation and ECM remodeling within 24 weeks regardless of completion of bone healing of the defect in the experimental model.

2W-02
Effect of Collagen Tripeptide of Type I Collagen on Proliferation, Migration and Collagen Synthesis in Human Aortic Smooth Muscle Cells

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Objectives: Collagen tripeptide (CTP) is a tripeptide fraction containing Gly-X-Y sequence which is a degrading product of type I collagen by a bacterial collagenase and showed many biological activities in recent studies. The objective of this study is to evaluate the effect of CTP on the proliferation, migration and the synthesis of type I and IV collagens in cultured human aortic smooth muscle cells (AoSNCs).

Methods: Three different concentrations of CTP (3, 30, 300μg/ml) were used as stimuli and the culture of human AoSMCs were performed, and then, immunohistochemistry, Western-blot, ELISA and Polycarbonate Membrane kit were used to investigate the effect of CTP.

Results: We found CTP can prominently inhibit the proliferation of AoSMCs by down-regulating PCNA protein expression level (P<0.05) and PCNA-positive cell ratio (P<0.01). The inhibitory effect of CTP on cell migration was also shown (P<0.05). The obvious dose-dependence has not been shown among these three concentrations. CTP also can accelerate the fibrillogenesis of type I collagen and promote the expression of type IV collagen as extracellular matrix around the AoSMCs.

Conclusion: These findings verified the effect of CTP on the proliferation, migration and synthetic activity of AoSMCs in vitro for the first time and suggested its pharmacologic value for some cardiovascular diseases such as atherosclerosis.