

LOCAL DELIVERY OF LENTIVIRAL BONE MORPHOGENETIC PROTEIN 6 IMPROVES DIABETIC FRACTURE HEALING IN RATS

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BACKGROUND & OBJECTIVES

Diabetes is a worldwide health problem. Among the diabetic-related co-morbidities, low-energy-trauma fracture is one of the major causes of morbidity and mortality due to increased fracture risk in comparison with normal group and impaired healing procedure after fracture. Bone morphogenetic protein 6 (BMP-6) is one of the newly discovered members from the transforming growth factor super family. It has been reported that BMP-6 not only shows more potent oestrogenic function than other BMPs, but also takes part in glucose and fat metabolism.

1. Build closed femoral transverse fracture animal model and study the role of BMP-6 in normal fracture healing.
2. Build diabetic fracture model and investigate the association between BMP-6 and impaired fracture healing.
3. Local BMP-6 gene delivery to rats with diabetic fracture. Investigate effects of gene therapy on diabetic fracture.

METHODS AND RESULTS

1. Closed femoral transverse fracture model

A self-designed three-point bending system (Fig. 1A) was built to induced fracture in long bones. Cadavers of adult male Sprague-Dawley rats were collected to test its repeatability to produce transverse fracture in rats' femurs (Fig. 1B). In vivo experiment was carried out under general anaesthesia (Sevoflurane). Femurs were internally fixed by 1mm K-Wire before fracture was induced (Fig. 1C).

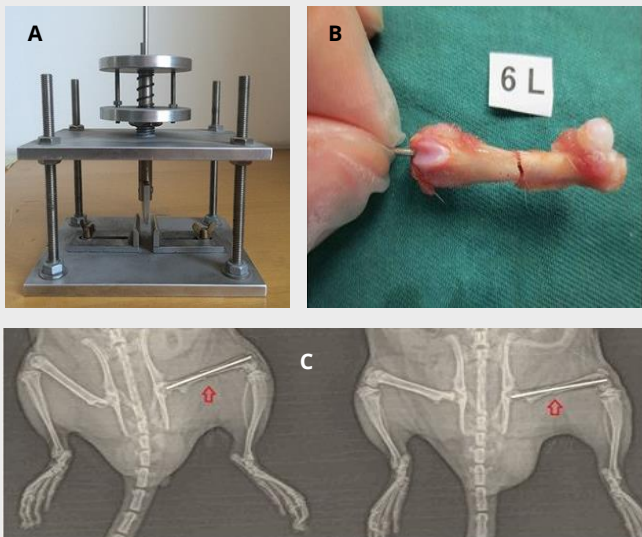


Fig. 1

Western Blot of callus extraction showed that BMP-6 was dramatically up-regulated during fracture healing (Fig. 2).

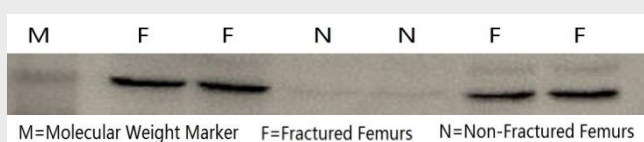


Fig. 2

2. Diabetic fracture model

Type 1 diabetes mellitus (T1DM) was induced by intraperitoneal Streptozocin (STZ) injection. Body weight, water and food intake, blood and urine glucose level were monitored (Fig. 3).

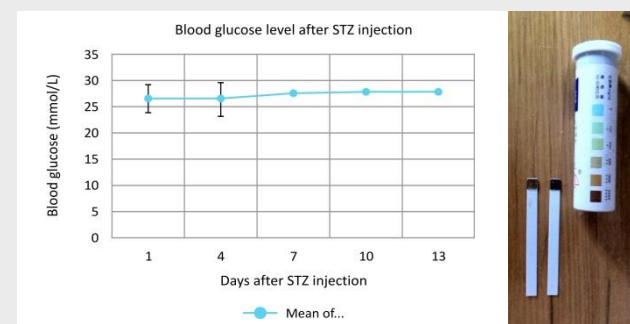


Fig. 3

Femoral fracture was induced 2 weeks after STZ injection. Impaired fracture healing and down-regulation of BMP-6 were found 4 weeks after fracture (Fig. 4).

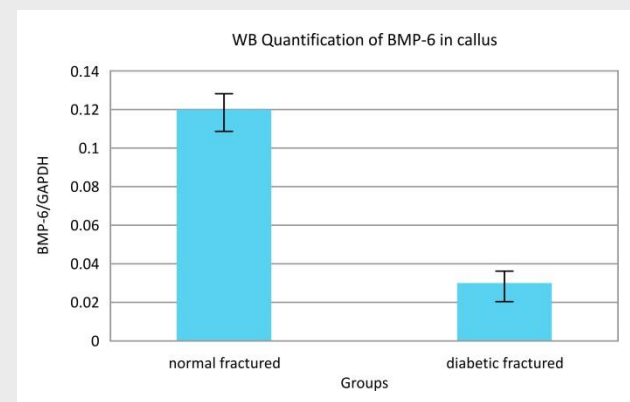


Fig. 4

3. Gene therapy for diabetic fracture

The Lentiviral BMP-6 (LV-BMP-6) overexpressing vectors were firstly applied to cultured rat's cells (ex vivo). 3FLAG positive spots were detected with Immunohistochemistry (Fig. 5A). Western Blot showed an increase in BMP-6 (Fig. 5B).

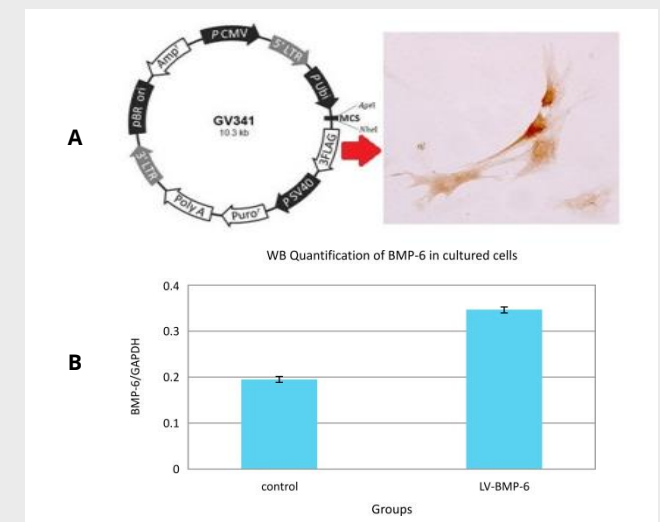


Fig. 5

STZ-induced diabetic rats received intra-medullary gene delivery right before fracture. During the 4 post-fracture weeks, water and food intake, urinary production and blood glucose level were down-regulated significantly in the gene therapy group compared with the diabetic control group. Enhanced fracture healing was also detected (Fig. 6).

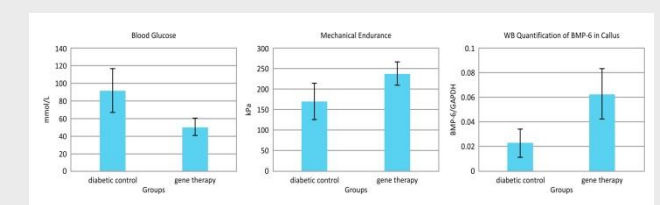


Fig. 6

CONCLUSIONS & RECOMMENDATIONS

BMP-6 took active part in normal fracture healing. Down-regulation of BMP-6 was found associated with impaired fracture healing in diabetic rats. Local delivery of LV-BMP-6 achieved improvement of diabetic fracture healing and correction of metabolic disorders at the same time.

The mechanism of BMP-6's effects on fracture and metabolism need to be investigated further. Insulin sensitivity of peripheral tissue, especially skeletal muscles and liver which are important to glucose storage, should be tested to see whether or not BMP-6 is a factor independent from insulin that lowers blood glucose. Gene therapy could also be applied to T2DM animal model in further studies.