MUSCLE BASED GENE THERAPY AND TISSUE ENGINEERING FOR TIBIAL PHYSEAL DEFECTS

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Introduction: Injuries of the growth plate can lead to bony bridge formation and result in limb-length discrepancies and angular deformities. The treatment of physeal injuries is challenging and the long-term prognosis unclear. Various treatment modalities of physeal injuries, including epiphyseodesis and interposition of fat, bone wax, muscle, silicone, bone cement, iliac apophyseal autograft or cultured chondrocytes were used in the past with various success rates. We investigated the efficacy of muscle based gene therapy using the insulin growth factor I (IGF-1) and bone morphogenetic protein 2 (BMP-2) for treatment of tibial physeal defects in rabbits.

Method: Forty-four 6 week-old New Zealand White rabbits with wide open physes were selected for the experiment. The policies and procedures of the animal laboratory are in accordance with those published by the USA Department of Health and Human Services. The research protocols used for these experiments were approved by the Animal Research and Care Committee at the authors’ institutions. Following an anteromedial incision, the medial half of the proximal tibial growth plate was excised using #21G needles and small curettes. The deep fascia and skin were sutured layer-by-layer. The right tibia was chosen as the experimental leg; left legs served as sham controls. The animals were divided into four groups: (1) physeal defect without treatment; (2) physeal defect and interposition of a free autologous muscle flap; (3) physeal defect, interposition of a free autologous muscle flap and injection of adenovirus-IGF-1 (ad-IGF-1; 1.5x10^7 pfu) and (4) physeal treatment, interposition of a free autologous muscle flap injection of adenovirus –BMP-2 (ad-BMP-2; 1.5x10^7 pfu). Autologous muscle flap (2x3 mm) was harvested from the tibialis anterior muscle. PA roentgenograms of both legs in prone position were obtained at 2-week intervals up to 12 weeks postoperatively in 6 rabbits of each group. Twelve weeks following surgery, the rabbits were sacrificed. The proximal tibiae harvested, fixed in formaline 4% for 1 week, decalcified in 10% EDTA for 6 weeks and snap frozen. The specimen were sectioned at 10 µm and H/E stained for histological assessment. Additional 2 rabbits of each group were sacrificed 4 and 8 weeks following surgery to obtain specimens for histological examination. The leg-length discrepancy ratio(left tibia length-right tibia length)/left tibia length and epiphyseal-diaphyseal angle (EDA) difference (right EDA-left EDA) of the medial tibial proximal epiphysis were used as main parameters to assess the growth of the physeal plate.

Result: In all groups, a slight tibia lengthening was observed 2 weeks following surgery (Fig. 1). While a significant leg shortening was demonstrated in the control and BMP-2 group, there was no leg length discrepancy in the muscle group and a slight leg lengthening in the IGF-1 group between 4 and 12 weeks following the injury of the physeal plate (Fig. 1).

The injury of the medial tibial physis caused a varus angulation of the medial tibial epiphysis in all groups (Fig.2). The first effect of the physeal injury was shown radiographically 2 weeks following surgery with increase of the right medial EDA compared to the left medial EDA in all groups. While the angulation of the right medial epiphysis increased up to 30° in the control and BMP-2 group, the muscle (15°) and IGF-1 (5°) group had significantly less angulation in the course of 12 weeks following physeal plate injury (Fig. 3).

Discussion: Untreated defects of the median tibial physis caused significant varus angulation of the proximal tibial epiphysis and a shortening of the Tibia in rabbits. In accordance to other authors, the treatment of physeal defects with autologous muscle tissue resulted in a significant reduction of the tibial angulation and tibia shortening. The addition of an adenoviral vector expressing the BMP-2 gene caused a significant increase and adenoviral vector expressing IGF-1 gene a significant decrease of the growth disturbance in the injured physeal plates treated with autologous muscle tissue. The capability of BMP-2 to produce bone within skeletal muscle was already proven in vivo. We assume that early closure of the injured physeal plates was caused by bone formation following adBMP-2 in our experiment. IGF-I is known to play a central role in the regulation of mitotic and metabolic activities of the growth plate chondrocytes.

The positive effect of adIGF-1 in treatment of physeal defects indicates that this growth factor may be supportive in the restoration of the growth plate following injuries.

For the first time, a biological effect of muscle based gene therapy was demonstrated in physeal defects. Although nearby restoration of angular deformities was demonstrated by the treatment of physeal defects using autologous muscle tissue in combination with adIGF-1 in rabbits, clinical application in human situation will require further investigations.

References:

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