Doxycycline Inducible Adenoviral Delivery Of Bmp-2 With Mesenchymal Stem Cells And A Calcium Phosphate Ceramic For The Repair Of Critically-sized Bone Defects In A Rat Model.

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Introduction: Gene therapy offers many potential advantages over the use of recombinant proteins for the repair of bone defects including localised, sustained and controllable transgene expression in physiologically relevant quantities. However, an effective 'off-the-shelf' delivery system has yet to be devised. Pre-clinical and clinical studies have demonstrated the regenerative potential of mesenchymal stem cells (MSCs) in non-union fracture, large bone defects and osteogenesis imperfect. Bone morphogenetic proteins (BMPs) are key modulators of fracture healing; with the efficacy of BMP-2 in bone regeneration being reported in many animal and clinical studies. Here, we report the use of a doxycycline tet-on adenoviral vector (AdTetBMP-2) in combination with MSCs, fibrin and a micro and macroporous biphasic calcium phosphate ceramic (MBCP®) to repair critically-sized bone defects in nude rats. The hypothesis is that AdTetBMP-2 enhances bone repair and precoating the ceramic granules with virus is an effective delivery system.

Methods:
Human bone marrow aspirate was obtained with full ethical approval (KEK-ZH-NR:2010-0444/0) and the mononuclear fraction obtained by ficol density separation. The adherent fraction was passaged to P2. The efficiency of transgene expression by an AdTetBMP-2 vector was investigated in vitro with human MSCs encapsulated in fibrin gels containing MBCP® granules (60% hydroxyapatite and 40% calcium phosphate). Secreted BMP-2 was quantified by ELISA over 16 days. The effect of adenoviral gene transfer of BMP-2 upon bone healing was investigated in vivo in 4mm critically-sized, internally fixated, femoral defects in nude rats. MSCs were transduced in 3D, either by direct application of AdTetBMP-2 or by pre-coating MBCP® granules with the virus, and combined with fibrin directly prior to transplantation. Doxycycline was administered in the animals feed. Control groups without virus and/or doxycycline were included. Animals were housed in groups in individually ventilated cages and euthanized after 12 weeks. Radiographs were performed directly post-operatively, and at 6 and 12 weeks. Ex-vivo CT and histological analysis was performed post-mortem.

Results:
BMP-2 was detected in the culture media of AdTetBMP-2 transduced MSCs at day 2 (2.1.±0.0528ng/ml) which increased during culture to 5.73ng/ml ±0.59 at day 16. No secreted BMP-2 was detected in either doxycycline or un-transduced controls. Radiological assessment scores of the defect site at 12 weeks, were higher in the AdTetBMP-2 + doxycycline group by direct application (4.50 ±1.30) compared to
controls (2.92 ±1.15) (Figure 1 A-C). Significantly higher radiograph scores were seen when MBCP®
granules were pre-coated with the virus (4.08 ±1.32) compared to doxycycline controls (2.80 ±0.27)
(Mann Whitney, p=0.0325). No adverse reactions or ectopic ossification in the surrounding tissue of the
defect site was seen histologically in any of the animals at post-mortem. MBCP® granules integrated
with nascent bone, fibrous tissue and bone callus within the defect site. The percentage of bone within
the defect site was greater in animals that had received direct in vivo delivery of AdTetBMP-2 (30±10%)
or when MBCP® were pre-coated with the virus (28±7%) compared to corresponding doxycycline
controls (both 22±5%) (Figure 1 D-F).

Figure 1. Doxycycline inducible Adenoviral delivery of BMP-2 promotes bone repair in critically sized
defects.
(A) Radiograph scores of bone defect healing after 12 weeks as determined by two, blinded,
independent assessors. (B) Post-operative and (C) 12 week radiographs from group +Ad+Dox. (D) De
novo bone as a percentage of the total defect site area at 12 weeks, semi-quantified from Giemsa Eosin
stained sections. Giesma Eosin stained sections from (E) +Pre-C Ad and (F) +Pre-C Ad +Dox groups. Box
plots present scores from each animal with the interquartile range, *p<0.05. Groups: +Ad: AdTetBMP-2
(n=6), +Pre-C Ad: MCBP® pre-coated with AdTetBMP-2 (n=5), +Dox: doxycycline control (n=4), +Ad+Dox:
AdTetBMP-2 +doxycycline (n=6), +Pre-C Ad +Dox: MCBP pre-coated with AdTetBMP-2 +doxycycline
(n=6).

Discussion:
Here, we show that delivery of BMP-2 using a doxycycline inducible adenoviral vector can improve bone
regeneration induced by the transplantation of MSCs, fibrin and MBCP® in vivo. The precise amount and
temporal availability of BMP-2 required for the repair of bone defects is not known, however, our in
vitro and in vivo data suggests that this can be achieved with relatively low (ng/ml) levels of the growth
factor. In addition no complications were observed. Moreover, the efficiency of the virus when pre-
coated onto our ceramic biomaterial suggests the potential for an 'off-the-shelf' application.

Significance:
Inducible viral gene delivery systems may allow controlled growth factor release in a temporal manner,
in order to achieve a clinically effective repair of critically-sized bone defects and non-healing fractures.