The Effect of Photodynamic Therapy (PDT) on Femur Fractures with Critically Sized Defects

Margarete K. Akens, Dr. med. vet., PhD1,2, Joshua Bernick, MSc3, Sadiya Yousef4, Diane Nam, MSc MD FRCSC3,5, Albert J. Yee, MSc MD FRCSC1,5, Brian C. Wilson, PhD6,7, Cari M. Whyne, PhD3,5.

1TECHNA Institute, University Health Network, Toronto, ON, Canada, 2Department of Surgery, Faculty of Medicine, University of Toronto, ON, Canada, 3Sunnybrook Research Institute, Toronto, ON, Canada, 4University Health Network, Toronto, ON, Canada, 5Department of Surgery, Toronto, ON, Canada, 6Ontario Cancer Institute, Toronto, ON, Canada, 7Department of Medical Biophysics, Toronto, ON, Canada.

Disclosures:

Introduction: The majority of long bone fractures heal successfully without complications, however compound and/or comminuted fractures resulting from high impact trauma can result in delayed healing or non-union. Early intervention in high risk fractures could decrease patient morbidity and reduce health care system costs (1). Photodynamic therapy (PDT) is a non-surgical, non-ionizing minimally invasive local treatment, which has been successfully applied to treat multiple types of cancer, skin diseases and age related macular degeneration. This treatment involves the local or systemic administration of a photosensitizing drug, which is activated by non-thermal laser light at a photosensitizer specific wavelength. This light activation leads, in the presence of oxygen, to the generation of cytotoxic species (e.g. singlet oxygen) (2), which can induce apoptosis and/or necrosis of targeted cells and tissue and also influence immune responses (3). PDT treatment of metastatically involved vertebrae resulted in improved vertebral bone strength, stiffness and architecture, motivating the investigation of PDT as an approach to augment bone healing (4). In our previous work, applying PDT treatment to comminuted rat tibia fractures yielded an increase of bone formation after 4 weeks (5). The aim of this study was to evaluate the ability of PDT treatment to enhance healing and/or prevent the development of non-union in femoral fractures exhibiting critically size defects.

Methods: Femoral fractures with critically sized defects (6 mm in length) were generated in 20 adult female Sprague-Dawley (SD) rats. Under general anaesthesia the femur was exposed and an 8-hole PEEK plate attached laterally with 6 screws (RISystem, Davos, Switzerland). Using a Gigly saw, a 6-mm mid-shaft bone piece was then removed beneath the middle of the plate. The musculature and skin incision was then closed. The positioning of the plate and screws was confirmed using high resolution x-ray imaging (Faxitron X-Ray LLC, Lincolnshire, IL). Rats were randomly allocated to 3 groups: control (no treatment); PDT applied 1 day (1d) post fracture and PDT applied 7 days (7d) post fracture. For PDT treatment prior to the application of light, a photosensitising drug (Visudyne, Novartis, Dorval, QC, Canada) was injected intravenously at a concentration of 1mg/kg. Fifteen minutes later, light energy of 75J was delivered at 690 nm using a 1 cm diffuser fibre placed subcutaneously parallel to the fracture under fluoroscopic guidance (6). Weekly faxitron images and blood samples were taken and serum stored for further analyses. Institutional animal care committee approval was obtained for all procedures. Vascular endothelial growth factor (VEGF) serum concentration was determined using Quantikine® RatVEGF Immunoassay (R&D Systems, Minneapolis, MN, USA), the C-terminal telopeptide of type I collagen (PICP) using the RatLaps™ EIA and osteocalcin using the RatMID™ Osteocalcin EIA (Immunodiagnostic Systems Ltd., Fountain Hills, AZ, USA). The rats were euthanized 7 weeks after induction of the fracture and their femora harvested. µCT images at an isotropic 13.3 µm/voxel resolution (Inveon MicroCT, Siemens, Erlangen, Germany) were acquired of the fracture site and callus for 3D architectural analysis (AmiraDev 5.2, FEI Visualization Science Group, Burlington, USA). Thereafter, the bone was decalcified and processed for histology. Statistical analysis of the bone volume measurements was performed using a 1-way analysis of variance.

Results: All rats recovered well from the fracture generation and PDT treatments; however three animals were euthanized early due to plate displacement. The PICP concentration in the control group was generally stable, decreasing slightly after surgery and returning to pre-surgical levels after 4-5 weeks. In the PDT treated groups, the PICP concentration fluctuated greatly at both day14 and day 42 with half of the specimens demonstrating increases in concentration compared to pre-op values and half reductions. There were no differences found in the serum osteocalcin levels or the VEGF serum level concentrations between groups. The total bone volume (TV) was evaluated from µCT images in a region of interest extending incorporating the fracture gap and extending to the first screw placements on either side of the gap (9mm). No significant differences were found in TV, however a trend toward lower volumes was seen with PDT treatment and longer time until treatment (control: 58 ± 29 mm3; 1d PDT: 49 ± 17 mm3; and 7d PDT: 38 ± 9 mm3). In contrast, BMD (gHA/cm2) trended toward higher values in the PDT treated groups (1d PDT: 0.86 ± 0.02; 7d PDT: 0.85 ± 0.08) compared to controls (0.79 ± 0.09). The fracture gap measured on histology slides/µCT images demonstrated a trend toward smaller gaps in the PDT treated groups (control: 3.68 ± 0.94 mm; 1d PDT 2.81 ± 1.17 mm; 7d PDT 3.43± 0.59 mm). The histology slides of the control group showed more cartilage and woven bone formation in contrast to the PDT treated groups which exhibited more structured and mature bone (Fig 1). Two of the control rats showed
histological signs of the development of non-unions, which was not seen in the PDT treated groups.

**Discussion:** PDT treatment of rat femur fractures led to lower overall formation of bone, but the bone had higher density with a decrease in the size of the fracture gap. The increase in bone density in the PDT treated groups may suggest formation of better quality bone (vs. quantity of bone). Histologically, with more cartilage and woven bone present in the control group in contrast to more mature bone and in the PDT group, the fracture healing seems to follow different pattern, which requires further investigation. The high variance seen in the serum measurements of bone markers is not uncommon and has previously reported within the literature\(^1\). Further extension of the time course of the study is needed to determine if PDT can reduce the incidence of non or mal unions in critically sized defects.

**Significance:** PDT may provide a cost-effective local minimally invasive treatment to enhance long bone fracture healing, by improving bone quality and reducing the development of non-unions in fractures with critically sized defects.

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**References:**
5. Akens MK, et al.; Orthopaedic Research Society; 2012; San Francisco

![Figure 1: Sagittal histology sections (H&E) of a femur fracture gaps 7 weeks after fracture generation (A: control group; B: 1dPDT group). Lines indicate the original gap size (6 mm).](image)

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