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ENHANCEMENT OF FRACTURE HEALING BY NITROSOALBUMIN

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Introduction:

Nitric oxide (NO) is a short-lived free radical gas produced from L-arginine by the nitric oxide synthases (NOSs). NO plays an important role on fracture healing. NO is expressed during fracture healing in rats and in humans and systemic suppression of NOSs impairs fracture healing. The current study evaluated the effects of locally administered nitric oxide on fracture healing by using a novel slow release NO donor nitrosoalbumin.

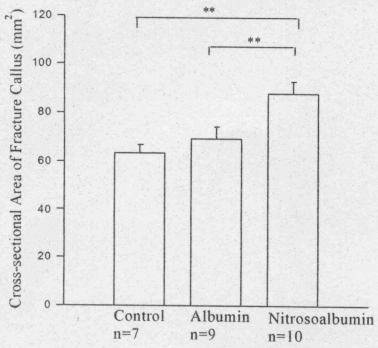
Methods:

Nitrosoalbumin was synthesized by reducing sulfhydril groups of albumin followed by the removal of reducing agent and nitrosylation of reduced albumin with sodium nitrite in an acid environment. Nitrosothiol and protein content of nitrosoalbumin solution was then measured. As intermolecular disulfides of albumin were disassembled before nitrosation, nitrosothiol content was approximately three times higher than protein content.

Animal Model: 26 male Sprague-Dawley rats weighing 358±25 g underwent a right femoral mid-shaft osteotomy performed using a gigli saw and fixed with a 1.6mm diameter K-wire across the fracture site. A gelatine sponge (5mm diameter x 1mm thick) was implanted between the fracture ends. Prior to the implantation, the gelatine sponge was soaked with 25µL phosphate buffered saline (PBS) containing either 4 nmol albumin (albumin group), or 12 nmol nitrosoalbumin (nitrosoalbumin group). Control group received PBS alone with the gelatine sponge carrier. All rats were sacrificed on day 18 and their right and left hind limbs harvested.

Cross sectional area (CSA): CSA of the callus was determined by measuring the callus diameter across in the medio-lateral and antero-posterior plane using a vernier caliper. CSA of the un-operated left femur mid-shaft was determined as an internal control.

Fig 1. Mean values and standard errors of callus cross-sectional area on day 18 (**= p=< 0.01).



Bone mineral density (BMD): Dual energy x-ray absorptiometry (DEXA) analysis was performed to estimate BMD (g/cm^2). An 8 mm x 7 mm rectangle region of interest (ROI) was centred on the fracture site as a representative of callus mineral density. Mineral density of left un-operated femur was measured as an internal control.

Biomechanics: Using a specialised jig, each end of the bone was centralised in concentric aluminium tubes (set apart by a constant distance of 15 mm) and fixed with PMMA cement. Specimen were gripped in a biaxial INSTRON testing system with consistent orientation, and loaded to failure torsionally at a rotational rate of 10 deg/sec. Maximum torque (Nm), torsional stiffness (Nm/deg), and total energy (Nm.deg) were determined from the torque-rotation plots.

Statistics: All data are presented as mean±SE. Differences between experimental groups were assessed using unpaired two-tailed Student's *t*-tests, analysis of variance (ANOVA), and Fisher's LSD post hoc test. The level of significance was set at $p<0.05$.

Results:

The half-life of 12 nmol nitrosoalbumin/ 25µL PBS solution was determined as 5 hours at 37 °C, pH 7.4, in vitro and 12 nmol NO had been released over 240 hours.

Local administration of a single dose of the nitric oxide donor nitrosoalbumin increased callus CSA by 40% compared to the control ($p<0.01$) and 30% compared to the albumin group ($p=<0.01$). There was no significant difference between control and albumin groups (Fig. 1).

Callus mineral density of the albumin group showed a downward trend by 10% to 15% when compared to the control ånd the nitrosoalbumin group respectively.

Torsional failure of right femora occurred through the original fracture site. The initial loading, stiffness, and maximum torque were lower than intact femur (~1/10), consistent with early stage fracture healing. The nitrosoalbumin group showed 70% greater maximal torque than the control group ($p=<0.01$), 130% and 60% greater energy absorption than the control ($p=<0.001$) and albumin ($p=<0.01$) groups respectively, signifying the improved fracture healing (Table 1). There were no differences in the biomechanical properties of the contralateral intact femora.

Table 1. Biomechanical properties of operated femora on day 18 shown as mean ± SE. [C=Control, A=Albumin, N=Nitrosoalbumin, **=p<0.01 (C/N), ***= p<0.001(C/N), ††= p<0.01 (A/N)].

Parameters	Control n=7	Albumin n=9	Nitrosoalbumin n=10
Max Torque x10^{-3} (Nm)	65 ± 10	83 ± 9	109 ± 1 **
Deformation (degrees)	88± 31	76 ± 23	84.0 ± 29
Stiffness x 10^{-3} (Nm / degree)	0.8 ± 0.2	1.3 ± 0.3	1.5 ± 0.2
Total Energy (Nm x degrees)	4.2 ± 2.3	5.9 ± 2.6	9.5 ± 3.3 *** ††

Discussion:

A single intra-operative local administration of the NO donor nitrosoalbumin via gelatine sponge carrier improved callus cross-sectional area by 40% and torsional mechanical properties two fold at 18 days rat femoral healing fracture. This improvement was due to NO, since albumin alone had no significant effects on fracture healing. Nitrosoalbumin may be a cost-effective treatment to accelerate fracture healing in the future.

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