AKI AND STEM CELLS

**Introduction and Aims:** Adult renal progenitor cells (RPCs) may contribute to repair processes featuring acute kidney injury (AKI). Bone morphogenetic proteins (BMPs) regulate differentiation, modeling and regeneration in several tissues. Aim of the study was to evaluate the biological actions of BMP-2 in ARPCs in an human model of AKI in vitro and in vivo.

**Methods:** BMP-2 and BMP-2 receptors gene (RT-PCR) and protein (ELISA/immunoblotting/immunoﬂuorescence) expression were evaluated in ARPCs isolated from human adult kidney (magnetic cell sorting). Intracellular reactive oxygen species (ROS) generation was measured by 2',7'-DCF. Nox4 protein expression was studied by immunoblotting and gene silencing (siRNA). BMP-2, CD133, a-SMA and Nox4 protein expression was evaluated by confocal microscopy in graft biopsies of renal transplant recipients with delayed graft function (DGF), a model of AKI.

**Results:** BMP-2 was expressed in ARPCs of normal adult human kidney and it was up-regulated in vivo after DGF (p=0.02). ARPCs expressed BMP Receptors suggesting their potential responsiveness to BMP-2. Incubation of ARPCs with this growth factor enhanced ROS production (p=0.03), NADPH oxidase activity (p=0.01) and fibronectin (p=0.04) protein expression in cultured ARPCs. Moreover, in vivo we observed an increased co-localization of a-SMA with CD133 after DGF along with a significant decrease of CD133/CD24 co-expression, featuring ARPCs. The halving of the CD133 stem cell marker expression following BMP-2 stimulation along with a parallel increase in the expression of alpha-smooth muscle actin (αSMA) confirmed that ARPCs can undergo a real myofibroblastic transformation. In vitro, the oxidative stimulus (H2O2) induced a-SMA expression in ARPCs (p=0.03), while the anti-oxidant N-acetyl-cysteine inhibited BMP-2-induced a-SMA expression (p=0.04). Nox4 silencing abolished BMP-2-induced NADPH oxidase activation and myofibroblastic phenotype induction (p=0.01).

**Conclusions:** We demonstrated that: a) ARPCs express BMP-2; b) this expression is increased in a model of AKI; c) BMP-2 may induce the commitment of ARPCs towards a myofibroblastic phenotype in vitro and in vivo; d) this pro-fibrotic effect is mediated by Nox4 activation. Our ﬁndings suggest a novel mechanism linking AKI with progressive renal damage.

**FO002 ENDOTHELIAL-TO-MESENCHYMAL TRANSITION IN SWINE RENAL ISCHEMIA-REPERFUSION INJURY IS MEDIATED BY COMPLEMENT AND AKT PATHWAY**

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**Introduction and Aims:** EndMT significantly contributes to the development of tissue fibrosis. However, the pathogenic factors and signaling pathways regulating EndMT are poorly understood.

**Methods:** In an experimental model of I/R, 10 pigs underwent 30 min of renal warm 1 and 24h of R. Recombinant human C1-inhibitor was administered in 5 pigs before R. CD31, alpha-SMA and FSP1 protein expression were investigated by confocal microscopy.

**Results:** Renal I/R injury reduced the density of peritubular capillaries (24h 1.2±0.41 vs 24h 1.4±0.31, p=0.03), signiﬁcantly increased the interstitial expression of the myofibroblast marker a-SMA (24h 5.1±0.58 vs t0 1.7±0.13, p=0.04) and induced the generation of CD31+/FSP1+ cells (24h 18.9±4.4 vs t0 8.2±3.0, p=0.04) after 24h of R. These events were associated with a signiﬁcant increase in Akt phosphorylation in endothelial cells. Inhibition of the Complement system activation by C1-inhibitor, reduced CD31+/FSP1+ cell number (24h C1-inh 7.7±2.1, p=0.03 vs t24h ctr), preserved peritubular capillaries density (24h C1-inh 2.15±0.2 vs p=0.05 vs t24h ctr) and abolished a-SMA interstitial expression.

Interestingly, incubation of cultured endothelial cells with C3a led to EndMT, as shown by a decrease in vonWillebrand-Factor expression (C3a 4.6±0.8 vs basal 7.3±0.6, p=0.04) and an increased a-SMA expression (C3a 0.9±0.1 vs basal 0.3±0.1, p=0.03) along with a signiﬁcant increase of Akt phosphorylation. C3-induced a-SMA expression was signiﬁcantly reduced by Akt inhibition (C3a+Akt inhibitor IV 0.6±0.1 vs C3a 0.3±0.1, p=0.03). Accordingly, inhibition of Complement in vivo by C1-inhibitor led to the abrogation of Akt phosphorylation in renal endothelial cells. **Conclusions:** Our data demonstrate a critical role for Complement in the acute induction of the EndMT via the Akt pathway. Therapeutic inhibition of these pathways may be essential to prevent the development of vascular-derived tissue fibrosis.

**FO003 RENOPROTECTIVE EFFECTS OF IN VIVO TREATMENT WITH DNASE IN A RAT MODEL OF ISCHEMIA-REPERFUSION-INDUCED ACUTE KIDNEY INJURY.**

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**Introduction and Aims:** Acute renal injury (AKI) is a major complication of systemic lupus erythematosus (SLE). SLE is manifested by impaired apoptosis, resulting in massive DNA damage and cell death within the injured organs. In healthy tissues, the remnants of the destroyed nuclear acids are cleared by DNases, the enzymes responsible for degradation of damaged DNA and elimination of DNA debris. However, in SLE patients, including those with lupus nephritis-induced AKI, the activity of DNase is inhibited. In early sixties, exogenous administration of DNase has been proposed as a therapeutic measure for SLE patients with low DNase activity. By analogy, DNase might also accelerate the elimination of dead cells and cell debris from severely injured rat kidneys. We tested the possibility that in vivo administration of DNase might exert renoprotective effects, preventing AKI development in a model of experimentally induced ischemia/reperfusion.

**Methods:** 48 Sprague-Dawley rats were subjected to left unilateral nephrectomy, with simultaneous clamping of contralateral kidney aorta for 60min. Control rats underwent surgery of similar duress and duration, but without clamping; thus, their contralateral kidneys remained non-ischemic. Part of the rats was injected i.p. with DNase prior to discontinuing anesthesia. Concentrations of the injected DNase varied from 16mg up to 96mg per rat. On sacrifice, blood samples were procured for DNase activity and renal function evaluation. The kidneys were allocated for pathologic examinations. In some animals, renal perfusion was evaluated by Lazer Doppler technique.

**Results:** Cystatin-C was significantly augmented in ischemic animals not treated with DNase. In DNase-receiving rats, a significant dose-dependent decrease of cystatin-C was evident. Renal perfusion, evaluated by Lazer Doppler technique, was significantly increased in DNase treated animals. At a fixed dose (32 mg/mg DNase injection), serum DNase activity was time-dependently increasing. DNase activity peak was recorded after 6h, gradually decreasing thereafter. No surplus DNAase activity was detected following 48h. Histopathologic evaluation revealed significantly decreased percentage of tubular necrotizing cells, intra-tubular epithelial sloughing, significant decrease of extracellular matrix deposition and number of damaged tubule per total tubule count in kidneys from DNase-treated animals. Immunohistochemical staining with anti-single strand DNA antibody demonstrated significantly decreased presence of damaged DNA strands in DNase treated animals.

**Conclusions:** Exogenous DNase injection brought about enhanced DNase activity and attenuated AKI in kidneys of ischemic rats. Concomitantly, their renal functioning significantly improved.

At optimal DNase concentrations, well tolerated both by healthy and ischemic animals, renal functioning of ischemic rats was close to the values of healthy controls. 3. Histopathologic evaluation demonstrated decreased signs of renal tissue damage, in accordance with the improvement of renal functioning and the increase in renal perfusion.
THE EFFECT OF BONE MARROW MESCENHYMAL STEM CELLS (BMSC) OR CONDITIONED MEDIUM (CM) IN RATS WITH ACUTE KIDNEY INJURY (AKI) INDUCED BY LIPOPOLYSACCHARIDE (LPS).

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Introduction and Aims: Sepsis is characterized by a severe inflammatory response to infection, and its complications, including AKI, can be fatal. Studies indicate that the BMSC can act on several levels of endogenous repair to bring about resolution of diseases and its mechanism of action may be due to paracrine modulation and conditioned medium. The aim of this study is to evaluate the BMSC or CM effect at cellular modulation and/or repairment on LPS in rats.

Methods: The BMSC were collected from the tibia and femur of adult male Wistar rats, characterized by flow cytometry and differentiated into osteocytes and adipocytes. For the LPS protocol, the female Wistar rats received LPS (10mg/Kg/BW) (LPS group) or water (CTL) in a single dose i.v. (N=10/group). After 24 or 72 hr, the female rats received i.v. BMSC (1X106cells) injection or CM (500μl), 1 or 3 doses via the tail vein. Samples of blood and urine 24 hours were collected to creatinine (Cr), urea (U) and FENa evaluations. The kidneys were perfused and removed for HE, Ki67, caspase 3 and Y chromosome analysis.

Results: There was an increased in Cr (1.7±0.04; 2.2±0.04) and a decrease in FENa (0.7±0.03; 0.6±0.02) in the groups LPS24or72h when compared to CTL24,72h (0.6±0.04; 0.6±0.02; 2.6±0.02; 2.5±0.01; p<0.05; respectively).

In the animals treated with BMSC or CM, the creatinine decreased significantly (0.9±0.01; 1.1±0.03) followed by an increase in FENa (1.7±0.04; 2.0±0.03). There was no difference in Cr (0.9±0.01 vs. 0.9±0.03) and in FENa (1.7±0.04 vs. 2.2±0.02) between the groups receiving BMSC and CM, respectively. However the protective effective shown in these groups was intensified when the doses were given 3 times. In LPS-group the kidneys showed a small marked Ki67 and intense caspase 3 expression but it was highly marked for Ki67 and lower expression for caspase 3 in LPS-BMSC and LPS-CM groups. However, a striking difference was observed in the BMSC or CM treated animals where the presence of Ki67 and Y chromosome was detected and no histological ATN lesions were observed. This effect was maximized when the doses of BMSC or CM were higher.

Conclusions: These results strongly suggest that BMSC or CM can minimize AKI in this sepsis model. This therapeutic effects have a significant impact on renal function observed holds substantial promise for its use in this pathological situation with high morbidity and mortality.