AHDMRT

Australasian Hyperbaric & Diving Medicine Research Trust

FINAL REPORT

PROJECT TITLE

Effects of hyperbaric oxygen and aminoguanidine treatment on femoral head osteonecrosis in a rat model

Grant Agency: AH+DMRT

Total support: $10,000

INVESTIGATORS

Chief Investigator

Professor Ross Crawford, MBBS (QLD) 1987, FRACS 1997, DPhil (Oxon) 2000
Chair in Orthopaedic Research IHBI, QUT
Orthopaedic consultant at The Price Charles Hospital
Address: Kelvin Grove, Q Block - IHBI Level 7, 703G T
Telephone: +61 7 3139 4481 Fax: 3139 4043 Email: r.crawford@qut.edu.au

Other investigators:

- Professor Yin Xiao, PhD, (UQ), 2000, MDSc (Wuhan) 1991, BDSc (Wuhan) 1986
  Leader, Bone Biology group, IHBI, QUT
  Address: Q Block – IHBI level 7, Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, Qld 4059
  Telephone: 073138 6240 Fax: 3138 6030 Email: yin.xiao@qut.edu.au
- Dr Ali Kalhor Mogahddam, MD (shaheed Beheshti medical University), Master of research student (Queensland university of technology)
  Orthopaedic registrar, The Prince Charles hospital
  Address: The Prince Charles hospital, Rode Rd, Chermside, QLD, 4032
  Telephone: 0731395168 Fax: 0731394250 Email: akalhormoghaddam@yahoo.com
- Professor Dietmar Hutmacher, *PhD* (Natl Univ Singapore), MBA (Henley)
  Chair in Regenerative MedicineIHBI, QUT
  Address: 60 Musk Avenue, Kelvin Grove QLD 4059
  Telephone: 3138 6077 Fax: 073138 6030 Email: dietmar.hutmacher@qut.edu.au
- Dr Travis Klein, PhD (University of California – San Diego, USA 2005)
  Postdoctoral Research Fellow, IHBI, QUT
  Address: 60 Musk Ave, Kelvin Grove, Qld 4059, Australia
  Telephone: 3138 6142 Fax: 3138 6030 Email: t2.klein@qut.edu.au

AIMS OF THE PROJECT

1. Hyperbaric oxygen (HBO) and/or Aminoguanidine (AMG) reduce osteonecrosis in idiopathic osteonecrosis of rat femoral heads.
2. HBO and AMG will act to reduce osteonecrosis by reducing osteoblast apoptosis and expression of inducible nitric oxide synthase (iNOS)

Specific Aims:

1. To determine if HBO and/or AMG prevent idiopathic osteonecrosis in a rat model
2. To elicit mechanisms of HBO and AMG action in osteonecrosis by investigating
   a. osteocyte apoptosis in an established model of osteonecrosis
   b. expression of eNOS and iNOS in an established model of osteonecrosis

PROGRESS AND RESULTS

Apoptosis is the final destiny of many cells in the body, though this process has been observed in some pathological processes. One of these pathological processes is femoral head non-traumatic osteonecrosis. Among many pro/anti-apoptotic factors nitric oxide has been recently an area of further interest. The osteocyte apoptosis and its relation to proapoptotic action of inducible form of nitric oxide synthase (NOS) which produce high concentration of nitric oxide have been flagged. The aim of this study was to investigate the effect of hyperbaric oxygen (HBO) and inducible NOS suppressor (Aminoguanidine) in prevention of femoral head osteonecrosis in a experimental model of osteonecrosis, spontaneous hypertensive rats (SHRs).

After animal ethic approval 34 SHR rats were divided into four groups. Ten rats were allocated to control group (who did not received any treatment), and eight rats allocated to three treatment groups namely: HBO, Aminoguanidine (AMG), and the combination HBO and AMG treatments (HBO+AMG). The HBO group received 250 kPa of oxygen through hyperbaric chamber for 30 days started at their 5th weeks of life, the AMG group received 1mg/ml of AMG in drinking water from 5^{th} weeks till 17^{th} weeks of life, and the last group received combination of these treatments. Rats were sacrificed at the end of 17^{th} weeks of life and both femurs were analysed for evidence of osteonecrosis using Micro CT scan, and H&E staining. Also, osteocyte apoptosis and presence of two different forms of NOS (inducible (iNOS) and endothelial (eNOS)) were analysed by immunostaining and apoptosis staining (Hoechst and TUNEL).

Bone morphology of metaphyseal and epiphyseal area of all rats were investigated and analysed. Micro CT findings revealed significantly higher mean fractional trabecular bone volume (FBV) of metaphyseal area in untreated SHRs compared with all other treatments (HBO, $P<0.05$, HBO+AMG, $P<0.005$, and AMG $P<0.001$). Bone surface to volume ratio also significantly increased with HBO+AMG and AMG treatments when compared with control group (18.7 Vs 20.8, $P<0.05$, and 18.7 Vs 21.1, $P<0.05$). Epiphyseal mean FBV did not change significantly among groups. In metaphyseal area trabecular thickness and numbers significantly decreased with AMG treatment, while trabecular separation significantly increased with both AMG and HBO+AMG treatment.

Histological ratio of no ossification and osteonecrosis was 37.5%, 43.7%, 18.7% and 6.2% of control, HBO, HBO+AMG and AMG groups respectively with only significant difference observed between HBO and AMG treatment ($P<0.01$). High concentration of iNOS was observed in the region of osteonecrosis while there was no evidence of eNOS activity around that region.

In comparison with control group, ratio of osteocyte apoptosis significantly reduced in AMG treatment ($P<0.005$). We also observed significantly fewer apoptotic osteocytes in AMG group comparing with HBO treatment ($P<0.05$).

CONCLUSION

None of our treatments prevents osteonecrosis at histological or micro CT scan level. High concentration of iNOS and significant reduction of osteocyte apoptosis with AMG treatment were supportive of iNOS modulating osteocyte apoptosis in SHRs.

Budget:
A total of $10,000 has been used for animal experiments, micro-CT, immunohistochemistry, and histology.

A research paper will be submitted and the AHDMRT foundation will be informed and acknowledged.

---

1 August 2013