Canine Adipose-derived MSCs are Neurotrophic and Angiogenic: Pre-clinical Assessment of their Regenerative Activity Leading to Clinical Trials in Dogs

*[1]IR Al-delfi, [1]X Zhang, [2]CA Sharp, [2]P Myint, [2]JF Innes, [1]WEB Johnson

[1]School of Life & Health Sciences, Aston University; [2]Veterinary Tissue Bank, UK

*aldelirt@aston.ac.uk

Introduction

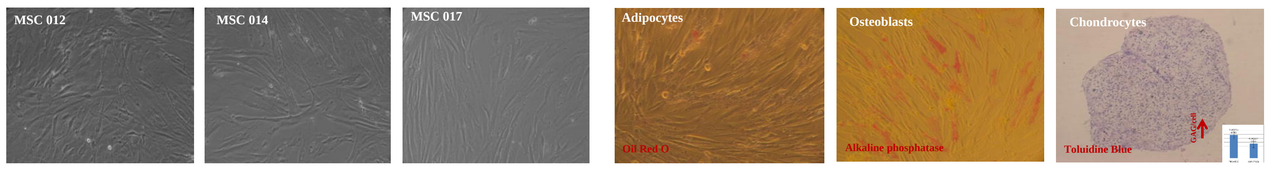
- Mesenchymal stem/stromal cells (MSCs) are multipotent cells with capacity to differentiate along mesodermal lineages, which can be sourced from adult bone marrow (BM) and adipose tissue (AD)
- MSCs possess important regenerative activity through paracrine effects on endogenous cells present at wound sites, which has been well documented for BM MSCs, but is less well known for AD MSCs [1,2]
- The paracrine activity of MSCs has advantage in cell therapies for CNS damage, such as spinal cord injury (SCI) and stroke[2-3]
- Dogs suffering from natural SCI represent a target patient group in which to examine the safety and efficacy of MSC transplantation therapies

Materials & Methods

- Stromal cells were cultured from inguinal fat pads of dogs and characterised according to ISCT guidelines by appearance, flow cytometry and differentiation potential.
- These MSC cultures were used to generate serum-free culture conditioned medium (MSC CM) at low and high passage
- E10 chick DRG neurons or SHSY5Y neuroblastoma cells were seeded in 24 well plates containing MSC CM versus serum-free control medium
- Scratch assays of EaHy926 endothelial cells were performed in the presence of MSC CM versus control medium. EaHy-926 cells were also seeded onto culture wells pre-coated with MSC CM or control medium

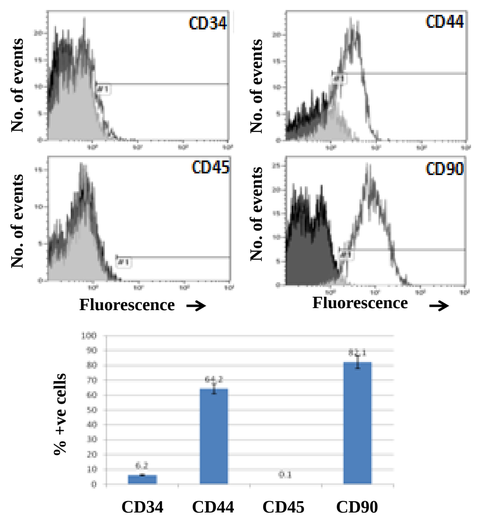
STUDY AIM: With a view to developing an MSC transplantation therapy for dogs with SCI, this study has investigated the paracrine activity of canine AD MSC on neurons and endothelial cells. In addition, we have examined the influence of MSC ageing in culture on such paracrine activity

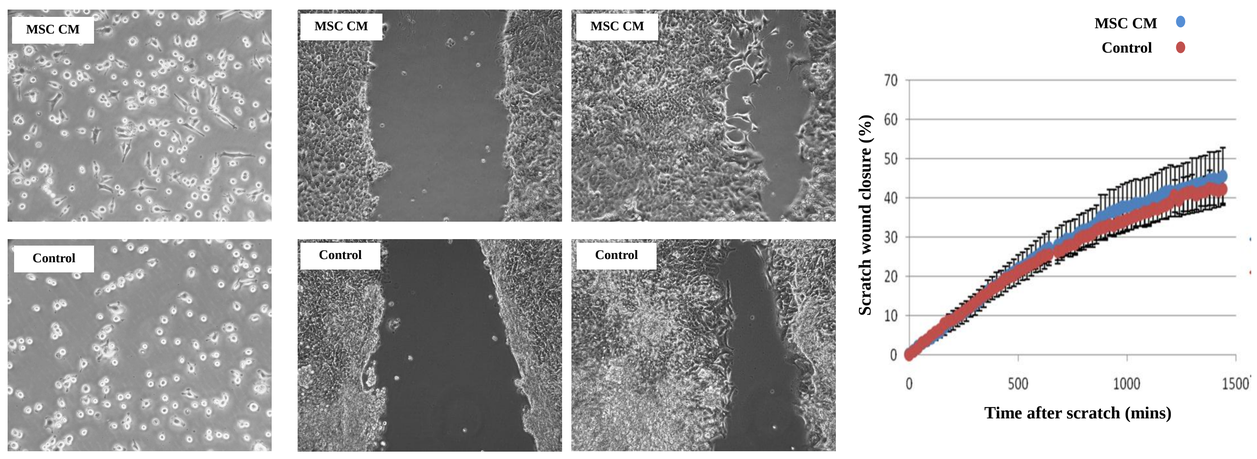
Results



Characterisation of canine AD MSCs

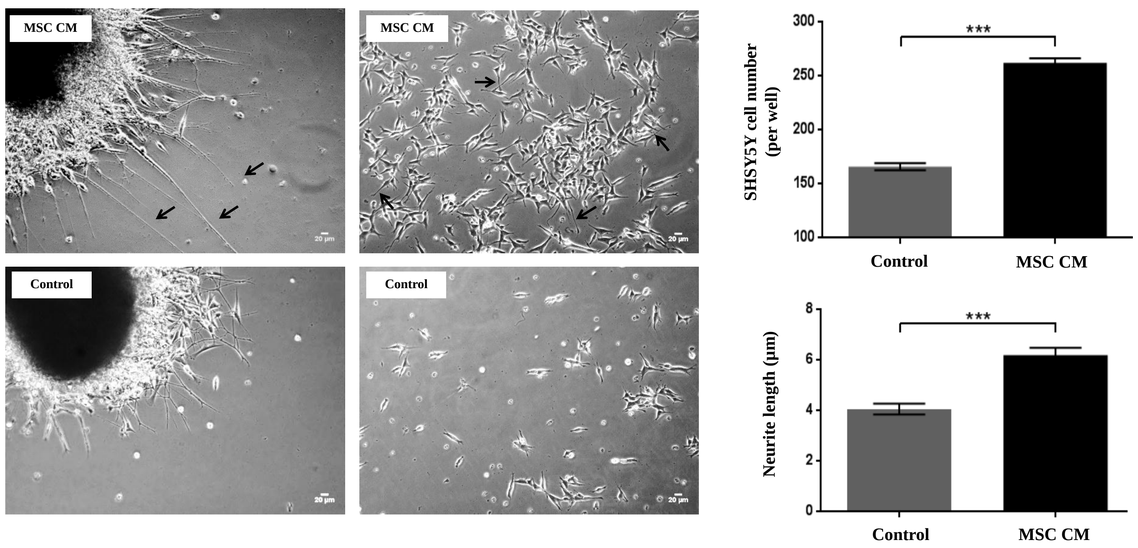
By passage 2-3, canine adipose-derived cells were from 3 donors were plastic adherent and stromal (above left panels), largely CD34-ve/CD45-ve, CD44+ve and CD90+ve (right panels). These cells differentiated to some extent along 3 mesenchymal lineages (above right panels; inset GAG/cell - a quantitative measure of chondrogenic differentiation. Data shown are means ± SD.





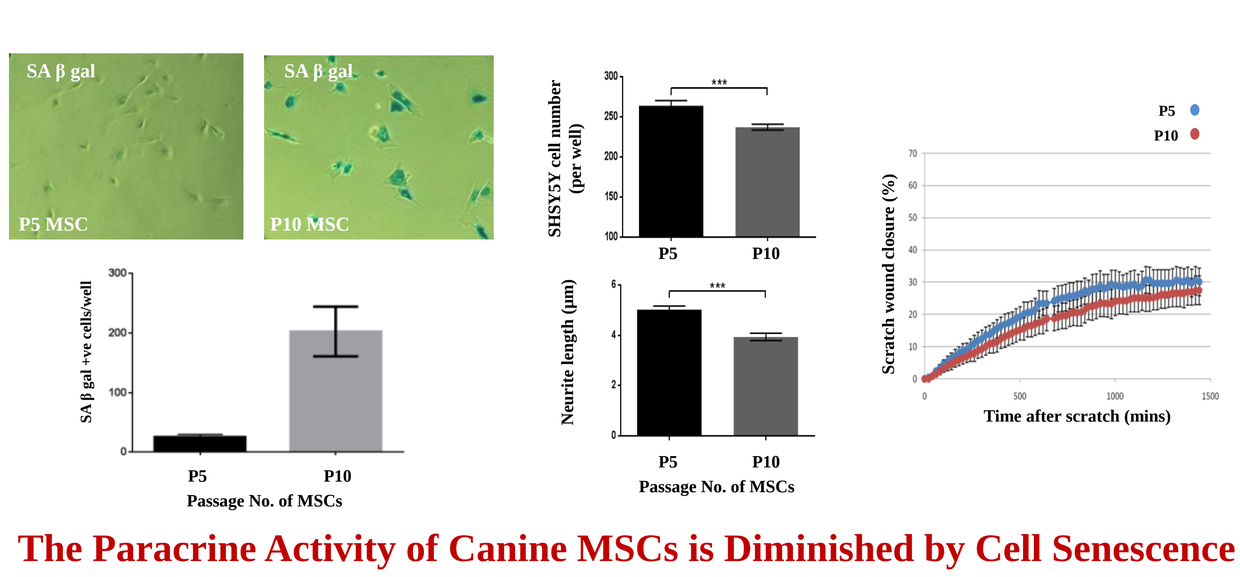
Canine AD MSCs secrete angiogenic factors

Conditioned medium from cultures of canine AD MSCs stimulated EaHy-926 endothelial cell adhesion (above left panels) and EaHy-926 cell migration/proliferation in scratch wound assays (above middle and right panels). The MSC CM was coated to culture plastics to enhance cell adhesion, whilst MSC CM was added in solution to the scratch assays. There was a significant increase in the rate of wound closure in MSC CM versus control medium ($p<0.001$; non parametric ANOVA). Data shown are means ± SD.



Canine AD MSCs secrete neurotrophic factors

Conditioned medium from cultures of canine AD MSCs stimulated neurite outgrowth from DRG neurons (above left panels, arrowed) and SHSY5Y cells (above middle panels, arrowed). There were significant increases both in the number of SHSY5Y cells present in MSC CM versus control medium and also in the extent of neurite outgrowth (above right panels). Data shown are means ± SD. ***$p<0.001$



The Paracrine Activity of Canine MSCs is Diminished by Cell Senescence

Culture expanding canine AD MSCs was associated with decreased proliferation and increased prevalence of SA β galactosidase positive cells (above left panels). Furthermore, the conditioned medium from these senescent MSC cultures was significantly less stimulatory for SHSY5Y neurons (above middle panels; ***$p<0.001$; Mann Whitney *U* test) and EaHy-926 endothelial cells in scratch wound assays (above right panels; $p<0.001$; ANOVA). Data shown are means ± SD.

Discussion

- **These data support the use of adipose-derived MSCs for cell therapies in dogs with CNS damage, e.g. SCI.**
- **Secreted factors from canine MSCs were neurotrophic for DRG neurons and SHSY5Y neuroblastoma cells. In addition, MSC secreted factors enhanced the adhesion and migration/proliferation of EaHy926 endothelial cells, which would also be expected to enhance angiogenesis and hence would healing.**
- **MSCs are known to secrete soluble growth factors/chemokines, e.g. NGF, BDNF, VEGF, as well as permissive ECM components[3,4], which may account for their trophic activity. MSCs are also immunodulatory through their secretion of cytokines[5,6]. Potential cross species problems of ligand-receptor engagement/effect, have not emerged when translated into single species studies.**
- **However, the study has demonstrated for the first time that the culture expansion of canine MSCs diminishes their paracrine secretome activity. This suggests that earlier treatment with MSCs would likely maximise potential benefit.**

References: [1]: Caplan AI & Corea D (2010) *Cell Stem Cell* 9: 11-15. [2] Paul G & Anisimov SV (2013) *Biochimie*, 95: 12. 2246–2256. [3] Wright KT et al., (2011) *Stem Cells* 29, 169-178. [4] Walter *et al.*, (2010) *Exp. Cell Res.* 316: 1271-81. [5] Bai *et al.*, (2012) *Nature Neuroscience* 15: 862-70. [6] Nakajima *et al.*, (2012) *J Neurotrauma* 29: 1614-1625.