PROCEEDINGS OF THE SIXTH CONFERENCE ON CURRENT VETERINARY PRACTICES

In collaboration with:

frontiers Abstract Book

The Conference on Current Veterinary Practices aims at bringing equine veterinarians together to discuss the latest insights in veterinary surgery, medical imaging, drug treatment and horse care. In the present conference we highlight the latest developments in veterinary regenerative medicine. There will be a focus on the use and the mode of action of these novel treatment modalities in correlation with the different stages of osteoarthrosis development based on results from clinical trials.

The conference is registered with the Royal Dutch Veterinary Society (KNMvD), and therefore, study points will be acquired. Partially supported by an educational grant from GST-Anacura, Eickemeyer, and Orthopaedics.be.

PROCEEDINGS OF THE SIXTH CONFERENCE ON CURRENT VETERINARY PRACTICES

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First Conference on Current Veterinary Practices: Tom Mariën, Equitom Equine Hospital, Meldert-Lummen, Belgium

Second Conference on Current Veterinary Practices: Jan H. Spaas, Global Stem cell Technology (GST) - part of Anacura, Evergem, Belgium

Third Conference on Current Veterinary Practices: Eickemeyer BV, BJ Culemborg, The Netherlands

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Fifth Conference on Current Veterinary Practices: Katilyne Ghijselings, Orthopaedics, Oostkamp, Belgium

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Sixth Conference on Current Veterinary Practices:
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ORAL PRESENTATIONS

Venue: Europaboulevard 10, 1083 HH Amsterdam, The Netherlands

Dealing with Inflammation – the Effect on Stem Cells and Relevance for OA in Horses

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Inflammatory cytokines are present in the osteoarthritic joint of horses and their negative effects on chondrocytes are well documented. Treatments are therefore often based on methods that aim to modulate inflammation. Our work on equine tendon injury has demonstrated that different types of stem cells have different fates and mechanisms of action in injured tissues. Pluripotent equine embryonic stem cells (ESCs) differentiate into tenocytes and exhibit a high survival following injection (Barsby et al., 2014) but, in contrast, mesenchymal stem cells (MSCs) exhibit very low survivals (Barsby et al., 2014). MSCs are now believed to function by modulating the inflammatory reaction (Bavin et al., 2015, 2017) rather than direct differentiation (Becerra et al., 2013; Broeckx et al., 2014a).

In the UK only autologous MSCs are approved for use. The generation of autologous MSCs requires the isolation of a tissue sample from the horse, followed by 2-4 weeks of culture expansion to produce sufficient numbers of MSCs, prior to their injection back into the horse from which they were derived. This prevents the immediate treatment of acute injuries and makes it very difficult to standardise, given the donor variability of MSCs (Broeckx et al., 2014b; Caplan, 2017).

We and others have demonstrated that equine MSCs are immune privileged and fail to induce the proliferation of allogeneic lymphocytes *in vitro* (Bavin et al., 2015, 2017; Carmona et al., 2016). This suggests that they would be safe to use in allogeneic transplantations *in vivo* and supports the data available to date on the clinical application of allogeneic MSCs which have not been associated with any negative effects (Frisbie et al., 2007; Guest et al., 2008; Carrade et al., 2012; Carrade Holt et al., 2014; Carter-Arnold et al., 2014; D'Angelo et al., 2017). However, other work has reported that MHC mis-matched MSCs can produce immune responses *in vitro* (Guest et al., 2010) and *in vivo* (Hraha et al., 2011; Ionita et al., 2016) and it remains to be determined if the clinical efficacy of MSCs might be impaired by using allogeneic cells.

IL-1β is an inflammatory cytokine that is upregulated in both the OA joint and following a tendon injury. Our work has demonstrated that, as in chondrocytes (Lacey et al., 2009; Kol et al., 2015; Jacobsen et al., 2016), IL-1β has negative effects on equine tendon cells, producing a dramatic upregulation in matrix metalloproteinase (MMP) gene expression and causing a significant decrease in the expression of tendon associated genes scleraxis and cartilage oligomeric matrix protein (COMP). Using our established 3D culture model to generate artificial tendons (Manning et al., 2015; Martino et al., 2016; Mirza et al., 2016) we have further demonstrated that IL-1β exposure results in reduced matrix remodelling by adult equine tenocytes. This effect is neutralised by the addition of an IL-1 receptor antagonist (IL-1Ra).

We have previously demonstrated that equine MSCs can reduce the expression of inflammatory cytokines such as IFN-γ and IL6 by stimulated peripheral blood mononuclear cells (PBMCs) (Bavin et al., 2017). However, work by others has shown that MSCs are not able to modulate IL-1β production by inflammatory macrophages or protect tendon cells from its effects (Owens et al., 2016). IL-1β has negative effects on MSCs themselves (Paterson et al., 2014; Schnabel et al., 2014; Pezzanite et al., 2015) and inhibition of IL-1β signalling can promote MSC mediated tissue regeneration (Sullivan et al., 2014). Therefore it is not clear if the clinical application of MSCs to inflammatory OA will be sufficient to protect cartilage cells from the damaging effects of IL-1β.

In horses, IRAP (interleukin receptor antagonist protein, also known as autologous conditioned serum (ACS)) and PRP (platelet rich plasma) have been used to treat OA. However, the results have been variable (Textor, 2011; Van Loon et al., 2014; Svala et al., 2015; Textor and Clark, 2018) and as biological products the actual amount of IL-1Ra has been shown to vary between donors, IL-1β levels themselves are increased compared to non-conditioned serum, and many other cytokines are also present (Wehling et al., 2009; Williams et al., 2013; Xie et al., 2014).

In contrast to adult tendon cells, ESC-derived tendon cells appear to be protected from the effects of IL-1β. They exhibit no change in their expression of MMPs or tendon-associated genes, and their ability to remodel a collagen matrix to generate artificial tendons *in vitro* is not impaired by the presence of IL-1β. Mouse ESCs and their differentiated derivatives have also been shown to be unaffected by IL-1β (Zayed et al., 2017).

Understanding the molecular basis for these observations may lead to the future development of novel inhibitory synthetic compounds that would be cheaper to manufacture, standardised and offer a more targeted approach than a cell based therapy. Of course other inflammatory cytokines also have a role in OA and future work to

determine their specific effects is required to develop treatments that can modulate their levels or interfere with their signalling pathways.

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Author Biography

Debbie Guest did her undergraduate degree in Genetics at the University of Leeds. She remained at the University of Leeds to do her PhD, studying the transcriptional control of neural stem cell differentiation with Professor's Noel Buckley and Alan Handyside. Following the completion of her PhD in 2005 she started a post-doctoral position at the Equine Fertility Unit in Newmarket with Professor Twink Allen. In this post she continued previous work to derive and characterise equine embryonic stem cells and began a project to determine their therapeutic potential in tendon repair. In 2007, following the closure of the Equine Fertility Unit, Dr Guest moved her project to the Animal Health Trust in Newmarket where she is now the Head of Stem Cell Research. Her research group continues to work on equine embryonic stem cells and their potential in tendon repair, but they have also derived induced pluripotent stem cells from horses and dogs. These cells are currently being used establish laboratory disease-modelling tools for fracture in horses and corneal dystrophies in dogs. http://www.aht.org.uk/cms-display/science_stemintro.html

A Placebo Controlled Study Evaluating the Effects of Allogeneic Chondrogenic Induced Mesenchymal Stem Cells to Treat Osteoarthritis in Horses

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To date osteoarthritis (OA) is still a debilitating disease in horses with a high morbidity and hallmarked by cartilage degeneration (van Weeren and de Grauw, 2010; Mcilwraith et al., 2012). The associated pain often forces the owner to retire its horse early from an athletic career or pleasure riding (Goodrich and Nixon, 2006; de Souza, 2016). Current treatment modalities focus on assessing the symptoms of the disease, but do not present a durable solution (Goodrich and Nixon, 2006). Therefore, regenerative medicine is gaining increased interest as a new treatment modality for OA (Wilke et al., 2007; Frisbie et al., 2009).

Regenerative medicine comprises among others stem cell based therapy (Malone, 2002; Whitworth and Banks, 2014). From a legal perspective stem cells are defined as an active substance and are therefore a veterinary medicinal product according to the European law. Thus, not only their production, and marketing are subjected to strict regulations, but clinical trials using stem cell based products are also heavily regulated (e.g. they have to be performed according to good clinical practice (GCP), presence of placebo control group, random allocation of the treatments, etc.) (Faltus and Brehm, 2016). From a scientific perspective stem cells are defined as cells with a capacity of self renewal, which are able to differentiate into different cell types (Horvitz and Herskowitz, 1992; Fortier, 2005; Semedo et al., 2011; Spaas et al., 2013; Whitworth and Banks, 2014). Based on their origin they can be divided into two categories: embryonic stem cells (ESCs), which are pluripotent, and adult stem cells, which are multipotent. However, ESCs pose a risk for teratoma formation *in vivo* and their use and production is subjected to various ethical issues. Therefore, most research has focused on the use of adult stem cells as potential therapy (Herberts et al., 2011).

Equine mesenchymal stem cells (MSCs) are a type of adult stem cells, which can be isolated from various tissue sources such as bone marrow, umbilical cord tissue, umbilical cord blood, peripheral blood and adipose tissue (Carrade et al., 2011). They are mostly used to treat orthopedic injuries and for the regeneration of cartilage, bone and tendons (Carrade et al., 2012). In this light, the use of equine MSCs for the treatment of cartilage defects and osteoarthritis (OA) has been the topic of several studies over

recent years (Wilke et al., 2007; Frisbie et al., 2009; McIlwraith et al., 2011; Spaas et al., 2012; Broeckx et al., 2014a,b; Ferris et al., 2014; Whitworth and Banks, 2014) and with their increased use, the safety of MSCs has also been progressively investigated (Carrade et al., 2011, 2012; Pigott et al., 2013a,b; Broeckx et al., 2014a,b, 2018; Ardanaz et al., 2016; Colbath et al., 2017). However, a randomized, double blinded, placebo controlled study investigating the efficacy of allogeneic MSCs to treat OA in the horse is currently lacking.

To be able to investigate the efficacy of any medicinal product on OA in a standardized manner, an experimental model simulating the natural occurring OA is necessary. Presently, the carpal fragment model is the most frequently used experimental OA model in the horse (Foland et al., 1994; Mcilwraith et al., 2012). However, the metacarpophalangeal (MCP) joint is more frequently plagued with OA compared to the carpal joint (Mcilwraith et al., 2012), making it an interesting joint to investigate in clinical studies. Various techniques exist to induce OA in the MCP joint (Gustafson et al., 1992; Simmons et al., 1999; van Harreveld et al., 2002; Goranov, 2011; Boyce et al., 2013; Maninchedda et al., 2015). However each technique has it's own disadvantages, such as slow onset of OA (Boyce et al., 2013), severe inflammation (Gustafson et al., 1992; Goranov, 2011) or permanent instability (Simmons et al., 1999).

Therefore, a study was performed using an improved MCP OA model, to asses the efficacy of equine allogeneic chondrogenic-induced MSCs (ciMSCs) combined with equine allogeneic plasma (EAP) as a treatment for OA. Moreover, to fulfill the requirements of the European Medicine Agency (EMA) the study was performed according to GCP guidelines and all relevant national and international regulations.

The OA model was applied in the right MCP joint of 12 healthy horses and consisted of the creation of an osteochondral (OC) fragment on the dorsomedial aspect of the first phalanx combined with an opposing groove lesion in the cartilage of the third metacarpal bone. All horses were box rested for 1 week, after which they were trained daily on a treadmill for the subsequent study period of 11 weeks. Five weeks after surgery, horses randomly received either the investigational veterinary product (IVP = ciMSCs +EAP) or a 0.9% saline solution (= placebo control: CP) intra-articularly in the right MCP joint. From surgery until the study end (11 weeks), horses underwent a weekly joint assessment, a visual lameness assessment and inertial sensor-based lameness assessment. Pressure plate analysis was performed before surgery, at week 5 and 11 and X-rays were taken of the MCP joints before surgery, day 1 after surgery, at week 5 and 11. Additionally, synovial fluid was collected before surgery, at week 5, week 5 + 1 day, week 7, week 9, and week 11 for biomarker analysis and cytology. At the end of study, all horses were euthanized, and the MCP joints were evaluated macroscopically and histologically.

No serious adverse events or suspected adverse drug reactions were seen during the study. The IVP significantly reduced visual lameness compared to the CP as demonstrated by a significant decrease in AAEP scores and response to flexion. Moreover, a significant decrease of the mean vector sum was seen in the IVP group for the treadmill and after flexion on a straight line as measured with the inertial sensor-based system. There was no significant difference in heat sensation at the local injection site or pain to palpation between the treatment groups. The joint effusion scores were significantly lower in the IVP group compared to the CP group. There were no significant changes in joint circumference or number of radiographic changes between the treatment groups. At the two latest time points a significant higher joint fluid viscosity was noted in the IVP group compared to the CP group. Additionally, a significantly higher glycosaminoglycan concentration was present in the synovial fluid of the CP group. The other cytology parameters or biomarkers were not significantly different between the two treatment groups. Post mortem examination revealed significantly less wear lines and synovial hyperemia in the MCP joint of the IVP group compared to the CP group. Additionally, significantly more Alcian blue uptake was seen in the cartilage adjacent to the OC in the IVP group, together with a higher area percentage of COMP and collagen II. The area percentage of collagen II was also significantly higher in the cartilage adjacent to the groove lesion in the IVP group.

Based on these results, ciMSCs combined with EAP seems a promising treatment for OA in the horse. However, since an experimental model of OA was used to evaluate treatment under standardized circumstances, further research should be done on a larger number of horses with naturally occurring OA.

Keywords: horse, mesenchymal stem cells, osteoarthritis

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- Obtained her degree in Veterinary Medicine at the Ghent University in 1993.
- Worked since 1993 as an assistant at the department of Surgery and Anaesthesiology of the Ghent University, to qualify as an equine and bovine surgeon.
- Obtained her PhD degree in 2000 with a thesis on the diagnosis and treatment of equine sarcoids.

- Became a Diplomate of the European College of Veterinary Surgeons in 2002.
- Present position: full professor at the Department of Surgery and Anaesthesiology of Domestic Animals, Ghent University. Responsible for the Large animal surgical clinic.
- ECVS president 2015–2016. Former member of the ECVS board, program and examination committee.
- Governor of Ghent University 2014–2018.
- Author or co-author of more than 100 publications in peer-reviewed journals and invited in a regular basis as speaker on national and international conferences.
- Present research interests: equine sarcoids, equine wound management and minimal invasive surgical techniques (arthroscopy and laparoscopy).

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