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Equine allogeneic chondrogenic induced mesenchymal stem cells: A GCP target animal safety and biodistribution study

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ABSTRACT

The safety of the intra-articular use of mesenchymal stem cells (MSCs) is scarcely reported. Therefore, the goal of this study was to investigate the safety of a single intra-articular injection with allogeneic chondrogenic induced MSCs combined with equine plasma (= the investigational product: IVP) compared to a saline (0.9% NaCl) placebo control (= control product: CP). Sixteen healthy experimental horses were randomly assigned to receive a single intra-articular injection with either the IVP (n = 8) or the CP (n = 8) in the left metacarpophalangeal joint. All horses underwent a daily clinical assessment throughout the entire study period of 42 days to assess adverse events. Additionally, a local joint assessment and a lameness examination were performed daily during the first two weeks, and weekly the following 4 weeks. Blood samples were taken weekly for hematological and biochemical analysis. At the end of the study period, horses of the IVP group were euthanized for a thorough necropsy and to check for biodistribution. Tissue samples of the injected joint were collected for histological examination. In both CP and IVP treated horses a mild transient subjective increase in periarticular temperature and lameness was noted after the intra-articular injection with no significant differences between the treatment groups. No distribution of the cells was found using immunohistochemistry and no ectopic tissue formation or signs of inflammation were found on histology. A single intra-articular injection of allogeneic chondrogenic induced MSCs combined with allogeneic plasma in horses had the same clinical side effects as an intra-articular injection with saline solution.

1. Introduction

Osteoarthritis (OA) is a well known joint disease in veterinary medicine. The typical characteristics of OA are pain, degeneration of the articular cartilage and changes in the bone and periarticular soft tissues (Goodrich and Nixon, 2006; Malone, 2002; Mcilwraith et al., 2012; van Weeren and de Grauw, 2010). In horses, OA is a frequent cause of lameness, diminished athletic performance and early retirement from an athletic career or pleasure riding (de Souza, 2016; Ferris et al., 2011; Goodrich and Nixon, 2006; Mcilwraith et al., 2012). The high prevalence of the disease and its associated morbidity generates demand for an effective treatment.

Currently, OA is mainly treated by administration of NSAIDs or corticosteroids to reduce symptoms of pain and inflammation (Goodrich and Nixon, 2006; Malone, 2002). However, the use of disease modifying therapies, which prevent, retard or even reverse progression of OA, is gaining popularity. In line with this trend, research on the use of mesenchymal stem cells (MSCs) as a more durable therapy to treat OA in horses has become increasingly prominent recent years (Broeckx et al., 2014a,b; Ferris et al., 2014; Frisbie et al., 2009; McIlwraith et al., 2011; Spaas et al., 2012; Whitworth and Banks, 2014; Wilke et al., 2007).

Promising findings for the treatment of OA with MSCs have been reported (Fortier and Travis, 2011; Whitworth and Banks, 2014), and recently, research has been done on the influence of carriers and chondrogenic priming of MSCs on clinical outcome (Broeckx et al., 2014a,b). Our previous work (Broeckx et al., 2014b) indicated that the combined use of MSCs and plasma to treat OA significantly increased

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clinical improvement in horses compared to plasma alone. Additionally, another study from our group showed a higher rate (albeit not statistically significant) of horses returning to work after treatment with a combination of chondrogenic induced MSCs and plasma (86%) compared to horses treated with a combination of native MSCs and plasma (78%) (Broeckx et al., 2014a). The positive influence of chondrogenic priming was also seen in an *in vitro* study where chondrogenic induction of the MSCs significantly improved the overall adherence of the MSCs to cartilage surface and the penetration of the MSCs into cartilage lesions in cartilage explant cultures (Spaas et al., 2015).

The studies of Broeckx et al. (2014a,b) mainly focused on the efficacy of the MSCs for treating OA in patients, which did not allow an in depth safety examination. However, with the increasing popularity of MSCs as a therapy, their safe application is becoming more and more important. The safety of autologous, allogeneic and sometimes xenogenic MSCs derived from bone marrow, placental tissue, cord blood, cord tissue, adipose tissue and peripheral blood has been investigated to some extent (Ardanaz et al., 2016; Broeckx et al., 2014a,b; Carrade et al., 2011, 2012; Colbath et al., 2017; Pigott et al., 2013a,b). Both the *in vitro* and *in vivo* studies suggest that an intra-articular injection of allogeneic MSCs can be safely used in horses. However, *in vitro* studies cannot always be extrapolated *in vivo* and the *in vivo* studies were not always controlled, blinded and often 1 horse received multiple different treatments. Moreover, to the authors' knowledge, a randomized, double blinded and placebo controlled *in vivo* study performed according to good clinical practice (GCP) guidelines (VICH GL9) investigating the safety of an intra-articular injection of allogeneic MSCs has not been performed.

Therefore, this target animal safety study assessed the safety of a single intra-articular injection of a novel equine allogeneic MSC preparation, consisting of chondrogenic induced MSCs derived from peripheral blood combined with allogeneic plasma in healthy horses. The latter investigational veterinary product (IVP) was hypothesized to lead to no more side effects than an intra-articular injection with a 0.9% saline solution.

2. Materials and methods

2.1. Study design and animals

This study was a randomized, double blinded, blocked and placebo-controlled clinical study using healthy experimental horses, free of lameness or any visible systemic disease. None of the horses had a history of pregnancy or received a blood transfusion before recruitment based on their available medical history. A total of 16 horses were enrolled in this study, and randomly allocated to one of two following treatment groups using Randomizer_2 (2.1.0).xls: group 1 received the investigational product (IVP) ($n = 8$), while group 2 received the placebo control ($n = 8$). The groups were blocked on gender, meaning both groups consisted of an equal amount of males (geldings $n = 4$) and females (mares $n = 4$). The age of the horses ranged from 5 to 24 years and they displayed a body weight ranging from 454 to 670 kg. Different breeds were used: Belgian Warmblood ($n = 7$), Hanoverian ($n = 1$), KWPN ($n = 1$), Rheinlander ($n = 1$), Selle Français ($n = 1$) and Warmblood nonspecific ($n = 4$). The total study period was 42 ± 1 day from treatment until euthanasia/adoption.

During the study, animals were housed in individual boxes. Hay and water were available *ad libitum*. Horses were fed with 1 kg concentrate each morning and evening. The protocol of this study was approved by the local ethics committee of Global Stem cell Technology (approval number EC_2015_001; Permit Number: LA1700607). The entire study was conducted according to GCP (Good Clinical Practice) guidelines (VICH GL9) and in compliance with the VICH guidelines 41 and 43 (Target Animal Safety), as applicable. This target animal safety study is necessary to obtain a marketing authorization in Europe according to Directive 2004/28/EC and 2009/9/EC amending 2001/82/EC, thus the data of this study were submitted to the European Medicines Agency (EMA).

2.2. The investigational and control product

2.2.1. Investigational product

The IVP consisted of a proprietary formulation of allogeneic (the donor animal is a different individual of the same species as the acceptor animal) chondrogenic induced MSCs (ciMSCs) derived from the peripheral blood harvested from a single donor horse combined with equine allogeneic plasma (EAP) harvested from a single donor horse, different from the MSC donor. Blood collection of the donors was approved by the local ethical committee (approval number: EC_2012_001). The donors were screened for 29 equine pathogens per agreement with the Belgian Federal Medicines Agency and were further not included in any part of this study.

The production of ciMSCs and EAP was performed according to good manufacturing practice (GMP) conditions in a GMP certified site (nr. BE/GMP/2015/082). Cells were grown in tissue culture flasks, characterized and frozen at P5 as an intermediate cell stock as previously described (Spaas et al., 2013). After characterization (Koerner et al., 2006; Martinello et al., 2010), cells were thawed, cultured and chondrogenic induced in a two dimensional tissue culture system from P9 to P10 using proprietary media (Spaas et al., 2015). At final harvesting ciMSCs were trypsinized, resuspended at a concentration of 2×10^6 cells/ml in 1 ml of Dulbecco's Modified Eagle Medium low glucose with 10% of dimethyl sulfoxide (DMSO, Sigma) and frozen at − 80 °C in cryovials until use. Before usage, cells were assessed for cell number, viability, sterility, mycoplasma, purity and identity.

EAP was produced out of peripheral blood taken in a citrate phosphate dextrose adenine-1 (CPDA-1) single blood bag (Terumo). Eight samples of 1 ml EAP were prepared as previously described by our group (Beerts et al., 2013). Each sample contained approximately $124 * 10^6$ platelets and was frozen and stored at − 80 °C before administration.

2.2.2. Control product

The placebo control consisted out of a sterile 0.9% saline solution (B. Braun). The control product (CP) was stored at room temperature until use. The volume of the CP per treatment was matched to that of the IVP, namely 2 ml.

2.3. Treatment administration

For treatment administration, all horses were intravenously sedated with detomidine hydrochloride. Both the IVP and CP were administered once in the left metacarpophalangeal (MCP) joint using the collateral sesamoidean approach. For the IVP, the thawed ciMSCs and EAP were drawn into one syringe, so a total volume of 2 ml was obtained. Due to the nature of the products (color difference), the treatment was administered by a separate person (the dispenser, FP), who did not participate in any of the clinical or laboratory examinations. This way, blinding of the personnel who assessed the safety outcome parameters (SYB, JHS, MD, AMM) and performed the *post mortem* evaluation (LVB, KC) as described below was ensured.

2.4. Safety outcome

Throughout the study period, horses were checked regularly for (serious) adverse events and suspected drug reactions as described below. Following definitions were adhered:

- An adverse event was any observation in the animals that was unfavorable and unintended and occurred after the application of an IVP or CP, whether or not it was considered to be product related
- A serious adverse event was any adverse event which resulted in