CHAPTER N0.

12

Regenerative Medicine in Equine Injuries

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Summary: Equine are valuable animals which are the stimulant for activity, entertainment and pleasure for people all age groups. Owing to their involvement in various sports they get insulted and injured routinely. Major injuries occur to their legs and most of them are musculoskeletal in nature. Substantial injuries include bone fractures, tenodesmic injuries, wounds on lower limbs, osteoarthritis etc. To treat such crucial problems, regenerative medicine is being taken into consideration as an effective option. Regenerative medicine is a revolutionizing, rapidly emerging, progressing and developing branch of alternative veterinary medicine which aims to regenerate damaged tissues and cells and involves in the resumption of their normal function. Moreover, it encompasses various therapeutic techniques including the use of mesenchymal stem cells, Platelet-rich plasma, autologous conditioned serum and autologous protein solution. Mesenchymal stem cells are derived from multiple sites of the body such as bone marrow, adipose tissue etc. and have this remarkable ability to differentiate into osteoblasts and chondrocytes. In addition to this, platelet-rich plasma induces its healing effect by releasing a plethora of growth factors from platelets. Moreover, autologous conditioned serum is obtained from blood and it acts as a cytokine antagonist by releasing interleukins. Lastly, the autologous protein solution provides its therapeutic effect by combining the merits of both platelet-rich plasma and autologous conditioned serum. These miraculous therapies are being used and delivering their promising results by reducing pain and instigating healing for the above mentioned problems. Most of the animals regain their previous performance potential and resume their normal athletic activities yet investigations are still being conducted to get optimized outcomes from regenerative medicine.

Keywords: Regenerative medicine, Injuries, Therapies, Horse

INTRODUCTION

Equine is one of the most precious families among animals which is a source of recreation, pleasure and entertainment for people. They keep people mentally and emotionally healthy by being their companions. Since they are athletic animals and take part in sports, they suffer from multiple issues for instance wounds on lower limbs and musculoskeletal injuries etc. Out of aforementioned problems, musculoskeletal injury is the main cause to make horses lose their sporting career earlier in life because of damage to their ligaments and tendons (Bonilla-Gutierrez et al., 2018). Due to musculoskeletal problems, one of the major and obvious clinical signs in equine is lameness (Murray et al., 2010).

However, the nature and site of injuries depend on the age and gaming areas of equine. To treat such injuries, surgery, shock waves, hyaluronic acid and thermo-cautery are the traditional methods being used. Nonetheless, the shortcomings of formerly mentioned therapies are that they do not make the damaged tissue regain its previous normal activity and chances of relapse are always there (Marco et al., 2014). In order to resolve such problems, regenerative medicine has been introduced which is considered as an advancing and rapidly growing field focuses on regeneration of cells and revival of normal function of ruptured tissue (Mason & Dunnill, 2008; Katari et al., 2015). In equine veterinary practice, Mesenchymal Stem Cells (MSCs), Autologous Conditioned Serum (ACS), Autologous Protein Solution (APS) and Platelet-Rich Plasma (PRP) are various regenerative techniques being used to treat injuries (Iris et al., 2021).

OVERVIEW OF REGENERATIVE MEDICINE

Regenerative medicine (RM) is an advancing, developing and rapidly growing branch aims to regenerate cells and resumption of normal function of injured and damaged cells and tissues due to mechanical injury etc. (Mason & Dunnill, 2008; Katari et al., 2015). RM incorporates the field of tissue engineering (TE) or aids in the self-recovery of damaged and injured tissues by the inducement of endogenic mechanism (Pezzanite et al., 2023).

In equine practice, various methods of regenerative therapies are being used such as mesenchymal stem cells (MSCs), autologous conditioned serum (ACS), platelet-rich plasma (PRP), autologous protein solution (APS) etc. (Choudhary & Choudhary, 2022). The main role of regenerative medicine is to encourage the healing of the injured tissues and organs of the body. Since horses are weight bearing animals and used in multiple sports, the primary reason for their injuries is a mechanical overload which ultimately damages their ligaments and tendons, bones and menisci (Millar et al., 2017).

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TYPES OF EQUINE INJURIES

Wounds on Lower Limbs of Equine

Wounds on the lower limbs of equine are quite common because of their involvement in various sports. Distal limbs get routinely affected as they are close to the ground and have a prolonged healing time due to poor vascularization. Whenever a serious injury occurs, repair mechanism involves the formation of a scar tissue which is quite hard as well as functionally impaired rendering a horse loses its athletic performance resultantly (Godwin et al., 2012). Wounds which occur below the knee or hock do not heal and show resistance as there is a great distance between lower limbs and trunk. In addition to this, healing becomes delayed and sometimes no healing occurs because of improper blood supply which ultimately leads to low oxygen distribution to cellular level, disparity of growth factors and reduced temperature (Dyson, 1997; Theoret et al., 2001).

Osteoarthritis

Osteoarthritis is a diverse and complicated disease of entire joint characterized by inflammation, degeneration and ageing of cartilage which consequently damages all articular tissues of joint (Loeser et al., 2012). Occurrence of osteoarthritis depends on multiple factors such as age, genetics, physical status as well as nature of mechanical injury (Goldring et al., 2011; Berenbaum, 2013). It is a major reason for lameness which makes horses unable to perform their sporting activities and around 60% of lameness in horses is associated with osteoarthritis (McIlwraith et al., 2012). The pathological phenomenon of disease is initiated by synovial macrophages (which are responsible for inflammation), chondrocytes and synoviocytes which cause an activation of innate form of immune response and cytokine production (Kraus et al., 2016). While pathogenesis and disease development, there is a complex form of exchange and communication between osteocytes, chondrocytes, fibroblasts, synovial macrophages, penetrating white blood cells and synovial fluid along with extracellular matrix of articular tissues (Berenbaum, 2013; Mobasheri et al., 2017). In retaliation to injury, the normal inactive and inert chondrocytes become functional due to phenotypic shift which in return represented by proliferation of cells, formation of clusters as well as high production of matrix degrading enzymes and proteins of extracellular matrix (Houard et al., 2013). The inflamed synovium has a marked role in subchondral bone's osteoclastogenesis. Subchondral bone is an origin of inflammatory mediators which in turn are involved in pain, hypertrophy of chondrocytes and degeneration of inner layer of cartilage (Berenbaum, 2013). The communication between penetrating immune cells and destructed articular tissues promote reduction and depletion of cellular homeostasis, causes chronic inflammation and creates imbalance between degradation and formation of matrix which eventually leads to disease progression (Sherwood, 2018).

Teno-Desmic Injuries

Besides damage to osteoarticular tissues, ligaments and tendons when get affected become the major reason for morbidity and early retirement of horses from sports and athletic career. The superficial and deep digital flexor tendons and suspensory ligaments are regularly affected structures in teno-desmic injuries (Clegg, 2012).

Tendons: Tendons are strong and flexible connective tissues which join muscles to bones. They are composed of type I collagen (Cooper & Valentine, 2016). Tearing of tendon's collagen fibres occur when regular and strenuous exercise is performed or when extreme stretching of tendons occur due to mechanical overload (Thorpe et al., 2010). During gallop or at high speed, there is an intense stress on tendons which resultantly leads to the micro-damage of collagen fibrils. Ergo, if it is not resolved then it will eventually transforms into clinical injury. In particular, micro-damage of tendons occur when there is a decrease in metabolic activity of tenocytes (Thorpe et al., 2010). Consequently, the damage engenders an increased activity of cytokines and degrading enzymes of matrix. During tendon injuries, the most common catabolic molecules involved are tumour necrosis factor alpha (TNF-a), interleukin-1 (IL-1), matrix metalloproteinases (MMPs) etc. (Gaida et al., 2016). In damaged tissue, an increase in blood supply as well as release of stress signals promotes the penetration of inflammatory cells and allows removal and debridement of proteolytic enzymes (Dakin, 2017). During repair, there is a formation of type-III collagen which is able to endure stretching better than type-I collagen. However, following remodelling results in the conversion of type-III collagen to type-I and becomes able to stretch and contract but the result is still a weak tendon (Dakin et al., 2011; Walker et al., 2013).

Suspensory Ligaments: The suspensory ligaments are short band of flexible fibrous connective tissues which connects two bones together. They are mainly made up of type-I collagen fibres and minute portions of types III, V, VI, XI, XII, and XIV collagen fibres (Alsook et al., 2015). Damage to suspensory ligaments is the reason for rupture of collagen fibres as well as small blood vessels which ultimately lead to the formation of hematoma and signs of inflammation such as swelling and pain (Gibson & Steel, 2004). Proximal suspensory desmitis (PSD) can occur either in forelimbs or hind limbs or in both concurrently. Nonetheless, there are differences between forelimbs and hind limbs in PSD. When there is an acute phase in forelimbs, localized heat, and minor oedema can be seen in proximal region of metacarpal and enlargement of medial palmar vein as well (Genovese et al., 2011). However, in acute form of PSD in hind limbs, there may be the signs of localized swelling, heat and pain but such signs are not always obvious (Dyson, 1994). Moreover, another type of desmitis is degenerative suspensory ligament desmitis (DSLD). The pathognomonic sign of DSLD is an increase in the branches and diameter of suspensory ligament's body. As far as the histological appearance is concerned, multiple changes can be seen for instance acellular areas, fibrosis, deposition of hemosiderin, hypercellularity chondroid metaplasia and hyalinization of collagen (Dyson, 2007). The reason for increase in the diameter of body of suspensory ligaments is that during injury there is a marked rise in type-III and V collagen fibres and reduction in type-I (Alsook et al., 2015). In insulted and damaged suspensory ligaments, there is a marked rise in the activity of cytokines and matrix metalloproteinases (MMPs). MMPs and TNF-a are gauged and evaluated in

damaged ligaments as they are the indicators of tissue catabolism (Plaas et al., 2011). Even so, MMP-3 and MMP-13 are the frequently estimated enzymes in horses. The proinflammatory action in ligaments occur when macrophages secrete interleukins e.g. IL-1b and IL-6 (Kaplan et al., 2017).

Bone Fractures: In equine medicine, physiology, pathology and repair of bone has a significant role because of difficulties in repair of fractures. Bone cysts have grabbed attention in current years but bone fracture repair is still an area of keen interest (Govoni, 2015; Diloksumpan et al., 2020). Fractures in horses cause trouble in treatment and has complications as well. Moreover, their treatment is not economically feasible which generally leads to the eutahnization of horses (Payne & Compston, 2012; Ball et al., 2019). The Management as well as repair of fractures is quite troublesome and challenging in horses as compared to other species as they are huge and heavy weight animals. During osteosynthesis, various kinds of complications for instance, instability, infection and angular limb deformities can occur. Moreover, while post-surgery recovery phase, there are chances of refracture which is a paramount problem (Richardson, 2008).

Meniscus: Menisci are C shaped wedges in pair (medial and lateral) made of fibrocartilage and located between the bones of hind limbs (femur and tibia) which are connected together by stifle joint. Menisci perform some supreme roles such as joint stabilization, facilitates lubrication of joint, reduces stress and absorbs shock etc., (Renström & Johnson, 1990; Makris et al., 2011). Such type of lameness is common in horses which arises from stifle joint and pain occurs because of injury of menisci. The usual and frequent lameness occurs when cranial horn of medial meniscus and its correlated cranial meniscotibial ligament get ruptured (McCoy et al., 2019). Diagnosis of the injuries to stifle joint's soft tissues is difficult because of anatomical location, size of the joint and vague and non-obvious clinical signs. Resultantly, diagnosis is made by several imaging techniques such as magnetic resonance imaging, radiographs and ultrasounds (Walmsley, 2005; Daglish et al., 2018). Because of an inconvenient location of menisci, it is not possible to use repair techniques namely replacements and suturing other than endoscopy for partial meniscectomy. However, traditional treatment consisting of rest and non-steroidal anti-inflammatory drugs are available. Moreover, the prognosis for restoration of normal previous athletic activity entirely depends on the severity of an injury e.g. if a horse has less severe injury, then he may regain his performance activities and vice versa (Walmsley, 2005; McCoy et al., 2019).

REGENERATIVE THERAPIES

Mesenchymal Stem Cells

Mesenchymal Stem Cells are also called as multipotent mesenchymal stromal cells, multipotent stromal cells and medicinal signalling cells. Mesenchymal Stem Cells are such kind of cells which have this special ability to differentiate into chondroblasts, osteoblasts and adipocytes (Dominici et al., 2006). MSCs can be taken from various sites of the body for instance umbilical cord, amniotic membrane and amniotic fluids. However, stem cells derived from adipose tissues and bone marrow are the supreme sources (Smith et al., 2003; Arnhold et al., 2007). There are evidences that MSCs induce their therapeutic effect by secreting a substance called "secretome". Secretomes of MSCs which are derived from subcutaneous adipose tissues possess chemokines and cytokines as growth factors. These growth factors promote angiogenesis, enhance immune response and resultantly cause tissue as well as wound healing (Lavoie et al., 2013).

Autologous Conditioned Serum

Autologous Conditioned Serum is a biological product obtained from the blood of a patient used to treat several medical conditions (Wehling et al., 2007). Autologous Conditioned Serum is a product without cells collected after coagulation and centrifugation of blood. During coagulation, white blood cells and platelets which are present in blood sample are triggered to release cytokines and growth factors (Hraha et al., 2011). In ACS platelets are not that enriched as in platelet-rich plasma (PRP). However, its medicinal effect is associated with increased level of Interleukin-1 Receptor Antagonist Protein (IL-Ra) and Interleukin Growth Factor (IGF) as they both are considered as crucial factors in thwarting the outpouring of devastating cytokine in joint disease (Rutgers et al., 2015; Schnabel et al., 2019; Marques-Smith et al., 2020). Moreover, ACS is injected thrice as compared to PRP with one week gap (Lasarzik et al., 2018). Last but not least, ACS has benefit over PRP is that it can be easily stored and frozen as it is cell-free biological product (McClain & McCarrel, 2019; Hauschild et al., 2017).

Platelet-Rich Plasma

Platelets are also called as thrombocytes. Platelets are nucleus free cytoplasmic fragments engender from megakaryocytes, the cells form in bone marrow, which come in blood circulation and help in clotting of blood. Platelets are the birth place of multiple growth factors which are involved in repairing tissues, reducing inflammation and facilitating cell growth (Kawase, 2015). Unlike ACS, PRP is collected from uncoagulated blood without incubation. PRP induces its medicinal and remedial effect by degranulation of alpha granules on platelets which resultantly cause the release of cascade of cytokines and growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β1),), fibroblast growth factor (FGF), insulin-like growth factor (IGF) etc. (McLellan & Plevin, 2011; Sundman et al., 2011). Moreover, PRP is a concentrate which contains both platelets and white blood cells in a similar concentration approximately 300-400×103 per ml of plasma (Mishra et al., 2012).

Autologous Protein Solution

Autologous Protein Solution (APS) is a new biological product which has been launched of late to integrate the benefits of both ACS and PRP. It strengthens growth factors, platelets and anti-inflammatory cytokines. Interestingly, APS is a more alluring choice for equine practitioners over PRP as it does not require an incubation of 24 hours (Linardi et al., 2019).

REGENERATIVE THERAPIES BASED ON DISEASE

Osteoarthritis

Mesenchymal stem cells: At present, few techniques are being used for the treatment of osteoarthritis such as bone marrow stimulation and debridement, matrix-induced autologous chondrocyte implantation (MACI) and autologous chondrocyte implantation (ACI). However, they are not involved in the regeneration of articular and hyaline cartilage (Cokelaere et al., 2016). As their replacement, MSCs are considered an encouraging type of cells for the repair of cartilage (Caplan, 1991). Clinical studies in humans, goats and rats have shown the promising ability of MSCs to decrease pain, improve function of joint, and reduction in the size of lesions of cartilage (Bekkers et al., 2013). However, to fully understand and achieve the milestone of hyaline cartilage regeneration by MSCs, both in vivo as well as in vitro trials are required (Cokelaere et al., 2016).

Platelet-Rich Plasma: PRP is an optimistic therapeutic choice for the treatment of osteoarthritis because of its anticatabolic and anabolic effects on articular chondrocytes (Carmona et al., 2016; Kisiday et al., 2012). An advantageous impact of PRP is reported in several clinical trials in equine right after ephemeral aggravation of inflammation of joint followed by PRP injection (Smit et al., 2019). However, there is also a difference of results in various studies. In one study, there was a great improvement in range of movement, grade of lameness and gait after PRP, but in another trial where platelet lysate was used to induce therapeutic effect in osteoarthritis, no remarkable improvements were shown and horses returned to their previous degree of lameness unfortunately (Tyrnenopoulou et al., 2016). In a nut shell, type of research model and cellular content of the product are the two main factors which cause the therapeutic effect of PRP differ greatly (Garbin & Olver, 2020).

Autologous Conditioned Serum: Likewise PRP, ACS also demonstrated different results in different trials. It is considered that ACS induces its therapeutic effect due to high level of Interleukin-1Ra (IL-1Ra) (Rutgers et al., 2015; Marques-Smith et al., 2020). The main plan of action of ACS is to antagonize Interleukin-1 beta (IL-1B) since it is the main factor behind development and exacerbation of disease. Moreover, in few other in vivo and in vitro studies it was shown that ACS enhanced the effect of Interleukin-10 (IL-10) in synovium and type-II collagen (Alvarez et al., 2020).

Teno-Desmic Injuries

Mesenchymal stem cells: Mesenchymal stem cells are considered an appealing therapeutic option in tenodesmic injuries since they are capable of differentiating themselves into a cell of many different tissues together with ligaments and tendons (Renzi et al., 2013; Youngstrom et al., 2016). The mechanism which has been understood so far after MSCs transplantation is that it escalates the activity of anti-inflammatory cytokines and reduces proinflammatory cytokine concentration as well as penetration of immune cells into the damaged tissue (Linard et al., 2013; Usunier et al., 2014). Moreover, both autologous and allogeneic MSCs are being used in tenodesmic injuries. In one study it was investigated that autologous MSCs encourage angiogenesis while healing of damaged tendon tissue. Besides, it was also discovered of late that injection of autologous MSCs in blood vessels (intra-arterially or intravenously) can have advantageous impact during treatment of tendinopathies (Durgam & Stewart, 2016). Likewise, horses suffering from tendonitis of SDFT, DDFT and SL desmitis returned to their normal athletic performance when treated with allogeneic MSCs obtained from umbilical cord (Van-Loon et al., 2014). In another study, it was revealed that racehorses going through desmitis of suspensory ligament and inflammation of superficial digital flexor tendon showed alleviation in degree of lameness following peripheral blood-derived stem cells injection (Marfe et al., 2012; Vandenberghe et al., 2015).

Platelet-Rich Plasma: Platelet-Rich Plasma is a popular and widely used kind of therapeutic technique for the treatment of tenodesmic injuries as it contains multiple growth factors. Many studies have demonstrated encouraging results following PRP injection such as improved arrangement of collagen network, better angiogenesis and high durability of regenerated tissue (Geburek et al., 2016; Schnabel et al., 2019). In one study, there was better outcome in 150 lame horses as 81% horses showed healing and regeneration after receiving PRP intralesionally (Scala et al., 2014). Moreover, likewise results were acquired from horses with tendonitis of SDFT and DDFT after 50 days of therapy (Pietro et al., 2013). Nonetheless, in some trials when the effect of PRP and other treatment methods were compared then it was found that some horses retorted better to PRP and those treated with shock wave therapy also responded better and resumed their normal activity (Giunta et al., 2019).

Meniscus

In an in vivo trial, the regeneration of injured meniscal tissue due to induction of MSCs to injured joints in a caprine sample raised expectations which were also supported by some in vitro studies for meniscal cells of equine and explants (Kisiday et al., 2012; Kremer et al., 2017). However, a meniscus implant should be similar to natural meniscus in terms of many characteristics such as of shape, size, biochemical properties and vascularity to meet the load bearing requirements of knee (Coninck et al., 2014; Sun et al., 2017). Moreover, in a different study, meniscus parts of equine were attached with fibrin glue and fibrin glue including equine bone marrow mesenchymal stem cells (bmMSCs) inserted into mice subcutaneously. Implants with bmMSCs demonstrated increased angiogenesis and enhanced attachment of meniscus parts than fibrin alone (Ferris et al., 2012). Besides fruitful results in different trials, there are still some challenges with equine meniscus implantation which need to be addressed.

Distal Limb Wounds

Platelet-Rich plasma: In one of the studies PRP gel was used to treat cutaneous wounds on limbs as it has a capability of regenerating damaged tissues by releasing growth factors. Wounds which were treated with PRP gel showed well organized arrangement and nexus of collagen fibres and demonstrated angiogenesis as well (Charleata et al., 2003).

Mesenchymal stem cells: Though bmMSCs have a great potential of regenerating damaged tissue but in one of the studies equine umbilical cord MSCs (e-ucMSCs) were used for the treatment of wounds. It was seen that wounds were healed due to enhanced activity of the equine HLA-G ortholog protein found in ucMSCs (Iribarne et al., 2024).

Bone Fractures

Platelet-Rich Plasma: PRP has a great capability to stimulate regeneration of bones as platelets have this remarkable feature of releasing cascade of growth factors when degranulation of alpha-granules on platelets occurs (Liebig et al., 2020). An evidence on healing property of PRP can be seen when a tibia fracture with prolonged healing in donkey showed rapid and swift healing after injection (Faillace et al., 2017).

Mesenchymal stem cells: Many reconstructive therapy techniques are grounded on stem cells because of their notable role in bone healing. MSCs can be obtained from various sources nonetheless, those derived from bone marrow have superior osteogenic characteristics (Toupadakis et al., 2010). The aforementioned statement can be supported with a study in which MSCs obtained from equine bone marrow injected along with fibrin glue in mice assisted in bone development far better than MSCs derived from muscles (McDuffee et al., 2014). Therefore, till yet autologous bone grafting is being considered the best method of its kind while working with equine fractures.

CONCLUSION

In a nutshell, equine are precious animals and their injuries render them lose their athletic career. To address this problem, regenerative medicine is being used and doing a remarkable job by treating their various injures including bone fracture, tendon and ligament injuries, wounds on lower limbs etc. through the use of multiple techniques e.g. mesenchymal stem cells, platelet-rich plasma and autologous conditioned serum. Due to miracles of regenerative medicine, most of the equine return to their normal performance but some therapies are still under observation and researches are going on to gain maximum results from them.

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