学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
The title of the doctoral dissertation

Disease Modifying Osteoarthritic Drug, Pentosan Polysulfate Sodium Modulates Cytokine-Induced Osteoarthritic Changes and Promotes Articular Cartilage Tissue Regeneration *in vitro*

(変形性関節症病態修飾薬である多硫酸ペントサンは、関節軟骨におけるサイトカイン誘導性関節症病態を変化させ、培養関節軟骨の再生を刺激する)

Pentosan polysulfate (PPS) is a semi-synthetic sulfated polysaccharide derived from wood of beech plant, *Fagus sylvatica* that is available for the relief of various medical conditions including thrombi and interstitial cystitis in humans, and osteoarthritis (OA) in dogs and horses. Its mechanism of action as well as its effects on some novel therapeutic targets for OA remains to be fully elucidated. The objectives of the study were to investigate the effects of PPS on cytokine-induced iNOS, c-Jun and HIF-α isoforms expression, redifferentiation of dedifferentiated monolayer expanded canine articular chondrocytes (CACs) and chondrogenic differentiation potential of canine bone marrow-derived mesenchymal stem cells (cBMSCs) *in vitro*.

The first study, demonstrated that PPS is a novel inhibitor of IL-1β-induced iNOS, c-Jun and HIF-1α mRNA upregulation and iNOS protein induction which may be beneficial for OA treatment. CACs were recalcitrant to single cytokine-induction of iNOS protein including to a combination of IL-1β + TNF-α, IL-1β + LPS except to TNF-α + LPS and IL-1β+ TNF-α + LPS suggestive of a protective mechanism from iNOS detrimental effects on perpetuating OA. PPS significantly abrogated IL-1β + TNF-α + LPS-induced iNOS protein expression.

For successful cartilage tissue regeneration and repair of OA defects by autologous chondrocytes transplantation (ACT), redifferentiation of dedifferentiated chondrocytes following *in vitro* monolayer expansion has long been proposed as the best hope for returning chondrocytes to their native articular cartilage mode of expression prior to implantation. The second study,

demonstrated that combined alginate beads culture of dedifferentiated monolayer P1 CACs in 20% DMEM supplemented with only PPS for 18 days results in a full retain to their 'native' cartilage phenotype as verified by enhanced synthesis of type II collagen, aggrecan and proteoglycan (PG) deposition with complete suppression of type I and X collagen. HIF-1α and HIF-2α proteins were detected for the first time under normoxia condition indicating that culture of chondrocytes in alginate may stabilize the HIF-α isoforms at the protein level.

In the third study, the independent chondrogenic potential of BMSCs sourced from young dogs in monolayer expansion cultures without known chondroinductive factors was evaluated. The results showed that cBMSCs exhibit independent chondrogenic differentiation in monolayer cultures in the absence of chondroinductive factors as verified by the expression of Sox-9, type II collagen and aggrecan. However, independent chondrogenic potential and phenotype retention of cBMSCs is passage-dependent and decreases with extensive passaging.

In the fourth study, the effects of PPS and polysulfated glycosaminoglycan (PSGAG) on chondrogenic differentiation of cBMSCs in alginate and micromass culture (MMC) were evaluated. Both PPS and PSGAG failed to promote chondrogenesis in alginate culture model although PSGAG significantly upregulated type I collagen. In contrast, PPS significantly enhanced chondrogenesis in cBMSCs in MMC whereas PSGAG inhibited chondrogenesis and promoted a fibrocartilage-like phenotype. The findings of the studies will contribute to the body of knowledge on the effects of PPS on novel OA therapeutic targets. Furthermore, the demonstrated *in vitro* chondroinductive effects of PPS on CACs and cBMSCs will influence the future focus for cartilage tissue regeneration and repair efforts.