

TITLE: Therapeutic Efficacy of mitoprotective SS-31 peptide in Osteoarthritis: *in vitro* and *in vivo* studies

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INTRODUCTION: Osteoarthritis (OA), a condition causing significant joint impairment, lacks effective disease-modifying drugs to halt or mitigate posttraumatic osteoarthritis (PTOA) progression. Emerging research indicates that treatments focused on mitigating the acute biological responses following joint injuries could hold promise in preventing ongoing chondrocyte cell death and the progressive degradation of the extracellular matrix. Furthermore, there is substantial evidence highlighting the involvement of mitochondrial dysfunction in advanced stages of osteoarthritis. Key pathological alterations in the early stages of OA, such as oxidative stress and chondrocyte cell death, are largely mediated by the mitochondria. This underscores the critical role of mitochondrial health in the pathogenesis of the condition.

Elamipretide/Szeto-Schiller peptides (SS-31) work by improving mitochondrial function, reducing oxidative stress, and increasing energy production within the cell. It has shown promise in clinical trials for various medical conditions, where mitochondrial dysfunction plays a significant role in the disease's progression. This drug represents a promising avenue for treating conditions linked to mitochondrial impairments and holds potential in improving overall cellular health and vitality. Present study delves into the evaluation of SS-31 as a therapeutic mitoprotective agent in both animal as well as cell line models of osteoarthritis.

METHODS: SS-31 is known mitoprotective agent. To validate SS-31's mitoprotective effects in OA, an *in vitro* osteoarthritis cell line model was established using the SW1353 human chondrosarcoma cell line. Chondrocytes were exposed to IL-1 β and TNF- α (10ng/ml each for 48 hours) to induce an osteoarthritis-like condition. Different concentrations of SS-31 (1 μ M and 10 μ M) were administered for 24 hours, and mitochondrial biogenesis marker gene expression was assessed via RT-PCR

To assess the preventive and therapeutic effects of SS-31, two guinea pig models were employed. In a chemically induced osteoarthritis model, Monosodium iodoacetate (MIA) was injected into the right knee joints (n=6), followed by SS-31 treatment (0.5mg/kg) in a treatment group (n=3) and saline in a control group (n=3). SS-31 was administered once weekly for four weeks. In a spontaneous model, guinea pigs aged 6 months (n=5) received weekly SS-31 injections (0.5mg/kg) over a 12-week period whereas placebo group (n=4) was administered with saline. Osteoarthritis symptoms were assessed via radiography using Osteoarthritis Research Society International (OARSI) scoring system. Knee joints were extracted, fixed, and histopathologically evaluated using MANKIN scoring system.

RESULTS: Human chondrogenic cell line SW1353 treated with inflammatory cytokines (IL-1 β and TNF- α) showed gene expression characteristics of OA-like conditions. Study of mitochondrial gene expression also revealed a decrease in the expression of genes associated with mitochondrial biogenesis (ATP synthase, PGC-1, NRF-1, and NRF-2), suggesting a decline in mitochondrial function during OA progression. Treatment with SS-31 at varying concentrations (1 μ M and 10 μ M) in the cell line model led to an increase in the expression of genes associated with mitochondrial biogenesis, particularly ATP synthase, PGC-1, NRF-1, and NRF-2. These results suggest the potential therapeutic effect of SS-31 in mitigating mitochondrial dysfunction in an *in vitro* cell line model of OA.

In the chemically induced OA guinea pig model, treatment with SS-31 at a dosage of 0.5mg/kg resulted in a significant reduction in cartilage damage and OA symptoms, as confirmed by radiographic and histopathological investigations. In the spontaneous OA model, 6-month-old guinea pigs received SS-31 at 0.5mg/kg over 12 weeks, leading to a substantial decrease in OA symptoms based on radiographic OARSI and histopathological MANKIN grading. These findings indicate the therapeutic efficacy of SS-31 in both chemically induced and spontaneous models of OA in guinea pigs, suggesting its potential for clinical applications.

DISCUSSION: The promising dual role of SS-31 in preventing and treating OA in guinea pig model by decreasing cartilage damage and its potential for ameliorating mitochondrial dysfunction by enhancing mitochondrial activity associated with OA progression in an *in vitro* settings underscore the potential clinical significance of SS-31 in the management of OA.

CLINICAL RELEVANCE: SS-31 emerges as a promising therapeutic candidate for osteoarthritis characterized by mitochondrial dysfunction. Additionally, the ongoing phase 2 and phase 3 clinical trials for other mitochondrial diseases underscore the potential repurposing of SS-31 as a valuable and expedited intervention in the management of osteoarthritis.