Micellar Delivery of a SIRT6 Activator Alleviates DNA Damage in Human Chondrocytes

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Introduction: Osteoarthritis (OA) is a progressive degenerative joint disease marked by chondrocyte senescence, genomic instability, and extracellular matrix degradation. Current therapeutic strategies largely focus on pain management rather than underlying cellular pathology. Recent studies have implicated activation of SIRT6 as a promising mechanism to mitigate cellular senescence and DNA damage in aging tissues. The small-molecule activator MDL-800 has shown potential to upregulate SIRT6 activity and restore homeostasis [1]. However, MDL-800 exhibits poor aqueous solubility and dose-limiting cytotoxicity, impeding its translational potential. To address these limitations, we developed MDL-SKiF, a micelle-encapsulated MDL-800 formulated with Pluronic F127. This formulation markedly enhances water solubility and biocompatibility [2]. This study investigates the in vitro therapeutic efficacy and cytotoxic profile of free MDL-800 and MDL-SKiFs in primary human chondrocytes derived from both Osteoarthritis (OA) and cadaveric (AM) donors.

Methods: MDL-SKiFs were synthesized through drug solubilization and a surfactant-stripping process [2]. Primary chondrocytes were isolated from surgical waste cartilage of patients undergoing total knee replacement for end-stage OA or from ankle joint cartilage of cadaveric donors exhibiting normal morphology and no reported joint pathology. Chondrocytes were maintained in monolayer culture before the treatment (n=6 total donors, 3 OA and 3 AM). Primary chondrocytes from 3 OA (1 male aged 69; 2 females aged 76 and 78) and 3 healthy donors (2 males aged 62 and 56; 1 female aged 73) were treated with free MDL-800 or MDL-SKiF at matched concentrations (20 µM, 40 µM, 80 µM, and 160 µM) for 24h and 72h. DNA strand breaks were quantified by alkaline comet assay and expressed as Tail DNA%. Alkaline comet assay image quantification was performed using blinded analysis in ImageJ, utilizing the OpenComet plugin to assess tail percentage. Cell viability was assessed using calcein-AM/ethidium homodimer-1 staining via fluorescence microscopy imaging and flow cytometry. Quantitative flow data were normalized to methanol-treated cells as a negative control to enable dose-dependent viability comparisons across treatment conditions. Statistical significance was evaluated using paired or unpaired t-tests, with $p < 0.05$ considered significant.

Results: After 24 h of treatment, MDL-SKiFs significantly reduced DNA damage at the 40 µM concentration in OA chondrocytes compared to untreated controls (p=0.0267), whereas free MDL-800 did not reach significance (p = 0.1275) (**Fig.B**). Across 4 OA donors, the protective trend was consistent, with donor-to-donor variation partially attenuated in the MDL-SKiF group (**Fig.A**). After 72 h of treatment at 40 µM, pooled data from 3 OA and 3 AM donors showed sustained DNA damage reduction effect by MDL-SKiFs (p=0.0102 vs. Control), comparable to free MDL-800 (p = 0.0215) (**Fig.C**). However, at 160 µM, MDL-800 induced significant cytotoxicity, while MDL-SKiFs preserved >90% viability, as demonstrated by fluorescence staining and flow cytometry (**Fig.D**). In a follow-up dose comparison across 5 donors, MDL-SKiFs at both 40 µM and 160 µM significantly protected against DNA damage (p<0.0001 vs. Control). Moreover, the 160 µM dose yielded greater efficacy than 40 µM (p=0.0083) without compromising cell survival (**Fig.E**).

Discussion: This study introduces a therapeutic strategy that modulates key molecular pathways underlying osteoarthritis progression. MDL-SKiFs retained its therapeutic efficacy and provided an expanded therapeutic window. The results highlight the enhanced safety profile and sustained efficacy of the MDL-SKiFs by enhancing solubility and minimizing cytotoxicity. Although sustained intra-articular drug retention remains a challenge, MDL-SKiFs show potential to overcome this barrier. While overall trends were consistent, donor-to-donor variability was observed in treatment response and dose sensitivity. For evaluating potential clinical translatability of this delivery approach, our in vivo studies involving animal models will also consider biological differences between human and murine chondrocytes as different dose-response characteristics would be encountered.

Significance/Clinical Relevance: This study introduces MDL-SKiFs as a novel delivery platform and evaluates its safety and therapeutic efficacy in protecting primary human chondrocytes from DNA damage, in comparison to free MDL-800. By addressing the unmet need for disease-modifying osteoarthritis therapies, this platform holds strong promise for the translational advancement of SIRT6-targeted therapeutics in degenerative joint disease.

Reference: 1. Copp, M. E. et al. SIRT6 activation rescues the age-related decline in DNA damage repair in primary human chondrocytes. bioRxiv (Cold Spring Harbor Laboratory) (2023). https://doi.org/10.1101/2023.02.27.530205, 2. Zhang, Y. et al. Therapeutic surfactant-stripped frozen micelles. Nature Communications 7, 11649 (2016). https://doi.org:10.1038/ncomms11649.
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