Suramin Enhances WIF1 Expression to Suppress Inflammasome Signaling in Osteoarthritis

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INTRODUCTION: Wnt signaling dysregulation accelerates cartilage matrix degradation in osteoarthritis (OA). Wnt inhibitory factor-1 (WIF1), which binds extracellular Wnt ligands to limit receptor activation, is consistently diminished in osteoarthritic cartilage[1]. Concurrently, pyroptosis, an inflammatory cell death pathway driven by NF-κB–primed NLRP3 inflammasome activation, caspase-1 cleavage, and gasdermin-D pore formation, contributes to chondrocyte loss and IL-1β/IL-18 releases. While WIF1 deficiency in OA is established, whether restoring WIF1 directly inhibits NF-κB/NLRP3-mediated inflammasome signaling remains unclear. Moreover, clinically applicable strategies to elevate WIF1 expression are lacking. Suramin, an antiparasitic drug known to modulate both Wnt signaling and NF-κB activity, has not been evaluated for WIF1 regulation [2]. We hypothesized that augmenting WIF1 suppresses inflammasome activation in chondrocytes and tested whether suramin can upregulate WIF1 to achieve this protective effect.

METHODS: Animal study: After IACUC approval, OA was induced in male Sprague–Dawley rats (11 weeks old, 400 g; n=6 per group) by anterior cruciate ligament transection (ACLT). Male rats were selected to limit variability from sex hormones. Rats received weekly intra-articular injections of saline or suramin (0.4 mg, MedChemExpress) for 12 weeks. Contralateral knees served as controls. Joints were harvested for histology and immunohistochemistry (IHC) of WIF1, NLRP3, ASC, caspase-1, and GSDMD (ThermoFisher). Human OA chondrocytes: After IRB approval, primary OA chondrocytes were isolated from discarded cartilage of total knee arthroplasty patients (2 females,1 male, age range 65-75 years). Molecular assays: qRT-PCR, Western blot, and immunofluorescence were used to analyze WIF1, inflammasome markers (NLRP3, ASC, caspase-1, GSDMD, IL-18), and ECM-related genes (COL2, SOX9, MMP3, MMP13, ADAMTS4). WIF1 modulation: Chondrocytes were transfected with the pcWIF1 plasmid or control vector for gain-of-function, and with the shWIF1 plasmid for loss-of-function, before IL-1β stimulation to assess the role of WIF1 in mediating suramin's effects. Statistics: Data are presented as mean ± SD from ≥3 independent experiments. One-way ANOVA with Tukey's post hoc test was used; $p < 0.05$ was considered significant.

RESULTS SECTION: In ACLT rats, WIF1 expression decreased by 40.2 ± 5.8% ($p<0.001$), while suramin increased WIF1 expression levels toward to sham controls (Fig. 1). The inflammasome-related proteins (NLRP3, ASC, caspase-1, GSDMD) were significantly elevated in articular cartilage compared with contralateral joints. Suramin administration increased WIF1 staining intensity ($p<0.001$) and reduced expression of inflammasome-related proteins, compared with ACLT group ($p<0.05$-0.01, Fig. 1). In IL-1β-stimulated human OA chondrocytes, WIF1 was markedly downregulated with concomitant increases in NLRP3, ASC, caspase-1, GSDMD, and IL-18 ($p<0.05$-0.01, Western blot, Fig. 2a). Suramin significantly increased WIF1 expression of human OA chondrocytes in a dose-dependent manner, with 5 and 10 μM both showing significant elevation compared with untreated controls ($p<0.05$, Western blot, Fig.2b). Immunofluorescence confirmed diminished accumulation of NLRP3 and GSDMD in suramin-treated cells (Fig 2c). Overexpression of WIF1 (pcWIF1, 2 μg) in human OA chondrocytes reproduced suramin's protective effects, significantly reducing inflammasome proteins ($p<0.05$–0.01, Western blot, Fig.3a) Conversely, WIF1 knockdown (shWIF1) aggravated IL-1β–induced inflammasome protein expression and abrogated the anti-inflammatory effects of suramin ($p<0.05$–0.01, Western blot, Fig. 3b). Together, these results identify WIF1 upregulation as a key mediator of for suramin's chondroprotective effects.

DISCUSSION: Although WIF1 downregulation is a consistent finding in OA cartilage, the extent to which enhancing WIF1 expression can mitigate inflammatory signaling and matrix degradation has not been fully elucidated. In this study, suramin increased WIF1 expression in a dose-dependent manner in human OA chondrocytes and concurrently reduced inflammasome activation both in vitro and in vivo. Gain- and loss-of-function experiments further demonstrated that WIF1 elevation reproduced, whereas WIF1 depletion attenuated, the inflammasome-suppressive and chondroprotective effects of suramin. These results suggest that WIF1 upregulation is linked to suppression of NF-κB/NLRP3 signaling and improved extracellular matrix homeostasis. Study limitations include the single animal model, limited human donor samples, and the potential for suramin to exert effects independent of WIF1. Future studies employing genetic manipulation of WIF1 and optimized local delivery strategies are warranted to validate WIF1 as a therapeutic target.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings advance the understanding of WIF1 from a passive disease marker to an active therapeutic target in OA. Suramin, an established drug with known safety data, emerges as a practical pharmacologic means to enhance WIF1 expression, suppress inflammasome-related signaling, and preserve cartilage matrix integrity. This work highlights WIF1 modulation as a potential disease-modifying strategy warranting further preclinical and translational evaluation.

REFERENCES: [1] Cheng et al. Front Physiol. 2022 (PMID:36117702); [2] Shen et al. Int Immunopharmacol. 2023 (PMID:37182454)

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IMAGES AND TABLES:

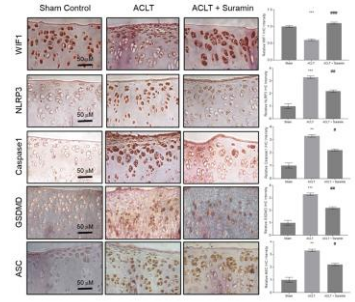

Fig. 1 Suramin increases WIF1 expression and suppresses inflammasome activation in ACLT rat cartilage. $p<0.05$ vs. control (#); vs. ACLT (*)

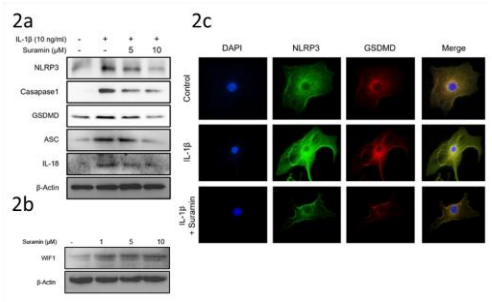

Fig. 2 Suramin dose-dependently increases WIF1 and attenuates IL-1β–induced inflammasome activation in human OA chondrocytes. (a–b) Western blots: (a) IL-1β ± suramin on inflammasome proteins; (b) dose-dependent WIF1 upregulation **(c)** Immunofluorescence: reduced NLRP3 and GSDMD with suramin

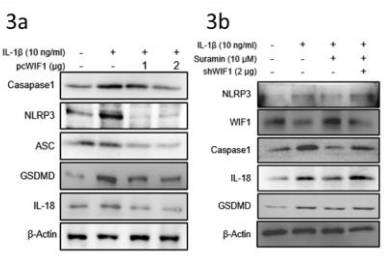

Fig. 3 WIF1 is required for suramin-mediated chondroprotection. (a–b) Western blots: (a) pcWIF1 overexpression mimics suramin, reducing inflammasome activation; (b) shWIF1 knockdown abolishes suramin's inhibitory effects