Dual Inhibition of TNFα and IL-1β Signaling Attenuates Osteoarthritis in Mouse and Non-Human Primate Models

Wenyu Fu[1,2], Xin Liu[1], Ronghan Liu[2], Jin Wo[3], Shuya Wang[2], Guodong Sun[2,3], Chuan-ju Liu[1,2]
Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, CT, USA
Department of Orthopaedic Surgery, New York University Medical Center, New York, NY, USA
Department of Orthopaedics, The First Affiliated Hospital of Jinan University, Guangzhou, China
wenyu.fu@yale.edu

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Introduction: Osteoarthritis (OA) is driven in part by chronic low-grade inflammation, with TNFα and IL-1β as key mediators. Clinical trials targeting either cytokine alone have failed, likely due to their concurrent and redundant roles in cartilage catabolism. We hypothesized that dual inhibition of TNFα and IL-1β signaling would be more effective. Screening an FDA-approved drug library, we identified dabigatran (DAB) as a compound that simultaneously suppresses both pathways without systemic immunosuppression. We tested dabigatran in mouse and cynomolgus macaque OA models, demonstrating disease-modifying potential. Mechanistically, dabigatran targets BIG2, attenuating inflammatory catabolic signaling in chondrocytes. This study highlights dual cytokine targeting as a promising therapeutic approach for OA.

Materials & Methods: THP-1 cells stably expressing an NF-κB luciferase reporter were used to screen FDA-approved drugs for inhibitory activity. The selected compound's effects were evaluated in vitro and in vivo by Western blot, qRT-PCR, immunofluorescence, Safranin O, and IHC staining. Mass spectrometry and co-immunoprecipitation identified its molecular target and interaction with TNFR1 and IL1R. Animal studies were approved by the IACUC or Yale University and Jinan University.

Results: **Discovery of dabigatran as a dual inhibitor of TNFα- and IL-1β-mediated signaling in chondrocytes.** A library of 1,046 FDA-approved drugs was screened using an NF-κB-bla stable cell line. Three hits, DAB, daunorubicin, and penfluridol, inhibited TNFα- and IL-1β-induced NF-κB activation. Secondary screening in C28I2 chondrocytes confirmed consistent inhibition by all three compounds. In NF-κB luciferase transgenic mice with destabilization of the medial meniscus (DMM)-induced OA, all three drugs reduced NF-κB luminescence in affected joints, with DAB showing the strongest effect. Subsequent studies focused on DAB. DAB inhibited IL-1β- and TNFα-induced NF-κB activation in a dose-dependent manner. Functionally, DAB blocked TNFα- and IL-1β-induced expression of catabolic enzymes MMP13, ADAMTS4, NOS2, and COX2. Collectively, these results establish DAB as a potent dual inhibitor of TNFα- and IL-1β-driven NF-κB activation and chondrocyte catabolism, underscoring its therapeutic potential in OA.

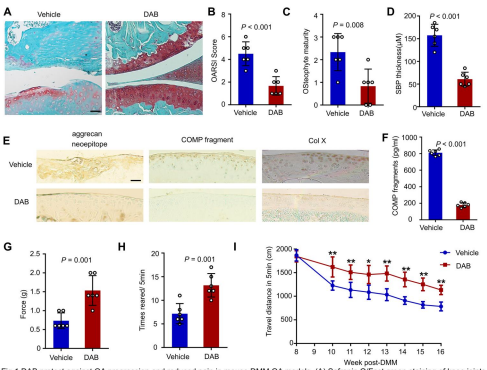

Fig.1 DAB protect against OA progression and reduced pain in mouse DMM OA models. (A) Safranin O/Fast green staining of knee joints from mice with indicted treatment. (B-D) Quantification of OARSI scorer, osteophyte development, ans subchondral chondral bone plated thickness in A. (E) IHC staining for Aggrecan neoepitope, COMP fragment, and COIX in knee joinsts. (F) Serum COMP fragment levels in indicated mice. (G-I) von Frey assay, 5min time reared, and travle didtance in indicated mice.

DAB attenuates OA progression and alleviates OA associated pain in the mouse DMM OA model. Using the surgical DMM model, DAB was administered via intra-articular injection every other day for 16 weeks. DAB treatment significantly reduced articular cartilage degradation, osteophyte formation, and subchondral bone plate thickening compared to vehicle controls (**Fig. 1A-D**). Immunohistochemistry showed decreased levels of aggrecan neoepitope, COMP fragments, and collagen X in DAB-treated joints (**Fig. 1E**), and serum COMP fragment levels were also significantly lowered (**Fig. 1F**). Importantly, DAB alleviated DMM-induced OA pain behaviors (**Fig. 1G-I**). These findings support the potential of DAB as a repurposed FDA-approved drug for OA therapy.

DAB ameliorats OA progression in non-human primates. To evaluate the therapeutic potential of DAB in a large animal model, partial medial meniscectomy (PMM) surgery was performed on the left knee of 16 male non-human primates, macaca fascicularis, aged 7.6 to 8.2 years, followed by treated with DAB for 7 months starting from 1month after PMM surgery (**Fig 2A**). Both μCT and MRI analysis revealed that DAB significantly reduced subchondral bone mass and alleviated cartilage loss compared to vehicle treated controls (**Fig. 2B-E**). Moreover, kinematics-based analysis, which was highly sensitive for decting subtle locomoteo abnormalities, was used to record the average trajectory and knee joint motion range, and found that DAB-treated animals demonstrated improved overall gait performance and a successive gait cycle (Stepping almost without straightening). In contrast, vehicle treated animals tended to straighten their left legs most of the time and limit knee joint movement, indicative of discomfort and impaired function (**Fig. 2F**).

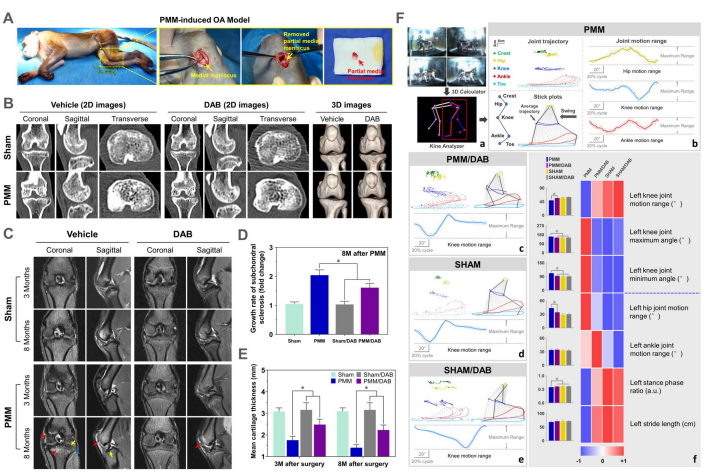

Fig.2 DAB alleviates PMM-induced OA in hon-human primates. (A) Study design: PMM or sham surgery with vehicle or DAB treatment (n=4 per group). (b) 2D and 3D CT images at 8 months after PMM surgery. (C) MRI monitoring of knee OA at 3 and 8 months after surgery (red arrows: effusion of knee joint and inflammatory edema; yellow arrow: cartiage damage; blue arrow: osteophytes). (D) Quantification of subchondral sclerosis by CT. (E) Articular cartilage thickness measured by MRI. (F) Hindlimb gait analysis. a. Fluorescence dues traced in 3D during treadmil locomotion. b-e. Hindlimb kinematics koint trajectory, and stick plots trajectory during locomotion for each group. f. Clustering matrix of clinically relevant gait parameters for four groups of animals.Techinical measurements were repeated at least three times.

DAB protected against OA through targeting BIG2. Biochemical co-purification identified brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2) as a novel molecular target of DAB. Mechanistic studies revealed that DAB modulates BIG2 to suppress both IL-1β and TNFα inflammatory pathways via distinct mechanisms. DAB enhanced the interaction between BIG2 and TNF receptor (TNFR), promoting the release of membrane-bound TNFR into the extracellular medium, thereby inhibiting TNFα activity. In contrast, DAB facilitated the binding of IL-1 receptor (IL1R) to BIG2, accelerating IL1R degradation through the autophagy-lysosome pathway (**Fig. 3**). Functional assays demonstrated that BIG2 deficiency completely abolished DAB's inhibitory effects on both IL-1β and TNFα signaling, underscoring BIG2's essential role in mediating DAB's anti-inflammatory actions. **Conclusions:** Our findings demonstrate that DAB effectively inhibits OA progression in both mouse and non-human primate models. As a novel dual inhibitor of IL-1β and TNFα signaling, DAB holds promise as a therapeutic agent for OA and potentially other diseases driven by these pro-inflammatory cytokines.

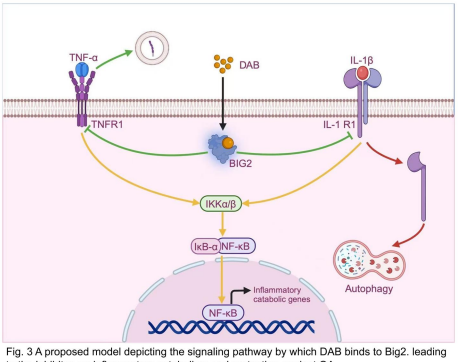

Fig. 3 A proposed model depicting the signaling pathway by which DAB binds to Big2. leading to the inhibiton og inflammatory catabolism and protection against OA.

Significance: This study establishes BIG2-mediated inflammatory modulation as a novel mechanistic and therapeutic network and positions DAB as a promising disease-modifying candidate for OA and other TNFα/IL-1β-driven disorders and conditions.