

# Preliminary In Vivo Evaluation of the Glycolysis Inhibitor 2DG in a Murine Arthritis Model: Potential for Cartilage

Yoshifumi Ohashi<sup>1</sup>, Kenya Terabe<sup>2</sup>, Shiro Imagama<sup>2</sup>, Cheryl B. Knudson<sup>3</sup>, Warren Knudson<sup>3</sup>, Nobunori Takahashi<sup>1</sup>,

<sup>1</sup>Department of Orthopedic Surgery, Aichi Medical University, Nagakute, Japan

<sup>2</sup>Department of Orthopedic surgery, Nagoya University, Nagoya, Japan

<sup>3</sup>Department of Anatomy & Cell Biology, Brody School of Medicine, East Carolina University, NC, USA

Email of Presenting Author: yoshifumi000227@gmail.com

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[INTRODUCTION]: Recent in vitro studies using IL-1 $\beta$ -stimulated human and bovine chondrocytes have demonstrated that osteoarthritic cartilage exhibits enhanced anaerobic glycolysis and suppressed oxidative metabolism [ref]. Inhibition of glycolysis with 2-deoxy-D-glucose (2DG), a glycolytic inhibitor, normalized these metabolic alterations and exerted protective effects on cartilage matrix integrity (1). Similar changes were reproduced through overexpression of hyaluronan synthase 2 (HAS2-OE) and by treatment with the HA synthesis inhibitor 4-methylumbelliferone (4MU), further suggesting that glucose metabolic reprogramming contributes to OA progression (2). However, the in vivo effects of glycolytic inhibition on joint inflammation and cartilage preservation remain unverified.

[METHODS]: We used SKG/Jcl mice (8-week-old, BALB/c background) harboring a point mutation in ZAP-70 (W163C), which develop spontaneous arthritis due to autoreactive CD4+ T cells (3). Under SPF conditions, arthritis was induced with a single intraperitoneal injection of the  $\beta$ -glucan curdlan. Mice were divided into three groups: ①**Control group**: BALB/c mice, untreated, regular water, ②**Curdlan group**: SKG mice, curdlan injection + regular water, ③**Curdlan + 2DG group**: SKG mice, curdlan injection + 1% 2DG in drinking water. Treatment continued for 12 weeks. Arthritis severity was assessed weekly using a validated arthritis scoring system targeting digits, wrists, and ankles (ref). Matrix metalloproteinase (MMP) activity was visualized using a fluorescent probe (IVISense MMP 645 FAST, Revvity) administered intraperitoneally, followed by imaging with IVIS (PerkinElmer). At week 12, serum was collected, and IL-6 levels were measured using an ELISA kit (Dialone). Data are presented as relative changes normalized to the control group (set as 1.0).

[RESULTS SECTION]: The curdlan group developed significant arthritis from week 1 (arthritis score: control 1.0 vs. curdlan 4.8), with a peak at week 6 (curdlan: 15.8). In contrast, the curdlan + 2DG group showed a more gradual increase, with a significantly lower peak score at week 6 (11.2,  $p < 0.05$ ) (Figure 1). IVIS imaging revealed strong MMP signal in the wrist, ankle, and tail joints of the curdlan group (The blue glowing area). In the curdlan + 2DG group, MMP expression was markedly reduced, especially in the tail region (Figure 2). Serum IL-6 levels were significantly elevated in the curdlan group compared to controls (1.22 vs. 1.0,  $p < 0.05$ ), while the curdlan + 2DG group exhibited lower IL-6 levels (1.16 vs. 1.22,  $p < 0.05$ ) (Figure 3)

[DISCUSSION]: This preliminary study suggests that glycolysis inhibition via 2DG can attenuate arthritis progression and associated MMP activity in vivo. These findings are consistent with our previous in vitro data, supporting the hypothesis that altered glucose metabolism plays a role in inflammation-induced cartilage degradation. While the effects observed here are promising, further validation through histopathological and molecular analyses is warranted.

[SIGNIFICANCE/CLINICAL RELEVANCE]: Our results provide in vivo evidence that 2DG may serve as a potential disease-modifying therapeutic agent for inflammatory arthritis and secondary osteoarthritis. Targeting glycolytic metabolism could represent a novel therapeutic strategy for joint preservation in inflammatory joint diseases. Conditions that alter the dependence of OA or OA-like chondrocytes on glycolysis pathways for the protection of ATP, are clinical to reversing the altered metabolism associated with this pathology and provide a means to return these cells to a more steady-state, quiescent phenotype.

## [REFERENCES]:

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3. Yoshitomi H, Sakaguchi S. [SKG mice as a model of RA]. *Nihon Rinsho.* 2005;63 Suppl 1:40-5.

## [IMAGES AND TABLES]:

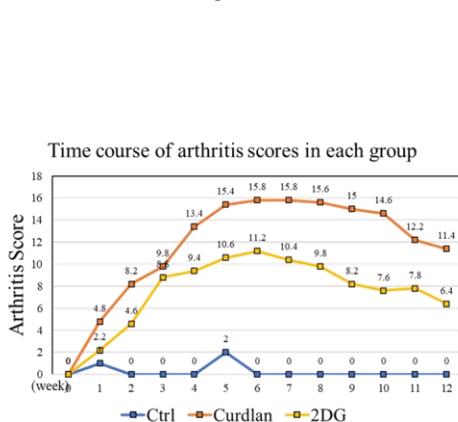


Figure 1: Time course of arthritis scores in each group

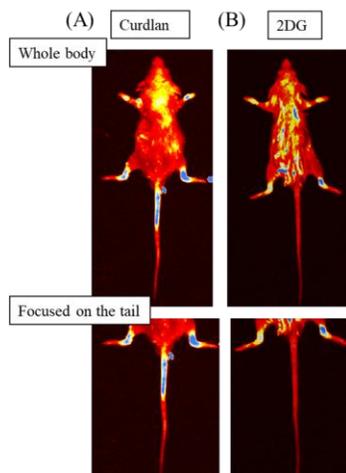


Figure 2: Representative IVIS Images Visualizing MMP Expression in Arthritic Joints

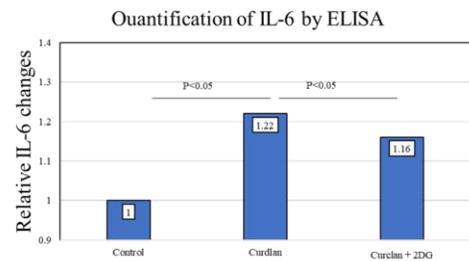


Figure 3: Quantification of IL-6 by ELISA