Intra-articular Injection Of Magnesium Chloride (MgCl2) Attenuates The Progression Of Osteoarthritis Through Suppressing The Expression Of Nucleostemin

Jerry Jiankun XU, PhD1, Yifeng ZHANG, PhD1, Jiali WANG, PhD1, Kelvin HO, MD3, Bruma Saichuen FU, PhD1, Kaiming CHAN, MD1,2, Ling QIN, PhD1,3.

1The Chinese University of Hong Kong, Hong Kong, China, 2The Jockey Club Sports Medicine and Health Sciences Centre, Hong Kong, China, 3Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China.


Introduction: Osteoarthritis (OA) is a degenerative joint disease with strong genetic component projected to affect 67 million adults in USA by 2030 [1]. Recently, the results of a large genome wide association study (7,473 cases and 42,938 controls) for OA have been reported and identified nucleostemin (NS) as a novel susceptibility locus for the disease [2]. It is also well known that NS plays a critical role in maintaining the pluripotency of stem cells and its expression level is down-regulated when entering differentiation stages [3]. Mg2+ could stimulate not only the osteogenesis of bone marrow mesenchymal stem cells (BMSCs) [4], but also the cartilage formation of synovial mesenchymal stem cells [5]. Moreover, intra-articular injection of Mg2+ containing solution (5 mol/L, 100μL) has shown encouraging effects on reducing collagenase induced OA as well as release of pain [6]. Based on above evidence, we specifically hypothesis that intra-articular injection of Mg2+ containing solution can attenuate the progression of a more common kind of OA (induced by anterior cruciate ligament transection, ACLT) via a previously unrevealed mechanism, i.e. suppression of NS.

Methods: Rabbit anti-nucleostemin and anti-actin antibodies were from Abcam (ab70346). The goat anti-rabbit HRP-linked secondary antibody was from CST (#7074s). Other products of high purity were purchased from local commercial stores.

The current study included two parts with specified objectives: 1) to test if there is higher expression of NS in ACLT induced rat OA; 2) to test the efficiency of MgCl2 in attenuation of OA progression and the expression levels of NS post treatment at different time points. Animals: 5-month-old male SD rats (weight average: 450 g) were used to establish unilateral ACLT models. Sham group received only skin incision and exposure of ACL but without transection. To archive objective 1, paraffin sections from sham and ACLT groups (n=5/group) were used for Safranin O staining and immunohistochemistry (antibody concentration, 1:200) determination of NS (to compare NS (+) cell number). Another 3 samples from each group were used to extract protein from cartilage for quantitative determination of NS using western blotting (antibody concentration, 1:1000). After confirming OA model was developed 2 months post operation, we performed planned experiments as illustrated in Fig.2 (addressed objective 2). 32 rats received ACLT were randomly divided into saline and MgCl2 groups (n=8/group/time point). Above solutions (5 mol/L, 100μL) were injected into the OA knee joint, respectively (twice per week, for two weeks). Samples were harvested at 4 and 8 weeks after the treatment. Test methods were described at objective 1. In addition, we also tested NS expression in rat BMSCs with and without Mg2+ treatment.
Statistical analysis was done using Graphpad Prism (version 6.01). Student’s t test was used to compare the difference between specified two groups. Significant difference was determined at P < 0.05.

Results: No rat was dead during the treatment. OA developed 2 months after surgery as characterized with obvious loss of cartilage matrix (Fig. 1A). IHC result indicated that NS positive cells were significantly more in ACLT group as compared with sham (Fig. 1A, D). It was consistent with the result from western blotting (Fig. 1B, C). Results from in vitro experiments showed that the expression levels of NS in BMSCs were significantly lower when supplementation of 10 mM Mg2+, at both 14 and 21 days, as compared with control group without supplementation of Mg2+ (Fig. 1E). The said treatment protocol showed injection of MgCl2 promoted the synthesis of cartilage matrix at both week 4 and 8 post treatment (Fig. 3A). The expression of NS in cartilage was profoundly down regulated after MgCl2 treatment, as compared with saline group (p<0.01, Fig. 3B, C, D). However, it is worth our attention that at week 8 post MgCl2 injection, the NS expression was much higher than week 4 MgCl2 group, though significantly lower than saline group at the same time point (Fig. 3D).

Figure legends:
Fig.1 OA models were developed 2 months after ACLT (A). NS expression was significant higher in rat OA samples (A-D). Supplementation of 10 mM Mg ions suppressed NS expression in BMSCs (E).
Fig.2 Schematic representation of the experimental design.
Fig.3 Intra-articular injection of MgCl2 attenuated the loss of cartilage matrix (A) and inhibited the expression of NS (B-D).

Discussion: In this study, for the first time, we have confirmed significantly higher expression of NS in ACLT induced OA of rat. Furthermore, we provide direct and crucial evidence supporting that Mg2+ attenuates the progression of OA at least via targeting NS. NS is expressed in both synovial tissue and cartilage. Although the role of NS in human OA is recognized, there is limited approach for suppressing its expression. Current data establishes a solid scientific basis for further exploration of not only the functional outcome (mechanical properties and behaviors), but also the upstream and down-stream signaling pathways involved in the alternation of NS induced by supplementation of Mg2+. As we noted that current treatment required repeat injections and the beneficial effect may not last after longer time point, sustained and controlled release Mg2+ delivery systems (encapsulated with hydrogel-like biomaterials) will be developed to improve the long-term efficacy.

Significance: This simple, widely available and inexpensive administration of magnesium ions containing product i.e. MgCl2 has the potential to reduce the massive health economic burden of OA.
**A** Safranin O staining of Femur and Tibia from Sham and ACLT groups.  
**B** Western blots showing NS and β-actin expression levels in Sham and ACLT groups.  
**C** Graph showing fold change of NS expression post-operation.  
**D** Bar graph comparing NS (+) cell number in Sham and ACLT groups.  
**E** Western blots showing NS and β-actin expression levels across different MgCl₂ concentrations.

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<th>Grouping</th>
<th>Week 4</th>
<th>Week 8</th>
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<tr>
<td>Saline</td>
<td>n=8*</td>
<td>n=8</td>
<td>32</td>
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<tr>
<td>MgCl₂</td>
<td>n=8</td>
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Note: *5 samples were harvested for histological studies, another 3 samples were prepared for western blotting.