

# The therapeutic effects of silver nanoparticle on the inflammatory disease of the temporomandibular joint

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## ABSTRACT

The objective of this study was to exam the therapeutic effects of silver nanoparticle on the inflammatory disease of the temporomandibular joint (TMJ). Mustard oil was injected to TMJ of the mouse for inducing the arthritis. The mice of negative control were received the Vaseline application and the positive controls were received indomethacin ointment on the preauricular area. The experimental groups were received the ointment contained the silver nanoparticle. The topical application was done every 24 hours after inducing the arthritis. The amount of food intake was checked every 2 hours. For the histological analysis, the mice were sacrificed at 8 and 72 hours after inducing arthritis. The comparison of the functional recovery between the experimental group and the positive control were similar at 72 hours after inducing the arthritis, but they were higher than the negative control ( $P < 0.05$ ). In the histological exam, the thickness of fibrous disc at 72 hours was statistically significantly lower in the experimental group than the negative control ( $P < 0.05$ ). The silver nano-ointment showed faster recovery from the TMJ arthritis in the animal model.

**Keywords:** silver nanoparticle, temporomandibular joint, anti-inflammatory

## 1 INTRODUCTION

The arthritis of the temporomandibular joint (TMJ) is found in the patients having TMJ disorders. In some cases, it is caused by the septic arthritis [1]. In other cases, it is the terminal stage of TMJ internal derangement [2]. Both cases may show pain in TMJ and the pain is a chief complain of the patients who visit the hospital [3]. Therefore, the pain control and the functional recovery are a key issue in treating the TMJ arthritis.

The painful response is caused by the inflammatory process of TMJ. Therefore the cytokines related to the inflammatory process mediate the painful response of TMJ. Among them, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin (IL) are well known mediators [4]. Leukotriene and prostaglandin are important in producing TNF and IL from inflammatory cells [5, 6, 7]. From these reasons, non-

steroidal anti-inflammatory drug (NSAID) or steroid has been used for the treatment of TMJ arthritis [8, 9].

The silver nanoparticles have anti-microbial effect [10] and anti-inflammatory effect [11]. As TMJ arthritis can be caused by the septic arthritis [1], the anti-microbial effect may be beneficial for the treatment of TMJ arthritis. Furthermore, the anti-inflammatory effect may reduce joint inflammation and help early functional recovery.

The objective of this study was to exam the therapeutic effect of the silver nano-ointment in the experimentally induced TMJ arthritis in mice.

## 2 MATERIALS AND METHOD

### 2.1 Arthritis inducing and measurement of food intake

Eighteen-month-old female IcrTacSam: ICR (Samtako, Osan, Korea) mycoplasma-free mice were used in this study. Mice were kept in standard plastic cages at 20°C with a 12/12-hour dark/light cycle [4]. Fifteen healthy mice were included in this experiment. They were divided as three groups (control, NSAID, and silver). For inducing TMJ arthritis, mustard oil (Sigma, St. Louis, MO) and mineral oil (Sigma) were mixed as 1 to 4 ratios. After diluting mixture to 20%, 15  $\mu$ l of diluents was injected to each preauricular area. NSAID ointment (indomethacin; VI-GEL<sup>®</sup>, Chodang Pharm., Seoul, Korea) as a positive control or silver nano-ointment (T-II<sup>®</sup>, NPC Inc., Iksan, Korea) as an experimental group was applied to the preauricular area using cotton ball every 24 hours. For the negative control, Vaseline was applied on the preauricular area. The ointment application was started immediately after injecting oil.

Mice received laboratory food (NIH#31M, Samtako, Osan, Korea) and tap water. The reference amount of food intake was measured from 48 hours before injection. The food pellet was given for every 2 hours and it was removed after 5 minutes eating. The weight of food pellet before and after eating was recorded as the amount of food intake for 5 minutes. The recorded data were averaged and set as a baseline food intake for each mouse. It was compared to the

amount of food intake after injection and calculated as a ratio. It was also recorded every 2 hours. If the ratio was 100, it would mean 100% functional recovery to pre-injection state. The ratio was used for comparing the functional recovery between groups.

## 2.2 Histological analysis

Thirty-five mice were used for the histological analysis. The method for inducing TMJ arthritis was in accord to previous section. They were also divided as three groups. Grouping criteria were in accord to the previous section. Fifteen mice were sacrificed at 8 hours after injection. Another fifteen mice were sacrificed at 72 hours after injection. TMJ was removed en bloc and all samples were fixed at 4% paraformaldehyde for 8 hours at 4°C. Then, the decalcification was done in formic acid for 24 hours. They were embedded in paraffin. The samples were prepared as 5 μ m thickness. They were stained at hematoxylline and eosin. The thickness of fibrous cartilage was measured using software. The thickness was measured in the cut which showed the widest dimension between medial to lateral pole of the condyle.

## 2.3 Statistical analysis

The difference between groups was compared by the independent samples t-test. The significance level was set as  $p < 0.05$ .

# 3 RESULTS

The functional recovery was shown in Fig. 1. The silver group showed fastest recovery and two out of five animal started to eat at 4 hours after inducing arthritis. The NSAID group showed that one out of five animal started to eat at 8 hours after.

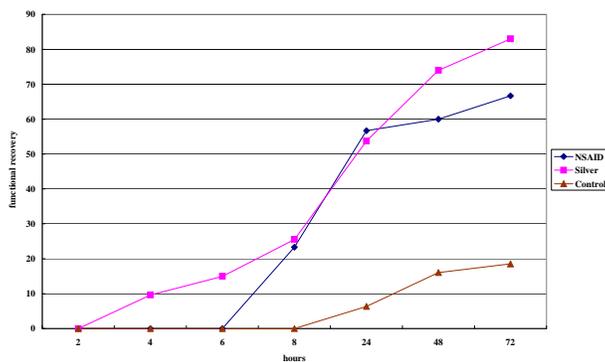


Figure 1. The functional recovery of each group

The control showed to start eating at 24 hours after (Fig. 1). The functional recovery at 72 hours showed 66.7%, 83.0%, and 18.5% in NSAID, silver, and control, respectively.

When compared to the control, both silver and NSAID group showed statistically significant difference ( $P < 0.05$ ).

The thickness of the disc was shown in Figure 2. The values at 8 hours after inducing arthritis were  $127.78 \pm 29.22 \mu m$ ,  $126.48 \pm 26.75 \mu m$ , and  $130.60 \pm 28.55 \mu m$  in control, silver, and NSAID group, respectively. When compared to the control, there was no significant difference ( $p > 0.05$ ). The values at 72 hours after inducing arthritis were  $168.01 \pm 26.83 \mu m$ ,  $144.70 \pm 29.74 \mu m$ , and  $157.57 \pm 22.91 \mu m$  in control, silver, and NSAID group, respectively. When compared to the control, there was significant difference between silver group and control ( $p < 0.05$ ).

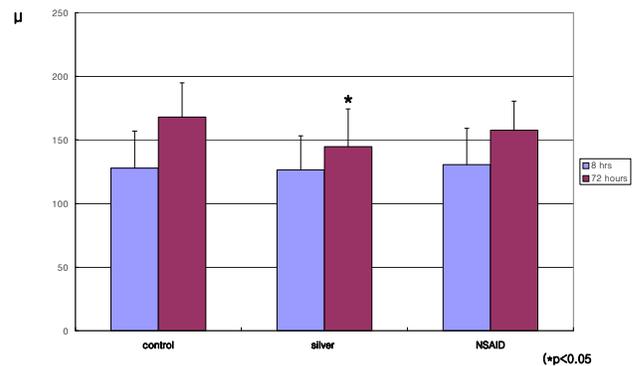


Figure 2. The thickness of the fibrocartilage.

The histological views were shown in Figure 3. The normal TMJ showed dense and intimate fibrous tissue (Fig. 3A). The control was 72 hours after injection and Vaseline application (Fig. 3B). It showed loose fibrous bundle and enlarged thickness compared to the negative control (Fig. 3A). Though all three samples (Fig. 3B, C, D) showed increased thickness when compared to negative control (Fig. 3A), NSAID group (Fig. 3C) and silver group (Fig. 3D) showed less looseness in fibrous bundle than the control (Fig. 3B).

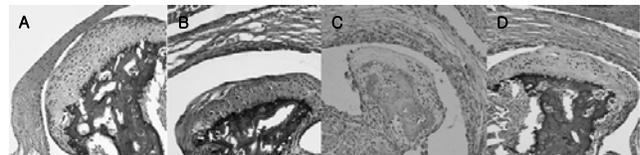


Figure 3. The histological view of each group (A: normal TMJ of mouse, B: control, C: NSAID, D: silver)

# 4 DISCUSSION

The therapeutic effect of the ointment containing silver nanoparticles in the TMJ arthritis was evaluated in the animal model. They showed significantly faster recovery in the masticatory function than the control (Fig. 1;  $p < 0.05$ )

and least swelling in the TMJ disc at 72 hours after inducing arthritis (Fig. 2;  $p < 0.05$ ). It showed similar therapeutic effect to the NSAID topical application.

TMJ inflammation may change the chewing patterns and food intake [12]. The silver group showed fastest recovery. Two out of five animals started to eat at 4 hours after inducing arthritis. The NSAID group showed that one out of five animals started to eat at 8 hours after. The control showed to start eating at 24 hours after (Fig. 1). Therefore, the functional recovery was fastest in the silver group in terms of the food intake. The functional recovery at 72 hours showed 66.7%, 83.0%, and 18.5% in NSAID, silver, and control group, respectively. When compared to the control, both silver and NSAID group showed statistically significant difference ( $P < 0.05$ ). The control showed only 18.5% of recovery when compared to the pre-injection standard level. In case of experimentally induced TMJ arthritis, the inflammatory reaction can last up to 6 weeks [13]. Therefore, low functional recovery in the control at 3 days after injection might be due to the inflammatory reaction of TMJ and resultant pain.

The acute biologic marker for the TMJ arthritis may be condylar cartilage thickness, soft tissue swelling, and chromodacryorrhea [14]. The thickness of the disc at 8 hours after inducing arthritis were  $127.78 \pm 29.22 \mu\text{m}$ ,  $126.48 \pm 26.75 \mu\text{m}$ , and  $130.60 \pm 28.55 \mu\text{m}$  in control, silver, and NSAID group, respectively (Fig. 2). There was no significant difference between the control and the experimental groups. The values at 72 hours after inducing arthritis were  $168.01 \pm 26.83 \mu\text{m}$ ,  $144.70 \pm 29.74 \mu\text{m}$ , and  $157.57 \pm 22.91 \mu\text{m}$  in control, silver, and NSAID group, respectively (Fig. 2). When compared to the control, there was significant difference between silver group and control ( $p < 0.05$ ). The decreased swelling in the silver group might be due to the anti-inflammatory effect of the silver nanoparticles [11, 15]. The silver nanoparticles show the rapid healing and improved cosmetic appearance in the skin wound [15]. When the dressing contained silver nanoparticles is used, the wound site is shown a reduction of bacteria and neutrophilic inflammation [16].

The histological findings were also similar to the other biologic marker. Normal disc showed intimate and dense fibrous tissue (Fig. 3A). After injecting oil, the fibrous tissue became loose due to edema and swelling (Fig. 3B, C, D). The looseness of fibrous network was prominent in the control (Fig. 3B). Though the loose fibrous network was also found in NSAID and silver groups (Fig. 3C, D), they were less prominent compared to the control (Fig. 3B).

The silver nanoparticle has been shown the antimicrobial effect [10] and the anti-inflammatory effect [11]. The mechanism of the anti-inflammatory effect of the silver nanoparticles has been largely unknown. The silver nanoparticle inhibits the expression of TNF- $\alpha$  and IL-12

and induces apoptosis of inflammatory cells [11]. The silver nanoparticles can reduce the erythema in the dermatitis and the therapeutic efficacy is similar to the steroid [17]. The reduced inflammation by the silver nanoparticles in our experiments might be partly explained by previous publications. However, further studies must be encouraged to unveil the anti-inflammatory mechanism by the silver nanoparticles.

## 5 CONCLUSION

In conclusion, the ointment containing the silver nanoparticles showed faster recovery from the TMJ arthritis. It might be due to the anti-inflammatory effects of the silver nanoparticles.

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