Can Platelet-Rich Plasma Protect Rat Achilles Tendons From the Deleterious Effects of Triamcinolone Acetonide?

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Background: Triamcinolone acetonide (TA) injections are widely used for tendinitis but have deleterious effects, including tendon degeneration or tendon rupture.

Purpose: To investigate whether adding platelet-rich plasma (PRP), a blood fraction that participates in tissue repair processes, to TA can prevent its deleterious effects.

Study Design: Controlled laboratory study.

Methods: Rat Achilles tendons were injected with TA, TA + PRP, PRP alone, or saline (control). Biomechanical testing and histological analyses were performed on Achilles tendons 1 week after injections.

Results: The maximum failure loads in the control, TA, TA + PRP, and PRP groups were 31.7 ± 2.3, 19.0 ± 3.6, 31.0 ± 7.1, and 30.2 ± 6.8 N, respectively. The tendon stiffness in the control, TA, TA + PRP, and PRP groups was 12.1 ± 1.8, 7.5 ± 1.8, 11.0 ± 2.8, and 11.3 ± 2.5 N/mm, respectively. The maximum failure load and stiffness were significantly lower in the TA group compared with the other 3 groups. There was no significant difference between the TA + PRP and control groups. Cell invasions, vacuolation, collagen attenuation, and increased type III collagen expression were histologically observed in the TA group; however, these changes were prevented by the simultaneous administration of PRP.

Conclusion: Administering PRP may prevent deleterious effects caused by TA; therefore, PRP may be used as a protective agent in clinical situations.

Clinical Relevance: PRP can be useful as a protective agent for sports injury patients receiving local corticosteroid injections.

Keywords: triamcinolone acetonide; platelet-rich plasma; rat Achilles tendon; corticosteroid injection

Corticosteroid injections are widely used for tendinitis because of their anti-inflammatory effects. Improvements in joint function and pain relief after corticosteroid injections were observed in a clinical study,[1] while several cases of tendon degeneration or rupture after corticosteroid injections have been reported.[9] There are several in vivo and in vitro studies regarding the deleterious effects after corticosteroid injection. According to in vitro studies, corticosteroids decreased cell number, suppressed cell proliferation, and reduced collagen synthesis.[23,24] In an earlier in vivo study, we reported that triamcinolone acetonide (TA) decreased mechanical strength and induced histological changes in the rat Achilles tendon.[12]

Platelet-rich plasma (PRP) is an autologous concentration of platelets, which contains many growth factors, including platelet-derived growth factor, transforming growth factor–β, basic fibroblastic growth factor, vascular endothelial growth factor, insulin-like growth factor–1, and epidermal growth factor. PRP is prepared from autogenous blood and considered to be safe from transmissible diseases,[13] and it has been reported to stimulate the regeneration of tendon tissue[3,19] or cell proliferation and to enhance

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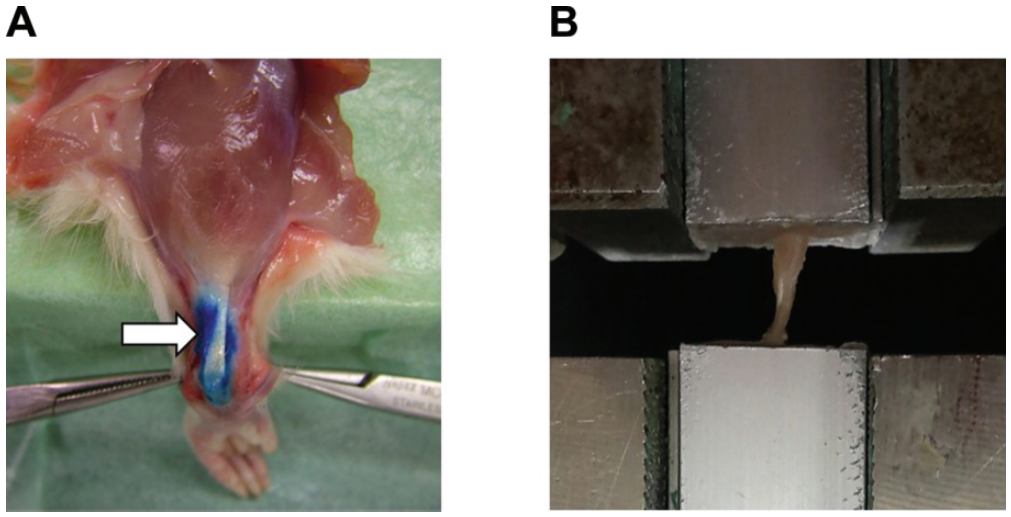

Figure 1. Percutaneous reagent injection and biomechanical test. (A) Toluidine blue stain was observed only around the surface of the Achilles tendon (white arrow). (B) The tendon and the calcaneus were placed in specially designed devices, using polymethyl methacrylate resin, and placed vertically in a tensile strength sensor.

matrix gene expressions.[6] Muto et al[13] previously reported that TA decreased the viability of cells derived from the human rotator cuff by causing apoptosis, and these deleterious effects were prevented by administering a combination of TA and PRP in vitro. However, the combination of TA and PRP on the rat Achilles tendon has not yet been elucidated in vivo. The purpose of this study was to investigate whether adding PRP to TA injections can prevent the deleterious effects of TA in the rat Achilles tendon.

METHODS

All animal procedures were performed under the approval and guidance of the Animal Care and Use Committee of Kobe University.

Animal Model and Surgical Technique

A total of forty 10-week-old male Sprague-Dawley rats (CLEA Japan) were used. All solute was diluted by saline into final concentration as follows: 0.2 mg/kg TA (0.1 mg TA) (TA group; n = 10), 0.2 mg/kg TA (0.1 mg TA) and 50% vol/vol PRP (TA + PRP group; n = 10), 50% vol/vol PRP (PRP group; n = 10), and saline (control group; n = 10). The TA + PRP solution was not a significant dilution of TA alone. The rats were sedated by inhaling diethyl ether and anesthetized with a 50-mg/kg intraperitoneal injection of pentobarbital (Kyoritsu). The ankles of all rats in each group were shaved, and 50 μL of the appropriate solution was injected percutaneously at the Achilles tendon–calcaneus junction into the surface of the right Achilles tendon with a 26-gauge needle.[12] To confirm whether the solution was delivered to the surface of 4 Achilles tendons, toluidine blue (50 μL) was injected to the Achilles tendon in a similar manner as described above (Figure 1A). The hind limbs were dissected as in a preliminary study.[12] After the injections, all animals were housed in standard cages with unrestricted food, water, and activity; the animals were monitored according to a standardized protocol. One week after the injection, the rats were sacrificed by an intraperitoneal injection of a fatal amount of pentobarbital, and their Achilles tendons were harvested. Four tendon specimens from each group were analyzed histologically. After the Achilles tendon–calcaneus complex was isolated, tendon specimens were stored in saline-soaked gauze at –80°C until biomechanical testing.

Platelet-Rich Plasma Preparations

Whole blood was collected by cardiac puncture from 3 healthy rats and centrifuged at 220*g* for 10 minutes to separate the red blood cell fraction from whole plasma. Afterward, a second centrifugation was conducted at 330*g* for 15 minutes to separate PRP from platelet-poor plasma.[17] The platelet count in the PRP was about 3 times higher than that of whole blood. The donors' platelet counts in whole blood were 880,000 to 979,000 platelets/μL. The concentrated platelet counts were 3,336,000 to 3,592,000 platelets/μL.

Biomechanical Testing

The specimens were thawed. The proximal Achilles tendons were covered with gauze and sutured with nylon (6&66) monofilament yarn (Alfresa Pharma). The tendon and calcaneus were placed in specially designed devices using polymethyl methacrylate resin and placed vertically in a tensile strength sensor (AG-I; Shimadzu) (Figure 1B). Prior to performing the tensile test, the tissues were preconditioned with a static preload of 0.2 N for 1 minute, followed by 5 cycles of loading and unloading at a strain amplitude of approximately 0.5% at 60 mm/min.

Immediately after preconditioning, the maximum failure load was recorded at a uniaxial tension of 60 mm/min. The maximum failure load was measured as the primary outcome, and the tendon stiffness was calculated from the load-deformation curve (n = 6 per group). This number was backed up by a power analysis ($\alpha = 0.05$; power level, 80%; SD, 20%; effect size, 0.68).

Histological Analysis

Tissue samples were fixed in 4% paraformaldehyde for 24 hours, decalcified with 0.25 mol/L ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline, dehydrated in a graded series of alcohol solutions, and embedded in paraffin wax. Long-axis sections (6 μm thick) were stained with hematoxylin-eosin and assessed.[12] The histological findings regarding the cellular responses were assessed using a light microscope (Keyence). The tissues were evaluated with fiber alignment scoring using the microscope (n = 4 per group).[14] Collagen fibers were analyzed using Picrosirius red staining because it binds directly to the fibers. Long-axis sections (6 μm thick) were stained with a Picrosirius Red Stain Kit (Polyscience) according to the manufacturer's protocol. Deparaffinized sections were stained in Picrosirius red for 60 minutes and washed in acidified water. The sections were dehydrated, cleared, and mounted. Under polarization microscopy, orange and green fibers indicated type I and type III collagen, respectively.

Statistical Analysis

All data are expressed as mean ± SD. Groups were compared using 1-way analysis of variance. Post hoc analyses were performed using the Student-Newman-Keuls test; *P* values of <.05 were considered statistically significant.

RESULTS

Mechanical Evaluation

All samples ruptured at the Achilles tendon–calcaneus junction. The maximum failure loads in the control, TA, TA + PRP, and PRP groups were 31.7 ± 2.3, 19.0 ± 3.6, 31.0 ± 7.1, and 30.2 ± 6.8 N, respectively (Figure 2A). The tendon stiffness in the control, TA, TA + PRP, and PRP groups was 12.1 ± 1.8, 7.5 ± 1.8, 11.0 ± 2.8, and 11.3 ± 2.5 N/mm, respectively (Figure 2B). The maximum failure load and stiffness in the TA group were significantly lower than the control group. The maximum failure load and stiffness in the TA + PRP group were significantly greater than the TA group; the same measurements in the PRP group were not significantly greater than in the control group.

Hematoxylin-Eosin Staining

Macroscopically, the control (ie, saline-injected) and PRP groups showed normal tendon appearance. Cell invasions and vacuolations at the surface layers of the tendons were observed in the TA group; however, these apparent changes were not observed in the TA + PRP group (Figure 3).

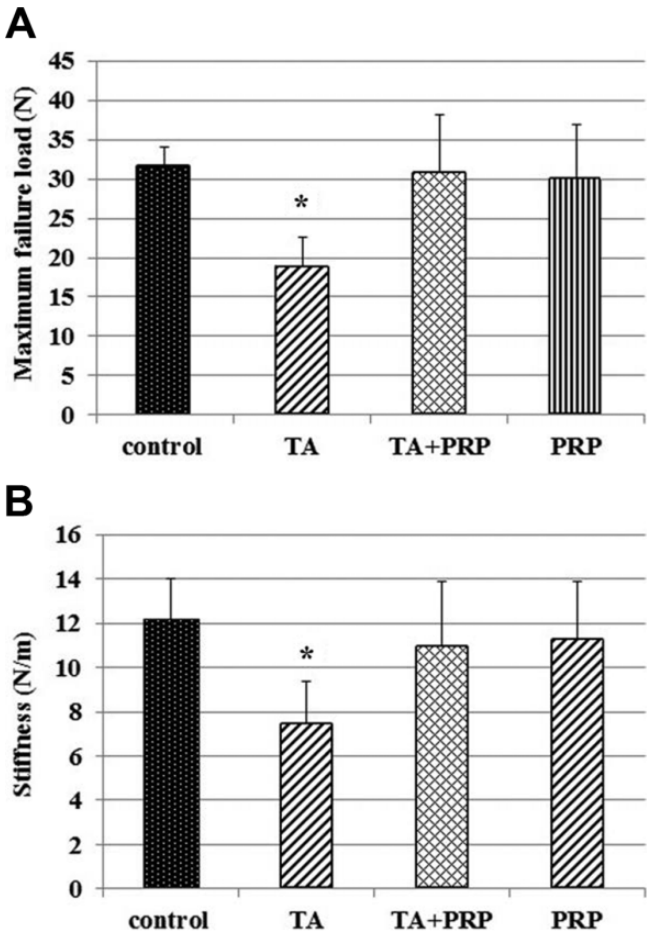

Figure 2. (A) The maximum failure load was significantly lower in the triamcinolone acetonide (TA) group than the control group. (B) Stiffness was significantly lower in the TA group than the control group. There were no significant differences in the maximum failure loads or stiffness values between the TA + platelet-rich plasma (PRP) and control groups (*$P < .05$).

Histological Scoring

The fiber alignment scores in the control, TA, TA + PRP, and PRP groups were 2.9 ± 0.3, 1.3 ± 0.7, 2.6 ± 0.4, and 2.7 ± 0.5, respectively (n = 4 per group). There was a significant difference in the fiber alignment score between the TA and the control groups. The fiber alignment score for the TA + PRP group was significantly greater than that for the TA group. There was no significant difference between the PRP and the control groups (Figure 4).

Picrosirius Red Staining

In the TA group, collagen fiber bundles were aligned irregularly with fiber attenuation at the surface of the tendon.

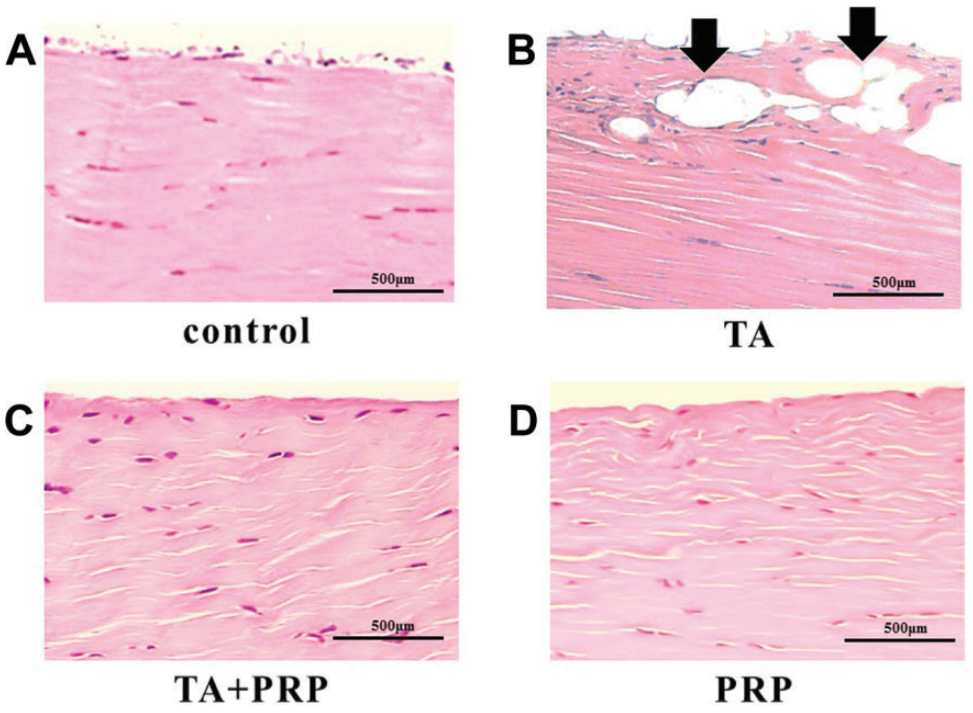

Figure 3. Hematoxylin-eosin-stained histologic images from the control, triamcinolone acetonide (TA), TA + platelet-rich plasma (PRP), and PRP groups. Cell infiltration and vacuolation were observed in the TA group (black arrowheads) but not in the TA + PRP group. Scale bars = 500 μm.

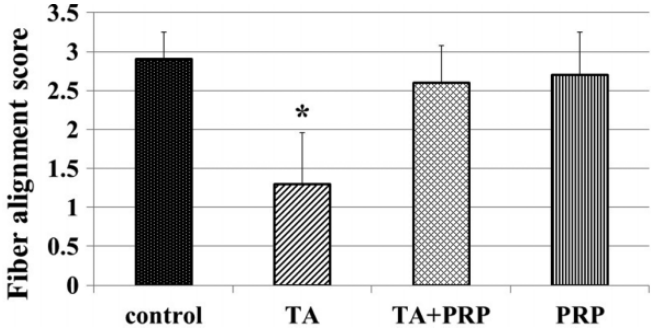

Figure 4. The fiber alignment score was significantly lower in the triamcinolone acetonide (TA) group than the control group. However, there were no significant differences between the TA + platelet-rich plasma (PRP) and control groups (*$P < .05$).

Type I collagen expression in the tendon was observed in all groups using polarization microscopy. Type III collagen expression at the surface of the tendon was observed in the TA group. However, little type III collagen expression was observed in the TA + PRP group (Figure 5).

DISCUSSION

Corticosteroid injections are commonly used for soft tissue disorders and a wide range of inflammatory disorders.[16] However, local corticosteroid injections have been related with causes of tendon degeneration or rupture in many case reports.[9] Muto et al[13] previously reported that TA had deleterious effects in cultured human rotator cuff–derived cells, which were prevented by the simultaneous administration of PRP in vitro. However, there are no reports regarding the effects of combined TA and PRP in an in vivo model. As expected, we observed that exposure to TA decreased mechanical strength and caused histological changes; these deleterious effects were also prevented by the administration of PRP in the in vivo study. Type I collagen is the most abundant collagen (>90% of the total collagen content) found in normal tendons. When tendons are injured, an increased proportion of type III collagen is produced during the early injury response.[22] A low expression of type III collagen was detected in the surface layers of tendons in the control group of this study. In the TA group, type III collagen localization was detected in the surface layers of the tendons. However, type III collagen expressions in the TA + PRP group did not increase. According to these results, PRP protected the rat Achilles tendons from the early injury response of TA.

Platelet-rich plasma is a blood fraction containing a high concentration of platelets, which actively play a role in healing processes.[13] Each growth factor, with its own characteristic actions, stimulates angiogenesis, cell migration, and cell growth and increases protein synthesis. These processes lead to better and faster tissue healing.[5,20] It is unclear whether PRP has anti-inflammatory effects and reduces local inflammation. According to a study regarding

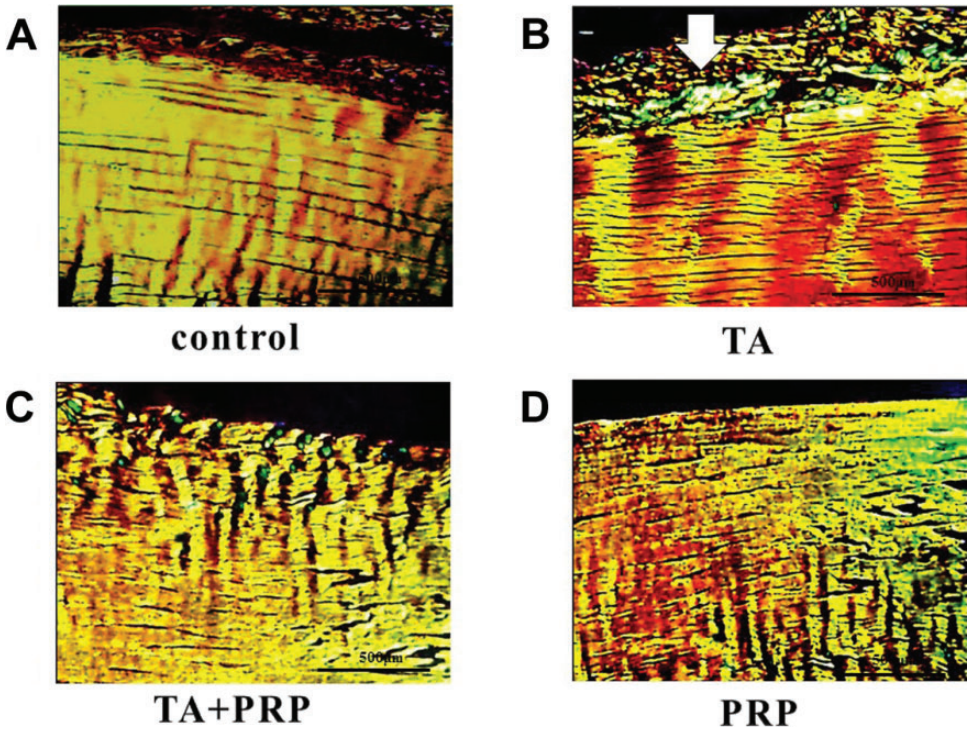

Figure 5. Picrosirius red–stained histologic images of the control, triamcinolone acetonide (TA), TA + platelet-rich plasma (PRP), and PRP groups. Attenuation of the collagen fibers and type III collagen were observed in the surface layers of the tendons of the TA group (white arrow). The changes in the TA + PRP group were not increased. Scale bars = 500 μm. Orange and green fibers indicate type I and III collagen, respectively.

the effect of PRP on inflammatory pain relief, PRP was observed to reduce the level of discomfort.[7] Sampson et al[18] reported that intra-articular injections of PRP in patients with knee osteoarthritis were safe and effective for reducing pain. Moreover, PRP reduced pain in patients with total shoulder and knee arthroplasty. They considered that PRP could reduce pain because it augmented the inflammation cascade and allowed earlier hemostasis and repair after surgical treatment.[10] However, PRP contained undesirable pro-inflammatory cytokines and induced an inflammatory cytokine response in joints.[21] Therefore, we combined corticosteroids that have an anti-inflammatory effect with PRP, which could promote tissue repair in this study. Mechanical strength and fiber alignment scores for the TA + PRP group were significantly greater than those for the TA group, while those for the PRP group showed no significant difference compared with the control group. It was reported that PRP increased mechanical strength and protected histological changes in injured rat Achilles tendons.[8] However, PRP was injected into the injured tendons in these studies, whereas PRP was injected into normal tendons in our study. The administration of PRP to normal tendons did not increase the mechanical strength or improve the histological appearance. Therefore, we were able to determine that PRP could protect injured tendons from the deleterious effect of TA administration.

A previous report showed the tendon ruptured at the Achilles tendon–calcaneus junction.[12] Other reports showed that the tendon failure point was midsubstance.[2] In this study, tendons ruptured at the Achilles tendon–calcaneus junction. We injected each solution at the Achilles tendon–calcaneus junction in reference to the previous report. This location might be the most damaged by the injections, which may have caused tendons to rupture at Achilles tendon–calcaneus junction in this study.

There were several limitations in this study. First, the corticosteroid dose was determined to be equivalent to a human dose. A corticosteroid dose of approximately 0.2 mg/kg was used for the experiments. However, there may be differences in the effective corticosteroid dose between rats and humans; further examinations at various dose levels are required. Second, the corticosteroid injection may have adverse effects to the tendons depending on the timing of evaluations. According to the present data, corticosteroid injections decreased mechanical strength in the rat Achilles tendon at 7 days. Alternatively, it has also been reported that no biomechanical strength effects were observed after corticosteroid injections in a previous study[15]; however, these evaluations were performed >1 month after corticosteroid injection. The study of the rat tendon was evaluated at 55 days.[11] The rabbit patellar tendon was evaluated at a mean 33 days after corticosteroid injection.[11] In a rotator cuff injection model, corticosteroids reduced the biomechanical properties of the rotator cuff tendon at 1 week. However, the corticosteroid affected the biomechanical properties of the

supraspinatus tendon temporally, which returned to control levels by 3 weeks.[11] Therefore, we evaluated tendon 1 week after the injection; however, the long-term effect of TA and PRP need to be evaluated in the future because the deleterious effects of corticosteroid may be initiated in the early phase after injection and eventually subside after a longer period. Third, the corticosteroids were injected around normal tendons in this study. In clinical situations, corticosteroids are usually injected around inflamed tendons. Further examination in a tendinopathy model will be needed. Fourth, PRP can be categorized into subtypes; however, we did not assess the subtype. Dohan Ehrenfest et al[4] classified PRP into 4 categories depending on leukocyte and fibrin content of PRP: leukocyte-poor or pure platelet-rich plasma, leukocyte- and platelet-rich plasma, leukocyte-poor or pure platelet-rich fibrin, and leukocyte- and platelet-rich fibrin. However, we did not count leukocytes and classify the type of PRP in this study. Fifth, we injected toluidine blue only in a pilot study and did not blindly inject each treatment in this study. These injections might not be performed in an identical fashion and harm the Achilles tendons.

CONCLUSION

Triamcinolone acetonide significantly decreased mechanical strength and caused histological changes in the rat Achilles tendon; this deleterious effect was prevented by the simultaneous administration of PRP.

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