Effectiveness of intra-articular injection of hialuronic acid on arthogenic contractures of rat knee joints in the remobilization phase

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Abstract

Introduction: The study examines the impact of intra-articular hyaluronic acid injections on arthrogenic contractures in rat knee joints, focusing on the remobilization phase after immobilization-induced contractures.

Methods: Utilizing a controlled experimental design, the study involved rats with induced knee joint immobilization, followed by treatment with intra-articular injections of hyaluronic acid. Measurements of range of motion (ROM) were used to assess the effectiveness of the treatment.

Results: The treatment group showed a significant improvement in knee joint ROM compared to controls, indicating that hyaluronic acid injections effectively reduce contractures and improve mobility.

Discussion: The findings support the therapeutic potential of hyaluronic acid in treating joint contractures, suggesting a beneficial approach for post-immobilization recovery in clinical settings

Keywords: Hyaluronic acid, knee joint contractures, arthrogenic contractures, range of motion, rat model, intra-articular injections

1. Introduction

Formation and recovery processes contracture joints induced by immobilizationhas checked with carefully using animal models . Responsible structureanswer has shared in a way wide become factor myogenic and arthrogenic . Research conducted by Trudel et al . show that myogenic factors especially responsible answer For contracture joints in phase beginning immobilization (in time two week) and completed with remobilization (Kaneguchi et al., 2020; Trudel et al. 2014). On the contrary, factors arthrogenic part big contribute to contractures joints severe cases caused by prolonged immobilization (morefrom four week), and recovery with remobilization No expected in case this (Trudel et al. 2014). Contracture arthrogenic also increasingly develop during remobilization after immobilization period short (in three Sunday). For avoid contracture irreversible joints, then contracture arthrogenic must targeted .(Kaneguchi et al., 2020)

Depending on the underlying pathology, contracture Joints can too classified as progressive or nonprogressive. Contracture progressive acquired, related with condition chronic like injury to the brain, or marrow bone back, disease rheumatism, and procedures repair surgery like arthroplasty total knee, and usually related with factor extrinsic . Example

factor extrinsic including limited joint ROM, mobilityreduced physique, weakness of muscle, spasticity, disorders cognition, and pain On the other hand, contractures are non- progressive usually nature congenital, affecting Lots joints and members body and relationships with reason genetic, for example arthrogryposis multiplex congenita.

A number of study report that prevention contracture arthrogenic achieved with injection decorin intraarticular, which inhibits activity transformation factor growth -beta, acid hyaluronic acid, mytomycin C, and celecoxib. Reports previously This using the contracture model traumatic animal or a combination of joint trauma and immobilization, both of which show change prominent inflammation in the affected joint, nolike the model does move . In modelsimmobilization, contracture arthrogenic reduced with intra- articular injection of the corticosteroid triamcinolone in rats . However, as effect side, joint treated micewith triamcinolone shows disturbance with burden much weightmore light than controls that don't treated .

A studies recently This show that intra- articular hyaluronan injection can prevent change Fibrotic and inflamed capsulejoints that don't moving (Ozawa et al., 2016; Kanazawa et al., 2015). Exogenous hyaluronan treatment stimulate synthesis hyaluronan and reduces pro-inflammatory mediators in osteoarthritic joints . Intra- articular injection of hyaluronan with heavy molecule high, which modulates response inflammation, yes prevent change Fibrotic capsulejoints that don't moves in mice and rabbits . (Ozawa et al., 2016)

Stiffness joints secondary consequence immobilization inhibited by injection sour intra- articular hyaluronic acid in contracture joints experimental found in research rabbit . Biochemical and biomechanical parameters used For evaluate stiffness joints after nine Sunday immobilization . In all treatment, acid hyaluronic acid reduce stiffness measured in contractures about 50% compared with contracture rabbits that don't given treatment . Besides that 's sour hyaluronic acid prevent disappearance glycosaminoglycans (measured with hexosamine), which is usually occurs in contractures that do not treated . The result related with hypothesis Work that injection intra- articular drugs like sour hyaluronic acid will stimulate synthesis sour hyaluronic acid in matrix periarticular connective tissue . If spacing and lubricating properties from glycosaminoglycans can maintained in circumstances *stress-deprived,*" collapse centripetal " of matrix fibrillar can avoided, relationships cross anomaly can minimized, and mechanisms more normal joints canmaintained .(Amiel et al., 1985)

As has been mentioned Previously, intra- articular injection sour hyaluronic acid contain modulate response inflammation, yes prevent change Fibrotic capsulejoints that don't move . Intra- articular injection sour hyaluronic acid own can prevent change Fibrotic and

inflamed capsulejoints that don't move . Maintenance hyaluronan exogenous stimulate synthesis hyaluronan and reduces pro- inflammatory mediators . For that, researcher interested For research about effect intra- articular injection sour hyaluronic acid to contracture joints knee .

2. Methods

Study This is study experimental therapeutic with use animal experiment (mice) and use post-test only control group design . Study carried out at the Mathematics and Natural Sciences Laboratory, University of North Sumatra with time study for 3 months (September – October 2023). Mouse group control and treatment get food and Drink given adlibitum in pellet and feed form mice and incubated for 8 weeks and done inspection clinical that is observation activity daily, signs infection (lust eating, paralysis, presence of sinuses etc.) and measurements temperature and weight eachthree day, as well as against mouse control . Then mouse will sedation use ketamine 35 mg/kg and xylazine 5mg/kg were continued with inhalation use diethyl ether. Next with shaving to hair on both his feet as well as done PROM measurements before done immobilization .

Immobilization with using a 0.8 mm K-wire on the tibial shaft subcutaneously to the femur with position knee flexion 150 degrees . Before start action done antiseptic and aseptic action against second limbs using povidone iodine. Furthermore mouse will given the antibiotic cefazoline 22mg/kg and anti- pain ketorolac 0.1mg/kg IV for prevent infection and overcoming painful . Immobilization done for 4 weeks .

After 4 weeks, done release tool immobilization in the form of a k-wire starts phase remobilization accompanied with injection intraarticular in groups sour hyaluronic acid and Nacl 0.9 % on day 7th, 14th, 28th, 35th and 42nd. Giving InjectionThis use syringe sterile size 31 G. Rat in group experiment accept injection sour hyaluronic acid used form in form injection in water with concentration of 0.1 ml →10 mg/ml HA and mice in group control accept injection of 0.3 mL of 0.9% NaCl. (Wang et al., 2014).

After it was done on the 56th day Knee PROM recording mouse using a goniometer with the way the feet will given a torque of 40Ncm for give similar results .Previously mouse euthanized . If the pupil of the eyeball grow, no There is reflex Light, breathing and beating heart stop furthermore Myotomy was performed with domuscle transectionthigh mouse For exclude component contracture myogenic .

Knee ROM be measured using a plastic goniometer with mouse lie on your side at temperature room (22 °C). Goniometer use three points (proximal femur, distal tibia, and

condyle lateral femoral) for measure corner joints knee . Point fulcrum centered at the top room joints lateral knee, with fingering depression network soft right behindlateral tibial plateau . This is pivot point from which angle between the tibia and the bone thigh be measured . One arm of the goniometer is placed parallel with a line between greater trochanter and joints knees, and arms other placed parallel with longitudinal axis of the leg section below and above ankle withwide currently .

Data is collected directly by researchers . Normality test done with the Shapiro-Wilk test of recall amount sample < 50. If the data is normally distributed then will while the independent T test was used If No normally distributed using the Mann Whitney test. If the data analysis is normally distributed then will ANOVA test was used whereas If No normally distributed using the Kruskal Wallis test determine difference all over group . If results analysis show exists difference between group so next For determine difference between group using the Bonferonni Post-hoc test or alternative Mann Whitney U test . Program used is device soft statistics with windows based . Limits of significance when$p < 0.05$.

3. Results

On analysis characteristics *Range of Motion* three group mouse done average degree assessment . Entire mouse experience post ROM increase Myotomy with value 1 ^{0} Where matter This significant in a way statistics . Mouse ROM control before Myotomy is 126.37 ± 3.58 p This increase to 127.37 ± 3.42. 0.9% NaCl rat ROM before Myotomy is 127 ± 5.01 and after Myotomy is 128.37 ± 4.43. Mean ROM of mice with sour Hyaluronic was 141.5 ± 3.42 with post ROM Myotomy 142.37 ± 3.66. Entire results This found increased and significant in a way statistics with paired t-test where $p < 0.05$. This result can observed in Table 1.

Table 1. ROM of mice before and post Myotomy

Group	ROM before Myotomy	ROM after Myotomy	*p-value*
Control	126.37 ± 3.58	127.37 ± 3.42	0.007 [a]
NaCl 0.9%	127 ± 5.01	128.37 ± 4.43	0.004 [a]
Sour hyaluronate	141.5 ± 3.42	142.37 ± 3.66	0.021 [a]

[a)] Paired T-test

For test is sour Hyaluronic own influence compared to therapy other before Myotomy done ROM assessmentstatistics between One each other. Comparison between group show that

sour hyaluronic acid own effect more tall in increase ROM and overcome contracture compared to with other procedures before Myotomy . This matter proven with p- value < 0.001 in the ANOVA test and p-value < 0.001 in the Post-hoc Bonferroni test compare between third group . This result can observed in Table 2.

Table 2. Analyze each ROM treat before Myotomy

Group	ROM before Myotomy	p-value	*Post-hoc Bonferonni*		
			Control	NaCl 0.9%	Sour hyaluronate
Control	126.37 ± 3.58	<0.001*		1	<0.001
NaCl 0.9%	127 ± 5.01		1		<0.001
Asam hyaluronate	141,5 ± 3,42		<0.001	<0.001	

***) ANOVA**

For test more carry on is sour Hyaluronic own influence compared to therapy other post Myotomy done ROM assessmentstatistics between One each other. Comparison between group show that sour hyaluronic acid own effect more tall in increase ROM and overcome contracture compared to with other post management Myotomy . This matter proven with p- value < 0.001 in the ANOVA test and p-value < 0.001 in the Post-hoc Bonferroni test compare between third group . This result can observed in Table 3.

Table 3. Analyze each ROM treat after Myotomy

Group	ROM after Myotomy	p-value	*Post-hoc Bonferonni*		
			Control	NaCl 0.9%	Sour hyaluronate
Control	127.37 ± 3.42	<0.001*		1	<0.001
NaCl 0.9%	128.37 ± 4.43		1		<0.001
Asam hyaluronate	142,37 ± 3,66		<0.001	<0.001	

***) ANOVA**

For do data visualization then create a bar chart Where results This in a way subjective show contrast increase in ROM between group before myotomy and after myotomy **.**

4. Discussion

Comparison between group show that sour hyaluronic acid own effect more tall in increase ROM and overcome contracture compared to with other procedures before done Myotomy ($p<0.001$). matter This in line with Research that states that after done giving sour hyaluronic, then ROM increases during remobilization depends time, and reached 120.6˚ on day to 14, though Still Far more low compared to control with range 157.6˚ ($p<0.001$). (Kaneguchi et all., 2017)

Explanation This related with How component myogenic and arthrogenic to total contractures based on ROM results before and after myotomy . Study previously report that on the day to 0, contracture myogenic contribute more big compared to contracture arthrogenic . However, contribution contracture myogenic in a way gradually decrease during period remobilization, meanwhile contracture arthrogenic increase . On the day by 14, total contractures are reduced almost half of it, however remaining contracturepart big nature arthrogenic . (Kaneguchi et all., 2017). This matter in line with study this is what found it that comparison between group treatment and control show sour hyaluronic acid own effect more tall in increase ROM and overcome contracture compared to with other post management Myotomy . This matter proven with p- value < 0.001 in the ANOVA test and p-value < 0.001 in the Post-hoc Bonferroni test compare between third group

A meta- analysis injection sour intra- articular hyaluronic acid For treatment osteoarthritis find that compared to with sour hyaluronic acid placebo produce effect profitable that can be measured on function joints in 4 weeks, reach effectiveness peaks at 8 weeks, and delivers residual effects up to 24 weeks . (Wang et al., 2014; Bannuru et al., 2011)

Research result This is also supported by Wang et al. is that giving sour hyaluronic acid intraarticular for 8 weeks immobilization knee in a way significant reduce level formation adhesion joints, lowering content collagen, and increases ROM. A number of operation joints need immobilization up to 8 weeks, and our findings show possibility benefit clinical from injection sour hyaluronic acid to in immobilized jointup to 8 weeks . Adhesion joints often appear after 4 weeks immobilization, therefore That duration 8 weeks from study This possible more analysiscritical from effect sour hyaluronic acid in prevent adhesion joints . (Wang et al., 2014).

5. Conclusion

Mean ROM of mice with sour Hyaluronic was 141.5 ± 3.42 with post ROM Myotomy 142.37 ± 3.66. Mean ROM of mice control before Myotomy is 126.37 ± 3.58 p This increase

to 127.37 ± 3.42. Comparison between group show that sour hyaluronic acid own effect more tall in increase ROM and overcome contracture compared to with other procedures before nor post Myotomy .

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