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The Combination Effect Of Bovine Collagen And Plasma Rich Platelet On Biomechanical Strength Of Repaired Achilles Tendon Rat Model With Diabetes Mellitus Type - 1 (Rattusnovergicus Strain Wistar)

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Abstract: *Diabetes Mellitus (DM) alters normal biologic process of tendon healing through inadequate of growth factors, abnormalities in the production of cytokines, impaired neovascularization and collagen synthesis. Plasma Rich Platelet (PRP) have the ability to increase growth factor concentration which results in the increase of collagen production and tissue healing. Bovine collagen type I play role in intrinsic pathway which result in more elastic and stronger tendon. The purpose of this research is to evaluate the combination effect of bovine collagen type I and PRP in Achilles tendon ruptures of rat with DM type 1 through biomechanical and histopathological examination.This is an experimental study, performed in 25 adult male rat, divided into 5 groups. The first group was normal, second group was DM type 1, third group was DM type 1 treated with bovine collagen, fourth group was DM type 1 treated with PRP, and fifth group was DM type 1 treated with combination of collagen and PRP. The biomechanical evaluation was performed by traction machine until totally ruptured (ultimated strength) and through histopathological evaluation by count of tenoblast cell. Wefound there is significant difference in the third group and fifth group and no significant difference in the second and four group by biomechanical and histopathology examination (p-value <0.05).Combination of bovine collagen type I and PRP showed better biomechanical results on early ultimate strength of ruptured tendon on diabetes mellitus type 1.*

Keywords: *Bovine type I collagen, Plasma Rich Platelet, Tendon healing, Diabetes Mellitus type - 1.*

I. Introduction

Achilles tendon rupture is a common tendon rupture than other tendons [1,2]. The Fremantle diabetes study in Australia, Diabetes Mellitus (DM) patients with spontaneous rupture of 6,25% obtained in the case of Achilles tendon rupture [3]. Achilles tendon has a very minimal vascularity especially in wathershed zone 2 – 6 cm from the calcaneus so if there is degeneration and rupture, the healing process is not as good in many areas of vascularization [4,5,6]. On the Achilles tendon who had DM associated with an increase in glucose levels cause inadequate production of growth factor, cytokine production, disruption of neurovascular and disrupted the formation of the collagen matrix that increasingly hinder the healing process and decrease the Achilles tendon ruptures biomechanical forces [7].Conditions of acute hyperglycemia on tendon structure effect both biomechanics and histopathology. This is due to the accumulation of Advanced Glycation End Products (AGEs) in the tendon that ruptured causing this increase in arterial stiffness, changes in collagen cross-link that ruptured causing this increase in arterial stiffness, alter the structure, mechanical properties, decrease in collagen content rich and disrupt the formation of fibrils [7]. In general, the tendon healing process occurs via the intrinsic and extrinsic pathways. In biomechanics, tendon intrinsic pathway produce a stronger and more elastic, while the extrinsic pathway result in callus formation and adhesion in the tendon. Collagen scaffold on tendon healing role in the intrinsic pathway, whereas the PRP role in the intrinsic and extrinsic pathways [8,9,10]. Suture technique and an early rehabilitation can improve the quality of tendon grafting. Suturing techniques are expected to maintain a good approximation of the end of the stump so as to allow the healing process. Early rehabilitation will increase the number and thickness of the collagen network and improve the alignment of cells and tissues that will increase the amount of tendon fibers, the tendon fibers and the direction of the bond between networks [11]. The addition of collagen type 1 is expected to lead the formation of the mature tendon, induces fibroblast infiltration, as a biological scaffold as the growth of fibroblast cells and growth factors [12,13,14]. Patients whose DM, growth factors are not produced adequately, thus giving plasma concentrations of PRP that have high plasma concentrations (5 times than normal) and growth factors are expected to affect the healing of Achilles tendon rupture by stimulating the proliferation of fibroblast to form collagen[15,16]. Until now, no study has used a combination of PRP with collagen type 1 in the treatment of tendon rupture in DM. Healing

process in the Achilles that was not good especially in the DM condition in which the healing process twice as long and biomechanical strength was significantly lower in DM compared to the normal Achilles tendon [7],a matter considerable importance. It is expected that the provision of triple helix collagen and PRP can speed tendon healing with good quality, reduce the duration of immobilization, speed up the rehabilitation program and can return to daily activities earlier. The aim of this study was to determine the effect of combination of PRP and bovine type 1 collagen on healing of Achilles tendon rupture through an increased number of fibroblast proliferation and biomechanical strength early in wistar rats with DM type 1 condition.

II. Methods

This research is experimental study, randomizedpost testcontrol group designto determine the effect of combination of bovine type I collagen and PRPon repaired Achilles tendon DM type 1 rat. Samplesdivided into2 groups, normoglycemic rat (negative control group, consist of 5 rats), and DM Type 1 rat (rat injected with streptozotocin to create DM type 1, consist of 20 rats). The DM type 1 rat then divided into 4 groups : the positive control group, collagen group, PRP group, and collagen + PRP group, each consist of 5 rats. After a week of adjusment, rat's Achilles tendon was transected and repaired with 2 strand modified Kessler and was treated accordingly to the group (no treatment, collagen treatment, PRP treatment, and collagen + PRP treatment). Evaluation was performed through histopathological evaluation count of fibroblast and biomechanically, 10 days after treatment

Research was conductedat the Laboratory of Physiology, Pathology Anatomy, Pathology Clinic, Pharmacology and Biomaterial BrawijayaUniversity from 01 February– 21 February 2014. The populationin this study wereadultmale rat Rattus novergicus strain Wistar, 150 – 170gramsandaged6 - 8weeks.Samplewere25 male rat aged 6-8weeks, 150-170 grams (Blood Sugar > 200mg/dl 24 hours after STZ injection). Sample selected by simplerandom sampling technique .Based onthe formulaFederer then obtaineda minimumestimate ofthe sample sizeis 5foreach group.To anticipate theunwanted exclusion of the sample, we addedas much as 20% of thenumber of samplesineach group.

Inclusion criteria for the research is male Rattus novergicus strain Wistar, age 6 – 8 weeks, weight 150 – 170 grams, and healthy. Exclusion cirteria is rat with infection, dead during experiment, and re-rupture.

III. Results

After 10 days of treatment, the rats were sacrificed, and the Achilles tendon were taken, processed histologically and biomechanically.This research conducted at fibroblast cell counts per field of view, using a microscope equipped with screen magnification 400x, measurement of the by counting the number of fusiform shaped fibroblast with one or more nuclei, basophilic and stain purple in HE [15].

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Ultimate Load	Between Groups	3561.015	4	890.254	112.364	.000
	Within Groups	198.074	25	7.923		
	Total	3759.089	29			
Ultimate Strength	Between Groups	47.237	4	11.809	66.508	.000
	Within Groups	4.439	25	.178		
	Total	51.676	29			
Jumlah Fibroblas per lapangan pandang	Between Groups	2295.061	4	573.765	88.745	.000
	Within Groups	161.633	25	6.465		
	Total	2456.695	29			

Jumlah Fibroblas per lapangan pandang

Tukey HSD[a]

		Subset for alpha = .05			
Perlakuan	N	1	2	3	4
PRP	6	13.6000			
DM (Non PRP+Collacure)	6	13.6000			
Collacure	6		24.6000		
Collacure+PRP	6			30.4000	
Normal	6				35.2333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Ultimate Strength

Tukey HSD[a]

Perlakuan	N	Subset for alpha = .05			
		1	2	3	4
PRP	6	2.2860			
DM (Non PRP+Collacure)	6	2.3058			
Collacure	6		3.3783		
Collacure+PRP	6			4.6495	
Normal	6				5.4224
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Annova analysis of data showed difference significantin the number of biomechanics, ultimate load, ultimate strength, amount fibroblast per field of view ($p < 0,05$). Post Hoc Test with Tukey HSD showed ultimate Load and ultimate Strength in PRP group have non-significant result with DM group, but PRP group significant result with Collagen + PRP, Collagen and Normal. Amount Fibroblast per field of view showed that PRP group have non-significant result with DM group, but PRP group significant result with Collagen + PRP group, Collagen group and normal group.

Correlations

		Ultimate Load	Ultimate Strength	Jumlah Fibroblas per lapangan pandang
Ultimate Load	Pearson Correlation	1	.962**	.963**
	Sig. (2-tailed)		.000	.000
	N	30	30	30
Ultimate Strength	Pearson Correlation	.962**	1	.956**
	Sig. (2-tailed)	.000		.000
	N	30	30	30
Jumlah Fibroblas per lapangan pandang	Pearson Correlation	.963**	.956**	1
	Sig. (2-tailed)	.000	.000	
	N	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation between fibroblast per field of view with ultimate strength variables have sig $0,000 < 0,05$, so it has a significant correlation between fibroblast with ultimate load and ultimate strength.

Regression Analysis was to calculate the magnitude of influence between fibroblast cell count to ultimate load and ultimate strength, when fibroblast cell count has increased, then the ultimate load and strength will also increase.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	8.025	1.585		5.063	.000
	Jumlah Fibroblas per lapangan pandang	1.191	.063	.963	18.916	.000

a. Dependent Variable: Ultimate Load

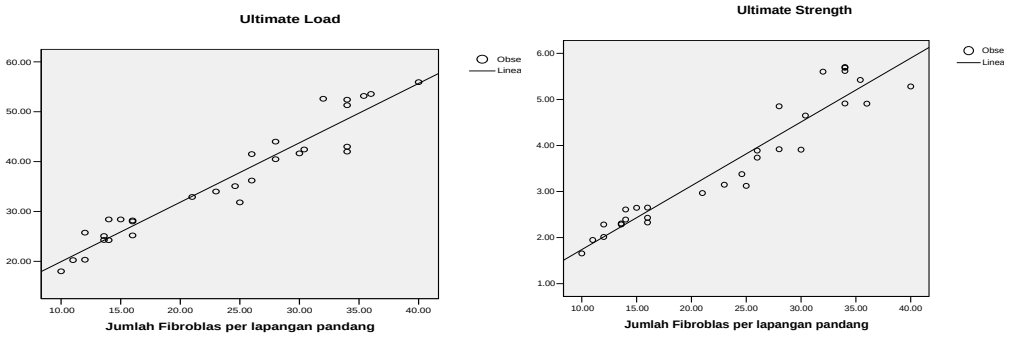
$$Y = 0,353 + 0,139\ X$$

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.353	.203		1.738	.093
	Jumlah Fibroblas per lapangan pandang	.139	.008	.956	17.160	.000

a. Dependent Variable: Ultimate Strength

$$Y = 8,025 + 1,191\ X$$



We also calculate the coefficient of determination and correlation coefficient with variable results 92,7% ultimate load and 91,3% ultimate strength variables will be affected by the independent variable (the number of fibroblast cell), while the rest will be influenced by other variables that are not addressed in this study.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.963[a]	.927	.925	3.12141

a. Predictors: (Constant), Jumlah Fibroblas per lapangan pandang

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.956[a]	.913	.910	.40031

a. Predictors: (Constant), Jumlah Fibroblas per lapangan pandang

We did F Test and the result was Ultimate Load and Ultimate Strength can be affected significantly by fibroblast cell number.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3486.280	1	3486.280	357.817	.000[a]
	Residual	272.809	28	9.743		
	Total	3759.089	29			

a. Predictors: (Constant), Jumlah Fibroblas per lapangan pandang

b. Dependent Variable: Ultimate Load

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	47.189	1	47.189	294.468	.000[a]
	Residual	4.487	28	.160		
	Total	51.676	29			

a. Predictors: (Constant), Jumlah Fibroblas per lapangan pandang

b. Dependent Variable: Ultimate Strength

IV. Discussion

This study aims to measure the biomechanical strength of the Achilles tendon with DM type 1 rats after grafting. Measurement of the ultimate load and strength showed that a large force was found in the group given collagen type 1 (Collacure) and also in combination of collagen group and PRP with the group without given either PRP or not getting PRP and collagen. On the post hoc analysis test (Tukey HSD test), treatment with PRP had no significant difference with DM group without any treatment, because being in the same column, but the treatment of PRP group had a significant difference compared with the group of collagen and PRP, collagen group and normal groups. It is due to each one having a different average at ultimate load, ultimate strength and

the number of cells per fields of view fibroblast. The meta-analysis study conducted by evaluating 23 randomized trial and 10 prospective cohort, said that there was no significant benefit in the use of PRP [16]. In another study with the use of a randomized double blind, placebo-controlled trial showed no significant difference among patients given injections of PRP with injection saline[17]. While the use of collagen combined with PRP, which is performed on the tendon gap obtained that PRP effectively enhance the healing process in the tendon guinea pigs [18]. Things mentioned above can be explained, that the use of collagen aims to provide room and bridge the fibroblast to proliferate, with the space created by collagen, the PRP can work effectively in the tendon, because the media and the room given that either to trigger an increase in the proliferation of fibroblast, so the combination of collagen and PRP, can provide a better biomechanical outcome compared with the others group on the strength of tendon rupture after repair. In this study also found a relationship between variables fibroblast per field of view with the ultimate load and ultimate strength, with a very strong category, because it is on an interval of 0,8-1,0. Correlation between fibroblast per field of view with the ultimate load and ultimate strength, also has a sig value. 0,000 < 0,05, so it has a significant correlation. The regression analysis, which calculates the magnitude of the influence of fibroblast cell with ultimate load and strength , concluded that the ultimate load and strength is influenced by the number of fibroblast. This research focused on early biomechanical forces on the Achilles tendon with DM type 1 condition after repair. Increase in ultimate load and ultimate strength assumes that the tendon will be more resistant to loads, making it more convincing to be done early rehabilitation. The use of type 1 collagen as a scaffold is a media and a good space to PRP to improve tendon healing process, resulting in a stronger biomechanical strength compared with no use of a combination of collagen and PRP.

V. Conclusion

Combination of bovine collagen type I and PRP showed better biomechanical results on early ultimate strength of ruptured tendon on DM type 1.

References

[1]. Leppilahti,J.: Puranen J,; and Orava,S : Incidence of Achilles tendon rupture. Acta Orthop. Scandinavica, 67: 277-279. 1996

[2]. Maffuilli N, Longo UG, Maffuilli GD, Khana A, Denaro V. Achilles tendon ruptures in diabetic patients. Arch Orthop Trauma Surg2011; 131:33-38

[3]. M.H.B. Zakaria, W.A. Davis, T.M.E Davis, Incidence and predictors of hospitalization for tendon rupture in type 2 diabetes: the Freemantle Diabetes Study, Fremantle Hospital, Fremantle, Australia

[4]. Azar FM,Sport Injury : Traumatic Disorders (In Canale ST, Beaty JH Eds. Campbell's Operative Orthopaedics. 11th Ed. Mosby-Elsevier. Philladelphia. 2007)

[5]. Maffuli N. Current Concepts Review Rupture of the Achilles Tendon. The Journal of Bone& Joint Surgery Am, 1999 Jul 01;81(7):1019-36

[6]. Rosalyn T, Nguyen, MD, Joanne Borg-Steinj, MD, Kelly Mcinnis, DO. Apllications of Platelet-Rich Plasma in Musculoskeletal and Sports Medicine: An Evidence-Based Approach. American Academy of Physical Medicine and Rehabilitation. USA. 2011

[7]. Egemen, Onur; Ozkaya, Ozay; Ozturk, MuhammedBesir; Sen, Ebru; et al. The Biomechanical and Histological Effects of Diabetes on tendon Healing: Experimental Study in Rats. J Hand Microsurgery. July-December 2012; 4(2): 60-64.

[8]. Roshan James et al. Tendon: Biology, Biomechanics, Repair, Growth Factors, and Evolving Treatment Options. J Hand Surg 2008;33A:102–112

[9]. Kajikawa Y et al. Platelet – rich Plasma enhances the initial mobilization of circulating physiol derived cells for tendon healing. Am J Physiol Cell Physiol 215 (3) : 837 – 845. 2008

[10]. Longo UG, Lamberti A, Maffuli N, et al. Review Article Scaffolds in Tendon Tissue Engineering. Hindawi Publishing Corporation Stem Cells International Volume 2012

[11]. Nunley JA. The Achilles Tendon : Treatment and Rehabilitation(Springer Science+Business Media, LLC. New York. 2009)

[12]. Hampson K, Forsyth NR, Maffulli N. Tendon Tissue Engineering. Topics in Tissue Engineering, Vol. 4. Eds. N Ashammakhi, R Reis, & F Chiellini. 2008.

[13]. Chen, Jimin; Xu, Jiake; Wang, Allan; and Zheng, Minghao. Scaffolds for tendon and Ligament Repair: Review of The efficacy of Commercial Products. Expert Rev. Med. Devices 6. 2009; 61-73.

[14]. Longo, Umile Giuseppe; Lamberti, Alfredo; Maffulli, Nicola; Denaro, Vincenzo. Tendon Augmentation Grafts: A Systematic Review. British Medical Bulletin. Januari 2010; 1-24.

[15]. Indraswary, Recita. Effect of topical Foeniculumvulgare Mill extract on epithelial phase of wound healing in gingival of Sprague Dawley rats. Sultan Agung Scientific Journal Vol. XLIX. 2011.

[16]. De Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, Verhaar JA, Weinans H, Tol JL,. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA. 2010;303:144-9

[17]. Patrick Sadoghi, Claudio Rosso, Victor Valderrabano. The Role of Platelets in the Treatment of Achilles Tendon Injuries. Harvard School of Public Health, Boston,2012

[18]. AtemehNazhvani, AbotorabNaenini, SeifollahDehghani. Evaluation of the Effect of Platelet Rich Plasma (PRP) in Tendon Gap Healing by Measuring Collagen Synthesis in Guinea Pig. Shiraz University, Shiraz, Iran. 2013.