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RESEARCH ARTICLE

Comparison of Intralesional Platelet Rich Plasma and 10% Dextrose Effect towards Injured Muscle Healing

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ABSTRACT

Platelet Rich Plasma (PRP) and Dextrose 10% have prolife rant effect by promote growth factor so that it could be use to promote healing of soft tissue. By using PRP and dextrose 10% injection intralesion in muscle injury were hoped fasten muscle healing process and improve muscle quality. This is experimental comparative research, was done on 27 rats that divided into 3 groups. After All of subject got muscle injury grade II, The first group was administered with PRP, , the second group with dextrose 10%, the third group with NaCl 0,9% injection intralesion. After one week, the subjects were sacrificed, and their gastrocnemius muscles were examined to see the level of myoblast through immuno histochemical technique. The result show increase level of myoblast in PRP and dextrose 10% group than control, and the level of myoblast was better in PRP group than dextrose 10% group (PRP:Dextrose 10%:NaCl 0,9% = 12,33: 8,00: 5,67). In conclusion, usage PRP and dextrose 10% injection intralesion can increase level of myoblast in muscle injury grade 2, and usage PRP was better than dextrose 10%.

INTRODUCTION

Muscle injury is a quite common injury often occured in acute or recurrent situation and leads to a decreasedability of activity performance. It could be due to a direct impact of a blunt trauma, punctured wounds, or excessive use in an exercise.[1,2] Managing a muscle injury conservatively includes five steps known as PRICE: Protect, Rest, Ice, Compression, and Elevation. PRICE method effectively carried out for the first 1-2 days to reduce inflammation and edema at the site of injury. Start from the 3rd day, physiotherapy can be done by Trans Electrical Nerve Stimulation (TENS) or with a neodymium-YAG laser. Isometric contractions can also be started and continued with concentric and eccentric contractions gradually.[3] Surgery might be required in certain patients, such as in athletes with large intramuscular hematoma, grade III muscle injury where the muscle can not contract, grade III injuries when the rupture of the muscle more than 50%, and there is persistent pain in more than 6 months.[3-5] Normal movement can usually be achieved at week 4. The formation of connective tissue because of myofibroblast will hinder the movement and increase the risk of recurrence. Recurrence within 2 months after returnedto daily activitiesshows that rehabilitation program does not progress properly. This becomes a problem for professionals such as injured athletes who need a good recovery to get back to theirprevious performance. Therefore, a variety of methodsand therapy modalities are developed to improve muscle injury recovery. [3-5] In the last decade, several methods are developed such as prolotherapy with the use of growth factors as therapeutic modality in the treatment of muscle injuries. Administration of growth factors can be directly, for example by the administration of platelet rich plasma (PRP) which contains a variety of growth factors, or indirectly by administering growth factor stimulants that can stimulate the body to produce growth factors.[6] Platelet rich plasma has been applied locally on diabetic ulcers to speed up the healing process.[7,8] However, direct use of growth factors is actually impractical because it requires special tools and preparation as well as costly price.[9] Dextroseisa simple carbohydrate which is an indirect growth factor. It increased 12-lipoxygenase pathway (12-LO) on arachidonic acid metabolism which has the effect of angiogenesis and increase the level ofgrowth factorsonmuscle cell.[12] Besides its advantage because it is proliferanto the cell, liquid10% dextroseis also easily foundin the marketat an affordable price, so thatit canbean effective,efficient, practical additional treatment modality for muscle injury treatment.Therefore,

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this research of comparing the effects of plateletrich plasma and10% dextrose towards injured muscle healing is proposed.

METHODS

This study is a comparative experimental study using wistar strain rats. The first group which is administered PRP, the second group which is administered10% dextrose, and the control group which is given 0.9% NaCl, were compared.

Inclusion criteria were rats which meets the following requirements:

1. male, age 12-16 weeks
2. weigh 180-250 grams
3. in healthy conditions

Exclusion criteria were as follows:

1. infected during adaptation or during the study
2. died during adaptation or during the study

This research was conductedwithseveralstagesasfollows:

1. Preparation ofrats as the experimentsample
- MaleWistarratsaged12-16 weekswith weight of180-250grams
- Quarantinedfor 7daysforadaptation of newenvironmentbefore being given the treatment.
- The ratswere randomized into3 groups, each group consistingof9rats, and were put into a cagewith certain treatment.

2. Platelet rich plasma preparations
- Platelet rich plasma preparations in this study use the methods by Sonnleiter et al., 2000 in Messora et al., 2011.
- A blood sample is taken from the four rat's heart, 3.5 mleach using a 5 ml sterile syringe.
- Prior to blood sampling, the rats were sedated with HCl ketamine intramuscularly.
- The blood sample then was put into a 4.5 ml tube Vaccutainer BD-Citrate.
- The tube containing the blood is then taken to the Clinical Pathology Laboratory of Molecular Biology department for the manufacturing of platelet rich plasma.
- By using a centrifuge machine MRi 23-Jouan, the blood tube was centrifuged 160 xg for 20 minutes at 22^{o}C.
- After centrifuged, the blood will be separated in three layers: plasma, platelet concentrate and red blood cells. Then the top phase (at the top mark of 1.4 ml) was transferred into a new tube.
- The tube is then centrifuged again at 400 xg, for 15 minutes at 22^{o}C.
- After centrifuged, the blood will be separated in two layers: the supernatant phase - platelet poor plasma (above the mark of 0.35 ml) and platelet rich plasma which are below the line.
- Platelet-poor plasma inside the tube was removed to left only platelet rich plasma.

3. Treatment inrat's gastrocnemius muscle with grade II muscle injury.
- The sample rats were sedated with HCl ketamine intramuscularly.
- The hair of rat's lower limbs were shaved.
- Aseptic and antiseptic procedure with 70% alcohol and 10% povidone iodine was done.
- Cutis and subcutis incisions were made at the posterior part of the lower leg rats for 2 cm.
- Diameter of the rat's gastrocnemius muscle was measured.
- Incision was done perpendiculary towards the gastrocnemius muscle fibers by 50% of the diameter.
- The group then divided into two groups where the first group was given platelet rich plasma and the second group was given NaCl as placebo.
- After receiving treatment, the wound is closed using 4/0 nylon threadand immobilized by a circular cast.

4. Maintenance of rats
- In the first 3 days, the rats were given antibiotics cefazolin 200 mg intramuscularly.
- During maintenance, all the rats weregiven the same food and drink.
- If during maintenance are rats experiencing an infection or death, then the rats will be dropped out.

5. Taking the examination material from rats
- After 1 week, the sample material of injured muscle will be taken.
- The sample material with a size of 0.5 x 0.5 x 0.5 cm was put into a tube containing formalin.
- The sample is then brought to the Laboratory of Pathology for preparations.

6. Immuno histochemistry examination
- The sample prepare mixture will be added with reagent myoD1, to perform the binding with cells mioblas.

- Then under the microscope CX-21, the intensity ofMyoD1 reagent binding with myoblast cells and myobast cells distribution formed in each prepare will be counted.

7. Analysis of data and statistics

- Data is then processed with SPSS 18.0 for Windows.
- The data were analyzed with univariate analysis. This analysis is done to get a general idea of frequency distribution by describing each of the variables used in the research
- Then the normality of the data is tested with the Shapiro-Wilk test.
- Then proceeded with the homogeneity test using Levene test.
- Then proceeded with post hoc analysis using difference test and Fisher's Least Significant difference (LSD).

RESULTS

This research was conducted using experimental animals of 27malerats Wistar strain which are divided into 3 groups. The first group was treated with PRP, the second group was treated with10% dextrose in 1cc of intralesional, and the third group was treated with0.9% NaCl. One week after treatment, samples were taken from the rat's gastrocnemius muscle with 0, 5x0, 5x0, 5 cmlesion and immuno histochemical examination using my oD1 reagent was done, and the myoblast distribution was calculated with Histoscore method by scoring the intensity and myoblast cells distribution. [29]

MyoblastcellintensitywithMyoD1divided into three scoring: [27]

1. (+) weak with score of 1,
2. (+) moderate with score of 2, and
3. (+) strong with score of 3.

Myoblast cell distribution was divided into four scoring: [27]

1. <20% with a score of1,
2. 20-50% with a score of2,
3. 51-80% with a score of3, and
4. >80% with a score of4.

The immuno histochemical examination results were scored and compared between two groups, where the value of the score is based on Hscore scoring system with formula: **Hscore=(intensity +1) xmyoblast distribution.**[27] Assessment was given in the form of ordinal scale scores with a total score ranging from 2-16. Figure 1.1, 1.2 and 1.3 shows a picture of the myoblast cell anatomical pathology binded to the reagent myoD1at various intensities

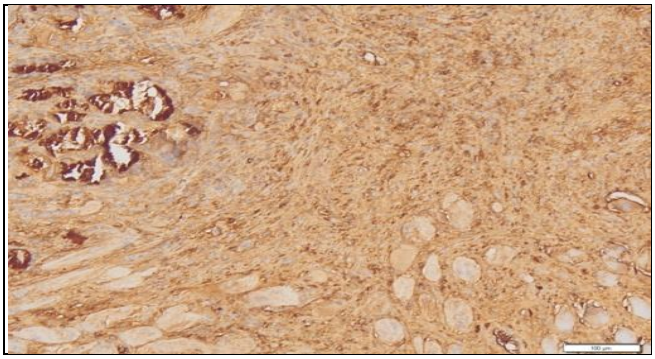

Figure 1.1. Picture of myoblast cells that bind myoD1 on strong intensity

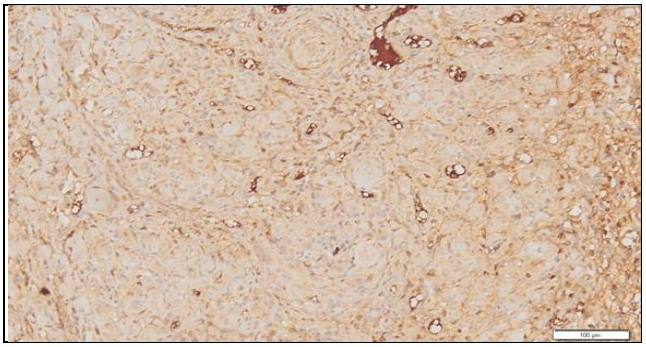

Figure 1.2. Picture of myoblast cells that bind myoD1 at moderate intensity

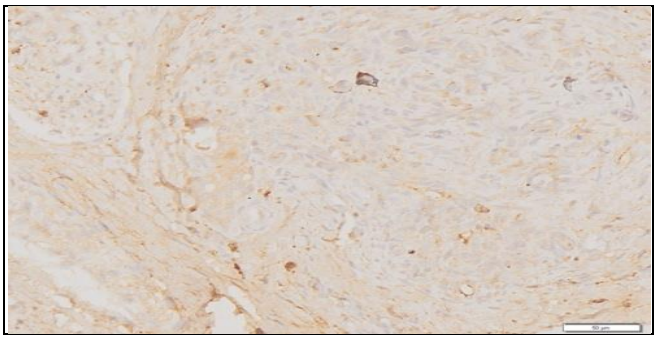

Figure 1.3. Picture of myoblast cells that bind myoD1 on weak intensity

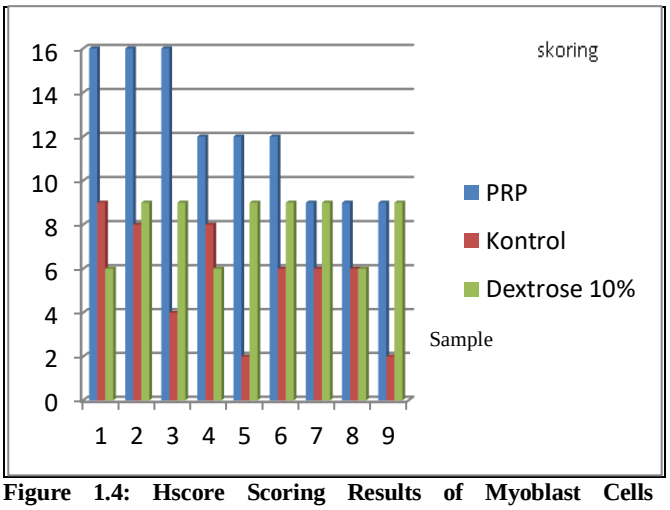

Figure 1.4: Hscore Scoring Results of Myoblast Cells distribution in each rat

Research results for the three groups of rats were descriptively shown in the following tables and figures (table 1.1 - table 1.2):

Table 1.1: Scores intensity and distribution of myoblast cells in Group 1sample (NaCl 0.9%) based on the Hscore scoring system

Sample	No	PRP	Dextrose 10%	NaCl 0,9%
H Score	1	16	6	9
	2	16	9	8
	3	16	9	4
	4	12	6	8
	5	12	9	2
	6	12	9	6
	7	9	9	6
	8	9	6	6
	9	9	9	2
Mean data		12,33	8,00	5,67

Table 1.2: Calculation Results of Fisher LSD test

		Mean Difference			95% Confidence Interval	
(I) Kelompok	(J) Kelompok	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Kontrol	PRP	-6,66667*	1,15470	,000	-9,0499	-4,2835
	Dextrose 10%	-2,33333	1,15470	,055	-4,7165	,0499
PRP	Kontrol	6,66667*	1,15470	,000	4,2835	9,0499
	Dextrose 10%	4,33333*	1,15470	,001	1,9501	6,7165
Dextrose 10%	Kontrol	2,33333	1,15470	,055	-,0499	4,7165
	PRP	-4,33333*	1,15470	,001	-6,7165	-1,9501

Table 1.1and Figure 1.4 illustrates the scoring of myoblast from muscle healing in rats. Based on these data it can be seen that the lowest score in the PRP group is 9, the highest score is 16and the mean score is12.33; lowest scoreat10% dextrosegroupis6, the highest scoreis12andthe mean score is 8; whereas the lowest score in the control group is 2, the highest score is 9 and the mean score is 5.67, indicating that PRP group mean score was higher than 10% dextrose and control group, as well as the mean score ofdextrose10% group is higher than the mean score of the control group.

Then the Fisher Least Significant Difference (LSD) test was done to see whether there is significant difference in the number of myoblast cells in the rat's muscles between the PRP, dextrose group and the control group (table 1.2)

Based on the statistical calculation, it was obtained that Mvalue between PRP and control groups of 6.67 with a P value of 0.0. Mvalue between 10% dextrose group and control is 2.33with a P value of 0.05. Mvalue between groups PRP and dextrose10% is 4.33 with a P value of 0.01. This statistical analysis showed that the value of P<0.05.Therefore, we can conclude that there are differences in the number of myoblast cells in the rat's muscles in the PRP and10% dextrose group towards the control group, and there are differences in the number of myoblast cells in the rat's muscles in the PRP towards 10% dextrose group.

DISCUSSION

These results shows that administration of intralesional injection of PRP and dextrose 10% on the injured muscle is proved to be effective by increasing the number of myoblast cells in the muscle ($p < 0.05$) with a mean difference between groups PRP, dextrose and control groups is 12, 33: 8.00: 5.67.

Increased number of myoblast cells in the muscles injected with PRP and dextrose occurs because PRP and dextrose are prolife rant to the cell. PRP contains direct growth factors, while Dextrose can provide a stimulant to increase the level of growth factor that plays an important role in muscle cell migration and proliferation. The cells will produce growth factors in a few minutes to a few hours when exposed with dextrose with concentrations above 0.6% (the normal cell glucose concentration is 0.1%). Extracellular hyperglycemic condition will increase 12-lipoxygenase pathway (12-LO) on arachidonic acid metabolism which has the effect of angiogenesis and increasing the level of growth factors on muscle cells.[12]These growth factors, among others: Platelet Derived Growth factors, transforming growth factors beta, Epidermal Growth factors, Basic Fibroblast Growth factors, Insulin Like Growth factors and Connective Tissue Growth factors. All of these factors have been identified as the key to stimulate the healing of muscle injuries 10% dextrose injections induce proliferation without an increase in inflammatory reactions, making it suitable to be used in cases of acute muscle injury that occurs when the inflammatory process increases significantly. The results are consistent with other studies that stated that administration of Platelet Rich Plasma is proven to stimulate cell migration and my fibroblasts differentiation and enhance the healing process of muscle injuries. [10, 11] Platelet rich plasma has been applied locally on diabeticulcersto accelerates the wound healing process. [7, 8] The injection of 10% dextrose can also be used in cases of injury and laxity of the ligaments. Provision of intra-articular dextrose injection can clinically help patients with osteoarthritis, and 3-year study in patients with ACL laxity injected with dextrose10% -25% showed gains of strength in ACL laxity. [15, 16] Increasing number of myoblasts cells in the administration of PRP is much higher than that of dextrose 10%, because PRP contains direct growth factors, so it will work directly to increase cell proliferation, whereas 10% dextrose does not contain growth factors directly, but it stimulates growth factors in the body to work, so that the

stimulated growth factor will increase the cell proliferation. Increasing number of myoblast cells in this study assessed based on scoring system of Hscore. Hscore scoring system evaluates two important variables shown in healing of the injured muscle, the intensity and distribution of myoblast cells. Assessment was carried out by a specialist consultant of anatomical pathology to minimize the possibility of bias.[28]

The weakness in this study was about the difficulties in terms of uniformity of rat's gastrocnemius muscle diameter, so there is a possibility of bias at the time of sampling for immuno histochemical examination.

CONCLUSIONS AND SUGGESTIONS

CONCLUSIONS

1. PRP and 10% dextrose intralesional injection on injured muscle may increase the number of myoblast cells and improve muscle healing.
2. Administration of PRP on injured muscle is more effective than the administration of 10% dextroseto improve muscle healing

SUGGESTIONS

The results of this study may become one of the additional modalities recommendation that can be used for healing in injured muscles, which by the administration ofPRPand10% dextrose. The findings can be applied to human where intralesional PRP and10% dextrose can be injected in the injured muscle, as the dose is adjusted with the width of the injury.

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