RESEARCH ARTICLE

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

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ABSTRACT
Platelet Rich Plasma (PRP) and Dextrose 10% have proliferant 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 occurred 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. 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. Surgery might be required in certain patients, such as in athletes with large intramuscular hematoma, grade III muscle injury where the muscle cannot contract, grade III injuries when the rupture of the muscle more than 50%, and there is persistent pain in more than 6 months. 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 activities shows 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 their previous performance. Therefore, a variety of methods and therapy modalities are developed to improve muscle injury recovery. 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. Platelet rich plasma has been applied locally on diabetic ulcers to speed up the healing process. Dextrose is a 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 of growth factor on muscle cell. Besides its advantage because it is proliferant to the cell, liquid 10% dextrose also easily foundin the market at an affordable price, so that it can be an effective, efficient, practical additional treatment modality for muscle injury treatment. Therefore,

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this research of comparing the effects of plateletrich plasma and 10% 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 conducted with several stages as follows:

1. Preparation of rats as the experimentsample
   - Male Wistar rats aged 12-16 weeks with weight of 180-250 grams
   - Quarantined for 7 days for adaptation of new environment before being given the treatment.
   - The rats were randomized into 3 groups, each group consisting of 9 rats, 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 ml each 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 Vacutainer 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°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°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 in rat’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 perpendicular 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 thread and 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 were given 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 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.

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• Then under the microscope CX-21, the intensity of MyoD1 reagent binding with myoblast cells and myoblast 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 27 male rats Wistar strain which are divided into 3 groups. The first group was treated with PRP, the second group was treated with 10% dextrose in 1cc of intralesional, and the third group was treated with 0.9% NaCl. One week after treatment, samples were taken from the rat's gastrocnemius muscle with 0, 5x0, 5x0, 5 cm lesion and immuno histochemical examination using myoD1 reagent was done, and the myoblast distribution was calculated with Histoscore method by scoring the intensity and myoblast cells distribution. [29]

Myoblast cell intensity with MyoD1 divided 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 of 1,
2. 20-50% with a score of 2,
3. 51-80% with a score of 3, and
4. >80% with a score of 4.

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: 

\[ H_{\text{score}} = (\text{intensity} + 1) \times \text{myoblast distribution} \]  

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 myoD1 at 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):
DISCUSSION
These results show 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. 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. Platelet rich plasma has been applied locally on diabetic ulcerostro accelerates the wound healing process. 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. Increasing number of myoblast 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. 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% dextrose to 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 of PRP and 10% dextrose. The findings can be applied to human where intralesional PRP and 10% dextrose can be injected in the injured muscle, as the dose is adjusted with the width of the injury.

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