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Research article

Our experience regarding withdraw blood from rats and preparation of platelet-rich plasma

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ABSTRACT

Aim: Withdraw blood from rats is an important, but not easy, invasive procedure in experimental research on these animals. In addition, the preparation and standardization of platelet-rich plasma (PRP) is a more difficult process in rats. In this study, we presented our experiences about rat blood collection and PRP preparation technique.

Methods and Result: This experimental study was performed with ten male Wistar rats weighing 250–300 g. Under anesthesia, the blood was obtained by percutaneous puncture from the right ventricle of the rats. The blood obtained from rats was rapidly transferred to tubes containing anticoagulants such as sodium citrate or acid citrate dextrose solution A. After the first centrifuge, the all plasma was collected by a pipette after a second spin in a sterile tube. As a result of all these processes, PRP at the desired concentration was obtained.

Conclusions: Blood withdraw from rats is not an easy method, and when large amounts of blood are required cardiac blood intake is necessary. In order to achieve the therapeutic intensity in PRP preparation, usually a double spin is required and the concentration obtained with the base number of platelets should be compared.

Keywords: Rat, blood withdraw techniques, platelet rich plasma.

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Introduction

Many developments in the medical world are carried out through animal experiments. Among the clinical studies using animals are platelet-rich plasma (PRP) studies of plastic surgery, orthopedics, and dentistry specialists. In this research mice, rabbits, rats, and guinea pigs are frequently used as laboratory animals. With the many techniques used during blood collection the researcher's choice of method, which should be convenient and easily reproducible, will facilitate the course of the study and reduce variability between data generated by providing standardization. Among small laboratory animals, rodents are

the most studied subject because of the ease of supply, low cost, and proximity to the human model. There are many studies in the literature about blood supply from rats. In this study, we present our own experience, our rat blood collection and PRP preparation technique.

Methods and Results

Ten male, Wistar rats (250-300 g) were used in this study, which were obtained from the Bolu Abant İzzet Baysal University (BAIBU) Animal Care and Research Laboratories (Bolu, Turkey). The BAIBU Ethics Committee approved all procedures to be performed on the experimental animals. Routine animal care guidelines and the Guide to the Care and Use of Laboratory Animals (1996) were essential in the practice of this study's procedures. Free access to water and food for the rats along with a 12-hour dark/light cycle in a temperature-controlled room were also provided. All of the rats were first placed on a homeothermic table to maintain a 37° C body temperature. Anesthesia was administered with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine hydrochloride (100 mg/kg of Ketalar [Eczacibasi, Turkey]). Approximately 7 ml of blood was collected from each rat, mean amount of thrombocytes was 700000 per mm3, during first spin an average of 2.5 cc of plasma was collected and measurements showed that mean thrombocyte yield was %69. A mean concentration of 1.36 million per mm3 was found. For an effective PRP, a concentration of approximately 3 million per mm3 (3-4 times of blood count) must be obtained, and this shows us that usually a second spin is needed to reach the desired concentration.

Rat blood obtaining

Possible blood collection areas of the rat include the heart, jugular vein, tail vein, tail artery, orbital sinus, and lateral saphenous vein. Peripheral areas provide a lower volume of blood (2–3 ml) than the cardia, allowing blood collection without sacrificing the animal. However, in such a case, the volume of blood drawn should be replaced with an equal volume of serum physiologic (SF) in order to prevent the experimental animal from entering into hypovolemic shock (2).

If the study plan involves sacrifice of the animal, the heart as the central blood supply can be used to increase the volume of blood drawn. At this point inexperienced researchers should perform a thoracotomy to visualize the heart and puncture the right ventricle. If the researcher is experienced a percutaneous puncture may be performed to reach right ventricle (Figure 1A-D). Although the maximum volume of blood to be considered is 10–13 ml, it is practically possible to take approximately 5-7 ml of blood from a 250–300 g rodent (1, 2).

Platelet-rich plasma (PRP) preparation

The quick transfer of blood to the tube containing the anticoagulant is vital at this point. Unlike human blood, rat blood tends to coagulate quickly and will be wasted if the transfer to the tube is not swift. It is not practical to withdraw it directly to the tube containing the anticoagulant. The anticoagulant must be in the injector when the blood is first drawn into the syringe and then transferred to the citrate tube. In our experience, we generally prefer the use of sodium citrate or acid citrate dextrose solution A (ACDA) for anticoagulation.

Rat blood contains high amounts of platelet compared to human blood. Although mean values differ between rat subspecies, rats have an average of 700 thousand thrombocytes in mm3. It is important to note that the PRP obtained from a rat should have a much higher

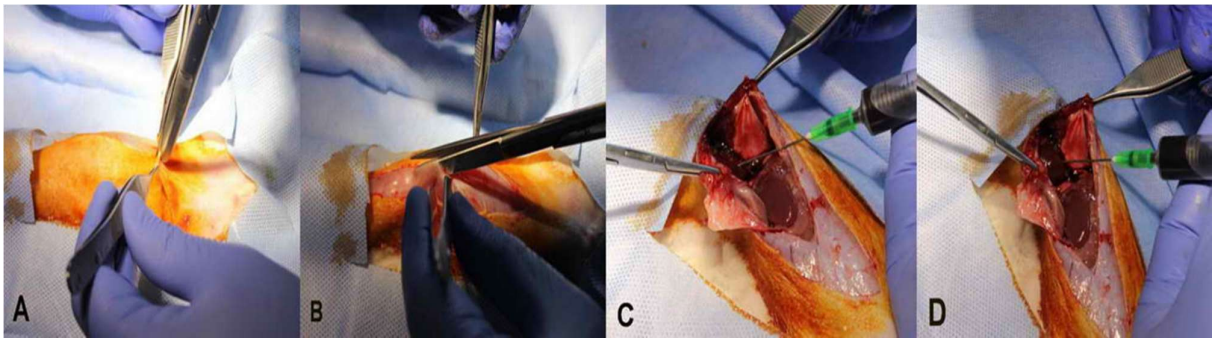

Figure 1 A-D. Blood collection directly from the rat hearth.

number of platelets compared to human PRP with the same concentration ratio. In terms of appropriate concentration, when human PRP is in the range of 1–1.5 million, this number increases to 2.5–3 million for rat PRP acquisition. About 6 ml of blood must be used to obtain a high concentration of 0.7 ml PRP (3). A single-stage centrifuge will usually not be sufficient when preparing rat PRP. After the first centrifuge all plasma is collected by a pipette after a second spin in a sterile tube. Platelets should be collected and homogenized by shaking the plasma without measuring the PRP values (3).

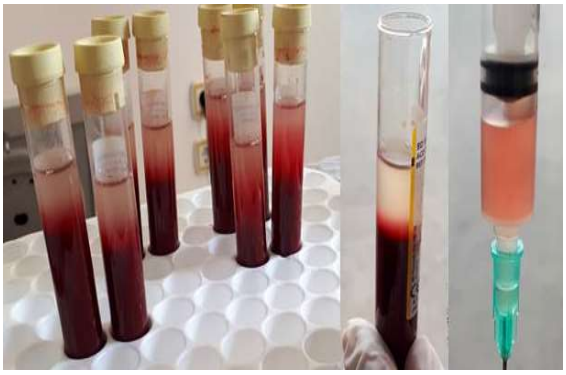

Figure 2. Appearance of the rat platelet-rich plasma.

Discussion

Although a number of blood supply areas can be used to withdraw blood from rats, the anatomy should be familiar and the blood obtained by an experienced hand. The easiest method to draw blood is intracardiac puncture. The disadvantage is the sacrifice of the animal. In our study, the blood taken from rats should be transferred immediately to the tube containing ACDA or taken with injectors containing anticoagulant, otherwise the blood will be coagulated without being transferred from the injector to the anticoagulant tube (4). Often it is not possible to achieve sufficient density with a single spin in the PRP. In these cases, a second spin should be performed.

After the second spin, it is possible to reach the desired concentrations by controlling the dilution amount. Although many of the g (a unit for centrifugation steps) and duration values selected in the centrifuge are prepared by various firms, many researchers use homemade PRP because it is more economical. It is important the platelets are activated early when preparing the PRP. During the second spin, especially when high g is used, a denser PRP is obtained, but growth factors are released early (5). Early release of growth factors is not always a desired outcome.

The standardization of PRP will be more difficult in rats. Due to the lack of rat blood, studies requiring a large amount of PRP may obtain it from rats sacrificed as other test

subjects. Immunological problems would not be expected because PRP contains only platelets as well as genetic identically of animals.

There are some limitations of this experimental study. Both blood collection and PRP preparation technique was studied on the same group of rats. Therefore, difference groups and statistical comparisons could not be made. We think that further experimental studies may be needed on the subject.

Conclusion

Blood withdraw from rats is not an easy method, and when large amounts of blood are required cardiac blood intake is necessary. In order to avoid early coagulation blood should be taken directly to the injector containing anticoagulant and transferred swiftly to the tube. In order to achieve the therapeutic intensity in PRP preparation, usually a double spin is required and the concentration obtained with the base number of platelets should be compared.

Conflict of Interest: *No conflict of interest was declared by the authors.*

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