Short Communication

Proposal for Point-of-Care Testing of Platelet-Rich Plasma Quality

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Abstract

Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) regenerative therapy lacks evidence to make clinical decisions. This weakness cannot be addressed only by standardization of preparation protocols and devices. In this article, we have emphasized on the necessity for quality testing to obtain strong evidence supporting PRP/PRF therapy.

Keywords: Platelet counts, platelet-rich fibrin, platelet-rich plasma, point-of-care testing, quality assurance

Introduction

One of the critical challenges faced by platelet-rich plasma (PRP)/platelet-rich fibrin (PRF) therapy is the lack of clinical evidence. Once a therapeutic method is reported to have outstanding clinical outcomes, it should be tested by the clinicians in severe cases. They can even challenge the method based on the outcome. However, such clinical outcomes are not considered as "evidence" by the national regulatory authorities. Evidence can be obtained by randomized clinical trials (RCTs), systematic reviews, and meta-analysis.

Medical Evidence is ranked on the basis of validity, i.e., the strength of evidence. It forms a pyramid called hierarchy of medical evidence [Figure 1].[1-3] In this hierarchy, case series, trials without controls, commentaries, and basic sciences generate low or insignificant evidence and therefore, they are ranked at the bottom. Case-control and cohort studies are ranked in the middle, and the RCT-based systematic reviews and meta-analysis form the top group.

In case of PRP/PRF study, increasing numbers of systematic reviews and meta-analyses have been published.[4-13] In the last 3 years (2016 − 2018), using PubMed and manual sorting by keywords and contents, it was found that the number of review articles reporting positive effects and those reporting negative effects were almost the same. However, most of the positive reviews have indicated that there is a lack of long-term, high-quality clinical trials and standardized treatment protocols.

Meta-analysis is not ideal or perfect because it contains an inherent limitation such as clinical, methodological, or statistical heterogeneity,[3] and thereby causes uncertainty and error. Despite this limitation, meta-analysis has been applied as a standard method for the evaluation of the clinical effectiveness of the synthetic drugs. Since the clinical outcomes of PRP/PRF therapies are influenced by various factors, regenerative therapy using home-made PRP/PRF preparations as well as synthetic drugs should be analyzed.

Figure 2 illustrates the difference between industrial products including low-molecular weight drugs and home-made cell-based medicinal products such as PRP/PRF preparations. It is meaningless to perform clinical trials and evaluate their clinical effectiveness without standardization of quality. Although the standardization of preparation protocol,[14] including the use of designated, genuine terminologies,[15] minimizes the heterogeneity in the quality of the product, it does not provide assurance about its quality. To conduct

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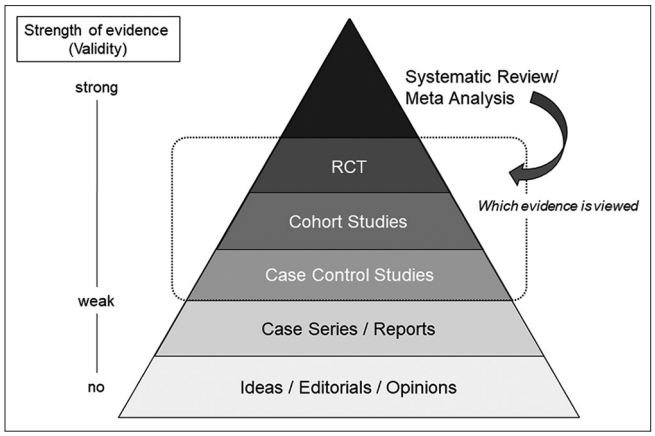

Figure 1: The evidence-based medicine pyramid

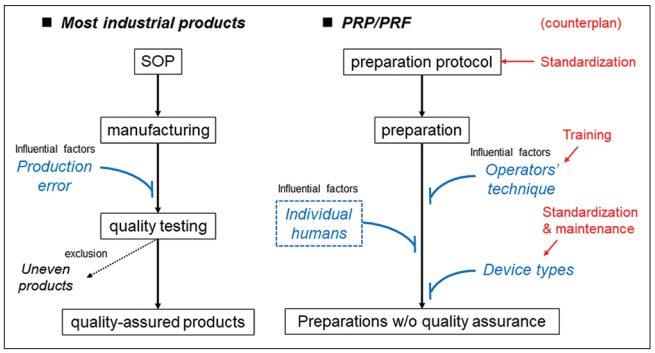

Figure 2: Differences between industrial products and home-made platelet-rich plasma/platelet-rich fibrin

reliable RCTs of PRP/PRF therapy, the quality of the PRP/PRF preparations should be standardized, a method of point-of-care (POC) testing of the quality should be developed, and quality of the individual PRP/PRF preparations should be examined before use.

Historical Background of Platelet-Rich Plasma Therapy and Research

Since Marx *et al*. first reported that the clinical effectiveness of PRP in sinus bone regeneration,[16] PRP therapy has been modified and spread to various medical fields as promising biomedicine. In case of PRP therapy, clinical study has preceded basic research. Basic research has always followed clinical studies to find and establish the scientific foundation to support the clinical use. However, it would be helpful to reevaluate the historical background to understand and interpret PRP/PRF therapy. Here, we roughly divide the history of basic research into three phases.

1. Proof of correlation between concentrated platelet counts and growth factor levels in PRP: This phase includes modification and comparisons of PRP preparation protocols
2. Development of PRP derivatives: This phase produced PRF from noncitrated blood samples without the aid of coagulation factors. In addition, this phase included debates regarding the necessity of leukocyte inclusion, which is continuing to date
3. Comparative study among PRP derivatives: The PRP derivatives were ranked by their ability of controlled release of growth factors. It is a kind of "competition of brands" which sometimes creates a conflict of interest. We believe that such competition will not allow rapid progress in the better understanding and use of PRP/PRF therapy.

Among all the events, the introduction of PRP in regenerative therapy and the development of PRF can be considered as epoch-making events.[16,17] All the other findings and developments are considered unimportant in improving the predictability of PRP/PRF therapy, which is unfortunate. Notwithstanding the failures, the accumulation of the research findings and clinical experiences may have contributed to the gradual understanding of the essence of PRP/PRF therapy.

Essence of Platelet-Rich Plasma/Platelet-Rich Fibrin Therapy

To ascertain the essence of PRP/PRF therapy, two major points need to be discussed: (1) PRP/PRF therapy is an adjuvant therapy.[18,19] This concept indicates that even though PRP/PRF may not be capable of producing significant regenerative effects on their own; PRP/PRF, in combination with preceding or simultaneous surgical operation or medication, can be expected to elicit synergistic effects.[20-22] This concept is similar to the tissue engineering triad, an equilateral triangle illustrating three important components of tissue engineering, which are cells, growth factors, and scaffolds.[23] The PRP/PRF preparations contain growth factors and scaffolding materials, but no stem cells or progenitor cells are directly involved in tissue regeneration.[24] Therefore, the possibility of combinational treatments with stem cells/progenitor cells has recently been investigated to compensate for the shortcomings of PRP/PRF.[12] (2) Successful PRP/PRF therapy depends on initial angiogenesis.[24] In soft tissue, such as muscle and connective tissue, blood vessels are easily formed by recruiting endothelial progenitor cells. In contrast, for the anatomical reason, it is relatively difficult to efficiently recruit progenitor cells into skeletal tissue and induce neovascularization. It should be noted that these characteristics are largely dependent on the platelet counts and the levels of related growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF).

Problems Solved by Platelet-Rich Plasma Therapy

As mentioned above, the primary factor that delayed the progress of PRP/PRF therapy is the unexpected negative results that are attributed to the wide differences in individual samples. To reduce and to minimize the differences, in the initial phase, we proposed for standardization of preparation protocols.[14] Several groups have agreed on or independently raised similar proposals.[25-27]

However, this is not sufficient. When well-trained operators independently prepare PRP (or its derivatives) from the same blood samples according to the standardized protocols, they may be able to produce very similar PRP preparations, which are routinely assessed by platelet and leukocyte counts. However, such a preparation protocol does not guarantee uniform results in PRP preparations when produced by independent operators with various skills or from various donors. In addition, even from the same donors, as many clinicians may have experienced, the order of blood collection sometimes influences the coagulation rate in individual tubes.

The differences in individual preparations result not only from varied blood samples but also from varied operators' technique. Thus, it is not surprising that different clinical outcomes could be obtained by PRP preparations that are independently prepared from the same donors by the same protocols. Knowledge about these differences will increase the clinical predictability and effectiveness of the PRP preparations.

In contrast, manual pipetting or transfer of fractions is not required in PRF preparation, making it less sensitive to the operators' technique. Moreover, in PRP preparation, automated preparation machines are available. However, a comprehensive comparison of the preparation systems shows that the PRF preparation system is somewhat superior to other PRP preparation systems regarding reproducibility. Although PRF is not free of quality variation, the factor influencing PRF quality can be limited to the difference in individual blood samples.

Quality Assurance of Individual Platelet-Rich Plasma/Platelet-Rich Fibrin Preparations

In the earlier phases of PRP study, PRP had been classified solely by the types of preparation protocols, and this classification system is widely accepted even now. Mishra *et al*. and Milants *et al*. introduced new classifications of PRP preparations.[28,29] The advantage of classification by Mishra *et al*. is that criteria are limited to platelet and leukocyte counts and activation. The platelet-derived white blood cell (PAW) classification further simplified the classification by Mishra *et al*. On the other hand, Lana *et al*. proposed a detailed classification, MARSPILL.[30]

The common factor in these classifications is the requirement of blood cell counts in the starting whole-blood samples. Although blood testing is important to evaluate the physical conditions, the importance of baselines for platelet and leukocyte counts and the concentration-dependent reaction rates of blood cells for quality assurance are not known. The relation between concentration-dependent reaction rates and potency of blood cells needs to be elucidated although it is generally accepted that the growth factors stored in platelets are the main factors in the regenerative capability of PRP. We believe that platelet and leukocyte counts in the final products, which are practically limited to PRP reparations at present, are essential criteria for clinical potency. Although it is difficult to count blood cells in the insoluble PRF clots, platelet and leukocyte counts are the most reliable and convenient quantitative criteria also for PRF quality.

Based on this concept, we have developed a system for the determination of platelet counts using a spectrophotometer.[31] An inexpensive, palm-top spectrophotometer (~$800) was demonstrated to be useful in platelet counting. The procedure requires only a standard curve, but not the operators' skill, labor, or time for testing. The disadvantage is that the accuracy of this method is often disturbed by the inclusion of significant numbers of leukocytes and erythrocytes. Therefore, this method can be applied to pure-PRP including plasma rich in growth factors.

We also developed the method to determine platelet counts in PRF preparations.[32] Since PRF is insoluble and platelets aggregate to tightly adhere to the fibrin fibers, platelet counting is impossible without efficient digestion. Almost all the proteolytic enzymes, we tested digested the fibrin fibers but also simultaneously injured or deformed the platelets because of which they could not be accurately counted by Coulter principle-based automated hematology analyzers. After many trials and errors, we found that tissue-plasminogen activator (t-PA) efficiently digested the fibrin clots without sacrificing platelets and released single platelets from aggregates. The t-PA is a serine protease that converts the proenzyme plasminogen to proteinase plasmin, is specifically bound to fibrin along with plasminogen, and its activity can be enhanced over 100-fold through specific binding.[33,34] The disadvantages of this method are the cost of the t-PA reagent and the time required for digestion (~4 h). We look forward to a breakthrough modification in this method that will enable an easy determination of platelet and leukocyte counts, thereby assuring high-quality PRF.

Regulatory Framework for Platelet-Rich Plasma/Platelet-Rich Fibrin Therapy and Self-Guidelines for Platelet-Rich Plasma/Platelet-Rich Fibrin Quality in Japan

As reported earlier,[35] PRP/PRF therapy is regulated by the newly established regulatory framework in Japan. According to this regulation,[36] the shipping criteria for PRP/PRF is not indicated by the law or other guidelines; it is dependent on the independent decisions of each certified committee for regenerative medicine. In Niigata University Hospital (Niigata, Japan), its own committee decided that PRP and PRF preparations should be evaluated by platelet counts ($4 \times 10^5/\mu L$) in the final products and by morphological stability, respectively. Due to the latter criterion is qualitative rather than quantitative, we propose that even after shipping, platelet counts should be determined by the t-PA digestion method to examine possible correlations between platelet counts and clinical outcomes. We hope that many clinicians will introduce blood cell counting to their clinics and hospitals.

Summary and Concluding Remarks

The earlier phases of PRP/PRF therapy and research lacked the aspect of standardization. Individual clinicians and operators independently chose PRP preparation protocols that they thought were the best. This situation has been gradually corrected as the suspicion against the potency and predictability of the PRP/PRF therapy increased. Development of automatic preparation devices was expected to contribute to the standardization of the preparation protocol. However, against our expectation, development has only resulted in encouraging the competition between brands and impeding the standardization.

Similar situations are often observed in PRF preparations. Anticoagulants can be used for distinguishing PRF and PRP preparations. However, in comparison to these PRF-like materials, the original Choukroun's PRF and its derivatives, such as advanced PRF (A-PRF) and concentrated growth factors (CGF), have similar biological, biomechanical, and biochemical characteristics.[24,37,38] The crucial differences within the PRF family is not understood. Except for the inclusion of leukocytes, these variations, as well as variations of PRP, act on injured tissues essentially by similar mechanisms of action. Although important for each manufacturer, competition between brands reduces the sound and significant progress of PRP/PRF therapy and research. To improve the situation, we have introduced the general classification of synthetic drugs and proposed the use of generic names (e.g., PRF) instead of brand names (e.g., A-PRF, CGF) while investigating the common mechanisms of action.[15]

Finally, we want to emphasize the necessity of quality assurance of individual PRP/PRF preparations. The same preparation protocols do not necessarily produce PRP/PRF preparations of similar quality. We think that in many cases, unexpected clinical outcomes result mainly from their poor quality. Therefore, we recommend all clinicians to perform point-of-care testing of each PRP/PRF preparation, such as determination of platelet counts and assure its quality before use. Even if point-of-care testing is difficult for PRF at present, it will become possible in the near future. This testing is indispensable not only for better predictable clinical outcomes but also for reliable RCTs to obtain strong evidence for PRP/PRF therapy.

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Conflicts of interest

There are no conflicts of interest.

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