

Effects on platelets of acute inflammatory area: an experimental study

Abstract

Introduction

It is known well that inflammation may affect the platelets. However, there are inconsistencies between the results of observational studies investigating changes in platelet indices in inflammatory conditions. This study aimed to investigate the possible effects of acute inflammation on platelet indices in plantar inflammation model in rats.

Materials and methods

A total of 10 rats, 5 in each group, were used in the study. Lambda-carrageenan and saline were applied subcutaneously to the right hind paw of the rats in the inflammation group and in the control group, respectively. Six hours after the administration, blood samples were taken from femoral arteries and femoral veins, and platelet indices were measured by a hematology analyzer. In addition, plantar tissue samples belonging to the control and inflammation groups were evaluated histopathologically.

Results

On histopathological examination, no pathological condition was observed in the control group, while there were changes consistent with acute inflammation in the lambda-carrageenan-injected group. There was no significant difference in terms of platelet indices between both the arterial and vein samples and between the control and inflammation groups.

Conclusions

Our results suggest that platelet indices cannot be used in the diagnosis of acute inflammatory conditions. However, in our opinion, these findings must not be interpreted as that acute inflammation does not affect platelet number and volume. Instead, we believe that it may be more appropriate to say that acute inflammation does not produce a quantitatively significant change in platelet indices due to the combination of the opposite effects.

Keywords

acute inflammation, rats, carrageenan, blood platelets, mean platelet volume

Introduction

It has been demonstrated that inflammation may generate a prothrombotic environment being likely to affect platelets (1–3). There are numerous studies suggesting that platelet indices can be used as a biomarker to evaluate the severity of acute inflammatory diseases (4,5). The most commonly used indices are mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) (5). Because of that the measurement of platelet indices is performed as part of routine complete blood count and does not create additional costs, the prognostic and predictive value of these indices in various diseases has become an increasingly popular research topic (5). However, there are serious inconsistencies between the results of observational studies investigating changes in platelet indices in inflammatory conditions, possibly due to methodological limitations of observational studies (6). The aim of our experimental study was to investigate the artery supplying the acute inflammatory area and the vein collecting blood from it and so on, was to evaluate whether there is a change in platelets in terms of number, volume, and distribution width.

Materials and methods

Materials

This experimental study was carried out in 2020 after ethical approval from Kafkas University Animal Experiments Local Ethics Committee (2019/10-144). Adult female Wistar Albino rats weighing 200-250 g, taken from Kafkas University Experimental Animals Application and Research Center were used for this study. A total of 10 rats were included in the study, with 5 rats in each group. All rats were fed and housed ad libitum in a 12-hour light and 12-hour dark cycle.

Methods

To create inflammation, 1% lambda-carrageenan solution with a volume of 50 μ L by a 26-gauge injector was administered subcutaneously to the plantar tissue in the right hind paw of the rats (7). In the control group, the same volume of saline was administered in the same way instead of lambda-carrageenan. Edema and redness, which are the cardinal signs of acute inflammation, were observed at the end of six hours after lambda-carrageenan administration (Figure 1).

A simple experimental setting was prepared to give the rats a suitable position for blood collection procedures (Figure 2). By uncovering the relevant area (Figure 3), approximately 0.5 mL of whole blood sample was taken from the right femoral vein and artery of rats under xylazine-ketamine anesthesia (Figure 4) and placed into tubes with ethylenediaminetetraacetic acid (EDTA). Platelet indices (platelet count, MPV, PDW, and plateletcrit) were measured using the rat kit on the MS4e device (Melet Schloesing Laboratories, France).

To confirm inflammation, sequential sections were taken from the plantar regions of the feet, and these samples were fixed in 10% neutral formalin for 24 hours. Then, paraffin blocks were prepared. Hematoxylin-Eosin staining was performed by taking 5 μ m sections from the paraffin blocks and these preparations were examined under a light microscope.

Statistical analysis

Statistical analyzes were performed using SPSS 20.0.0 (IBM, USA). It was decided whether the data fit the normal distribution or not by examining the histogram graphs. Two independent groups were compared with the Mann-Whitney U test, and two dependent groups with the Wilcoxon Signed Ranks test. Data were expressed as median (minimum-maximum). A value of $p < 0.05$ was considered statistically significant.

Results

It was observed that the lambda-carrageenan-administered foot was macroscopically edematous and hyperemic. Microscopically, intense neutrophil infiltration was detected in the subcutaneous tissue, especially around the vessels. Also, neutrophil adhesion was found in the vessel lumen. Both neutrophil infiltrations and adhesions indicated the presence of acute inflammation (Figure 5A). The saline-injected foot had a normal appearance both macroscopically and microscopically (Figure 5B).

The results of platelet indices in arterial and venous blood samples are shown in Table 1. There was no significant difference in platelet indices between artery and vein samples in both the control group and the inflammation group. Also, platelet indices were similar between arterial samples and between vein samples of the two groups.

Discussion

As an integral part of the complete blood count, platelet indices are widely and inexpensively analyzed in laboratories. Although numerous clinical studies have been conducted on the use of platelet indices in the diagnosis and prognosis of various acute inflammatory diseases, there are serious inconsistencies between the results of these studies. For example, it has been reported that the platelet count decreases in acute cholecystitis (8) and acute appendicitis (9)

and increases in acute cholecystitis (10) and perforated appendicitis (11). Similarly, some researchers have suggested that MPV is decreased in acute appendicitis (9,12–15), acute cholecystitis (8,10), and acute pancreatitis (16,17), while others have claimed that it increases in acute appendicitis (18), perforated appendicitis (11), and acute cholecystitis (19). There are also conflicting results about PCT (10,20) and PDW (10,11,14–16,20,21).

We found no change in platelet indices in an experimental model of acute inflammation. Consistent with the results of our study, in some previous clinical studies, it has been reported that the platelet count (12,13,15–17,19–21), MPV (14,15,20), PCT (20), and PDW (15,16,20) are unchanged in various acute inflammatory diseases. In our opinion, these findings cannot be interpreted as that acute inflammation does not affect platelet indices. Instead, we believe that it may be more appropriate to interpret that acute inflammation does not produce a quantitatively significant change in platelet indices due to the combination of the opposite (i.e., both enhancer and reducer) effects. Hypercoagulability is known as an important hallmark of inflammation (22) and platelets can aggregate at the site of inflammation (23). In other words, there is platelet consumption in the area of inflammation. Since platelets reduce blood levels of thrombopoietin (TPO) by internalizing TPO in the plasma (24), in any case where the platelet count is reduced, TPO levels rise in the plasma, stimulating new platelet production in the bone marrow and thus maintaining the platelet count in the blood. Also, various inflammatory cytokines, mostly in synergy with TPO, may stimulate the proliferation or the differentiation of megakaryocytes (25). Larger platelets contain more granules and prothrombotic substances, have greater thrombogenic potential, and are more prone to aggregate and consumption (26,27). The newly produced and released young platelets in the bone marrow have also a larger volume and reactivity (28). Moreover, inflammation activates platelets, increasing their volume (29). On the one hand, the consumption of large platelets in the inflammatory area, on the other hand, the production of large platelets in the bone marrow

and platelet activation induced by inflammation may be the reason why MPV remains quantitatively unchanged.

In this study, we also did not find a significant difference in terms of platelet indices between arterial and venous blood in the control group. In a very old study by Tocantins (30), it has been stated that the platelet count in mammals is slightly higher in arterial blood than in venous blood. In the study of Downie et al. (31) on pigs, it has been determined that the platelet count in arterial blood did not differ statistically from that in venous blood. It has also been reported in humans that there is no difference with regard to platelet counts between arterial and venous blood samples (32). In another recent study in humans, it has been found no significant difference in both platelet count and MPV between artery and vein samples (33).

In conclusion, in our experimental study, we could not find any evidence that platelet indices can be used in the diagnosis or the management of an acute inflammatory disease. In previous clinical studies, it is possible to come across reports that the same platelet index increases, decreases, or does not change in any acute inflammatory disease. It is not clear what the cause of these discrepancies is. It seems that many factors may cause an increase or decrease in platelet indices in the inflammatory process. Perhaps, the results of clinical studies may reflect discrete periods in which the balance between opposite effects in the different stages of acute inflammation is disrupted and reestablished. Therefore, we think that there is a need for more comprehensive studies in which the acute inflammatory process will be followed step by step.

Data availability statement

The data generated and analysed in the presented study are available from the corresponding author on request.

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Figure Legends

Figure 1. Edema and redness after lambda-carrageenan administration.

Figure 2. The experimental setting used in blood collection procedures.

Figure 3. The uncovering of the femoral artery and vein area.

Figure 4. The drawing blood from the femoral vein.

Figure 5. A. Histological image of the inflamed foot: neutrophil adhesion in the vessel lumen (indicated by arrow) and perivascular dense neutrophil infiltration. H&E painting. Bar: 50 μm . **B.** Histological image of the intact foot: normal histological appearance. H&E painting. Bar: 200 μm .