Effects of autohemotherapy on hematological responses in Wistar female rats
Autohemotherapy in rats

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Abstract

Introduction: Autohemotherapy is a type of treatment that have acquired an opposite role and have presented its efficiency strived by the medical community for many reasons. In this study we aimed to evaluate the effects of authohaemotherapy on hematological response.

Method: We used Wistar female rats (300g). The study consisted in a control group and a treatment group, blood samples were collected at the first day and at the eighth day after the application. In the both groups we collected 300 µl of blood from each rat through a syringe with a previously prepared solution of 30 µl of sodium citrate 3.2%. In the autohemotherapy group the blood sample was immediately injected in the quadriceps muscle on the back of the thigh hind limb. Rats from the control group did not receive intramuscular blood application. The cellular count was done through flow cytometry and the samples were dosed for immunoglobulin.

Results: In the both groups we observed increased production of erytrocites, hemoglobin and platelet ($p<0.05$). However, there was reduction of basophil in the control group and reduction of lymphocyte, monocyte and neutrophil in the both groups. No effects were observed in IgA, IgG and IgM levels.

Conclusion: Autohemotherapy did not influence hematological responses in Wistar female rats.

Key words: Therapy; Blood; Cytokines, Chemotactic; Hemoglobins; Blood platelets.

Introduction

The literature investigated some components of the blood as adjunctive therapy in some treatments [1-4]. The procedure in which the blood is removed and the same blood is administrated through intra muscular or subcutaneous injection is known as autohemotherapy. This procedure may still be accomplished with the application of whole blood [5].

The degradation products of erythrocytes are erythropoiesis [6] and immune [7] stimulators. It was observed an increase in 10% or more in the number of platelet-forming cells in the blood and organs from 15 to 30 minutes after application of this procedure [8]. It was also observed an increase of antibodies responsible for neutralizing the cytotoxic products of cell degradation [5].

The immune stimulation caused by the blood was also observed in allergic diseases [7]. A positive clinical effect was obtained in patients with bronchial asthma induced by infection. The production of antibodies against antigens in the lung tissue was suppressed [9].

Autohemotherapy was applied in patients with systemic lupus erythematosus resistant to costicoesteroids. The patients presented clinical remission with decreased activity of the DNase enzyme, the same effect of immunosuppressors treatment [10]. Triquet et al performed autohemotherapy in HIV-negative patients presenting chronic ulcers in the lower limbs. The blood collected from the patient was heparinized and applied to the wound. The result was the ease of removal of the fibronecrotic tissue and faster formation of granulation tissue on the wound [11].

One simple report of clinical outcomes is not enough to establish the actual effects of this technique and there are no clinical studies that have sufficient data to discard the side effects or complications of this procedure.

In 2007 the National Agency of Sanitary Surveillance (ANVISA) through the Management of Blood and Blood components (GGSTO) banned this procedure in Brazil. Autohemotherapy is included in the item V, Article 2 of Decree 77.052/76, and it constitutes a sanitary infraction, subject to the penalties provided in Section XXIX of Article 10 of Law no. 6437, 20 August 1977 [12]. One of the arguments for the prohibition contained in the note was the absence of scientific evidence and indexed reports to prove the efficacy and safety of this procedure. In fact, the literature has not been able to conclude whether autohemotherapy may cause immediate or delayed adverse reactions. In addition, it is not confirmed yet the physiological responses of female subjects treated with autohemotherapy. Therefore, we aimed to evaluate the effects of autohemotherapy on hematological responses in female rats.

Method

Animals

The experiments were performed in Wistar female rats (n=15, 300 g). Rats were housed individually in plastic cages under standard laboratory conditions. We divided the animals into two groups: Control (n=5) and autohemotherapy (n=10) groups. They were kept under a 12-h light/dark cycle and had free access to food and water. Housing conditions and experimental procedures were approved by our institution's Animal Ethics Committee (protocol number 009/2008). Efforts were made to minimize the number of animals used.

Protocol Procedures

Blood samples was collected through the tail vein with a needle injection of 1 ml with a needle (35x0.7 mm) with a previously prepared solution of sodium citrate 3, 2%, looking for a 1: 10 ratio of the volume of anticoagulant in the volume of blood collected. The animals were anesthetized in order to avoid stress during the procedure using a combined solution of 1ml of ketamine (100mg/ml), 0.5 ml of xylazine (20mg/ml) and 8.5 ml of saline 0.9%. The volume of blood collected from each animal did not exceed 0.05 ml/kg or 7.5% of blood volume from each animal.

In the autohemotherapy group we collected 300 µl of blood from each rat through a syringe with a previously prepared solution of 30 µl of sodium citrate 3.2%. The volume was immediately injected in the quadriceps muscle on the back of the thigh hind limb. The application of blood was considered the autohemotherapy treatment, this procedure was performed once in each animal.

In the control group we collected 300 µl of blood from each rat, however, we did not apply the intramuscular injection.

We collected blood samples for laboratory analysis equivalent to 7.5% of volume of each animal before the treatment and on the 8th day after the treatment, respecting the period of one week for hematopoietic recovery.

Blood samples were analyzed through complete blood count and it was measured immunoglobulin (Ig) G, Ig A and Ig M.

Cells were counted with the flow cytometer (ABX Pentra 60) and blades were also performed using the technique of blood smear and subsequent staining with Leishman (eosinophilic stain basophilic) for the qualitative and quantitative morphological evaluation of peripheral blood cells.

After the complete blood count, the samples were subjected to centrifugation in order to determine the concentrations of immunoglobulins IgG, IgA and IgM through radial immunodiffusion. After inoculation of the plasma above the plates, halos were measured for 72 h incubation at 25 ° C.

Statistical Analysis

To verify the normality of the distributions we applied the Kolmorogov-Smirnov normality test. We applied the parametric paired Student T test to compare the variables between before and after the treatment, because all distributions were parametric. Differences were considered significant when the probability of a Type I error was less than 5% ($p < 0.05$). We used the statistical package GraphPad Prisma®.

Results

Hematocrit, Hemoglobin and Platelet

Figure 1 presents data regarding hematocrit, hemoglobin and platelet levels in the control group before and after blood collection. We observe that all parameters were increased ($p<0.05$) in the second blood sample.

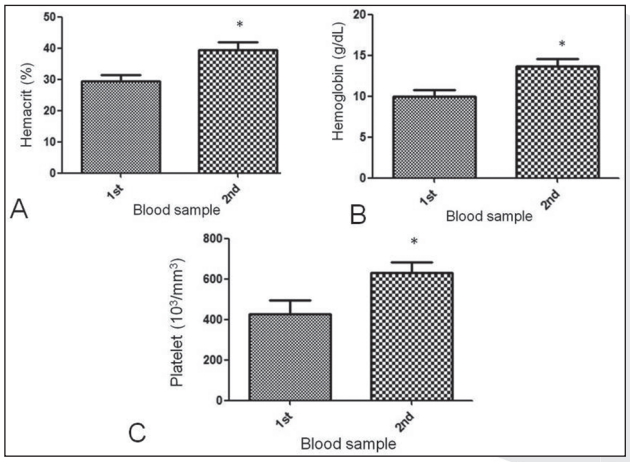

*Figure 1. Hematocrit (A), hemoglobin (B) and platelet (C) before (1st) and after (2nd) blood collection in the control group. *p<0.05: 1st vs. 2nd*

Similar to the control group, hematocrit, hemoglobin and platelet parameters were also increased after autohemotherapy treatment (Figure 2).

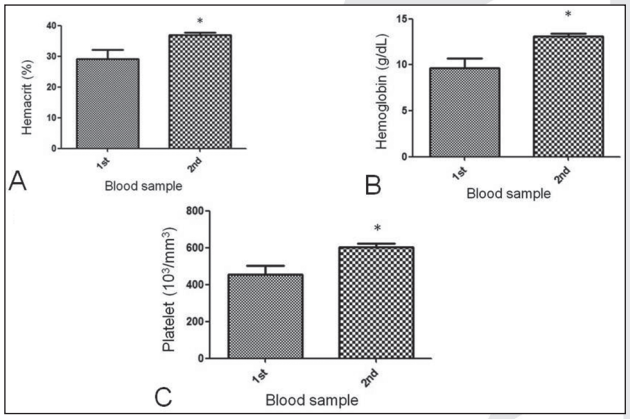

*Figure 2. Hematocrit (A), hemoglobin (B) and platelet (C) before (1st) and after (2nd) autohemotherapy treatment. *p<0.05: 1st vs. 2nd*

Basophil, Eosinophil, Lymphocyte, Monocyte and Neutrophil

According to Figure 3, the control group presented lower values after the 1st blood collection in relation to plasma basophil, lymphocit, monocyte and neutrophil levels.

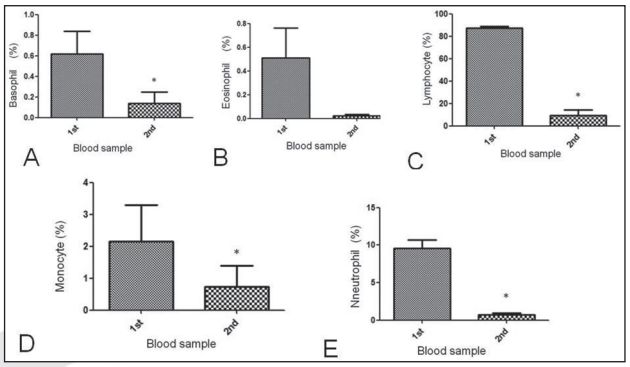

*Figure 3. Basophil (A), eosinophil (B), lymphocyte (C), monocyte (D) and neutrophil (E) before (1st) and after (2nd) blood collection in the control group in the control group. *p<0.05: 1st vs. 2nd*

In relation to the treated group, basophil levels tended to be decreased after autohemotherapy treatment, however, it did not reach statistical significance. Similar to the control group, eosinophil was not significantly reduced after autohemotherapy. Lymphocyte, monocyte and neutrophil were significantly decreased after autohemotherapy treatment (Figure 4).

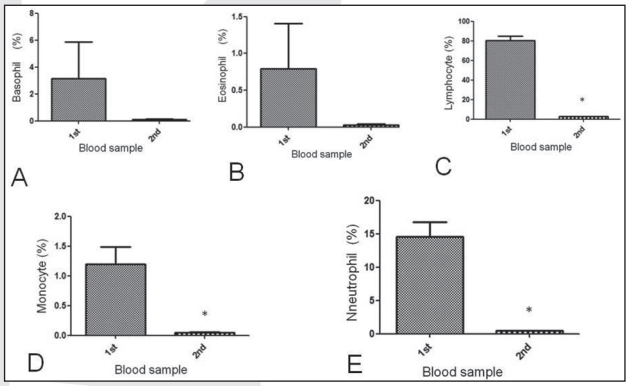

*Figure 4. Hematocrit (A), hemoglobin (B) and platelet (C) (1st) and after (2nd) autohemotherapy treatment. *p<0.05: 1st vs. 2nd*

IgA, IgG and IgM

IgA, IgG and IgM were not changed after blood collection in the treated group (Figure 5). In the treated group, the same cells were not changed after authohemotherapy treatment (Figure 6).

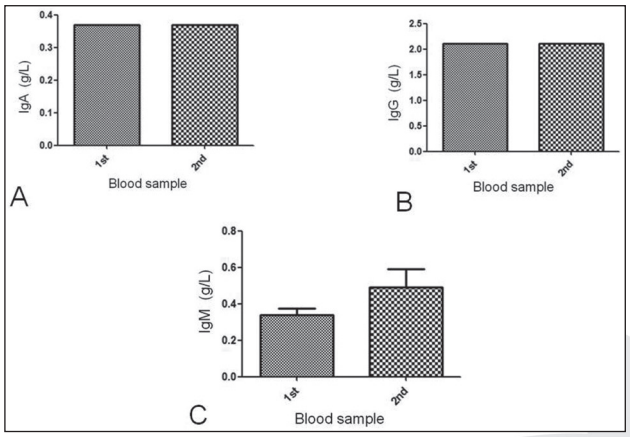

Figure 5. IgA (A), IgG (B) and IgM (C) before (1st) and after (2nd) blood collection in the control group in the control group

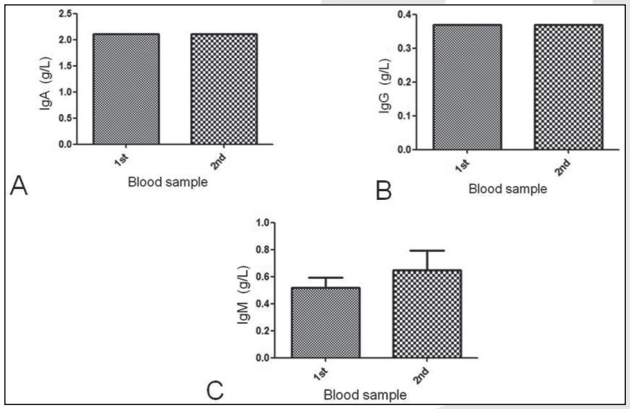

Figure 6. IgA (A), IgG (B) and IgM (C) (1st) and after (2nd) autohemotherapy treatment

Discussion

This investigation was undertaken to evaluate the effects of autohemotherapy on hematological responses in female rats. As a main finding, there was no difference between the control and treated groups regarding hematocrit, hemoglobin, platelet, basophil, eosinophil, lymphocyte, monocyte, neutrophil, IgA, IgG and IgM. We suggest that the treatment presented the same effects of the control group. Blood removal is responsible for inducing blood cell production, since blood loss lead to decreased tissue oxygenation and it is a stimulus for erythropoiesis [13]. This mechanism also occurred with a small volume of blood applied, such as the procedure used in our study.

In relation to the leukocyte count, we observed no difference between the autohemotherapy and control groups. Our finding indicates that blood withdrawal reduced the production of leucocyte. Moreover, a previous investigation concluded that ozonated autohemotherapy in a dose of 50 mg/mL does not present significant influence on natural killer cell function in hemodialyzed patients [14]. No differentiation was made between the types of lymphocytes, we suggest future studies to perform this procedure. To the best of our knowledge, no previous study specifically examined the induction of leukocyte cell by cell. Thus, there is no guesswork in the literature regarding the pathophysiology of this mechanism.

A possible explanation for the phagocyte behavior found in our study is that intramuscular application of blood as well as blood withdrawal cause a local inflammatory process by stimulating phagocytosis through tissue macrophages [15]. Tissue macrophages present antigens to T lymphocytes that are activated and are involved in the process of B cells activation that initiate the process of clonal division with the production of antibodies and globulins [16]. Neutrophils are recruited from peripheral blood after inflammatory mediators secreted by damaged tissues [15]. The result of the process is a decreased number of lymphocytes induced by local stimulation, which is responsible for the response modulation and is detected in peripheral blood. Since neutrophils and macrophages recruited into the tissues phagocytize the injured tissues and blood cells inserted in place with cleaning function until the damaged cells and tissues are properly removed [15], it is observed a lower removal to the lymphatic system are poorly detected in peripheral blood.

Bocci et al [17] published the first study to show that autohaemotherapy may activate an immunological marker in normal subjects without procuring any toxic effects. However, according to our data, in the comparison between control and autohemotherapy groups regarding immunoglobulins, there was no difference. We noted that in the both groups immunoglobulins production was not changed. It indicates that autohemotherapy does not induce the production of acute-phase immunoglobulin. Furthermore, induced desensitization plays an important part in the mechanism of action of autohaemotherapy. The administration of large doses of erythrocytes or erythrolysate results in immunosuppression [5]. Nevertheless, according to Klemparskaya et al [5], autohaemotherapy does

not cause side effects and is feasible both on an in- and out-patient basis.

In this study, the maximum amount of blood that could be injected into the muscles of the animals was collected. This volume of blood did not affect blood oxygenation because the animals did not present symptoms related to this mechanism such as cyanosis and reduced blood hemoglobin levels. However, although this volume is not dangerous to blood oxygenation, it may have been responsible for the induction of hematopoietic responses observed in the autohemotherapy and in the control groups.

Based on our findings, Wistar female rats treated with autohemotherapy presented the same responses described in previous studies such as induction of erytropoiesis [5]. Also, similar responses were observed related to Ig M production. Nonetheless, we found no reference regarding this mechanism in female rats. Observing the principles of erythropoiesis induction, this mechanism occurs when there is any condition that decreases tissue oxygenation such as low blood volume, anemia, low hemoglobin levels, reduced blood flow, low atmospheric oxygen or lung disease [18]. In these cases, renal hypoxia stimulates the production of erythropoietin inducing factor from bone marrow to produce erytrocites [19]. Removal of 5 ml of blood during autohemotherapy performed in humans does not induce renal hypoxia [20].

We observed that autohemotherapy and blood removal increased blood platelet levels. However, a previous study reported opposite findings in humans [21]. Tylick et al evaluated the effects of ozonated autohaemotherapy on the platelet function in chronically haemodialysed patients with peripheral arterial disease. They found that autohemotherapy with ozone concentration of 50 microg/ml and citrate as an anticoagulant does not induce platelet aggregation.

Our data demonstrated that autohemotherapy presented the same effects of no treatment regarding hematological responses. This data does not provide evidence enough to implement changes to human treatment. We suggest further studies to justify the application of this technique in humans for treatment or cure of any disease.

These data provide useful information given that currently many types of animals are widely studied in order to develop new therapies for the prevention of several cardiovascular disorders in humans [22-25].

Conclusion

Autohemotherapy did not influence hematological responses in Wistar female rats.

Acknowledgement

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