Autohemotherapy: hematological and histological changes in wistar rats

Auto-hemoterapia: alterações hematológicas e histológicas em ratos wistar

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Abstract

Objective – To evaluate the hematological and histological changes in Wistar rats submitted to autohemotherapy. **Methods** – Twelve Wistar rats were divided into 2 groups: case and control. This last one was submitted to autohemotherapy for 4 weeks. Hematological parameters were analyzed and a fragment of the thigh muscle which received injections of blood was submitted to histological analysis. **Results** – The hematological parameters analyzed did not suffer changes with autohemotherapy. In the histological analysis was observed that there were inflammatory infiltrate and blood in the muscle of animals which belonged to the case group, while the control group showed full muscle without any signs of change. **Conclusions** – The risk/benefit in the use of autohemotherapy for increase blood cell count shows non-compensatory, due to lack of knowledge about the consequences that the autohemotherapy can cause in the organism.

Descriptors: Histology; Leukocyte count; Hematology

Resumo

Objetivo – Avaliar as alterações hematológicas e histológicas em ratos Wistar submetidos à auto-hemoterapia. **Métodos** – Doze ratos Wistar foram distribuídos em 2 grupos: caso e controle. Este último foi submetido à auto-hemoterapia por 4 semanas. As alterações hematológicas foram analisadas a partir de hemograma e a análise histológica por meio da remoção de um fragmento do músculo da coxa direita, que recebeu as injeções de sangue. **Resultados** – Os parâmetros hematológicos analisados não sofreram alterações com a auto-hemoterapia. Na análise histológica foi observada presença de infiltrado inflamatório e sangue tecidual nos cortes dos animais pertencentes ao grupo caso enquanto que o grupo controle apresentou músculo íntegro, sem quaisquer sinais de alterações. **Conclusões** – O risco/benefício do uso da auto-hemoterapia para aumentar a contagem de células sanguíneas mostra-se não compensatório devido a falta de conhecimento acerca das consequências que a auto-hemoterapia pode causar no organismo.

Descritores: Histologia; Contagem de leucócitos; Hematologia

Introduction

In 1912, French physician Paul Revaut defined autohemotherapy as a therapeutic method[1] in which retires by venipuncture of a small amount of blood from the patient and applies in his muscle tissue[2].

When the blood is injected into the donor, his body recognizes as a foreign and recruits immune cells[2]. The autohemotherapy is able to increase the differential count of leukocytes from an initial value of 5% of macrophages to 22% after eight hours of application, clearly demonstrating the contribution to the defense system of the organism[2-3].

This technique assists in the prevention and cure of various diseases, is indicated for the treatment of scleroderma, atherosclerosis, lupus, thrombocytopenic purpura, asthma, ovarian cysts and fibroids, gangrene due to spider bite, and others[2].

Existing for over 100 years, the autohemotherapy is used, disseminated and defended by many of his followers and totally rejected by those who not believe in its effectiveness, but both do not have enough arguments to defend, in fact, their opinions. Little studied, this technique no presents scientific evidence of its effectiveness, or even its harms, although its users cite benefits and cures promoted by it.

In Technical Note n.º 1 of 13 April 2007 the Agência Nacional de Vigilância Sanitária (ANVISA, National Agency of Health Surveillance) warns about the autohemotherapy, because its a procedure that is not on the law n.º 153 of June 14, 2004[4], which establishes the technical regulations for conducting procedures of hemotherapy. Another factor for the aversion of the technique is that there is no scientific evidence, indexed studies supporting its safety and efficacy. It is not known if this practice can cause adverse reactions with unexpected severity[5]. The procedure of autohemotherapy is not recognized by the Brazilian Society of Hematology and Hemotherapy[6] and ultimately, the procedure can be framed in item V, Section 2 of Decree 77.052/76, which states that its practice is understood as a health violation[7] subject to the penalties provided for in item XXIX, Article 10 of Law n.º 6437 dated August 20, 1977[8].

The Federal Counsel of Medicine (FCM) has issued an opinion stating that the autohemotherapy was not subject to genuine tests, it has not been confirmed and there is nothing, besides suspicion, isolated cases without scientific evidence, being its current use in humans an irresponsible adventure[9]. The law of CFM Nº. 1,499 of August 26, 1998, prohibits physicians to make use of

therapeutic practices not recognized by the scientific community[10].

Knowing that the autohemotherapy may present risks to users, such as injuries, tissue necrosis, hematoma and phlebitis[11] and with so many controversies opinions about this procedure, arises the need for an experiment in vivo that contributes to the clarification and demystification of autohemotherapy. Therefore this study aimed to evaluate the hematological and histological changes in Wistar rats subjected to this technique.

Methods

Ethical Procedures

This project was submitted to the Ethics Committee on Animal Experimentation (EAEC) from Faculdade Integrado de Campo Mourão and approved in accordance with the protocol n.º 370, according to the ethical principles of animal experimentation established by the Brazilian College of Animal Experimentation (COBEA).

Animals

Twelve male Wistar rats, with approximately 60 days and 200g of body weight, from the Central Animal Laboratory of the State University of Maringa were used. These animals were kept in a vivarium of the Faculdade Integrado de Campo Mourão, at room temperature and light/dark light cycle of 12 hours, in cages with galvanized grid with deposit to ration and water bottle. These went through a week of adaptation and already acclimated, the experiments were started. The animals were divided into 2 groups: case and control. Those in the case group underwent autohemotherapy and in the animals of control group, blood was collected and administered sterile saline (0.85%) in the thigh muscle.

Autohemotherapy

Was collected 100µL of blood from the tail vein of each animal and immediately the blood was aseptically injected in the quadriceps muscle on the back of the right thigh of hind limb of the respective animals, without any treatment of the material[12]. The volume of blood collected from each animal did not exceed 0.05ml/kg or 7.5% of of whole blood volume.

The autohemotherapy was performed once a week for four weeks. Two days after each session, the blood was collected by the same way described above, with EDTA anticoagulant, from the animals of the case and control group for the blood count[13].

Hematological analysis

Blood counts were performed in hematological counter (Sysmex KX – 21N), which examined the following parameters: total leukocyte, lymphocyte, erythrocyte and platelets count, dosage of hemoglobin (Hb), hematocrit (Ht)and determination of blood indices: Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular hemoglobin concentration (MCHC) and Anisocytosis Index (RDW).

Histological analysis

After the end of the experiment (4th week), all rats were sedated/anesthetized using a dose of 120 mg/kg of ketamine 2% and 10 mg/kg of xylazine 2% intraperitoneal. When the rats were completely anesthtized we proceeded with euthanasia by intracardiac administration of potassium chloride. Immediately after, were removed the fragments muscle of the right thigh of each animal for the preparation of histological sections. The fragments were fixed with 10% buffered formalin for 24 hours and preserved in 70% alcohol.

The specimens were submitted to dehydration, leaf clearing and paraffin embedding melted at 60°C. For these processes the pieces were immersed in alcohol solutions of increasing percentages (80%, 90% and 100%), then in a solution of xylene and finally in melted paraffin. Were made cuts with 5µm thick, separated by intervals of 15µm (semi-serial). The prepared slides were stained with hematoxylin-eosin.

The entire length of the cut was examined with 10X objective lens and, when necessary, with a 40X objective. We sought to analyze aspects related to the presence of inflammation and signs of tissue damage. The readings were performed in duplicate.

Statistical analysis

In order to test whether there were significant differences in mean blood cell count between the case and control groups, was made a unpaired t-test in the R program (R Development Core Team). The functions were written according to the package "The R Stats Package".

Results

The analysis of the blood cell counts obtained four weeks after the autohemotherapy revealed that this procedure has no influence ($p>0,05$) in the amount of these cells (Table 1).

With respect to the total leukocyte count was observed only after the second week of autohemotherapy a little evolution. In the fourth week of the procedure the case group showed the greatest difference (18.4%) in leukocyte counts compared to the "control" group, however the values have no statistical relevance ($t = 0.32$, $p = 0.77$). It is also possible to note that the total count of leukocyte in both groups did not developed steadily. The same result was found for the lymphocytes, without significant differences between case and control groups ($t = 0.41$, $p = 0.70$) e cell count inconstant, with high values of standard deviation.

In histological analysis, it was noted that the animals of case group showed intense inflammatory infiltrate in the evaluated sections (Figure 1). It is noteworthy that were identified focal infiltrators. Also it was observed in animals of the same group presence of blood in tis-

Table 1. Means and standard deviations of the items evaluated in the blood count of the animals of case and control groups according to period of evaluation.

Analyzed parameters	1st week		2nd week		3rd week		4th week	
	Case group	Control group	Case group	Control group	Case group	Control group	Case group	Control group
Leukocytes/ µl	11,067 (± 2,988)	16,700 (± 4,762)	8,525 (± 3,415)	8,300 (± 2,927)	11,180 (± 7,664)	10,300 (± 1,956)	9,440 (± 2,952)	7,700 (± 3,024)
Lymphocytes (%)	66.83 (± 8.5)	59.5 (± 7.9)	75.05 (± 10)	63.7 (± 15.2)	69.96 (± 21.89)	94.3 (± 22.36)	79.84 (± 11.29)	89.4 (± 17.99)
Erythrocyte (million)	6.89 (± 1.49)	6.52 (± 1.12)	5.98 (± 0.9)	7.95 (± 1.87)	6.57 (± 1.04)	8.04 (± 2.1)	7.48 (± 0.5)	7.72 (± 0.9)
Hb (g/dL)	12.93 (± 2.64)	11.7 (± 1.79)	11.5 (± 1.99)	16 (± 3.1)	12.78 (± 1.3)	13.9 (± 2.02)	14.06 (± 1.19)	13.6 (± 1.93)
Ht (%)	41.77 (± 8.82)	38 (± 6.21)	39.02 (± 5.98)	51.6 (± 4.36)	40.42 (± 6.68)	47.9 (± 7.66)	45.48 (± 3.31)	45.2 (± 7.43)
MCV (fL)	60.7 (± 0.76)	59 (± 1.11)	65.1 (± 1.19)	64.9 (0.97)	61.52 (± 1.08)	59.6 (1.05)	60.72 (± 1.35)	58.5 (± 1.42)
MCH (pg)	18.8 (± 0.64)	17.9 (± 0.99)	19.2 (± 0.87)	20.1 (± 1.67)	19.68 (± 1.95)	17.3 (± 1.74)	18.8 (±0.76)	17.6 (± 0.85)
MCHC (g/dL)	30.9 (± 0.73)	30.4 (± 2.14)	29.4 (± 1.14)	31 (± 1.58)	31.96 (± 3.2)	29 (± 0.96)	30.94 (± 0.65)	31.1 (± 1.44)
RDW (%)	20.45 (± 5.36)	18.8 (± 3.64)	21.01 (± 2.47)	19.6 (± 4.91)	27.12 (± 10.54)	23.4 (± 6.11)	16.18 (± 2.4)	19.4 (± 4.59)
Platelets µl	570,000 (± 341,734)	735,000 (± 425,269)	65,700 (± 39,584)	471,000 (± 255,968)	205,000 (± 353,322)	382,000 (± 244,826)	447,000 (± 269,870)	598,000 (± 497,355)

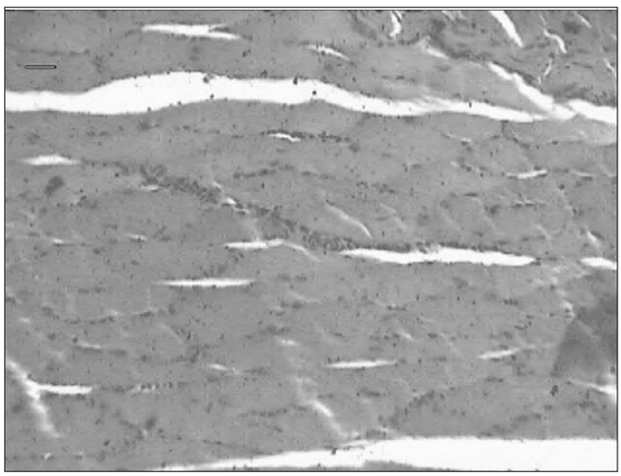

Figure 1. Histological section of the right thigh muscle of an animal of the case group. H & E 100X. Bar: 300µm

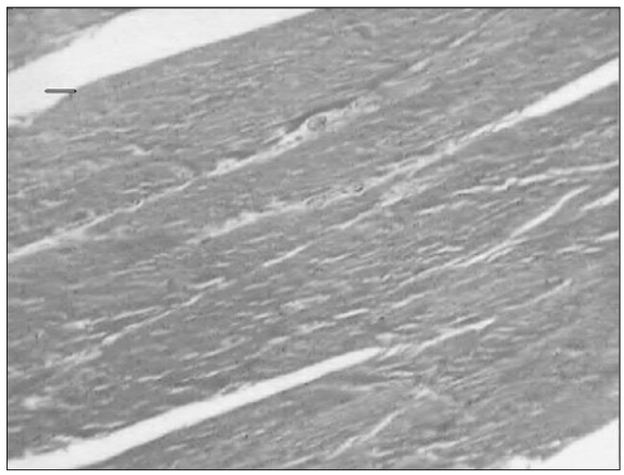

Figure 2. Histological section of the right thigh muscle of an animal of the control group. H & E 100X. Bar: 300µm

sue. The control group showed the intact muscle tissue, without any changes or signs of inflammatory response (Figure 2). There was the presence of macrophages in tissue sections and was also not possible to differentiate another leukocytes.

No animals died during the experiment, nor macroscopic lesions were observed at the sites of injection in both groups.

Discussion

The practice of autohemotherapy has become more popular over the decades of its existence, however there are few scientific studies that bring their real benefits. This study evaluated hematological parameters and how the muscle tissue responds to weekly injections of blood in rats who did or not autohemotherapy.

Existing studies have evaluated the use of autohemotherapy as an adjunct in the treatment of optic neuropathy[14], type 2 diabetes mellitus[15-16], increase of immunity[17], treatment of age-related macular degeneration[18], transmissible venereal tumor[19] and obtained satisfactory results. Other authors[12,20-21] found no advantages in the use of autohemotherapy, as Brewer[22], who claim that the apparent clinical benefits of autohemotherapy are not statistically reliable. More randomized trials are re-

quired to clarify the effectiveness of autohemotherapy. With regard to the harmful effects of the autohemotherapy, scientific researchs are scarce.

These differences of opinion are responsible for the rejection of the technique by many medical professionals, but also encourages studies about the discovery potential risks and benefits of it.

The blood count is a highly useful review of several laboratory parameters. In this test it is possible to analyze the erythrocyte, leukocyte and platelet profile of the patient[13]. In this work the focus was primarily to determine the leukocyte portion of the animals, since according to Teixeira[3] self-hemotherapy influences the white blood cell count, directly contributing to increasing the immunity of users of the technique.

In the present study the autohemotherapy not influenced the blood count, according as Ibanes[12]. Using the same methodology, another authors found an increase in leukocyte count in mice compared to the first day of the experiment and the control group[23]. Maybe this difference in the result is due to the species of the animals used. Biedunkiewicz *et al.*[24] found no influence of the ozone autohemotherapy in the action of Natural Killer (NK) lymphocytes. But Trevisanil *et al.*[17] observed a tendency for stimulate the immune response of humans.

The leukocytes are known by promote the body's defense, especially the lymphocytes. However, not always the amount of leukocytes directly influences in the effectiveness of the immune system, because there is also action of mediators, cytokines, growth factors, antibodies[25-26]. By some limitation we could not detected these substances, but this would be interesting for complement our results.

The autohemotherapy sessions were held in the quadriceps muscle on the back of the right thigh of hind limb of animals. The skeletal muscle consists of very long cylindrical multinucleated fibers, which have transverse striations. It is not typical of this tissue the find of defense cells, so their presence usually indicates an inflammatory tissue reaction[27].

In the histopathology review of the muscle tissue of the animals, it was observed that the animals in the case group showed inflammatory response throughout the length of the assessed cuts, compared with the control group, which showed full musculature and without the presence of leukocyte infiltration in tissue. This tissue response is expected, considering that a biological material belonging to another location was injected in the tissue[28]. However this reaction was not sufficient to significantly increase the total count of circulating leukocytes, was only a local reaction. But the excess of intramuscular administrations of blood can injure tissue irreversibly and promotes necrosis[29].

In this study the autohemotherapy did not change the hematological responses and have promoted damages in tissue. This data does not provide evidence enough to include this method as an alternative therapeutic. We suggest further researches about the mechanism of action whereby the autohemotherapy promotes the cure the diseases in humans.

Conclusions

With the results obtained in this experiment, we noted that the autohemotherapy not influenced the blood cell count and damaged the mucle of the rats. So we conclude that the risk/benefit of using autohemotherapy is not compensatory, due to lack of knowledge about the consequences that this therapy can result in the organism.

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