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EXPERIMENTAL STUDY

Primary and secondary thrombocytosis induced by exercise and environmental luminosity

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Abstract: *Objectives:* We investigated the exercise and different environmental luminosities effects on blood platelets count in order to identify primary and secondary thrombocytosis, respectively.
Background: Platelets alteration has been associated with important pathological events, such as neurodegenerative diseases, and the count of these cells in bloodstream is influenced by several effects, including physical and chemical. Owing the difficulty to study the aetiology of thrombocytosis in human models, we employed acute and chronic free drug interventions in order to identify these two types of this important disease in laboratory animals.
Methods: Forty rats were exposed to standard (SI) or experimental (EI) illumination from 45 days-old. Both groups were exposed to 12 h daylight (2700 K; 565–590 nm; < 60 lux; from 06:00 h to 18:00 h). During dark period SI animals were kept in total darkness while EI remained under red light (> 600 nm, < 15 lux). At 92 days-old, exercised animals were submitted to an acute bout of swimming at individualized intensity and control animals remained at rest.
Results: Blood samples were collected immediately after the exercise for platelets count, which were among 849000 ± 115817 and 1085600 ± 177089/mm³ of blood. Exercise ($F = 6.91$; $p = 0.01$) and EI ($F = 6.66$; $p = 0.01$) increased platelets count, showing no interaction between effects ($F = 0.01$; $p = 0.89$).
Conclusion: Primary thrombocytosis was detected owing an acute exercise and the secondary thrombocytosis due to the constant red light during dark period, without any pharmacological interventions and strongly respecting the ethical aspects, enabling future studies on aetiology of thrombocytosis through this model *(Fig. 2, Ref. 35)*. Text in PDF *www.elis.sk.*
Key words: thrombocytosis, platelets, exercise, animal model, environmental luminosity.

Megakaryocytopoiesis is the production of megakaryocytes, responsible for platelets release (1). Platelets are important disc-shaped small anuclear cells of blood that play an important role repairing wounds and the vascular damage naturally generating during all life, beyond the participation on innate immune response (2) and clotting process (3) widely investigated currently due to the important source of information for biological science (4–6). Platelets alteration has been associated with some important pathological events, such as neurodegenerative diseases (7). The blood platelets count in blood stream is influenced by several effects, including physical and chemical (8). The primary thrombocytosis (increased platelets count) is acute-phase related, while secondary is usually associated to chronic events (9).

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It is quite known that the exercise transiently increases platelet count (10), which are initially released by the spleen and lungs (8) probably due to the muscular microtrauma (11). This primary thrombocytosis is characterized by short lived increase, considered as a normal organism response toward benefit exercise adaptation. Nevertheless, secondary thrombocytosis is considered abnormal and usually related to chronic inflammation, myocardial infarction, cancer, thrombosis and cardiovascular diseases (1, 8, 12).

In clinical routine it is very difficult to determine if the thrombocytosis is related to primary or secondary mechanisms in human beings (9). Thus, the animal model provides a unique tool to better understand some diseases and healing process (1) and could be useful for aetiology and pathogenesis of thrombocytosis, however, this possibility should be more studied. The rat is the most used animal on experiments, however, this rodent are nocturnal (13) and possess a high light sensibility (14). This sensibility associated to variations on environmental luminosity could generate stress, which could lead to secondary thrombocytosis (15).

In light of the above findings, our study investigated the acute effect of a swimming bout at anaerobic threshold intensity until exhaustion and the chronic effect of different environmental illuminations on blood platelets count. Our hypothesis was that with this design we would be capable of distinguish the primary and secondary thrombocytosis. Accepting this, the

primary and secondary thrombocytosis could be induced without any pharmacological interventions and strongly respecting the ethical aspects.

Methods

Animals

Forty albino Wistar rats were kept in polyethylene cages at 22 ± 2 °C, 45–55 % of relative humidity and noise below 85 decibels, being a light/dark cycle of 12/12 h with lights switched on at 06:00 h. The animals were fed with rat-specific standard chow (70 % carbohydrate, 23.5 % protein, 6.5 % fat; Labina-Purina®, Purina 5008, St. Louis, MO) and water *ad libitum*. The institutional review board approved the experiment (protocol 018/10), which followed the current National Legislation and Standards for Animal Experimentation.

Environmental luminosity and study design

During the light period all animals were exposed to an incandescent lamp (Phillips®, Soft, 100 W, 2700 K, 565–590 nm, < 60 lux). At 45 days old, forty rats were equally divided into 2 groups: standard (SI) and experimental illumination (EI). From 18:00 h to 06:00 h, EI groups were exposed to constant red light (Rosco®, Supergel, Fire # 19, > 600 nm, < 15 lux), while SI groups remained at total darkness and were submitted to the same red light only during procedures. Lamps were carefully positioned to assure equal distance among light source and cages.

Handling, cage cleaning, water and food addition and testing were begun at 20:00h in order to respect the high values for body core temperature (16) and spontaneous activity of rats (17), leading the animals to less stress at this time of day (18). In order to perform two weeks of an aquatic and swimming testing adaptation (AA) the animals were subdivided into control (ct) and exercised (ex) groups at 76 days-old. At the end of AA and at 90 days-old, the lactate minimum test (LMT) were conducted for all animals. After 48 hours, exercised groups were submitted to a swimming bout until exhaustion at lactate minimum intensity (*tlim*), while control groups remained at rest (Fig. 1).

Swimming testing

Routines were performed in cylindrical PVC tubes (30 cm diameter and 120 cm depth) containing clean water at 31 ± 1 °C, to elicit individual swimming and warrant the continuous swimming pattern.

The lactate minimum test (LMT) consisted in three steps: 1) hyperlactatemia induction by two short bouts of high intensity exercise (13 % of body weight – % bw); 2) passive recovery period of 9 minutes to allow the release of the lactate produced by muscles toward the bloodstream; and 3) incremental phase (4, 4.5, 5, 5.5, 6, 7 % bw), in which the blood lactate was collected and analyzed for the minimum value identification. This nadir was calculated through second order polynomial adjust and indicated the maximal intensity when the release and consumption of blood lactate was equal, representing the maximal lactate steady state phenomena in swimming rats (19). The lactate minimum intensity (LMi) and

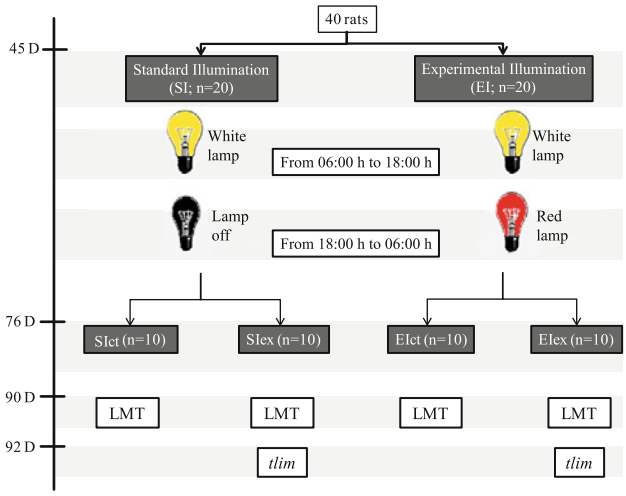

Fig. 1. Schematic summary of study design. SIct – standard illumination control; SIex – standard illumination exercised; EI – experimental illumination; LMT – lactate minimum test; *tlim* – time to exhaustion.

lactatemia corresponding to LMi (LLM) were determined as described elsewhere (20).

Forty-eight hours after perform LMT, exercised animals were submitted to a swimming trial at lactate minimum intensity, being recorded the time to exhaustion (*tlim*). The exhaustion criteria was accept unanimously by two experienced researchers, when the animal was incapable to return to the surface during 10 seconds submerged, even with vigorous trying, as previously described (21).

Blood collection and analytical procedures

Blood samples were collected from rat´s distal tail during LMT, before ([lac]pre) and after ([lac]post) *tlim*. After prepared, samples were used to determine lactate concentration by enzymatic method against calibration curve, as described elsewhere (22). Immediately after *tlim* for the exercised group, or at rest and same time of day for control group, animals were euthanized in carbonic gas chamber, being blood collected by cardiac puncture method. Blood samples of each rat was immediately transferred to a plastic tubes containing k3EDTA (fl Medical®, Torreglia, PD, Itália) for platelets counts, which were analyzed by hemocromocytometric flux method with semiconductor laser performed on automatic counter (MAXM Coulter).

Statistical procedures

Data were expressed as the mean ± standard deviation. Statistical analysis was carried out employing a software package (Statistic 7.0). We used the t test to determine the differences between the exercised groups for LMi, LLM, [lac]pre and [lac]post and *tlim*. Two-way analysis of variance were used for the investigation of luminosity and exercise effects (and their interaction) on platelets count. The Newmann–Keuls post hoc test was used to locate differences when pertinent. The significance level was set at 5 % ($p < 0.05$).

Results

LMi corresponded to 4.78 ± 0.27 and 4.98 ± 0.27 % bw for SI and EI (p = 0.12), respectively, being the lactatemia for these intensities corresponding to 5.81 ± 1.00 and 4.38 ± 0.52 mmol/L (p < 0.01), respectively.

The *tlim* corresponded to 113.58 ± 46.67 for SI and 51.93 ± 24.65 min for EI (p < 0.01). [lac]pre corresponded to 1.99 ± 0.42 mmol/L for SI and 1.21 ± 0.25 mmol/L for EI (p < 0.01) and [lac] post were 5.78 ± 0.87 and 5.63 ± 1.21 mmol/L for SI and EI, respectively (p = 0.76).

Platelets count for SIct, SIex, EIct e EIex were 849000 ± 115817, 974400 ± 146827, 972200 ± 114645 and 1085600 ± 177089/mm³ of blood. The exercise significantly increased platelets counts (F = 6.91; p = 0.01) as well as the experimental illumination (F = 6.66; p = 0.01), with no interaction between these main effects (F = 0.01; p = 0.89), as illustrated by Figure 2.

Discussion

The main finding of the present study was the identification of primary and secondary thrombocytosis through acute and chronic effects owing to exercise and environmental luminosity, respectively, accepting our initial hypothesis.

Concerning the lactate minimum test, we found no luminosity effect for LMi, revealing the protocol´s robustness. Moreover, the LLM and [lac]pre were sensible to identify the adverse condition, at which the animals were exposed when under EI, considering that in some previous studies on malnourished rats the same lactatemia results were found (23). Probably the adverse conditions increased adrenocorticotropic hormone (24) and consequently adrenaline and corticosterone (25). This situation led to an increase in resting glycolytic metabolism, decreasing glycogen content (26).

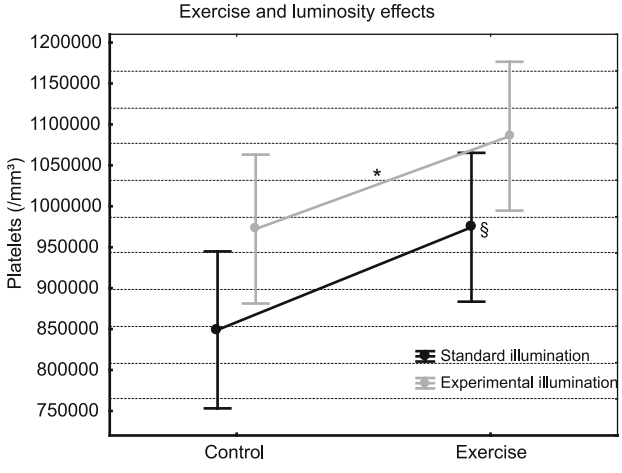

Fig. 2. ANOVA results for exercise (control vs exercise), luminosity (standard vs experimental illumination) and their interaction for platelets count. * F = 6.66 and p = 0.01 in relation to SI; § F = 6.91 and p = 0.01 in relation to control. The effects showed no significant interaction as described in the text.

Considering that less glycogen is available, glycolytic substrate is reduced and also the lactate concentration (23, 27). According to these authors and in agreement of this logic, low lactate concentration could indicate low glycogen stores. Since the glycogen is the main substrate for the required intensity (28) the lower *tlim* for EI is a reasonable result.

Regarding the exercise effect on platelets count, even with no statistical difference among control and exercise groups for SI and EI, we found an increment of 12.87 % and 10.45 %, respectively, which was enough to lead to a significant effect of exercise when analyzed by analysis of variance (F = 6.91; p = 0.01). Animal model experiments showed that despite the platelets decreased due to exercise training in *Wistar* rats (29), an acute exercise increased these counts (30). Moreover, there was a great variation on platelets activation regarding to endurance exercise in literature (31), being the activation associated to the factors explained in the introduction section, as muscular microtrauma releasing platelets from the spleen and lungs (8, 11). Considering these known platelets variation owing to a specific exercise intensity, we individually prescribed the exercise through lactate minimum test, a robust and valid protocol contained in swimming rats literature (19, 20, 32).

In addition, higher platelets counts were found for animals exposed to experimental illumination (secondary thrombocytosis), in other words, chronically. This finding could be explained due to the possible stress originated by constant red light during dark period. Stressful conditions has been related to modulations in platelet count and some disorders related to platelet functionality in humans (15, 33, 34). Regarding the above described, our study showed an evidence in the same direction for rats.

We could observe in the results section that both exercise and luminosity generated significant increase on platelets count. Moreover, there was no interaction between effects, which demonstrated that the exercise or experimental luminosity could modulate the platelets count singly. According to our interpretation, this result confirms the presence of acute and chronic effects on platelets count owing exercise and luminosity, respectively.

Despite the fact that our study discriminated the primary and secondary thrombocytosis using a rodent model, our design is not out of criticism. The platelets activation and aggregation certainly could conduct us to more robust affirmations, however, platelet scores could be related to platelets activation and aggregation (35). For instance, with the platelets count we could investigate our initial hypothesis, nevertheless, future studies are necessary to deeply explore the mechanisms of platelets activation and aggregation for primary and secondary thrombocytosis generated by the studied effects.

In summary, we found higher platelets counts in animals exercised and submitted to an experimental illumination when compared to control and standard illumination. The primary thrombocytosis was found due to an acute strenuous exercise and the secondary thrombocytosis was found owing the stress caused by the constant red light during dark period for these nocturnal animals. We accepted the initial hypothesis, determining a useful rodent model to induce primary and secondary thrombocytosis without drugs administration and respecting the ethical aspects for animal experimentations, allowing future studies supported

by molecular analysis the view to enhancing the knowledge about this important disease.

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