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HEMATOLOGICAL ALTERATIONS INDUCED BY *Tityus discrepans* SCORPION VENOM IN MICE

Alteraciones Hematológicas Inducidas por el Veneno del Escorpión Tityus discrepans en Ratones

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ABSTRACT

In order to evidence the hematological changes induced by the venom of the scorpion *Tityus discrepans*, a sublethal dose of *Tityus discrepans* venom (Tdv, 1 μg/g) in a total volume of 0.1 mL was intraperitoneally injected in BALB/c female mice (20±2 g; n=20). Mice were anesthetized and blood samples were withdrawn by cardiocentesis at 0 h, 3 h, 6 h, and 12 h after Tdv administration. Hematologic analyses were performed by routine procedures. A significant ($p<0.05$) increase in hematocrit, hemoglobin concentration and total protein concentration was observed at 3 h and 6 h, possibly due to dehydration, splenic contraction and acute-phase protein induction. The red blood cell count in envenomed mice was significantly ($p<0.05$) higher in comparison with the control only at 12 h. *Tityus discrepans* venom caused neutrophilia and lymphopenia probably as a result of catecholamine release, without significant ($p>0.05$) changes in absolute leukocyte count. Neither platelets number nor hematimetric indexes significantly ($p>0.05$) changed. Altogether, these results suggest that Tdv administration induces alterations in the hematologic profile in mice.

RESUMEN

Para evidenciar los cambios hematológicos inducidos por la inyección intraperitoneal de una dosis subletal (1 μg/g) de veneno del escorpión *Tityus discrepans* (VTd), se usaron ratones hembras de la raza BALB/c con un peso de 20±2g (n=20). Los ratones fueron anestesiados y se obtuvieron muestras de sangre por cardiocentesis a las 0 h, 3 h, 6 h y 12 h después de administrado el VTd. Los análisis hematológicos fueron realizados por procedimientos rutinarios. Se observó un aumento significativo ($p<0,05$) en el hematocrito, la concentración de hemoglobina y la concentración de proteínas plasmáticas a las 3 h y 6 h, posiblemente debido a deshidratación, contracción esplénica e inducción de proteínas de fase aguda. El conteo de glóbulos rojos en ratones envenenados fue significativamente ($p<0,05$) mayor en comparación con el control sólo a las 12 h. El VTd causó neutrofilia y linfopenia probablemente debido a la liberación de catecolaminas, sin observarse cambios significativos ($p>0,05$) en el conteo total de leucocitos. Ni el número de plaquetas ni los índices hematimétricos fueron afectados significativamente ($p>0,05$) por la administración de VTd. Considerando los hallazgos, la administración de VTd induce alteraciones en el perfil hematológico en ratones.

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(**Key words**: Scorpion venom; toxic substances; hematology; *Tityus discrepans; haematocrit;* blood proteins; psychomotor agitation; mice)

(**Palabras clave**: Veneno de escorpión; sustancias tóxicas; hematología; *Tityus discrepans;* hematocrito; proteínas sanguíneas; agitación psicomotora; ratón)

Introduction

Tityus discrepans stings are responsible for severe and potentially lethal envenomations in the North Central region of Venezuela [1]. Clinical and experimental studies have shown that victims of *T. discrepans* stings could develop several disturbances, including pain in the site of the sting, nausea, vomiting, syalorrhea, abdominal pain, sweating, pallor, priapism, diarrhea, hypotension or hypertension, coagulation disorders, acute pancreatitis, and cardiopulmonary complications [1-4]. Scorpion venom is a complex mixture of components, containing several neurotoxic peptides that modify the gating mechanisms of sodium channels [5, 6]. These peptides induce a massive release of neurotransmitters such as acetylcholine, noradrenaline and adrenaline, leading to an autonomic dysfunction [7, 8]. Both, the autonomic hyperactivity and the direct effect of scorpion venom on cells of the immune system, contribute to a widespread inflammatory reaction and multiorgan failure [9, 10]. Consecutively, these pathophysiological alterations could induce changes in the hematological parameters. Although the effects of *T. discrepans* venom (Tdv) have been studied at the clinical and pathological level [3, 11], the information about hematological changes induced by this venom in mice is scarce. Therefore, an experimental study was conducted to evidence these hematological alterations in the mouse model.

Materials and Methods

Mice and Venom

BALB/c female mice (20±2 g, n=40) were obtained from the Instituto Venezolano de Investigaciones Científicas (IVIC) and received water and food *ad libitum* up to the day of the experiment, when only access to water was allowed. Adult *T. discrepans* scorpions were collected near San Antonio de Los Altos, the State of Miranda (10°20'N, 67°45'W), and identified according to the criteria of González-Sponga [12]. The scorpions were fed *Gryllodes sigillatus* (Orthoptera, Gryllidae) once a week. *T. discrepans* venom was extracted by manual stimulation of the telsons [13] from 20 scorpions, freeze-dried at 50 mbar and -40 °C, and stored at -80 °C. The venom was resuspended in 0.9 % NaCl, centrifuged at 12,000 x *g* for 10 min to eliminate insoluble matter, and maintained at 4 °C in the dark until use. The protein content was determined in the supernatant according to the method of Lowry *et al.* [14].

Experimental Procedures

All experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals set by the Bioethics Committee of the Facultad de Ciencias Veterinarias, Universidad Central de Venezuela. The mice were randomly assigned to control (n=20) or venom (n=20) groups, with four subgroups (n=5) each, corresponding to the blood sampling arrangement after the administration of the *T. discrepans* scorpion venom (Tdv): 0 h, 3 h, 6 h, and 12 h. Control animals were intraperitoneally injected with 0.1 mL of saline (0.9% NaCl), whereas mice from the venom group were intraperitoneally injected with a sublethal dose of Tdv (1 μg/g) in a total volume of 0.1 mL [15]. At the corresponding time, the mice were anesthetized with dietylether for 3 min, and blood samples were withdrawn by cardiocentesis and rapidly stored in EDTA-containing tubes. A small aliquot was used for blood smears.

Hematological Parameters and Total Protein Determination

Hematological parameters were determined according to routine methods [16]. Briefly, hematocrit was determined using the microhematocrit method and a MB® microcentrifuge. Red blood cells count was performed in blood samples diluted in saline (0.85% NaCl) in a Hayem's pipette, and

counted in a Neubauer chamber placed in an Olympus CX41® microscope. For those samples with hemoconcentration, additional dilutions were necessary. Hemoglobin was determined through the cyanmethemoglobin method, measuring absorbance at 540 nm in a Leitz Model M® photometer, and total protein concentration was measured by refractometry [16]. White blood cells count was performed in blood samples that were diluted in a 1:20 Turk's solution and counted in a Neubauer chamber, as previously described. Platelet number was determined using the Diagnopette® system. The hematimetric indexes were determined through routine calculations and the blood smears were stained with Hemacolor®.

Statistical Analysis

Results are shown as the arithmetic mean ± standard error of the mean (mean ± SEM) and comparisons between treatments were analyzed using the unpaired Student's t test or ANOVA, followed by the Bonferroni *post hoc* test (Graphpad Prism®). The results were considered statistically significant when $p \leq 0.05$.

Results

Mice injected with *T. discrepans* scorpion venom (Tdv) showed moderate agitation, piloerection, diarrhea, neuromuscular alterations, and priapism.

Hematocrit, Red Blood Cell Count (RBC), Hemoglobin, and Total Protein Concentration

As shown in Figure 1 A, the hematologic analysis showed a significant ($p<0.001$) increase in the hematocrit over the control values at 3 h and 6 h (Tdv: 59.40±1.43 % *vs.* control: 48.60±0.40%; Tdv: 59.40±1.91 % *vs.* control: 49.40±0.51 %, respectively). The hematocrit values returned to basal levels at 12 h. The RBC count did not significantly change ($p>0.05$) at 3 h and 6 h, as shown in Figure 1 B. However, the RBC count significantly increased ($p<0.05$) at 12 h when compared with the control (Tdv: 12.57±2.02 x 10^6 RBC/mm^3 *vs.* control: 7.58±1.51 x 10^6 RBC/mm^3). Figure 1 C shows that hemoglobin concentration at 3 h and 6 h also significantly ($p<0.01$) increased (Tdv: 16.50±0.76 g/dL *vs.* control: 14.50±0.21 g/dL; Tdv: 16.82±0.18 g/dL *vs.* control: 14.66±0.27 g/dL, respectively), returning to control values at 12 h. Figure 1 D shows that total protein concentration significantly increased ($p<0.05$) at 3 h in comparison with the control (Tdv: 5.20±0.14 g/dL *vs.* control: 4.44±0.14 g/dL).

Hematimetric Indexes

The hematimetric indexes in envenomed mice did not significantly change ($p>0.05$) in comparison with the control (Table 1).

Total and Differential White Blood Cell (WBC) Counts

Figure 2 A shows that envenomed mice did not show total WBC count changes ($p>0.05$). However, these mice did show significant ($p<0.05$) changes in the differential WBC count. Neutrophilia and lymphopenia were observed at 3 h (Figure 2 C, polymorphonuclears; Tdv: 73.80±2.15% *vs.* control: 21.40±4.12 %; lymphocytes; Tdv: 25.40±2.31 % *vs.* control: 78.60±4.12%), 6h (polymorphonuclears; Tdv: 53.20±4.64% *vs.* control: 24.00±3.95%; lymphocytes; Tdv: 46.40±4.45% *vs.* control: 74.80±3.93%), and 12h (polymorphonuclears; Tdv: 57.40±5.30% *vs.* control: 33.20±7.12%; lymphocytes; Tdv: 42.60±5.30% *vs.* control: 70.80±3.61%).

Number of Circulating Platelets

The number of circulating platelets in envenomed mice did not significantly change in comparison with the control ($p>0.05$), as shown in Figure 3.

Discussion

Mice injected with Tdv showed hematological alterations, which could be related to concomitant and complex pathophysiological mechanisms induced by the different components of the venom [11, 17]. Firstly, the increase in hematocrit, hemoglobin and total protein may derive from scorpion venom-induced hemoconcentration, which could be partly a consequence of dehydration. In this regard, Ribeiro *et al.* [18] showed an increase in hemoglobin and hematocrit in dogs injected with *T. serrulatus* venom (Tsv), and these authors suggested that spleen contraction induced by pain and catecholamine release could be an important factor involved. They also showed an increase in RBC count after Tsv injection in three out of six

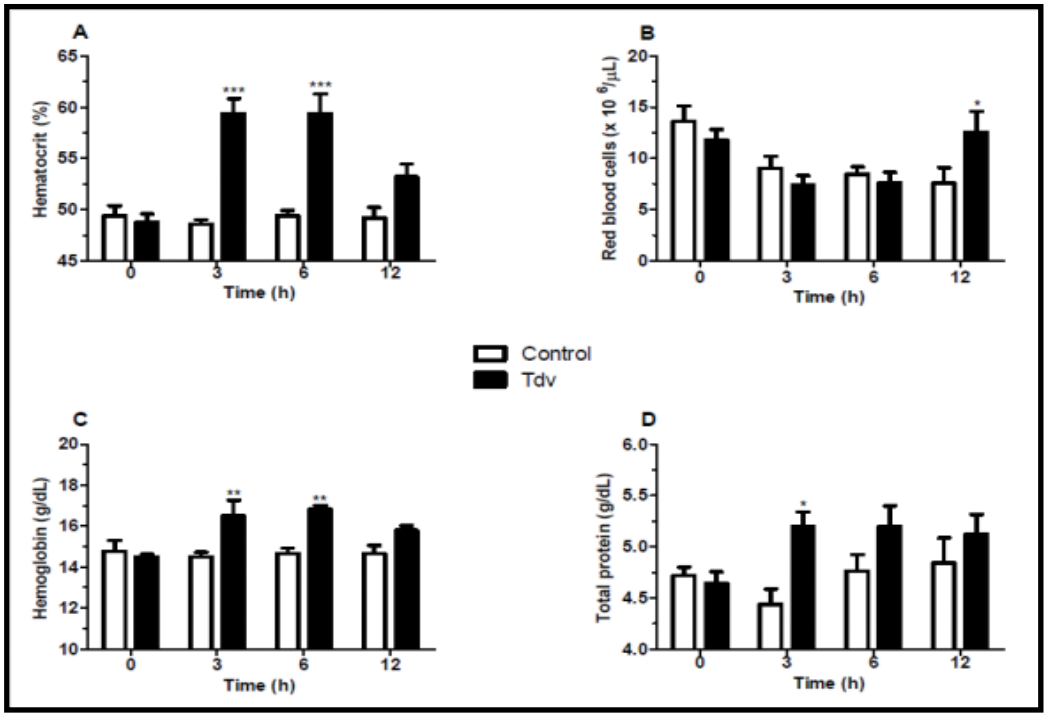

Figure 1. Effect of *Tityus discrepans* scorpion venom on (A) hematocrit, (B) red blood cell (RBC) count, (C) hemoglobin, and (D) total protein in BALB/c mice. BALB/c female mice were intraperitoneally injected with *T. discrepans* venom (Tdv); blood samples were withdrawn at 0 h, 3 h, 6 h, and 12 h, and analyzed as described in Materials and Methods. The values correspond to arithmetic mean ± standard error of the mean (SEM) of five (5) animals (n=5). Significant differences in comparison to the control: $^{***}p<0.001$

Table 1. Effect of *Tityus discrepans* scorpion venom on hematimetric indexes in BALB/c mice. Hematocrit, red blood cell (RBC) count, and hemoglobin values were used to calculate the hematimetric indexes. MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Tdv: *T. discrepans* venom (1 µg/g). The values correspond to the arithmetic mean ± standard error of the mean (SEM) of five (5) animals (n=5). There were no significant differences (p>0.05)

GROUP (TIME)	MCV (µm³)	MHC (pg)	MCHC (g/dL)
Control (0 h)	38.12±4.47	11.43±1.48	29.88±0.85
Tdv (0 h)	42.67±3.81	12.67±1.11	29.74±0.34
Control (3 h)	58.71±9.34	17.41±2.59	29.86±0.52
Tdv (3 h)	87.37±15.08	24.04±3.82	27.70±0.74
Control (6 h)	60.20±4.65	17.94±1.66	29.68±0.72
Tdv (6 h)	83.71±10.74	23.61±2.78	28.40±0.67
Control (12 h)	75.96±14.50	22.82±4.57	29.78±0.47
Tdv (12 h)	49.08±10.71	14.66±3.35	29.74±0.46

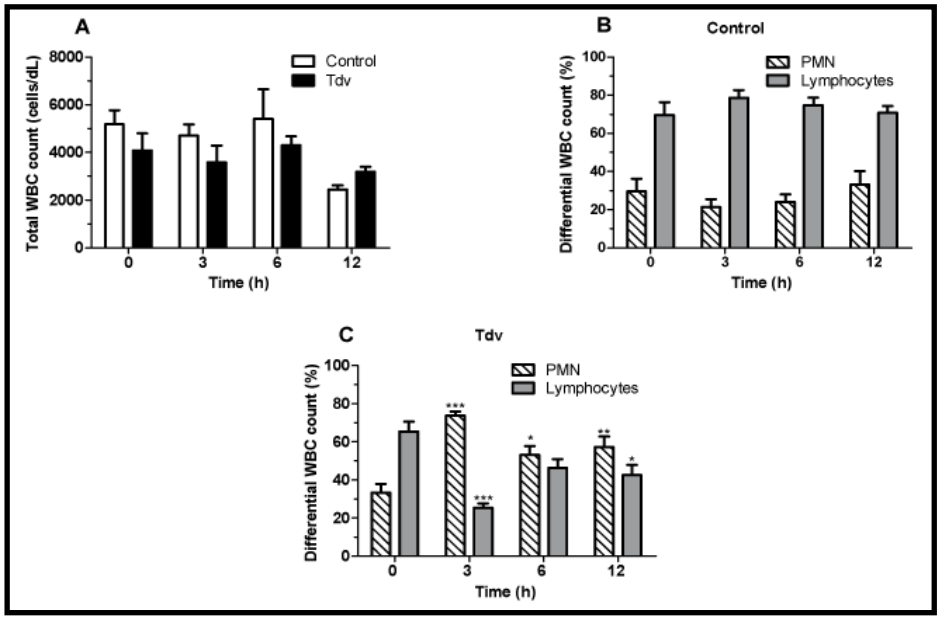

Figure 2. Effect of *Tityus discrepans* scorpion venom on total (A) and differential (C) white blood cell count (WBC) in BALB/c mice. BALB/c female mice were intraperitoneally injected with *T. discrepans* venom (Tdv), blood samples were withdrawn at 0 h, 3 h, 6 h, and 12 h and analyzed as described in Materials and Methods. The values correspond to the arithmetic mean ± standard error of the mean (SEM) of five (5) animals (n= 5). Significant differences in comparison to the control (B): *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

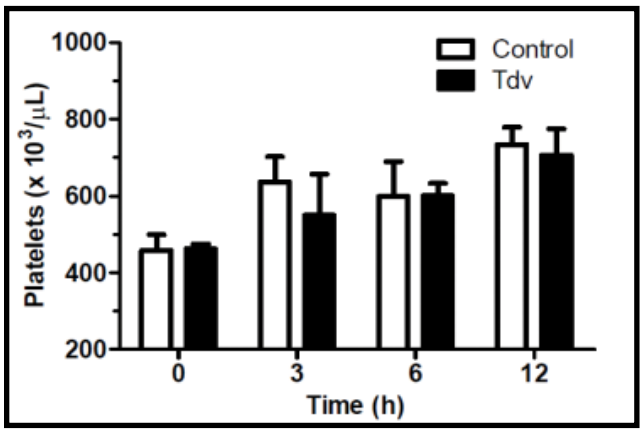

Figure 3. Effect of *Tityus discrepans* scorpion venom on the number of circulating platelets in BALB/c mice. BALB/c female mice were intraperitoneally injected with *T. discrepans* venom (Tdv), blood samples were withdrawn at 0 h, 3 h, 6 h, and 12 h, and analyzed as described in Materials and Methods. The values correspond to the arithmetic mean ± standard error of mean (SEM) of five (5) animals (n = 5). There were no significant differences ($p > 0.05$)

dogs. Moreover, in another study, an increase in hematocrit, RBC count, hemoglobin concentration, and total protein concentration in rats injected with Tsv was shown [19]. Similarly, in our study, the RBC count in envenomed mice at 12 h was higher in comparison with the control. Diarrhea is a common consequence of Tdv injection in mice, and can be considered as an important cause of dehydration and hemoconcentration. Increased vascular permeability, salivation, urinary volume, and lacrimation are also responsible for the severe dehydration observed in scorpion envenomation [20].

In agreement with Ribeiro *et al.* [18], the hematimetric indexes did not significantly change, which might suggest that cell number and water content of blood shifted concomitantly during the course of the experiment. The significant increase of total protein concentration argues in favor of the development of hemoconcentration. Although it was not determined, the rise in total protein concentration may also result from an increase in the synthesis of acute-phase proteins, induced during the exacerbation of the inflammatory response generated by the scorpion venom [10, 20].

Although leukocytosis was not evidenced, neutrophilia and lymphopenia did occur, as has been observed in mice under the effect of *Androctonus australis* venom [21]. A transient lymphocytosis (30 min) followed by lymphopenia and neutrophilia (2-4 h) after catecholamine administration in humans, has been shown [22]. Therefore, it is possible that the relative changes in the number of circulating leukocytes reported herein could result, at least in part, from the massive release of catecholamines [23]. Furthermore, it has been suggested that mice and

rats injected with Tsv develop leukocytosis probably as a consequence of recruitment of neutrophils from bone marrow or demargination [20, 24], and some complement factors such as C3a and C5a could be involved in this process [25]. In our study, neutrophilia and lymphopenia were observed even at 12 h, which suggest an alteration of slow resolution, as has been noticed by Pessini *et al*. [20], who showed neutrophilia even 168 h after Tsv injection in mice.

The number of circulating platelets did not significantly change in envenomed mice in comparison with the control, which was an unexpected result. Concerning this, it has been shown that adrenaline increases the number of circulating platelets [26] and potentiates the ADP- or collagen-induced aggregation [27]. Since Tdv increases adrenaline in plasma [28] and also activates platelets directly [29], then the possibility that these effects could affect the number of circulating platelets, due to changes in platelet aggregability during envenomation by Tdv, should be explored in future studies.

In summary, the experimental administration of a sublethal dose (1 μg/g) of Tdv in female mice clearly developed a transient acute envenomation, characterized by hematological changes related to hemoconcentration and stress neutrophilia. This envenomation worsened up to 6 h and was followed by a fast resolution, since some of the evaluated parameters returned towards control values at 12 h. Whether different hematological alterations will be observed with a different dose of Tdv, requires further study.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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