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BRIEF COMMUNICATION

Ascaris lumbricoides: ALTERATION OF THE ERYTHROCYTE SUPERFICIAL CHARGE USING THE PARTITION METHOD IN AQUEOUS TWO-PHASE SYSTEM

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SUMMARY

Sialic acid is responsible for the negative charge of the erythrocyte. The decrease of sialic acid has hemodynamical and hemorheological importance. The aim was to study the effect of *A. lumbricoides* on the erythrocyte superficial charge using the Partition Method in aqueous two-phase system in order to indirectly evaluate the alteration of sialic acid in the red cells.

We worked with five parasite extracts (AE) and larvae concentrate (LC). Erythrocyte superficial charge was studied by working with non-treated (Controls) and treated erythrocytes. The treatment consisted of incubating the erythrocytes with AE or LC for 30 minutes at 4 ºC, 20 ºC and 37 ºC. The red cells were separated in a sensitive charge two-phase system (Dx/ PEG). The partition coefficient (P) of treated and untreated erythrocytes were calculated. The results showed a P decrease at the three temperatures for red cells treated with four of the AE. The remaining extract did change P values at any of the temperatures studied. The erythrocytes treated with LC showed a decrease in the P value at 37 ºC and 4 ºC but no change was observed at 25 ºC. Statistical analysis concluded that P values were significantly lower in treated erythrocytes than in their corresponding untreated ones ($p < 0.05$). The Partition Method showed that this parasite alters the erythrocyte superficial charge which may indicate that it can catch sialic acid.

KEYWORDS: *Ascaris lumbricoides;* Erythrocyte charge; Partition method.

Sialic acid is responsible for the negative charge of the erythrocyte membrane and contributes to the structural properties of the glycophorines. A decrease in the contents of sialic acid in the membrane promotes erythrocyte aggregation, rouleaux formation, decrease in the blood flow and increase in blood viscosity, which results in a greater interaction between erythrocytes and vascular endothelium[8]. Cellular mechanisms of many infectious processes are not known with accuracy and they may involve host and parasite surface glycoconjugates. *Trypanosoma cruzi* takes sialic acid from the host glycoconjugates and transfers it to molecules on its surface. Taking hold of sialic acid makes *T. cruzi* resistant to the attack of complement proteins and antibodies of cytolitic capacity[5]. In trypanosomiasis caused by *T. congolense*, *T. brucei* and *T. evansi* the adhesion phenomenon of the parasites to erythrocytes and endothelial cells is mediated by structures which contain sialic acid residues[2,4,9]. The existence of invasion ways through acid sialic receptors in *Plasmodium falciparum* has been communicated[7]. The occurrence of sialic acid in different parasite infections with endocellular replication has been reported but there are no reports of its role in *Ascaris lumbricoides*. The aim was to study the effect of *A. lumbricoides* on the erythrocyte superficial charge using the Partition Method (PM) in aqueous two-phase system in order to indirectly evaluate the alteration of sialic acid in the red cells.

Five parasite extracts (AE) and a larvae concentrated (LC) (500-600 larvae/mL) were used. AE were obtained by surgical remotion of the cuticle from adult worms[13] and LC was obtained from eggs eclosion[6] and larvae collection by Baerman Morales Method[16]. Erythrocyte superficial charge was studied by working with non -treated human erythrocytes (controls) and treated (T) ones, both washed in phosphate buffer saline (PBS) and resuspended to a 75% hematocrit. The red cells were obtained from normal individuals O Group. The treatment consisted of incubating the erythrocytes with AE or LC for 30 minutes at 4 ºC, 20 ºC and 37 ºC. Then, they were washed three times with PBS. The red cells were separated in an aqueous system containing 5% (w/w) Dx and 4 % (w/w) PEG in PBS, pH 7.4. The erythrocytes are retained of the upper phase according to the negative superficial charge[1,15]. The partition coefficient (P) is defined as the number of cells in the upper phase with respect to the totality of added cells. P of untreated erythrocytes was compared to the P obtained from the same treated red cells. The P values were simultaneously calculated in treated and untreated erythrocytes. Fifteen measurements were made for the 5 AE at the three temperatures and six measurements were made for LC which was incubated in two erythrocyte suspensions at each temperature.

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25 ºC:	PNT = 0.91 / PT $[EA]_1$ = 0.15	4ºC:	PNT = 1 / PT $[EA]_1$ = 0.31	37 ºC:	PNT = 1 / PT $[EA]_1$ = 0.60
	PNT = 0.91 / PT $[EA]_2$ = 0.08		PNT = 0.92 / PT $[EA]_2$ = 0.56		PNT = 1 / PT $[EA]_2$ = 0.43
	PNT = 1 / PT $[EA]_3$ = 0.27		PNT = 0.92 / PT $[EA]_3$ = 0.17		PNT = 1 / PT $[EA]_3$ = 0.17
	PNT = 0.91 / PT $[EA]_4$ = 0.50		PNT = 0.92 / PT $[EA]_4$ = 0.18		PNT = 1 / PT $[EA]_4$ = 0.59
	PNT = 1 / PT $[EA]_5$ = 0.97		PNT = 0.96 / PT $[EA]_5$ = 0.90		PNT = 0.90 / PT $[EA]_5$ = 0.90
	PNT = 0.95 / PT CL = 0.95		PNT = 1 / PT CL = 0.02		PNT = 0.98 / PT CL = 0.01
	PNT = 0.98 / PT CL = 0.98		PNT = 1/ PT CL = 0.01		PNT = 0.98 / PT CL = 0.02

PNT: value of the partition coefficient of non- treated erythrocytes; PT: value of the partition coefficient of treated erythrocytes

The results showed a decrease in P value at the three temperatures for erythrocytes treated with four of the five AE. The remaining AE did not modify P values at any of the temperatures assayed. The erythrocytes treated with LC showed a decrease in P value at 37 ºC and 4 ºC. No change was observed at 25 ºC. The observed P values were lower in red cells treated with LC than in erythrocytes treated with AE.

In order to compare PT values and their respective PNT, Wilcoxon Test[17] was used in paired samples for each temperature. The results of statistical analysis concluded that P values were significantly lower in treated erythrocytes than in their corresponding untreated ones ($p < 0.05$). At 4 ºC PNT median was 0.96 and the interquartile range was 0.08 while PT values were 0.18 and 0.54, respectively. At 25 ºC, the P median for the controls was 0.95 and the interquartile range 0.09, while PT were 0.50 and 0.82, respectively. At 37 ºC, PNT median was 1 and the interquartile range 0.02, while PT values were 0.43 and 0.58, respectively. P values showed a greater variation in treated red cells than in untreated ones at the three temperatures.

PM is a simple technique used to separate cells having slightly different surface properties and to characterize biologically important molecules[1,15]. Authors of this experience have used PM to study changes in erythrocyte superficial charge[8,10,11,12,14]. The results have shown that *A. lumbricoides* can alter the erythrocyte superficial charge which may indicate that it can catch sialic acid both in its adult and larval stages. The larvae stages circulate into the blood stream. The adult stage, even if it is located in intestine, might have contact with blood when it pierces the intestinal wall or penetrates into ducts and veins[3]. This finding could be significant to explain the evading mechanism of the host´s immune response.

RESUMEN

Ascaris lumbricoides: alteración de la carga superficial eritrocitaria usando el Método de Partición en sistemas bifásicos acuosos

La disminución de ácido sialico, responsable de la carga negativa del eritrocito, tiene importancia hemodinámica y hemorreológica. El objetivo fue estudiar el efecto de *A. lumbricoides* sobre la carga superficial eritrocitaria aplicando el método de partición en sistemas bifásicos acuosos, a los fines de evaluar de manera indirecta la alteración de acido sialico de los eritrocitos. Se trabajó con 5 extractos del parásito adulto (EA) y con un concentrado de larvas (500-600 larvas/mL) (CL). Se estudió la carga superficial eritrocitaria, trabajando con eritrocitos no tratados y tratados. El tratamiento consistió en la incubación de los eritrocitos con EA o CL durante 30 minutos a 4 ºC, 25 ºC y 37 ºC. Los eritrocitos fueron sometidos a la separación en un sistema bifásico carga sensible constituido por Dx / PEG. Se calculó el coeficiente de reparto (P), de los eritrocitos sin tratar y tratados. Los resultados mostraron disminución de P a las 3 temperaturas, en hematíes tratados con 4 de los EA. El EA restante no modificó los valores de P a ninguna de las temperaturas estudiadas. CL produjo la disminución de P a 37 ºC y 4 ºC, pero no se observó modificación a 25 ºC. Los análisis estadísticos concluyeron que los valores de P son significativamente menores en los eritrocitos tratados que en los respectivos eritrocitos sin tratar ($p < 0.05$). El método de partición demostró que *A. lumbricoides* altera la carga superficial eritrocitaria lo que indicaría que el parásito, tanto en su estado adulto como en sus fases larvales, puede captar acido sialico.

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