Aminoquinolone WR6026 as a feasible substitute for gentian violet in Chagas' disease prophylaxis in preserved blood for transfusional purposes

O emprego da aminoquinolina WR6026 como substituto incolor da violeta de genciana na profilaxia da doença de Chagas transfusional

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Abstract *The search for a colorless, nontoxic and efficient drug to prevent transfusion-associated Chagas' disease (TACD) has been underway unsuccessfully since 1953 when gentian violet was preconized and to date is still being used as the only* in vitro *trypanocidal agent. The recent findings of aminoquinolone "WR6026" as a trypanocidal agent, led the authors to study the metabolism of red cells stored with this compound, the main objective of which was to define its applicability in TACD control. Ten units of human whole blood collected in CPDA-1 were divided into two equal satellite bags. One had "WR6026" (final concentration 62.5µg/mL) added and the other was used as a control, both were stored at 4ºC. At baseline, day 7, 14, 21 and 28, samples were taken for the following measurements: adenosine triphosphate (ATP), hemoglobin, electrolytes (sodium and potassium), gases (pO_2 and pCO_2) and osmotic fragility. The results of tests and control were analyzed through parametric t-student test. The results were similar in both groups throughout the experiment except for the level of ATP on day 14, which presented significantly higher values in the tests when compared with the controls (p = 0.012). It was concluded that WR6026 does not interfere in the preservation and probably the viability of the erythrocytes also until day 28 of storage. Consequently the authors suggest that WR6026 could emerge as a colorless substitute for gentian violet in the control of TACD in endemic areas.*

Key-words: *Transfusion-associated Chagas' disease. Chemoprophylaxis. Aminoquinolone. Blood transfusion.*

Resumo *A inexistência de uma droga eficiente e incolor para esterilizar* in vitro *o sangue chagásico faz com que a violeta de genciana continue sendo desde 1953 a única substância empregada no controle da doença de Chagas transfusional. A recente descoberta da ação tripanosomicida da aminoquinolina WR 6026, estimulou-nos a pesquisar eventuais efeitos deletérios deste sal sobre o eritrócito preservado, com o objetivo se avaliar a conveniência de seu emprego no controle da doença de Chagas transfusional. Dez unidades de sangue total foram coletadas em bolsas duplas CPD-A1 e divididas em volumes iguais. Em uma das bolsas foram colocados 62.5µg/mL de WR 6026 e ambas as bolsas estocadas a 4ºC. À zero hora, dos dias 7,14, 21 e 28 amostras foram retiradas de cada bolsa com e sem WR6026 e submetidas aos seguintes testes: dosagem de ATP, hemoglobina, eletrólitos (Na e K), gases (pO2 e pCO2) e curva de fragilidade osmótica. Os resultados foram similares nos dois grupos, com exceção do ATP que, no grupo teste, apresentou valores significamente maiores que os do grupo controle no 14º dia de estocagem. Os resultados permitem concluir que o WR6026 não interfere na preservação e possivelmente na viabilidade do eritrócito durante a estocagem normal, sugerindo que este sal pode vir a ser o substituto incolor da violeta de genciana, no controle da doença de Chagas transfusional.*

Palavras-chaves: *Doença de Chagas associada à transfusão. Quimioprofilaxia. Aminoquinolina. Transfusão de sangue.*

Chagas' disease (American trypanosomiasis) is a zoonosis caused by the flagellate protozoan parasite *Trypanosoma cruzi* and affects approximately 18 million people in Central and South America[32 49]. It is estimated that up to 60 percent of these infected individuals have migrated to urban areas in both endemic and non-endemic countries and that approximately 100,000 *T. cruzi*-infected individuals are now living in North America, where three cases of transfusion-associated Chagas' disease (TACD) have been recently notified in immunocompromised patients[22 24 32 38 49].

The prevalence of *T. cruzi* infection among Latin American blood donors during the early 1980's and 1990's was approximately 7 and 3%, respectively[29 30 36 49]. Nowadays, this prevalence has fallen to nearly one percent[25 36]. Despite a gradual reduction in the seroprevalence of antibodies to *T. cruzi* in blood donors in regions such as Brazil, Uruguay and Guatemala, there has reportedly been a steady prevalence in other countries, for examples Bolivia, Argentina (north region), Paraguay and Mexico[9 15 25 29 32 37 49 50]. With the eradication in some countries of the blood-sucking triatomine insects that transmit *T. cruzi*, allogeneic blood transfusion has become the main mechanism underlying the continuity of the endemic disease[49 50].

Current strategies to prevent TACD include identification of potentially infected blood donors by questionnaire and serological screening tests, treatment of collected blood with gentian violet (GV) and also alternative prophylactic measures, such as filtration and irradiation of blood products[2 21 31-33 36 40 42 44].

Although having shown some promising initial results, photo irradiation (ultraviolet A light and gamma-irradiation), did not demonstrate satisfactory data in further studies[1 23 33 51]. Leukodepletion, using leukocyte filters, has reduced *T. cruzi* concentration in infected blood, but was not able to completely eliminate all the blood trypomastigotes[31 32].

The application of a questionnaire prior to donation to identify individuals who might be infected with *T. cruzi* has been done with success in nonendemic areas, but may significantly reduce the blood supply in endemic areas[2 19 32]. Although there are more than ten available methods, serology can present conflicting results. Hence, strict quality control procedures are needed to rule out false results[27 32 34 46].

In areas of high endemicity or in regions where serologic screening is not feasible, the risk of TACD may be reduced by the addition of GV in the collected blood[40 44 49]. However, many restrictions have been made to the use of GV mainly due to its violet color; and possible mutagenic action[18]. Consequently, in the last four decades, over a thousand different drugs or natural products have been studied in order to identify a colorless, nontoxic, and efficient drug to prevent TACD that could eventually substitute gentian violet, however with no success up until now[11 36 41].

An aminoquinolone, Walter Reed n⁰ 6026 (WR6026) developed by the Walter Reed Institute (U.S.A) is a promising drug for visceral leishmaniosis treatment[48] and has also been tested by a group of Brazilian scientists that reported its *in vitro* action at 62.5µg/mL against bloodstream forms of *T. cruzi*[10]. More recently, another group of Brazilian researchers showed that this same salt tested at different concentrations (ranging from 1 to 3.5mg/kg/day) for a period of 28 days had an increasing efficacy up to 2mg/kg/day after which it decreased with higher doses[16].

In view of these studies, the authors considered it would be useful to investigate a hypothetical effect of WR6026 on red cell metabolism, in order to determine its applicability against TACD.

MATERIAL AND METHODS

Ten units of human whole blood were collected from ten normal donors (Hemocentro Regional de Uberaba) in double bags with citrate-phosphate-dextrose-adenine (CPDA-1) (Baxter, USA). After blood collection, 200mL of whole blood were transferred to the satellite bag (control). To the 300mL remaining 18.75mg of WR6026 was added, with a final concentration of 62.5µg/mL (test). Both bags were stored at 4ºC, and at day zero, 7, 14, 21 and 28 samples were taken for the same determinations utilized in the previous study of metabolism and preservation of the erythrocyte added with GV[35]. These were:

a) adenosine triphosphate (ATP): measured in duplicate by Beutler's method (1975)[6].

b) hemoglobin: by automated method using a Coulter T-890.

c) electrolytes (sodium and potassium), pH and gases (pO_2 and pCO_2): automatic analyzer model IL1640 pH/blood gas/electrolytes (Instrumentation Laboratory SA - Milan, Italy).

d) osmotic fragility: using Dacie and Lewis' technique (1975)[12].

Statistical analysis. The continuous numeric variables showed a normal distribution and were expressed as mean ± S.D. Parametric T-student test was employed to compare the samples of control and those treated with WR6026 at each time of the experiment. Results were considered significant when $p < 0.05$[39].

RESULTS

The results are summarized in Tables 1 and 2 and Figures 1 to 3. To compare these results with the authors' previous study, as mentioned above, they were grouped as follows: organic phosphates (Table 1) and, since the

values of sodium, potassium, pH, pO_2, pCO_2 and hemoglobin were uniform and without statistical significance, they were presented as mean values in a single table (Table 2). Osmotic fragility was presented in Figures 1 to 3.

Organic phosphate. *Adenosine triphosphate (ATP)*: a gradual decrease on the ATP values was observed during the time of storage between both groups (control and test). However, on day 14, the ATP figures were significantly higher in samples added with the compound (Test) ($p = 0.012$) (Table 1).

Electrolytes. *Potassium (K):* this electrolyte showed a gradual increase during storage in both groups. Comparison between the two groups showed no significant differences with similar means throughout the experiment (Table 2).

Table 1 - *Adenosine triphosphate in µM/g Hb on fresh (time zero) and stored blood (day 7, 14, 21 and 28), collected in CPD-A1, treated with WR6026 (Test) and without WR6026 (control).*

Sample	Baseline		Day 7		Day 14		Day 21		Day 28	
	C	T	C	T	C	T	C	T	C	T
1	6.539	6.80	6.317	6.60	5.705	6.015	5.012	4.785	2.890	2.776
2	6.387	6.610	6.170	6.386	5.525	6.333	4.980	5.012	2.228	3.319
3	6.363	6.420	6.147	6.202	5.580	5.950	4.875	4.625	2.510	3.012
4	6.428	6.459	6.210	6.240	5.690	5.985	5.015	4.530	3.280	3.152
5	6.573	6.138	6.350	5.930	5.820	5.765	4.680	4.120	3.255	2.830
6	6.150	5.988	5.970	5.735	5.235	5.420	NP	NP	NP	NP
7	6.330	6.260	6.120	6.035	5.575	5.625	NP	NP	NP	NP
8	6.135	6.920	5.930	6.800	5.390	6.585	NP	NP	NP	NP
9	5.950	5.997	5.715	5.758	5.208	5.376	NP	NP	NP	NP
10	6.280	6.420	6.035	6.280	5.480	5.895	NP	NP	NP	NP
Mean	6.314	6.369	6.103	6.163	5.521	5.895*	4.912	4.614	2.833	3.018
Stand. Deviation	0.192	0.286	0.201	0.317	0.199	0.377	0.142	0.331	0.461	0.225

C = control; T = test; NP = not performed

* $p = 0.012$

Table 2 - *Mean values of potassium, sodium, pH, partial pressure of oxygen, partial pressure of carbon dioxide and hemoglobin, on fresh blood (baseline) and stored (day 7, 14, 21 and 28), collected in CPD-A1 and treated with WR6026 (Test) and without WR6026 (Control).*

Parameter	Baseline		Day 7		Day 14		Day 21		Day 28	
	C	T	C	T	C	T	C	T	C	T
Potassium	3.48	3.72	10.75	11.72	16.06	16.56	18.40	18.10	23.42	24.14
Sodium (mEq/L)	151.6	150.6	145.8	144.2	143.8	142.7	137.6	135.8	140.4	137.0
pH	6.995	6.980	6.741	6.736	6.591	6.584	6.498	6.526	6.398	6.412
pO2 (mmHg)	30.5	29.3	35.3	27.1	39.5	36.3	49.6	51.4	60.6	55.2
PCO2 (mmHg)	83.6	86.6	132.1	135.3	146.7	149.0	155.2	143.3	129.6	116.4
Hemoglobin (g%)	14.2	15.12	15.02	15.48	14.52	13.42	12.8	11.80	12.24	12.36

C = control; T = test

Sodium (Na): The mean Na values presented a slight decrease during storage. On comparing the two groups, slightly lower levels were observed, although these were not statistically significant, between the samples with and without added WR6026 (Table 2).

pH, Gases and Hemoglobin. *pH:* the pH presented a discreet and gradual decrease during storage. Both groups showed a similar behavior during the entire experiment (Table 2).

Partial pressure of oxygen (pO₂): in both groups pO_2 presented gradual increase reaching duplicate values at the end of week 4 of storage. However, there were no differences between the two groups (Table 2).

Partial pressure of carbonic gas (pCO₂): the pCO_2 showed a gradual increase at the end of the 3rd week with a discreet fall on day 28 of storage. Comparison between the two groups did not show any significant difference during the experiment (Table 2).

Hemoglobin: the mean values of hemoglobin presented a discreet and gradual fall throughout the experiment. There were no statistical significant differences between the groups (Test and Control) (Table 2).

Osmotic fragility. Osmotic fragility was tested at concentrations of 0.00 to 0.80% and of sodium chloride (NaCl). Similar levels of hemolysis were presented throughout the experiment in both groups with no evidence of a statistically significant difference. Figures 1 to 3 show the mean values of hemolysis at each dilution, at baseline, day 14 and 28 for both groups (test and control) and also in a standard group at baseline.
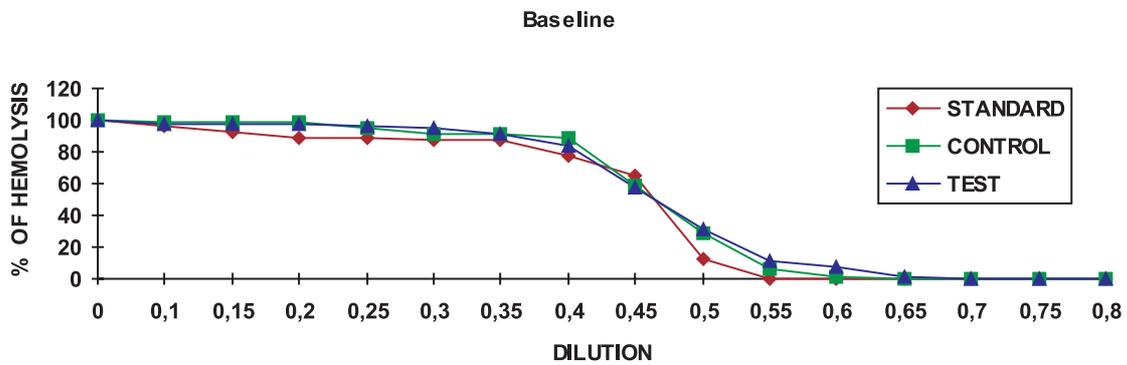
Baseline

Figure 1 - Osmotic fragility - Curve of medium values of hemolysis of fresh blood collected in heparin (standard), stored in CPD-A1 with the compound WR6026 (Test) and without it (control) at baseline.
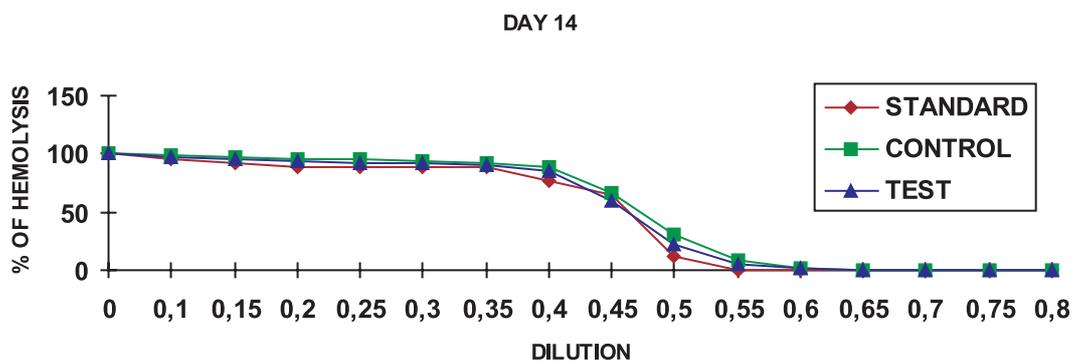
DAY 14

Figure 2- Osmotic fragility - Curve of mean values of hemolysis of fresh blood collected in heparin (standard), stored in CPD-A1 with the compound WR6026 (Test) and without compound (control) on day 14.
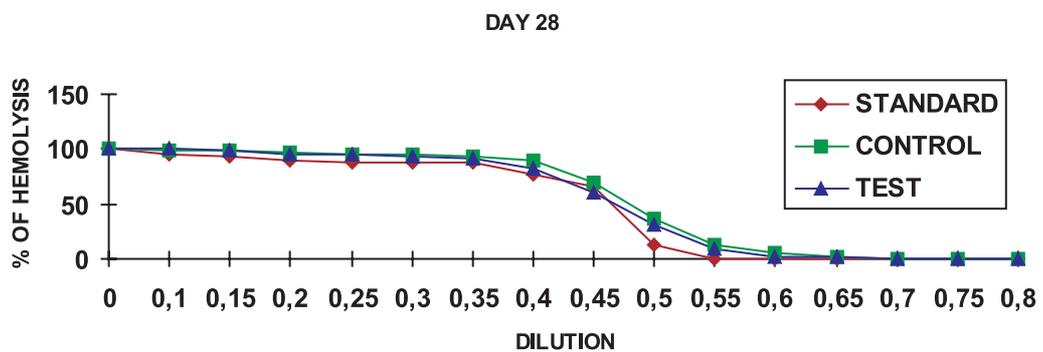
DAY 28

Figure 3 - Osmotic fragility - Curve of mean values of hemolysis of fresh blood collected in heparin (standard), stored in CPD-A1 with the compound WR6026 (Test) and without compound (control) on day 28.

DISCUSSION

As previously mentioned, the current strategies to prevent TACD include the identification of infected blood donors or treatment of collected blood[2 32 37 51].

The lack of a safe and efficient method for the control of TACD and the evidence that WR6026 has been shown to have a strong trypanocidal action, motivated the present study, with the main objective of investigating possible metabolic alterations on the red cells stored for transfusions and with the compound WR6026 added, in order to determine the possibility of its use in TACD control.

The following parameters were studied: ATP, K, Na, pH, pCO_2, pO_2, hemoglobin and osmotic fragility. These parameters have been widely used to investigate red cell preservation and viability in stored blood[8 20 26 37]. Furthermore, the same parameters have been used in a previous study with gentian violet[35]. The relationship between ATP levels and viability of erythrocytes *in vitro* was emphatically defended in 1967, by Dern and cols who did not consider stored erythrocytes with ATP levels below 1.5µM/g Hb to be viable[14]. The present study demonstrated a decrease in the ATP levels, but at the end of week 4, they were approximately two times superior to the lower limits established by the authors, suggesting a good level of erythrocyte viability.

In this study, a variation was observed in potassium that ranged from 1.5 to 8 mEq/L (mean 5mEq/L), with an expected normal increase between 5 to 10mEq/L per week, through blood storage[4 5 6]. Such a result may be considered indirect evidence of a poor hemolysis state in blood storage.

The pH values found in the authors' experiments were slightly inferior to those presented in standard tables regarding component preservation[3]. However, they were similar to those found in the authors' previous studies[5 35]. A possible hypothesis to explain these results is the fact that the analyses were performed at room temperature (24°C), whereas in the literature the same analysis was carried out at a storage temperature of 4°C; a condition which increases pH by approximately five decimeters[6 45].

When studying gases, the experiment showed concordant results in both groups and also with those of previous studies[3 17]. The gradual elevation of pO_2 values is possibly explained by the increase of fixation of O_2 by hemoglobin since there was a 2.3DPG decrease, and also by oxygen diffusion through the bags[20 26 47]. The increased pCO2 level at the end of week 3 is most probably due to the release of HCO^-_3 and its decrease by day 28 may be explained by the permeability of the plastic bags to CO_2, thus allowing its diffusion to the environment[20].

A discreet fall in the mean values of hemoglobin in both groups was noticed. However, the slight potassium increase, similar or inferior to those values reported in the literature, is strong evidence of absence of important hemolysis in the samples. Additionally, the free hemoglobin analyzed in samples up to week 2 was within normal values (data not shown). Furthermore, Chiari and cols[10] did not notice any sign of hemolysis in samples utilized when studying the trypanocidal activity of WR6026[10].

As demonstrated, the osmotic fragility did not present any significant alteration during the four weeks of storage. Based on the studies, which correlate curves of normal fragility to an adequate preservation and good viability of stored blood[13], the present results reinforce the evidence that WR6026 does not interfere with the viability of the erythrocytes.

Based on the absence of significant alterations over the eight parameters analyzed, it was possible to conclude that WR6026 does not interfere with red cell preservation and possibly nor in the viability of the erythrocyte until day 28. Recent studies have showed a slight and moderate increase of methahemoglobin and also nephrotoxicity related to doses and time of utilization of the salt, generally superior to 2mg/kg/day for a period of 28 days[16 28 43 48]. This dosage *in vivo* corresponds to 140mg/kg/day or 3920mg for 28 days for an adult of 70kg. In the authors' studies each blood bag with 300ml of whole blood contained 18.75mg of WR6026, which corresponds to a dose 20 times smaller than that demonstrated to be nephrotoxic. These findings enable the prediction that this compound may become a useful and colorless substitute for gentian violet in the control of TACD.

ACKNOWLEDGEMENTS

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