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Photoinactivation of *Trypanosoma cruzi* in red cell suspensions with thiopyrylium

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Abstract

Chagas disease, endemic in rural areas of Mexico, Central and South America, is caused by the protozoan parasite, *Trypanosoma cruzi*, which is spread by the Reduviid bug and also by transfusion or organ transplant. Transmission of the organism from asymptomatic donors to immunocompromised recipients, leads to clinically apparent disease. With recent immigration patterns, *T. cruzi* is now becoming an increasing problem in non-endemic areas of North America and Europe. Blood screening tests for *T. cruzi* are being developed, and one test is currently licensed by the United States Food and Drug Administration and has been implemented in some U.S. blood centers. This study alternatively investigates the potential for a novel DNA-intercalating photosensitizer, thiopyrylium (TP), to inactivate *T. cruzi* in red cell suspensions. With complete inactivation using 6.3 μM of TP and 1.1 J/cm² red light treatment, results suggest that the organism is highly sensitive to photoinactivation under conditions much less stringent than those that have been previously demonstrated to maintain red cell (RBC) properties during 42 day storage.

Introduction

Trypanosoma cruzi (*T. cruzi*) is a vector-borne protozoan parasite which causes Chagas' disease, affecting 18–20 million people. Infection by the organism is often asymptomatic in immunocompetent individuals; but 10–30% of infected individuals progressively develop heart or intestinal disease decades later. It is estimated that 50,000 infected individual die of Chagas' disease annually. Although the disease was primarily transmitted to humans by the excrement of the Reduviid bug in rural areas of Mexico, Central, and South America, transfusion of infected blood products accounts for a significant proportion of transmissions [1–4]. Since 1987, six cases of transfusion-transmitted *T. cruzi* and five cases of organ-transmitted *T. cruzi* have been reported in North America [5,6]. Blood screening immunoassays for *T. cruzi* are under development [7,8]; one assay has recently been licensed by the FDA and has been implemented in some U.S. blood centers, including the American Red Cross and Blood Systems. From January 29 to March 9, 2007, 139 repeat reactive units were identified with 15 of 81 units confirmed by RIPA (9). Even with the availability of antibody screening, the proportion of seronegative donors who can still transmit the disease is unknown. Two drugs, nifurtimox and benznidazole, are considered effective in treating acutely infected individuals; however, their use is controversial in chronically infected patients and the toxicities of treatment are often a drawback to their use [2].

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Virus inactivation methods for blood have been explored as a means to reduce risk of routinely tested agents and from emerging agents for which no deferral or screening methods are available. Previous studies have demonstrated that a DNA-intercalating flexible photosensitizer, thiopyrylium (TP), has potent activity against many viral and bacterial pathogens as well as the parasite, *Leishmania donovani infantum*, with little alteration of RBC storage properties under phototreatment conditions considerably more harsh than those required for robust pathogen inactivation [10,11]. In this study, we investigated whether TP can photoinactivate *T. cruzi* in RBC suspensions.

Materials and Methods

T. cruzi were cultured at $22 \pm 2^\circ\text{C}$ in Schneider's Drosophila medium (Invitrogen, Carlsbad, CA) supplemented with 30% heat-inactivated fetal calf serum (Invitrogen). Parasites were induced into logarithmic phase by inoculating cultures with organisms to yield inoculums of approximately $2 \times 10^6/\text{mL}$. Stationary phase trypomastigotes, the extracellular form of the organism, were obtained when cultures approached $2 \times 10^7/\text{mL}$, as determined by hemacytometer count.

On the day of each experiment, 50 mL of ABO-identical whole blood was collected from three donors in CPD-containing tubes. One half of one mL of the infected inoculum, containing 1×10^8 trypomastigotes was added to each of three 50 mL donor whole blood samples. Infected red blood cells (RBCs) were prepared by centrifugation ($2700 \times g$ for 10 minutes at 22°C) and removal of platelet poor plasma. Two-mL samples of infected RBCs were subsequently phototreated with TP at a final concentration of 3.1 and 6.3 μM and 1.1 J/cm^2 of 670 nm red light according to a previously described method [10]. Non-illuminated samples without dye served as controls for each donor.

Measurement of parasite titers was performed in triplicate for each experimental condition. Following phototreatment, treated and control samples were 10-fold serially diluted in Schneider's Drosophila medium containing 30% FBS and incubated at $22 \pm 2^\circ\text{C}$ for 28 days, and microscopically examined for growth. Parasite titers were determined by the median tissue culture infective dose method with 50% endpoint [12].

Results and Discussion

Results of the experiment are given in Table 1. Control $\log_{10}$ titers ranged from 4.6–6.6 parasites/mL, with an average of 5.3 ± 0.9 parasites/mL. Samples treated with 3.1 μM TP and 1.1 J/cm^2 light had no organisms present in two of three cultures, with an average $\log_{10}$ titer of 1.3 ± 0.9 CFU/mL. No organisms were recovered in any of the three flasks containing samples treated with 6.3 μM thiopyrylium and 1.1 J/cm^2 light, corresponding to an average $\log_{10}$ titer of 0.6 parasites/mL.

T. cruzi trypomastigotes, which required 6.3 μM of TP and 1.1 J/cm^2 light for complete inactivation, appears to be slightly more sensitive to phototreatment than *Leishmania donovani infantum* amastigotes, which required 12 μM of the dye and 1.1 J/cm^2 light for complete inactivation. Differences between the sensitivities of these similar organisms may be due to greater access of dye to extracellular *T. cruzi* than to intracellular *Leishmania donovani infantum*. Both double stranded DNA parasites are considerably more sensitive to photoinactivation than single stranded RNA viruses, such as HIV, which require 80 μM TP for complete inactivation [10].

Inactivation methods may be useful in reducing the infectivity of *T. cruzi*, but any potential method must also be shown not to damage the blood component, and to be safe for recipients. Inactivation of *T. cruzi* in red cells using a DNA alkylating agent, Inactine, has been reported,

but the development of antibodies to the treated red cells has halted further clinical development [13]. Psoralen and long-wavelength treatment has also been shown to inactivate *T. cruzi* in platelet concentrates and plasma [14]. Methylene blue and light has been demonstrated to photoinactivate *T. cruzi* in plasma [15]. A previous study [10] demonstrated that phototreatment using concentrations of TP 25-times those used in this study maintained most of the in vitro properties, including hemolysis, ATP, lactate, glucose, pH and morphology, of treated red cells during storage and that freshly infused canine red cells phototreated with 160 μ M TP had similar 24 hour recovery and survival as untreated cells [16]. However, much work remains to develop a practical method for RBC photoinactivation by TP. There will be a need to demonstrate adequate 24 hour in vivo recovery following infusion of treated and stored human RBC suspensions, to assess whether phototreated red cells are immunogenic, and to conduct the myriad of studies necessary to demonstrate an adequate safety margin for recipients of this compound.

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Table 1

T. cruzi culture results in control and phototreated red cells

Tube Serial Dilution	Untreated			3.2 μ M TP + light			6.3 μ M TP + light		
	1	2	3	1	2	3	1	2	3
0	+	+	+	+	-	-	-	-	-
1	+	+	+	+	-	-	-	-	-
2	+	+	+	-	-	-	-	-	-
3	+	+	+	-	-	-	-	-	-
4	-	+	-	-	-	-	-	-	-
5	-	+	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-