

NOTA PRÉVIA

TREATMENT OF *T. CRUZI* INFECTED HUMAN PLATELET CONCENTRATES WITH AMINOMETHYLTRIMETHYL PSORALEN (AMT) AND ULTRAVIOLET A (UV-A) LIGHT: PRELIMINARY RESULTS

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The present measures adopted to prevent transfusion-associated Chagas' disease include screening of blood donors, and/or the inactivation of T. cruzi in collected blood using gentian violet (GV), as a trypanocidal agent. In this study, we investigated the efficacy of the combined use of AMT and UV-A in inactivating T. cruzi in infected human platelet concentrates. Human platelet concentrates were infected with T. cruzi (2x10⁸/ml) of the Y strain, transferred to PL 269 (Fenwal Laboratories) containers, and treated with GV (250µg/ml), and ascorbic acid (1mg/ml); GV, ascorbic acid and UV-A; GV and UV-A; AMT (40µg/ml) and ascorbic acid; AMT, ascorbic acid and UV-A; AMT and UV-A; UV-A alone; and untreated (control). All UV-A treated platelet concentrates were exposed to UV-A doses of 24, 92, 184, 276, 368 and 644 kJ/m², and the microscopical research of active T. cruzi was performed, using the microhematocrit technique, 1, 6 and 24 hours after each treatment. A high number of active forms of T. cruzi was observed in all conditions, except when GV was used as the trypanocidal agent, providing evidence of the failure of AMT and UV-A in inactivating T. cruzi in infected human platelet concentrates.

Key-words: Transfusion-associated Chagas' disease. Photodynamic treatment. UV-A and Chagas' disease. Chemoprophylaxis. Blood transfusion.

American trypanosomiasis (Chagas' disease) is a zoonosis caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). It has been estimated that approximately 60% of the 18 million people infected with *T. cruzi* have migrated from rural endemic areas of Latin America to urban areas of endemic and non-endemic countries^{1, 2}. Since most of these individuals are silent chronic carriers they potentially jeopardize the local blood supply, and blood recipients are still being infected by *T. cruzi*.

The prevention of the transfusion-associated Chagas' disease (TA-CD) has been done by clinical and serological screening of blood donors³, and/or inactivation of *T. cruzi* in collected blood using gentian violet (GV) as a trypanocidal agent⁴.

Recently, the combination of ultraviolet light (UV-A) and aminomethyltrimethyl psoralen (AMT) has been shown to be able to inactivate virus infecting cellular blood products⁵. In this study, we investigated the efficacy of the combined use of AMT and UV-A in inactivating *T. cruzi* in contaminated human platelet concentrates.

MATERIAL AND METHODS

Human platelet concentrates were infected with *T. cruzi* of the Y strain (2 x 10⁸ parasites per ml), transferred into UV-permeable platelet storage PL269 containers (Baxter Health Corporation, Fenwal Division) and treated under one of the following conditions:

- 1) Untreated
- 2) GV (250µg/ml) + Ascorbic Acid (1mg/ml)
- 3) GV ((250µg/ml) + Ascorbic Acid (1mg/ml) + UV-A
- 4) GV (250µg/ml) + UV-A
- 5) AMT (40µg/ml) + Ascorbic Acid (1mg/ml)
- 6) AMT (40µg/ml) + Ascorbic Acid (1mg/ml) + UV-A

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7) AMT (40µg/ml) + UV-A

8) UV-A only

The UV-A irradiation was delivered at doses of either 24, 92, 184, 276, 368, or 644kJ/m² using a prototype ultraviolet irradiator (Fenwal Laboratories) containing two banks of six UV-A bulbs (BLE-1T151); (Spectronics Corporation).

The microscopic examination of active circulating forms of *T. cruzi* in platelet suspensions was performed using the microhematocrit technique⁹ at 1, 6, and 24 hours after protocol incubation.

RESULTS

As expected, the inactivation of *T. cruzi* parasites as defined by absence of active circulating parasites, was observed in all platelet concentrates treated with protocols containing GV (protocols 2, 3 and 4) immediately after 1 hour of treatment incubation. This *T. cruzi* inactivation was confirmed by animal experiments in which Swiss mice inoculated with platelet suspensions submitted to such treatment protocols did not develop a *T. cruzi* infection.

In contrast, active forms of *T. cruzi* were observed, until after 24 hours of incubation, in platelet concentrates left untreated (protocol 1); treated with AMT plus AA (protocol 5); or treated with UV-A without association with GV (protocols 6, 7 and 8).

The results of the study are summarized in Table 1.

Table 1. Treatment of T. cruzi contaminated human platelet concentrates with AMT and UV-A light

Treatment protocol	Active circulating forms of <i>T. cruzi</i>		
	1 hour after treatment	6 hours after treatment	24 hours after treatment
Untreated	Positive	Positive	Positive
GV + AA	Negative	Negative	Negative
GV + AA +UV-A	Negative	Negative	Negative
GV + UV-A	Negative	Negative	Negative
AMT + AA	Positive	Positive	Positive
AMT + AA +UV-A	Positive	Positive	Positive
AMT + UV-A	Positive	Positive	Positive
UV-A only	Positive	Positive	Positive

UV-A light dose tested = 24, 92, 184, 276, and 644 kJ.m⁻².

DISCUSSION

Allogeneic blood transfusion is the main route of transmission of Chagas' disease in urban areas; of both endemic and non-endemic

countries. The exclusion of donors who immigrated from endemic areas by using a questionnaire can significantly reduce the blood supply in certain areas. Moreover, the serologic diagnosis of Chagas' disease is complex yielding both false positive and false negative results. Thus, the investigation of methods to inactivate *T. cruzi* that might be present in blood product is essential. We could show recently, that the association of GV, AA and photoirradiation with visible light efficiently sterilized *T. cruzi*-infected blood after treatment incubation time shorter than 30 minutes⁵. In the present study, we investigated the effectiveness of the combination of AMT and UV-A irradiation in inactivating *T. cruzi* in contaminated human platelet concentrates, since it has been demonstrated, in a rabbit model, that AMT/UV-A treated platelet suspensions retain hemostatic function⁸.

The data from our experiments confirm the trypanocidal activity of the gentian violet, alone or combined with ascorbic acid. In contrast, identical numbers of active circulating forms of *T. cruzi* were observed under all treatment conditions including the various doses of UV-A irradiation ranging from 24 to 644 kJ/m². The present data provide evidence, therefore, of the lack of trypanocidal activity of AMT and UV-A in inactivating *T. cruzi* present in contaminated human platelet concentrates.

Against our results, Gottlieb et al⁵ showed that AMT together with UV-A were able to inactivate 5 log of *T. cruzi* in platelet concentrates.

Further studies are being developed in our laboratory in order to elucidate the role of UV-A in *T. cruzi* inactivation.

RESUMO

As medidas adotadas atualmente para prevenir a doença de Chagas transfusional incluem a seleção dos doadores de sangue e/ou a inativação do T. cruzi no sangue coletado através do uso da violeta de genciana (VG) como agente tripanosomicida. Neste estudo, nós investigamos a eficácia do uso combinado do AMT e da UV-A para a neutralização do T. cruzi em concentrados de plaquetas humanas infectados. Os concentrados de plaquetas infectados com cepa Y de T. cruzi (2x10⁶/ml) foram transferidos para recipientes PL 269 (Fenwal Laboratories) e tratados com VG (250µg/ml) e ácido ascórbico (1mg/ml); VG, ácido ascórbico e UV-A; GV e UV-A;

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AMT (40µg/ml) e ácido ascórbico; AMT, ácido ascórbico e UV-A; AMT e UV-A; somente UV-A; e não tratado (controle). Todos os concentrados de plaquetas foram expostos a doses de 24, 92, 184, 276, 368 e 644kJ/m² de UV-A, e a pesquisa microscópica do *T. cruzi* foi realizada pela técnica do microhematócrito, 1, 6 e 24 horas após cada tratamento. Grande número de formas ativas de *T. cruzi* foi observado em todos os experimentos, exceto quando foi usada a VG como o agente tripanosomicida, evidenciando a ineficácia do AMT e da luz ultravioleta em inativar o *T. cruzi* nos concentrados de plaquetas humanas infectados.

Palavras-chaves: Doença de Chagas transfusional. Quimioprofilaxia. Tratamento fotodinâmico. Luz ultravioleta e doença de Chagas. Transfusão de sangue.

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