Experimental Parasitology 128 (2011) 301–308

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

# *Trypanosoma cruzi*: Correlation of muscle lesions with contractile properties in the acute phase of experimental infection in mice (*Mus musculus*)

Mario V. Ramirez-Archila [a,c,1], Jesús Muñiz [a,1], Adolfo Virgen-Ortiz [a,d,1,3], Oscar Newton-Sánchez [b,2], Valery G. Melnikov [b,2], Oxana R. Dobrovinskaya [a,*]

[a] Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio 965, C.P. 28045 Colima, Col., Mexico
[b] Facultad de Medicina, Universidad de Colima, Av. Universidad # 333 Col. Las Viboras, C.P. 28040 Colima, Col., Mexico
[c] Facultad de Ciencias de la Educación, Universidad de Colima, Av. Universidad # 333 Col. Las Viboras, C.P. 28040 Colima, Col., Mexico
[d] Departamento de Ciencias Químico Biológicas, Unidad Regional Sur, Universidad de Sonora, Blvd. Lázaro Cárdenas, No. 100 Col Francisco Villa, 85880 Novojoa, Sinaloa, Mexico

ARTICLE INFO

*Article history:*
Received 8 October 2010
Received in revised form 10 February 2011
Accepted 14 February 2011
Available online 18 February 2011

*Keywords:*
*Trypanosoma cruzi*
Muscular histotropism
Contractile properties
Rectus abdominis
Plantaris
Single twitch
Tetanus

ABSTRACT

Parasitism in skeletal muscles and myositis are commonly observed during experimental *Trypanosoma cruzi* infection. The effect of *T. cruzi* infection on contractile properties of skeletal muscles in consecutive periods of the acute infection in BALB/c mice was studied. Albarrada strain (clone 4) which was isolated in Mexico and has demonstrated a high level of blood parasitemia and parasitism in skeletal muscles was used. Isolated strips of rectus abdominis muscle were subjected to direct electrical field *in vitro*. Alternatively, plantaris muscles were stimulated *in situ* through the sciatic nerve. The peak amplitudes of a single twitch and tetanus contractions were considered to estimate the mechanical properties of muscles. Histopathological analysis was performed to correlate functional changes with the evolution of tissue parasitism and tissue injury. Contractile properties of muscles were significantly attenuated during acute *T. cruzi* infection. The percentage of damaged muscles rather than the character of tissue pathology affected their contractile properties significantly.



## 1. Introduction

Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, represents the major cause of the heart pathology in the endemic regions of Latin America. Over 15 million people are affected, and approximately 28 million people are at risk of *T. cruzi* infection (WHO, 2007). In recent years, due to tourism and migration, the cases of Chagas' disease were also reported in non-endemic countries (Kirchhoff, 1993; Gascón et al., 2007).

In infected mammals, including humans, Chagas' disease goes through three characteristic phases: the acute, indeterminate and chronic. The main feature of the acute phase is the presence of free trypomastigotes in the bloodstream (parasitemia). Subsequently, bloodstream forms invade the hosts' tissues and convert into intracellular proliferating forms (amastigotes). The majority of *T. cruzi* strains possess cardiomiotropism, and 10–30% of *T. cruzi*-infected individuals develop varying degree of acute to chronic myocarditis (Acquatella, 2008). Skeletal muscles were also shown to be readily infected by *T. cruzi*. Muscular pain and weakness were reported in patients with Chagas' disease (Köberle, 1968). Myositis of deltoid and gastrocnemius muscle was demonstrated in patients with chronic and acute Chagas' disease and cardiopathy (Cenget and Rojas, 1959; Ponce, 1972). Cases of patients developing chagasic polymyositis have also been reported (Cossermelli et al., 1978). Furthermore, structural alterations in myofibrils were found in muscle biopsies of chronically infected individuals (Laguens et al., 1975). These alterations coincided with the presence of circulating antibodies against striated muscle fibers and the plasma membrane of endothelial cells (Laguens et al., 1975; Laguens and Cabeza Markert, 1991). Parasitism in different muscle groups, myositis, degeneration and necrosis of myofibrils were commonly observed during both acute and chronic phases of experimental infection in mice (Bijovsky et al., 1983; Molina et al., 1987; Losavio et al., 1989; Monteón et al., 1996). Myofibrillar breakdown and cytoskeleton alterations are most likely to be the result of different pathogenic processes taking place in affected muscles, including direct destruction of myofibrils by the parasites, tissue damage caused by inflammation and production of the nitric oxide (NO) and pro-inflammatory cytokines, microvascular lesions and an indirect effect of cross-reactive antibodies (revised in: Scharfstein

* Corresponding author. Address: Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio 965, C.P. 28045 Colima, Col., Mexico. Fax: +52 312 41 61 129.
*E-mail address:* dobrovinskaya@gmail.com (O.R. Dobrovinskaya).
[1] Fax: +52 312 41 61 129.
[2] Fax: +52 312 31 61 099.
[3] Fax: +1 642 425 9952.

0014-4894/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2011.02.012

et al., 2009). There is also evidence of neuromuscular junction damage (Mirkin et al., 1994). In the late period of infection the parasitism and inflammation in tissues decreased. However, the process of muscle regeneration in this period involves the replacement of muscle tissues by fibrosis (Buckner et al., 1999). Although the detailed mechanism of this phenomenon is not known yet, studies on cell culture models *in vitro* revealed that the myotropic Brazil strain of *T. cruzi* profoundly affected the ability of L6E9 myoblasts to differentiate into mature muscle myotubes (Rowin et al., 1983). The expression of genes coding for differentiated muscle-specific proteins was inhibited as well. One can expect that the functional properties (contractility) of affected muscles could not be restored completely in *T. cruzi* infected animals and individuals. Indeed, it was shown previously that the contractility and pharmacological response were altered dramatically in myocardium isolated from *T. cruzi* infected mice (Fernández-Culasso et al., 1991;Fernández et al., 1992). The changes in heart muscle contractility seem to be intimately related to structural alterations of mitochondria and oxidative phosphorylation deficiency in *T. cruzi* infected murine hearts (Garg et al., 2003; Báez et al., 2008).

The question, how *T. cruzi* infection affects the contractility of skeletal muscles was not addressed yet, neither in the acute nor in the chronic phases of the disease. Therefore, the aim of the present work was to study the effect of *T. cruzi* infection on contractile properties of skeletal muscles in consecutive periods of acute experimental infection in mice. Thereafter, histopathological analysis of muscle samples was performed to correlate functional changes with evolution of tissue parasitism and injury.

## 2. Materials and methods

Details of the experimental protocol were submitted to and approved by the Committee for Bioethics and Biosafety at the University of Colima. All procedures were carried out in compliance with the ethical standards for investigation of experimental pain in animals (Zimmerman, 1983).

### 2.1. Animals and parasites

Six to eight weeks old male BALB/c mice (weighing 25 ± 5 g) were obtained from our breeding facilities and housed in light and temperature controlled conditions with water and food *ad libitum*.

Albarrada strain of *T. cruzi* was isolated by our group from the *Triatominae* intra-domestic vector recollected in urban area of the state of Colima (Melnikov et al., 2005). The strain was cloned, and clone 4 (cl4) which demonstrated high parasitemia and parasitism in skeletal muscles in BALB/c mice was used in the present study. Blood stream trypomastigotes were obtained from previously infected BALB/c mice by cardiac puncture and used for infection of experimental animals.

### 2.2. Experimental models and groups

Albarrada cl4 clone caused severe parasitism and lesions in rectus abdominis muscle (previous experiments, not shown). However, anatomical features of rectus abdominis muscle precluded its use for *in situ* measurements. Then this muscle was considered for *in vitro* experiments. Plantaris muscles were less affected by the parasite than rectus abdominis muscles, but the former is more appropriate for *in situ* measurements. Both preparations, *in situ* and *in vitro*, were used to determine whether *T. cruzi* infection affects contractile properties of affected muscles. First, 64 animals were randomized into two sets, for *in vitro* (rectus abdominis) and *in situ* (plantaris) studies. Taking into account the data of our preliminary experiments on muscle injury during different periods of infection (not shown), the evaluation of contractile properties of muscles were performed in the following periods: 22–24 days post-infection (p.i.), when the peak of parasitemia was reached and severe tissue parasitism was observed (Maximum Parasitemia Phase, MPP); 30–32 days p.i., when the abundant inflammatory infiltrates were present in muscles (Inflammatory Phase, IP); and 45–47 days p.i., when extensive areas of affected muscles were substituted with fibrous tissue (Fibrotic Phase, FP). Taking into account these findings, the animals of each set were then divided in four groups (eight animals each): a control (C) and three experimental groups corresponding to three periods of acute infection: MPP, IP, and FP. Mice in experimental groups were injected intraperitoneally (i.p.) with $10^5$ of bloodstream trypomastigotes per mice. Parasitemia was monitored every three days as previously described (Melnikov et al., 2005). Animals in control groups were injected i.p. with equal volume of physiologic solution.

### 2.3. Muscle isolation and contractility experiments in vitro

The animals were anesthetized with sodium pentobarbital (30 mg $kg^{-1}$, i.p.). Tendon-to-tendon strips (2 mm wide, 1.0–1.5 mm thick and 3 mm long) from rectus abdominis muscle were quickly isolated and immersed in warm (37 °C) bath solution containing 154 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, and 11 mM glucose; pH 7.4 (Stum et al., 2008). After muscle isolation, the animals were immediately killed with an overdose of sodium pentobarbital (150 mg $kg^{-1}$, i.p.). Each isolated muscle strip was mounted horizontally in a temperature-controlled chamber containing 4 ml of bath solution. The chamber was perfused continuously with a gas mixture (95% $O_2$–5% $CO_2$) and maintained at a temperature of 37 °C. One tendon of the muscle strip was anchored inside the organ chamber and the other was connected to a force transducer (Kent Sci. Corporation, TRN011) mounted on computer-commanded step motor, in order to change accurately the muscle length. The muscles were subjected to electrical field stimulation (EFS). Responses to EFS were elicited by applying square wave pulses (1 ms duration) of supramaximal voltage (80 V), single for twitch and of varying frequencies (40–100 Hz, 2 s) for tetanus, delivered with an electric stimulator (Grass S88 plus stimulus-isolation unit Grass SIU5) through two platinum electrodes (2 mm × 10 mm) placed longitudinally 1–1.5 mm on either side of the muscle strip. The rest interval between successive stimuli was 2 min. The force signal was amplified, digitized (Digidata 1200 series, Axon Instruments), and saved for analysis using Axoscope and Sigmaplot software. At several muscle lengths, isometric twitches were elicited by single supramaximal stimuli until the maximal twitch amplitude corresponding to the optimal length ($L_o$) was obtained. With the muscle length set to the $L_o$, repeated stimulations at frequencies of 40–100 Hz were applied. The fusion of mechanical response was obtained at 50 Hz. This frequency was used for tetanic contractions in all *in vitro* protocols (Huerta et al., 1986; Tatsuya et al., 1999). At the end of experiments, the muscles strips were dried with absorbent paper and weighed on an analytical balance (Sartorius, Edgewood, NY, USA). All muscles used in contractility experiments, were fixed and processed for subsequent histological analysis.

### 2.4. Surgical preparation and contractility measurements in situ

The mice were anaesthetized with sodium pentobarbital (30 mg $kg^{-1}$, i.p.). Throughout the experiment, animals were kept at a surgical level of anesthesia with supplemental injections. The right leg plantaris muscle was liberated from the surrounding connective tissue, leaving the muscle proximal insertions and