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

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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

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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

blood supply intact. For indirect stimulation via the motor nerve, the sciatic nerve was isolated from the surrounding connective tissue and cut as far apart as possible from its entry into the muscle. During the surgery procedure, saline solution (containing in mM: 125 NaCl; 5.4 KCl; 1.05 $MgCl_2$; 1.8 $CaCl_2$; 11 glucose; pH 7.4) was applied to keep the tissues moist. A transverse hole was made in the femur using a micro-drill (F.S.T. 18,000–17; Fine Science Tools, Foster City, CA, USA), and the distal tendon of the muscle was tied to a hook. Subsequently the animal was transferred to a mechanical recording apparatus consisting of a plate mounted on an inclined base that allowed the muscle to be placed perpendicularly to a load transducer (FT10; Grass Co., Quincy, MA, USA). The plate had two posts to fix the steel rod passing through the hole in the femur. The transducer was mounted on an effector that was driven by a computer-controlled stepper-motor and wired to an A/D converter to permit the force responses to be display and saved. The tendon hook was attached to the transducer. Sciatic nerve was placed on stimulating electrodes wired to a stimulator (Grass S88 plus stimulus-isolation unit Grass SIU5) through two platinum electrodes (0.5 mm × 10 mm each). At several muscle lengths, isometric twitches were elicited by single supramaximal (1.6 V) stimulation of the motor nerve until the maximal twitch amplitude was obtained, corresponding to the optimal length, L_o. From the L_o, tetanic contractions of all muscles were elicited by repetitive stimulation at frequencies of 40–100 Hz. The frequency of stimulation at which fusion of mechanical response was obtained was 50 Hz. Then the motor nerve was stimulated to produce twitches (single 1.6 V supramaximal electrical stimuli of 1 ms duration) and tetanic contractions (1.6 V supramaximal stimuli of 40–100 Hz frequency and 0.5 s duration). At the end of experiments, the mice received an overdose of sodium pentobarbital (150 mg kg^{-1}, i.p.) (Virgen-Ortiz et al., 2008), and the muscles were excised from the animals, dried with absorbent paper, weighed on an analytical balance (Sartorius, Edgewood, NY, USA) and processed for following histological analysis.

2.5. Data analysis

The parameters measured were the peak tension of twitches and tetani (P_0) in isometric contraction at L_0 that allows the evaluation of the maximum tension that could develop in the muscle (Gordon et al., 1966). In both cases, the force results were expressed as force/CSA (N cm^{-2}), where CSA was the cross-sectional area. CSA was calculated using the equation CSA = MW/1.056L_o, where MW was the weight of the muscle (g); L_o, the optimal length of the muscle (cm); and 1.056 is the muscle density (g cm^{-3}). Finally, the results were expressed in kilopascals (kPa), where 1 N cm^{-2} = 10 kPa (Virgen-Ortiz et al., 2008).

2.6. Morphometric studies

Muscle strips were fixed in 10% buffered formalin solution for 3 days, and processed for paraffin embedding. Four micrometers of thick tissue sections were cut, stained with haematoxylin–eosin and analyzed by optical microscopy using digital imaging (Microscope Axioscop Carl Zeiss, 20× Achroplan Objective, Digital Camera MR5 Carl Zeiss). Morphometric studies of tissue parasitism, structural damage, inflammation, vascular damage and fibrosis were performed by analyzing 20 images per animal taken in randomly selected fields of tissue fragment sections (2.56 × 10^6 μm^2 of total analyzed surface). All analyses were performed using a 20× Achroplan objective, applying automatic scanning technique (Carl Zeiss). The images were captured and analyzed with Axiovision 4.8 software (Interactive measurements and automatic measurements modules). Muscle fibers were considered as damaged if the following pathologic changes were observed: edema, color change, parasitism (nests of amastigotes o single amastigotes inside the fibers), loss of fibers' striation and integrity. Inflammatory infiltrations in the muscles were quantified by counting the mononuclear cell nuclei, where accumulation of at least five mononuclear cells in the area of 20,000 μm^2 was considered as infiltrate. The area of infiltrate was measured, and the infiltrate type (focal, multifocal, and diffuse) was determined. When the infiltrates occupied less than 40% of the field of view (at objective 20×), they were considered as focal, and in the case of their extension more than 40% as diffuse. Fibrotic areas were determined and measured in semiautomatic mode with use of Automeasure Plus module (Carl Zeiss). To estimate vessel injury, the following pathologic changes were considered: loss of integrity of vessels' wall, presence of intravascular thrombi, inflammatory infiltrates and fibrosis in perivascular area. Areas affected by pathologic processes, and no-damaged areas were expressed as percentage of all tissue area analyzed.

2.7. Statistical analysis

All values were reported as mean ± SE. Comparative analysis between experimental groups was performed using the Mann–Whitney test. In all cases, differences were considered statistically significant when P values were <0.05.

3. Results

3.1. Parasitemia profile

Parasitemia profile of BALB/c mice infected with Albarrada strain is shown on the Fig. 1. Bloodstream trypomastigotic forms started to be detected in the peripheral blood on the 11–12 days after inoculation. Peak of parasitemia was reached on 20th–22nd post-infection day (88.33 ± 3.74 × 10^5 parasites/ml), then decreased, and finally stabilized at a low level (0.2–5 × 10^3 parasites/ml) at 35th–40th day of infection. The first 40 days post-inoculation were considered as acute phase.

3.2. Histopathological findings in rectus abdominis and plantaris muscles of infected mice

3.2.1. Rectus abdominis

Maximum Parasitemia Phase, MPP. Tissue parasitism and damage of rectus abdominis in different phases of acute infection is

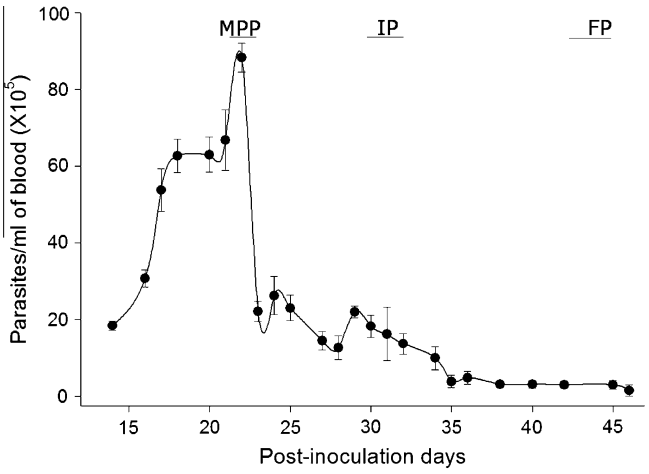

Fig. 1. Parasitemia curve in male BALB/c mice infected i.p. with 10^5 of bloodstream trypomastigotes of *T. cruzi* (Albarrada cl4 clone). Results are expressed as the mean ± SE (n = 3). *Abbreviations:* MPP, phase of maximal level of parasitemia and tissue parasitism; IP, inflammatory phase; FP, fibrotic phase.

demonstrated in Fig. 2 and in the Table 1A. On 22–24 days of infection when the peak of parasitemia was reached, numerous nests of amastigotes (1 nest per every 1.94 mm^2) were present (Fig. 2A). Focal or multifocal mononuclear infiltrates were detected in the majority of analyzed areas. Fibroblasts and a few macrophages were present, indicating that the process of tissue remodeling had begun. Fibrosis occupied about 11% of the analyzed area compared to 1% in healthy mice of the same age. Only 63% of rectus abdominis were considered as unimpaired tissue in this phase of infection.

Inflammatory Phase, IP. As the disease progressed, on 30–32 days post-infection, parasitism in tissue was similar to MPP. At the same time, significant areas of muscle fibers were destroyed with intense multifocal or diffuse inflammatory infiltration (Fig. 2B and Table 1A), with presence of lymphocytes, fibroblasts and macrophages.

Fibrotic phase, FP. The process of replacement of damaged muscles with fibrous and adipose tissue increased progressively, and 23% of observed areas were occupied by fibrosis in this phase (Fig. 2C and Table 1). Once the parasitemia has been stabilized at the very low level (45–47 days post-infection), nests of amastigotes were less numerous (1 nest per every 2.87 mm^2) and inflammatory lesions became less intense. Infiltrates were in general scarce, focal or multifocal.

Vascular damage. Serious abnormalities in the vascular system were revealed in all periods of observation in rectus abdominis. The percentage of affected vessels gradually increased as the disease developed (Table 1). Mainly, abundant perivascular infiltrate was present. Henceforth, intravascular infiltrations were found in the IP and FP phases, indicating a progressive character of vascular damage.

3.2.2. Plantaris

In general, plantaris muscle was less affected than rectus abdominis (Table 1). In MPP, about 90% of muscles retained their normal structure. In IP, the presence of inflammatory infiltrate was characteristic, however it was less abundant than in rectus abdominis of the same period. The remodeling process seems to be slower in plantaris muscles, because fibrosis areas occupied 12% of the total muscle in the FP, and inflammatory infiltrates were still relatively extensive. Vessels were significantly less damaged than in rectus abdominis muscle, and, contrary to rectus abdominis, there were no changes in this parameter during all experimental phases.

3.3. Comparative analysis of contractile properties of rectus abdominis muscle isolated from infected and healthy mice

3.3.1. Single muscular twitch

Since the rectus abdominis muscle was affected significantly during acute *T. cruzi* infection, the changes in its contractile properties were expected. First, we have evaluated the capability of the isolated muscle fibers to develop tension in response to stimulation by a single electric pulse. Isometric twitch contractions were elicited by applying of single pulse of supermaximal voltage (80 V, 1 ms). The results of these experiments are shown in Fig. 3A. The curve obtained in the experimental series with muscles isolated from healthy animals represents a classical twitch contraction curve. After a short latent period, the tension is increased and reaches the maximum. The level of maximal tension depends on the stimulus force. The mechanism involves actin and myosin interaction and depends on concentrations of Ca^{2+} and ATP in the sarcoplasm. In the absence of subsequent stimulus, Ca^{2+} is removed from the sarcoplasm, the muscle relaxed, and tension decreased gradually (Pollack, 1990). The maximal tension of 4.36 ± 0.01 kPa was reached in the muscles from the control group ($n = 8$). In the muscles isolated during MPP, the peak tension was only slightly lower (3.65 ± 0.01 kPa, $n = 8$), without statistically significant difference from the control ($P > 0.05$). In the muscles isolated in the subsequent periods of infection, the maximal tension was considerably weaker than in the control and MPP groups: 1.5 ± 0.1 kPa ($n = 8$) and 1.4 ± 0.2 kPa ($n = 8$), in IP and FP groups respectively, what could be estimated as 24–30% of control level. Statistical analysis revealed the difference between control and IP, and control and FP groups ($P < 0.05$), without any difference between IP and FP groups ($P > 0.05$). Time courses of muscle contractions were very similar for all groups of infected mice. Contraction time was 10–11 ms in all experimental groups, without a significant difference from control.

3.3.2. Fused tetanus

If a muscle fiber is stimulated so rapidly that it does not relax between stimuli, a smooth, sustained contraction termed tetanus occurs. As far as the fiber is restimulated while there is still some

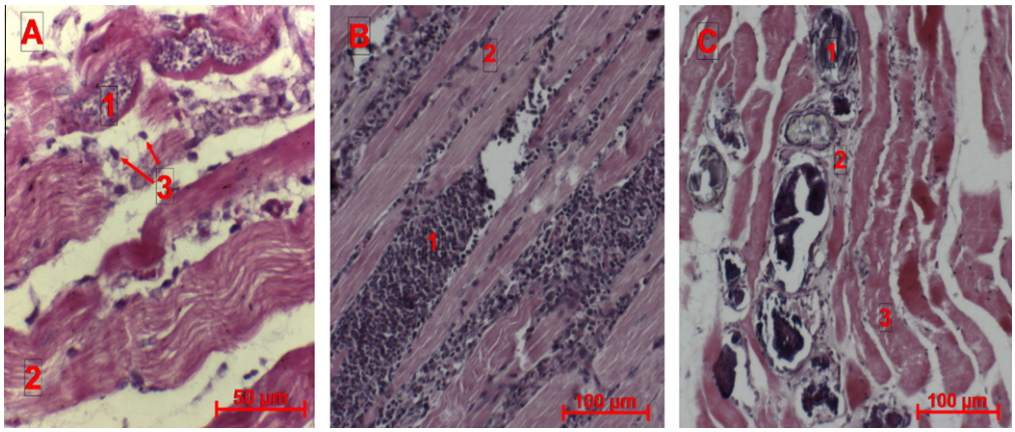

Fig. 2. Digital photomicrographs of typical (present in more than 80% of specimens) pathological changes provoked by *T. cruzi* (Albarrada cl4 clone) infection in rectus abdominis muscle in BALB/c mice (Haematoxylin and Eosin staining). (A) Phase of maximal level of parasitemia and tissue parasitism (MPP): 1. Parasite nest observed in completely destroyed muscular fiber. 2. Unaffected muscular fibers. 3. Scarce inmunocompetent cells in immediate proximity to infected fiber. (B) Inflammatory phase (IP) characterized by presence of mononuclear infiltration in muscle: 1. Focal infiltration mononuclear cells and fibroblasts 2. Unaffected muscular fibers. (C) The fibrotic phase (FP). Fibrosis and calcifications are characteristic. 1. Calcifications usually replace the necrotized fibers. 2. Fibrotic tissue always encapsulates the calcifications and lies around; fibrosis replaces the destroyed muscular fiber and forms broad areas or narrow stripes between the unaffected muscular fibers, or around the vascular and neural bundles. 3. Unaffected muscular fibers.

Table 1
Histopathological changes in rectus abdominis and plantaris skeletal muscles infected with *T. cruzi* from BALB/c strain male mice.

Phase	Unimpaired muscle	Fibrosis	Inflammatory infiltrate			Vascular damage	
			Focal	Multifocal	Diffuse	Impaired vessels	Unimpaired vessels
Rectus abdominis							
C	99	1	0	0	0	0	100
MPP	63	11	23	3	0	48.4	51.6
IP	42	23	0	17.5	17.5	68.6	31.4
FP	52	35	5	8	0	84.7	15.3
Plantaris							
C	99	1	0	0	0	0	100
MPP	90	5	1.5	3	0.5	36	64
IP	67	3	13	17	0	35	65
FP	53	12	11	21	3	31	69

C: Control; MPP: Maximum Parasitism Phase; IP: Inflammatory Phase; FP: Fibrotic Phase. All values for the "Unimpaired muscle", "Fibrosis" and "Inflammatory infiltrates columns" are presented in% of total specimeń surfaces analyzed. The values for the "Vascular damage" columns are presented in% of total vessels number analyzed per the total surface.

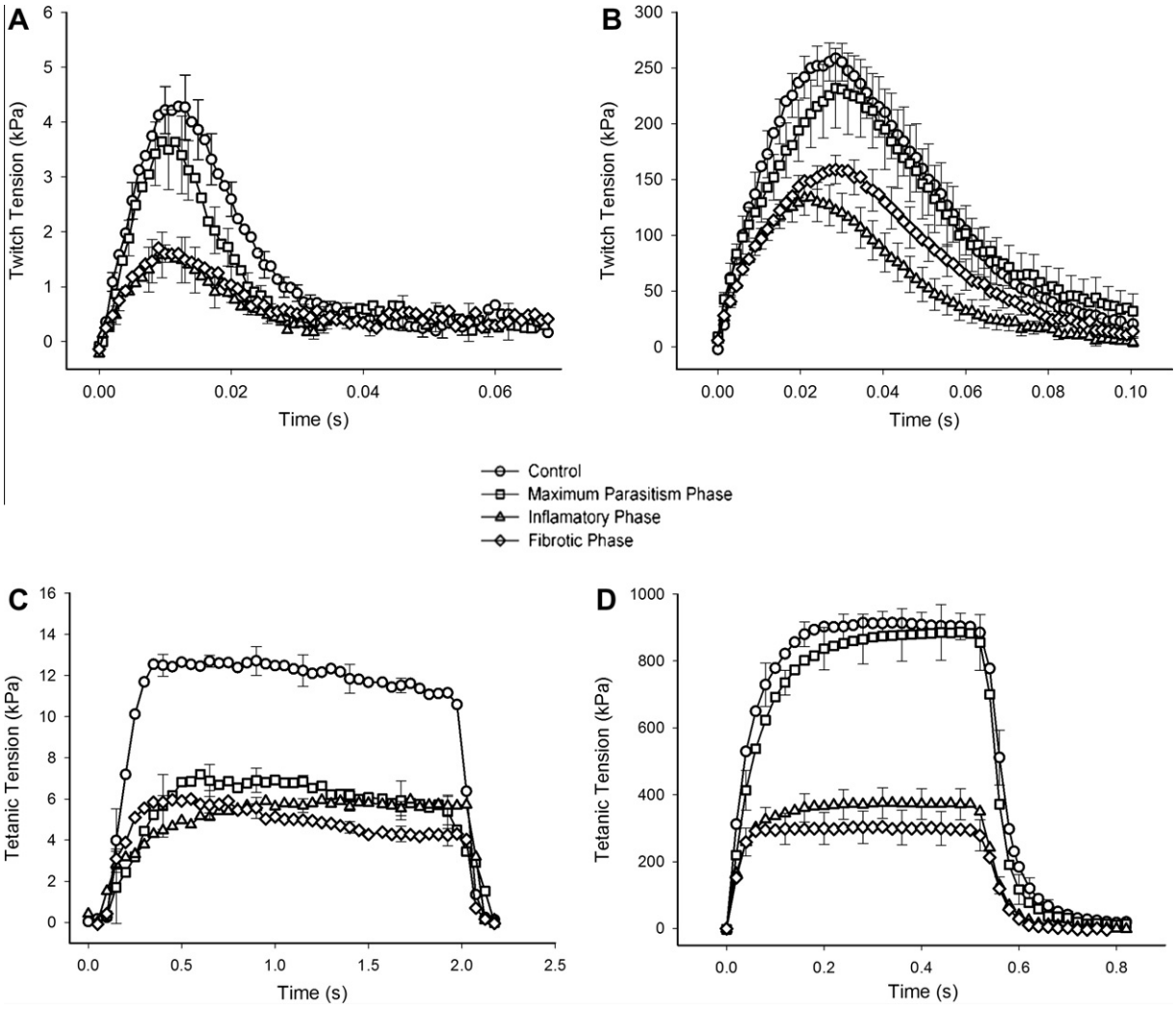

Fig. 3. Evaluation of contractile function of skeletal muscles isolated from BALB/c mice in different phases of infection with *T. cruzi*. (A and B) Records of twitches (A) and tetani (B) obtained in *in vitro* experiments with rectus abdominis muscle. (C and D) Records of twitches (C) and tetani (D) obtained in *in situ* experiments with plantaris muscle. In all cases the control groups are non-infected mice. The data are means ± standard error (SE), $n = 8$ for each group.

contractile activity, the amplitude of tension produced in fused tetanus is significantly higher than in single contraction. During repetitive electric stimulations, Ca^{2+} is not removed from the sarcoplasm, and the concentration of intracellular Ca^{2+} increases, maintaining strong contraction (Pollack, 1990). Then, to further test the influence of *T. cruzi* infection on muscle function, the maximum force-generating capacity was assessed by producing 60 Hz tetani for 2 s. Peak tetanic tension developed by rectus abdominis isolated from healthy mice was 12.61 ± 0.02 kPa, about five times higher than during single contraction. This parameter

was significantly lower in muscles isolated from all groups of infected mice (6.89 ± 0.04, 5.84 ± 0.02, 5.82 ± 0.04 kPa for MPP, IP, and FP groups, correspondingly (Fig. 3B), with statistically significant difference from the control ($P < 0.05$).

3.4. Comparative analysis of in situ contractile properties of plantaris muscle in infected and healthy mice

The amplitude of peak tension of a single muscular twitch of control plantaris muscle was 269.5 ± 17.5 kPa and the contraction time was 30.37 ± 3.42 ms (Fig. 3C). Infection did not change the time course of response, but the amplitude decreased considerably in IP and FP groups (131.1 ± 9.7 and 155.5 ± 16.7 kPa, respectively, Fig. 3C). Similarly, the amplitude of tetanus was decreased in IP and FP, but not in MPP groups (Fig. 3D).

4. Discussion

In general, different *T. cruzi* strains demonstrate tropism for heart, skeletal muscles and nervous tissue. Polymyositis observed in patients with Chagas' disease is usually overshadowed by the more clinically evident cardiac disease. Whereas correlations between pathologic changes in tissue and functional abnormalities are described in relative detail for chagasic hearts, no studies were undertaken to reveal the relationship between skeletal muscles injury with their contractile properties. In the present work we have correlated contractile properties of rectus abdominis and plantaris muscle with the degree and character of tissue damage analyzed on histological level, in three different stages of acute experimental trypanosomiasis in mice. To test the mechanic properties of muscles, isolated strips of rectus abdominis were subjected to direct electrical field stimulation *in vitro*, or, alternatively, plantaris muscles were stimulated *in situ* through the sciatic nerve. Single twitch and tetanus protocols were applied.

It was shown that contractile properties of muscles were significantly attenuated during acute *T. cruzi* infection. The phenomenon was confirmed in both *in vitro* (rectus abdominis) and *in situ* (plantaris) experiments, indicating that it was due to muscle rather than to motor nervous damage.

The peak of parasitemia was reached at 22–24 days of infections (MPP stage). In the rectus abdominis severely affected by the parasite, we have found numerous nests of amastigotes accompanied by mononuclear inflammatory infiltrates, mostly scarce (87.5%), but moderate in rare cases (12.5%). More than 60% of analyzed areas were unaffected, and 25% and 12% were occupied by inflammation or fibrosis, correspondingly. Half of the vessels were involved in the inflammatory process (perivasculitis). However, on the functional level, the amplitudes of single contractions showed only a slight decrease compared to the control level, with the differences being statistically insignificant ($n = 8$, $P > 0.05$). At the same time, the amplitude of tetanic contraction in this period was already considerably decreased (Fig. 3B). As far as the mitochondrial activity is the important factor for sustained muscular activity (Curtin and Woledge, 1978), both mitochondrial dysfunction and oxidative imbalance in skeletal tissue reported recently for acute *T. cruzi* infection in mice (Wen et al., 2008) could contribute to this phenomenon.

On 30–32 days (PI stage) of infection, parasitemia decreased, but the extension of parasitism and inflammation in muscles increased drastically. More than 35% of analyzed areas were occupied by inflammatory infiltrate, and its degree shifted from mainly scarce in MPP stage to moderate (75%) and even severe (12.5%). 22% of muscle tissue was replaced by fibrous and adipose tissue. The percentage of unaffected tissue diminished from 63 (in PP stage) to 42%. This change has had a strong impact on the functional characteristics of rectus abdominis muscle, and the amplitudes of single contractions were two times lower, than in the MPP phase. However, the amplitudes of tetanus were similar in MPP and IP phases. It is likely that the critical level of damage for tetanic contraction was reached already in the first period of infection. On 45–47 days (PF stage) of infection, parasitemia stabilized at the lowest level, inflammatory reaction in tissues was significantly less pronounced (only 12.5% of examined areas were occupied by inflammatory infiltrate). On the other hand, the muscles were replaced by fibrous and adipose tissue in large areas (more than 30%), and the percentage of unaffected muscle tissue was about 40–45%, similar to IP group. Contractile properties of muscles in this period were also very similar to those in the inflammatory stage (IP) despite the fact that the processes taking place in tissues were different, with the prevalence of inflammation in PF stage and of fibrosis in IP stage.

The above results show that skeletal muscles are capable, to some extent, to maintain their basic functions during the infection process. Although the degree of damage was significant in the first stage of infection characterized by maximal peak of parasitemia, it does not reach the critical level at this period, and the amplitude of a single twitch was practically unaffected. The shift to anaerobic metabolism in the affected skeletal muscles with decreased oxidative capacities could be one of the feasible adaptive mechanisms, as was described in patients with advanced Chagas' disease (Montes de Oca et al., 2004). At the same time, serious changes in functional properties were observed just overcoming this critical level of tissue damage in the following stages of Chagas' disease. The ability to develop tetanic contraction seems to be more affected by pathologic processes in muscles, so this function was changed already in MPP phase.

Similar processes took place in the plantaris muscle. However tissue damage developed slower in this muscle (Table 1), and both single and tetanic amplitudes were not affected in MPP phase, when only 10% of tissue was damaged. The variations in parasitism and pathologic changes between rectus abdominis and plantaris muscles might be correlated with different factors, the more important of which are the structure of muscular fibers and differences in regional blood supply. The diversity of the content and composition of heavy myosin chain in the skeletal muscular fibers was reported earlier (Eng et al., 2008). The muscle fibers are divided into fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG) and slow-twitch oxidative (SO), which present significant differences in oxidative-glycolytic metabolism. FOG and SO fibers show high activity of enzymes of aerobic metabolism when comparing to FG fibers (Schwartz-Giblin et al., 1983). At the same time, *T. cruzi* is known to be very sensitive to oxidative stress (Bachega et al., 2009). Then the muscles with high percentage of SO and/or FOG fibers are likely to be less suitable for parasite survival and, consequently, would be less affected during infection. Indeed, as was shown earlier, plantaris muscle in rats contains more than 55% of SO + FOG fibers (Ishihara et al., 1998), whereas this value in rectus abdominis is less than 30% (Alvarez Rosa et al., 2007).

Furthermore, muscle contractions are well known to be regulated by intracellular Ca^{2+} ions, which switch thin filaments into an active state by binding to troponin. ATP is important for the contraction process as well, since Ca^{2+} activates the myosin–ATP complex, which drives the sliding action between actin and myosin (Szent-Györgyi, 1975). Although it is widely accepted that the process of the host cell invasion by *T. cruzi* depends on the energy accumulated by parasite (Schenkman et al., 1991), which specific source of energy is used is not known yet (Martins et al., 2009). At the same time, no changes in the activity of the mitochondrial ATP synthase was observed in myocardium of rats infected with the Colombian strain of *T. cruzi* (Rendón et al., 2007). Ca^{2+} mobilization at the single cell level was detected in cultured

L6E6 myoblasts during their interaction with *T. cruzi* trypomastigotes (Moreno et al., 1994). Subsequent studies reported that *T. cruzi* infection induce repetitive cytosolic-free Ca^{2+} transients in rat kidney fibroblasts (Tardieux et al., 1994), affects intracellular Ca^{2+} levels in neonatal cardiomyocytes (Taniwaki et al., 2006), and may involve Ca^{2+} mobilization from intracellular stores in different mammalian cells (Yoshida and Cortez, 2008). Despite several published reports about effect of *T. cruzi* infection on Ca^{2+} signaling in different types of host cells, more studies are needed to link these changes to muscle contractile properties.

In the present work we have shown that *T. cruzi* infection impaired significantly contractile properties of skeletal muscles in mice. The percentage of damaged muscles was important, independently on the character of tissue pathology. Even during late acute infection, when parasitism and inflammation were decreased, and the process of tissue remodeling took place in the muscles, the contractile properties remained attenuated significantly due to the fact that muscles were replaced by fibrous tissue and fat.

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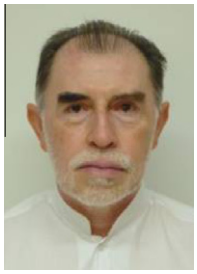

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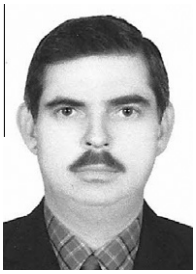

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