LA ULTRAESTRUCTURA DE *LEISHMANIA VENEZUELENSIS* EN HUMANOS Y GATOS NATURALMENTE INFECTADOS CON LEISHMANIASIS CUTÁNEA

THE ULTRASTRUCTURE OF *LEISHMANIA VENEZUELENSIS* IN HUMANS AND CATS NATURALLY INFECTED WITH CUTANEOUS LEISHMANIASIS

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RESUMEN

La ultraestructura de la *Leishmania venezuelensis* en humanos y gatos naturalmente infectados con leishmaniasis cutánea fue similar a la de otras especies conocidas de *Leishmania* que producen enfermedad en humanos. Sin embargo, se observaron algunos hallazgos peculiares, tales como el diámetro promedio, el

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número y la distancia media entre los microtúbulos peliculares, la presencia de grandes megasomas, el desarrollo de vacuolas fagocíticas gigantes, la disposición de los túbulos en el corpúsculo parabasal, la presencia de microtúbulos por debajo de la membrana de la bolsa flagelar y la existencia de una organela excretoria en el polo posterior.

Palabras claves: Ultraestructura, *Leishmania venezuelensis*, leishmaniasis cutánea, organela excretoria.

ABSTRACT

The ultrastructure of *Leishmania venezuelensis* in humans and cats naturally infected with cutaneous leishmaniasis was similar to other known species of *Leishmania* producing disease in humans. However, some peculiar findings were observed, such as the mean diameter, the number of and mean distance between pellicular microtubules, the presence of large megasomes, the development of giant phagocytic vacuoles, the disposition of tubules at the basal body, the presence of microtubules beneath the membrane of the flagellar pocket and the existence at the posterior pole of an excretory organelle.

Key words: Ultrastructure, *Leishmania venezuelensis*, cutaneous leishmaniasis, excretory organelle.

INTRODUCTION

Cutaneous and mucocutaneous leishmaniasis are the most widespread forms of the disease in Venezuela. Visceral leishmaniasis is less frequent.

In rural areas of the central western region of Venezuela, with high humidity, the causal agents of cutaneous and mucocuta-

neous leishmaniasis are a variant of the WHO *Leishmania braziliensis* reference strain and a hybrid between *L. braziliensis* and *L. guyanensis*[4,5]. In peripheric suburbs of towns located in xerophitic areas, near rivers and creeks, *L. venezuelensis* produces most of the cases of cutaneous leishmaniasis[3]. The endemic areas for visceral leishmaniasis are near macrotermic forests at the foot of hills, being continued by planes of tropical climatology. Its causal agent in Venezuela has not yet been characterized, but it is presumed to be *L. chagasi*. In the central western region of Venezuela there are then four parasites, which normally differ in their morphology, biology, ecology, epidemiology, clinical, biochemistry and immunology. We have therefore started a comparative electron microscopic study on these organisms.

In this article we describe the ultrastructural features of *L. venezuelensis* in ten patients and three cats naturally infected with cutaneous leishmaniasis in Barquisimeto, Lara State, Venezuela.

MATERIALS AND METHODS

Biopsies were taken from ten patients with ulcerous and nodular lesions on the skin and from three cats with nodular lesions on the nose and ears. Samples both from humans and cats were taken from the infiltrated edges of the lesions, prior to treatment and then processed for electron microscopy. Parasites isolated both from humans and cats were identified, based on molecular criteria, as *L. venezuelensis* [4,5].

All material removed from humans and cats was cut in 1 mm3 pieces and fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, for 3 h at 4 C, washed several times in the buffer containing 7.5% sucrose, and then postfixed 1 h with 1% osmium tetroxide in cacodylate buffer at room temperature. The tissues were then alcohol dehydrated and embedded in Poly-bed B 12. The blocks were sectioned with a Dupont Sorval MT-2B ultramicroto-

me, then stained with uranyl acetate and lead citrate, and observed in a Hitachi H-500 electron microscope. Sections of more than 200 *L. venezuelensis* amastigotes were studied.

RESULTS

In humans and cats, amastigotes of *L. venezuelensis* were frequently found whithin macrophages, free in the cytoplasm or inside a large phagocytic vacuole (Fig. 1). Inside the vacuole they were normally located at the periphery, in close contact with the vacuolar membrane either on the flagellum side (Fig. 2) or often opposed. Parasites in the cytoplasm vary in size from 2.7 x 2.0 to 5.5 x 4.0 in median section (Figs. 1,2). Amastigotes were surrounded by a 10 nm thick unit membrane. Subpellicular microtubules in cross sections look like circles of about 25 nm in diameter; the mean distance between them is 15 nm and they are linked to each other by mean of thin fibrils (Fig. 3).

The flagellum measuring from 250 to 333 nm in diameter is located at the anterior part of the body within a large flagellar pocket, varying in cross sections from 277 to 833 nm (Fig. 4). The pocket and the flagellum are lined by the unit membrane continuous with that of the body. The flagellum contains nine pairs of peripheral microtubules and two central microtubules with a diameter of 37 nm (Fig. 5). The two central microtubules arise from the axosome (Fig. 6). The flagellum extends for a short distance beyond the flagellar pocket, but it is thinner in the funnel-like part of the pocket (Fig. 4). Associated with the flagellar pocket there are four microtubules (Fig. 3).

A large part of the cell is occupied by a round or ovoid nucleous. It is surrounded by a unit membrane with large pores (Fig. 7).

The kinetoplast appears as a bar of 611-1,388 x 67-111 nm. It is surrounded by two unit membranes (Fig. 2,6,8,10).

Fig. 1. A giant phagocytic vacuole (PV) containing six amastigotes. x 5.400.

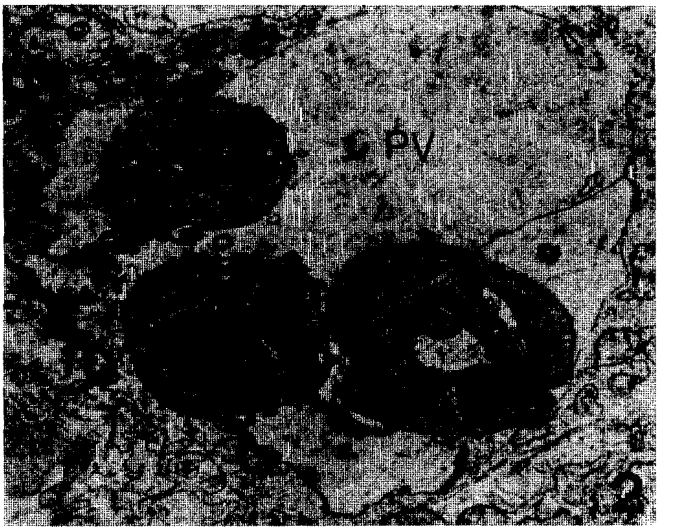

Fig. 2. Three amastigotes within a phagocytic vacuole (PV). Note the direct aposition of the flagellum (F) to the vacuolar membrane; K: Kinetoplast; N: nucleus. x 15.000.

Fig. 3. A transverse section of an amastigote shows four microtubules (mt, arrow), associated with the flagellar pocket. M: mitochondrion; FP: flagellar pocket. x 67.500.

Fig. 4. A longitudinal section of an amastigote is shown. See the shape of the flagellar pocket (FP). An electron dense material is observed in its anterior part. K: kinetoplast; arrow: lipid granules; F: flagellum. x 18.000.

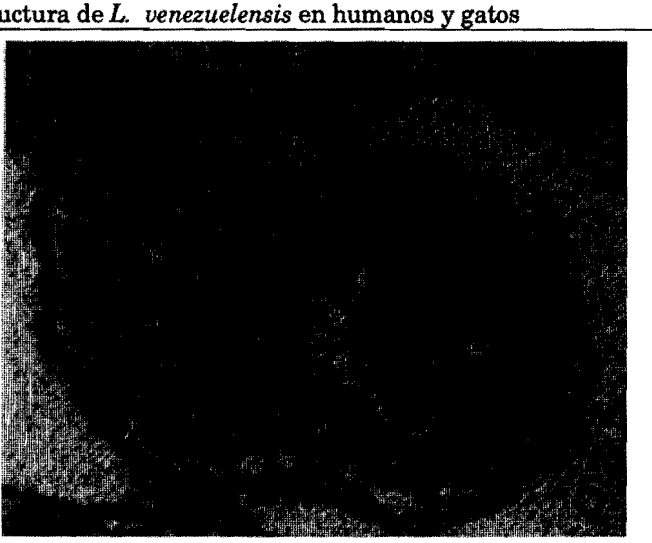

Fig. 5. A cross section of the flagellum in the funnel-like part of the flagellar pocket with nine pairs of peripheral microtubules (pt) and two central microtubules (ct). um: unitary membrane. x 22.500.

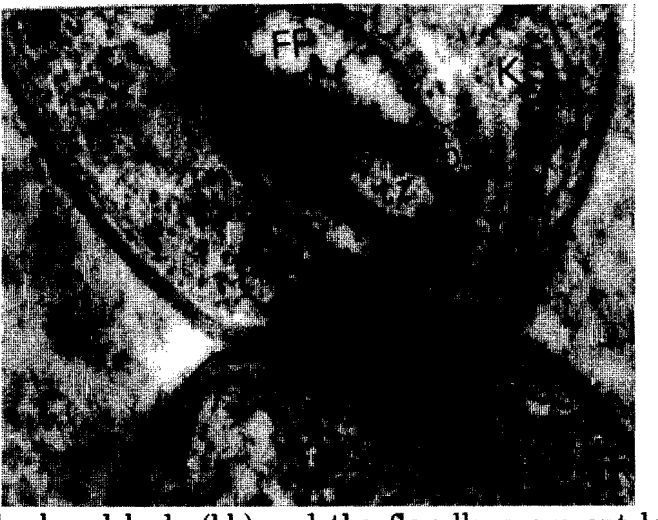

Fig. 6. The basal body (bb) and the flagellum are cut longitudinally. The basal body is close to the kinetoplast (K). See the transitional zone (tz) between the basal body and the axosome (a) and the flagellar pocket (FP). x 54.000.

The basal body, with a diameter of 156-185 nm and a length of 111-130 nm is anterior to kinetoplast, at the base of the flagellum (Fig. 6). A transitional zone measuring 278-333 nm length and a diameter of 185 nm is located between the axosome and the basal body. The basal body contains only nine tubules, and the two central tubules are absent (Fig. 9).

The Golgi apparatus appears like small vesicles of varied size, frequently found between the nucleus and the kinetoplast. Lysosome-like structures and large phagosomes are often seen in the vecinity of the Golgi apparatus. The endoplasmic reticulum consists of vesicles and tubules, having smooth membranes (Fig. 10).

In the cytoplasm there are ribosomes, polysomes, and also large megasomes from 222 nm to 2.5 u in diameter containing numerous vesicles from 233 to 250 nm. There are also lipid-like round or oval bodies, from 367 nm to 1.8 u. As many as nine such bodies may be encountered in a single section of an amastigote (Figs. 7,8,11,12).

One of the outstanding features found in the amastigote of *L. venezuelensis* is a small opening, like a cytopyge, in the cell membrane, located at the posterior pole of the parasite, opposite to the flagellar pocket, measuring from 333 to 389 mm width (Fig. 7,11,12). This structure is lined by two pellicular membranes continuos with the unit membrane of the amastigote. The underlying layer of microtubules is apparently absent in this site. Near this structure, inside the parasite cytoplasm, there is usually a large megasome, while outside the parasite a large vesicle contai-nig a material similar to those found in the megasomes has been observed. This vesicle is lined by a membrane continuous with that of the phagocytic vacuoles (Figs. 7,12).

Some of the amastigotes were found inside a phagocytic vacuole containing a homogeneous electrodense material .

Fig. 7. An amastigote apparently discharging through the excretory structure. Note the neigbouring megasomes (m). See also nuclear material through a nuclear membrane pore. N: nucleus. x 54.000.

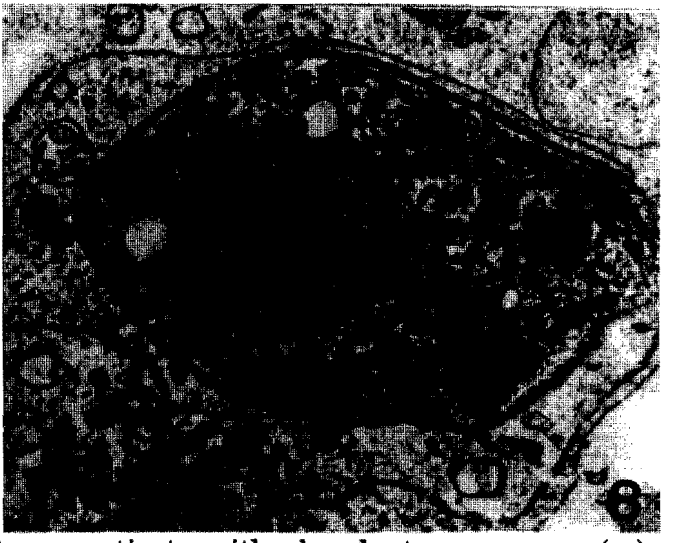

Fig. 8. An amastigote with abundant megasomes (m) and lipid granules (LG). M: mitochondrion; K: kinetoplast. Note the excretory organelle at the posterior pole (arrow). x 21.000.

Fig. 9. A cross section of the basal body with only nine doublet tubules. FP: flagellar pocket; V: vacuole.

Fig. 10. An amastigote showing the Golgi apparatus (GA), the endoplasmic reticulum (ER), kinetoplast (K), mitochondrion (M) and megasomes (m). x 18.000.

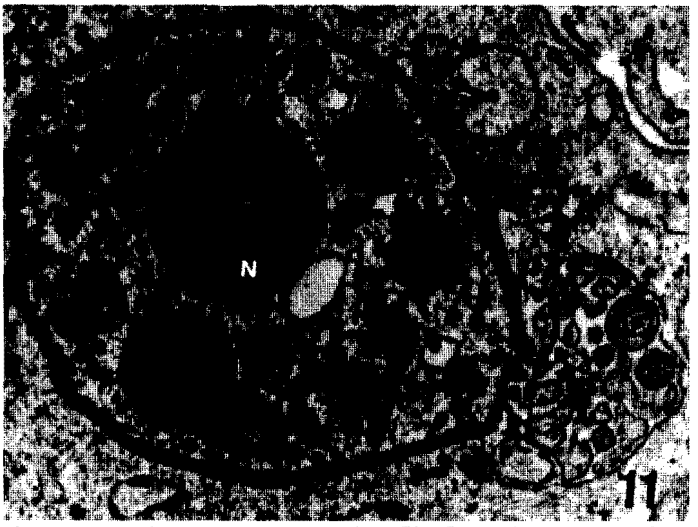

Fig. 11. An amastigote apparently discharging a multivesicular material through the excretory structure. N: nucleus; LG: lipid granules. x 36.000.

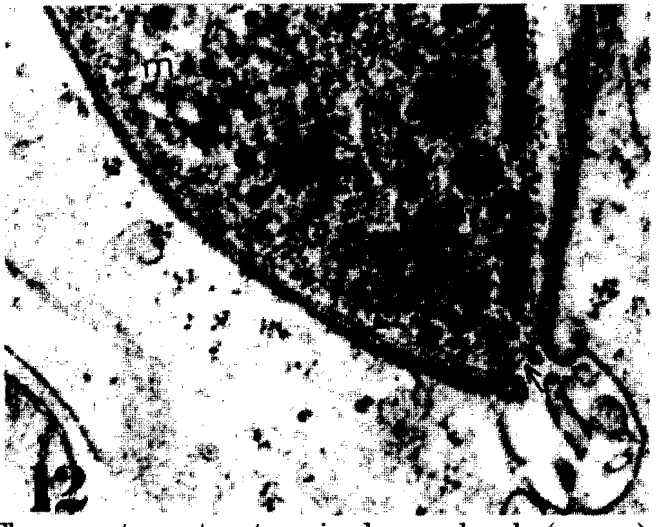

Fig. 12. The excretory structure is shown clearly (arrow). Note the absence of microtubules in this site. m: megasomes. x 60.000.

DISCUSSION

Leishmania venezuelensis appears to have the same basic ultrastructure as other *Leishmania* species. However, the opening in its cell membrane found at the posterior pole seems to be typical of this parasite. Pham *et al.* [17] observed at the posterior pole of *L. tropica* a cup-like invagination of the pellicle to engulf food particles, as the expression of a pinocytic activity. However, the feeding mechanisms of some *Leishmania* species has been investigated by Jadim & Creemers in both *L. tropica* and *L. mexicana*[14,15] and they suggested that the microorganism must be feeding through the flagellar pocket. A similar structure was found in *Trypanosoma brucei*, *T. cruzi* and *Crithidia fasciculata*[20].

Based on our ultrastructural finding we suggest that the structure found at the posterior pole of *L. venezuelensis* is probably excretory.

The megasomes have been also found by Djaczenko *et al.* [10] in *L. donovani*, Alexander & Vickerman[1] in *L. mexicana*, and in other members of the family Trypanosomatidae such as *Crithidia*[7], *Herpetomonas*[9], *Leptomonas*[21], *Phytomonas*[2] and *T. brucei*[16]. These megasomes were found to be a storage site of proteins, lipids and / or lipoproteins [20].

The microtubules associated with the flagellar pocket have been found also in *L. braziliensis*[8], *T. cruzi*, *T. brucei* and *Crithidia*[21] and seem to be a general feature of trypanosomatids.

The different species of *Leishmania* have various numbers of microtubules in their pellicle. In *L. mexicana* there are 130-200[12], in *L. donovani* 80-120[19], in *L. tropica* 90[6], while in *L. venezuelensis* there are 96 to 132. The number of microtubules is probably related to the diameter of the cell. The fibrils that link the microtubules were mentioned by Rudzinska *et al.*[18] in *L. donovani*, by Hentzer & Kobayasi[13] in *L. tropica* and by Souto-Padron *et al.*[21] in trypanosomatids. This association is probably responsible for the rigidity of the cell.

The flagellum of *L. venezuelensis* has the classical axoneme structure (9 + 2); however, near the tip it has nine doublet tubules disposed irregularly. Hentzer & Kobayasi[13] demonstrated in *L. tropica* that the central tubules arising from the axosome do not extend to the end of the flagellum. The central tubules are replaced by one of the peripheral doublet tubules.

Basal bodies occur at the base of both cilia and flagella[6,11]. Hentzer & Kobayasi[13] claim that the basal body contain nine triplet fibrils, while the observation of Garnham[12] with *L. mexicana* shows that it has nine doblet fibrils.

It may be concluded that the minor ultrastructural differences between *L. venezuelensis* and other *Leishmania* species infecting man pointed out in this study do not provide per se any morphological tool for their differentiation, except for the new structure probably involved with excretion.

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REFERENCES

1. ALEXANDER, J. & VICKERMAN, K. Fusion of host cell secondary lysosomes with the parasitophorous vacuoles of *Leishmania mexicana*-infected macrophages. **J. Protozool.**, 1975;22:40-53.

2. ATTIAS, M. & DE SOUZA, W. Fine structure and cytochemistry of *Phytomonas davidi*. **J. Submicroscop. Cytol.**, 1985;17:205-212.

3. BONFANTE-GARRIDO, R.; BARROETA, S.; MEJIA DE ALEJOS, M.A.; MELENDEZ, E.; ARREDONDO, C; URDANETA, R. & URDANETA, I. Urban cutaneous leishmaniasis in Barquisimeto, Venezuela. **PAHO Bull.**, 1987;21:149-155.

4. BONFANTE-GARRIDO, R.; URDANETA, I., URDANETA, R. & ALVARADO, J. Natural infection of cats with *Leishmania* in Barquisimeto, Venezuela. **Trans. R. Soc. Trop. Med. Hyg.**, 1991;85:53.

5. BONFANTE-GARRIDO, R.; MELENDEZ, E.; BARROETA, S.; MEJIA DE ALEJOS, M.A.; MOMEN, H., CUPOLILLO, E., MCMAHON-PRATT, D. & GRIMALDI, G. Jr. Cutaneous leishmaniasis in Western Venezuela caused by infection with *L. venezuelensis* and *L. braziliensis* variants. **Trans. R. Soc. Trop. Med. Hyg.**, 1992;86:141-148.

6. BOURLOND-REINERT, L. & Nicolay, M. Leishmaniose cutanée: Etude ultrastructurale. **Dermatologica (Basel)**, 1975;151:113-124.

7. BROOKER, B.E. The fine structure of *Crithidia fasciculata* with special reference to the organelles involved in the ingestion and digestion of proteins. **Z. Zellforsch**, 116:532-563.

8. CAMPO-AASEN, I.; ALEMAN, C. & CONVIT, J. New organelle observed in *L. braziliensis*. **Trans. R. Soc. Trop. Med. Hyg.**, 1973;67:122-124.

9. DE SOUZA, W.; ROSSI, M. A.; KITAJIMA, E. W.; SANTOS, R. & ROITMAN, I. An electron microscopic study of *Herpetomonas* sp (*Leptomonas pessoai*). **Can. J. Microbiol.**, 1976;22:197-203.

10. DJACZENKO, W.; FILADORO, F. & PIZZI, R. On the presence of multivesicular bodies in *Leishmania donovani*. **Experentia**, 1969;25:666-667.

11. GARDENER, P. J. Pellicle associated structures in the amastigote stage of *Trypanosoma cruzi* and *Leishmania* especies. **Ann. Trop. Med. Parasitol.**, 1974;68:167-176.

12. GARNHAM, P.C.C. The genus *Leishmania*. **Bull. WHO**, 1971;44:477-489.

13. HENTZER, B. & KOBAYASI, T. The ultrastructure of *L. tropica* in skin lesions. **Act. Path. Microb. Scand. Sect. B**, 1977;85:153-160.

14. JADIM, J. M. & CREEMERS, J. L'ultrastructure des formes en rosaces de *L. tropica*, Wright, 1903. **Annals Soc. Belge Med. Trop.**, 1966;46:349.

15. JADIM, J. M. & CREEMERS, J. Etude de l'ultrastructure et de la Biología de *Leishmania mexicana*, Biagi, 1953. II Le formes en rosaces. **Bull. Soc. Path. Exot.**, 1967;60:58.

16. LANGRETH, S.G. & BALBER, A.E. Protein uptake and digestion in bloodstream and cultures forms of *Trypanosoma brucei*. **J. Protozool.**, 1975;22:40-53.

17. PHAM, T.D.; AZAR, H.A.; MOSCOVIC, E.A. & KURBAN, A.K. The ultrastructure of *Leishmania tropica* in the oriental sore. **Ann. Trop. Med. Parasitol.**, 1970;64:1-5.

18. RUDZINSKA, M.A.; D'ALESSANDRO, P.A. & TRAGER, W. The fine structure of *Leishmania donovani* and the role of the kinetoplast in the Leishmania-leptomonad transformation. **J. Protozool.**, 1964;11:166-191.

19. SANYAL, A.B. & SEN GUPTA, P.C. Fine structure of *Leishmania* in dermal leishmanoid. **Trans. R. Soc. Trop. Med. Hyg.**, 1967;61:211-216.

20. SOARES, M. J. & DE SOUZA, W. Cytoplasmic organelles of trypanosomatids: a cytohemical and stereological study. **J. Submicroscop. Cytol. Pathol.**, 1988;20:349-361.

21. SOUTO-PADRON, T.; LIMA, V.M.Q.G.; ROITMAN, I. & DE SOUZA, W. An electron microscopic and cytochemical study of *Leptomonas samueli*. Z. **Parasitenkd.**, 1980;62:127-143.