Journal of Cell and Animal Biology Vol. 2 (6), pp. 129-133, June, 2008
Available online at http://www.academicjournals.org/JCAB
ISSN 1996-0867 © 2007 Academic Journals

Full Length Research Paper

Study on ultrastructure of *Leishmania major* and *Lizard leishmania*

Bahram Kazemi[1, 2]*, Mohammad Hassan Heidari[1, 3], Mehdi Naderi[2], Abbas Piryaei [1, 3] and Mohammad -Reza Nazari -Pouya[2]

[1]Cellular and Molecular Biology Research Center -Shahid Beheshti University M.C., Tehran, I.R. Iran.
[2]Parasitology Department - Shahid Beheshti University, M.C., Tehran, I.R. Iran.
[3]Anatomy Department - Shahid Beheshti University M.C., Tehran, I.R. Iran.

Accepted 16 May, 2008

We compared ultrastructure of *Lizard Leishmania* and *Leishmania major* promastigotes by electron microscope. *Leishmania major* (MRHO/R64/Nadim1) and *Lizard leishmania* promastigotes were mass cultured, collected by centrifugation and washed by PBS buffer three times, the ultrastructural features of both *Leishmania* promastigotes were observed by transmission electron microscope. Their subpellicular microtubules diameter and cytoplasmic membrane thickness are identical, but there is difference in two parasites in respect of distance between kinetoplast and flagella. We could not find golgi apparatus in *Lizard Leishmania*.

Key words: *Lizard Leishmania*, *Leishmania* major, Transmission Electron Microscope.

INTRODUCTION

Leishmaniasis is the result of infection with *Leishmania* species differently manifested due to infection with different types of the parasite (Modabber 1991). The disease is considered as one of the important health problems in 82 countries, since 12 million people are already affected and 1.5 - 2 million people get involved every year (WHO, 1997).

There are some studies (Lewis, 1975, al-Shammary et al., 1995, Anderson and Ellis 1965, Angelopoulos 1970 and Burton 1966) on ultrastructure of *Leishmania* spp. We have isolated *a Leishmania* promastigote by lizard cardiac blood culture (Kazemi et al., 2004b) and used for mice immunization by whole lysate (Kazemi et al., 2002) or fractionated antigen (Kazemi et al 2004a). This *Leishmania* have different habitation in its host as compared with human *Leishmanias* (*Leishmania major*, *Leishmania tropica* and *Leishmania infatum*) which are prevalent in Iran (Kazemi et al., 2004b) and some investigators reported *Lizard leishmania* and its importance in epidemiogical studies of leishmaniasis (Paperna et al., 2001; Belova, 1971; Heish, 1958; Seyedi-Rashti, 1971; Telford, 1979; Belova, 1966; Edeson and Himo, 1973, Pozio et al., 1983; Elwasilia, 1988). For this reason we interested to study ultrastructuraly differences between *Leishmania major* and *Lizard leishmania* promastigotes. We have designed to study ultrastructuraly differences between *Leishmania major* and *Lizard leishmania* promastigote in respect to Golgi apparatus, distance from kinetoplast to flagella, subpellicular microtubules diameter and cytoplasmic membrane thickness by electron microscopy.

*Corresponding author. E-mail: bahram_14@yahoo.com and kazemi@sbmu.ac.ir. Tell: 009821 23872552. Fax: 009821 22439956

MATERIALS AND METHODS

Parasites

L. major (MRHO/IR/64/Nadim 1) has given from School of Public Health, Tehran University of Medical Sciences and *Lizard leishmania* (was frozen) has given from Cellular and Molecular Biology Research Center (Kazemi et al 2004b). *Leishmania* promastigotes have been cultured on NNN medium and mass culture has carried out on $RPMI_{1640}$ culture medium supplemented with 20% fetal calf serum, but *Lizard Leishmania* promastigote has been mass cultured on LB broth bacterial culture medium. *Leishmania* promastigotes have collected by centrifugation and washed by PBS buffer three times.

Electron microscopy processing

The centrifuged promastigotes were fixed by 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) at 4ºC for 24 h, then washed by

Table 1. Comparison between means of subpllicular microtubules diameter of *L. major* and *Lizard leishmania*.

Variables	df	Mean	SD	SDE	T	Significant level	
						90%	99%
Subpellicular microtubule measurement of *lizard leishmania*	90	0.0025	0.0004	0.00005	1.39	1.98	2.86
Subpellicular microtubule measurement of *L. major*	90	0.0024	0.0002	0.00003	1.39	1.98	2.86

SD – standard deviation; SDE- standard deviation error; df – degree of freedom; T = t-test

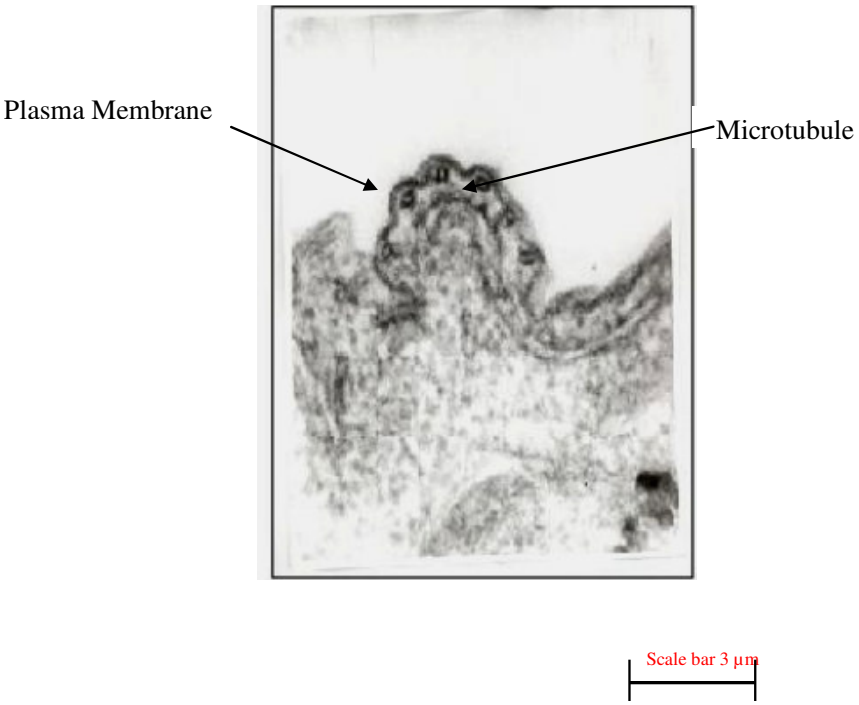

Figure 1. Electron micrograph of *Leishmania major*. It shows the bilayer plasma membrane and subpellicular microtubules. Original magnification: 35000X

phosphate buffer 3 times, post fixed in 1% osmium tetroxide in the same buffer for 1:30 h, dehydrated in graded acetone and embedded in epon 812 resin. Thin sections (50 - 70nm) were prepared and stained with uranyl acetate and lead citrate, and then used a Zeiss EM 900 transmission electron microscope operating at 80 KV for ultrastructural evaluation.

RESULTS

Transmission electron micrographs of both parasites were taken and some ultrastructural features have been measured by Motic software carefully and analyzed by informatics methods. (Motic software; National Optical and Scientific Instruments Inc, San Antonio, Texas, a software developed for digital cameras for vast assortment of functions allowing to view live video, capture images, manipulate, and measure). Subpellicular microtubules diameter of *Lizard leishmania* in respect to T= 1.39 and fd 95 is shorter than of *L. major* with T=1.98 and α = 0.05, with 99% assurancy, their subpellicular microtubules diameter are identical (Table 1, Figure 1 and 2). Thickness of *Lizard leishmania* cytoplasmic membrane in respect to T = 1.985 and fd 147 is larger than of *L. major* with 1.98 and α = 0.05, with 99% assurancy there are difference between measurement of cytoplasmic membrane of both parasites. But in respect to measurement method this difference is described to error of worker and they are identical. (Table 2, Figures 1 and 2). Table 3 and Figures 3 and 4 show that distance from kinetoplast to flagella in *Lizard Leishmania* in respect to T = 1.12 and fd 88 is larger than of *Lizard major* with 2.86 and α = 0.01. We didn't show Golgi apparatus for *Lizard leishmania*.

DISCUSSION

Because the lizard Leishmania can be maintained in laboratory culture medium without any risk of human, it is a useful model organism for studies in field of molecular biology and biochemistry of the genus *Leishmania* (Noyes et al. 1998).

Some investigators suggested that reptilian *Leishmania*

Table 2. Comparison between means of cytoplasmic membrane thickness of *L. major* and *lizard leishmania* promastigotes.

Variables	df	Mean	SD	SDE	T	Significant level	
						90%	99%
Measurement of cytoplasmic membrane of *lizard leishmania*	147	0.030	0.001	0.00035	1.985	1.98	2.86
Measurement of cytoplasmic membrane of *L. major*	147	0.0031	0.001	0.00040	1.985	1.98	2.86

SD – standard deviation. SDE- standard deviation error. df – degree of freedom. T = t-test

Table 3. Comparison of means distance from kinetoplast to flagellum in *L. major* and *Lizard leishmania* promastigote.

Variables	df	Mean	SD	SDE	T	Significant level	
						90%	99%
From kinetoplast to flagellum in *lizard leishmania*	88	0.0039	0.002	0.00001	1.12	1.98	2.86
From kietoplast to flagellum in *L. major*	88	0.0014	0.0003	0.00003	1.12	1.98	2.86

SD – standard deviation SDE- standard deviation error df – degree of freedom. T = t-test

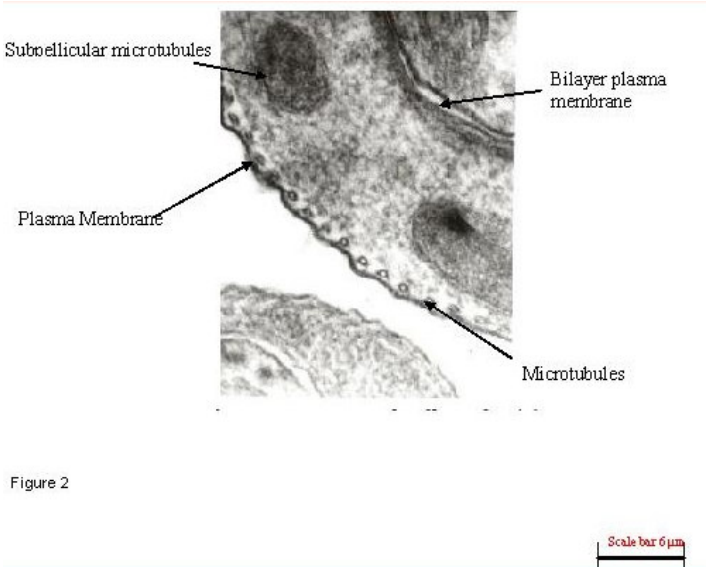

Figure 2. Electron micrograph of *lizard Leishmania*. It shows the bilayer plasma membrane and subpellicular microtubules. Original magnification: 85000X.

evolved from mammalian leishmania besed on DNA and RNA polymerase gene sequences (Croan et al., 1997) and studies of DNA and lipid composition (Simpson and Holz, 1988). There are some reports on electron microscopy studies on some aspects of Leishmania (Ueda-Nakamura et al., 2007; Correa et al., 2007; Castro et al., 2006; Hiam et al., 2006; Uezato et al., 2005; Yoneyama et al., 2006), but there are little informations on comparative study between human Leishmania and *Lizard leishmania*. Some investigators like: Lewis (1975), al-Shammary et al. (1995), Anderson and Ellis (1965), Angelopoulos (1970), Burton (1966) were studied on ultrastructure of *Leishmania* sp. Lewis (1975) have been studied 4 species of reptilian *Leishmania* and one species of human *Leishmania*. He suggests that there are no differences on ultrastructure of mentioned *Leishmania* but he showed differences in diameter of subpellicular microtubules. Lewis (1975) suggested that it can be classified *Leishmania* species by this difference. Lewis (1975) measured reptilian *Leishmania* microtubules at 26.5 nm but 45.6 nm for mammalian *Leishmania*, while we measured *Lizard leishmania* microtubules at 25.8 nm and of *L. major* at 24 nm, while of *plasmodium* sporozoite is 26 ± 1.2 nm (Cyrklaff et al., 2007) and for *Toxoplasma gondii* is 32 nm (Morrissette et al., 1997). Lewis` findings are different from our results, because there are many different species of reptilian Leishmania and Iranian *Lizard leishmania* is different from Lewis report.

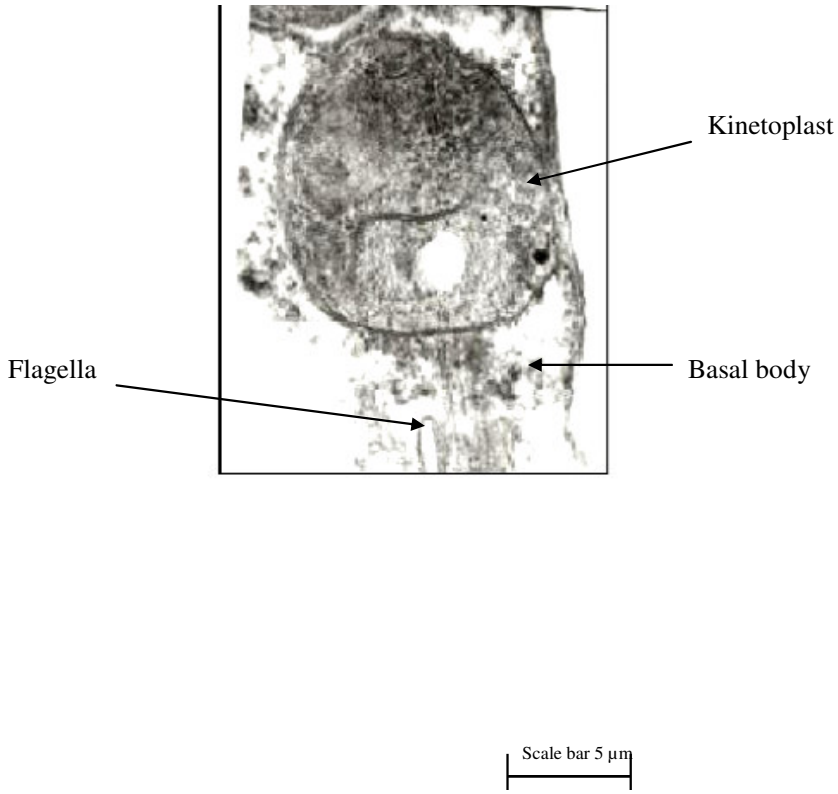

Figure 3. Electron micrograph of *Leishmania major*. It shows the distance from flagella to kinetoplast. Original magnification: 50000X.

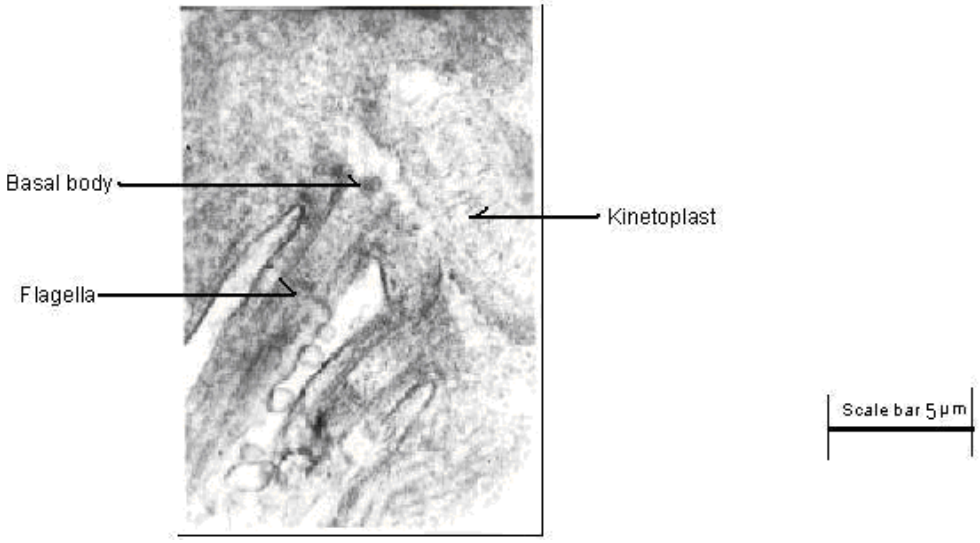

Figure 4. Electron micrograph of *lizard Leishmania.* It shows the distance from flagella to kinetoplast. Original magnification is 50000X.

There is no study on measurement of distance from kinetoplast to flagella in literature, but we showed that this parameter in *Lizard leishmania* promastigote is larger than of *L. major*.

Conclusion

We compared some parameters in *Lizard leishmania* and Leishmania major by electron microscope. Their subplli-

cular microtubuls are identical, but *Lizard leishmania* cytoplasmic membrane is thicker than *L. major and* distance from kinetoplast to flagella in *Lizard leishmania* is larger and there is no Golgi apparatus for *Lizard leishmania* promastigote.

ACKNOWLEDGEMENT

This study was supported by a grant (No 3/685) from Shahid Beheshti University, M.C. and was conducted in Cellular and Molecular Biology Research Center. The authors wish to thank to Mr. Hamidreza Niazi for technical expertise.

REFERENCES

Anderson WA, Ellis RA (1965). Ultrastructure of *trypanosome lewisi*: flagellum, microtubules and kinetoplast. J. Protozool. 12: 483 – 499.

al-Shammary FJ, Shoukrey NM, al-Shewemi SE, Ibrahim EA, al-Zahrani MA, al-Tuwaijri AS (1995). *Leishmania major*: *in vitro* ultrastructural study of the paraxial rod of promastigotes. Int. J. Parasitol. 25: 443-452.

Angelopoulos F (1970). Pellicular microtubules in the family trypanosomatidae. J. Protozool 17: 39-51.

Belova EM (1966). Detection of leptomonads in some species of Lizards in the Turkmen SSR. Med. parazitol (Mosko). 35: 281- 283.

Belova EM (1971). Reptiles and their importance in epidemiology of leishmaniasis. Bul. World Org. 44: 553- 560.

Burton PR (1966). A comparative electron microscopic study of cytoplasmic microtubules and axial unites tubules in a spermatozoan and a protozoan. J. Morphol. 120: 397-424.

Correa JR, Brazil RP, Soares MJ (2007). Leishmania (Viannia) lainsoni (Silveira et al. 1987): ultrastructural aspects of the parasite and skin lesion in experimentally infected hamster (Mesocricetus auratus). Parasitol. Res. 100: 1227-1232.

Croan DG, Morrison DA, Ellis JT (1997). Evolution of the genus *Leishmania* revealed by comparison of DNA and RNA polymerase gene sequences. Mol. Biochem. Parasitol. 89: 149-159.

Cyrklaff M, Kudryashev M, Leis A, Leonard K, Baumeister W, Menard R, Meissner M, Frischknecht F (2007). Cryoelectron tomography reveals periodic material at the inner side of subpellicular microtubules in apicomplexan parasites. J. Exp. Med. 204: 1281-1287.

Edeson JF, Himo J (1973). Leishmania sp in the blood of Lizard (Agama stellio) from Lebanon. Trans. Roy. Soc. Trop. Med. Hyg. 67: 27.

Elwasilia M (1988). Leishmania tarentolae Wenyon,1921 from the gecko taretola annularis in the Sudan. Parasitol. Res. 74: 591-592.

Heish RB (1958). On the Leishmania adleri Sp. from lacertid lizard (Latastid sp) in Kenya. Ann. Trop. Med. Parasit. 52: 63-71.

Hiam A, Sebastien D, George B, Arlette F, Kalil J, Le Pape P (2006). Microtubule target for new antileishmanial drugs based on ethyl3-haloacetamidobenzoates. J. Enzyme Inhib. Med. Chem. 21: 305-312.

Kazemi B, Moazzen F, Abadi A, Ghadjari A, Bandehpour M, Seyed N (2004a). Immunization of Balb/C mice by protein fragments of *lizard Leishmania* promastigote. Pak. J. Biol. Sci. 7: 1699-1702.

Kazemi B,. Tahvildar- Bideroni Gh, Hashemi Feshareki SR, Javadian E (2004b). Isolation a *Lizard Leishmania* promastigote from its natural host in Iran. J. Biol. Sci. 4: 620-623.

Kazemi B, Bayat M, Labibi F (2002). Studies on immunization of Balb/c mice by saroleishmania and resistant to *L. major*. Iranian J. Basic Med. Sci. 5: 164-167.

Lewis DH (1975). Ultrastractural study of promastigotes of *Leishmania* from reptile. J. Protozool. 22: 433 – 452.

Modabber F (1991-1992). Leishmaniasis.TDR Progress.11th Program report. pp: 77-87.

Morrissette NS, Murray JM, Roos DS (1997). Subpellicular microtubules associate with an intramembranous particle lattice in the protozoan parasite Toxoplasma gondii. J. Cell Sci. 110: 35-42.

Noyes HA, Chance M, Croan DG, John T, Ellis JT (1998). *Leishmania (Sauroleishmania)*: A Comment on Classification. Parasitol. Today 14: 167.

Paperna I, Boulard Y, Hering-Hagenbeck SH, Landau I (2001). Description and ultrastructure of Leishmania zuckermani n. sp. amastigotes detected within the erythrocytes of the South African gecko *Pachydactylus turneri* Gray, 1864. Parasite. 8: 349-353.

Pozio E, Gramicia M, Gradoni L, Maroli M (1983). Hemoflagellates in cyrotodactylus kotoschyi) Reptilian Gekkonidae) in Italy. Acta Trop. 40: 399-400.

Seyedi Rashti MA, Nafici A, Nadim A (1971). Further report on Lizard Leishmania in the north part of Iran. J. Trop. Med. Hyg. 74: 70-71.

Simpson L, Holz GJr (1988). The status of Leishmania tarentolae/Trypanosoma platydactyli. Parasitol. Today 4: 115-118.

Telford SRJR, (1979). Evolutionary implication of Leishmania amastigotes in circulating blood cells of Lizards. Parasitolgy 79: 317-324.

Ueda-Nakamura T, Attias M, Souza W (2007). Comparative analysis of megasomes in members of the Leishmania mexicana complex. Res. Microbiol. 158: 456-462.

Uezato H, Kato H, Kayo S, Hagiwara K, Bhutto AM, Katakura K, Nonaka S, Hashiguchi Y (2005). The attachment and entry of Leishmania (Leishmania) major into macrophages: observation by scanning electron microscope. J. Dermatol. 32: 534-540.

WHO (1997). Leishmaniasis. TDR. Progress 13th Program Report. pp: 101-105.

Yoneyama KA, Tanaka AK, Silveria TG, Takahashi HK, Straus AH (2006). Characterization of Leishmania (Viannia) braziliensis membrane microdomains, and their role in macrophage infectivity. J. Lipid Res. 47: 2171- 2178.