Ultrastructural Changes on *Entamoeba histolytica* HM1-IMSS Caused by the Flavan-3-Ol, (−)-Epicatechin

Jacqueline Soto[1], **Consuelo Gómez**[1], **Fernando Calzada**[2], **María Esther Ramírez**[1]

[1] Laboratorio de Biomedicina Molecular 1, Escuela Nacional de Medicina y Homeopatía-IPN, México City, México

[2] Unidad de Investigación Médica en Farmacología de Productos Naturales, CMN, S. XXI, IMSS, México City, México

Abstract

The flavan-3-ol, (−)-epicatechin has been previously identified as the most important antiamoebic compound among the extracts from two medicinal plants: *Rubus coriifolius* and *Geranium mexicanum.* Here we report the effects of epicatechin on *Entamoeba histolytica* morphology, analyzed by electronic microscopy. *E. histolytica* trophozoites were incubated for 48 h at 37 °C in the presence of 1.9 µg/mL epicatechin and processed for electronic microscopy analysis. Epicatechin induced nuclear and cytoplasmic changes in the treated trophozoites. These morphological alterations are identical to the cellular changes experienced by *E. histolytica* trophozoites undergoing programmed cell death (PCD), suggesting that epicatechin could be an alternative compound to treat amoebiasis.

Key words

Entamoeba histolytica · flavan-3-ol, (−)-epicatechin · *Geranium mexicanum* · Geraniaceae · electronic microscopy · morphology

Abbreviation

PCD: programmed cell death

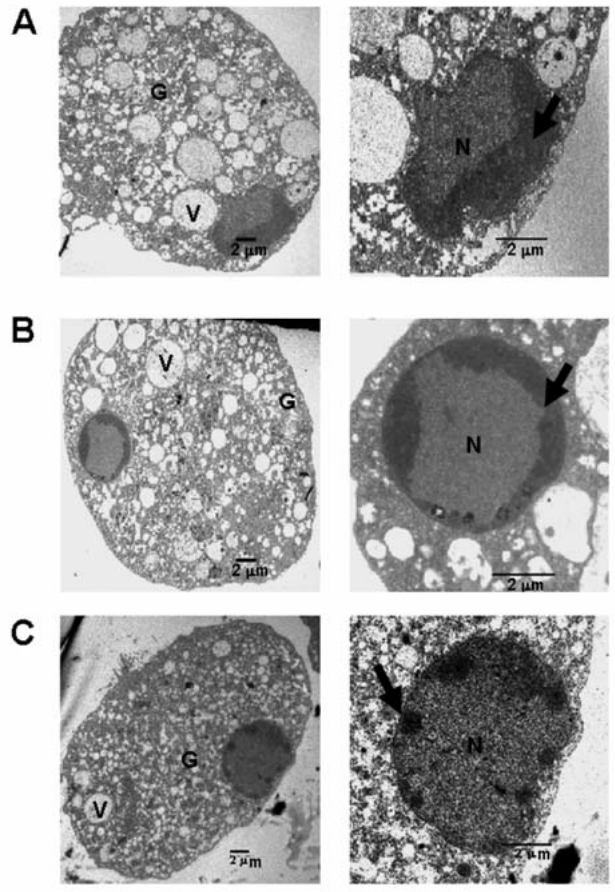

Fig. 1 Ultrastructural analysis of *E. histolytica* trophozoites. Electronic microscopy of trophozoites: **A** without treatment; **B** treated with DMSO; **C** treated with (−)-epicatechin. N, nucleus; V, vacuoles; G, glycogen deposits. Arrows indicate chromatin distribution. Bars 2 µm. Right panels show nuclei close-up.

Amoebiasis is an important worldwide public health problem. The disease is maintained under control with drug treatment; however, there are reports of drug resistance in *E. histolytica in vivo* [1] and *in vitro* [2,3] studies. Therefore, and due to the fact that México is an endemic country for amoebiasis, there is an urgent need to find alternative and safe drugs with a high efficacy in treating amoebiasis. A potential source of new drugs is from plants. There are several studies demonstrating that flavonoids have a potent activity against *E. histolytica*. Calzada et al. isolated antiprotozoal flavonoids from *Helianthemum glomeratum, R. coriifolius,* and *G. mexicanum* [4–6]. In the last two, flavan-3-ol, (−)-epicatechin was the main active compound against *E. histolytica* trophozoites with a 50% inhibitory concentration (IC_{50}) value of 1.9 µg/mL [4]. In an experimental model of *Giardia lamblia* infection, it was also demonstrated that epicatechin has a higher activity than metronidazole and emetine [7]. In this work, we demonstrated that epicatechin induced dramatic morphological changes in *E. histolytica* HM1-IMSS trophozoites.

Epicatechin, obtained from the roots of *G. mexicanum* (Geraniaceae) [4], was incubated with *E. histolytica* trophozoites to determine the ultrastructural alterations associated with its antiamoebic effect. Approximately 95% of the trophozoites experienced morphological changes. Parasites incubated in the presence of epicatechin displayed significant morphological alterations in the nucleus region (**Fig. 1 C**) when compared to untreated and dimethyl sulphoxide (DMSO)-treated trophozoites (**Fig. 1 A** and **B**, respectively). Epicatechin induced chromatin redistribution, forming small clumps around the nuclear membrane. Trophozoites also showed two main cytoplasmic alterations: a significant increase in the number of glycogen deposits and a substantial reduction in the number and size of vacuoles (**Fig. 1 C**). In addition, treated trophozoites maintained their nuclear and plasma membrane integrity (**Fig. 1**, right panels).

The morphological changes associated with a 50% epicatechin inhibitory concentration were identical to the ones produced in *E. histolytica* trophozoites undergoing a PCD phenomenon induced under stress conditions by exposure to G418 aminoglycoside antibiotic [8] and to nitric oxide species [9]. In several studies it has been demonstrated that the mechanisms of catechins in the inhibition of cancer cell growth are cell cycle arrest and cell apoptosis [10–12].

In summary, it was shown that IC_{50} of epicatechin induced morphological changes in *E. histolytica* trophozoites. Further studies are required to establish if such changes could be related with a

PCD phenomenon induced by this flavan-3-ol and to confirm the potential of this molecule as a possible candidate for amoebiasis chemotherapy.

Material and Methods

Epicatechin was isolated from *G. mexicanum* (Voucher Calzada 14405). The extraction and isolation procedure was performed according to the protocol previously reported by Alanis et al. [13]. The plant was collected in Ozumba, State of México, and identified by Ms. Abigail Aguilar (Herbarium IMSSM of the Instituto Mexicano del Seguro Social). A voucher specimen was deposited at the IMSSM Herbarium of the Instituto Mexicano del Seguro Social. The purity of the compound was > 99.9% as determined by HPLC.

E. histolytica HM1-IMSS strain was axenically maintained in TYI-S-33 medium, supplemented with 10% bovine serum and it was used in the log phase of growth [14]. *E. histolytica* trophozoites were incubated for 48 h at 37 °C in the presence of 1.9 µg/mL (IC_{50}) epicatechin in DMSO. The experiments were performed in duplicate. Each test included two control groups: trophozoites treated with DMSO (1%) and trophozoites without treatment [4]. After 48 h incubation, trophozoites were fixed with 3% glutaraldehyde for 2 h and washed three times with 0.1 M glutaraldehyde, 1% $CaCl_2$. Cells were post-fixed with osmium tetraoxide for 2 h. Then trophozoites were washed three times and were dehydrated using increasing ethanol concentrations (10 to 100%) for 10 min. Specimens were treated with different proportions of propylene oxide: alcohol mixtures (2 : 1, 1 : 1, 1 : 2, 1 : 0) for 15 min. Pre-inclusion was done using propylene oxide: resin 2 : 1, 15 min; 1 : 1, 1 h; 1 : 2, 15 min, 0 : 1, 15 min. Polymerization was performed by incubating at 60 °C for 48 h. Thin sections were stained with uranyl acetate followed by lead citrate (both from SP1 Supplies; SP1-Chem) and examined in a JEOL-10-10 transmission electron microscope.

Acknowledgements

MER thanks Dr. María Esther Sánchez, Microscopy Central, ENCB-IPN, México.

This study was supported by CONACyT, México, Project 83384, and by SIP-IPN, México, Project 2008045.

References

1 *Hanna RM, Dahniya MH, Bard SS, El-Betagy A.* Percutaneous catheter drainage in drug-resistant amoebic liver abscess. Trop Med Int Health 2000; 5: 578–581
2 *Orozco E, Hernández F, Rodríguez MA.* Isolation and characterization of mutants resistant to emetine. Mol Biochem Parasitol 1985; 15: 49–59
3 *Prabhu R, Sehgal R, Chakraborti A, Malla N, Ganguly NK, Mahajan RC.* Isolation of emetine resistant clones of *Entamoeba histolytica* by petri dish agar method. Indian J Med Res 2000; 111: 11–13
4 *Calzada F, Cervantes-Martínez JA, Yépez-Mulia L. In vitro* antiprotozoal activity from the roots of *Geranium mexicanum* and its constituents on *Entamoeba histolytica* and *Giardia lamblia.* J Ethnopharmacol 2005; 98: 191–193
5 *Calzada F, Meckes M, Cedillo-Rivera R.* Antiamoebic and antigiardial activity of plant flavonoids. Planta Med 1999; 65: 78–80
6 *Calzada F, López R, Meckes M, Cedillo-Rivera R.* Flavonoids of the aerial parts of *Helianthemum glomeratum.* Pharm Biol 1995; 33: 351–352
7 *Barbosa E, Calzada F, Campos F. In vivo* antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. J Ethnopharmacol 2007; 109: 552–554
8 *Villalba JD, Gómez C, Medel O, Sanchez V, Carrero JC, Shibayama M, Pérez DG.* Programmed cell death in *Entamoeba histolytica* induced by the aminoglycoside G418. Microbiology 2007; 153: 3852–3863
9 *Ramos E, Olivos-García A, Nequiz M, Saavedra E, Tello E, Saralegui A, Montfort I, Pérez Tamayo R. Entamoeba histolytica*: apoptosis induced *in vitro* by nitric oxide species. Exp Parasitol 2007; 116: 257–265
10 *Yang GY, Liao J, Li C, Chung J, Yurkow EJ, Ho CT, Yang CS.* Effect of black and green tea polyphenols on c-jun phosphorylation and H_2O_2 production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction. Carcinogenesis 2000; 21: 2035–2039
11 *Ahmad N, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H.* Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997; 89: 1881–1886
12 *Hibasami H, Komiya T, Achiwa Y, Ohnishi K, Kojima T, Nakanishi K, Akashi K, Hara Y.* Induction of apoptosis in human stomach cancer cells by green tea catechins. Oncol Rep 1998; 5: 527–529
13 *Alanis AD, Calzada F, Cedillo-Rivera R, Meckes M.* Antiprotozoal activity of the constituents of *Rubus coriifolius.* Phytother Res 2003; 17: 681–682
14 *Diamond LS, Harlow DR, Cunnick CC.* A new medium for axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans R Soc Trop Med Hyg 1978; 72: 431–432

received August 13, 2009
revised October 14, 2009
accepted October 19, 2009

Bibliography
DOI http://dx.doi.org/10.1055/s-0029-1240599
Published online November 16, 2009
Planta Med 2010; 76: 611–612

ISSN 0032-0943

Correspondence
Prof. Dr. María Esther Ramírez
Lab. de Biología Molecular 1
Escuela Nacional de Medicina y Homeopatía-IPN
Guillermo Massieu Helguera, No. 239, Fracc. La Escalera,
Ticomán, CP 07320
Mexico City
Mexico
Phone: + 55 57 29 60 00 ext. 5 55 34
Fax: + 55 57 29 60 00 ext. 5 55 34
estherramirezmoreno@yahoo.com