Effect of bovine lactoferrin in a therapeutic hamster model of hepatic amoebiasis[1]

Cynthia Ordaz-Pichardo, Nidia León-Sicairos, Verónica Ivonne Hernández-Ramírez, Patricia Talamás-Rohana, and Mireya de la Garza

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

Abstract: *Entamoeba histolytica* is the causative agent of amoebiasis, a disease that produces dysentery as a result of the perforation of the large intestine. This parasite often invades other organs, primarily the liver, leading to an amoebic liver abscess (ALA), which can cause death. Metronidazole is the drug of choice for the treatment of ALA; however, it produces toxic side effects in patients. Lactoferrin (Lf) is a glycoprotein of the innate immune response that sequesters iron in the mucosae. Lf possesses immune-regulatory properties, such as antiinflammatory and antioxidant activities. Moreover, the microbicidal activity of apoLf, which lacks bound iron, has been shown. In this study, we evaluated the therapeutic effect of bovine Lf (bLf) against ALA in a model of hepatic amoebiasis in hamsters. Interestingly, hamsters treated intragastrically with Lf (2.5 mg/100 g mass) over a period of 8 days showed no clinical signs of disease and ALA was effectively decreased, with only 0.63% detectable lesion, compared with 63% in untreated animals. Furthermore, liver function and blood cells approached normal levels among those receiving bLf treatment. These results suggest that bLf may aid in the therapy of amoebiasis, likely without producing undesirable effects in patients.

Key words: amoebic liver abscess, *Entamoeba histolytica*, bovine lactoferrin, metronidazole.

Résumé : *Entamoeba histolytica* est l'agent causal de l'amibiase, une maladie qui produit une dysenterie qui résulte de la perforation du gros intestin. Ce parasite envahit souvent d'autres organes, surtout le foie, produisant des abcès hépatiques (amibiase hépatique ou cutanée) qui peuvent causer la mort. Le métronidazole est un médicament de choix pour le traitement de l'amibiase hépatique; cependant il produit des effets secondaires toxiques chez les patients. La lactoferrine (Lf) est une glycoprotéine de la réponse immune innée qui séquestre le fer dans les muqueuses. La Lf possède des propriétés régulatrices immunitaires, notamment des activités anti-inflammatoires et antioxydantes. De plus, l'activité microbiocide de l'apoLf qui est dépourvue de fer lié, a été démontrée. Dans cette étude, nous avons évalué l'effet thérapeutique de la Lf bovine envers l'amibiase hépatique chez le hamster. Fait intéressant, les hamsters traités à la lactoferrine (2,5 mg/100 g poids) intragastrique pendant 8 jours ne montraient pas de signes cliniques de la maladie, et l'amibiase hépatique était effectivement diminuée avec seulement 0,63 % de lésions détectables, comparativement à 63 % chez les animaux non traités. De plus, les fonctions hépatiques et la formule sanguine des animaux traités à la bLf s'approchaient de la normale. Ces résultats suggèrent que la bLf pourrait contribuer à la thérapie de l'amibiase, probablement sans produire d'effets indésirables chez les patients.

Mots-clés : amibiase hépatique, *Entamoeba histolytica*, lactoferrine bovine, métronidazole.

[Traduit par la Rédaction]

Introduction

Amoebiasis is a parasitic disease that is caused by the protozoan *Entamoeba histolytica.* This disease affects approximately 50 million people and causes 40 000–100 000 deaths annually worldwide. All people are at risk of infection, although infection/disease occurs predominantly among infants and travelers to endemic countries (Haque et al. 2003; Stanley 2003; Tannich et al. 2003). At the onset of amoebiasis, trophozoites invade the mucosa of the large intestine, causing colitis, dysentery, and ulcers. In severe cases, amoebae spread to the liver, causing an amoebic liver abscess

Received 1 August 2011. Revision received 17 October 2011. Accepted 25 October 2011. Published at www.nrcresearchpress.com/bcb on 14 February 2012.

C. Ordaz-Pichardo. Laboratorio de Biología Celular y Productos Naturales, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera No. 239 Fraccionamiento La Escalera, Ticomán, D.F. 07320, México.
N. León-Sicairos. Unidad de Investigación de la Facultad de Medicina, Universidad Autónoma de Sinaloa. Av. Cedros y Sauces Fraccionamiento Los Fresnos, Culiacán, Sinaloa 80246, México.
V.I. Hernández-Ramírez and P. Talamás-Rohana. Departamento de Infectómica y Patogénesis Experimental, Centro de Investigación y de Estudios Avanzados del IPN. Apdo. 14-740, México, D.F. 07000, México.
M. de la Garza. Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del IPN. Apdo. 14-740, México, D. F. 07000, México.

Corresponding author: Mireya de la Garza (e-mail: mireya@cell.cinvestav.mx).

[1]This article is part of a Special Issue entitled Lactoferrin and has undergone the Journal's usual peer review process.

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

(ALA). Therefore, treatment of amoebiasis should include luminal and (or) extraluminal agents, depending on the site of infection (Roe 1983; Martínez-Palomo 1987; Dobiás et al. 1994).

Lactoferrin (Lf) is a cationic iron-binding glycoprotein belonging to the mammalian nonspecific innate immune response. Lf is secreted in the mucosae and by neutrophils at sites of infection to sequester iron and deprive invaders of this element that is essential for their multiplication and invasion. The concentration of human Lf (hLf) naturally increases in the blood during infections (Arnold et al. 1982; Valenti et al. 2004). Human and bovine Lf (bLf) interact with pathogen membranes causing cytolysis, including multidrug resistant strains (Baveye et al. 1999; Flores-Villaseñor et al. 2010). In addition, Lf can boost the effect of drugs and antibiotics against pathogens (Kuipers et al. 1999; Diarra et al. 2002; van der Strate et al. 2003).

Previous studies have shown that Lf causes 2 contradictory effects on *E. histolytica* trophozoites cultured in vitro. Human holoLf, the iron-loaded form, is used by amoebae as an iron source (León-Sicairos et al. 2005). However, human apoLf (without iron) binds to the amoebic cytoplasmic membrane and displays amoebicidal activity. Additionally, human Lf synergizes with IgA and lysozyme to inhibit the growth of amoebae in vitro (León-Sicairos et al. 2006*a*). Given that Lf, IgA, and lysozyme are secreted together in the mucosa, they could protect against amoebiasis. Interestingly, both hLf and bLf exhibit a synergistic effect with metronidazole (Mtz) on cultured in vitro amoebae (León-Sicairos et al. 2006*b*). The ingestion of Lf produces lactoferricin peptides (Lfcins), which reach the blood and all body organs and are often more active than the native protein (Yamauchi et al. 1993; Wakabayashi et al. 2003; Gifford et al. 2005). Because bLf and bLfcin are amoebicidal in vitro (León-Sicairos et al. 2006*a*; León-Sicairos et al. 2006*b*; López-Soto et al. 2010), oral doses of bLf may be useful in the treatment of amoebiasis.

Mtz has remained the drug of choice for the treatment of extra-intestinal amoebiasis. However, many patients do not tolerate this medication because of its side effects. Moreover, Mtz is toxic and mutagenic for microorganisms by inducing DNA breaks and could be carcinogenic because it produces chromosomal aberrations in mouse peripheral lymphocytes (Roe 1983; Dobiás et al. 1994). In addition, strains resistant to Mtz have been found among cultured *E. histolytica* (Upcroft and Upcroft 2001). Thus, the need for an effective and safe antiamoebic agent is increasing.

Lf is normally present in the intestinal mucosa, and thus, this protein should interact with amoebic trophozoites to prevent their reproduction; however, in many cases, amoebae are able to multiply and cause disease. The aim of this study was to determine the effect of intragastrically administered bLf on amoebic liver abscesses induced in hamsters, in comparison with Mtz treatment.

Materials and methods

Reagents

bLf was obtained from DMV International; it was 90% pure. Mtz (Vertisal 500) was purchased from Silanes Laboratory, Mexico.

Amoeba strain and growth conditions

Trophozoites from the *E. histolytica* HM-1:IMSS strain were axenically grown in BI-S-33 medium (Diamond et al. 1978) supplemented with 17% bovine serum (*v*/*v*) (Biofluid, Rockville, Md.). They were cultured in glass screw-cap tubes at 37 °C for 48 h. Tubes were placed in an ice bath for 10 min to detach amoebae from the tube walls, and trophozoites were harvested by centrifugation at 500*g* for 5 min and washed twice with PBS. Cell viability was determined by trypan blue dye exclusion in amoebae observed under a light microscope in a Neubauer chamber.

Model of ALA in hamster

Care of animals

The induction of hepatic amoebiasis in hamsters was performed in accordance with the International Norms of Care and Use of Laboratory Animals (NOM 062-200-1999). Experiments were conducted at the Animal Care Unit at the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Mexico. In all cases, 2-month-old male Syrian golden hamsters (*Mesocrisetus auratus*) with an average mass of 100 g were used.

Preservation of amoeba virulence

To activate the virulence of the amoebae to produce disease, 3 hamsters were intraperitoneally anesthetized with sodium pentobarbital (Anestesal, Smith Kline, Mexico), and 1 hepatic lobe was inoculated with 1.5×10^6 trophozoites in 0.2 mL of Diamond medium (from a culture at 48 h) (Tsutsumi et al. 1992). They were humanely sacrificed at day 7. Subsequently, a liver fragment was excised under sterile conditions and transferred to a tube with culture medium supplemented with serum, streptomycin (500 µg/mL), and penicillin (500 U/mL) and cultured for 24–48 h at 37 °C. Trophozoites were recovered from the infected liver tissue and reinoculated into hamsters 6 times, with a gradual reduction in antibiotic concentration until its elimination. These virulent trophozoites were resuspended in culture medium without serum prior to use in healing assays.

Assays for ALA healing

The method of Ordaz-Pichardo et al. (2005) was used for the induction and the treatment of ALA. Eight groups of 15 hamsters were formed. Hamsters were housed individually for 4 days and starved for 12 h before surgery. All treatments were initiated on the fourth day after the induction of ALA. Hamsters were orally intubated daily using a gastric tube with 0.2 mL water (controls) or with treatments over a period of 8 days. All groups were monitored daily, and alterations in the animals' behavior were recorded. Several parameters (food and water ingestion, fecal characteristics, etc.) and the initial and final masses were documented for all animals.

Group I was the negative control for ALA; hamsters were intrahepatically inoculated with culture medium alone with no treatment. Group II was the positive control for ALA development at the onset of treatment; hamsters were inoculated with 1.5×10^6 amoebae, without treatment, and sacrificed at day 3. Group III was the positive control for ALA development without treatment; hamsters were inoculated with amoebae and sacrificed at day 12. Group IV was

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

the positive control for ALA healing; hamsters were inoculated with amoebae and treated with a high dose of Mtz (5 mg/100 g animal mass; this dose has been standardized by several researchers in healing experimental infections of ALA in hamsters (Hernández-López and Escobedo-Salinas 1970; Martínez-Gigena et al. 1992). In Group V, hamsters were inoculated with amoebae and treated with a low dose of Mtz (0.5 mg/100 g mass). In Group VI, hamsters were inoculated with amoebae and treated with a high dose of bLf (10 mg/100 g mass). In Group VII, hamsters were inoculated with amoebae and treated with a low dose of bLf (2.5 mg/100 g mass). In Group VIII, hamsters were inoculated with amoebae and treated with a combination of Mtz (0.5 mg/100 g mass) and bLf (2.5 mg/100 g mass).

Hamsters were sacrificed by cardiac puncture 24 h after the final treatment. Hepatic lesions were macroscopically characterized, and the livers were dissected and weighed. Representative samples from lesions and from healthy tissues were fixed in 10% formaldehyde in PBS, and several sections were stained with hematoxylin–eosin for histopathological analysis. The person reading the histology slides was blinded as to the intervention group.

Liver enzymatic activities and other blood parameters

Sera from 10 hamsters from each group were analyzed for liver proteins and an enzymatic profile (Sánchez-Ramírez et al. 2001). The enzymes tested were lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT). Additionally, bilirubins, albumin, globulin, and total protein were measured. All tests were analyzed with a Hitachi 917 instrument (Roche Diagnostics). Glucose, cholesterol, and triglycerides were analyzed with a Cobas Mira System (Roche Diagnostics). Additionally, several blood parameters were quantified (leukocytes, lymphocytes, monocytes, granulocytes, erythrocytes, hemoglobin, hematocrit, and platelets) using a Beckman Coulter Ac-T instrument.

Statistical analysis

An analysis of variance was performed to detect significant differences among groups with distinct antiamoebic treatments.

Results

Induction of ALA and treatments

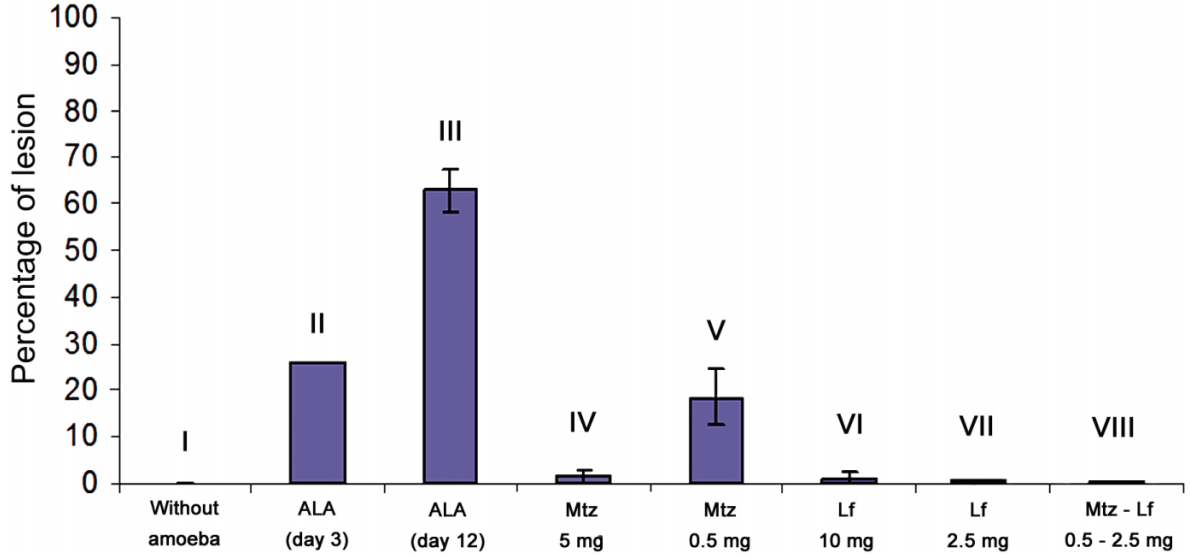
First, to acquire a virulent strain of *E. histolytica,* trophozoites were passaged 6 times through hamster livers, and this strain was used to produce ALA in the treatment groups. Figure 1 shows the results of all the different treatment schemes. The first bar shows Group I (negative control without amoebae and treatment with 0% ALA). Bar II shows Group II, at day 3 postinfection; an ALA of ~1 g was obtained (26% lesion on average), and this percent increased over the following days (63% lesion in Group III at day 12). In the latter case, hamsters displayed abdominal distension, weight loss, dyspnea, and lethargy. Using these data, treatment schemes were established and the percent lesion was calculated. In Group IV, which was treated with Mtz (5 mg/100 g mass), ALA was 1.5% on average. Hamsters in this group exhibited stress, slight tachycardia and several animals had diarrhea during the course of treatment and showed slightly aggressive behavior. Hamsters in this group were nervous, with ruffled fur; at sacrifice, the levels of intestinal gas had increased and the fecal content was watery. Therefore, Mtz was toxic to these animals. In Group V, which was treated with a 10-fold lower concentration of Mtz (0.5 mg/100 g mass), hamsters did not show clinical signs, but ALA was present at a level of 18%. In the group treated with bLf (Group VI, 10 mg/100 g mass), there was 100% survival, and hamsters did not show clinical signs of disease development; ALA in this group was 1.2%. Histopathologically, these animals did not show any alterations in the kidney, lung, spleen, and heart (data not shown). Unexpectedly, in the group treated with a minor dose of bLf (Group VII, 2.5 mg/100 g mass), hamsters showed no clinical signs and ALA was 0.63%. Finally, hamsters treated with the combination of Mtz (0.5 mg) and bLf (2.5 mg) (Group VIII) presented with neither clinical signs nor altered behavior and ALA was effectively eliminated (~0.15% lesion). Thus, bLf significantly diminished the amoebic hepatic abscess. The combination of a reduced concentration of both Mtz and Lf also had a positive effect in hamsters and nearly abolished ALA.

Hepatic lesions in hamsters treated with antiamoebics

Figure 2 displays representative samples of livers from hamsters treated with 8 doses of each antiamoebic: Lf, Mtz, or a mixture of the two. The lower panels in each group show the histopathological analysis of the liver samples. As expected, in panel I, a liver from a healthy hamster (Group I) demonstrates that the intraperitoneal inoculation of culture medium does not affect the liver lobes; the hepatic tissue is intact, exhibiting connections between hepatocytes, with well-defined nuclei and a typical staining pattern for the cells. In panel II, a liver from a hamster infected with amoebae and sacrificed at day 3 (Group II) exhibits an encapsulated ALA (arrow) that was white-yellow and lumpy and cut hard upon dissection. Some cellular infiltrates and amoebae (arrows, lower panel) are present. In panel III, a liver from a hamster from Group III infected with amoebae for 12 days exhibited yellowish, lumpy ALA that released fluid when dissected. Cellular infiltrates of acute and chronic types are present, indicating an amoebic invasion of the tissue (arrows). Moreover, the intercellular connections are lost, with erythrocytes in the intervening space. In some microscopic fields (not shown), fibroblasts were observed, corresponding to the abscess capsule. In panel IV, which displays a liver from the higher Mtz dose (Group IV), a very small abscess (arrow) is observed at the diaphragmatic edge, with fibrillar material surrounding the lesion. In this group, livers exhibited hepatomegaly, and the breakage of cellular junctions, amoebic detritus, cellular infiltration, erythrocytes, and vascularization were commonly observed by histological analysis. In contrast, when one-tenth of the Mtz dose was applied (Group V, panel V), ALA was not completely healed, with lesions present primarily on the surface of the right lobe (arrow). In this case, the lesion was also yellowish and lumpy in appearance and smooth when it was dissected. Vascular congestion, the loss of cellular junctions, and a small number of polymorphonuclear cells are present. Panel VI includes a liver from an animal treated with 10 mg bLf/100 g mass

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

Fig. 1. Percent liver lesion in hamsters exposed to different treatments. Amoebic liver abscesses (ALAs) were induced by intrahepatic inoculation of 1.5×10^6 trophozoites in 0.2 mL of medium. Treatments were initiated at 4 days postinfection, when the abscess reached an average of 26% of the total liver mass. Group I, negative control for ALA; hamsters without treatment were inoculated with culture medium without serum and without trophozoites. Group II, positive control for ALA development at the onset of treatment; hamsters were inoculated with amoebae, without treatment, and sacrificed at day 3. Group III, positive control for ALA development without treatment; hamsters were inoculated with amoebae and sacrificed at day 12. Group IV, positive control for ALA healing; hamsters were inoculated with amoebae and treated with a high dose of metronidazole (Mtz) (5 mg/100 g animal mass). Group V, hamsters were inoculated with amoebae and treated with a low dose of Mtz (0.5 mg/100 g mass). Group VI, hamsters were inoculated with amoebae and treated with bovine lactoferrin (bLf) (10 mg/100 g mass). Group VII, hamsters were inoculated with amoebae and treated with bLf (2.5 mg/100 g mass). Group VIII, hamsters were inoculated with amoebae and treated with a combination of Mtz (0.5 mg/100 g mass) and bLf (2.5 mg/100 g mass).



(Group VI). In this case, a small liver lesion is shown in the left lobe (arrow), which was beige in appearance. Panel VII (Group VII) shows a nearly imperceptible lesion. Finally, panel VIII shows the liver of an animal treated with the combination of Mtz and Lf, which is apparently healed. In both Lf treatments (panels VI, VII, and VIII), there were no alterations of the hepatic morphology, trophozoites, or inflammatory cells.

Proteins, enzymes, and blood cells return to normal levels in hamsters treated with Lf

To analyze whether the induction of ALA and treatment with Lf and Mtz altered hamster health, a hepatic profile was performed for several proteins among hamsters in each group. Data from all experimental groups are presented in Table 1. In the second and third columns, reference values for healthy humans and for ALA patients are shown, respectively, corresponding to ranges found in the literature. The fourth and fifth columns report values obtained at day 12 for healthy hamsters and for those in which a hepatic abscess was induced, respectively, corresponding to the ranges found in this work. The sixth seventh columns list values obtained in hamsters treated with Mtz (5 and 0.5 mg/100 g mass, respectively). The eighth and ninth columns show values among hamsters treated with bLf (10 and 2.5 mg/100 g mass), and the tenth column lists values among hamsters treated with the combination of Mtz–bLf (0.5:2.5 mg/100 g mass).

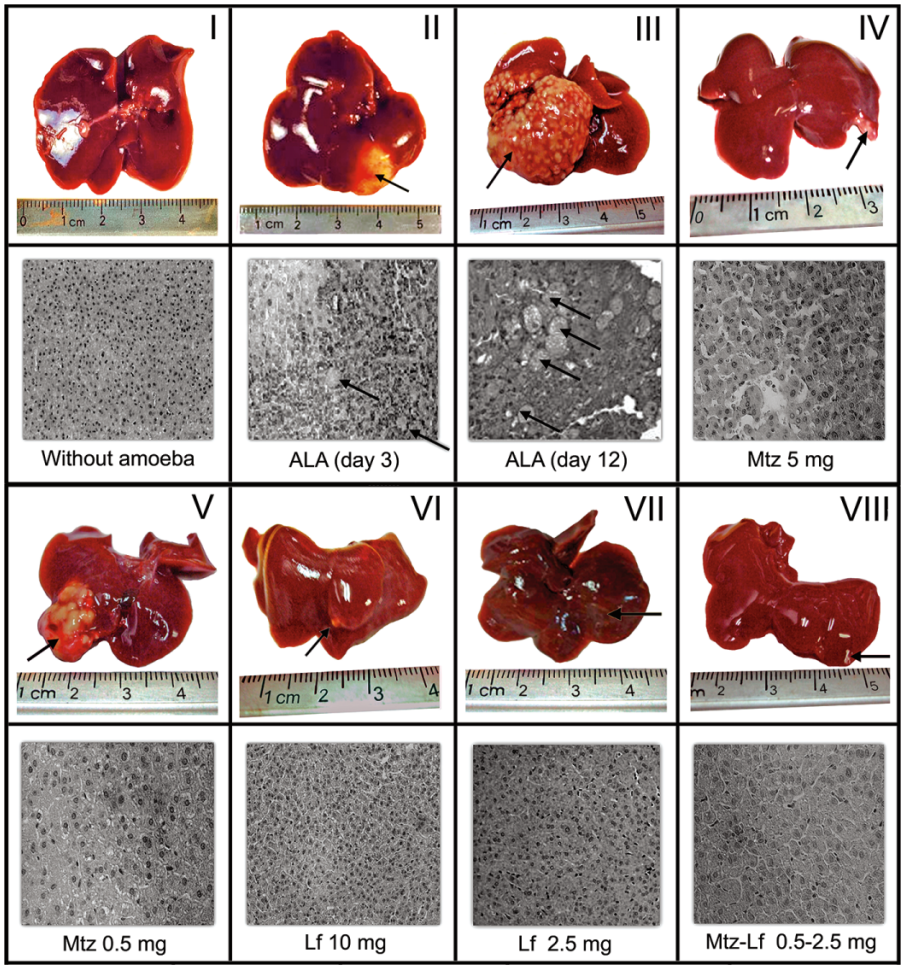
As expected, we found differences in the values of the hepatic protein profile between healthy humans and hamsters. In humans suffering from ALA, these values increase ~2-fold, except for albumin, which declines considerably. However, in hamsters in which ALA was induced, only ALP and total and indirect bilirubin levels increased. Among animals treated with 5 mg/100 g mass of Mtz, enzyme levels were similar to those of healthy hamsters, except for albumin, which remained depleted as occurs in humans. Interestingly, with 0.5 mg Mtz/100 g mass, the level of ALP increased to the value exhibited by untreated hamsters with ALA, suggesting that a lower dose of Mtz compared with the therapeutic dose is not sufficient to result in the complete recovery of the liver.

Both treatments with Lf (10 and 2.5 mg/100 g mass) led to normal hepatic enzyme levels, suggesting that bLf modulates the healing of ALA. However, albumin remained depleted. In the group of animals treated with the combination of Mtz–bLf (0.5:2.5 mg/100 g mass), all of the values were augmented to ranges found among healthy hamsters, and albumin levels increased slightly. Taken together, these results suggest that during the establishment of ALA, the protein profile is metabolically altered because of the infection; however, when Lf is applied, hepatic function is restored.

Leukocyte and granulocyte levels increased 2- to 3-fold during infection. However, treatments with bLf returned these cells to normal values whereas hamsters treated only with Mtz maintained slightly increased levels until sacrifice. The number of all other cells analyzed was not changed by the abscess or treatments (data not shown). Furthermore, glucose levels decreased and triglycerides increased (each 3- to 4-fold), although both reached the normal values with the Mtz and bLf treatments.

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

Fig. 2. Representative samples of livers from hamsters treated with 8 doses of each of the following antiamoebic: bovine lactoferrin (bLf), metronidazole (Mtz), or the combination of both (Mtz–bLf), corresponding to the groups of hamsters described in Fig. 1. The lower panels in each group show the histopathological analysis of the liver specimens. ALA, amoebic liver abscess.



Discussion

Amoebiasis is a parasitic disease that is transmitted by cysts through the fecal-oral route; thus, it is a serious problem in developing countries with poor sanitary conditions. In some regions of the world, the virulence of *E. histolytica* strains leads to a high incidence of intestinal amoebiasis and ALA. A high prevalence of amoebiasis has been reported in Mexico and several countries in South America, Africa, and Asia (Barreda et al. 2002*a*).

Different types of drugs have been tested for use in treating amoebiasis. According to their site of action, these drugs have been classified as luminals (e.g., quinfamide) and both luminals and extraluminals (e.g., Mtz and emetine) (Barreda et al. 2002*b*). All of the drugs currently employed produce unfavorable side effects in patients, and some of them induce severe tissue damage. In addition, when they are used at high doses and (or) for long-term management of infection, selection for drug-resistant parasites can occur. Mtz, which remains the drug of choice for treating ALA, is toxic, causing cephalea, nausea, vomiting, abdominal pain, and other side effects. More important, Mtz may be carcinogenic to patients because it causes DNA breakage and chromosomal aberrations in cultured cells and is carcinogenic in animal tests (Browman and Rand 1980; Roe 1983; Mudry et al. 1994; Dobiás et al. 1994; Kapoor et al. 1999; el-Nahas and el-Ashmawy 2004).

Therefore, it is of primary importance to discover new drugs to eradicate *E. histolytica* from intestinal and hepatic abscesses without causing injury to patients. In this regard, one alternative compound is Lf and its derived peptides. Lf has the benefit of not causing deleterious side effects in people; on the contrary, it is an immune modulator that promotes the antiinflammatory response (Baveye et al. 1999; Actor et al. 2009). hLf and bLf have been assayed as antimicrobials against a broad range of pathogenic species, including viruses, bacteria, fungi, and parasites (Brock 2002; Jenssen and Hancock 2009). In addition, combining Lf with other milk proteins, such as IgA and lysozyme, increases its bactericidal and amoebicidal effect (Leitch and Willcox 1999; Vaerman 1984; León-Sicairos et al. 2006*a*). Lf also provides adequate nutrition to breastfed infants, aids in defense against infections, and facilitates the optimal development of important physiological functions in newborns, such as iron absorption, bifidogenic action, immune protection, and the rapid development of the intestinal mucosa (Lönnerdal 2003).

Lf has already been a helpful option against diverse enteric pathogens in clinical trials. Supplementation with bLf prevented

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

Table 1. Profile of serum from hamster after different treatments.

	Humans			Hamsters		
	Healthy	With ALA	Reference	Healthy (n = 10)	With ALA* (n = 10)	Metronidazole* (5 mg) (n = 10)
LDH (U/L)	240–480	207 (n = 1)	Gill et al. 2002	473–826	118–415	127–673
		347–2587 (n = 2)	Tamez et al. 2009			
ALP (U/L)	39–117	Twice the normal value (n = 67)	Katzenstein et al.1982	131–166	214–468	131–146
		116 (n = 1)	Gill et al. 2002			
		407.68±343.42(n = 39)	Wiwanitkit 2002			
		>120 (n = 589)	Wells and Arguedas 2004			
		259 (n = 14)	Graillet et al. 2008			
		50–371 (n = 2)	Tamez et al. 2009			
GGT (U/L)	5–39	25–103 (n = 2)	Tamez et al. 2009	1–8	0–5	0–5
GOT (UI/L)	5–40	>40 (n = 67)	Katzenstein et al.1982	67–166	55–154	47–112
		>95 (n = 9)	Villalobos et al. 1982			
		92.62±118.74 (n = 39)	Wiwanitkit 2002			
		1 to 2.5 times as that the normal value (n = 459)	Wells and Arguedas 2004			
		28–783 (n = 2)	Tamez et al. 2009			
GPT (UI/L)	5–40	>95 (n = 9)	Villalobos et al. 1982	55–131	27–73	37–64
		57 (n = 1)	Gill et al. 2002			
		83.74±107.84 (n = 39)	Wiwanitkit 2002			
		89–664 (n = 2)	Tamez et al. 2009			
DB (mg/dL)	0–0.3	7.5 (n = 1)	Villalobos et al. 1982	0.14–0.34	0.05–0.4	0.01–0.1
		2.5–11.2 (n = 2)	Tamez et al. 2009			
IB (mg/dL)	0.1–1			0.01–0.11	0.16–0.50	0.02–0.5
TB (mg/dL)	0.1–1.2	Twice the normal value (n = 67)	Katzenstein et al.1982	0.22–0.41	0.25–0.90	0.03–0.5
		>3.5 (n = 135)	Sharma et al. 1996			
		2.44±2.08 (n = 39)	Wiwanitkit 2002			
		>3.5 (n = 509)	Wells and Arguedas 2004			
		3.7–13.1 (n = 2)	Tamez et al. 2009			
Albumin (g/dL)	3.5–5	<2.0 (n = 135)	Sharma et al. 1996	5.7–5.9	1.84–1.94	2.99–3.18
		2.86±0.61 (n = 39)	Wiwanitkit 2002			
		<2.0 (n = 404)	Wells and Arguedas 2004			
		1.7 (n = 14)	Graillet et al. 2008			
Globulins (g/dL)	2.3–3.5			2.9–3.1	2.7–4.5	2.7
Relelation A/G (g/dL)	1.5–2.5			2.6–2.9	0.41–0.72	1.11–1.19
Total protein (g/dL)	6–8			6.32–7.76	4.63–6.13	5.68–5.86

Note: ALA, amoebic liver abscess; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; GOT, glutamic-oxa-
TB, total bilirubin; A, albumin; G, globulins; and ND, not determined.

*Data correspond to hamster sera at the day of sacrifice (day 12).

sepsis in very low birth mass infants in neonatal intensive care units in Italy. Furthermore, no adverse effects or intolerance to treatment occurred with Lf (Manzoni et al. 2009). In another study, Japanese children who consumed 100 mg of bLf daily for 3 months had no incidence of rotavirus-induced gastroenteritis (Egashira et al. 2007). Concerning the use of Lf in the management of parasitic diseases, a study with Peruvian children demonstrated a lower prevalence of colonization with *Giardia* species when 2 daily doses of 500 mg bLf were administered for 9 months (Zavaleta et al. 2007; Ochoa et al. 2008; Ochoa and Cleary 2009).

Interestingly, beginning 30 years ago, observations among milk-drinking African nomads of the Maasai tribe showed an unusual freedom from infection with *E. histolytica* compared with similar nomads consuming a mixed diet (Murray et al. 1980). The Maasai mainly consumed the milk of Cebu cattle, which demonstrates a lower iron concentration than what is typically reported for bovine milk. Accordingly, when iron sulfate was orally administered to the Maasai people, amoebiasis increased among the population; the authors proposed that the amoebicidal effect was due to the host's iron-binding proteins, transferrin and lactoferrin.

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

Metronidazole* (0.5 mg) (n = 10)	Lactoferrin* (10 mg) (n = 10)	Lactoferrin* (2.5 mg) (n = 5)	Metronidazole/lactoferrin* (0.5 / 2.5 mg) (n = 10)
495–732	207–932	154–237	172–518
232–324	108–357	ND	106–143
1–8	2–8	ND	2–9
67–118	58–171	48–226	54–107
48–80	43–68	40–90	35–61
0.01–0.2	0.02–0.2	0.63–1.48	0–0.1
0.01–0.4	0.05–0.4	0.02–0.15	0.05–0.5
0.1–0.5	0.07–0.5	0.71–1.62	0.08–0.5
3–3.13	2.56–3.23	3.85–4.67	3.1–3.34
3.1–3.3	2.7–3.5	ND	2.9–3.0
0.9–1.01	0.74–1.15	ND	1.09–1.12
6.17–6.33	5.42–6.34	7.35–7.87	6.06–6.35

lacetic transaminase; AST, ; GPT, glutamic-pyruvic transaminase; ALT, ; DB, direct bilirubin; IB, indirect bilirubin;

The therapeutic dose of Mtz in hamsters with amoebic hepatic lesions has been established as 5 mg/100 g mass over a period of 10 days (Hernández-López and Escobedo-Salinas 1970; Martínez-Gigena et al. 1992; Ordaz-Pichardo et al. 2005). Martínez-Gigena et al. (1992) analyzed histological changes in the liver throughout Mtz treatment, reporting that lesions effectively disappeared and trophozoites were not observed in tissues at days 7 and 9. In this work, we employed the same dosing over a period of 8 days; with this treatment regimen, all animals were nearly healed (1.54% lesion on average). However, undesirable side effects were confirmed among all animals, perhaps because of both the virulence factors secreted by the amoebae and Mtz toxicity.

Mtz has traditionally been used against *Helicobacter pylori* and other anaerobic bacteria, generally in a triple cocktail in combination with antibiotics (Suerbaum and Michetti 2002). Recent studies include the use of Lf as an adjuvant with the triple therapy (Calvet 2006). In a previous study, our group demonstrated a synergistic effect between Mtz and both hLf and bLf against *E. histolytica* in vitro (León-Sicairos et al. 2006*b*). This finding has since been confirmed using a mixture consisting of one-third of the inhibitory concentration of

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

bLf plus one-third of that of Mtz, with >95% of trophozoite growth inhibited. All of these reports encourage us to study the effect of bLf and its combination with Mtz in the healing of amoebic hepatic abscesses. In this work, we characterize the antiamoebic effect of apoLf in a model of hepatic amoebiasis in hamsters. Furthermore, because Mtz is an efficient amoebicidal, with the purpose of diminishing its toxic effects in hepatic amoebiasis treatment, we studied the amoebicidal effect of a low Mtz dose in combination with Lf in this model.

The administration of a high or low dose of Lf alone and in combination with Mtz did not cause any clinical signs in hamsters, and ALAs were nearly eliminated, validating the use of this glycoprotein in vivo. A 10-fold lower dose of Mtz resulted in 18.3% lesion. In contrast, by using bLf alone (2.5 mg/100 g mass), we obtained an inhibitory effect similar to that obtained with a high dose of Mtz. More studies must be performed to optimize the dose of bLf or the Mtz–bLf combination to be successful in treating hepatic amoebiasis in patients.

Mtz is extensively metabolized by the liver to form 2 primary oxidative metabolites, hydroxy and acetic acid metabolites (Lau et al. 1992; Björnsson et al. 2002). Thus, it was important to distinguish between the damage caused to the liver by amoebae and by Mtz treatment alone and with Lf. One of the approaches to measure hepatic damage is the enzyme and protein profile. In untreated patients with ALA, all serum protein levels are generally increased, except for albumin, which is considerably reduced (Sharma et al. 1996; Wiwanitkit 2002; Wells and Arguedas 2004; Graillet et al. 2008). In hamsters in which ALA was induced, albumin and LDH levels declined and ALP and total bilirubin levels increased. Bilirubin, a product of heme catabolism, is an indicator of erythrocyte destruction and, together with the other increased values, is a hallmark of the metabolic disorder caused by the amoebae per se because they can obstruct biliary pathways and damage hepatocyte junctions.

Interestingly, hamsters treated with a therapeutic dose of Mtz exhibited normal values for almost all enzymes, perhaps by the diminution of liver damage and amoeba death. However, with a 10-fold lower dose of Mtz, ALP levels were similar to those of hamsters with abscesses present. In the case of Lf alone or in combination with a drug or antibiotic, there are no parameters in the literature to compare the liver protein profile in humans or hamsters. Importantly, with the administration of Lf to hamsters, alone or in combination with Mtz, a general recovery of liver function was demonstrated because blood enzyme values were similar to those of healthy hamsters. This healing is perhaps due to the immunostimulatory functions of this protein.

In conclusion, bLf is a promising pharmaceutical therapy alone or as an adjunct to Mtz for the treatment of amoebic hepatic abscesses. Because bLf was well tolerated by hamsters, their liver tissue and cellular architecture were regenerated, and their serum protein profiles returned to normal values, we can recommend the use of bLf in patients, likely without causing toxicity and undesirable effects.

Acknowledgments

We thank QFB Amelia Angel for her valuable assistance in cultivating the virulent strain of *Entamoeba histolytica* and MVZ Rafael Leyva Muñoz and Ricardo Gaxiola Centeno for hamster care. We also thank DMV International for their donation of bLf. This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACyT), México (grant no. 60102). This work was presented at the Xth Conference on Lactoferrin, Structure, Function and Applications, with the sponsorship of CONACyT and the Instituto de Ciencia y Tecnología del Distrito Federal (ICyTDF), México.

References

Actor, J.K., Hwang, S.A., and Kruzel, M.L. 2009. Lactoferrin as a natural immune modulator. Curr. Pharm. Des. **15**(17): 1956–1973. doi:10.2174/138161209788453202. PMID:19519436.

Arnold, R.R., Russell, J.E., Champion, W.J., Brewer, M., and Gauthier, J.J. 1982. Bactericidal activity of human lactoferrin: differentiation from the stasis of iron deprivation. Infect. Immun. **35**(3): 792–799. PMID:6802759.

Barreda, A.R., Díaz, B.A., and Chávez, M.B. 2002*a*. Amibiasis. Una perspectiva actual (Amoebiasis. A current perspective. Chapter 7, Epidemiology). Labs. Pharmacia, I, Mexico.

Barreda, A.R., Díaz, B.A., and Chávez, M.B. 2002*b*. Amibiasis. Una perspectiva actual. (Amoebiasis. A current perspective. Chapter 9, Treatment). Labs. Pharmacia, I, Mexico.

Baveye, S., Elass, E., Mazurier, J., Spik, G., and Legrand, D. 1999. Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. Clin. Chem. Lab. Med. **37**(3): 281–286. doi:10.1515/CCLM.1999.049. PMID:10353473.

Björnsson, E., Nordlinder, H., and Olsson, R. 2002. Metronidazol as a probable cause of severe liver injury. Hepatogastroenterology, **49**(43): 252–254. PMID:11941968.

Brock, J.H. 2002. The physiology of lactoferrin. Biochem. Cell Biol. **80**(1): 1–6. doi:10.1139/o01-212. PMID:11908632.

Browman, W.C., and Rand, M.J. 1980. Textbook of Pharmacology. Blackwell Mos, II.

Calvet, X. 2006. *Helicobacter pylori* infection: Treatment options. Digestion, **73**(Suppl. 1): 119–128. doi:10.1159/000089787. PMID:16498260.

Diamond, L.S., Harlow, D.R., and Cunnick, C.C. 1978. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba.* Trans. R. Soc. Trop. Med. Hyg. **72**(4): 431–432. doi:10.1016/0035-9203(78)90144-X. PMID:212851.

Diarra, M.S., Petitclerc, D., and Lacasse, P. 2002. Effect of lactoferrin in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis. J. Dairy Sci. **85**(5): 1141–1149. doi:10.3168/jds.S0022-0302(02)74176-3. PMID:12086049.

Dobiás, L., Cerná, M., Rössner, P., and Srám, R. 1994. Genotoxicity and carcinogenicity of metronidazole. Mutat. Res. **317**(3): 177–194. PMID:7515153.

Egashira, M., Takayanagi, T., Moriuchi, M., and Moriuchi, H. 2007. Does daily intake of bovine lactoferrin-containing products ameliorate rotaviral gastroenteritis? Acta Paediatr. **96**(8): 1242–1244. doi:10.1111/j.1651-2227.2007.00393.x. PMID:17590195.

el-Nahas, A.F., and el-Ashmawy, I.M. 2004. Reproductive and cytogenetic toxicity of metronidazole in male mice. Basic Clin. Pharmacol. Toxicol. **94**(5): 226–231. PMID:15125692.

Flores-Villaseñor, H., Canizalez-Román, A., Reyes-López, M., Nazmi, K., de la Garza, M., Zazueta-Beltrán, J., et al. 2010. Bactericidal effect of bovine lactoferrin, LFcin, LFampin and LFchimera on antibiotic-resistant *Staphylococcus aureus* and *Escherichia coli.* Biometals, **23**(3): 569–578. doi:10.1007/s10534-010-9306-4. PMID:20195887.

Gifford, J.L., Hunter, H.N., and Vogel, H.J. 2005. Lactoferricin: a

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.

lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell. Mol. Life Sci. **62**(22): 2588–2598. doi:10.1007/s00018-005-5373-z. PMID:16261252.

Gill, J.K., Vincent, A.L., Greene, J.N., Sandin, R.L., and Sniffen, J.C. 2002. Amebic liver abscess. Infect. Med. **28**(12): 548–552.

Graillet, R., Sánchez-Aguilar, M., Morán-Mendoza, A.O., Hernández-Sierra, J.F., Gordillo-Moscoso, A., and Tapia-Pérez, J.H. 2008. Analysis of factors associated to failure of medical treatment of amoebic liver abscess. Cir. Esp. **84**(2): 83–86. doi:10.1016/S0009-739X(08)72139-0. PMID:18682186.

Hague, R., Huston, C.D., Hughes, M., Houpt, E., and Petri, M.A., Jr. 2003. Amebiasis. N. Engl. J. Med., **348**: 1565–1573. PMID: 12700377.

Hernández-López, H., and Escobedo-Salinas, A. 1970. Efectos del metronidazol sobre el absceso hepático amibiano del hámster (Effects of metronidazole on amoebic hepatic abscess in hamster). Arch. Invest. Med. (Mex.), **1**(Suppl.): S125–S128.

Jenssen, H., and Hancock, R.E. 2009. Antimicrobial properties of lactoferrin. Biochimie, **91**(1): 19–29. doi:10.1016/j.biochi.2008.05.015. PMID:18573312.

Kapoor, K., Chandra, M., Nag, D., Paliwal, J.K., Gupta, R.C., and Saxena, R.C. 1999. Evaluation of metronidazole toxicity: a prospective study. Int. J. Clin. Pharmacol. Res. **19**(3): 83–88. PMID:10761537.

Katzenstein, D., Rickerson, V., and Braude, A. 1982. New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis, and hepatic enzymes in 67 consecutive cases in San Diego. Medicine (Baltimore), **61**(4): 237–246. PMID:6806561.

Kuipers, M.E., de Vries, H.G., Eikelboom, M.C., Meijer, D.K., and Swart, P.J. 1999. Synergistic fungistatic effects of lactoferrin in combination with antifungal drugs against clinical *Candida* isolates. Antimicrob. Agents Chemother. **43**(11): 2635–2641. PMID:10543740.

Lau, A.H., Lam, N.P., Piscitelli, S.C., Wilkes, L., and Danziger, L.H. 1992. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. Clin. Pharmacokinet. **23**(5): 328–364. doi:10.2165/00003088-199223050-00002. PMID:1478003.

Leitch, E.C., and Willcox, M.D. 1999. Elucidation of the antistaphylococcal action of lactoferrin and lysozyme. J. Med. Microbiol. **48**(9): 867–871. doi:10.1099/00222615-48-9-867. PMID:10482299.

León-Sicairos, N., Reyes-López, M., Canizalez-Roman, A., Bermudez-Cruz, R.M., Serrano-Luna, J.J., Arroyo, R., and de la Garza, M. 2005. Human hololactoferrin: endocytosis and use as an iron source by the parasite *Entamoeba histolytica.* Microbiology, **151**(12): 3859–3871. doi:10.1099/mic.0.28121-0. PMID:16339932.

León-Sicairos, N., López-Soto, F., Reyes-López, M., Godínez-Vargas, D., Ordaz-Pichardo, C., and de la Garza, M. 2006*a*. Amoebicidal activity of milk, lactoferrin, sIgA and lysozyme. Clin. Med. Res. **4**(2): 106–113. doi:10.3121/cmr.4.2.106. PMID: 16809402.

León-Sicairos, N., Reyes-López, M., Ordaz-Pichardo, C., and de la Garza, M. 2006*b*. Microbicidal action of lactoferrin and lactoferricin and their synergistic effect with metronidazole in *Entamoeba histolytica.* Biochem. Cell Biol. **84**(3): 327–336. PMID:16936803.

Lönnerdal, B. 2003. Nutritional and physiologic significance of human milk proteins. Am. J. Clin. Nutr. **77**(6 Suppl.): 1537S–1543S. PMID:12812151.

López-Soto, F., León-Sicairos, N., Nazmi, K., Bolscher, J.G., and de la Garza, M. 2010. Microbicidal effect of the lactoferrin peptides Lactoferricin 17–30, Lactoferrampin 265–284, and Lactoferrin chimera on the parasite *Entamoeba histolytica.* Biometals, **23**(3): 563–568. doi:10.1007/s10534-010-9295-3. PMID:20140481.

Manzoni, P., Rinaldi, M., Cattani, S., Pugni, L., Romeo, M.G., Messner, H., et al.Italian Task Force for the Study and Prevention of Neonatal Fungal Infections, Italian Society of Neonatology 2009. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates. JAMA, **302**(13): 1421–1428. doi:10.1001/jama.2009.1403. PMID:19809023.

Martínez-Gigena, M.P., Shibayama-Salas, M., Tsutsumi, V., and Martínez-Palomo, A. 1992. Histological changes during healing of experimental amebic liver abscess treated with metronidazole. Arch. Med. Res. **23**(2): 209–212. PMID:1340296.

Martínez-Palomo, A. 1987. The pathogenesis of amoebiasis. Parasitol. Today, **3**(4): 111–118. doi:10.1016/0169-4758(87)90048-2. PMID:15462926.

Mudry, M.D., Carballo, M., Labal de Vinuesa, M., González-Cid, M., and Larripa, I. 1994. Mutagenic bioassay of certain pharmacologycal drugs: Metronidazole (MTZ). Mutat. Res. **305**(2): 127–132. doi:10.1016/0027-5107(94)90230-5. PMID: 7510021.

Murray, M.J., Murray, A., and Murray, C.J. 1980. The salutary effect of milk on amoebiasis and its reversal by iron. BMJ, **280**(6228): 1351–1352. doi:10.1136/bmj.280.6228.1351. PMID:7388537.

Ochoa, T.J., and Cleary, T.G. 2009. Effect of lactoferrin on enteric pathogens. Biochimie, **91**(1): 30–34. doi:10.1016/j.biochi.2008.04.006. PMID:18472012.

Ochoa, T.J., Chea-Woo, E., Campos, M., Pecho, I., Prada, A., McMahon, R.J., and Cleary, T.G. 2008. Impact of lactoferrin supplementation on growth and prevalence of *Giardia* colonization in children. Clin. Infect. Dis. **46**(12): 1881–1883. doi:10.1086/588476. PMID:18462105.

Ordaz-Pichardo, C., Shibayama, M., Villa-Treviño, S., Arriaga-Alba, M., Angeles, E., and de la Garza, M. 2005. Antiamoebic and toxicity studies of a carbamic acid derivative, and its therapeutic effect in the hamster model of hepatic amoebiasis. Antimicrob. Agents Chemother. **49**(3): 1160–1168. doi:10.1128/AAC.49.3.1160-1168.2005. PMID:15728919.

Roe, F.J. 1983. Toxicologic evaluation of metronidazole with particular reference to carcinogenic, mutagenic and teratogenic potential. Surgery, **93**(1): 158–164. PMID:6336861.

Sánchez-Ramírez, B., Mata-González, S., Valdez, A., Ramos-Martínez, E., and Talamás-Rohana, P. 2001. Liver function test during amoebic liver abscess formation in indomethacin-treated hamsters. J. Exp. Zool. **290**(3): 201–206. doi:10.1002/jez.1050. PMID:11479899.

Sharma, M.P., Dasarathy, S., Verma, N., Saksena, S., and Shukla, D.K. 1996. Prognostic markers in amebic liver abscess: a prospective study. Am. J. Gastroenterol. **91**(12): 2584–2588. PMID:8946991.

Stanley, S.L., Jr. 2003. Amoebiasis. Lancet **361**(9362): 1025–1034. doi:10.1016/S0140-6736(03)12830-9. PMID:12660071.

Suerbaum, S., and Michetti, P. 2002. *Helicobacter pylori* Infection. N. Engl. J. Med. **347**(15): 1175–1186. doi:10.1056/NEJMra020542. PMID:12374879.

Tamez, A., Guillén, N., and Castorena, G. 2009. Absceso hepático amibiano múltiple (Multiple amoebic hepatic abscess). Rev. Asoc. Mex. Med. Crit. Ter. Int. **23**(3): 165–172.

Tannich, E., Mirelman, D., and Petri, W.A., Jr. 2003. Meeting report: EMBO workshop "Pathogenesis of amoebiasis: From genomics to disease". Institut Pasteur, Paris, France 19-22 May 2003 Protist **154**(3-4): 293–298. PMID:14658490.

Tsutsumi, V., Ramírez-Rosales, A., Lanz-Mendoza, H., Shibayama, M., Chávez, B., Rangel-López, E., and Martínez-Palomo, A. 1992. *Entamoeba histolytica*: Erythrophagocytosis, collagenolysis and liver abscess production as virulence markers. Trans. R. Soc. Trop.

Med. Hyg. **86**(2): 170–172. doi:10.1016/0035-9203(92)90555-Q. PMID:1440779.

Upcroft, P., and Upcroft, J. 2001. Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin. Microbiol. Rev. **14**(1): 150–164. doi:10.1128/CMR.14.1.150-164.2001. PMID: 11148007.

Vaerman, J.P. 1984. Effector mechanisms of IgA. Ann. Biol. Clin. (Paris), **42**(1): 61–70. PMID:6375472.

Valenti, P., Berlutti, F., Conte, M.P., Longhi, C., and Seganti, L. 2004. Lactoferrin functions: current states and perspectives. J. Clin. Gastroenterol. **38**(6 Suppl.): S127–S129. doi:10.1097/01.mcg.0000128941.46881.33. PMID:15220678.

van der Strate, B.W., De Boer, F.M., Bakker, H.I., Meijer, D.K., Molema, G., and Harmsen, M.C. 2003. Synergy of bovine lactoferrin with the anti-cytomegalovirus drug cidofovir *in vitro*. Antiviral Res. **58**(2): 159–165. doi:10.1016/S0166-3542(02)00211-5. PMID:12742576.

Villalobos, J.J., García, P., Maqueo, M., Campos, A., and Hervella, L.M. 1982. Absceso hepático amibano en 84 pacientes estudiados en el Instituto Nacional de la Nutrición Salvador Zubirán, en los últimos 5 años (Study of 84 patients with hepatic amebic absces in the National Institute for Nutrition Salvador Zubiran, in the last 5 years). Rev. Invest. Clin. **34**(1): 39–41. PMID:7089398.

Wakabayashi, H., Takase, M., and Tomita, M. 2003. Lactoferricin derived from milk protein lactoferrin. Curr. Pharm. Des. **9**(16): 1277–1287. doi:10.2174/1381612033454829. PMID:12769736.

Wells, C.D., and Arguedas, M. 2004. Amebic liver abscess. South. Med. J. **97**(7): 673–682. doi:10.1097/00007611-200407000-00013. PMID:14746412.

Wiwanitkit, V. 2002. A note on indirect hemagglutination (IHA) antibody titers among hospitalized patients in Thailand with amebic liver abscess. MedGenMed, **4**(3): 5–10.

Yamauchi, K., Tomita, M., Giehl, T., and Ellison, R., III.1993. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. Infect. Immun. **61**(2): 719–728. PMID: 8423097.

Zavaleta, N., Figueroa, D., Rivera, J., Sánchez, J., Alfaro, S., and Lönnerdal, B. 2007. Efficacy of a rice-based oral rehydration solution containing recombinant human lactoferrin and lysozyme in Peruvian children with acute diarrhea. J. Pediatr. Gastroenterol. Nutr. **44**(2): 258–264. doi:10.1097/MPG.0b013e31802c41b7. PMID:17255841.

Biochem. Cell Biol. Downloaded from www.nrcresearchpress.com by INSTITUTO POLITECNICO NACIONAL on 07/03/18
For personal use only.