Artículo:

Clinical significance of the redefinition of the agent of amoebiasis
Clinical significance of the redefinition of the agent of amoebiasis

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ABSTRACT. Entamoeba histolytica is the pathogenic species of Entamoeba that causes amoebic dysentery and other invasive disease. The morphologically similar species, E. dispar, is non-pathogenic and accounts for about 90% of the previously estimated 500 million E. histolytica infections world-wide. Because of the recent redefinition of E. histolytica and E. dispar, and the limited number of drugs available to treat amoebiasis, a new approach to treatment of individuals carrying these parasites is necessary. A meeting of eminent scientists has recently agreed that on no account should prophylaxis against amoebiasis be given, and no treatment without symptoms should be administered. The expense of treating asymptomatic individuals, both monetary and at the risk of over-use of precious drugs, does not appear to be justified. It would seem wise that we preserve currently effective anti-amoebic drugs and avoid the development of drug-resistant E. histolytica.

Key words: Entamoeba histolytica, Entamoeba dispar, metronidazole, amoebiasis, anti-amoebic drugs, metronidazole resistance.

INTRODUCTION

Invasive amoebiasis is one of the world’s most prevalent and fatal infectious diseases. Primarily a problem of the developing world, around 500 million people are infected worldwide while 75,000 die of the disease annually. Behind malaria and schistosomiasis, amoebiasis ranks third on the list of parasitic causes of death worldwide.1-3

There are four species of the protozoan genus Entamoeba which are commonly found in the human gastrointestinal tract, 4 namely E. coli, E. dispar, E. hartmanni and E. histolytica. E. histolytica is the agent of invasive amoebiasis and hence the only one of medical importance.5 The recent division of E. histolytica into non-pathogenic E. dispar and pathogenic E. histolytica,5 has rekindled a long dormant hypothesis put forward by the French parasitologist, Emile Brumpt in 1925,7 who suggested that there could be two morphologically identical, but genetically separate, species of Entamoeba. Recent mounting evidence and consensus of opinions indicates that he was correct. We now recognize E. histolytica as the only pathogenic species. While the implications of this development have been summarized in the WHO report of a meeting of experts,8 the WHO/PAHO/UNESCO Consultation on Amebiasis,9 and by Martinez-Polomo and Espinosa-Cantello,10 this review aims to present the background and implications for treatment of amoebiasis.

HISTORY

As far back as 1875, the Russian physician Fedor Lösch identified what he believed to be the causative agent (motile amoebae containing erythrocytes which he named Amoeba coli) of a case of dysentery but doubted its lone role in pathogenesis when it failed to produce disease in three of four dogs experimentally inoculated with it (see ref. 11). Kean,12 however, states of the experiment at that time, that one of three dogs exhibited the disease from which amoebae were retrieved, thus almost completely fulfilling Koch’s postulates. In 1886, while in Egypt, Robert Koch identified, and stained for the first time, amoebae from colonic and hepatic lesions but a Greek colleague of his, Stephanos Kartulis, failed to reproduce the disease in rabbits and guinea pigs (see ref. 11). In 1893 the German pair Quincke and Roos identified an important mode of transmission when they described 15 diseased patients who all shared the same drinking source. They also differentiated between one species of non-pathogenic (NP) amoeba which could not phagocytose erythrocytes and a pathogenic (P) one which could. The latter, described meticulously by Councilman and Lafleur was...
referred to as *E. dysenteriae* at the time (see ref. 11). The
great protozoologist Schaudinn, omitting the work of
Quinke and Roos, also described the differences between the
two organisms and renamed them *E. histolytica* and *E. coli*
(see ref. 12). A later study by Walker 13 demonstrated that
oral ingestion of *E. dysenteriae* cysts by human volunteers
led to invasive disease while *E. coli* cysts did not; a conclusi-
ve argument, one would think but one which was not given
deserving attention, especially after the discovery of *Shige-
lla* in 1898 as another cause of dysentery in man.

In 1925, Brumpt proposed that humans can be infected by
two morphologically identical species of *Entamoeba* produ-
cing quadrinucleate cysts measuring 10 µm or greater in di-
diameter;7 the P organism was identified as *E. dysenteriae* and
Brumpt named the NP one *E. dispar*. However, due to diffi-
culties in proving the existence of two visually similar yet pa-
thogenically different species, and because of human and ani-
mal studies suggesting that amoebae from asymptomatic ca-
riers could produce disease,14-16 Brumpt’s hypothesis gained
little favour. It took almost 70 years and the advent of modern
biochemical techniques to re-establish Brumpt’s idea.

In 1973, P and NP species were differentiated by their rela-
tive agglutinability with the lectin Con A.17 This was followed
in 1978 by the discovery that pathogenicity could also be cor-
related with the enzymatic profile of the organism. Sargeaut
and colleagues18 used thin-layer starch-gel electrophoresis to
separate isoenzyme variants of the glycolytic enzymes gluco-
sephosphate isomerase (GPI), phosphoglucomutase (PGM)
and L-malate: NADP oxidoreductase and classified 85 stocks
of *E. histolytica* into four groups according to their enzyme
profile. Enzyme Group II, which contained a faster migrating
band of PGM, was found in all cases of clinical amoebiasis
but was not found in any asymptomatic individuals, although
Sargeaut pointed out that in a larger study such persons (in a
preclinical state of disease) may be found. Diamond and
Clark5 presented a host of data to confirm the existence of *E.
histolytica* and *E. dispar*, and to honour the hypothesis put
forward by Brumpt. Biochemically, they cited thousands of
samples which correlated zymodeme class to pathogenicity.

Monoclonal antibodies have also provided much immu-
nological evidence, demonstrating the difference in surface
antigens between the two species19,21 but genetic evidence
has provided the most compelling argument, with DNA
probes distinguishing between differing gene sequences and
divergence in amino acid sequences.22

**STRUCTURE AND LIFE-CYCLE OF E. HISTOLYTICA**

*E. histolytica* exists in two forms: the motile and invasive
trophozoite and an infective cyst. The diameter of the tro-
phozoite varies between 10 and 60 µm; its variability is
affected by changes in temperature, pH, osmolarity and re-
dox potential, as well as feeding conditions actively inva-
ding amoebae tend to be larger.4 The cyst has been far less
studied than the trophozoite since encystation has proved di-
ficult in axenic cultures. In the past, the presence of cysts in
the stool has been used as a diagnostic tool for amoebiasis.

Cysts can remain viable outside the body for several days
and infection usually occurs by ingestion of water or food
contaminated by faecal matter. The cyst wall is dissolved in
the upper gastrointestinal tract and the organism excysts in
the terminal ileum, giving rise to 8 uni-nucleated trophozo-
ites. Trophozoites of *E. histolytica* as the name suggests, are
one of the most powerful tissue invaders known. Once pene-
tration of the intestinal mucosa is achieved, dissemination to
other organs, usually the liver, can occur. Trophozoites which
dwell in the colon multiply, encyst and are passed in the
stool from where further spread is possible.4

**PATHOLOGY AND CLINICAL MANIFESTATIONS**

*E. histolytica* causes pathology by invading intestinal epi-
thelium and producing intestinal lesions which may later
spread to extraintestinal sites. Four intestinal forms of
amoebiasis have been described. Amoebic dysentery, also
known as amoebic colitis, accounts for c.90% of intestinal
amoebiasis. Clinical presentation is usually subacute and of
less than one month’s duration, with symptoms ranging from
mild diarrhoea to classic dysentery.3 Dysentery is an inflam-
atory condition of the (usually lower) intestine accompanied
by abdominal pain, tenesmus and frequent stools containing
both blood and mucus.4 Fever and systemic manifestations are
generally absent and the clinical course is moderate, with
symptoms disappearing rapid with treatment. The remaining
three forms tend to have a rapid course and are very severe
conditions requiring immediate medical care. Fulminating
amoebic colitis consists of widespread necrotic ulcerous leso-
nons which may perforate and lead to peritonitis. Amoebic ap-
pendicitis is similar to its bacterial counterpart and amoeboma
is a pseudotumoural lesion whose formation is associated with
necrosis, inflammation and oedema of both mucosa and sub-
mucosa of the colon.4 Extraintestinal amoebiasis, brought
about by haematogenous spread of trophozoites, can infect the
liver, brain, lung, skin and rarely genitourinary structures3 but
amoebic liver abscess is by far the most common complica-
tion. Single or multiple abscesses are formed by local necrosis
and liquefaction.23,24

**EPIDEMIOLOGY**

While *E. histolytica* has been found in mammals such as
cats, dogs and primates, man is thought to be the only impor-
tant reservoir. Eichinger25 goes as far as saying that there is no
zoonotic reservoir of *E. histolytica* and the absence of having
to conform to multiple hosts has resulted in a simple life cycle involving only two stages. Amoebiasis has been described as a third world disease due to its high prevalence in poor countries. Low standards of hygiene and sanitation, in particular those related to crowding, contamination of food and water, and inadequate disposal of faeces, are all high risk factors for infection with *E. histolytica*. While *E. histolytica* is found worldwide, the highest prevalence rates are in developing countries and regions such as the Indian subcontinent, parts of central and South America, and tropical regions of Africa.

Amoebic liver abscess is more frequent in males than females and 10 times more common in adults than children. When prevalence of *Entamoeba* alone is considered, the 5-14 year age group was most affected as shown in a study in the Philippines where 1872 individuals were sampled, with a prevalence rate of 1% for *E. histolytica* and 7% for *E. dispar*. In Australia, *E. histolytica/dispar* has been found in 8% of 62 Aboriginal children examined in one study, while 28 was not present in 1683 Western Australian Aboriginals. One group found to have an increased prevalence of *E. histolytica/dispar* compared to the general population is homosexual males. In their study of 128 Sydney homosexual men, Law et al. found that 37% were infected with *E. histolytica* but that there was no evidence of pathogenicity, nor any association with GI symptoms. This led them to believe the organisms found were NP (*E. dispar*), a conclusion supported by Sargeaunt and Allason-Jones et al., who found the majority of *E. histolytica* isolates from homosexual males to be of the NP zymodeme.

**STOOL DETECTION TESTS**

The diagnosis of intestinal amoebiasis primarily relies on the detection of parasites in the stools, and the epidemiological implications of *E. histolytica versus E. dispar* infection can be fully realized only if there are methods to distinguish the two species among large numbers of samples. The “simplest” (in terms of technology) form of detection is light microscopy. Gonzalez-Ruiz et al. used the observation of erythrophagocytic trophozoites in the case of bloody diarrhoea as a marker of *E. histolytica* infection and found this method to be 100% specific (using isoenzyme electrophoresis as the reference).

Haque et al., using a stool ELISA based on monoclonal antibodies to the galactose-specific adhesin of *E. histolytica*, found that this method was 97% specific and 100% sensitive in a small (12P and 22NP) number of samples. They also stated their disapproval of microscopy on the grounds that three or more separate stool samples were frequently required for detection.

Troll et al. found that their PCR-based detection of *E. histolytica* and *E. dispar* had good sensitivity and specificity as long as fresh faecal samples were used. They propose the assay as a complementary test to microscopy in special patients such as pregnant women and the immunocompromised in whom it would be important to differentiate *E. histolytica* from *E. dispar*. Other reports of successful PCR assays include that of Rivera and colleagues and Britten et al., who successfully used defined primer sets to determine the prevalence of *E. histolytica* and *E. dispar* in formalin fixed stool specimens. Although inexpensive assays to distinguish *E. histolytica* and *E. dispar* in a clinical environment are not commercially available as yet, several tests are under development.

**ANTI-AMOEBIC DRUGS**

Anti-amoebic drugs may be classified into three groups: luminal, tissue and mixed amoebicides. The drugs of choice belong to the latter and include metronidazole and their nitroimidazole derivative analogues, tinidazole and omedazole; these drugs are not only more effective therapeutically, than any others, but also have the advantage of oral administration.

The introduction of nitroheterocyclic drugs in the late 1950’s and 1960’s heralded a new era in the treatment of Gramm negative and positive bacteria and a range of pathogenic protozoan parasites. The antibiotic, azomycin (a 2-nitroimidazole compound) isolated in Japan from a streptomycin, was the first active nitroimidazole to be discovered and acted as the main impetus for the systematic search for drugs with activity against anaerobic protozoa. This lead to the synthesis of the 5-nitroimidazole, metronidazole (1-??-hydroxethyl-2-methyl-5-nitroimidazole), and the demonstration of activity against *Trichomonas vaginalis* by Cosar and Julou. Subsequently metronidazole was shown to cure giardiasis, amoebiasis and Balantidium infections. Metronidazole is now the most widely used drug in the treatment of anaerobic protozoan parasitic infections by *T. vaginalis, Giardia duodenalis* and *Entamoeba histolytica*. It is remarkably safe compared with the toxic amoebicide, emetine, and is the recommended drug to treat amoebiasis.

Metronidazole and the related nitroimidazole, tinidazole (which is not available in some countries), are also the only effective drugs for the treatment of trichomoniasis and are the drugs of choice to treat giardiasis. In the latter cases clinical resistance to these drugs has been documented.

Laboratory induced metronidazole resistance in *E. histolytica* has been reported where metronidazole-resistant *E. histolytica* was maintained indefinitely in medium containing 1.7 mg/l. While serum concentrations of metronidazole reach a maximum of 17 mg/l with recommended treatment regimes the concentration within abscesses is likely to be less than this and for only a few days duration.

In the event of overt clinical resistance to metronidazole in the anaerobic protozoa, and while vaccine development,
at least for amoebiasis, is still in progress.47 There is no acceptable alternative to treat either invasive amoebiasis, or Trichomoniasis, keeping in mind documented cross-resistance between currently used and experimental nitroimidazole drugs41,48 and world-wide availability. Recent evidence indicates that resistance among micro-organisms has developed, contrary to hopes and expectations, microbes will remain resistant long after doctors stop prescribing the drugs.49 It is therefore imperative that every step is taken to preserve our most precious drugs, metronidazole included.

Diloxanide furoate, diiodohydroxyquin and paromomycin are examples of luminal amoebicides with the well tolerated diloxanide furoate the mainstay for treatment of asymptomatic cyst carriers.50,51 In the case of liver abscess, chloroquine which accumulates in high concentrations in tissues, may be used in addition to metronidazole.52,53

TREATMENT

All symptomatic patients with bloody stools containing motile trophozoites with ingested erythrocytes should be treated according to the severity of the disease. Severe cases may require surgery but for the majority of adult cases 7-10 days of 750 mg tid metronidazole will be sufficient.53,54 Since metronidazole is routinely used to treat amoebiasis, on no account should it be used prophylactically.55 Sargeaunt56 is quite adamant that only E. histolytica is pathogenic and that the non-pathogenic E. dispar is stable, thus removing the need to treat E. dispar carriers. However, Diamond and Clark,5 caution against withholding treatment to asymptomatic individuals since E. histolytica cysts have been found in such persons. Gatti et al.57 describe a case in Italy where they believe an asymptomatic Filippino housemaid transmitted the E. histolytica to the family employed by her, resulting in five cases of morbidity and one of mortality.

Similarly to, Sargeaunt, Burchard58 advocates treatment only for E. histolytica but in the absence of differentiation advises all cyst-passers should be treated. Further, an analysis of the cost-effectiveness of treating E. histolytica/E. dispar cyst carriers was carried out in Mexico using a control (placebo) group and a treatment (metronidazole) group.59 Results showed an absence of disease in the placebo group, an early acquisition of the carrier state in the treated group and a small difference in months free of the carrier state. The authors concluded that the high cost of treating these “patients” could be far better utilized in preventing amoebic disease. Cost in dollars cannot be the only consideration the cost of losing precious drugs, such as metronidazole, as a result of drug resistance to inappropriate or over-use must be avoided. If treatment of asymptomatic patients is considered necessary, diloxanide furoate should be used.

CONCLUSION

The redefinition of E. histolytica into two separate species, NP E. dispar and P E. histolytica, means that only patients carrying E. histolytica should be considered for treatment and that prophylactic treatment should not be given. Some experts go one step further and recommend no treatment without symptoms, including bloody stools and invasive disease. In light of the fact that there is only one family of drugs recommended for the treatment of amoebiasis and that cross resistance between drugs within this family is well documented, it would seem wise that we follow the latter recommendation. In the event of overt clinical resistance to the 5-nitroimidazole drugs in E. histolytica (and resistance to metronidazole has been induced in laboratory maintained E. histolytica) there is no safe alternative for treatment of the millions of sufferers of asymptomatic amoebiasis.

REFERENCES


