IN VITRO STUDIES ON THE SENSITIVITY OF LOCAL *ENTAMOEBA HISTOLYTICA* TO ANTI-AMOEBIC DRUGS

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INTRODUCTION

Entamoeba histolytica which normally lives in the intestinal lumen can cause amoebic dysentery and liver abscess. In Thailand, the prevalence of amoebiasis by parasitological surveys had been documented among population groups and varying percentages of infection determined. The rates varied from 1-6% depending on the regions (Na Bang Xang *et al.*, 1969; Chongsuphajaisiddhi *et al.*, 1971; Harinasuta and Charoenlarp, 1971; Chullarerk, 1977). Strains of *Entamoeba histolytica* may show difference in susceptibility to various anti-amoebic drugs. The susceptibility of local *Entamoeba histolytica* to amoebicidal drugs is necessary for effective treatment and management of amoebiasis.

This study was conducted to determine the minimum inhibitory concentration of drugs against the locally isolated strains of *E. histolytica*.

MATERIALS AND METHODS

The locally isolated amoebae from thirty hosts had been used in this study. Twenty three were obtained from amoebic dysentery cases who had blood mucous stool with trophozoites, two cases were isolated from sigmoidoscope and rectal swabs of the hospitalized patients. Another five isolates were obtained from the cyst-passers who were without any symptoms.

The amoebae were first culture in the modified Boeck and Drbohlav's diphasic medium in association with the normal flora, and *Clostridium perfingens* was added. After growing satisfactorily, of age from 24-48 hours, they were collected and then transferred into all-liquid medium (liver extract, marmite and horse serum in phosphate buffer at pH 7.2) with rice starch (Woolfe, 1957). Usually 5-6 subculture were required in the all-liquid medium to obtain luxurant growth and enough for testing the susceptibility. The amoebae were counted by using haemocytometer and suspended in fresh all-liquid medium in order to give approximately 1,500 trophozoites per 50 microlitre.

The various concentration of drugs dehydroemetine, ornidazole, metronidazole and tinidazole, in buffered saline pH 7.2 were dispensed in sterile test tubes in volumes of 2.0 millilitre. To each of these test tubes 2 ml of double strength all-liquid medium and a loopful of rice starch were added. A control was put up for each isolate tested by substituting the drug with 2.0 µl of buffered saline. Each tube was inoculated with 50 µl of the suspension containing 1,500 amoebae. Each isolate was tested repeatedly five times with each various concentration of drugs. The tubes were incubated at 37°C and the result were read at 48 hours after inoculation.

A criterion for arriving at the amoebicidal endpoints was recorded on the presence or absence of living amoebae by direct observation under the microscope and by subculture.

Table 1

The minimal inhibitory concentration μg/ml of *Entamoeba histolytica* to anti-amoebic durgs.

Isolated strains no.	Dehydroemetine	Ornidazole	Metronidazole	Tinidazole
1	0.5	0.0625	0.0625	0.0625
2	0.5	0.0625	0.0625	0.0625
3	0.25	0.125	0.125	0.0625
4	0.25	0.0625	0.125	0.125
5	0.5	0.0625	0.0625	0.0625
6	0.5	0.0625	0.0625	0.0625
7	0.25	0.125	0.0625	0.125
8	0.25	0.125	0.125	0.0625
9	0.125	0.0625	0.0625	0.0625
10	0.25	0.0625	0.125	0.0625
11	0.125	0.0625	0.125	0.0625
12	0.25	0.0625	0.0625	0.0625
13	0.25	0.125	0.125	0.0625
14	0.125	0.0625	0.125	0.0625
15	0.125	0.0625	0.0625	0.0625
16	0.25	0.0625	0.125	0.125
17	0.25	0.0625	0.125	0.0625
18	0.125	0.0625	0.0625	0.0625
19	0.5	0.0625	0.0625	0.125
20	0.25	0.0625	0.0625	0.0625
21	0.25	0.125	0.125	0.125
22	0.5	0.0625	0.125	0.125
23	0.25	0.125	0.125	0.125
24	1	0.125	0.125	0.125
25	1	0.125	0.125	0.125
26	0.5	0.25	0.125	0.25
27	0.25	0.125	0.125	0.125
28	0.125	0.125	0.125	0.125
29	0.5	0.0625	0.0625	0.125
30	1	0.0625	0.0625	0.0625

A tube was recorded as negative if no amoeba was seen and confirmed by subculture in modified Boeck and Drbohalv's diphasic medium. The positive was when one or more active trophozoites were seen. If the trophozoite was non-motile, vital stain (1% neutral red stain for 15-20 minutes) was used for differentiation, a living trophozoite takes on a light red stain.

RESULTS

The details of the minimal inhibitory concentration of *Entamoeba histolytica* to anti-amoebic drugs are summarized in Table 1.

The minimal inhibitory concentration (MIC) for dehydroemetine ranged from

0.125 to 1 μg/ml, ornidazole ranged from 0.0625 to 0.25 μg/ml, metronidazole ranged from 0.0625 to 0.125 μg/ml and tinidazole ranged from 0.0625 to 0.25 μg/ml.

The MIC of dehydroemetine was significantly different from ornidazole, metronidazole and tinidazole. Metronidazole was superior to that of dehydroemetine but there was no significant difference among ornidazole, metronidazole and tinidazole.

DISCUSSION

This study was carried out using *Entamoeba histolytica* grown along with bacterial associates. It was difficult to assess accurately whether the drug was directly acting on the amoebae or it was acting indirectly by inhibiting the bacterial associates. However, this method had some advantage as well, the environment was more or less comparable to the conditions of the intestine. The MIC of metronidazole to the locally isolated strains in this experiment was similar to that of studies in India, 0.125-0.625 μg/ml (Vinayak and Prakash, 1969; Vinayak and Prakash, 1970).

SUMMARY

The *in vitro* activity of drugs, namely dehydroemetine, ornidazole, metronidazole and tinidazole were determined against the locally isolated strains of *E. histolytica* in Thailand. The test was performed in liquid monophasic medium, i.e. liver marmite serum medium. In all, locally isolated strains from thirty hosts studied, the minimal inhibitory concentration (MIC) for dehydroemetine ranged from 0.125 to 1 μg/ml, ornidazole ranged from 0.0625 to 0.25 μg/ml, metronidazole ranged from 0.0625 to 0.125 μg/ml, and tinidazole ranged from 0.0625 μg/ml to 0.25 μg/ml.

The MIC of dehydroemetine was significantly different from ornidazole, metronidazole and tinidazole. Metronidazole was superior to that of dehydroemetine but was not significantly different among ornidazole, metronidazole and tinidazole.

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