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Comparative study of aminosidine, etophamide and nimorazole, alone or in combination, in the treatment of intestinal amoebiasis in Kenya

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Summary. 417 patients suffering from intestinal amoebiasis were randomly allocated to 6 different treatment groups in a controlled study in 3 District Hospitals in Kenya. The patients received either aminosidine (A), etophamide (E), nimorazole (N), or the combinations NA, NE, EA. Treatment in all cases was given twice daily for 5 days. Before and after treatment, rectosigmoidoscopy was done in each patient, and stool examination with characterization of invasive (IF) and non invasive (NIF) forms of amoeba was done daily throughout treatment, and on Days 15, 30 and 60 of follow-up.

Clinical cure was good after all the treatments, varying from 90 to 100%; parasitological cure at the end of treatment was 100% in the NA and EA treatments groups, and 98% in A group. The incidence of relapses was nil in the EA group, followed by 3% in NA and 6% in A groups. Anatomical cure (healing of ulcers) was 97.8% in the NA group, 95.5% in the N group and 88.5% in the A group. Drug tolerance was excellent or good after all the treatments, except that the EA combination produced diarrhoea in 76.5% of patients.

Overall analysis of the findings, including tolerance of the various treatments, showed that aminosidine either alone or in combination with nimorazole gave the best results.

Ulcers seen on rectosigmoidoscopy were more common in patients excreting invasive forms of amoebae in their stools.

Key words: Intestinal Amoebiasis, Aminosidine, Etophamide, Nimorazole; Drug combinations, adverse effects

Amoebiasis, especially in the invasive form, constitutes a major health and social problem in many tropical countries [1]. In Kenya the disease is common in all parts of the country, with an incidence varying from 10 to 30% of the general population [2]. Intestinal amoebiasis is the most widespread form; in highly endemic areas, approximately 90% of the affected subjects are asymptomatic carriers, while the remaining 10% show definite clinical evidence of the disease [3].

Drugs used for treating amoebiasis in general, and its intestinal form in particular, are numerous and chemically diverse [4, 5]. Several of them, however, are not widely used, at least as first choice therapy, partly because of their limited efficacy, and partly because of the adverse effects they produce [6, 7]. Some, such as nitroimidazole and dichloracetamide derivatives, and one aminoglycoside antibiotic (paromomycin, or aminosidine), are still widely used for the treatment of intestinal amoebiasis, but the literature remains vague about their particular indications in the various pathological manifestations of amoebiasis. Very little is known of the activity of such drugs when used in combination.

The aim of the present study was to assess the efficacy of three different antiamoebic drugs administered individually, or variously combined two at a time, in invasive and non invasive intestinal amoebiasis. At the same time, possible correlations were sought between the various test parameters measured before the start of treatment.

Patients and methods

The study was conducted at the District Hospitals of Kiambu, Machakos and Kilifi, in a total of 417 patients (183 m and 234 f), between the ages of 6 and 80 y, who were suffering from *E. histolytica* intestinal infection. Pregnant women, patients with known allergy to the drugs, those with coexisting extra-intestinal amoebiasis or other major diseases, and those treated with antiamoebic drugs in the 30 days prior to recruitment, were excluded from the study.

The patients selected were informed of the purpose and nature of the study and were entered after giving their consent in writing. They were hospitalized for the duration of treatment. Before treatment (Day 0), each patient were examined: clinical history and general physical examination; gross stool examination for mucus and/or blood; microscopical stool examination (three direct examinations and a concentration test) to detect invasive (IF) or non invasive (NIF) amoebic forms [8]; rectosigmoidoscopy to detect amoebic ulcers (classified as mild or severe according to their number and/or size). The same tests were repeated daily throughout the treatment period and again at follow up examinations after 15, 30 and 60 days,

Table 1. Details of the main patient characteristics and diagnostic features in the six treatment groups (Day 0)

Treatment	Number of patients	Mean Age (years)	Mean BW (kg)	Stool examinations NIF (No.)	IF (No.)	Rectosigmoidoscopy Patients with ulcers no. positive/no. examined
A	100	28.3	51.0	65	35	54/ 88
E	102	28.9	50.1	67	35	50/ 80
N	100	31.2	49.4	73	27	68/ 93
NA	49	22.2	49.2	37	12	32/ 48
NE	49	30.5	49.8	33	16	35/ 44
EA	17	27.3	54.2	11	6	11/ 16
Mean or Total	417	28.6	50.2	286	131	250/369

A = aminosidine; E = etophamide; N = nimorazole

Table 2. Patient distribution by invasive (IF) and noninvasive (NIF) amoebic forms in the stools according to the features observed at rectosigmoidoscopy (RSS)

RSS / DRUG	Not done		Normal		Mild		Severe	
	NIF (No.)	IF (No.)	NIF (No.)	IF (No.)	NIF (No.)	IF (No.)	NIF (No.)	IF (No.)
A	6	6	29	5	27	14	3	10
E	14	8	24	6	22	15	7	6
N	4	3	24	1	37	13	8	10
NA	1	0	14	2	20	4	2	6
NE	4	1	8	1	16	6	5	8
EA	0	1	5	0	6	1	0	4
Total	29	19	104	15	128	53	25	44

with the exception of rectosigmoidoscopy, which was repeated only at the end of treatment for logistic reasons. IF and NIF forms of *E. histolytica* were distinguished in accordance with WHO recommendations [9].

The patients were randomly allocated to 6 different treatment groups (Table 1), each of which was treated orally for 5 consecutive days. The doses of drugs given on their own and in combination were: aminosidine (A) 500 mg b. d. for adults, 15 mg · kg^{-1} body wt. b. d. for children; etophamide (E) 600 mg b. d. for adults, 15 mg · kg^{-1} body wt. b. d. for children; nimorazole (N) 1 g b. d. for adults, 20 mg · kg^{-1} body wt. b. d. for children. All drugs were administered under direct medical supervision. The persons in charge of stool examination and rectosigmoidoscopy were not informed of the drug being taken. All patients were monitored daily from the start of the trial for possible adverse events attributable to the test drugs.

The criteria adopted for assessing results at the end of the study were as follows [10]: *Clinical cure*, defined as the disappearance of all symptoms present on entry. *Parasitological cure* – disappearance of all parasitic forms from stools or ulcer scrapings. *Anatomical cure* – healing of previous ulceration. The persistence of any form of *E. histolytica* at the end of treatment was rated as a failure, and the reappearance of any form during the follow up period, after initial disappearance, was rated as a recurrence.

Drug tolerance was rated as excellent in the absence of any side effects, good in the presence of mild side effects, and poor if there were severe manifestations attributable to treatment.

Results

The distribution of the 417 patients into the six treatment groups is shown in Table 1. There was homogeneity for age, body weight, presence of invasive or non-invasive forms of *E. histolytica* in the stools, and ulcers at rectosigmoidoscopy. Overall, the prevalence of NIF (68.6%) was greater than that of IF (31.4%). Rectosigmoidoscopy before treatment revealed parietal ulcers in 67.8% of the 369 patients examined.

Rectosigmoidoscopy findings on Day 0 have been correlated with the amoebic forms found in the stools in Table 2. When the rectosigmoidoscopy was normal, there was a definite prevalence of NIF over IF, ratio approximately 7:1. When ulcers were present, the ratio dropped to 2.4 in the case of mild ulcers, and to as low as 0.6 with severe ulcers. Overall, IFs in patients subjected to rectosigmoidoscopy occurred most commonly in patients (86.6%) with amoebic ulcers.

The pattern of amoebic forms found in the stools over time is displayed in Table 3. During treatment, IFs disappeared more rapidly and more often than NIFs, regardless of the drug being administered. At the end of treatment the total prevalence of IFs in the faeces was 0.7% and that of NIFs was 7.7% relative to the initial findings. The incidence of failures, essentially reflecting the persistence of non-invasive forms, was nil in patients treated with the NA or EA combinations, as compared to 2% in patients treated with A alone, 6.1% in those treated with NE, 8% in those treated with N alone, and 9.8% in those treated with E alone.

The incidence of recurrence in the various treatment groups was judged from follow up data. With the proviso that the percentage of patients reporting for recheck was 88.5% at 15 days, 67.6% at 30 days and 51.3% at 60 days, the overall distribution of recurrences was fairly uniform over time, namely 8.4% after 15 days, 9.2% at 30 days, and 7.5% at 60 days of follow up. At all rechecks, however, the incidence of recurrence was nil in the EA group, 3% in the NA group, 6% in the A group, 6.8% in the E group, 14.6% in the N group, and 17.3% in the NE group. Only the reappearance of parasites on Days 15 and 30 should be considered as true recurrences, as reappearance at Day 60 could represent reinfection [4, 10].

Rectosigmoidoscopy was done in 88.5% of all patients before treatment and it was repeated in 83.2% at the end of treatment (Table 4). At the time of recruitment, rectosigmoid ulcers were present in 67.8% of patients, with a larger number of mild than of severe forms. In general, treatment seemed to promote the healing of such lesions, which were present at termination in only 9.2% of cases, and then with an even greater predominance of mild forms. The best results in terms of ulcer healing were obtained with the NA combination (97.8% cured), followed by N (95.5%), A (88.5%), NE (87.8%), E (87.5%), and EA (77.0%).

Table 3. Cumulative daily clearance of amoebic forms from stools during treatment and follow-up

Drug	Stool examination	Days of treatment						Follow-up		
		0	1	2	3	4	6	15	30	60
A	NIF	65	61	35	11	4	2	2	6	6
	IF	35	14	2	2	0	0	1	0	0
	clearance %	0	25	63	87	96	98	97	93	90.6
E	NIF	67	52	22	20	14	9	5	5	2
	IF	35	8	2	1	2	1	0	0	0
	clearance %	0	41.4	76.5	79.4	83.4	90.2	94	90.5	95
N	NIF	73	50	37	28	20	8	16	8	2
	IF	27	17	2	2	1	0	2	0	0
	clearance %	0	33	61	70	79	92	78.6	87.5	95.5
NA	NIF	37	38	17	6	1	0	1	3	0
	IF	12	4	1	0	0	0	0	0	0
	clearance %	0	42.3	63.3	87.7	98	100	98	95.3	100
NE	NIF	33	19	9	9	6	3	4	3	6
	IF	16	3	4	2	0	0	0	1	0
	clearance %	0	55	73.4	77.5	87.7	93.9	89.7	81.8	70
EA	NIF	11	8	5	0	0	0	0	0	0
	IF	6	4	0	1	1	0	0	0	0
	clearance %	0	29.4	70.5	94.1	94.1	100	100	100	100

Table 4. Evolution of mild and severe amoebic ulcers seen at rectosigmoidoscopy before and after the various treatments

Drug	None		Mild		Severe		Total	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
A	34	77	41	10	13	0	88	87
E	30	63	37	8	13	1	80	72
N	25	84	50	4	18	0	93	88
NA	16	45	24	1	8	0	48	46
NE	9	36	22	5	13	0	44	41
EA	5	10	7	2	4	1	16	13
Total	119	315	181	30	69	2	369	347
%	(32.2)	(90.8)	(49.1)	(8.6)	(18.7)	(0.6)		

Table 5. Clinical cure and drug tolerance in treated patients

Drug	Clinical cure (%)	Drug tolerance		
		excellent (%)	good (%)	poor (%)
A	99	61.0	38.0	1.0
E	98	92.2	7.8	0.0
N	98	100.0	0.0	0.0
NA	100	89.8	8.2	2.0
NE	98	95.9	4.1	0.0
EA	?	0.0	23.5	76.5

Mucus alone at gross stool examination was found in 59.7% of patients before treatment, more often in association with NIFs. Mucus and blood together were seen in 33.6% of cases, more often in association with IFs. At the end of treatment, no patient had either mucus or blood of the faeces.

The percentages of cases from whom all signs and symptoms present on Day 0 disappeared with treatment (clinical cures), and the tolerance of the various treatments are listed in Table 5. Clinical cure was achieved in the vast majority of patients (between 98 and 100%) with all the different treatments. The tolerance of individual drugs and their combinations was generally satisfactory in all treatment groups, except that treated with the EA combination. Indeed, the recruiting of patients into this group, originally planned to total 50 patients, was discontinued because of the high incidence of diarrhoea, which was quite severe in many cases, making it impossible to carry out a proper assessment of the clinical cure in this group.

Discussion

The initial observations in this study provide some interesting considerations. First, the prevalence of non-invasive over invasive forms of *E. histolytica* (Table 1) was not very marked compared to that generally observed in areas where *E. histolytica* is highly endemic, where it is reported to be 90% and 10% respectively [3]. This discrepancy probably reflects a consequence of patient selection, since all the patients in the study had come to hospital because of specific abdominal complaints.

Rectosigmoid ulcers, both mild and severe (Table 1), were found in 67.8% of cases, which made them more common than the presence of invasive forms in the stools (31.4%). While no data are available in the literature about a correlation between intestinal amoebic ulceration and the presence of invasive trophozoites in the stools, the general consensus is that only the latter form is responsible for ulceration [9, 10], although amoebic ulcers have been reported in patients showing only *E. histolytica* cysts in the stool [11, 12]. In the present cases a definite correlation was only found between severe ulcers and the invasive forms of *E. histolytica*, and there was a higher prevalence of non-invasive forms in patients with mild ulcers, and especially in those without ulcers (Table 2).

Certain continuing studies have suggested that some traditional ideas about the pathogenesis of intestinal amoebiasis stand in need of correction. The old belief that invasive forms do not make cysts [5], and that the detection of *E. histolytica* cysts in the stools rules out invasive disease, is now challenged by the recognized existence of pathogenic *E. histolytica* zymodemes, especially in some tropical areas, and of non-pathogenic zymodemes prevalent in temperate zones [13, 14]. Those strains (or species?) are said to produce cysts [15]. In the case of pathogenic strains there would be continuous contact (invasion) with the intestinal tissues of the host, even in the absence of clinical manifestations, as demonstrated by the constant coexistence of high antibody titres [16]. Thus, the detection only of cysts (classified as NIF) in the stools of patients in this study could not exclude their possible origin from pathogenic strains of *E. histolytica* in parietal amoebic ulcers, with cyst formation while the amoebae travelled along the gut [15]. The possibility cannot be disregarded, either, that some of the observed lesions might have had an origin other than amoebiasis. Other common pathogens such as *Yersinia enterocolitica, Campylobacter enteritis, Balantidium coli* and *Shigella* species, can produce lesions in the distal gut [17, 18, 19], which are not always easy to distinguish from amoebic ulcers at rectosigmoidoscopy [20].

The overall cure rates at the end of treatment were quite satisfactory, and did not differ much from published data for the individual drugs [4, 21]. Little appears to have been published about the activity of the anti-amoebic drug combinations. Relief of symptoms was obtained in 98 to 100% of cases (Table 5), parasitological cure in 90 to 100% (Table 3), and healing of ulcer in 77 to 98% (Table 4). It was decided to assess changes in clinical presentation and ulcer healing separately rather than together under the common heading of "clinical cure" [10], because these two aspects of intestinal amoebiasis behave differently during recovery. The rate of ulcer healing depends on the initial size and depth, its location in the gut wall, and the possibility of bacterial superinfection.

More rapid and complete disappearance of invasive than of non-invasive forms was seen with all six treatment regimens, albeit with some minor differences from one treatment to another. This is not surprising, as it is known that parasitic cells in a stage of active replication, notably the invasive forms of *E. histolytica*, are far more susceptible to the action of specific drugs than non-replicating forms [3, 22]. This has also repeatedly been demonstrated in allied fields of research, such as bacteriology [23] and oncology [24].

In contrast with published reports [5, 21], the nitroimidazole derivative administered alone was not found to be more active on the invasive forms than the dichloracetamide derivative or aminosidine, which are regarded as drugs with exclusive intraluminal activity. In reality, aminosidine and etophamide are practically non-absorbed from the gut, and the intraluminal concentrations obtained with therapeutic dosages are enormous, sometimes exceeding 1000 times the MIC for *E. histolytica*. Both drugs also definitely penetrate mucosal lesions [25, 26]. In contrast, the nitroimidazole derivative is absorbed quite extensively from the upper intestine and its concentration in the large gut, where *E. histolytica* lodges, is irrelevant. This can account for its poor efficacy against the non-invasive forms [21], well confirmed here. Further confirmation of this view comes from the incidence of recurrence, which was significantly lower in the patients treated with aminosidine or etophamide than in those treated with nimorazole.

The best results were obtained with the EA and NA combinations. With the former, however, problems of tolerance were encountered, and there were eventually few treated cases on which to base a final judgment. The latter therapy, therefore, can be proposed as first-choice antiparasitic treatment for invasive intestinal amoebiasis, where all agree that a drug active in the tissues must be given together with one that acts mostly in the intestinal lumen [3, 5, 21].

In agreement with some previous reports [11, 12, 27, 28], in each therapeutic group, with the exception of nimorazole group, the parasitological cure rate (Table 3) was higher than anatomical cure rate (Table 4) at the end of treatment. Average across all patients, the incidence of persistent parasites in the stools was 5,5%, while the persistence of ulcers was seen in 9,2% of cases. A possible explanation for the small discrepancy may be in the fact that the time required for ulcer repair depends very much on the initial depth and size of the lesions. In the patients, the higher frequency of mild ulcers at the end of treatment was largely a reflection of improvement in initially severe ulcers, as confirmed by the absence of *E. histolytica* in scrapings from residual ulcers in many of the patients, a finding already reported by others [27].

The tolerability of all treatments except the EA combination was quite good, especially in patients treated with nimorazole alone. Some mild side effects were seen in patients receiving aminosidine. Amongst those treated with the EA combination, however, there were frequent episodes of violent diarrhea. Aminosidine is known to produce loosening of the stools of short duration as a result of its inhibitory activity on the normal intestinal flora, and this was noted in some of the patients of the aminosidine treatment group. However, etophamide has no antibacterial activity at all [De Carneri, 1977, unpublished data], and mild nausea is its best known side effect. There is no ready explanation for the severe diarrhoea seen in many patients treated with the two drugs together.

Assessing the results of the present study on the basis of clinical, parasitological and anatomical cures, recurrence and safety, it can be concluded that the combination of nimorazole and aminosidine proved to be the most suitable treatment, followed by aminosidine alone. The combination of etophamide with aminosidine, while very active from the parasitologic point of view, was unacceptable in practice, because of its prominent adverse effects.

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