Treatment of hepatic encephalopathy with metronidazole

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SUMMARY Neomycin, an antibiotic which is primarily active against the aerobic gut flora and hence reduces the endogenous production of ammonia, is a well-recognised form of treatment for acute or acute on chronic hepatic encephalopathy. This study suggests that metronidazole may be a useful alternative or even adjunctive treatment for such patients. Theoretical and practical justifications for the use of this drug are presented. The results of a week's prescription of each drug have been assessed by changes in clinical and biochemical criteria, including electroencephalograms and arterial ammonia samples. In the treatment of a series of 11 mildly or moderately, and seven severely affected, patients with histologically confirmed cirrhosis, metronidazole is shown to be as effective as neomycin.

The treatment of hepatic encephalopathy includes reduction of protein intake, alleviation of possible precipitating causes such as haemorrhage, as well as the administration of an antibacterial agent to reduce excessive endogenous ammonia formation by bacterial decomposition in the gut. Oral neomycin, used to suppress the intestinal flora in both acute and chronic hepatic failure, has been shown experimentally to reduce blood ammonia levels.^{1 2} However, the use of neomycin is not without hazard, as diarrhoea may be precipitated and there is the slight but definite risk of acute enterocolitis due to an overgrowth of resistant staphylococci or *Clostridium difficile*.^{3 4} During chronic prophylactic administration systemic effects, such as deafness, are possible, as well as steatorrhoea, which can aggravate the nutritional problems.^{5 6}

Neomycin is primarily active against Gram-negative bacilli and staphylococci, and has very limited action against streptococci and anaerobes in general. Yet recent bacteriological studies have suggested the Gram-negative anaerobes make a major contribution to the generation of ammonia from peptides.⁷ On theoretical grounds it seems likely that metronidazole, which is active against bacteroides and other anaerobes, could also reduce the production of endogenous ammonia by its effect on the anaerobic intestinal flora. In order to test this hypothesis, and to ascertain whether any benefit might ensue in patients with hepatic encephalopathy, a clinical trial was undertaken to compare the effect of metronidazole with that of neomycin.

Methods

Neomycin 1 g, or metronidazole 0.2 g four times a day, were each prescribed separately for one week to patients with acute or chronic encephalopathy. The order of treatment was randomised and was not disclosed to the clinical assessor (MHM). Every patient acted as his own control, and initial baseline results were contrasted with those after each antibiotic regimen.

PATIENTS

A series of 11 patients (group A) had mild or moderate features of encephalopathy, and in every case this complicated cirrhosis, which was confirmed histologically from a liver biopsy. Clinical details are shown in Table 1. There were eight females and three males, whose mean age was 60.8 years and range 46 to 86 years. In four the diagnosis was primary biliary cirrhosis. Four had alcoholic cirrhosis, two cryptogenic cirrhosis, and in one the cirrhosis followed chronic active hepatitis. The preliminary study was later extended to include seven patients (group B) with signs of severe encephalopathy, of whom one was comatose. In this group there were three males, and the mean age was 68.3 years, while the range extended from 55 to 73 years. The aetiology of their histologically proven cirrhosis was cryptogenic in four, alcoholic in two, and due to primary biliary cirrhosis in one.

The mental state was assessed and graded according to criteria suggested by Parsons-Smith *et al.* in 1957⁸ and modified by Conn *et al.* in 1977.⁹ Grade 0—No abnormality detected

Grade 1—Trivial lack of awareness, shortened attention-span.

Grade 2—Lethargy, disorientation in time, clear personality change, or inappropriate behaviour

Grade 3—Very drowsy, semicomatose but responsive to stimuli, confused, gross disorientation in time or space, bizarre behaviour

Grade 4—Coma

Further quantification of the clinical state was achieved by grading the degree of asterixis, as described by Conn in 1960¹⁰ and Conn *et al.* in 1977.⁹ The grades were then as follows:

Grade 0—No flap

Grade 1—Rare flapping movement of fingers or hands

Grade 2—Occasional irregular flaps

Grade 3—Frequent flaps

Grade 4—Almost continuous movement

Foot or was graded: severe, grade 2; moderate, grade 1; or absent, grade 0.

With a single exception, the patients were hospitalised throughout the study. There was an initial observation period of at least a week for the patients in group A, when necessary changes in diet and medication were instituted, but there were no

changes during the subsequent trial period. This was intended to counteract any significant bias in favour of the first antibiotic administered. Bowel function was monitored and recorded. No laxatives were prescribed, although two patients in group B had enemas on admission.

Protein intake was restricted to 40 g daily for group A patients, and in only one did this represent a change on admission. In this group the only other medication was a diuretic, prescribed for seven, potassium, which was given to five, and lactulose. This was prescribed for three patients, but in every case had already been started at least six weeks before admission. These three patients, and two others, had been given a short course of neomycin in the preceding six months, but its reintroduction was incorporated into the trial on a 'blind' basis with metronidazole.

All but one of the patients in group B had been on a 30 g restricted protein diet before admission, but two immediately had this reduced to 20 g. Intravenous fluids were started for two, but only continued for less than one week. A third patient required parenteral fluids towards the end of the first week, and this was continued. Diuretics were used for five, but not changed during the trial. In three, lactulose had been started at least four weeks earlier,

Table 1 Clinical and biochemical status of all patients at onset of study

Patient	Age (yr)	Sex	Diagnosis	Surgical shunt	Portal hypertension	Recent bleed	Past history enceph.	Bilirubin* (μmol/l)	Albumin† (g/l)	Urea‡ (mmol/l)	Mental state	Asterixis	EEG MDF	Arterial ammonia
Group A														
1	67	F	Cryptogenic	0	+	+	—	64	30	10.5	0	1	8.3	0.7
2	65	F	Alcoholic	0	+	—	—	108		3.4	0	2	8.5	1.1
3	48	F	Primary biliary	0	+	—	—	123	29	4.0	2	2	6.7	1.6
4	69	M	Alcoholic	Porto-caval	+	—	+	132	30	4.2	1	2	6.9	1.7
5	61	F	Primary biliary	0	—	—	—	35	32	5.4	1	0	7.6	1.4
6	46	M	Alcoholic	0	+	+	+	36		2.1	0	1	9.0	0.8
7	53	F	Primary biliary	0	—	—	—		36	4.9	1	2	7.5	0.8
8	53	F	Alcoholic	0	+	+	—	152	27	6.4	0	1	9.3	1.3
9	47	M	Alcoholic	0	+	+	+	29	29	4.3	2	3	4.2	1.8
10	86	F	Cryptogenic	0	—	—	—	20	47	15.7	1	4	6.2	0.9
11	66	F	Primary biliary	Porto-caval	+	—	+	124	32	4.2	1	2	5.5	1.5
Group B														
1	73	M	Alcoholic	0	—	—	—	85	32	4.4	3	3	4.1	1.7
2	73	F	Cryptogenic	Porto-caval	+	—	—	29	43	4.7	3	3	3.1	1.6
3	71	M	Cryptogenic	0	—	—	—	43	23	7.4	4	4	2.9	1.4
4	65	M	Cryptogenic	Porto-caval	+	—	+	23	32	4.0	3	3	3.4	1.4
5	55	F	Alcoholic	0	—	—	—	166	25	13.5	4	4	2.9	1.9
6	75	F	Cryptogenic	0	+	—	+	180	29	10.2	3	4	4.7	1.6
7	66	F	Primary biliary	Spleno-renal	+	—	+	35	29	4.6	3	1	4.9	1.3

Normal range: *3–14 μmol/l; †35–50g/l; ‡3–7 mmol/l.

and a fourth was started on it at the time of admission, but there was no change during the trial. No other antibiotic was prescribed.

There were no obvious precipitating factors leading to admission for seven of the patients in group A. Mild chronic haemorrhage from varices had affected two, and one had had a single minor bleed one month before the trial. Only one patient in this group, who had bled at the time of admission, required transfusion. In group B the only clear precipitating factors were continued alcohol consumption in one, the use of loperamide (Imodium) for diarrhoea in one, and a bleed from varices one month earlier in a third patient. Deterioration over periods ranging from three weeks to one year had been noted in the remainder.

ELECTROENCEPHALOGRAPHIC RECORDINGS

Estimation of the mean dominant frequency (MDF) was performed according to the method of Read *et al.*¹¹ An inter-record variation greater than 0.6 Hz was considered to be outside the day-to-day variation of cirrhotic patients.¹²

ARTERIAL AMMONIA ESTIMATION

Fasting arterial ammonia was measured by a modified method of Seligson and Hirahara, in which a standard curve was constructed during each estimation.¹³ The percentage variation in a series of 20 standard estimations was plus or minus 10% at the 1.0 µg level. Arterial samples were obtained from the brachial artery, and all subjects had been fasting for at least 10 hours.

BACTERIOLOGICAL METHODS

Facilities for full bacteriological investigation of faecal flora were not available, but as a support to the clinical study specimens were processed by methods routinely available in the clinical laboratory. Faeces were collected from patients before entry to the trial and at weekly intervals thereafter, and processed as soon as possible in the laboratory. Approximately 0.5 g was collected from several sites in the interior of the specimen and a 10-fold dilution was made by shaking this sample with nutrient broth. The predominant bacteria were counted by the method of Miles and Misra¹⁴ with nutrient broth as the diluent, and spread plates were also prepared to assist in recognition of bacteria present. The following media and conditions of incubation (at 37°C for 40 h) were used: 5% horse-blood agar incubated in air with 10% carbon dioxide; 5% horse-blood agar with 150 mg/l neomycin, anaerobically with 4–10% carbon dioxide (GasPak Anaerobic Systems, BBL); MacConkey's bile salt-lactose agar, in air; Sabouraud's glucose-peptone agar in air; tomato juice agar (Oxoid), anaerobically with 4–10% carbon dioxide (GasPak).

Identification to genus of all distinct colonial types of bacteria was carried out. Anaerobic Gram-negative bacilli were called 'bacteroides' and were not further identified.

Results

Improvement in the mental state, reduction of asterixis and of foetor were noted during treatment with metronidazole as well as with neomycin, and

Table 2 Changes in clinical state of patients during trial period

	Mental state			Asterixis			Foetor		
	Baseline	Metronidazole	Neomycin	Baseline	Metronidazole	Neomycin	Baseline	Metronidazole	Neomycin
Group A	0	0	0	1	0	0	0	0	0
	0	0	0	2	1	2	1	0	0
	2	1	1	2	1	0	0	0	0
	1	1	1	2	2	3	0	0	0
	1	0	0	0	0	0	0	0	0
	0	0	0	1	0	0	0	0	0
	1	0	0	2	0	0	0	0	0
	0	0	0	1	1	1	0	0	0
	2	0	1	3	0	2	1	0	0
	1	0	1	4	0	2	1	0	0
Group B	1	1	1	2	2	2	1	1	1
	3	1	1	3	2	2	2	0	1
	3	2	1	3	3	3	2	0	0
	4	3	2	4	2	1	1	0	0
	3	2	1	3	2	2	1	1	1
	4	2	1	4	2	1	2	1	0
	3	4	4	4*	4*	4*	1	1	1
	3	2	2	1	1	1	1	0	0

*Could not be tested because of lack of co-operation.
For scales, see details in the text.

these results are shown in Table 2. In the moderately affected group (A) mental state scores improved during both treatment regimes, $P < 0.01$ for Wilcoxon-sign rank test. Asterixis was also diminished, $P < 0.01$ for metronidazole, $P < 0.05$ for neomycin. In group B there was significant improvement in mental state, $P < 0.05$, and asterixis $P < 0.01$, for both drugs. A prolonged effect due to neomycin might have influenced the results of the week's treatment with metronidazole, and to exclude this possibility an additional analysis was undertaken on the results from the 11 patients who had been given metronidazole before neomycin. Significant improvement ($P < 0.01$) in both mental state and asterixis could still be demonstrated for this combined series of both moderately and severely affected patients. Eleven of the patients had hepatic foetor and in seven this cleared after treatment. In one patient foetor was present at the end of the week on neomycin, whereas it had previously cleared after treatment with metronidazole. Thus, on clinical criteria alone, 14 of 18 patients (78%) improved during treatment, and no clear difference could be demonstrated between metronidazole and neomycin.

The Figure shows changes in EEG measurements using the mean dominant frequency as an indicator of encephalopathy. Thirteen of the 17 patients had an increase in their MDF after the week on metronidazole ($t = 2.99$, $P < 0.01$ for two-tailed Student's t test), while 11 improved during the neomycin treatment ($t = 3.114$, $P < 0.01$). No significant group differences could be demonstrated between values for the MDF after neomycin compared with those after metronidazole. Among patients in group A the MDFs were improved in seven while receiving met-

ronidazole and in five while receiving neomycin. In group B similar changes were observed in all of the seven patients with neomycin, and in six of the seven with metronidazole. In one female patient who remained severely ill, there was no change in the MDF after neomycin. Minor slowing from 4.7 Hz to 3.9 Hz was noted after the week on metronidazole, while during the month after this trial her clinical condition failed to improve and she eventually died.

Raised blood urea levels may also cause slowing of the EEG and for this reason additional comparison of the treatment regimes was made for 13 of these patients whose blood urea remained normal throughout the trial period. However, even in this smaller group of both severely and moderately affected patients, significant improvement had occurred in the MDF ($t = 3.00$, $P < 0.01$) for neomycin and for metronidazole ($t = 3.14$, $P < 0.01$). Once again it could also be shown that the results were as good after metronidazole as neomycin, with no significant difference evident between the final values for each treatment week.

Mean arterial ammonia levels were greater in group B than in group A, but these differences did not reach statistically significant levels (Mann Whitney U test). Table 3 shows the mean levels before and after treatment but no significant differences could be demonstrated for the results after metronidazole or neomycin.

Faecal cultures were obtained in 16 of the patients, of whom six were in a severely affected group. After neomycin treatment for a week the counts of coliforms were reduced to undetectable levels in three patients, but this effect was not seen in others. In seven patients at the end of a week of metronidazole

Figure Changes in the EEG mean dominant frequency after treatment. Histograms indicate the mean values, bar lines show the standard deviations.

Table 3 Arterial ammonia levels before and after week's treatment with either metronidazole or neomycin

Arterial ammonia levels	Baseline	After metronidazole	After neomycin
Group A			
Mean	1.26	1.16	1.28
SD	0.39	0.28	0.39
Group B			
Mean	2.56	1.43	1.44
SD	0.21	0.39	0.34

treatment, bacteroides could not be detected by the limited methods used. This was also true of one patient given the combination of neomycin and metronidazole. All three regimens were often followed by increases in yeasts and aerobic streptococci which would be resistant to the two agents.

Among the severely affected group B patients there were four who received metronidazole first compared with three who received neomycin first, a difference which is unlikely to have exerted a very significant effect on the overall assessment.

Seven of the moderately affected patients had metronidazole first, but in all these there was a delay of at least a week before starting any antibiotics, in order to reduce the possibility of bias.

Seven patients were also prescribed lactulose, but all except one had already received this for at least four weeks before the trial. In addition, four of them received neomycin rather than metronidazole as the first antibiotic. Bowel habit was carefully monitored, and was not significantly different in the metronidazole compared with the neomycin treatment weeks. Only two patients (group B) had enemas, each only one on admission, and one of these had neomycin before metronidazole.

Results of the clinical assessments, EEG analysis, and arterial ammonia estimations for five of the patients in group B who were given a week of combined treatment on neomycin as well as metroni-

dazole are seen in Table 4. The MDF values show a significant difference ($P < 0.01$ for Wilcoxon sign-rank test) between combined treatment and either neomycin or metronidazole alone, but differences in the clinical state do not reach statistically significant values. Arterial ammonia estimations, however, revealed no evidence of any improvement on the combined therapy.

Discussion

This study indicates that metronidazole may be as effective as neomycin in the treatment of acute, or acute on chronic hepatic encephalopathy. Improvement has been produced in quantified parameters of clinical and neuropsychological function used to assess the degree of encephalopathy. Various factors which may introduce bias in such a study have been clearly described by Conn and his colleagues,⁹ and these have been minimised by using each subject as their own control as well as allowing an initial assessment period before introducing antibiotics for the patients in the chronic, less severely affected group.

Although clinical and EEG improvement has been demonstrated, it is of interest that neither antibiotic has induced significant changes in the arterial ammonia levels. Initial blood levels were higher in the severely affected group of patients, but even here there was no demonstrable improvement. Although many authors, including White *et al.*,^{14a} have reported a correlation between blood ammonia levels and the degree of encephalopathy, others such as Phillips and his colleagues in 1952¹⁵ have failed to find this. The present results support the probability of some relationship, but do not indicate any invariable or close correlation. Differences in methodology have been cited as one possible reason but in the current study these have been in accordance with reportedly preferable procedures.^{13 16} The explanation may lie in a more complex relationship between blood ammonia and encephalopathy. In spite

Table 4 Changes in clinical state resulting from week's treatment with combination of neomycin and metronidazole, compared with changes after each antibiotic given separately for a week

Patient	EEG (Mean dominant frequency) (Hz)				Mental state/asterixis				Arterial ammonia ($\mu\text{g/ml}$)			
	Baseline	Metronidazole	Neomycin	Metronidazole and neomycin	Baseline	Metronidazole	Neomycin	Metronidazole and neomycin	Baseline	Metronidazole and neomycin	Neomycin	Metronidazole and neomycin
1	7.5	9.2	8.5	8.9	1/2	0/0	0/0	0/0	0.8	0.8	0.9	0.8
2	4.1	7.6	6.7	8.0	3/3	1/2	2/1	0/2	1.7	1.7	1.5	1.6
3	3.1	3.5	3.8	4.2	3/3	2/3	1/3	1/1	1.6	1.4	1.1	0.9
4	2.9	7.4	8.0	8.9	4/4	2/2	1/1	1/1	1.9	1.1	1.0	1.1
5	4.9	5.6	5.3	6.1	3/1	2/1	2/1	1/1	1.3	0.9	1.9	1.5
Mean	4.5	6.66	6.45	7.22	2.8/2.6	1.4/1.6	1.2/1.2	0.6/1	1.46	1.18	1.28	1.18
SD	1.86	2.18	1.94	2.04	1.10/1.14	0.89/1.14	0.84/1.10	0.55/0.71	0.43	0.37	0.41	0.36

of the conflicting evidence in respect of blood ammonia many authors have noted the deleterious effect of excessive protein intake, or a gastrointestinal haemorrhage in patients with cirrhosis. Van Caulaert, as early as 1932, reported that the administration of 5–10g ammonium chloride induced raised levels of blood ammonia, and a semistuporous state, in cirrhotic but not in normal subjects.¹⁷ Protein breakdown eventually gives rise to urea, the chief end-product of nitrogen metabolism. This can diffuse freely into all body fluids, including the intestinal lumen. Anaerobic bacteria, by far the most numerous of the flora of the large intestine and terminal ileum,¹⁸ contribute to bacterial urease activity in the gut¹⁹ which results in hydrolysis of the urea to ammonia. Recent studies *in vivo* have indicated that anaerobes such as bacteroides may indeed be major contributors to intestinal ammonia formation from proteins and their derivatives.⁷ Anaerobic bacteria are also capable of producing potentially toxic amines by decarboxylation of aminoacids.²⁰ It is known that methionine can induce coma in susceptible patients with liver disease, and that this is not related to any increase in ammonia production.²¹ For these reasons a beneficial effect of metronidazole might be anticipated in patients with hepatic encephalopathy, because of the known effect of this drug on anaerobic bacteria.^{22 23 24} In addition, some limited activity of metronidazole against aerobic bacteria in special situations has recently been demonstrated.^{25 26}

A reduction in the anaerobic flora of the small intestine after metronidazole has previously been demonstrated, but the fall in bacteroides in faeces that we observed in some patients was not seen in volunteers given a metronidazole regimen similar to the one used by us and studied with more elaborate anaerobic bacteriological methods.²⁷ These authors, however, demonstrated a marked reduction in faecal bacteroides after a combination of neomycin and metronidazole, such as we saw in one patient, and four of our patients who demonstrated a fall in bacteroides counts after metronidazole alone had received neomycin in the previous week. Arabi *et al.*²⁸ showed a reduction in the coliform count after neomycin in a similar proportion of patients to that seen in the present series.

Thus several theoretical possibilities exist to explain the efficacy of metronidazole in the treatment of hepatic encephalopathy, and the present investigation provides preliminary evidence to suggest that this is a practical alternative. It may, indeed, be a valuable innovation if pseudomembranous enterocolitis caused by *Clostridium difficile* proves to be a hazard of neomycin treatment.⁴ The addition of metronidazole to neomycin may further improve the clinical status of patients with encephalopathy. As

only a small number of patients has so far received treatment with the combination of drugs it is not possible to draw very firm conclusions. However, the demonstrable improvement in the EEG mean dominant frequency during treatment with the combination, compared with results for either drug alone, suggests that this could be a worthwhile regime to try when either drug alone has failed to produce an adequate clinical response, or when there is apparently diminishing benefit from neomycin.

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