PHASE 1 TOLERABILITY AND SEARCHING DOSE STUDIES WITH 4-ISOTHIOCYANATO-4'-NITRODIPHENYLAMINE (C.9333-Go/CGP 4540), A NEW ANTHELMINTIC

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1 Phase 1 and 2 studies were carried out in thirteen volunteers and thirty patients with hookworm anaemia with a new anthelmintic, 4-isothiocyanato-4'-nitrodiphenylamine (C.9333-Go/CGP 4540). Single and multiple doses were administered by mouth under careful supervision by the staff of the clinical pharmacology unit.

2 4-Isothiocyanato-4'-nitrodiphenylamine (C.9333-Go/CGP 4540) was well tolerated in volunteers up to a single dose of 1750 mg and in patients up to 3000 mg in divided doses. Side effects were rare, transient and mild. One patient had moderate giddiness for 24 h after the compound. The laboratory investigations did not show any adverse effects on organ functions. Transient non-specific changes in the T-wave were occasionally observed in the e.c.g.

3 Multiple doses (125 mg, 250 mg or 1000 mg $\times$ 3) of the compound were highly effective in eradicating hookworm infection, as judged by almost 100% reduction in faecal egg counts and significant recovery of parasites from 24 h stool samples. The compound also showed activity against *A. lumbricoides*, *T. trichiura* and *E. vermicularis*. The compound appears to be promising and more studies, in a field situation, are desirable.

Introduction

C.9333-Go/CGP 4540, 4-isothiocyanato-4'-nitrodiphenylamine, (Figure 1) is one of a series of synthetic organic isothiocyanates (Striebel, 1976) with an unusual anthelmintic activity against intestinal nematodes, filariae and schistosomes in experimental animals. These compounds were prepared in the Research Department of Ciba-Geigy Agrochemicals Division, Basle, and in the Chemical Laboratories of Ciba-Geigy Research Centre, Goregaon. C. 9333-Go/CGP 4540 was synthesized in the former laboratories. It eradicated both patent and non-patent infections with human hookworm *Necator americanus* in golden hamsters *Mesocricetus auratus*; the ED₅₀ in patent infection is $\sim$ 10 mg/kg (Sen, 1976). In acute anaesthetized dogs C.9333-Go/CGP 4540 at 30–50 mg/kg, intravenously, in DMA solution, produced a fall of 10–15 mmHg in blood pressure lasting for about 10 minutes. In chronic oral studies in dogs, a dose of 100 mg/kg once a day for four days, did not have any significant effect on blood pressure,

heart rate or e.c.g. No hypoglycaemic, antifertility or diuretic effects were observed with C.9333-Go/CGP 4540. In acute toxicity studies, the LD₅₀ in several species, rat, mouse, dog and monkey, was more than 5000 mg/kg, suggesting a poor absorption of the compound. The subacute toxicity studies in rats and dogs and teratogenic studies in rats, showed that the compound was fairly safe for human administration (Rao, 1976, personal communication).

We now present the results in Phase I and searching dose studies of the effects of C.9333-Go/CGP 4540 in volunteers and patients with hookworm infection.

Methods

Subjects

All subjects in the study were hospitalized in the Clinical Pharmacology Unit of K.E.M. Hospital,

Figure 1 The structural formula of C.9333-Go/CGP 4540.

Bombay. Thirteen hospital inpatients (12 males, one female), ranging in age from 24–60 years and weighing from 40–55 kg and recovered from minor intercurrent illness (Sheth, 1976) volunteered for Phase I studies. Careful history, physical examination and laboratory investigations were carried out to rule out any severe systemic disease. An informed written consent was obtained from each volunteer. The female subject admitted for the study was menopausal for five years, with no risk of teratogenicity.

Patients

Thirty hospital inpatients (27 males, three females) ranging in age from 18–67 years and with symptoms of hookworm anaemia (haemoglobin $<10\text{ g\%}$ $>5\text{ g\%}$) volunteered for the searching dose studies with C.9333-Go/CGP 4540. A careful history, thorough physical examination and essential laboratory investigations preceded the drug administration. An informed written consent was obtained from each patient. Three female patients included in the study were in menopausal period.

Tolerability profile

Baseline symptoms were evaluated and recorded before and after treatment to avoid non-drug adverse reactions (Vaidya, 1973); a check-list covered 32 symptoms and three blanks. Positive signs were recorded under each system. Pulse rate (PR), systolic (SBP) and diastolic (DBP) blood pressure in supine and standing positions were recorded basally and after drug administration. The following laboratory investigations to monitor organ functions were carried out basally, 24 h after and one week after drug administration: haemoglobin, haematocrit, red cell count, white cell count (total and differential), platelet count, sedimentation rate, serum uric acid, serum proteins, serum enzymes (transaminases, alkaline phosphatase and pseudocholinesterase), serum bilirubin (total and direct), serum cholesterol, fasting blood sugar, blood urea nitrogen, serum electrolytes (sodium, potassium and chloride), routine urinalysis and stool examination. An e.c.g. for standard and chest leads was taken before, 1 h, 2 h, 3 h, 24 h and one week after the drug administration.

The volunteers were carefully observed for a fortnight and the patients were observed for one month after the administration of C.9333-Go/CGP 4540. Even after discharge, the subjects were asked to report any unusual symptoms.

Drug administration

C.9333-Go/CGP 4540 (Tablets of 50, 100, 250, 500 mg) was administered orally, as single doses ranging from 50–1650 mg, to 13 volunteer subjects; the dose was given in the morning after an overnight fast and food was withheld for 4 hours. The following dosage schedules were studied in 30 patients, to evolve an optimal and safe regimen:

- Group A: Single doses ranging from 250–1750 mg administered on an empty stomach.
- Group B: $1000\text{ mg} \times 3$ every 12 h, the first and last doses administered on an empty stomach in the morning.
- Group C: $250\text{ mg} \times 3$ every 8 h, the first dose in a fasting state.
- Group D: $125\text{ mg} \times 3$ every 4 h, the first dose administered in the morning after overnight fasting.

No drugs were administered on the day of treatment with the anthelmintic. Patients with anaemia received iron and vitamin tablets before and after drug administration.

Parasitological methods

The methods used for the faecal egg counts were Stoll's technique (Stoll, 1925) and the modified stool homogenization and flotation technique (Faust, Sawitz, Tobie, Odom, Peres & Lincicome, 1939; Sen, 1972). The total 24-h samples on day 1, 2 and 3 after treatment were collected, homogenized, filtered and examined carefully by one of us (HGS) for the count and identification of the parasites. Coprocultures for larvae were carried out on post-treatment stool specimens (Hsieh, 1963).

Evaluation of efficacy

The clinical antihookworm efficacy of C.9333-Go/CGP 4540 was assessed by comparison of the pre-treatment faecal egg count (EPG) per gram (average of three daily consecutive stool samples) with the post-treatment EPG at weekly intervals for four weeks (Vaidya & Sen, 1974). The final EPG at four weeks was compared with the basal EPG for the calculation of percentage reduction in EPG. The recovery, count and identification of the parasites in 24-h stools for three consecutive days after treatment was also taken as an additional evidence of efficacy. The post-treatment stools showing zero EPG were double-checked by a negative coproculture.

Any concomitant intestinal parasitic infection was also studied by the methods for egg-count and worm count, but no special effort was made to evolve dosage schedules effective against other helminths in the gut; efforts were concentrated on the antihookworm efficacy of the compound.

Results

General tolerance

The general tolerance of C.9333-Go/CGP 4540 in hospitalized volunteers and patients was very good, even at high doses such as a single dose of 1750 mg or 1000 mg $\times$ 3. Table 1 shows the volunteer details, the doses of C.9333-Go/CGP 4540 and clinical and laboratory tolerability of the compound. Except for a transient, slight and asymptomatic reduction in blood pressure in two volunteers, no side effects or aggravation of baseline symptoms were observed in 13 volunteers. In one volunteer, at 1650 mg, an elevation in serum cholesterol from a basal value of 190 mg/100 ml to 275 mg/100 ml was seen; there was no significant change in serum transaminases. The laboratory investigations to evaluate organ functions after C.9333-Go/CGP 4540, did not show any other significant change.

A transient, mild, and non-specific reduction in the amplitude of T-wave was observed in seven volunteers at 3 h after the drug. One volunteer, on a dose of 1650 mg of the drug, showed a reduction in the amplitude of T-wave and an elevation of ST segment in leads V₁, V₂, V₃ at 3 hours. The changes returned to normal in 24 hours. No chest pain or alteration in serum transaminases were seen in any volunteer.

In 30 patients with hookworm infection, the general tolerance of the drug was good. The side effects reported were as follows: a delayed generalized maculopapular rash occurred in two patients, 10 days after discharge from the hospital ward. The rash disappeared in about 10 days. The patients had

received 350 mg and 400 mg of C.9333-Go/CGP 4540. Both patients were from the same area in a village; non-drug factors could not be ruled out. One patient developed diarrhoea after 1000 mg $\times$ 3 of the drug, but the stools showed vegetative forms of *Entamoeba histolytica*; the side effect cannot totally be ascribed to the drug. One patient, at a dose of 125 mg $\times$ 3 of the drug developed moderately severe giddiness; it was not accompanied by any changes in blood pressure. The symptom disappeared in 24 hours. A transient, mild and asymptomatic drop of SBP was observed in three patients. The laboratory investigations did not show any adverse effect on organ functions, at several dosage schedules of C.9333-Go/CGP 4540. No significant changes were seen in electrocardiogram except transient non-specific T-wave changes in an occasional patient. In one patient mild and transient (4 h) sinus tachycardia was observed; from a basal HR of 72 beats/min it was elevated to 98 beats/min at 2 h and 102 beats/min at 3 hours.

Antihookworm efficacy

Table 2 shows the results obtained with different dosage schedules of C.9333-Go/CGP 4540, in patients with hookworm infection. Single doses, though promising, did not eradicate the infection, except in one patient. Multiple doses 1000 mg $\times$ 3 at 12 hourly intervals and 250 mg $\times$ 3 at eight hourly intervals cured the infection, as shown by persistent zero EPG and a confirmation of cure by faecal culture for larvae. With a dose of 125 mg $\times$ 3 given at four hourly intervals, two out of 10 patients were not

Table 1 Phase 1 tolerability of C.9333-Go/CGP 4540 in volunteers

Subject	Sex	Age (years)	Weight (kg)	Total Dose (mg)	Dose (mg/kg)	Clinical tolerance	Laboratory findings
1	M	24	50	50	1.0	Good	NSC*
2	M	60	42	100	2.4	↓ SBP 16 mmHg	NSC
3	M	40	55	150	2.7	Good	NSC
4	M	30	41	200	4.8	Good	NSC
5	M	40	42	250	5.9	Good	NSC
6	M	25	49	400	8.1	Good	NSC
7	F	50	46	450	9.7	Good	NSC
8	M	24	52	500	9.6	Good	NSC
9	M	40	43	550	10.4	Good	NSC
10	M	30	40	850	21.2	Good	NSC
11	M	50	50	1000	20.0	Good	NSC
12	M	24	44	1250	28.4	Good	NSC
13	M	34	45	1650	36.6	↓ SBP 20 mmHg	Serum cholesterol raised from 190 to 275 mg/100ml

*NSC=No significant change.

completely cured. One of these patients had a pure *A. duodenale* infection.

Other parasites

Ascaris lumbricoides and *Enterobius vermicularis* parasites were recovered in several patients in post-treatment stools. A reduction in e.p.g. for these worms and *Trichuris trichiura* was also seen.

Discussion

C.9333-Go/CGP 4540 offers new possibilities for the eradication of hookworm infection. Our previous experience with tetrachlorethylene, iodothymol and bitoscanate made us wonder whether it would be ever possible to achieve zero post-treatment egg counts in a large population with a single course of an anthelmintic (Gupta, Vaidya, Karandikar & Sheth,

1967). The excellent efficacy of C.9333-Go/CGP 4540 in hospitalized patients, especially with multiple doses of 250 mg and above, makes it desirable to study this anthelmintic in the field on a large number of patients.

There was no evidence of any toxic effect on the bone marrow, liver and kidney as assessed by basal and repeated haemogram, liver and kidney function tests. The only side effect that was felt to be drug-related was giddiness in one patient, who did not have any baseline symptom of giddiness. The symptom was not related to any change in blood pressure. It is desirable to evaluate the incidence of this side effect in field trials. As hospitalized patients are usually resting and also receive good diet and drugs like iron and vitamins, the excellent tolerability seen in hospitalized patients cannot be extrapolated to patients in field situation. The marginal changes in blood pressure and occasional changes in T-wave in patients with anaemia are difficult to evaluate. Detailed studies for

Table 2 Anthelmintic efficacy of C.9333-Go/CGP 4540

Patient	EPG		Dosage (mg)	Worm recovery						% reduction in EPG
	Initial	Post treatment		N. americanus			A. duodenale			
				♂	♀	Total	♂	♀	Total	
1	7183	8467	250 × 1	0	0	0	0	0	0	0
2	3920	5713	300 × 1	0	0	0	0	0	0	0
3	3965	1760	350 × 1	25	16	41	0	3	0	56
4	7160	693	400 × 1	234	181	415	0	0	0	90
5	1820	760	550 × 1	0	0	0	23	12	35	60
6	4543	273	750 × 1	312	211	523	0	0	0	94
7	38360	21573	1000 × 1	8	3	11	0	0	0	44
8	16730	4120	1250 × 1	681	653	1334	0	0	0	75
9	9353	0	1500 × 1	166	189	355	0	0	0	100
10*	12927	13107	1750 × 1	—	—	—	—	—	—	0
11	1112	0	1000 × 3	63	69	132	0	0	0	100
12	5581	0	1000 × 3	63	86	149	0	0	0	100
13	3020	0	1000 × 3	217	220	437	0	0	0	100
14	6025	0	1000 × 3	243	192	435	0	0	0	100
15*	1907	0	1000 × 3	—	—	—	—	—	—	100
16	1440	0	250 × 3	1	1	2	0	0	0	100
17	3075	0	250 × 3	143	181	324	5	6	11	100
18	855	0	250 × 3	105	142	247	5	9	14	100
19	11350	0	250 × 3	562	270	832	0	0	0	100
20	2840	0	250 × 3	7	12	19	0	0	0	100
21	4490	0	125 × 3	29	50	79	0	0	0	100
22	9105	0	125 × 3	278	221	499	0	0	0	100
23	3107	0	125 × 3	47	40	87	0	0	0	100
24	5345	2760	125 × 3	78	58	136	0	0	0	48
25	5080	140	125 × 3	0	0	0	104	75	179	97
26*	5530	0	125 × 3	—	—	—	—	—	—	100
27*	7840	0	125 × 3	—	—	—	—	—	—	100
28	2020	0	125 × 3	208	217	425	0	0	0	100
29*	1572	0	125 × 3	—	—	—	—	—	—	100
30	1566	0	125 × 3	333	209	542	0	0	0	100

* Stools not checked for parasites; used for metabolic studies.

EPG — Faecal egg-count/g.

the cardiovascular tolerance of C.9333-Go/CGP 4540 are being conducted at present; early impressions do not cause any undue concern.

C.9333-Go/CGP 4540 has excellent anti-hookworm efficacy. The tolerability in hospitalized volunteers and patients is good. The drug also has anthelmintic activity against other parasites, *A. lumbricoides*, *T. trichiura* and *O. vermicularis*. The compound should be studied further in the field situation.

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