HB_sAg-binding to hydrophobic gels

Ligands coupled to Sepharose 4 B	μmol Ligand per ml adsorbent	HB_sAg titre of supernatant in	
		ID	Ausria II®
Hexylamine	2.8	1:32	
Octylamine	10.5	Absent	1:1024
Decylamine	10.8	Absent	1:512
Dodecylamine	8.0	Absent	1:128
Octadecylamine	2.7	Absent	1:32
5-Amino-nonan	2.8	1:32	
Glycyl-norleucine	8.2[a]	1:32	
Cykloheptylamine	5.3	1:32	
Hexamethylenediamine	12.8	1:32	
Hexamethylenediamine-benzoic acid	15.8	1:32	
Hexamethylenediamine-heptanoic acid	8.6	1:16	
Ethylenediamine	—[b]	1:32	
Ethylenediamine-succinic acid	2.5	1:32	
Ethylenediamine-octylsuccinic acid	8.9	Absent	1:128

[a] Determined by amino acid analysis. [b] Trinitrobenzenesulphonic acid reaction showed that the coupling functioned.

phobic ligand, we sometimes used organic solvents like ethanol, dioxane, dimethylformamide or solvents mixed with various amounts of water instead of buffered aqueous solutions. The amounts of ligand coupled was determined by NMR after hydrolysis of the gel adsorbent in concentrated formic acid or 6 *M* hydrochloric acid.

The adsorption experiments were performed by adding 2 ml of gel adsorbent to a test tube containing 2 ml of plasma having a HB_sAg titer of 1:64 in double immunodiffusion tests (ID). The tube was gently shaken for 15 min at room temperature. The gel was then removed by centrifugation and the supernatant was tested for the presence of HB_sAg. The determination of HB_sAg was performed by ID in agarose plates containing 2% dextrane to increase the sensitivity[7]. A rabbit HB_sAg-antiserum was used as precipitating agent. In some experiments, a sensitive radioimmuno assay method (Ausria II®) was used.

A number of different gel adsorbents were tested, and the results are shown in the Table. It is only the adsorbents that contain a straight hydrocarbon chain with more than seven carbon atoms that do bind HB_sAg. The other gel adsorbents do not show any affinity for HB_sAg, with the possible exception of heptanoic acid-hexamethylenediamine-Sepharose. The binding was very strong. The adsorbed material could not be eluted by changing pH or ionic strength of the eluting buffer. Elution with 6 *M* urea or 70 m*M* caprylate was not effective. As can be seen from the results in radioimmuno assay, there appears to be a correlation between the size of the straight hydrocarbon chain and the effectiveness of the adsorption of HB_sAg. The longer the hydrocarbon chain, the more effective the binding. The amount of coupled ligand seems to be of minor importance compared to the length of the hydrocarbon chain. It is established that albumins do bind to these types of gel adsorbents, but, despite this fact, HB_sAg binds to a very large extent to these gel adsorbents in the prescence of large amounts of albumin. This indicates that the competition of albumin for the affinity of HB_sAg is of minor importance.

The strong binding of HB_sAg to these gels suggests that they can be used for the removal of hepatitis virus from blood and blood products. It is fairly probable that the intact virus shows a similar behaviour to that of its coat component, but is cannot be ruled out that there may be a difference. Adsorption studies on the intact virus will answer that question. Another possible use of these adsorbents is in connection with HB_sAg experiments. For example, the amount of bound HB_sAg can be measured by incubating the gel with labelled HB_sAg-antibodies followed by suitable detection. The concentrating effect of the gel adsorbent would make such a test extremely sensitive.

[7] R. Berg, O. Ringertz and A. Espmark, Acta path. microbiol. scand. sect B. *79*, 423 (1971).

4-Isothiocyanato-4′-Nitrodiphenylamine (C 9333-Go/CGP 4540), an Anthelminthic with an Unusual Spectrum of Activity Against Intestinal Nematodes, Filariae and Schistosomes

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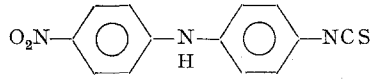
Summary. 4-isothiocyanato-4′-nitrodiphenylamine was found to possess activity against intestinal nematodes in mice, against schistosomes in various hosts including primates and against two filarial species in the mongolian jird. Upon administration in a single oral dose it is equally effective against *S. haematobium*, *S. mansoni* and *S. japonicum*.

Most of the known anthelminthics are active either against gastro-intestinal helminths or against systemic helminths. In our investigations with isothiocyanato compounds, we found substances displaying activity against both intestinal nematodes and systemic nematodes and trematodes. A 'broad-spectrum' anthelminthic would be of immense value in countries still in the throes of economic development, where intestinal helminthiasis, schistosomiasis and filariasis are not only highly endemic but often found simultaneously in one and the same

patient, and therefore constitute major public-health problems. The economic loss caused by these helminthic infestations is difficult to estimate, but is certainly considerable since these diseases are mostly incapacitating though not generally lethal. New and better antischistosomal and antifilarial drugs are urgently needed, and would do much to improve public health and hence contribute to the betterment of economic conditions in these countries.

From a great number of isothiocyanato compounds[1] tested, we have selected 4-isothiocyanato-4'-nitrodiphenylamine (C 9333-Go/CGP 4540) for detailed studies of its anthelminthic qualities on the basis of its high therapeutic index in experimental schistosomiasis and filariasis.



C 9333-Go/CGP 4540 is a yellow crystalline substance which is insoluble in water and most organic solvents and has a melting point of 198–199° when recrystallized from acetone. Its anthelminthic efficacy largely depends on the particle size and on the dosage form[2].

The first investigations of the anthelminthic properties of C 9333-Go/CGP 4540 were carried out with *Nippostrongylus muris*, *Nematospiroides dubius* and *Schistosoma mansoni* in mice. The material then used was relatively coarse, having a median particle size of 30–50 µm. At the end of the prepatent period of the various helminth species, the experimentally infested mice were given the compound orally or subcutaneously once or once daily for 3 days. The curative doses for the individual helminth species were found to be:

Nippostrongylus muris, 1000 mg/kg × 1 p.o.; 600 mg/kg × 3 p.o.
1000 mg/kg × 1 s.c., no activity.

Nematospiroides dubius, 300 mg/kg × 1 p.o.; 100 mg/kg × 3 p.o.;
1000 mg/kg × 1 s.c., no activity.

Schistosoma mansoni, 300 mg/kg × 1 p.o.; 50 mg/kg × 1 s.c.

Considering the limited predictive value of the mouse model for schistosomiasis[3,4] and the recent finding that antischistosomal drugs effective against *S. mansoni* are not necessarily active against *S. haematobium*[5,6], we extended our chemotherapeutic experiments to various host-parasite systems, including the 3 principal species of schistosome pathogenic for man. These studies were carried out with micronized C 9333-Go/CGP 4540 (median particle size: 4.23 µm; specific surface: 1.94 m²/g). For parenteral administration the compound was dissolved in polyethylene glycol 400 or dimethylacetamide. The results observed after treatment with single doses in experimental schistosomiasis are listed in Table I.

Table I.

Host	Parasite	Mode of administration	Curative dose (mg/kg)
Mouse	*S. mansoni*	p.o.	120
Mouse	*S. mansoni*	s.c.	50
Hamster	*S. mansoni*	p.o.	60
Hamster	*S. mansoni*	i.m.	50
Vervet monkey	*S. mansoni*	p.o.	50
Hamster	*S. japonicum*	i.m.	90
Dog	*S. japonicum*	p.o.	40
Dog	*S. japonicum*	i.v.	5
Hamster	*S. haematobium*	p.o.	40
Bird	*S. haematobium*	p.o.	30

It is noteworthy that C 9333-Go/CGP 4540 is almost equally active against *S. mansoni*, *S. haematobium* and *S. japonicum*. The dose-response curve is remarkably steep, (e.g. 3 mg/kg i.v. eliminated 10%, 4 mg/kg i.v. 99.7 and 5 mg/kg i.v. 100% of *S. japonicum* in beagle dogs with an average parasite load of > 2,000).

The antifilarial efficacy was tested in the mongolian jird (*Meriones unguiculatus*) infested with *Litosomoides carinii* or *Dipetalonema witei*. Oral treatment led to reductions in the parasite load compared with those found in untreated controls shown in Table II.

Table II.

Total dose (mg/kg)	Daily dose (mg/kg)	Duration of treatment (days)	% Reduction of Microfilariae	Macrofilariae
Litosomoides carinii				
200	100	2	93	100
250	50	5	80	16
300	300	1	50	54
500	100	5	99	100
600	300	2	100	100
1000	1000	1	96	100
1000	200	5	100	100
Dipetalonema witei				
200	100	2	97	50
500	500	1	98	60
600	300	2	100	37
1200	600	2	100	100

C 9333-Go/CGP 4540 was well tolerated in acute toxicity tests. No fatalities due to intoxication were recorded even after treatment with the compound in micronized form (1.94 m²/g) in the maximum quantities administrable orally, so that the median lethal dose (LD_{50}) could not be determined. The oral LD_{50} is certainly higher than 5,000 mg/kg in rats, mice, dogs, cats and rhesus monkeys.

Further experimental data on the activity of C 9333-Go/CGP 4540 against human hookworm in hamsters[7] and against schistosomes of various geographical origins, in mice[2], as well as the results of mutagenicity studies in bacteria[2], will be published elsewhere.

[1] These 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 by Dr. P. BRENNEISEN.

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