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CHEMOTHERAPEUTIC PROPERTIES OF SOME NEW QUATERNARY AMMONIUM COMPOUNDS; THEIR CESTICIDAL ACTION AGAINST *HYMENOLEPIS NANA*

BY

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This paper reports the results of screening some monoquaternary and bisquaternary compounds for cesticidal activity using *Hymenolepis nana* as the test parasite. Some of these compounds have antimicrobial and antifungal properties (Cox & D'Arcy, 1959, 1961). Others are effective against the cotton rat filarial parasite *Litomosoides carinii* (Hawking & Terry, 1959). The compounds tested were of the bisquinolinium, bisisoquinolinium, bis(7-aminoisoquinolinium), bis(4-aminoquinaldinium) and bis(4-aminocinnolinium) series, referred here for ease of reference as BQ*n*, BIQ*n*, BAQD*n* and BAC*n*, where *n* is equal to the number of methylene groups in the chain. The abbreviated nomenclature used is that of Barlow & Ing (1948). The other series consists of monoquaternary derivatives of 4-aminoquinaldine.

METHODS

The strain of *Hymenolepis nana* was obtained from the Wellcome Laboratories of Tropical Medicine, London, through the courtesy of Dr O. D. Standen and Mr J. E. D. Keeling. The infection was maintained in the laboratory as follows: ova were obtained by taking terminal gravid proglottids of worms from infected mice and crushing them lightly under a coverslip. The ova were washed in boiled and cooled tap water and allowed to stand for about 20 hr at about 30° C (room temperature). The ova were then counted in a haemocytometer chamber and an appropriate number (500 to 2,000) were given to each mouse along with 0.05 mg of morphine hydrochloride by a syringe and stomach tube. Ova from mature worms appeared in the faeces 14 to 16 days after intubation.

The compounds were tested against *Hymenolepis nana in vitro* using Sen & Hawking's (1960) method. The worms were obtained by killing the mice (infected 20 to 40 days earlier) and washing out their intestine. After two washes with Ringer solution the worms were transferred to nutrient broth containing penicillin and streptomycin. The worms were next transferred to small Kjeldahl flasks (10 ml. capacity) containing 3 ml. of medium. Suitable concentrations of compounds were added to these flasks before transferring the worms. The flasks were then kept in an incubator at 37° C and examined under a dissecting microscope after 24 hr to see whether the worms were living or dead.

The compounds which were found to be most active were then submitted to an acute toxicity trial. To determine the maximal tolerated dose (MTD) five mice were treated with a single oral dose at each dose level. The highest dose at which at least four out of five mice survived for 72 hr was taken as the MTD. Thereafter a dose lower than MTD was used to carry out *in vivo* tests. When a compound was found to be active *in vivo*, additional infected mice were treated at a fraction (usually half) of the previous dose. The minimum effective dose (MED) of a compound was the smallest dose which killed or removed a substantial amount of worm load from the treated mice compared to those from a control group of untreated mice.

To carry out *in vivo* tests the technique of Steward (1955) was followed with some modification. Groups of mice were taken on the nineteenth day after infection. The drug was given orally in a single dose by stomach tube. The mice were starved on the second day and killed on the third day after treatment. The worms were collected from the intestine and a score was made with the number of worms present multiplied by a factor for the size of the worm (worms more than 3 cm, 20; about 2.5 cm, 10; about 1.2 cm, 5; about 0.6 cm, 0.5; about 0.3 cm or less, 0.1). The results thus obtained were compared with those from a control group of untreated mice.

RESULTS

Table 1 summarizes the results of *in vitro* test. In the BIQ series sixteen compounds have been tested and it could be seen that there is a definite link between the cesticidal activity and the length of the polymethylene chain. Little activity could be detected in the BIQ

TABLE 1

RESULTS OF *IN VITRO* TEST OF COMPOUNDS AGAINST *HYMENOLEPIS NANA*

Drug	Minimum lethal concentration	Drug	Minimum lethal concentration
BIQ7	Inactive	BIQ32	Inactive
BIQ9	Inactive	BIQ34	Inactive
BIQ10	1 : 1,000	BIQ36	Inactive
BIQ11	1 : 1,000	BIQ40	Inactive
BIQ14	1 : 10,000	BAC16	1 : 10,000
BIQ15	1 : 1,000	BAC22	Inactive
BIQ16	1 : 1,000	BQ12	1 : 1,000
BIQ17	1 : 10,000	BQ20	1 : 1 million
BIQ18	1 : 10,000	BAIQ16	1 : 10,000
BIQ20	1 : 1 million	BAQD10	1 : 10,000
BIQ22	1 : 1·5 million	BAQD20	Inactive
BIQ24	1 : 10,000		

Drug	Minimum lethal concentration
4-Amino-1-heptylquinaldinium acetate	1 : 10,000
4-Amino-1-octylquinaldinium acetate	1 : 10,000
4-Amino-1-nonylquinaldinium acetate	1 : 100,000
4-Amino-1-decylquinaldinium acetate	1 : 100,000
4-Amino-1-undecylquinaldinium acetate	1 : 100,000
4-Amino-1-dodecylquinaldinium acetate (laurolinium)	1 : 1 million
4-Amino-1-tetradecylquinaldinium acetate	1 : 10,000
4-Amino-1-hexadecylquinaldinium acetate	Inactive
4-Amino-1-octadecylquinaldinium acetate	Inactive

compounds having less than ten methylene groups, but as the chain length increased from 11 to 22 the minimum lethal concentration decreased from 1 in 1,000 to 1 in 1.5 million. The peak cesticidal activity seems to have been reached at BIQ22 and thereafter the activity became less with the increasing chain length. The minimum lethal concentration for BIQ24 was 1 in 10,000 whereas BIQ32, BIQ36 and BIQ40 were all inactive.

The related BQ series also showed cesticidal activity against *Hymenolepis nana in vitro*. The most active compound of this series was BQ20 which killed the worms in 1 in 1 million drug concentration.

Several compounds of related BAC, BAIQ and BAQD series were also tested but none of them showed any appreciable cesticidal activity *in vitro*.

The activity of 4-aminoquinaldinium acetate compounds when tested *in vitro* increased with the increase in chain length, the maximum being for the dodecyl compound. The

minimum lethal concentration of 4-amino-l-dodecylquinaldinium acetate (laurolinium) was 1 in 1 million. With higher chain length the activity gradually decreased.

In acute toxicity tests, the maximal tolerated single oral dose (survival of 80% of mice for 72 hr) was 500 mg/kg for BIQ20 and for BIQ22; 250 mg/kg for BQ20; and 50 mg/kg for laurolinium. All the mice treated with BIQ20, BIQ22 and BQ20, whether dead or alive, were found to have their stomach and intestine inflated to a varying degree.

Table 2 gives the summary of *in vivo* tests. BIQ22 was the most active and safest of all the four compounds tested. Next came BIQ20, BQ20 and laurolinium, in that order.

TABLE 2

SUMMARY OF THE *IN VIVO* TESTS

The column headed response gives the number of mice freed from worms and the number of mice treated

Compound	Dose (mg/20 g)	Response	Average score	Remarks
BIQ20	8 mg	4/5	1·2	Active
	Control	0/5	34·9	
	5 mg	3/5	1·46	Active
	Control	0/5	36·22	
	3 mg	3/5	5·6	Active
	Control	0/5	40·3	
	3 mg	4/5	4·6	Active
	Control	2/10	51·0	
BIQ22	10 mg	5/5	0	Very active
	Control	2/10	52·5	
	5 mg	4/5	0·44	Very active
	Control	2/10	52·5	
	2·5 mg	8/10	1·15	Active
	Control	2/10	52·5	
	2 mg	9/10	0·5	Active
	Control	1/10	60·2	
	2 mg	5/6	1·8	Active
	Control	2/10	51·0	
BQ20	5 mg	3/3	0	Very active
	Control	2/10	51·0	
	2·5 mg	6/10	4·65	Active
	Control	2/10	83·95	
	2 mg	3/5	3·5	Active
	Control	2/10	51·0	
Laurolinium	0·5 mg	8/10	2·37	Active
	Control	3/10	63·42	
	0·2 mg	6/10	4·75	Active
	Control	3/10	63·42	

DISCUSSION

Quaternary ammonium compounds as a group have diverse biological properties. They have neuromuscular and ganglionic blocking activity, and antiacetylcholine, anticholinesterase, antimicrobial and antiparasitic actions. They are used as detergents and cosmetic adjuvants (D'Arcy & Taylor, 1962a,b). So far their anthelmintic actions have not been studied in detail. The absorption of these compounds from the gut is generally poor and unpredictable; and when injected parenterally most of them are toxic. Consequently it was thought that if used as anthelmintics they would have greater chances of acting directly on the intestinal worms without producing any ill effect on the host. The preliminary *in vitro* screening of these compounds would also be easy, as the test tubes would effectively simulate the intestines of the hosts. Compounds found active could then be tested *in vivo*.

The results of these experiments indicate that some members of the bisisoquinolinium, bisquinolinium and bis(4-aminoquinaldinium) acetate series possess considerable cesticidal activities *in vitro*. With the BIQ series, a peak of activity occurred at 22 carbon atoms and in BQ series at 20 carbon atoms.

From the *in vivo* test, BIQ22 was found to be the least toxic and most active compound. Yet all compounds of BIQ and related series produced abdominal distention due to an inflated stomach and small intestines in all the doses tried, though to various degrees. Detailed investigation to find out the minimum effective dose for each compound, particularly BIQ22, could not be carried out due to the small quantities of the compounds available. The preliminary investigations, however, showed that the compounds have definite cesticidal activities *in vitro* as well as *in vivo*. The cause of bloating of the small intestines and part of stomach, simulating " paralytic ileus," have been investigated with BQ20 in detail (Bhattacharya & Sen, 1962). The drug exerts its action on the preparatory phase and not on the emptying phase of peristaltic movement. Its site of action on the nervous structures is not on the ganglia but at the neuromuscular junction. The results of the mechanism of action of BIQ20 and 22 on the intestine will be reported separately.

Among the monoquaternary derivatives of 4-aminoquinaldine which resemble half the dequalinium molecule, maximum activity occurred at 12 carbon atom in laurolinium. The compound has marked antibacterial and antifungal properties (Cox & D'Arcy, 1959). Though very active against *Hymenolepis nana in vitro*, it is rather toxic when used orally.

It is difficult to say from the results of studies in mice only how far the active compounds will be useful as chemotherapeutic agents. Nevertheless, the present study at least brings forward evidence of a certain well-defined structure-activity relationship among the quaternary compounds. Studies in larger animals could not be undertaken due to insufficient material, but such an investigation will be necessary before the drugs could be considered suitable for clinical trial.

SUMMARY

1. Thirty-two monoquaternary and bisquaternary ammonium compounds have been tested for cesticidal activity using *Hymenolepis nana* as a test parasite. The compounds were first screened *in vitro* and those found active were tested *in vivo* in mice.

2. Compounds BIQ20, BIQ22, BQ20 and laurolinium were active *in vivo*, but the first three produced intestinal distension simulating " paralytic ileus."

3. A definite relation between chemical structure and cesticidal activity was observed.

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