CHEMOTHERAPEUTIC PROPERTIES OF SOME NEW QUATERNARY AMMONIUM SALTS

PART II. ANTIFILARIAL ACTION AGAINST *Litomosoides carinii*

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Several new quaternary ammonium salts have been tested for antifilarial activity against *Litomosoides carinii*, filarial parasite of the cotton rat. Many of these compounds are active in killing *L. carinii*; the most promising (BIQ 20) eicosane 1:20-bis(*iso*quinolinium iodide) and (BAC 20) eicosane 1:20-bis(4-aminocinnolinium iodide) possess an index (LD50 mice/minimum effective dose cotton rats) of 19 and 39 respectively. The compounds have a direct filaricidal action on the adult worms while the microfilariae are comparatively unaffected.

THIS paper describes the results of testing a series of quaternary ammonium salts against *Litomosoides carinii*, filarial parasite of the cotton rat. The chemistry and some of the pharmacological, and antimicrobial properties of these compounds are described in other papers of this series[1,2]. The abbreviated nomenclature of Barlow and Ing[3] is used in this paper; thus BQn, BIQn, BAIQn, BAQDn and BACn refer respectively to the bis-quinolinium, -*iso*quinolinium, -7-amino*iso*quinolinium, -4-aminoquinaldinium and -4-aminocinnolinium series, where n is equal to the number of methylene groups in the chain.

The cotton rats used in these tests were bred at this Institute and were infected in the laboratory by the method of Hawking and Sewell[4]. The tests were usually made with cotton rats which were in the early stages of infection (80–100 days after exposure to infected mites); this practice lessened the risk of the results being confused by the natural death of some of the worms. The compounds were synthesized by Dr. E. P. Taylor and his colleagues of Messrs. Allen and Hanburys Ltd.; the toxicity tests were carried out by Dr. H. O. J. Collier and his colleagues.

The *in vivo* antifilarial tests were based on the method of Sewell and Hawking[5]. Briefly, the rats were given five daily doses of the drug intraperitoneally at a level known to be non-toxic to mice. Soluble compounds were dissolved in sterile physiological saline; insoluble compounds were finely ground and suspended in 5 per cent gum acacia. Measured samples of tail blood were taken immediately before treatment and 7 and 14 days after the first dose to investigate the effect of the drugs on the numbers of circulating microfilariae. On the 14th day the rats were killed with coal gas and the worms were removed from the pleural cavities and placed in sterile Ringer's solution. If the worms were not actively moving, they were placed in an incubator at 37° for 30 minutes and re-examined. If again no movement was seen, the worms were deemed to have been killed by the drug. Where a compound was found

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to be active, additional rats were treated at a fraction (usually 0·5) of the previous dose. The minimum effective dose of a compound was taken to be the smallest dose which killed all the worms in two or more cotton rats.

Selected compounds were tested against the adults of *L. carinii in vitro*. The worms were removed from the pleural cavities under aseptic conditions and placed in Carrel flasks containing a simple medium consisting of one-third horse serum and two-thirds Tyrode's solution; penicillin and aureomycin were added to the flasks, each at a final concentration of 0·1 mg./ml. At least two male and two female worms were placed in each flask. In this simple medium *L. carinii* will remain alive and active for periods up to a fortnight, although the production of microfilariae ceases after about 24 hours.[6] The selected compounds were dissolved in sterile saline and added to the flasks in serial dilution. The minimum lethal concentration was arbitrarily taken as the lowest concentration of the drug which killed all the worms in the flask within 24 hours.

EXPERIMENTAL

Action on Adult L. carinii in vivo

Table I summarises the results of testing the several series of compounds against *L. carinii in vivo*. Table II illustrates the protocols of the tests made on two of the more active compounds. The most thoroughly explored series has been the BIQ compounds where there is a definite link between filaricidal activity and the length of the polymethylene chain.

TABLE I

THE ACTION OF QUATERNARY AMMONIUM SALTS ON ADULT *L. carinii in vivo*

Compound		LD50 mice mg./kg. i.p.	MED cotton rat mg./kg. daily × 5	Index LD50 mice/MED cotton rat
BIQ	12		>10·0	
	13	16·1	1·5	10·7
	14	9·8	0·8	12·2
	15	6·5	0·8	8·1
	16	5·8	0·8	7·2
	17	3·5	0·4	8·7
	18	2·3	0·2	11·5
	19	2·7	0·2	13·5
	20	2·8	0·15	18·7
	22	2·4	0·3	8·0
	24	2·6	0·3	8·7
	32	>64	>10·0	—
	40	>64	>10·0	—
BAC	20	19·5	0·5	39·0
	22	>40·0	4·0	>10·0
BQ	12	5·0	2·0	2·5
	14	4·0	1·6	2·5
	16	3·3	0·6	5·5
	18	2·8	0·8	3·5
	20	1·8	0·8	2·3
BAIQ	19	9·4	1·0	9·4
BAQD	10	20·9	10·0	2·1
	16	4·9	1·0	4·9
	20	—	1·0	—
BIQ	16 Suramin complex	400+	25·0 } single	16+
	20	400+	12·5 } dose	32+
	22	400+	15·0 }	27+

No activity could be detected in compounds having 12 or less methylene groups, but as the chain length increased from the 13 to 20, the total minimum effective dose decreased from 7·50 to 0·75 mg./kg. BIQ 20 seems to be the peak of antifilarial activity; BIQ 22 and BIQ 24 were slightly less active and the higher chain lengths BIQ 32 and BIQ 40 were inactive. The peak of acute toxicity in mice occurred at BIQ 18.

The related BQ series also showed antifilarial activity but to a lesser extent; the peak of activity occurred at BQ 16.

Individual BQ compounds were more toxic to mice than were the corresponding members of the BIQ series. Several compounds belonging to the BAIQ, BAQD and BAC series were tested; all series showed activity, but except for BAC 20 no compound was as promising as BIQ 20.

It is known that complexes of the trypanocidal compounds ethidium and pentamidine with suramin show decreased toxicity when compared with the parent compounds whilst at the same time the prophylactic activity is increased, presumably due to the formation of depots of insoluble drug which are slowly absorbed by the body. Williamson[7] has recently reviewed the work in this field. It was thought that the high toxicity of the quaternary ammonium compounds might be reduced in this way and accordingly, suramin compounds of BIQ 16, 20 and 22 were prepared. These compounds were much less toxic and yet were effective in killing adult *L. carinii* when given in single doses at the level shown in Table I.

TABLE II

ILLUSTRATIVE PROTOCOLS OF THE TESTS ON BIQ 20 AND BAC 20

Compound	Rat	Dose (mg./kg. × 5)	No. of worms killed/No. of worms present
BIQ 20	6431	0·3	14/14
	6916	0·2	all/50+
	6602	0·15	all/50+
	6605	0·15	16/16
	6520	0·15	5/5
	6526	0·15	all/50+
	6914	0·1	half/50+
	6467	0·1	8/8
	6665	0·1	7/16
BAC 20	6620	1·0	all 50+
	6621	0·5	all/50+
	6658	0·5	7/7
	6669	0·5	18/18
	6804	0·5	all/50+
	6801	0·2	about 20/50+
	6805	0·2	8/12
	6800	0·1	0/50+

Action in vitro *on Adult* L. carinii

A few compounds were tested *in vitro* against *L. carinii* to determine whether the drugs were killing the worms directly or whether their action was mediated through the host, as in the case of diethylcarbamazine. The results of these tests are shown in Table III. All compounds were effective in killing the worms at low concentrations and it would seem that the lethal action is a direct one. The minimum lethal concentrations of the drugs *in vitro* are of about the same order as the minimum effective

doses *in vivo*. The uterine contents of female worms, exposed to concentrations slightly less than the minimum lethal concentration, were examined with the phase contrast microscope. According to Dr. A. E. R. Taylor many malformed and aborted embryos were found; the primary oocytes appeared to be particularly affected by the BIQ compounds. The other tissues were apparently normal and the muscles were still actively contractile.

TABLE III

THE ACTION OF SOME QUATERNARY AMMONIUM SALTS ON *L. carinii in vitro*

Compound		Minimum lethal concentration (24 hr.) μg./ml.	MED *in vivo* mg./kg.
BIQ	16	8	0·8
	17	6	0·4
	18	1	0·2
BAIQ	19	10	1·0
BAQD	16	100	1·0

Action on the Microfilariae

Estimates of the numbers of circulating microfilariae before and after treatment showed only small differences. Seemingly the drugs do not directly affect the microfilariae which have reached the circulation. The small decreases in numbers which sometimes occurred (maximum reduction 20 per cent) are presumably due to the drugs killing the adult worms and so preventing the replenishment of those microfilariae which are eliminated by the host.

DISCUSSION

The results given show that some of the bis*iso*quinolinium and related compounds possess considerable antifilarial activity, the compounds with the most favourable relation between toxicity and activity being BIQ 20.

$$\frac{\text{LD50 mice}}{\text{MED cotton rats}} \quad \frac{2{\cdot}8}{0{\cdot}15} \text{ i.e. } 18{\cdot}7 \Big\} \text{and BAC } 20 \Big\{ \frac{19{\cdot}5}{0{\cdot}5} = 39{\cdot}0 \Big\} \text{(See Table I).}$$

This index of activity is not completely satisfactory as therapeutic tests and toxicity tests have not been made on the same species; cotton rats are too expensive to use for toxicity work. Nevertheless the index does provide a reasonable estimate of the therapeutic value of any compound which is sufficiently reliable for biological screening work of this type.

As regards the relation between chemical structure and antifilarial activity, it may be concluded that in the BIQ series, activity increases as the length of the carbon chain increases; the peak of activity probably occurs at a length of 20 carbon atoms. In the BQ series, the peak of activity may occur at a carbon length of 16 atoms. These peaks are not sharply defined and the data could also be interpreted as indicating a plateau of activity at 18–22 carbon atoms for the BIQ series and at 16–20 carbon atoms for the BQ series. The *iso*quinolinium group at the end of the chain produces somewhat greater activity than the quinolinium

group. With the other groupings, it is not known what the optimum length of carbon chain is, so that it cannot be definitely decided how the activity of the terminal groupings compares with those of the quinolinium and *iso*quinolinium.

The compounds have a direct filaricidal action upon the adult worms, while the microfilariae are comparatively unaffected. The most susceptible parts of the adult female worms seem to be the developing embryos and oocytes; this also occurs with many other filaricidal drugs (but not with diethylcarbamazine) and is probably due to these cell-groups having the highest metabolic turnover in the worm. Similarly in schistosomes, the part most susceptible to the lethal actions of lucanthone and of antimony salts is the gonad.

Besides being active, these compounds are also toxic, but in the most promising compounds (BIQ 20 and BAC 20) there is a fair margin between the minimum curative dose and the maximum tolerated dose. A dog tolerated four intravenous injections of BAC 20 at a dose of 10 mg./kg.; a rhesus monkey tolerated 10 mg./kg. but was killed by 12·5 mg./kg., death being apparently due to poisoning of the respiratory centre. The antifilarial activity deserves investigation in larger animals and ultimately (if possible) in man, but it is too soon at present to prophesy whether or not it will possess practical utility.

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