FURTHER OBSERVATIONS ON THE BIOLOGICAL PROPERTIES OF DEQUALINIUM (DEQUADIN) AND HEDAQUINIUM (TEOQUIL)

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The activity of dequalinium and hedaquinium against *Staphylococcus aureus* and *Candida albicans* is strongly antagonised by the sodium salts of fatty acids, but not by a number of detergents and a commercial liquid soap. Sufficient antagonism was shown by agar to render invalid the results obtained in assays of these antimicrobial agents by the agar plate method. Human saliva had only a slight antagonistic action on both substances. Twenty-one strains of *Candida albicans* showed some variation in sensitivity towards hedaquinium and dequalinium but none was particularly resistant. Both antimicrobial agents inhibited the growth of *Pityrosporum ovale* and *Trichomonas vaginalis*. Both compounds were adsorbed by human or bovine hair on which some remained after repeated washing with water. In cats dequalinium and hedaquinium blocked neuromuscular and ganglionic transmission when injected intravenously in amounts many times greater than the effective dose of suxamethonium chloride. When administered intravenously to mice, hedaquinium in relatively high doses exerted a brief paralysing action.

DEQUALINIUM and hedaquinium are synthetic antimicrobial agents discovered some few years ago in these laboratories[1–5]. Under the names "Dequadin" and "Teoquil" respectively, they are used in various pharmaceutical formulations in the treatment of a variety of non-systemic bacterial and fungal infections. Their antimicrobial activities and general pharmacology have been previously described, and the present paper summarises the results of subsequent investigations.

The possibility of using dequalinium in the form of lozenges for the prevention and treatment of throat and mouth infections made it essential to determine whether dequalinium was antagonised by human saliva. One great advantage of dequalinium over other antibacterial agents used for the treatment of infections of the mouth is that, unlike penicillin for example, it inhibits the growth of *Candida albicans*, and infection with this organism is not a sequel to therapy with lozenges of dequalinium as it frequently is to penicillin therapy. Indeed dequalinium has been used with great success in the treatment of *Candida* infections of the skin, tongue and vagina[6,7]. It was therefore of interest to examine the effect of dequalinium on a number of strains of *C. albicans*. Dequalinium was also shown to inhibit the growth of *Trichomonas vaginalis* and in the form of pessaries it was proposed for the treatment of vaginal infections due to *T. vaginalis* and *C. albicans*[8,9]. It was essential therefore to know the amounts that could be tolerated in the vagina and accordingly

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the local toxicity of dequalinium in the rabbit vagina was tested. The toxicity of dequalinium in the rabbit eye was also investigated, since in the form of eye drops it was proposed for the treatment of infections of the eyes. The adsorption of dequalinium and hedaquinium on hair was tested because these substances have given promising results in the treatment of ringworm in cattle and man[10,11], and preparations of hedaquinium are now widely used for this purpose. The inhibitory action of dequalinium and hedaquinium against *Pityrosporum ovale* was also determined, as this organism is believed by some to be the causative agent of dandruff. Finally, since both substances belong to a group other members of which are muscle relaxants, it was thought desirable to test the neuromuscular blocking activities of the compounds.

Material and Methods

Effect of Antagonists on the Antimicrobial Activity of Dequalinium and Hedaquinium

The following materials were used in these tests: sodium palmitate, sodium oleate, sodium stearate, a commercial liquid soap consisting mainly of sulphonated lorol and the potassium salts of coconut oil fatty acids, Cetrimide, "Teepol", sulphated castor oil, sulphonated lorol, Oxoid "Ionagar" No. 2, Oxoid agar-agar (New Zealand), shredded agar (Korean) and human saliva (collected by expectoration).

Antibacterial tests. The medium consisted of 1 per cent peptone (Difco), 0·5 per cent glucose and 0·5 per cent sodium chloride in distilled water. The antagonists were added to this solution in appropriate amounts and the pH was adjusted to 7·2. Dequalinium and hedaquinium were dissolved in the culture-medium and two-fold serial dilutions were made leaving a final volume of 5 ml. in each tube. The tubes were capped and autoclaved at 10 lb. pressure for 10 minutes. After being allowed to cool, each tube was inoculated with 0·02 ml. of a suspension of *Staph. aureus* CN491 containing approximately 12×10^6 organisms per ml. prepared from a culture grown for 18 hours in dextrose–peptone water. After incubation at 37° the amount of growth was assessed by eye after 5 and 8 days. The results were expressed in terms of minimal inhibitory concentration (M.I.C.).

Antifungal tests. The antagonists were added in suitable amounts to Sabouraud's broth consisting of 1 per cent peptone (Eupeptone No. 2, A. & H.) and 4 per cent glucose in tap water. Double strength aqueous solutions of dequalinium and hedaquinium were mixed with double strength broth and serially diluted two-fold leaving a final volume of 2 ml. in each tube. The tubes were capped and autoclaved at 10 lb. pressure for 10 minutes. A culture of *Candida albicans* 1549 was made by inoculating tubes of Sabouraud's broth and incubating overnight at 37°, and one drop of this culture was used to inoculate the tubes containing the antimicrobial agents and antagonists. The tubes were incubated at 37° for 6 to 8 days and the mean M.I.C. estimated visually.

Effect of dequalinium and hedaquinium on various strains of C. albicans. Cultures of other strains of *C. albicans* were prepared as described for

strain 1549 and the inhibitory activities of dequalinium and hedaquinium were compared with that of nystatin after incubation at 27° for 14 days.

Effect of dequalinium and hedaquinium on Pityrosporum ovale *and* Trichomonas vaginalis. A culture of *P. ovale* was made by inoculating a malt extract agar slope, moistened with (sterile) double cream, and incubated at 37° for 3 days. Dilutions of dequalinium and hedaquinium were prepared in malt extract broth. After autoclaving at 10 lb. pressure for 10 minutes double cream was added aseptically to each tube to give a final concentration of 0·1 per cent. The culture of *P. ovale* was emulsified in saline (20 ml.) and one drop was used to inoculate the tubes. M.I.C. values were determined after incubation at 37° for 7 days.

T. vaginalis was grown in a modification of Feinberg's medium[12] comprising horse serum, liver infusion and Hartley's digest broth at pH 6·8. Dilutions of the antimicrobial agents were made in this medium, but the horse serum fraction was added aseptically to the tubes after autoclaving. A drop of sediment from a 3-day culture was used to inoculate each tube and the M.I.C. values were determined microscopically after 3 days incubation at 37°.

Adsorption of dequalinium and hedaquinium on human and bovine hair. The hair was soaked for 60 minutes in 4 per cent solutions of dequalinium acetate and hedaquinium chloride (expressed as free base), washed by dipping in distilled water and dried for 30 minutes in air. The amount of antifungal agent still remaining in the hair, before and after washing, was roughly determined either by serial dilution of peptone water in which the hair had been soaked for 30 minutes or by serial bisection of individual pieces of hair which were then added to 5 ml. amounts of peptone water. *Staph. aureus* CN491 was used as test organism, the results being expressed as μg. of drug (base) adsorbed per mg. of hair.

Local toxicity of dequalinium and hedaquinium in rabbits. Vaginal. Pessaries containing the drugs were inserted into the vaginas of rabbits on five consecutive days or solutions of the drugs were introduced by means of a catheter. After three weeks the rabbits were killed, the vagina and uteri were examined and specimens of the tissues were submitted for histological examination.

Eyes. Eye drops containing the drugs were applied daily to the right eyes of a number of rabbits and control drops containing no drug were applied to the left eyes. After three weeks' administration the rabbits were killed and the appropriate tissues were examined histologically.

Neuromuscular blocking and ganglionic blocking activities. Cats. Neuromuscular blocking activity was tested by measuring the effects of the drugs when given intravenously on the response of the tibialis muscle to electrical stimulation of its motor nerve, and ganglionic blocking activity by measuring the effect on the nictitating membrane after pre-ganglionic stimulation when the drugs were administered by arterial injection close to the superior cervical ganglion[13].

Mice. Male white mice weighing 12 to 20 g. were injected intravenously with the drugs in saline and the paralysing activity was measured using a rotating drum[14].

RESULTS

Effect of Antagonists on the Inhibitory Action of Dequalinium and Hedaquinium

Soaps and detergents. The results are recorded in Tables I and II.

In concentrations of 0·05 per cent or more, sodium oleate, sodium palmitate and sodium stearate markedly antagonised both drugs, but the

TABLE I

ANTAGONISM BY VARIOUS SOAPS OF DEQUALINIUM AND HEDAQUINIUM AGAINST *Staph. aureus* CN491 IN DEXTROSE PEPTONE WATER

Soap	Percentage of soap in medium	M.I.C. (μg./ml.) at 5 days	
		Dequalinium	Hedaquinium
Sodium oleate	0·05	250	250
	0·1	>224	250
	0·2	>500	>500
Sodium palmitate ..	0·05	250	15·6
	0·1	250	31·2
	0·2	500	62·5
Sodium stearate.. ..	0·05	250	62·5
	0·1	>250	85·5
	0·2	>500	125·0
Liquid soap	0·005	0·312	0·312
	0·01	0·156	0·156
	0·05	0·156	0·625
None	—	0·36	0·125

TABLE II

ANTAGONISM BY VARIOUS DETERGENTS OF DEQUALINIUM AND HEDAQUINIUM AGAINST *Staph. aureus* CN 491 IN DEXTROSE PEPTONE WATER

Detergent	Concentration of detergent in medium	M.I.C. (μg./ml.) at 5 days	
		Dequalinium	Hedaquinium
Teepol	0 per cent	0·312	0·078
	0·0025 ,,	2·5	0·156
	0·005 ,,	2·5	0·625
	0·01 ,,	>10	2·5
Sulphated castor oil ..	0 ,,	0·156	0·078
	0·0025 ,,	5	5
	0·005 ,,	5	5
	0·01 ,,	5	5
Sulphonated lorol ..	0 ,,	0·156	0·078
	0·00125 ,,	0·078	0·312
	0·0025 ,,	0·156	1·25
	0·005 ,,	0·156	0·625
Cetrimide	0 μg/ml.	0·156	0·039
	0·025 ,,	0·312	0·078
	0·05 ,,	0·312	0·156
	0·1 ,,	0·156	0·078

commercial liquid soap did not. Teepol and sulphated castor oil slightly antagonised both dequalinium and hedaquinium, but sulphonated lorol and cetrimide did not.

Agar. The results of experiments with agar as an antagonist of dequalinium and hedaquinium, using *Staph. aureus* as test organism, are

given in Table III. At a concentration of 1·6 per cent comparable to that used in normal agar plates all samples antagonised both dequalinium and hedaquinium. The results of the experiments using *C. albicans* as test organism are shown in Table IV.

TABLE III

ANTAGONISM BY THREE SAMPLES OF AGAR OF DEQUALINIUM AND HEDAQUINIUM AGAINST *Staph. aureus* CN 491 IN DEXTROSE PEPTONE WATER

Agar	Percentage in medium	M.I.C. (μg./ml.) at 5 days	
		Dequalinium	Hedaquinium
Korean shredded agar ..	0·2	0·312	0·625
	0·4	0·442	1·25
	0·8	1·25	1·77
	1·6	2·5	1·77
Ionagar No. 2	0·2	0·625	1·25
	0·4	1·25	1·25
	0·8	2·5	2·5
	1·6	2·5	5·0
Agar-Agar (New Zealand).	0·2	1·25	1·25
	0·4	0·625	2·5
	0·8	2·5	2·5
	1·6	2·5	5·0
None	—	0·156	0·110

TABLE IV

ANTAGONISM BY AGAR OF DEQUALINIUM AND HEDAQUINIUM AGAINST *Candida albicans*

Agar, per cent	M.I.C. (μg./ml.) at 8 days	
	Dequalinium	Hedaquinium
0	1·25	0·31
0·1	2·5	0·62
0·2	5	0·32
0·4	5	0·16
0·8	20	1·25
1·6	40	2·5

The action of dequalinium and hedaquinium on *C. albicans* was antagonised by agar to about the same extent as with *Staph. aureus.*

Saliva. It will be seen from Table V that the addition of 10 per cent of human saliva to the medium slightly reduced the activity of both dequalinium and hedaquinium against *Staph. aureus.*

TABLE V

ANTAGONISM BY HUMAN SALIVA OF DEQUALINIUM AND HEDAQUINIUM AGAINST *Staph. aureus* CN 491 IN DEXTROSE PEPTONE WATER

Percentage of saliva in medium	M.I.C. in (μg./ml.) at 5 days	
	Dequalinium	Hedaquinium
0	0·221	0·055
5	0·625	0·156
10	0·878	0·878

Activity against various strains of C. albicans. In view of the importance of *C. albicans* as a surface pathogen, the activity of dequalinium and hedaquinium against 21 strains of *C. albicans* was compared with that of nystatin. It will be seen from Table VI that the M.I.C. of dequalinium varied between 0·63 and 5·0 μg./ml. and that of hedaquinium between 0·63 and 1·25 μg./ml.

TABLE VI

INHIBITORY ACTIVITIES OF VARIOUS COMPOUNDS AGAINST 21 STRAINS OF *Candida albicans* IN SABOURAUD'S BROTH

Strains	Mean M.I.C. (μg./ml.) at 14 days		
	Nystatin	Dequalinium	Hedaquinium
C. albicans Lab. strain 239	20	1·25	0·89
" " " 1549	20	2·5	0·89
" L.Sch of Hygiene "Vaginal" ..	40	1·25	0·89
" " " " "McGuiness" ..	40	1·25	0·89
" " " " "Mishra" ..	20	1·25	0·89
" " " " "Young" ..	20	1·25	0·89
" " " " Z 247a ..	80	0·63	0·63
" " " " Z 248a ..	80	2·5	0·89
" " " " Z 249	>40	2·5	0·89
" " " " Z 250	20	5·0	1·25
" " " " Z 251	40	1·25	0·89
" " " " Z 252	20	1·25	0·89
" " " " Z 253	40	2·5	0·89
" " " " Z 254	20	1·25	0·89
" " " " Z 255	40	2·5	1·25
" " " " Z 256	40	2·5	0·89
" " " " Z 257	40	2·5	0·89
" " " " Z 258	40	5·0	0·89
" " " " Z 259	40	1·25	1·25
" " " " Z 260	>40	2·5	1·25
" " " " Z 261	>40	2·5	0·89

TABLE VII

INHIBITORY ACTIVITIES OF DEQUALINIUM AND HEDAQUINIUM AGAINST *Pityrosporum ovale* AND *Trichomonas vaginalis*

Substance	Mean M.I.C. (μg./ml.)	
	P. ovale	*T. vaginalis*
Dequalinium chloride	5·1	18
Dequalinium acetate	—	18
Hedaquinium chloride	7·3	25
Diiodohydroxyquinoline ..	—	100

Activity against P. ovale *and* T. vaginalis. It will be seen from Table VII that dequalinium chloride was slightly more active than hedaquinium chloride against both *P. ovale* and *T. vaginalis* and that both were much more effective than diiodohydroxyquinoline against *T. vaginalis.*

Adsorption on human and bovine hair. It will be seen from Table VIII that human and bovine hair when soaked for 60 minutes in 4 per cent solutions of dequalinium and hedaquinium salts adsorbed appreciable quantities from the solutions. Although these amounts were reduced by three successive washings appreciable amounts remained on the hair. Samples of human hair from different sources gave different values, but

bovine hair adsorbed more. When bovine or human hair was stored for two to four weeks before being washed, the amounts of dequalinium and hedaquinium adsorbed on the hair were very little different from the amounts adsorbed by freshly washed hair. The amount adsorbed was, however, proportional to the time of soaking. Little dequalinium was adsorbed from solutions weaker than 4 per cent although detectable amounts of hedaquinium were adsorbed from a 1 per cent solution of the drug.

TABLE VIII

ADSORPTION OF DEQUALINIUM AND HEDAQUINIUM ON HUMAN AND BOVINE HAIR AFTER WASHING FOR 60 MINUTES

		Drug μg./mg. hair	
		Dequalinium 4 per cent	Hedaquinium 4 per cent
Human hair:	unwashed ..	3·2	6·4
	1 washing ..	0·8	3·2
	3 washings ..	0·8	1·6
Bovine hair:	unwashed ..	12	12·8
	1 washing ..	6	1·6
	3 washings ..	3	1·6

Local toxicity in rabbits. Eyes. The application to the eyes of rabbits of solutions containing 0·25, 1 or 4 per cent dequalinium acetate caused some closure or inflammation (Table IX); a 0·06 per cent solution had no detectable effect. Eye drops containing 0·1 per cent or 0·05 per cent of dequalinium chloride also caused no visible reactions.

TABLE IX

OCULAR TOXICITIES OF DEQUALINIUM AND HEDAQUINIUM

Substance	Type of preparation	Conc. per cent	Observations
Dequalinium acetate ..	Aqueous solution ..	4·00	Eye closed and did not recover
		1·00	Eye closed but recovered
		0·25	Slight reaction
		0·06	No reaction
Sodium acetate	Aqueous solution ..	1·90	No reaction
		0·47	"
		0·12	"
		0·03	"
Dequalinium chloride ..	Eye drops	0·10	No reaction
Dequalinium undecylenate	Oily solution	0·16	Eyelids encrusted but tissues normal
Control	Oil	—	
Dequalinium undecylenate	Aqueous solution ..	0·03	Eyes normal, and tissues normal
Control	Water	—	
Dequalinium chloride ..	Urea-sodium acetate solution	0·05	Eyes normal, tissues changed
Control	"	—	Eyes normal, tissues normal
Dequalinium chloride ..	Urea-glucose solution ..	0·05	Eyes normal, tissues changed
Control	"	—	Eyes normal, tissues normal
Dequalinium chloride ..	Sodium sulphatebenzyl alcohol	0·05	Eyes normal, tissues normal
Control	"	—	
Hedaquinium chloride ..	Aqueous infusion ..	0·01	Eyes normal
"	"	0·1	Eyes inflamed
Control	Saline solution	—	Eyes normal

Histological examination of eyes that had received 0·05 per cent of dequalinium chloride showed only slight changes or none. Dequalinium undecylenate in a 0·03 per cent aqueous solution had no adverse effect. A 0·1 per cent solution of hedaquinium chloride caused some inflammation of the eye, but a 0·01 per cent solution had no adverse effect.

TABLE X

VAGINAL TOXICITIES OF DEQUALINIUM AND HEDAQUINIUM

Substance	Type of preparation	Conc. per cent	Observations
Dequalinium acetate ..	Aqueous solution ..	1·00	Refused injections } vagina thickened and vascularised
		0·20	" " }
		0·04	No refusals
Sodium acetate	Aqueous solution ..	0·47	Normal
Dequalinium acetate ..	Aqueous solution ..	0·10	No refusals, vaginas inflamed and granulated
Dequalinium chloride ..	Pessary	0·10 }	Pessaries ejected within 5 minutes
		0·03 }	
		0·01 }	
Control	Pessary	—	Pessaries retained
Dequalinium chloride ..	Cones	0·2 mg./cone	Cones retained but only ¾ in. into vaginas
Dequalinium chloride ..	Urea solution, 50 per cent	0·02 }	No refusals, vaginas inflamed
		0·1 }	
Control	Urea solution, 50 per cent	—	Normal
Hedaquinium chloride ..	Aqueous infusion ..	0·01	Vaginas normal
"	" "	0·1	Vaginas vascularised
Control	Saline solution	—	Vaginas normal

Vaginal. Solutions of dequalinium salts were infused intravaginally in rabbits on successive days. The results are summarised in Table X. Solutions containing 0·2 per cent or 1 per cent of dequalinium acetate caused obvious discomfort, but solutions containing 0·1 per cent or less of dequalinium acetate and chloride were tolerated. On histological examination, however, inflammation was observed in all vaginas receiving a 0·02 per cent solution of dequalinium chloride or a 0·04 per cent solution of dequalinium acetate. Pessaries containing 0·01, 0·03 or 0·1 per cent of dequalinium chloride were ejected whereas control pessaries containing no drug were retained by the rabbits. Infusions of hedaquinium chloride did not cause visible reactions in the vagina at a concentration of 0·1 per cent but on histological examination some inflammation was noted. A 0·01 per cent solution did not cause inflammation.

Neuromuscular block. Dequalinium chloride administered intravenously to two cats caused some diminution in the response of the tibialis to intermittent electrical stimulation of its motor nerve, 1 mg./kg. giving a 78 per cent reduction in tension. Doses of 2 and 3 mg./kg. of hedaquinium methosulphate gave a 67 and a 99 per cent reduction respectively. In a direct comparison with suxamethonium chloride, hedaquinium methosulphate was found to have 1/200th the potency and about twice the duration of activity. When given by arterial injection close to the superior cervical ganglion, hedaquinium methosulphate at 2 mg./kg. produced a 50 to 90 per cent block in the contraction of the

nictitating membrane; recovery was complete. Hedaquinium methosulphate injected intravenously produced paralysis in mice, causing them to fall off the drum, the value of ED50 being 2·2 ± 0·3 mg./kg.

Discussion

The marked antagonistic action of soaps on both dequalinium and hedaquinium shows that soaps must not be used in the formulation of pharmaceutical preparations containing these antimicrobial substances, and that soaps must not be used in conjunction with such preparations. Where cleansing of skin, or hair is necessary, a detergent that does not reduce the antibacterial or antifungal activity of dequalinium or hedaquinium should be chosen.

The inhibitory effect of agar on the two substances explained some serious discrepancies observed when preparations were assayed by the serial dilution method and by the agar plate method. We recommend that the latter method should not be used, as low results will be obtained. Even with 10 per cent of human saliva in the medium dequalinium was found to be still effective against *Staph. aureus* in a concentration of one part per million.

Dequalinium has been found to be successful in the treatment of local infections with *Candida albicans* both in the mouth and in the vagina. It had been suggested that some strains might be more resistant than strain 1549 with which the initial investigations were carried out. The results reported here, however, show that although there is in fact a variation in the sensitivity of different strains, even the most resistant is inhibited by 5 parts per million. The observations with *Trichomonas vaginalis* showed that dequalinium chloride and acetate are more effective inhibitors of this organism in our tests than the widely-used diiodohydroxyquinoline.

Preparations with hedaquinium chloride as the active agent are being used with success in the treatment of fungal infections in man and in farm animals[10,11]. It was of interest to determine whether hair treated with hedaquinium would retain any of the antifungal agent after washing.

The experiments described above showed that solutions containing 0·1 per cent of dequalinium chloride had no irritant action on the eyes of rabbits. Accordingly the concentration of dequalinium chloride for eye drops was fixed at 0·1 per cent, clinical tests confirming that at this dilution the solution did not cause irritation in the human eye. The rabbit's vagina appeared to be more sensitive than the eye to dequalinium and the lowest concentration used in the animal tests and found to be only slightly irritant, namely 0·01 per cent, was selected in making pessaries for trials in women. Pessaries containing 0·01 per cent of dequalinium chloride were in fact well tolerated and gave satisfactory results in *C. albicans* and *T. vaginalis* infections[8,9].

The experiments on the neuromuscular blocking activities of the two antimicrobial agents showed that these were active by intravenous injection only when given in concentrations greatly in excess of the effective levels of a potent muscle relaxant such as suxamethonium. Dequalinium

and hedaquinium are recommended only for oral or topical therapy, their systemic toxicities precluding their use by parenteral administration. In an unpublished investigation of the metabolism of dequalinium it was observed that when large doses were given by mouth to rats none could be detected in the blood, all of the dose apparently being excreted in the faeces. It seems unlikely therefore that dequalinium will produce neuromuscular block when given orally.

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