SOME PHARMACOLOGICAL AND MICROBIOLOGICAL PROPERTIES OF CHLORHYDROXYQUINOLINE AND RELATED COMPOUNDS

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Chlorhydroxyquinoline, prepared by the chlorination of 8-hydroxyquinoline under controlled conditions, has been found to be active *in vitro* against a variety of micro-organisms. Its bacteriostatic activity appears to be greater than that of certain other halogenated derivatives and its oral toxicity in rats is low. After oral administration, chlorhydroxyquinoline is apparently excreted mainly in the faeces of rats and bacteriostatic levels have been observed in the stools of rats and dogs.

5-Chloro-7-iodo-8-hydroxyquinoline has been employed in the treatment of amoebic and bacillary dysenteries and 5:7-di-iodo-8-hydroxyquinoline has been used in intestinal amoebiasis. Also 5:7-dichlor-8-hydroxyquinaldine has been found to be effective clinically and *in vitro* against a variety of organisms encountered in the intestinal tract[1]. It seemed likely to us that chlorhydroxyquinoline, prepared by the chlorination of oxine under controlled conditions, would be active against organisms commonly responsible for intestinal infections.

A number of investigators have observed that oxine and several of its derivatives are effective against Gram-positive and Gram-negative bacteria and pathogenic fungi, while certain halogenated compounds are active against protozoa. There is some uncertainty about the fate of these substances in the body and the mode of their action against organisms in the intestinal tract. Leake[2] found that iodochlorhydroxyquinoline is rapidly excreted by man in the urine and Drill[3] concludes that the drug is absorbed from mucous membranes, excreted in the urine and detoxified in the liver, but Goodman and Gilman[4] suggest that most of an ingested dose probably passes through the intestinal tract without being absorbed. Haskins and his associates[5] studied the distribution of ^{131}I-labelled iodochlorhydroxyquinoline and found the compound seems to be absorbed and eliminated without marked decomposition. The drug may act in the lumen of the gut with an additional effect due to absorption into the intestinal circulation[4]. Di-iodohydroxyquinoline is absorbed to a less extent[6], but the blood-iodine levels[5,7] after oral administration indicate the possibility of some absorption occurring, although part may be iodine dissociated. Grabbe[8] reported that oxyquinoline sulphate is rapidly absorbed from dog intestines and excreted in the urine, mainly conjugated with sulphate, and in the bile.

It was, therefore, decided to compare the activity *in vitro* of chlorhydroxyquinoline with that of oxine, iodinated derivatives and various additional agents employed clinically and to investigate the toxicity and excretion of chlorhydroxyquinoline.

Antibacterial Activity

Many of the drugs tested are almost insoluble in water and the cup-plate technique has limitations owing to poor diffusion in solid media, but this method was considered useful in giving an indication of activity. In a series of preliminary tests, the bacteriostatic effects of various compounds and mixtures were investigated by placing the drugs in 5 mm. cups made with a cork-borer in nutrient agar and measuring the average annular zones of inhibition. The test-organisms were *Salmonella typhi*, *Salm. enteritidis*, *Shigella dysenteriae* (shigae), *Sh. sonnei* and *Sh. flexneri*.

The results indicated that under these conditions of testing, oxine exhibits effects comparable with those of the soluble salts of streptomycin, neomycin and tetracycline. In this series, chlorhydroxyquinoline was less active than oxine but more active than iodochlorhydroxyquinoline, whilst various mixtures of chlorhydroxyquinoline and phthalylsulphathiazole, streptomycin and neomycin appeared to show no additive effects.

Since solubility and diffusion are important factors in the measurement of antibacterial activity, a suitable solvent was sought and it was found that oxine, its halogenated derivatives and other drugs dissolve in dimethylformamide, although they precipitate on dilution with water. Filter paper discs were saturated with 0·4 per cent solutions of the compounds in dimethylformamide and placed while damp on agar plates, the test-organisms being *Salm. typhi*, *Salm. typhimurium*, *Salm. enteritidis*, *Sh. sonnei*, *Sh. flexneri*, *Sh. dysenteriae* (shigae) and *Escherichia coli*. The results, which are shown at Table I indicate that, under these conditions, the activities of oxine and chlorhydroxyquinoline are similar and that these two compounds are more active than iodochlorhydroxyquinoline and di-iodohydroxyquinoline. The solvent alone had no appreciable antibacterial activity.

TABLE I

ACTIVITY OF OXINE AND SOME HALOGENATED DERIVATIVES AGAINST THE TEST ORGANISMS USING FILTER-PAPER DISCS SATURATED WITH SOLUTIONS IN DIMETHYLFORMAMIDE AND PLACED WHILE STILL DAMP ON AGAR PLATES

Compound	Depth of Zone of Inhibition in mm.						
	Salm. typhi	*Salm. typhimurium*	*Salm. enteritidis*	*Sh. sonnei*	*Sh. flexneri*	*Sh. dysenteriae*	*E. coli*
Oxine	7·5	4	7	6	20	6·5	6
Chlor-oxine ..	7	6	5	6	21	8	5·5
Iodochlor-oxine ..	3·5	1·5	1·5	1·5	3·5	3	3
Di-iodo-oxine ..	1	1	2·5	1	3·5	1	3·5

Oxine and its derivatives were also made into pastes with water; porcelain cylinders were filled with these pastes and then placed on plates inoculated with other bacteria which may be associated with intestinal infections. The resulting zones of inhibition are recorded in Table II.

TABLE II

INHIBITION OF TEST ORGANISMS BY AQUEOUS PASTES OF VARIOUS AGENTS USING THE CYLINDER-PLATE METHOD

Organism	Depth of Zone of Inhibition in mm.				
	Oxine	Chlor-hydroxyquinoline	Iodochlor-hydroxyquinoline	Di-iodo hydroxyquinoline	Nystatin
Staph. aureus .. 47	13	13	2	<1	
Staph. aureus		9	2		
Proteus vulgaris	9	8	<1	<1	
Ps. aeruginosa	<1	<1	<1	<1	
Salm. paratyphi A	4	7	1	<1	
Candida albicans	11	10	2		9
Epidermophyton floccosum	17	3	3		1
Trichophyton mentagrophytes	10	6	2		2
Microsporum gypseum ..	12	7	2		2

Fluoro-Derivatives of Oxine

The 5-fluoro and 5:7-difluoro derivatives of 8-hydroxyquinoline were prepared for preliminary screening for bacteriostatic activity *in vitro* against *Staph. aureus, E. coli, P. vulgaris* end *Sh. sonnei* in comparison with chlorhydroxyquinoline. Results obtained by means of discs impregnated with dimethylformamide solutions indicated that the 5:7-difluoro-compound has a similar order of activity to chlorhydroxyquinoline, while 5-fluorohydroxyquinoline appeared to be less active than the other two substances.

ANTIFUNGAL ACTIVITY

The procelain cylinder method described above was used to give a preliminary estimation of activity of oxine, chlorhydroxyquinoline, iodochlorhydroxyquinoline and the antifungal antibiotic, nystatin, against certain pathogenic fungi. The results, shown in Table II, demonstrate the importance of diffusion because a large number of reports indicate that nystatin, which is insoluble in water, has high antifungal activity *in vivo* and that intense activity is exhibited *in vitro* by solutions in suitable organic solvents and by suspensions or other preparations which enable the antibiotic to be brought into intimate contact with the organisms.

PHARMACOLOGICAL PROPERTIES

Chlorhydroxyquinoline was given by mouth to groups comprising five male and five female rats for a period of 14 days. In one group of 10 animals, 0·1 g. was given per kg. of body weight daily, each member of another group received 0·2 g./kg. daily and the third group were controls. Each rat was weighed every third day, but only insignificant differences in body weight were observed in the three groups during and after the test-period. All animals survived and at the end of the fourteenth day, 2 rats

from each group were killed and examined, particular attention being paid to the livers and kidneys. No pathological changes were found.

The killed animals were also used in a preliminary study of the distribution and excretion of chlorhydroxyquinoline, based mainly on the ultra-violet absorption spectrum. No traces could be detected in the blood, but a compound appearing to have the quinoline nucleus was present in the intestinal contents.

In a second series of tests, groups of 4 adult rats were used. Four animals were given oxine 0·3 g./kg. daily by mouth, four received chlorhydroxyquinoline 0·3 g./kg. daily and four were left untreated as controls. No toxic effects were evident during the test-period of 3 days but it was noted that, although each rat in the control and oxine-treated groups excreted about 26 ml. of urine per day, the average output of animals in the chlorhydroxyquinoline-treated group was only about 3 ml. For this reason, particular attention was paid to the kidneys when the animals were killed at the end of the third day, but no pathological changes were evident.

Only small amounts of oxine or oxine-like substances could be found by the absorption spectrum method in the intestinal contents of the oxine-treated rats. The urine, however, consistently contained a compound resembling oxine in amounts which may represent 60–70 per cent of a daily dose; this substance was not unchanged oxine, but was most probably a conjugated compound[8].

The mass of the intestinal contents in the chlorhydroxyquinoline-treated group was much greater than that of the other groups and although no diarrhoea was evident, the faeces were soft. It is highly probable that the quantity and texture of the stools account for the reduced urinary output. A substantial amount of chlorhydroxyquinoline was present in the contents of the lower intestinal tract and the faeces had a yellow tinge which is likely to be due to break-down products. No unchanged chlorhydroxyquinoline appeared to be present in the urine, but a compound showing a similar, although not identical ultra-violet absorption peak was detected. Each rat received approximately 75 mg. of 8-hydroxyquinoline or chlorhydroxyquinoline daily and it was estimated that the average daily urinary excretion of changed oxine was about 52 mg. and that of changed chlorhydroxyquinoline only about 1·7 mg. Although various methods were used, the content of chlorhydroxyquinoline in the faeces could not be estimated satisfactorily and additional rats were given 0·16 g. of the drug per kg. daily in an endeavour to obtain more precise information. By means of modified methods of extraction and estimation, the average daily content in the urine at this dose-level was found to be approximately 0·5 mg., but the faecal content could not be estimated consistently.

Microbiological Tests on Faeces

Faeces were obtained daily for 3 days from four rats kept in metabolism cages; chlorhydroxyquinoline 0·16 g./kg. was given daily for 3 more days to two of these rats and the faeces were again collected. Faecal pellets of

approximately uniform weight and size were embedded in nutrient agar inoculated with an over-night culture of *Sh. flexneri* and the plates were incubated at 37° for 24 hours. No zones of inhibition were produced by pellets from untreated animals or by those obtained from the treated rats on the first day of medication. The second-day pellets of the treated rats, however, gave zones varying from 3 to 5 mm. in depth and those of the third day gave zones of 5, 6, 5 and 7 mm.

Small porcelain cylinders were filled with stools obtained from two dogs on 2 consecutive days and used to test for bacteriostatic activity against *Sh. flexneri.* No zones of inhibition were evident after incubation for 48 hours. The unused second-day faeces were mixed and a portion was sterilised and retained. The dogs were then given approximately 0·03 g. of chlorhydroxyquinoline per kg. daily as enteric coated tablets of 0·1 g., for 2 days and the faeces were collected for 4 days. This dose was selected because it would probably be similar to that which may be used clinically. Faeces obtained on the first day of medication produced no zones, but those collected on each of the next three days gave zones of 2–4 mm., 4–6 mm. and 0–3 mm. respectively. Chlorhydroxyquinoline was mixed evenly into the retained sterile faeces to give a range of concentrations and the zones of inhibition for *Sh. flexneri* were measured after incubation for 48 hours. Although the organism is highly sensitive to chlorhydroxyquinoline, this method is not satisfactory for estimating the faecal content.

The faeces of the last group of rats and of the dogs were dispersed in sterile Ringer's solution to give dilutions ranging from 1/100 to 1/10,000 and 1 ml. of each dilution was transferred to an agar plate and incubated aerobically for 72 hours. It was observed that the administration of chlorhydroxyquinoline modified the normal flora and reduced or even eliminated Gram-negative bacteria. Streptococci were not markedly affected.

Discussion

The results of these tests suggest that chlorhydroxyquinoline may be useful in bacillary infections of the intestinal tract. Under the conditions described this substance inhibited the multiplication of a variety of micro-organisms; it has a low oral toxicity in the rat and a substantial portion of an oral dose appears to be excreted in the faeces.

Methods of testing for bacteriostatic activity are limited by insolubility, and diffusion through solid media must be considered in interpreting the results obtained with the cup-plate techniques. Dimethylformamide has been found to be suitable for use by the filter paper disc method, but the oxine derivatives are precipitated from this and other solvents on adding water and so serial dilutions cannot easily be prepared in liquid media. We have employed suspensions in broth and found that, in a concentration of less than 0·1 per cent, chlorhydroxyquinoline is bacteriostatic for *E. coli*, but the minimal inhibitory concentration is influenced by several factors, notably size of particles.

Rats given chlorhydroxyquinoline by mouth in doses of 0·2 g./kg. daily for 14 days and 0·3 g./kg. daily for 3 days did not exhibit any toxic effects, while four rats given a single oral dose of 0·48 g./kg. showed normal gains in weight and no toxic symptoms over 14 days. By analogy with iodochlorhydroxyquinoline, di-iodohydroxyquinoline and chlorhydroxyquinaldine, it might be expected that chlorhydroxyquinoline would be largely excreted in the faeces, although the observations quoted in our introductory paragraphs suggest that there is some disagreement on the fate of certain of the halogenated derivatives of oxine in the body. Tests in this series have indicated that only a small part of an oral dose of chlorhydroxyquinoline is absorbed and excreted in the urine of rats and that the major portion remains in the intestinal tract, but the faecal content has not yet been estimated satisfactorily. In both rats and dogs treated with the substance, the levels in the stools are adequate to suppress the multiplication of *Sh. flexneri.* The term "chlorhydroxyquinoline" has been employed because spectrophotometry, chromatography, determination of melting points of fractions and other procedures have shown that the substance under consideration is not a single compound. An exhaustive study of individual chloro-derivatives of oxine has not yet been undertaken but tests so far conducted indicate that more highly chlorinated products and various fractions obtained by chromatographic separation are less active *in vitro* than chlorhydroxyquinoline.

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