Biochemical Pharmacology, 1964, Vol. 13, pp. 235–239. Pergamon Press Ltd., Printed in Great Britain.

THE *IN VITRO* EFFECT OF 8-HYDROXYQUINOLINE DERIVATIVES ON STRAINS OF *MYCOBACTERIUM TUBERCULOSIS*

P. JUHÁSZ,* I. BÉKÉSI† and J. URI†

(*Received* 26 *August*; *accepted* 23 *September* 1963)

Abstract—The *in vitro* action of oxine and 31 of its derivatives was tested against *M. tuberculosis* $H_{37}Rv$, Jensen-strain E_5, Streptomycin, INH, as well as for PAS-resistant variants of the $H_{37}Rv$ strain and the apathogenic M 607-strain. 5-aceto-oxine was found to be the most active compound studied though some activity was also seen with 5-propio-oxine, 5-ethyl-oxine, and 3, 4, 5, 6, 7-methyl derivatives of oxine. Streptomycin, INH and PAS-resistant strains disclosed no cross-resistance.

THE bacteriostatic effect of 8-hydroxyquinoline (oxine) and its derivatives has long been known. Since the studies made by Oettingen[12] and Albert *et al.*,[2] a great number of reports have been published on the antibacterial, antimycotic, antiamoebic, etc., effects of these substances. In his monography, Hollingshead[11] listed a great deal of experimental results reported in the literature.

Few workers have studied the action of oxine against mycobacteria and even these few studies include but a very small number of substances. Courmant,[9] Aitoff[11] found oxine sulphate inhibitory in dilutions 1:100,000 and 1:150,000, respectively. Bidault,[3] using dilutions 1:500–1:3·000, found a change in the virulence of *M. tuberculosis*. He could not, however, prevent experimental tuberculosis by oxine-sulphate. Wilstaedt[18] found 7-methyl-/ acetylphenylazo/-oxine, Schraufstätter,[13] 5–7-dichlor-oxine and Wiederkehr,[17] 5-chlor-oxine, to be active in vitro. Urbanski[14] reported the hydroxylamine of oxine as a drug of high toxicity, active both *in vitro* and *in vivo*. In England,[8] the thiosemicarbazone derivative of oxine was also used in human therapy. Recently, Brack[6, 7] has reported on the antibacterial effect of a number of oxine derivatives.

With Born, Kocka and Maron[14] and subsequently with Born and Szabó[5] we studied. the action of iodine-, chlor- and 5-methyl derivatives of oxine against *M. tuberculosis*. Good local results have been reported by Gyarmati, Born and Eidus.[10]

On the other hand, Uri and Szabó[15] reported that oxine, and Uri, Bognár and Békési[16] that its methyl derivatives have an inhibitory effect on the growth of dermatophytes. The results justified, from several aspects, the investigation of the action of the oxine derivatives available and synthetized mostly at this Laboratory against strains of *M. tuberculosis*.

* Present address; Laboratory of the County Sanatorium of Tuberculosis, Szolnok, Hungary.
† Present address: Institute for Antibiotic Research of the Hungarian Academy of Sciences and Department of Pharmacology, University Medical School, Debrecen, Hungary.

METHOD

In a series of experiments, we studied the effect of oxine and 31 of its derivatives on *M. tuberculosis* $H_{37}R_v$, on Hensen's strain E_5, and on variants of *M. tuberculosis* $H_{35}Rs$ resistant to streptomycin, isonicotinic acid hydrazide or para-amino-salicyclic acid. In order to obtain quick preliminary results, we also used the international apathogenic *Mycobacterium* sp. called M 607. As some of the compounds are badly or hardly soluble in water, they were dissolved in methylalkohol. In no case did the final concentration of the solvent attain the level inhibitory to the growth of *Mycobacteria* (1 per cent). Dilutions were made either exclusively with Šula's culture medium or with methylalkohol and Šula's medium combined.

Šula's medium was also used to determine the lowest inhibitory concentration.

The tubes contained 4·5 ml medium each. The test substances were measured into the tubes in adequate dilutions and in amounts of 0·5 ml, so that they contained a total amount of 5 ml. For inoculation of the medium, the $H_{37}R_v$ and M. 607 strains were cultivated in Šula's medium for 16 and 7 days, respectively. After homogenization in a bacterium grinding mill (Potter) followed by double washing with Šula's medium and centrifugation, the bacteria were weighed in a semi-humid state and each tube was inoculated with 0·05 mg ,that is 0·05 ml of a standardized suspension.

After inoculation, the tubes were covered with cellophane and fixed with rubber rings to reduce concentration of the medium through evaporation. In every instance, the virulent strains was incubated for 14 and 21 days and M 607 strain for 3 or 5 days at 37°. Growth equal to that of the control was marked +++; growth attaining 50 per cent of that of the control: ++; 20–30 colonies countable by naked eye were: +. The "—" mark refers to tubes revealing no visible growth.

RESULTS

The results, summarized in Table 1, illustrate the action of 32 substances against the $H_{37}R_v$-strain. They show that, with the exception of 12 compounds, the oxines under study act on virulent strains at a concentration below 10 μg/ml. The 3, 4, 5, 6, 7-methyl derivatives of oxine are unequivocally active. Among the keto- and alkyl derivatives of oxine, 5-acetooxine, 5-propio-oxine and 5-ethyl-oxine posses considerable activity.

Results obtained against were in perfect agreement with those obtained for $H_{37}R_v$.

When the streptomycin resistant variant/2000 μg/ml, INH/100 μg/ml/resistant and PAS/100 μg/ml/resistant strains used in the test series were inoculated, cross-resistance was not found for any of the oxine dreivatives tested.

Table 2 shows the inhibitory effect on M 607. As it can be seen, M 607 is acted upon by the same compounds as $H_{37}R_v$ and the other pathogenic strains. It is however, obvious that sensitivity of the M 607-strain is about 2–20 times lower than that of the virulent strains. It confirms our earlier experience and indicates that strain M 607 can be used only for screening and orientation purpose.

DISCUSSION

One of the most essential requirements for the prevention of tuberculosis is the research for newer effective inhibitors and their introduction in therapy. The two approaches to reach this aim (antibiotic and chemotherapeutic) have brought considerable results so far. Unfortunately, however, even though some new inhibitors

have been produced since the use of streptomycin and INH, they are not as good as these two drugs. Furthermore, we think the possession of these two highly active drugs have relegated such experiments in to the background to some extent. Owing to these results, the thorough and detailed study of a number of long known and more or less effective groups of compounds have been neglected.

TABLE 1. EFFECT OF OXINE DERIVATIVES ON *M. tuberculosis* $H_{37}R_V$-STRAIN

Compounds	Inhibitory conc., μg/ml				
	10	5	1	0·5	0·1
2-methyl-oxine	+++	+++	+++	+++	+++
3-methyl-oxine	−	−	−	+++	+++
3-methyl-2-ethyl-oxine	−	+++	+++	+++	+++
4-methyl-oxine	−	−	−	+++	+++
2-oxy-4-methyl-quinoline	+++	+++	+++	+++	+++
5-methyl-oxine	−	−	−	+	+++
6-methyl-oxine	−	−	−	(+)	+++
6-methyl-8-amino-quinoline	+++	+++	+++	+++	+++
6-methyl-8-nitro-quinoline	+++	+++	+++	+++	+++
7-methyl-oxine	−	−	−	+	++ +
Oxine	+++	+++	+++	+++	+++
Oxine-5-carbonic-acid	−	+++	+++	+++	+++
Oxine-6-carbonic-acid	−	+++	+++	+++	+++
Oxine-7-carbonic-acid	++	+++	+++	+++	+++
Oxine-7-carbonic acid ethylester	−	−	++	+++	+++
Oxine-7-carbonic acid-diethyl-amino ethylester	+++	+++	+++	+++	+++
Oxine-7-carbonic acid-hydrazide	+++	+++	+++	+++	+++
Oxine-7-carbonic acid diethyl-amino ethanol-penicillinate	+++	+++	+++	+++	+++
5-ethyl-oxine	−	−	−	++	+++
5-propyl-oxine	+++	+++	+++	+++	+++
5-butyl-oxine	+++	+++	+++	+++	+++
5-propio-oxine	−	−	−	−	+++
5-isovalero-oxine	−	+++	+++	+++	+++
5-butyro-oxine	−	+++	+++	+++	+++
5-aceto-oxine	−	−	−	−	(+++)
2-styril-oxine	−	+	+	+++	+++
4-styril-oxine	+++	+++	+++	+++	+++
Oxine-5-aldehyde	+++	+++	+++	+++	+++
Oxine-7-aldehyde	+++	+++	+++	+++	+++
5-7-dibrom-oxine	−	++	+++	+++	+++
Chinsol	−	−	++	+++	+++
Oxine-penillinate	−	++	+++	+++	+++

Our experiments are meant to call attention to the fact that the oxine derivatives include a number of compounds having a considerable bacteriostatic effect on *M. tuberculosis in vitro*. Of course *in vitro* results by themselves should not lead to excessive optimism. Several workers have suggested that the mechanism of action for oxines may be due their properties to form chelate-complexes. In the literature, however, there seem to be hardly any reports on animal experiments. Comparatively low toxicity may be listed among the good properties of the compounds. Promising results have been obtained for the majority of these compounds by tests of both acute and chronic toxicity. On the ground of past experiments, it may be interpreted as a favourable sign that action on the antibiotic-resistant strains is identical to action

on the sensitive $H_{37}R_V$. We also studied the inhibition of the most active oxine derivatives on some "wild" strains cultivated from routine pathological samples. The tested 17 strains showed similarly high sensitivity to substances which had been found active on $H_{37}R_V$. We have had as yet no experience regarding conditions of resistance developing against active compounds.

In view of the above results, we believe that a further study of these substances would not be aimless. It may be reasonably expected that a substance useful also in therapy will be found through tests for toxicity and protection made with the most active of these derivatives.

TABLE 2. EFFECT OF OXINE DERIVATIVES ON *M. tuberculosis* M 607-STRAIN

Compounds	Inhibitory conc., μg/ml				
	25	10	5	1	0·5
2-methyl oxine	− + + +	+ + +	+ + +	+ + +	+ + +
3-methyl oxine	− −	−	+ + +	+ + +	+ + +
3-methyl-2-ethyl oxine	− −	−	+ + +	+ + +	+ + +
4-methyl oxine	−	+	+ + +	+ + +	+ + +
2-oxy-4-methyl quinoline	+ + +	+ + +	+ + +	+ + +	+ + +
5-methyl oxine	−	−	+	+ + +	+ + +
6-methyl oxine	−	−	+ +	+ + +	+ + +
6-methyl-8-amino quinoline	+ + +	+ + +	+ + +	+ + +	+ + +
6-methyl-8-nitro quinoline	+ + +	+ + +	+ + +	+ + +	+ + +
7-methyl oxine	−	−	+	+ + +	+ + +
Oxine	−	+	+ + +	+ + +	+ + +
Oxine-5-carbonic acid	+	+ +	+ + +	+ + +	+ + +
Oxine-6-carbonic-acid	+ + +	+ + +	+ + +	+ + +	+ + +
Oxine-7-carbonic acid	+ + +	+ + +	+ + +	+ + +	+ + +
Oxine-7-carbonic acid ethylester	+ +	+ + +	+ + +	+ + +	+ + +
Oxine-7-carbonsav-diethylamino-ethylester	+ + +	+ + +	+ + +	+ + +	+ + +
Oxine-7-carbonic acid hydrazide	+ + +	+ + +	+ + +	+ + +	+ + +
Oxine-7-carbonic acid diethylamino ethanol pencillinate	+ + +	+ + +	+ + +	+ + +	+ + +
5-ethyl-oxine	−	−	+ +	+ + +	+ + +
5-propyl-oxine	+ +	+ + +	+ + +	+ + +	+ + +
5-butyl-oxine	−	+ + +	+ + +	+ + +	+ + +
5-propio-oxine	−	−	+	+ + +	+ + +
5-isovalero-oxine	−	+ +	+ + +	+ + +	+ + +
5-butyro-oxine	−	−	−	+ +	+ + +
5-aceto-oxine	−	−	−	−	+ +
2-styril-oxine	+ + +	+ + +	+ + +	+ + +	+ + +
4-styril-oxine	+ + +	+ + +	+ + +	+ + +	+ + +
Oxine-5-aldehyde	+ + +	+ + +	+ + +	+ + +	+ + +
Oxine-7-aldehyde	+ + +	+ + +	+ + +	+ + +	+ + +
5-7-dibrom-oxine	+ + +	+ + +	+ + +	+ + +	+ + +
Chinosol	+ +	+ + +	+ + +	+ + +	+ + +
Oxine-pencillinate	+ +	+ + +	+ + +	+ + +	+ + +

REFERENCES

1. J. AITOFF, *C. R. Soc. Biol., Paris* **124**, 949 (1937).
2. A. ALBERT, S. D. RUBBO, R. J. GOLDACRE and G. B. BALFOUR, *Brit. J. exp. Path.* **28**, 69 (1947).
3. P. BIDAULT, *C. R. Soc. Biol., Paris* **99**, 461 (1928).
4. J. BORN, I. KOCZKA and S. MARON, *Orv. Hétil.* **28**, 875 (1950).
5. J. BORN and I. SZABÓ, *Orv. Hetil.* **93**, 400 (1952).
6. A. BRACK, *Arzneimittel-Forsch.* **12**, 144 (1962).
7. A. BRACK, *Arzneimittel-Forsch.* **12**, 133 (1962).

8. *Brit. Pat.* 708. 013., Oxine thiosemicarbazon.
9. P. COURMONT, *C. R. Soc. Biol., Paris* **122**, 1110 (1936).
10. L. GYARMATI, J. BORN and L. EIDUS, *Orv. Hetil.* **41**, 1131 (1956).
11. R. G. W. HOLLINGSHEAD, *Oxine and its derivatives, London* (1954).
12. W. OETTINGEN, *Therapeutic Agents of the Quinoline Group* (1933).
13. E. SCHRAUFSTÄTTER, *Z. Naturf.* **5.b**, 190 (1950).
14. T. URBÁNSKI, *Nature, Lond.* **168**, 29 (1951).
15. J. URI and G. SZABÓ, *Acta physiol. hung.* **3**, 425 (1952).
16. J. URI, R. BOGNÁR and I. BÉKÉSI, *Acta .microbiol. hung.* **4**, 279 (1957).
17. F. WIEDERKEHR and E. HOFSTETTER, *Helv. chim. acta.* **35**, 468 (1952).
18. H. WILSTAEDT and M. BORGGARD, *Svensk. kem. Tidskr.* **57**, 254 (1945).