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NITROAKRIDIN 3582: A COMPOUND POSSESSING CHEMOTHERAPEUTIC ACTIVITY AGAINST THE VIRUSES OF PSITTACOSIS AND LYMPHOGRANULOMA VENEREUM

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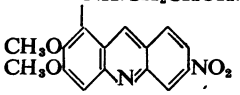
"Nitroakridin 3582" (Höchst) or 2,3-dimethoxy-6-nitro-9(3'-diethylamino-2'-hydroxypropyl)amino-acridine dihydrochloride



was developed by the Germans, who claimed that it possessed activity against rickettsiae. Smadel, Snyder, Hamilton, Fox, and Jackson (1946) found it to exert a beneficial effect on mice and embryonated eggs experimentally infected with murine or epidemic typhus, tsutsugamushi disease or Rocky Mountain spotted fever. Green, Rasmussen, and Smadel (1946) observed a chemotherapeutic effect of the compound in embryonated eggs infected with the Lee strain of influenza B virus. In most of their experiments they mixed the compound with virus and inoculated the mixture into the allantoic sac of 11-day embryos. In two experiments nitroakridin was injected an hour before virus. After 2–5 days they estimated virus in the allantoic fluid by means of the haemagglutination technique. Only 3 of 107 eggs receiving 1–10 M.I.D. of virus together with 0.5 mg. nitroakridin agglutinated red cells, and then to a lower titre than did infected control eggs. Against 100 or more M.I.D. of virus the compound exerted a less striking effect. The addition of nitroakridin to known positive allantoic fluids did not influence their titres; nor did the drug inactivate virus at room temperature in 15 minutes, about 5 minutes longer than the time occupied in inoculating eggs after preparation of a mixture.

This claim of therapeutic activity against one of the influenza viruses seemed sufficiently important to warrant investigation of the action of the compound against other viruses. My colleague, Dr. P. Gaubert, very kindly prepared a sample of nitroakridin, with which we obtained no favourable effect on influenza in mice or on infections caused by four neurotropic viruses. Against psittacosis and lymphogranuloma venereum, however, the compound showed moderate activity.

EXPERIMENTAL

In suitable chronic toxicity tests the maximal tolerated dose of nitroakridin in mice weighing 20 g. was 0.25 mg. daily, given intraperitoneally as a single dose dissolved in 0.5 c.c. sterile distilled water. In many experiments the animals were infected with a virus two hours after the second dose; variations from this procedure are indicated in the appropriate places. Dosing continued for a variable period according to the virus in use and the exact object of the particular experiment. Most six-day-old chick-embryos tolerated a single dose of 0.5 mg. injected into the yolk-sac two hours after virus had been given by the same route.

In comparative tests with other drugs we gave sulphonamides orally twice daily in doses of 5 mg. per 20 g., penicillin (96 per cent penicillin-II) by intraperitoneal injection of 500 units four times daily at 9.0 a.m., 12.45, 5.0 and 9.30 p.m. During the night, animals receiving penicillin were offered the drug dissolved in sterile distilled water from sterilized drinking bottles.

RESULTS

(a) Influenza

Table I sets forth the scores of the pulmonary lesions in mice infected intranasally with the PR-8 strain of influenza virus A and killed on the 7th day. The results in mice treated with nitroakridin do not suggest a therapeutic effect of the compound.

In the haemagglutination test nitroakridin did not inhibit agglutination of fowl erythrocytes by influenza virus; on the contrary, the stronger solutions themselves produced strong agglutination.

TABLE I

EFFECT OF TREATMENT WITH NITROAKRIDIN ON THE PULMONARY LESIONS OF MICE INFECTED INTRANASALLY WITH INFLUENZA VIRUS

5 = death with specific lesions on or before the 7th day. 4–0 = extent of pulmonary lesions recorded according to conventional practice.

Exp.	Number of doses of drug	Dilution of virus	Pulmonary lesions	
			Treated mice	Untreated mice
1	7	10^{-5}	4, 4, 3, 3, 3, 3, 3, 1, 0, 0 = 24	5, 5, 4, 4, 4, 3, 3, 3, 2, 1 = 34
		10^{-6}	4, 3, 3, 2, 1, 1, 1, 1, 0, 0 = 16	4, 3, 3, 2, 2, 1, 1, 1, 0, 0 = 17
		10^{-7}	1, 1, 1, 1, 0, 0, 0, 0, 0, 0 = 4	1, 1, 1, 1, 1, 0, 0, 0, 0, 0 = 5
2	7	10^{-4}	5, 5, 5, 5, 5, 5, 3, 2, 2, 0 = 37	5, 5, 5, 5, 5, 4, 4, 4, 3, 2 = 42
		10^{-5}	3, 3, 3, 2, 2, 2, 1, 1, 1, 0 = 18	5, 5, 4, 3, 2, 2, 2, 1, 1, 0 = 25
		10^{-6}	4, 3, 3, 2, 2, 1, 1, 1, 0, 0 = 17	3, 2, 2, 2, 2, 2, 2, 1, 0, 0 = 16
3	8	10^{-4}	5, 5, 5, 5, 5, 5, 5, 5, 4, 4 = 48	5, 5, 5, 5, 5, 4, 4, 4, 4, 3 = 44
		10^{-5}	5, 5, 4, 4, 4, 3, 3, 2, 0, 0 = 30	5, 5, 4, 4, 3, 3, 3, 2, 2, 0 = 31
		10^{-6}	3, 2, 2, 2, 2, 2, 1, 1, 1, 0 = 16	4, 3, 3, 3, 2, 2, 2, 1, 0, 0 = 20

(b) Equine encephalomyelitis, louping-ill, St. Louis encephalitis and rabies

Table II shows the mortality in groups of 30 mice treated or untreated with nitroakridin and infected with one or other neurotropic virus. In Eastern equine encephalomyelitis the compound apparently prolonged the mean period of survival slightly; clinically we noticed that several animals lingered on in a moribund state, an experience unusual with this infection. In louping-ill the compound produced a heavier mortality, which statistically was almost significant, and the average period of survival was shorter than in controls. With the virus of St. Louis encephalitis deaths were very much more numerous in treated animals, though the average period of survival appeared to be prolonged. In rabies the death-rate was slightly but not significantly greater than in control animals, the period of survival being unaltered.

None of these results suggests that nitroakridin may be a useful therapeutic agent for the diseases in question.

(c) Psittacosis

From the beginning it was clear that nitroakridin had a clear-cut beneficial effect on psittacosis. This effect was apparent not only from statistical consideration of the completed experiments; the

TABLE II

EFFECT OF TREATMENT WITH NITROAKRIDIN OF MICE INFECTED WITH NEUROTROPIC VIRUSES

Virus	Dose	Route of inoculation	Mortality in groups of 30 mice				
			Treated mice			Untreated mice	
			Number of doses of drug	Deaths	Mean period of survival in days*	Deaths	Mean period of survival in days*
Equine encephalomyelitis (Eastern strain)	1000 cerebral LD50	intramuscular	8	10	6.2	9	5.0
Louping-ill	1000 cerebral LD50	intramuscular	8	16	12.9	9	14.5
St. Louis encephalitis	1 cerebral LD50	intracerebral	14	25	18.0	8	14.9
Rabies (virus-fixe) ..	1000 cerebral LD50	intramuscular	14	25	15.1	19	14.9

* From time of inoculating virus.

TABLE III

EFFECT OF TREATMENT WITH NITROAKRIDIN OF MICE INFECTED WITH PSITTACOSIS

Exp.	Dilution of virus	Route of inoculation of virus	Day exp. ended	Mortality in groups of 30 mice					
				Treated mice				Untreated mice	
				Number of doses of drug	Time of first dose of drug	Deaths	Mean period of survival in days*	Deaths	Mean period of survival in days*
1	$10^{-6.5}$	intraperitoneal	22	8	26 hr. before virus	15	12.0	28	8.6
2	$10^{-7.0}$	intraperitoneal	26	8	26 hr. before virus	11	12.0	27	9.3
		,,	26	14	26 hr. ,, ,,	2	13.0	28	9.5
3	$10^{-7.0}$	intranasal	28	21	26 hr. before virus	20	15.2	25	15.2
		intraperitoneal	28	15	26 hr. ,, ,,	16	11.9	29	8.6
		,,	28	12	48 hr. after virus	22	11.9		
		,,	28	10	96 hr. ,, ,,	24	9.5		
4	$10^{-7.5}$=5 LD50	intraperitoneal	42	13	4 hr. after virus	13	14.3	28	7.1
	$10^{-7.0}$=10 LD50	,,	42	13	4 hr. ,, ,,	13	21.4	28	7.2
	$10^{-5.0}$=1000 LD50	,,	42	13	4 hr. ,, ,,	16	12.2		
	$10^{-7.0}$=10 LD50	,,	42	13	52 hr. ,, ,,	23	13.5		
5	$10^{-7.5}$=5 LD50	intraperitoneal	36	13	4 hr. after virus	11	19.1	27	15.4
	$10^{-7.0}$=10 LD50	,,	36	13	4 hr. ,, ,,	8	21.0	29	15.4
	$10^{-5.0}$=1000 LD50	,,	36	13	4 hr. ,, ,,	19	17.9		
	$10^{-7.0}$=10 LD50	,,	36	13	52 hr. ,, ,,	12	19.4		
6	$10^{-7.0}$	intraperitoneal	40	14	26 hr. before virus	26	19.7	26	11.5
	$10^{-6.0}$	,,	40	14	26 hr. ,, ,,	19	11.3	30	7.9
	$10^{-4.0}$	,,	40	14	26 hr. ,, ,,	27	10.3	26	7.5

* From time of inoculating virus.

casual visitor to the isolation-unit, at a time when untreated animals were dying, might see one cage of extremely sick (control) mice, and another containing more numerous and more active animals of which few or none were obviously ailing. Table III illustrates a number of points regarding this therapeutic action.

Experiment 1 demonstrated the decreased mortality and the longer survival time of treated as compared with untreated mice inoculated intraperitoneally.

Experiment 2 showed that while comparable control groups gave remarkably uniform results, extension of the period of treatment from 6 to 12 days after infection (8 to 14 doses including those before infection) greatly enhanced the therapeutic effect.

Experiment 3 failed to demonstrate an effect of similar magnitude when virus was instilled intranasally. Whereas when virus was injected intraperitoneally nitroakridin reduced mortality considerably and increased mean-survival-time, more prolonged administration of the drug had less effect upon the number of deaths and none on the mean-survival-time of mice inoculated intranasally. Even so, a definite effect was apparent in additional groups of mice treated exactly as the others, except that they were killed on the 8th day after intranasal inoculation and the focal lesions in the lungs counted. The foci in the control (untreated) group of 30 animals numbered 68, while those in the treated group of similar size numbered only 28. The other point established by Experiment 3 is that after intraperitoneal injection of virus delays in beginning treatment, of 48 and 96 hours respectively, resulted in a reduced favourable effect; even with the longer period of delay, however, the tendency was towards more frequent or more prolonged survival than in control mice. Confirmatory data on this point were obtained in Experiments 4 and 5. The partial benefit conferred by delayed treatment dispelled the suspicion that the greater efficacy of nitroakridin against intraperitoneally administered virus might be attributed to direct inactivation of the latter by drug still present in the abdominal cavity at the time of injection. Further evidence pointing to this conclusion will be

considered later. In *in vitro* experiments designed to elucidate its mode of action we found that concentrations of the drug of from 1 : 10,000 to 1 : 1,000 did not inactivate the virus fully within one hour ; the mixtures still killed most of the mice inoculated, though with the 1 : 1,000 concentration the incubation period of the disease was prolonged by one or two days.

In Experiments 4–6 the period of observation of the animals was extended and the effect of various doses of virus studied. If a large dose of virus be given (10^{-4}), nitroakridin is apparently capable only of increasing survival-time without preventing ultimate death. With a dilution of 10^{-5}, and often 10^{-7}, a considerable therapeutic effect is seen. When the dose of virus is very small, however, there is a tendency towards rather poorer therapeutic results, particularly evident in Experiment 6. In that experiment, at the time when administration of the drug ceased most of the controls were dead while nearly all the treated mice were alive and apparently quite well. About a week later many began to sicken, and the final mortality in this group was the same as among the controls. This reactivation of the disease, which we have seen on several occasions, did not come wholly as a surprise, because we had previously discovered that treatment with nitroakridin does not eradicate virus from the mice ; apparently healthy animals prevented from dying by such treatment carry fully virulent virus in a greatly enlarged spleen for at least a month. The fact that this reactivation tends to occur more often after small than after larger doses of virus would seem to merit further study ; it recalls the recent observation of Peterson and Fox (1947) in tsutsugamushi disease treated with methylene blue that treatment may safely be discontinued sooner after a massive infecting dose than after a smaller one.

Table IV shows the effect of varying the dosage of nitroakridin ; the best results appear to follow a dose of 0.25 mg. given once daily.

In Table V the effect of giving virus and drug by different routes is shown. It appears that intravenous administration of the drug is more effective against intraperitoneally inoculated virus than is intraperitoneal medication. Medication by the latter route, however, clearly has an effect on both intraperitoneal and intravenous infections. These observations furnish additional support for the conclusions reached previously, viz., that the action of nitroakridin is not purely a local one in the peritoneal cavity.

TABLE IV

CHEMOTHERAPY OF PSITTACOSIS

The effect of various doses of nitroakridin
All treatments began 4 hours after intraperitoneal injection of virus.

Dosage of Nitroakridin	Number of doses	Mortality in groups of 30 mice	
		Deaths	Mean period of survival in days
None	—	28	7.2
0.25 mg. in 0.5 c.c. water once daily	13	13	21.4
0.125 mg. in 0.25 c.c. water twice daily ..	26	21	10.8
0.1 mg. in 0.25 c.c. water once daily	13	26	10.2
0.5 mg. in 1.0 c.c. water once every three days	6	21	11.5

TABLE V

CHEMOTHERAPY OF PSITTACOSIS

The effect of varying the route of administration of virus and of nitroakridin
Infecting dilution of virus 10^{-6}. All treatments began 4 hours after injection of virus and continued daily for 16 doses. Animals observed for 37 days

Inoculation of virus	Injection of drug	Mortality in groups of 30 mice			
		Treated mice		Untreated mice	
		Deaths	Mean period of survival in days*	Deaths	Mean period of survival in days*
intraperitoneal	intraperitoneal	20	12.9	28	11.8
intraperitoneal	intravenous	8	17.6	—	—
intravenous	intraperitoneal	17	17.6	23	13.6

* From time of inoculating virus

Finally we made several comparisons of the activity of nitroakridin and other chemotherapeutic substances. Table VI presents the results of one such experiment. With our strain of psittacosis virus sulphadiazine and sulphamezathine consistently failed to prevent death and usually prolonged but slightly the mean period of survival ; sometimes sulphadiazine appeared to be the more active, at other times sulphamezathine. In the experiment shown in Table VI, nitroakridin, while failing to influence the final mortality, increased considerably the mean period of survival. It was, however, not nearly as efficacious as penicillin, though even after 18 days of therapy with this substance virus was not eradicated ; many of the mice, apparently well throughout the period of therapy, began to sicken and die five to ten days after treatment was discontinued.

TABLE VI

CHEMOTHERAPY OF PSITTACOSIS

Comparison of nitroakridin with other chemotherapeutic substances
Dosing began one hour after intraperitoneal injection of virus and continued for 18 days. Animals were observed for 35 days.

Mortality in groups of 30 mice		
	Deaths	Mean period of survival in days
Controls	26	8.3
Sulphadiazine .. .. .. (5 mg./20 g. twice daily, orally)	28	9.2
Sulphamezathine .. .. (5 mg./20 g. twice daily, orally)	26	8.7
Nitroakridin .. .. .. (0.25 mg./20 g. once daily, i.p.)	29	12.4
Penicillin .. .. .. (500 units/mouse 4 times daily, i.p.)	19	23.8

*(d) Lymphogranuloma venereum**

Our observations with this virus supply the data for Table VII. Virus inoculated into the yolk-sac killed all untreated embryos within 8 days, and most by the 6th day. A dose of 0.5 mg. nitroakridin injected into the yolk-sac two hours after virus killed a number of embryos within the first 24 hours. Of the remainder a few survived to the 20th day, while most of those dying did so at a later stage than the controls. A dose of 0.25 mg. per egg resulted in no toxic effects but all embryos succumbed to lymphogranuloma, albeit more slowly than the controls. Thus, against lymphogranuloma in chick-embryos nitroakridin exhibits moderate chemotherapeutic activity.

* Alternatively known as lymphogranuloma inguinale and climatic bubo.

COMMENT

It is well known that the virus of lymphogranuloma venereum is susceptible to chemotherapeutic attack by many sulphonamides. MacCallum and Findlay (1938), Findlay (1940), Jones, Rake, and McKee (1941), Rodaniche (1942), Felton, Hebb, and Oliphant (1943), van den Ende and Lush (1943) and others have dealt with various aspects of their action against the experimental disease in mice, and Meiklejohn, Wagner, and Beveridge (1946) in the chick-embryo. The last authors also showed that penicillin inhibited growth of this virus in the yolk-sac.

Reports of the activity of the commoner antibacterial agents against the psittacosis virus have not been unanimous. Rudd and Burnet (1941) failed to demonstrate in mice a therapeutic action of several sulphonamides, though Wiseman, Meiklejohn, Lackman, Wagner, and Beveridge (1946) and Early and Morgan (1946b) found sulphadiazine to be effective in mice, and Meiklejohn, Wagner, and Beveridge (1946) and Early and Morgan (1946a) in eggs. Heilman and Herrell (1944), Bedson and May (1945), Meiklejohn *et al.* (1946), Wiseman *et al.* (1946), and Early and Morgan (1946a and b), using mice, developing eggs, or tissue-cultures, have all observed activity on the part of penicillin. The last-named authors noted that the antibiotic was less effective against intranasal than against intravenous infection in mice, and ineffective against intracerebral inoculation; oral sulphadiazine was apparently less effective against intraperitoneal than against intravenous or respiratory infection.

Mauer (1938) reported that trypaflavin exerted a beneficial action in mice infected with psittacosis. With this exception the present appears to be the

TABLE VII

EFFECT OF NITROAKRIDIN ON CHICK-EMBRYOS INFECTED WITH LYMPHOGRANULOMA VENEREUM

Exp.	Dose per egg mg.	Results in groups of 15 eggs							
		Treated				Untreated			
		Non-specific deaths	Specific deaths	Survived	Mean period of survival in days†	Non-specific deaths	Specific deaths	Survived	Mean period of survival in days†
1	0.50	4	8	3	9.9	0	15	0	6.5
2	0.50	6	7	2	8.0	0	15	0	5.4
3	0.25	0	15	0	7.6	0	15	0	4.0

† Of embryos dying specifically.

first record of the activity of an acridine compound against viruses of the psittacosis—lymphogranuloma group.

We have shown that nitroakridin 3582 (Höchst) is moderately active against the viruses of psittacosis in mice and lymphogranuloma venereum in developing chick-embryos. Against our strain of psittacosis virus it appears more active than sulphadiazine or sulphamezathine (which indeed have only slight therapeutic properties) and much less active than penicillin. It is less active against intranasal than against intraperitoneal or intravenous infections.

Nearly all authors, including Rodaniche (1943), agree that treatment with current antibacterial agents does not sterilize infections with these viruses, and that despite clinical improvement or survival the animals continue to harbour fully virulent virus. In our experiments we have confirmed this observation for psittacosis and nitroakridin; we have further observed that after treatment for as long as 18 days with nitroakridin or penicillin apparently healthy survivors are apt, a week or more later, to develop symptoms of psittacosis and die.

Summary

Nitroakridin 3582 (Höchst) possesses moderate therapeutic activity against the viruses of psittacosis and lymphogranuloma venereum. It is more active than sulphadiazine and sulphamezathine but less active than penicillin. A week or more after the termination of a prolonged course of either nitroakridin or penicillin mice which have apparently been enabled successfully to withstand infection with psittacosis virus frequently develop symptoms and die. This observation accords with the general experience that the common antibacterial agents, while suppressing infections with viruses of the psittacosis–lymphogranuloma group, do not eradicate virus, which may still persist in a fully virulent state.

I am indebted to Prof. S. P. Bedson for the strain of psittacosis virus used in this work (M.O.H.154).

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