Brit. J. Pharmacol. (1952), 7, 473.

THE ACTION OF MEPACRINE AND TRYPAN RED IN A NUMBER OF VIRUS DISEASES

BY

E. WESTON HURST, J. M. PETERS, AND P. MELVIN

From Imperial Chemical Industries Ltd., Biological Laboratories, Hexagon House, Manchester, 9

(Received March 10, 1952)

The previous paper (Hurst, Melvin, and Peters, 1952) described the prophylactic effect of mepacrine, various bis-azo dyes, and other substances containing in the molecule numerous sulphonic-acid groups, and certain macromolecules against encephalitis induced by the viruses of equine encephalomyelitis and louping-ill. Of these substances mepacrine was by far the most active. It protected the majority of mice which otherwise would have succumbed to encephalitis after intramuscular injection of virus; this protection was associated with great reduction in the amount of virus circulating in the blood stream in the early, systemic phase of infection. The drug also exercised some protective effect when virus was injected intracerebrally. Its striking activity was not shared by a number of other acridine derivatives, several of which differed only in the nature of the side-chain attached in the 9-position. These observations encouraged us to hope for a similar action on other viruses, and the present report details our studies on many viruses in several different hosts. In addition to mepacrine we included trypan red in many of the tests, as, of course, we could not assume that their actions would run parallel over a whole range of infections.

We have already cited the relevant literature concerning trypan red and various neurotropic viruses, and mentioned that on the effect of nitroacridines in psittacosis and lymphogranuloma. Recently, Eaton, Cheever, and Levenson (1951) have investigated the action of two nitroacridines and a closely related chloroacridine on several viruses in the chick-embryo or in tissue-culture. The nitroacridines markedly inhibited growth of the viruses of meningo-pneumonitis and feline pneumonitis in the allantoic sac, but the chloroacridine produced negligible effects, as did also atabrin (mepacrine) given into the yolk-sac. Both types of compound slightly inhibited mumps virus, but only the nitro-compound influenza B. In tissue-cultures all compounds retarded the growth of the viruses of mumps, influenza A, and influenza B. The results with influenza B in eggs confirmed earlier findings of Green, Rasmussen, and Smadel (1946) with Nitroakridin 3582, though Hurst (1948) and Rasmussen and Stokes (1951) detected no effect of this particular nitroacridine on influenzal infections in mice. Thompson (1947) found that in tissue-cultures atabrin, proflavine, and 9-aminoacridine (as well as many other miscellaneous substances) reduced or prevented growth of vaccinia virus, while Briody and Stannard (1951) reported that proflavine inhibited growth of influenza B and vaccinia viruses in developing chick-embryos but exerted no effect on influenza A, mumps, or Newcastle disease. Effects of acridines on bacteriophages have been noted by Fitzgerald

and Lee (1946), Dickenson (1948), Foster (1948), and Smith (1949). On the negative side, Coggeshall and Maier (1942) observed no activity of two acridines against influenza or poliomyelitis in mice; Krueger *et al.* (1943) of several acridines against influenza in mice; Kramer, Geer, and Szobel (1944) of two acridines (one atabrin) against poliomyelitis in mice; Andrewes, King, and van den Ende (1943) of several acridines including mepacrine against influenza, lymphogranuloma, or vaccinia in mice or rabbits; and Cutting *et al.* (1947) against herpes simplex, vaccinia, or influenza in mice or chick-embryos. On clinical grounds Duančić (1942) claimed activity of atebrin (mepacrine) against mumps, measles, and influenza.

EXPERIMENTAL

Viruses and methods

We shall not attempt to describe in detail the numerous viruses used in this work. The great majority have been closely studied by us for many years; a few have been acquired recently from other laboratories and we accepted them without confirmation of what they purported to be. The working principles followed are sufficiently defined in the companion paper, and we would emphasize only one point concerning the " virulence " of the virus used for test. When weak therapeutic effects are encountered, results obtained consistently against a moderately active virus may easily be obscured or brought below the level of statistical significance by substituting a virus rendered more highly " virulent " by frequent serial passage. As far as possible we have of late tried to standardize the viruses used for infection by employing material taken at the first or second passage, depending on the particular virus, from stocks of lyophilized " master " virus.

Attempts at prophylaxis in mice

We examined the effect of mepacrine and of trypan red against 18 virus infections in mice. Tables I and II, which are largely self-explanatory, record the tests. Against the virus of *Western equine encephalomyelitis* injected by a peripheral route (Table I), mepacrine exerted the same marked effect as against the Eastern virus dealt with in the accompanying paper. Against *St. Louis encephalitis* virus injected intracerebrally it seemed to have some action which was very easily obscured by too large an infecting dose or too " virulent " a virus; a single large dose appeared more effective than repeated smaller doses. In the two tests with *rabies* intramuscularly we used the same sample of virus, on the first occasion as fresh mouse brain and on the second as glycerinated material six months old; during the interval the cerebral titre of the virus had fallen from $10^{-3.8}$ to $10^{-2.8}$. Whereas on the first occasion both treatments were unsuccessful, on the second it seemed that they might have shown a slight (but not significant) action. We obtained a similar inconclusive hint of activity with *neurotropic influenza* virus given intracerebrally. Against *Rift Valley fever* mepacrine produced a definite effect, either in merely lengthening the mean period of survival or in sparing mortality in addition; from many other experiments we can state that the first entry in Table I is not typical of its action, as almost always many animals were saved from death provided that the infecting dose of virus was not too large. Thus, with an infecting dose of approximately 1–10 LD100 virus, a single large dose of mepacrine usually reduced mortality to between one-third and two-thirds of that among the controls, whereas with 100 and 1,000 LD100 mortality was unaffected but the treated mice died after considerably lengthened mean periods of survival. The therapeutic effect against small

TABLE I

EFFECT OF TREATMENT WITH MEPACRINE ON VIRUS DISEASES IN MICE

Mepacrine was given orally (*a*) once in a dose of 10 mg./20 g. 24 hours before virus, or (*b*) twice daily in a dose of 2 mg. for the first 3 days and 1 mg. thereafter, beginning 24 hours before infection. The repeated doses continued for 12 days or until two-thirds of the controls had died, whichever was the shorter period. The figures in parentheses in this and subsequent Tables are the mean periods of survival of fatal cases in days

Virus	Infecting dilution and route	Number of mice	Deaths		
			Untreated	Mepacrine once	Mepacrine twice daily
Western equine encephalomyelitis	10^{-2} i.m.	30	23 (6.4)	2 (11.5)	4 (9.6)
St. Louis encephalitis	10^{-6} i.c.	30	14 (6.9)	9 (5.8)	12 (7.2)
	10^{-5} i.c.	30	29 (5.1)	11 (5.6)	26 (5.8)
	10^{-6} i.c.	30	27 (6.0)	10 (7.0)	19 (7.4)
Rabies—*virus-fixe*	10^{-1} i.m.	30	27 (12.7)	27 (13.5)	30 (12.5)
	10^{-1} i.m.	30	11 (15.2)	8 (14.6)	6 (17.8)
Rift Valley fever	$10^{-6\cdot5}$ i.p	30	23 (2.8)	25 (6.6)	24 (5.7)
	$10^{-6\cdot5}$ i.p.	30	28 (3.0)	18 (4.4)	—
	10^{-7} i.p.	30	30 (2.2)	19 (4.4)	—
Neurotropic influenza—W.S. strain	10^{-2} i.c.	20	13 (5.0)	13 (4.8)	10 (5.4)
	10^{-4} i.c.	20	10 (5.9)	8 (6.3)	7 (6.3)

TABLE II

EFFECT OF TREATMENT WITH MEPACRINE OR TRYPAN RED ON LYMPHOCYTIC CHORIOMENINGITIS AND HERPES FEBRILIS IN THE MOUSE

Mepacrine was given as described in Table I. Trypan red was administered intraperitoneally in a dose of 0.5 mg. twice daily, beginning 3 days before virus. Not all animals which developed symptoms died. With lymphocytic choriomeningitis a proportion showing at one stage the characteristic convulsive phenomena ultimately recovered. With herpes a few mice survived with transient or persistent paralysis of the hind limbs. Each group consisted of 30 mice

Virus	Infecting dilution and route	Results in groups of 30 mice											
		Untreated			Mepacrine once			Mepacrine twice daily			Trypan red twice daily		
		Died	Ill—recovered	No symptoms	Died	Ill—recovered	No symptoms	Died	Ill—recovered	No symptoms	Died	Ill—recovered	No symptoms
Lymphocytic choriomeningitis	10^{-3} i.c.	25 (7.9)	3	2	17 (10.2)	7	6	24 (9.8)	1	5	—	—	—
	10^{-4} i.c.	20 (8.6)	7	3	11 (10.0)	11	8	24 (10.0)	2	4	28 (7.8)	0	2
	10^{-4} i.c.	16 (12.1)	8	6	8 (13.5)	12	10	24 (10.5)	3	3	—	—	—
Herpes febrilis†	$10^{-1\cdot5}$ i.m.	17 (7.6)	3	10	—	—	—	—	—	—	12 (8.0)	2	16
											6 (13.8)	6	18
	10^{-1} i.m.	14 (8.6)	4	12	—	—	—	—	—	—	3 (12.6)*	2	25
											6 (9.3)	1	23
	10^{-2} i.m.	11 (8.7)	12	7	—	—	—	—	—	—	7 (8.3)*	1	22
											10 (11.0)	1	19
	10^{-1} i.m.	21 (8.4)	1	8	—	—	—	—	—	—	12 (10.0)*	1	17
	10^{-1} i.m.	21 (8.3)	1	8	14 (9.7)	2	14	12 (9.9)	1	17	19 (9.6)	0	11
	10^{-1} i.m.	28 (6.4)	2	0	19 (7.1)	2	9	21 (7.7)	1	8	26 (6.6)	1	3
	10^{-1} i.m.	27 (7.0)	0	3	15 (11.1)	3	12	22 (8.5)	2	6	27 (7.2)	1	2

* American sample of dye. † Testicular virus.

doses of virus was associated in the majority of animals with marked suppression of the pathological changes in the liver. Instead of the massive diffuse necrosis characteristic of Rift Valley fever in the mouse, many livers showed only small foci of midzonal necrosis which in a few mice were detected only after a careful search.

Many surviving animals subsequently did not resist a second dose of virus of around 1,000 LD100. None of these infections was influenced beneficially by treatment with trypan red; in view of the results with Eastern equine encephalomyelitis, however, it is possible that Western equine encephalomyelitis might have responded had the infecting dose been smaller.

Table II sets out the results obtained with two other viruses in mice. The virus of *lymphocytic choriomeningitis* injected intracerebrally was affected by a single large dose of mepacrine, but not by repeated smaller doses or by trypan red. Once more the effect was lost if too " virulent " a virus was used for infection.

We carried out the experiments with *herpes febrilis* at two stages—four years ago when we first began work with trypan red, and again quite recently. The virus infecting the earlier batches of animals was derived from rabbit brain which had been passed once intratesticularly to render it virulent by the intramuscular route. The virus used in recent experiments had been passed repeatedly intratesticularly. Against the earlier virus trypan red (both of English and of American origin) exerted a definite prophylactic action, whereas with the more recent virus we could detect no such effect; against the recent virus, however, the more potent mepacrine showed activity. It will be noted that in the earlier experiments more cases of non-fatal paralysis occurred.

In this and the companion paper we have repeatedly ascribed discrepancies in the results of treatment on different occasions to variations in the " virulence " of the sample of virus used for infection. Without making the papers inordinately long it is not possible to adduce all the evidence we have accumulated in favour of this interpretation of the facts. It does seem appropriate, however, to describe one observation illustrating the different results of treatment according to the " virulence " of the infecting virus. The relevant information appears in Table III. Herpes virus passed but once intratesticularly possessed a far higher intracerebral titre than did that passed repeatedly in the testis. Nevertheless, in therapeutic experiments against these viruses injected intramuscularly, the latter proved the more refractory to treatment.

Intraperitoneal infections in groups of 20 or 30 mice with the virus of *psittacosis*, intracerebral and intramuscular infections with *Russian spring-summer encephalitis* or the GD.VII and FA strains of *mouse encephalomyelitis* virus, and intracerebral infections with *Murray Valley encephalitis* (Anderson *et al.*, 1951), mouse-adapted *human poliomyelitis*, the TO *mouse poliomyelitis*, mouse-adapted *neurovaccinia*, or the *JHM* (Bailey *et al.*, 1949) viruses all resisted single or repeated dosage with mepacrine. Repeated doses of trypan red appeared to show slight activity against psittacosis, but here the results were perhaps suspect since both dye and virus were inoculated by the same route; in no other infection did trypan red influence the sequence of events unless for the worse.

In two experiments with *grey-lung* virus (Andrewes and Glover, 1945) introduced intranasally into groups of 20 mice, single or repeated doses of mepacrine produced no beneficial effect; the mean weights of the greatly enlarged lungs of animals surviving to the 20th day were the same as in control mice, and the titre of pulmonary virus (10^{-8}) was the same in all groups. The adverse influence of trypan red was evidenced by a very heavy mortality among infected mice treated with twice daily doses.

TABLE III

EFFECT OF "VIRULENCE" OF HERPES VIRUS ON THE RESULTS OF TREATMENT

The viruses used in this experiment were of the same basic strain. Herpes A was derived by one intracerebral and one intratesticular passage from an ampoule of lyophilized virus. Herpes B derived in the same manner four years previously had been passed repeatedly intratesticularly in the interval. Both viruses were fully neutralized by anti-herpes serum prepared with yet another derivative of the lyophilized "master" virus. The tests shown below were performed simultaneously on groups of 30 mice

Treatment	Infecting dilution of virus and route	Herpes A Intracerebral titre in mice $>10^{-6}$*			Herpes B Intracerebral titre in mice $10^{-2 \cdot 3}$*		
		Died	Paralysed but survived	No symptoms	Died	Paralysed but survived	No symptoms
None ..	$10^{-1 \cdot 5}$ i.m.	26 (6.3)	1	3	30 (5.1)	—	—
Mepacrine once		21 (7.7)	4	5	26 (6.5)	2	2
Mepacrine twice daily ..		26 (7.9)	1	3	30 (6.1)	—	—
Trypan red twice daily ..		22 (7.1)	4	4	30 (5.8)	—	—
None ..	10^{-1} i.m.	30 (6.5)	—	—	30 (5.8)	—	—
Mepacrine once		19 (7.4)	6	5	27 (6.6)	1	2
Trypan red twice daily ..		28 (5.9)	—	2	29 (5.4)	1	—

* The 50 per cent end-points given here and in Table IV are not 50 per cent fatalities, but were derived on the principles outlined by Hurst, Peters, and Melvin (1953)

The final infection studied in mice was *influenza*. In four experiments we infected groups of 10 mice intranasally with three dilutions of influenza A virus, and treated them with twice-daily doses of either mepacrine or trypan red. Neither treatment affected either mortality or the score of pulmonary lesions.

Having thus obtained evidence of the activity of mepacrine against some but not most of the viruses tested, it seemed possible that we might enhance the effect against the susceptible strains and demonstrate it against others if we could increase the amount of drug in the tissues. Accordingly, we dosed mice with 1 mg. mepacrine twice daily for 14 days, after which time they were distinctly yellow in colour. We then infected them intracerebrally with lymphocytic choriomeningitis, St. Louis, or G.D.VII viruses, or intramuscularly with rabies or G.D.VII viruses, and continued treatment. The results were similar to those above.

We performed two experiments each with the viruses of St. Louis encephalitis, lymphocytic choriomeningitis, and Rift Valley fever to determine the optimal time of administration of a single oral dose of mepacrine (10 mg./20 g.). With the first two the best results were obtained by dosing 24 hours or 1 hour before virus, but appreciable effects, even if usually only in the direction of a lengthened mean period of survival, resulted when treatment was applied at 24, 48, or 72 hours after virus. At 120 hours after virus there was a distinct tendency to more favourable results than at 24–72 hours; possibly at this time a check to viral growth was supplemented by developing immunity. With Rift Valley fever marked effects were observed from treatment at 48, 24, or 4 hours before virus, with the best result at 24 hours. Significant reduction in mortality also occurred with treatment at 4 or 18 hours

after infection in this very rapid developing disease, of which control mice begin to die in 48 hours.

Table IV shows that a therapeutic effect of mepacrine is associated with restricted multiplication of virus; although, with viruses injected intracerebrally, this is not very marked it is nevertheless reflected in a diminished mortality.

TABLE IV

TITRES OF VIRUS IN THE LIVERS OR BRAINS OF MICE TREATED OR UNTREATED WITH MEPACRINE

Groups of 5 livers (Rift Valley fever) or 4 brains (St. Louis encephalitis or lymphocytic choriomeningitis) were pooled and titrated at half-logarithmic intervals intraperitoneally or intracerebrally in groups of 6 mice. The $\log_{10}$ dilutions of virus giving 50 per cent end-points* are given below as positive quantities. Treatment consisted in a single oral dose of 10 mg. mepacrine 24 hours before infection

Virus and route of inoculation	Treatment	Days after infection							Mortality in duplicate groups of 30 mice
		1	2	3	4	5	6	7	
Rift Valley fever 10^{-7} i.p.	None	4.0	8.5	—	—	—	—	—	29 (2.3)
	Mepacrine	2.0	5.5	5.0	5.0	—	—	—	15 (3.5)
St. Louis encephalitis $10^{-6.5}$ i.c.	None	—	1.7	—	6.9	7.3	7.1	7.1	27 (7.4)
	Mepacrine	—	1.3	—	6.1	6.6	6.8	4.1	19 (8.8)
Lymphocytic choriomeningitis 10^{-4} i.c.	None	—	2.2	—	3.9	3.7	4.3	4.1	20 (8.2)+6 sick
	Mepacrine	—	2.8	—	3.3	2.5	1.9	2.4	15 (9.3)+4 sick
Lymphocytic choriomeningitis 10^{-4} i.c.	None	—	3.0	—	3.9	5.0	5.8	5.3	17 (8.7)+12 sick
	Mepacrine	—	2.2	—	3.4	4.5	4.4	4.6	14 (9.4)+6 sick

* See footnote Table III.

Tests in rabbits

Rabbits were given three oral doses each of 100 mg. mepacrine per kg. and thereafter 25 mg./kg. twice daily. After the third dose three were infected intradermally, together with an equal number of controls, with the virus of *infectious myxomatosis* at a dilution of 10^{7}. The treated animals developed smaller initial papules and a less abundant secondary eruption than did the untreated, but they all died on the 10th day whereas one control died on each of the 10th, 11th, and 12th days.

Six rabbits similarly dosed, together with the same number of controls, received intradermally each in three areas 1 and 10 skin infecting doses of *vaccinia* virus. The ensuing vaccinal reactions were weaker in the treated animals, but none was abolished. A similarly weak response may, of course, be obtained in rabbits sub-standard from many causes.

Four rabbits similarly treated, with four controls, were infected intradermally with a 10^{4} dilution of the virus of *Aujeszky's disease*. Two treated animals developed the characteristic symptoms at the same time as controls and died at the same times. The others developed symptoms and died 24 hours after the controls. We have observed similarly delayed death in animals poisoned by several substances.

Attempts to influence avian tumours

Twenty chickens around 500 g. in weight received oral doses of mepacrine (20 mg./100 g.) for four days before and seven days after a 10^{-3} dilution of glycerinated Rous sarcoma injected into one leg. They showed no difference from an equal number of controls in the time of development of tumours or in the number of birds developing tumours. A group of 12 chickens treated similarly, but infected with Fujinami sarcoma, likewise behaved as did an equal number of controls.

Other substances examined

Against Rift Valley fever we examined all the acridines mentioned in the accompanying paper except acriflavine and Safranine T. Most were inactive. Aminacrine, however, in a single test slightly but not significantly reduced mortality, while 2-methoxy-6-chloro-9-*n*-butylaminoacridine acetate and 6-chloro-9-piperidino-2-methoxyacridine prolonged the mean period of survival by 50 per cent or more, as did also 2: 6-diaminoanthrapyrimidine (Table V of the companion paper) which is a compound staining the liver more or less permanently (Hurst, 1952). D 51627 (Table I of the companion paper) was without appreciable effect. In the test in question mepacrine cut mortality by more than half.

Discussion

As already mentioned, the literature contains several suggestions that among the acridines there exist compounds with antiviral properties. Except for some nitroacridines, however, which undoubtedly influence the growth of the viruses of psittacosis and lymphogranuloma venereum in mice (Eaton, van Allen, and Wiener, 1947; Hurst, 1948), these suggestions relate to effects obtained on bacteriophage, in tissue-cultures, or in developing hens' eggs. All workers with considerable experience in the chemotherapy of diseases caused by the smaller viruses will probably agree that effects obtained *in vitro* or in the egg can rarely be transferred to the hatched chick or to the mammalian host. To this statement the acridines generally provide no exception; so far as our observations go, of a representative group of 14 acridines only one, mepacrine, possesses any considerable activity in the mouse. This activity is a specific attribute of mepacrine as a whole molecule; if showing traces of activity, the other acridines do not remotely approach mepacrine in efficacy, and the mepacrine side-chain when attached to the quinoline nucleus does not confer upon pamaquin or chloroquin the therapeutic properties which mepacrine shows in equine encephalomyelitis (see companion paper). If from the work on bacteriophage, etc., we conclude that antiviral activity is a characteristic of a number of acridines, we must believe that mepacrine possesses the appropriate pharmacological properties to enable this activity to become manifest in the mouse.

The viruses against which we have clearly demonstrated the activity of mepacrine run, in order of magnitude of the effect observed, somewhat as follows: the equine encephalomyelitis viruses injected intramuscularly; louping-ill injected intramuscularly, Rift Valley fever intraperitoneally, equine encephalomyelitis intracerebrally, herpes febrilis intramuscularly; lymphocytic choriomeningitis, St. Louis encephalitis, and louping-ill given intracerebrally. Considering, however, the fulminating course of Rift Valley fever in the mouse, and the presumptive difficulty in influencing it by therapy, we are probably justified in bringing this virus into the

same category as equine encephalomyelitis injected peripherally. The chief sites of multiplication of these viruses are: Rift Valley fever—liver; equine encephalomyelitis and louping-ill given intramuscularly—probably reticulo-endothelial system; herpes given intramuscularly—muscle and nervous system; equine encephalomyelitis and other viruses given intracerebrally—central nervous system. The tissue-concentrations of mepacrine run in the descending order—liver, spleen, brain. The effects described in this paper are, no doubt, the resultants of (*a*) the different inherent susceptibilities of the various viruses to mepacrine, (*b*) the different capacities of the viruses for growth in a particular tissue, and (*c*) what may well be the pharmacological property enabling mepacrine to demonstrate the acridine antiviral effect in the mouse, its tendency to concentrate in the tissues.

SUMMARY

This paper describes chemotherapeutic experiments with mepacrine against 21 viruses in mice, rabbits, or chickens. Against most viruses the action of trypan red was also observed, and against Rift Valley fever of a number of acridines and other compounds.

The following diseases were beneficially affected to some extent by mepacrine when treatment was begun before or soon after infection: Western equine encephalomyelitis, Rift Valley fever, herpes febrilis, lymphocytic choriomeningitis, St. Louis encephalitis; with the last two we obtained some evidence of a possible weak therapeutic effect of a single large dose of mepacrine given late in the infection. The weaker prophylactic effects in respect to lymphocytic choriomeningitis and St. Louis encephalitis were easily obscured if too " virulent " a virus were used for infection. Doubtfully influenced or not at all were: rabies, influenza (neurotropic and otherwise), psittacosis, Russian spring-summer encephalitis, Murray Valley encephalitis, vaccinia, and neurovaccinia, poliomyelitis, four mouse encephalomyelitis viruses, grey-lung virus, infectious myxomatosis, Aujeszky's virus, and two avian tumour viruses.

Trypan red reduced mortality in herpes febrilis and doubtfully in psittacosis.

2-Methoxy-6-chloro-9-*n*-butylaminoacridine acetate, 6-chloro-9-piperidino-2-methoxyacridine, and possibly aminacrine showed slight activity against Rift Valley fever, as did also 2: 6-diaminoanthrapyrimidine.

The unique properties of mepacrine among the acridines are discussed.

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