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Antiviral Activity and Induction of Interferon-Like Substance by Quinacrine and Acranil

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Several drugs with certain structural similarities (tricyclic ring system with dialkylaminoalkyl side chains) to tilorone, a potent interferon inducer, were screened for antiviral activity in vivo. Two acridine drugs, Acranil and quinacrine, were found to be effective, the former being almost as protective as tilorone and the latter less so. Both agents induced an interferon-like substance which could be detected in the serum of treated mice. The concentration of the inhibitory factor in the serum was highest after exposure to tilorone, followed in turn by Acranil and quinacrine, based on the administration of equal weights of drugs. Both tilorone and Acranil induced lower levels of circulating interferon-like substance in Balb/c mice than in other strains of mice. The serum factor induced by Acranil was shown to be stable at pH 2.

Tilorone is a highly potent, small-molecular-weight, orally active interferon inducer (11). Structure-activity studies have implicated polycyclic, mostly tricyclic, compounds bearing dialkylaminoalkyl side chains as those possessing antiviral and interferon-inducing capacity (R. F. Krueger et al., Int. Colloq. Interferon, Leuven, 1971, Abstr. no. 24). Several drugs with similar structures are used clinically for other than antiviral purposes. A number of such well-known drugs were investigated for possible antiviral and interferon-inducing activity.

MATERIALS AND METHODS

Mice. For the vaccinia virus mouse tail lesion test, 3- to 4-week-old outbred female CFLP mice weighing 10 to 18 g (breeding farm "LATI," Gödöllö, Hungary) were used. Similar mice weighing 17 to 29 g were used for studies of the kinetics of production of the serum interferon-like substance. The following mouse strains were employed in comparative studies: outbred 21- to 29-g Balb/c, outbred 30- to 41-g Swiss, outbred 18- to 23-g CFLP, and inbred 18- to 22-g C57BL. Only female Balb/c mice were used; all other groups consisted of males and females in equal number.

Vaccinia virus mouse tail lesion test. The vaccinia virus mouse tail lesion test was chosen for its simplicity. The method of Boyle, Haff, and Stewart (1) was followed with the exception that the lesions were counted on the 7th and 9th days after infection and averaged, rather than making a single counting on day 8. Groups consisted of 8 to 10 mice each, and statistical comparison of the numbers of lesions was done by Student's t test. Throughout the experiments, the same lot (L 40/2) of commercial freeze-dried vaccine of the Lister dermovaccinia virus strain was used for infection (kindly supplied by Miklós Koller, "Human" Institute for Serobacteriological Research and Production, Budapest, Hungary). The lyophilized vaccine was resuspended in physiological saline, pH 7, and was inoculated intravenously (0.2 ml per mouse) in the dosages indicated in the tables.

Drugs. Quinacrine (mepacrine, Atabrine) dihydrochloride was a product of Bayer A. G. and was kindly supplied by Chinoin Works, Budapest, Hungary. Acranil dihydrochloride solutions were prepared from tablets (Bayer) by extraction with hot water. Almost the total mass of the tablet dissolved, thus showing that solution of the active substance was probably completed. Tilorone hydrochloride was kindly supplied by R. F. Krueger (The Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio). Other drugs used are listed in Table 1. All test compounds were dissolved in tap water and given intragastrically. Unless indicated otherwise, all drugs were administered 19 to 22 h before infection in a dose of 200 mg/kg. This dose was also used for screening, because any antiviral effect of a drug acting at a higher dose might be considered as minimal.

Interferon assay. Groups consisting of five to nine (usually six) mice were treated as indicated in the text, and at various time intervals 12 to 20 drops of blood were taken from their tails and pooled. (To

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facilitate blood sampling, the mice were held for 30 min in a box heated to 37 to 38 C.) Interferon levels in the sera were titrated by the reduction of cytopathic effects (CPE) as follows. Mouse L-cell monolayers were grown in Parker medium 199 containing 10% calf serum at 37 C for 2 days. The tube cultures were then incubated with 0.5-ml samples of test serum dilutions at 37 C for 4 h. The serum dilution was then discarded and 100 TPD_{50} of Semliki Forest virus was added in a volume of 0.2 ml. After adsorption of the virus for 60 min at 37 C, 0.8 ml of Parker medium 199 containing 2% calf serum was added and the tubes were reincubated at 37 C for 3 days. The titer of interferon was expressed as the reciprocal of the highest serum dilution causing about 50% inhibition of CPE.

RESULTS

Screening of drugs for antivaccinia virus activity. A variety of drugs with a tricyclic ring system and bearing a dialkylaminoalkyl side chain were examined for antiviral activity in the vaccinia mouse tail lesion test. A few drugs with other cyclic ring systems and shorter (dialkylamino) side chains were also screened, as was diethylaminoethanol-HCl. All of these drugs are known to be readily absorbed from the gastrointestinal tract. The majority of drugs were inactive under the described test conditions and are listed in Table 1. However, two acridine derivatives with dialkylaminoalkyl side chains, quinacrine and Acranil, exhibited antivaccinia virus activity and were examined further. Their chemical structures, as well as that of tilorone, are depicted in Fig. 1.

Analysis of antivaccinia virus effect of quinacrine and Acranil. Table 2 shows the effectiveness of a single dose of quinacrine and Acranil as compared with that of tilorone, a known interferon inducer. The data are presented as the number of lesions per individual mouse in the treated and control groups. The latter consisted of untreated mice, since our previous experiments showed that placebo treatment with tap water did not influence the number of lesions significantly. In subsequent tables, only the average number of lesions and the statistical difference between the groups are presented.

The activity of different doses of tilorone, quinacrine, and Acranil in viral infection is shown in Table 3. The activity of Acranil approached that of tilorone, but quinacrine proved to be less potent, when compared on the basis of weight. The effect of time of pretreatment of mice on the activity exhibited by tilorone and quinacrine was then investigated. It can be seen in Table 4 that both drugs were effective when administered as long as 8 days prior to virus infection, although the protective effects were somewhat weaker than when the drugs were administered 1 day before infection.

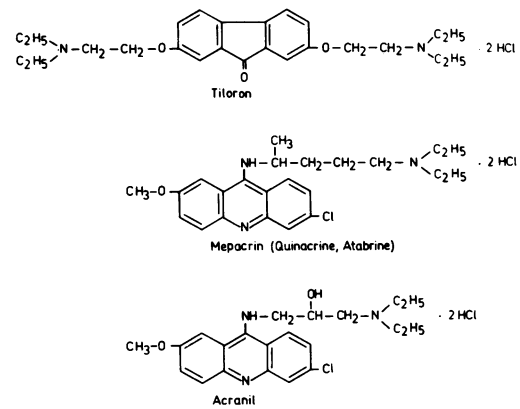

FIG. 1. *Structures of tilorone, quinacrine, and Acranil.*

TABLE 1. *Drugs with cyclic ring structures which failed to exhibit antiviral activity*

Nonproprietary name	Source	Ring system	Specific ring system	Side chain
Chlorpromazine-HCl	EGYT, Budapest	Tricyclic	Phenothiazine	Dialkylaminoalkyl
Promethazine-HCl	EGYT, Budapest	Tricyclic	Phenothiazine	Dialkylaminoalkyl
Diethazine-HCl	EGYT, Budapest	Tricyclic	Phenothiazine	Dialkylaminoalkyl
Imipramine-HCl	Farmitalia	Tricyclic	Dibenzoazepine	Dialkylaminoalkyl
Desipramine-HCl	Sigma Chemical Co.	Tricyclic	Dibenzoazepine	Dialkylaminoalkyl
Amitriptyline-HCl	EGYT, Budapest	Tricyclic	Dibenzocycloheptene	Dialkylaminoalkyl
Lucanthone-HCl	Bayer	Tricyclic	Thiaxanthone	Dialkylaminoalkyl
Methylene blue		Tricyclic	Thionine	Dialkylamino
Ethacridine-lactate	Hoechst	Tricyclic	Acridine	Dialkylamino
Chloroquine-diphosphate	EGYT, Budapest	Bicyclic	Quinoline	Dialkylaminoalkyl
Pamaquine-diphosphate	Bayer	Bicyclic	Quinoline	Dialkylaminoalkyl
Tripelennamine-HCl	EGYT, Budapest	Monocyclic	Benzene and pyridine	Dialkylaminoalkyl
Bencyclane-fumarate	EGYT, Budapest	Monocyclic	Cycloheptane	Dialkylaminoalkyl
Tetracycline-HCl		Tetracyclic	Naphthacene	Dialkylamino
Diethylaminoethanol-HCl[a]		—	—	—

[a] Administered also in seven daily doses, beginning 1 day before infection.

TABLE 2. *Effect of tilorone, quinacrine, and Acranil on the number of tail lesions induced in mice by vaccinia virus*[a]

Drug[b]	No. of lesions in individual mice	Avg no. of lesions	P
None	0, 1, 4, 6, 6, 7, 8, 13, 20, 23	8.80	
Tilorone	0, 0, 0, 0, 0, 0, 1, 3, 4, 4	1.20	<0.01
Quinacrine	0, 0, 0, 1, 1, 2, 2, 2, 3, 10	2.10	<0.02
Acranil	0, 0, 0, 0, 0, 0, 0, 1, 1, 2	0.40	<0.01

[a] Infecting dose: 2.8×10^6 plaque-forming units/mouse.

[b] Given intragastrically in a dose of 200 mg/kg, 22 h before virus infection.

TABLE 3. *Activity of tilorone, quinacrine, and Acranil as related to the drug and virus dose applied[a] in the mouse tail lesion test*

Expt no.	Drug[b]	Dose (mg/kg)	Avg no. of tail lesions	P
1	None		8.70	
	Tilorone	50	7.40	>0.60
	Acranil	50	9.40	>0.70
	Tilorone	100	2.30	<0.01
	Quinacrine	100	7.00	>0.50
	Acranil	100	2.80	<0.02
	Tilorone	200	0.70	<0.001
	Quinacrine	200	2.90	<0.05
	Acranil	200	2.10	<0.01
2	None		1.40	
	Tilorone	100	0.10	<0.05
	Quinacrine	300	0.80	>0.30
	Acranil	300	0.11	<0.05

[a] The infecting dose of virus was 2.8×10^5 plaque-forming units/mouse in experiment 1 and 2.8×10^4 plaque-forming units/mouse in experiment 2.

[b] Given intragastrically in a single dose, 19 to 21 h before infection.

Examination of sera for interferon activity. The interferon activity of pooled sera of CFLP mice taken at various times after administration of tilorone, quinacrine, and Acranil is shown in Table 5. Similar kinetics of production of circulating interferon-like substance were observed after exposure to all three drugs. The levels of interferon-like activity induced by Acranil and quinacrine in repeated experiments were lower than those induced by tilorone, and Acranil proved to be a stronger inducer than quinacrine, when applied at the same doses.

In a separate experiment, pooled sera obtained from mice 8 h after administration of Acranil or tilorone were dialyzed against pH 2 buffer at 4 C for 24 h, adjusted to pH 7.2 and assayed for interferon activity. The inhibitory titers of the sera before and after exposure to acidic conditions were identical. Further tests to identify the antiviral substance present in the sera were not done.

Induction of interferon-like substance in different mouse strains. Four mouse strains were compared for induction of interferon activity after administration of tilorone and Acranil (Table 6). The level of interferon-like substance induced by either drug at 18 h was lower in Balb/c mice than in the other strains tested. No significant differences in the responses were found among the other three mouse strains. Quinacrine failed to induce detectable levels of circulating interferon-like agent in Balb/c mice assayed at 2, 5, 18, and 48 h after administration of the drug.

DISCUSSION

Our results indicate that agents with antiviral activity exist among drugs with tricyclic structures to which dialkylaminoalkyl side chains are attached. Since not all compounds with this general structure were active, more studies are necessary to reveal the precise structure-activity relationship. The two active agents detected, quanacrine and Acranil, are acridine derivatives. The antiviral activity of some acridine derivatives has been well-known for a long time, Among the acridines examined previously, only quinacrine showed a substantial in vivo effect, and vaccinia infection was not among the viral infections against which it was found to be effective (7, 8). This divergence from our results might be due to differences (i) in the severity of virus infection, (ii) in the site of infection, or (iii) in the mouse strain used. It is of interest that in a detailed study on the in vivo activity of acridines (7) the importance of the specific structure of the side chain was also emphasized. It is also noteworthy that in the quinacrine and Acranil molecules only a single side chain is present, whereas tilorone possesses two.

The antiviral effectiveness of quinacrine and Acranil may be related to their capacity to induce circulating interferon-like substance shown in the present experiments. This assumption seems to be supported by the observation that, with respect to both antivaccinia activity and the capacity to induce serum interferon-like substance, the three compounds, compared on a weight basis, fell into the following order: tilorone > Acranil > quinacrine. Furthermore, the fact that quinacrine was effective when given as much as 8 days before infection also suggests that interferon may be

involved, in view of the prolonged antivaccinia action of several interferon inducers reported by De Clerq and De Somer (2). However, other phenomena, such as an effect on the host defense mechanisms, similar to that of tilorone and congeners (12), or a direct reaction with the infecting virus (4), which might modify the antiviral activity of the acridine compounds, cannot be excluded.

Since the exact nature of the serum antiviral substance induced by Acranil and quinacrine was not determined, the serum factor was referred to as an "interferon-like substance" throughout this paper. However, the stability of the serum factor at pH 2 indicates a relationship to interferons. The dialysis at pH 2 greatly lessened the possibility that any residual Acranil in the serum samples tested could have rendered the L-cell cultures resistant to the Semliki Forest virus used as challenge. Induction of interferon by quinacrine and Acranil has not been described previously.

TABLE 4. *Effect of time of pretreatment on the activity of tilorone and quinacrine in the mouse tail lesion test*[a]

Drug[b]	Dose (mg/kg)	Time of pretreatment (day before infection)	Avg no. of tail lesions	P
None			4.87	
Tilorone	200	1 (−21 h)	0.10	<0.001
Quinacrine	330	1 (−21 h)	0.60	<0.01
Tilorone	200	8	1.30	<0.02
Quinacrine	330	8	1.10	<0.02

[a] Infecting dose: 2.8×10^6 plaque-forming units of vaccinia virus/mouse.
[b] Given intragastrically, single doses.

TABLE 5. *Titers of interferon-like substance in pooled sera of CFLP mice at various intervals after oral administration of drugs*[a]

Expt no.	Blood collection (h)	Interferon titer[b]		
		Tilorone	Quinacrine	Acranil
1	2	10	<10	40
	6	80	40	80
	18	640	80	320
	42	40	40	40
2	18	>512	ND[c]	32
	24	ND	ND	32
	42	ND	ND	8

[a] A dose of 200 mg/kg was administered of each drug.
[b] In 0.5 ml of serum.
[c] ND, not done.

TABLE 6. *Comparison of different mouse strains for induction of interferon-like substance by tilorone and Acranil*[a]

Mouse strain	Interferon titer in 0.5 ml[b]	
	Tilorone	Acranil
Balb/c	>64[c]	8
Swiss	>400	64
CFLP	>512	32
C57BL	512	64

[a] Each drug was administered orally (200 mg/kg).
[b] Sera taken 18 h after drug administration and pooled.
[c] An average titer of 128 was observed in our several previous experiments.

It was interesting to note that in the Balb/c mice lower levels of serum interferon-like substance were induced by both tilorone and Acranil than in other mouse strains. This correlates with observations made with Newcastle disease virus as an interferon inducer (3). Our results should be considered as of limited value, however, since only one of the mouse strains studied was inbred.

Quinacrine and Acranil are used clinically as antiparasitic drugs. In protozoal infections, both interferon induction (5, 6, 14) and sensitivity to either interferon inducers (10) or exogeneous interferon (9, 13, 15) has been observed. It is possible that the induction of an interferon-like substance by quinacrine and Acranil might contribute to their antiprotozoal effect. However, other antiparasitic drugs examined in the present investigation, such as chloroquine, pamaquine, and lucanthone, exhibited no detectable antivaccinia activity.

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