THE THERAPY OF EXPERIMENTAL INFLUENZA IN MICE WITH ANTIBIOTIC LACTONES AND RELATED COMPOUNDS*

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The recent progress in the chemotherapy of infectious disease is notably deficient in regard to disorders of virus etiology. It has been suggested that this may be due to the intracellular nature and the metabolic interrelations with host cells that characterize virus infection; and that these characteristics serve to make the parasite invulnerable to chemical agents which are not at the same time toxic to the host cells. Although the view is no longer widely held, effective chemotherapeutic virucidal agents are significantly lacking.[1]

Numerous agents, e.g., urea, guanidine, propylene glycol, and various detergents, are known to inactivate readily or destroy certain viruses in vitro. These substances, however, have been uniformly ineffective in the host because of toxicity, inactivation in vivo, or failure to penetrate to the intracellular location of the virus in parasitized cells. There is no evidence that these drugs prevent the spread of the virus in the host; thus they are apparently ineffective even against the organism unprotected by cell barriers.[1]

The well-known approach through metabolite antagonists has been attempted, with but limited success. Several of the sulfonamides are reported to be somewhat effective in infections caused by the psittacosis-lymphogranuloma group.[2, 15, 17] This group has been shown to be susceptible also to the action of penicillin.[11, 17] It has been considered that the effectiveness of antibacterial agents against these large viruses is further evidence of their close phylogenetic relation to larger microorganisms. As for the smaller viruses, an attempt to alter the course of experimental poliomyelitis in mice by means of antagonistic analogs of naturally occurring pyrimidines yielded only questionable results.[9]

Another approach, made by Fitzgerald et al., pointed out the antiviral (phage) effect of certain of a group of acridines (especially phosphine GRN—2-amino-9- (p-aminophenyl) acridinium chloride).[3]

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The interesting feature of these compounds was the reversal of their activity by ribose nucleic acid.[4] Since the viruses maintain the serological specificity of their nucleoproteins even within the host cells, this was apparently a means of interfering with the nucleic acid metabolism (reproduction) of the organism. Andrewes suggests that the acidic phosphate groups of nucleic acids are the primary points of attachment of the acridines.[1] In practical application, however, the acridines have been disappointing.

Another attempt to affect the virus was made through specific enzyme inhibition by the use of certain arsenicals known to be -SH enzyme inhibitors. Some success in the treatment of experimental poliomyelitis in mice has been claimed for neoarsphenamine and several other arsenicals.[10]

The most recent contribution to the field of virus chemotherapy has resulted from an unique approach by Horsfall and McCarty.[6] These investigators have demonstrated that certain polysaccharide preparations of bacterial origin, as well as similar materials from other sources, are capable of modifying the course of infection with pneumonia virus of mice.

The recent demonstration of the interesting biological specificities of the antibiotic lactones and other synthetic analogs[8, 12] prompted an investigation of the activity of these agents in experimental virus infection. In view of the previous general lack of success in the field of virus chemotherapy, it seemed worthwhile to present the results obtained with some twenty-four compounds of this series, even though the study is yet in an early stage.

Methods and materials

The PR-8 strain of influenza A was obtained from Dr. E. W. Shrigley, Department of Bacteriology, Yale University School of Medicine. We are indebted to him for advice and assistance in the use of this organism.

The virus was carried in the Webster strain of white mice. Serial transfers were made on the fifth day after infection.

The inoculum for the experimental groups of animals was obtained in the following manner. Mice which had been infected five days previously were killed with chloroform and autopsied. Lungs which showed the characteristic plum-colored appearance of infection were excised and carefully ground in sterile sand and 2 cc. of saline for a period of 10 to 15 minutes. A total of 5 cc. of saline per lung was finally added and the mixture was allowed to sediment. The supernatant fluid was removed and centrifuged at 1000 r.p.m. for 5 minutes. Again the supernatant material was removed and then stored at 4° C. until needed.

The experimental animals were all of the same sex and age, and weighed between 18 and 22 gm. Individual mice were selected at random for infection from a single pooled group. Each mouse was lightly anesthetized with ether and 0.05 cc. of a 1:50 dilution of the virus suspension were administered intranasally at a rate designed to cause a minimum of respiratory difficulty. The mice used for virus potency titration, i.e., by giving 10-fold graded dilutions of the suspension, were infected last, in order to compensate for maximum deterioration of the virus preparation.

The infected test animals were segregated in a large cage and set aside for 24 hours. At the end of this time they were withdrawn in small groups at random and given the various drug treatments.

All the compounds freely soluble in water were used in 5 per cent aqueous solutions, injected intraperitoneally. The other agents were emulsified with a cholesterol-lecithin peanut oil medium* to form a stable 5 per cent suspension, and injected subcutaneously. The various regimens of administration are recorded in table 1.

The effects were measured in two ways. First, the times of death of the controls and of the treated groups were recorded up to 21 days, after which time the surviving animals were considered cured. Mice that died within the experimental period were examined for signs of pulmonary influenza. The mean per cent surviving was calculated in each of the ten-day intervals following the time of the first death in the control groups. This provided a second measure of delay in time of death for those groups not having a significant number of final survivors.

Experimentation and results

A summary of the results is presented in table 1. The data were obtained from five separate experiments each of which entailed an arbitrary level of infection resulting from an attempt to achieve 100 per cent mortality in the control group.

After the first experiment, the compounds most promising in therapeutic effect were retested, using different routes of administration and dosage schedules with the objective of producing high and prolonged blood levels.

Unfortunately, the level of infection was not constant, thereby increasing the number of variables and causing great difficulty in calculation of statistical reproducibility, because no satisfactory error term could be ascertained. The results must therefore be taken only as indications rather than as an exact measure of activity with a definite probability of chance occurrence.

As might be expected, the efficacy of the drugs decreased with an increased level of infection. But even when more than 10,000 $LD_{50's}$

* This material was freely supplied to us by Endo Products, Inc., under the trade name "Pendil."

of the virus were given, a distinct difference in survival time could be seen with some drugs, although all the animals eventually succumbed to the massive infection. With lesser infections, however, there always was a greater number of final survivors in groups which showed an average survival time greater than that of the controls.

In seven different experimental groups treated with butyrolactone a positive therapeutic effect was always observed. The most desirable results were obtained with repeated doses of 200 mg./kg. given intraperitoneally. Subcutaneous treatment with 150 mg./kg. twice daily for 5 days also produced effective cures.

Other compounds like gamma-valerolactone, 3-phenyl-2-butene-1,4-olide, parasorbic acid, and 6-methoxy-8-(2,5-dimethylpyrryl-1)-quinoline had some therapeutic effect in several trials.

Some of the agents like tetrahydrofuran, 5-nitro-2-furfuraldehyde semicarbazone,* 3-methyl-5-carboxy-2-pentene-1,4-olide, isoclavacin, and aspergillic acid showed promise in single trials, but have not yet been rechecked.

The other furans were without effect, as were the angelica lactones and the other gamma unsaturated lactones. In some cases, such as clavacin and methyl furan, there was noticeable evidence of drug toxicity at the dose level utilized so that the apparent inactivity of these drugs is questionable.

The most inconsistent results were obtained with anemonin, the antibiotic agent from the Ranunculaceae. This material is notably unstable in vivo and is fairly toxic. The more concentrated dosage regime probably produced some toxicity.

Discussion

The inability to produce 100 per cent mortality in all controls or at least to reproduce the same level of infection in all experiments made it impossible to compute the statistical significance of the results. Repeated experiments, however, tended to substantiate the therapeutic efficacy of some of these compounds.

From the data it is not possible to draw many biochemorphic conclusions. Generally, the most outstanding trend was in that the saturated compounds seemed to be more active than were the unsaturated ones. This is quite unexpected since it contrasts sharply with the results

* This material is marketed under the trade name "Furacin" by the Eaton Laboratories, Inc.

obtained with these compounds against bacteria and protozoa.[8, 12] It is also in disagreement with Veldstra's concepts about chemical structures which confer growth inhibitory properties.[16]

Methylation seems to cause a loss of activity as may be seen by comparing gamma-valerolactone with butyrolactone and dimethyl-isoclavacin with isoclavacin.

Wherever unsaturated compounds were effective it appears that the double bond in the 2-position is much more conducive to activity than that in the 3-position. This becomes evident by noting the activity of isoclavacin and 3-methyl-5-carboxy-2-pentene-1,4-olide as compared with clavacin and 3-pentene-1,4-olide.

Again in the furan series, it becomes apparent that the 5-nitro substituent is necessary for any pronounced biological activity.

The importance of the respective chemical groups, however, cannot be confidently stated before more careful quantitative techniques are utilized.

It is of interest to note that the therapeutic effects seen here were obtained after infection had been allowed to progress to a fairly advanced stage. Under these conditions, the efficacy of Horsfall and McCarty's polysaccharides,[11] which have definite prophylactic effect, would be reduced to insignificance.

The activity of butyrolactone is also interesting in view of its ability to cause complete suppression of cortical activity as measured by the EEG[5] and to produce in mice and chickens a reversible inhibition of voluntary movements at sublethal doses.* If these phenomena can be interpreted as a type of anesthetic property, then the rather marked efficacy of butyrolactone in these influenza virus infections bears a striking relationship to the antiviral properties of anesthetic agents, like diethyl ether, recently reported by Sulkin.[13, 14]

Conclusions

1. Twenty-four compounds were tested for their chemotherapeutic properties in experimental influenza (PR-8) infection in mice.

2. Most of these agents were of the group of antibiotic lactones and their synthetic analogs.

3. Butyrolactone, 6-methoxy-8-(2, 5-dimethylpyrryl-1)-quinoline, 3-phenyl-2-butene-1,4-olide, 3-methyl-5-carboxy-2-pentene-1,4-olide,

* Unpublished data of N. J. Giarman.

parasorbic acid, and isoclavacin seemed to produce marked therapeutic effects.

4. An attempt is made to relate chemical structure to chemotherapeutic activity.

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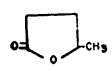
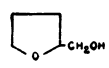
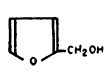
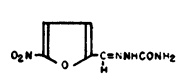
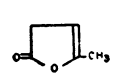
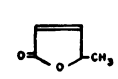
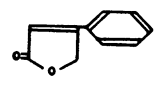
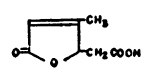
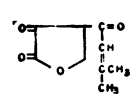
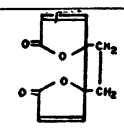
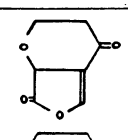
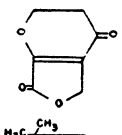
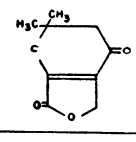
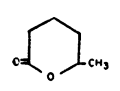
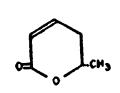
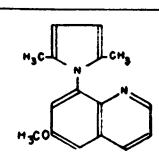
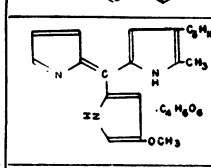
TABLE 1.

THE TREATMENT OF EXPERIMENTAL INFLUENZA IN MICE

Compound (source)	*Formula*	*Route-dose mg. per kg.*	*Duration of treatment*	*No. of mice*	*Effect, treated minus controls*		*L.D.$_{50}$ level of infection*
					% mean survivors	*% final survivors*	
γ-butyro-lactone (Cliffs-Dow)		i.p. — 400	single	8	18.0	10.0	——
		i.p. — 200	qd — 5x	7	41.9	47.1	100-1000
		i.p. — 100	tid — 15x	10	19.0	10.0	10-100
		i.p. — 100	tid — 15x	20	20.0	30.0	——
		i.p. — 150	bid — 10x	20	9.5	0	10000
		s.c. — 150	bid — 10x	20	3.3	0	10000
		s.c. — 150	bid — 10x	20	17.5	25.0	——
γ-valero-lactone (Monsanto)		i.p. — 500	single	5	30.0	30.0	——
		i.p. — 250	qd — 5x	7	18.0	18.6	100-1000
Tetrahydro-furan (DuPont)		i.p. — 100	single	5	12.0	0	10000
Tetrahydro-furfuryl alcohol (Quaker)		i.p. — 500	qd — 5x	10	2.0	-10.0	——
Methyl furan (Quaker)		s.c. — 100	single	5	-38.0	-30.0	——
Furfuryl alcohol (Quaker)		i.p. — 150	single	5	-12.0	-10.0	——
Furfural (Quaker)		i.p. — 150	single	7	-13.0	-30.0	——
5-nitro-2-furfuraldehyde semicarbazone (Eaton)		s.c. — 500	single	7	10.0	13.0	——
3-pentene 1,4-olide (Calco and Winthrop)		i.p. — 300	single	5	-28.0	-30.0	——
2-pentene 1,4-olide (Calco and Winthrop)		i.p. — 150	single	6	-8.0	-13.3	——
3-phenyl-2-butene-1,4-olide (Winthrop)		s.c. — 500	single	5	28.0	10.0	——
		s.c. — 100	qd — 5x	7	39.0	47.1	100-1000
		s.c. — 100	bid — 10x	10	7.0	10.0	10-100
3-methyl-5-carboxy-2-pentene-1,4-olide		i.p. — 100	single	5	34.0	50.0	——
		s.c. — 100	qd — 5x	7	18.0	18.6	100-1000
α-keto β(β,β' dimethylacryl) butyrolactone (Vick)		i.p. — 24	single	5	2.0	-10.0	——
Anemonin (Bergmann)		s.c. — 250	single	5	20.0	30.0	——
		s.c. — 100	qd — 5x	8	-8.0	-10.0	100-1000
Clavacin (Vick and Raistrick)		i.p. — 7.5	single	5	-46.0	-30.0	——
Isoclavacin (Vick)		i.p. — 25	single	5	12.0	10.0	——
Dimethyl-isoclavacin (Vick)		i.p. — 25	single	5	-8.0	-10.0	——
Dimethyl hydantoin (DuPont)		i.p. — 100	qd — 5x	10	5.0	0	10000
1,5-hexanolide (Calco)		i.p. — 500	qd — 5x	10	-43.0	-40.0	——
		i.p. — 250	bid — 10x	5	0	10.0	——
Parasorbic acid (Calco)		i.p. — 500	single	5	18.0	30.0	——
		s.c. — 100	qd — 5x	6	17.0	23.3	100-1000
6-methoxy-8-(2,5 dimethyl pyrryl-1) quinoline (Gilman)		s.c. — 500	single	5	36.0	50.0	——
		s.c. — 100	qd — 5x	7	37.6	33.0	100-1000
		s.c. — 100	bid — 10x	9	11.3	0	10-100
Prodigiosin tartrate		s.c. — 500	single	5	-2.0	10.0	——
Gramicidin		i.p. — 1.0	single	7	3.0	-1.4	——
Aspergillic acid		i.p. — 25	single	7	13.0	12.9	——