Br. J. Pharmac. Chemother. (1967), **31**, 19–25.

MODE OF ANTIVIRAL ACTION OF POTASSIUM BENZYLAMINOTHIOMETHANE SULPHONATE AND SOME DERIVATIVES*

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(*Received August* 6, 1965)

In our previous papers the synthesis and antiviral activity of diverse types of compounds, most of them containing the N–C–S sequence, was reported. Among the several compounds studied, potassium benzylaminothiomethanesulphonate (AB-3), its *o*-methyl derivative (AB-50) and amine-x (AB-44) were found to display marked antiviral effects. Results of experiments designed to obtain further insight into their possible mode of action are now described.

METHODS

AB-3, AB-50 and AB-44 were prepared as described earlier (Nageswara Rao, Narasimha Rao, Praphulla, Puttaswamy & Ramanathan, 1967). Egg-adapted vaccinia virus (Bangalore strain) and influenza virus PR_8 strains were used.

Micrococcus pyogenes var. *aureus,* Oxford strain, and *Candida albicans*–AB were used to study the effect of vitamins and amino acids on the inhibitory activity of the compounds, in Gale & Taylor's (1946) and Czapek-Dox sucrose media respectively.

Vitamins and amino acids were obtained commercially.

Procedures for cultivation of viruses in chick embryos and other details have been described earlier (Krishnamurthy, Nageswara Rao, Narasimha Rao & Praphulla, 1967).

RESULTS

In order to test the effect of the compounds on the infectivity of the vaccinia virus, each was mixed with the virus in a test tube and the mixture was inoculated on to the chorio-allantoic membranes, immediately or after incubation for different time intervals. AB-3 and AB-50, at a concentration of 1 mg/ml., did not prevent virus multiplication as measured by the production of good growth of the virus on chorioallantoic membranes. They reduced the effects of virus multiplication, however, affording protection to 67%,

* Based on a thesis submitted by S. Ramanathan, for the degree of Doctor of Philosophy of the Indian Institute of Science, Bangalore-12, India (1963).

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67%, 100% and 100% of the treated embryos when the virus-compound mixture was administered immediately or after incubation for 0.5, 1 and 2 hr. On the other hand, in a similar experiment, amine-x (concn: 2 mg/ml.), when administered at the same time intervals, produced survival rates of 100%, 100%, 83% and 83% with no visible growth on treated membranes.

To obtain more information regarding the interference by AB-50 and AB-44 with the maturation of viral components, the compounds were administered to eggs at different time intervals before or after inoculation with the virus. AB-50, at a dosage level of 1 mg/egg, displayed maximum inhibitory effect (83% survivors) at 2 to 3 hr after the infection. When the drug administration was delayed for 4 and 6 hr, the survival rates were 67% and 50% respectively. Significant antiviral activity (50% survivors) was observed when administered 1 to 6 hr before the infection. These results suggest interference with invasion or, more probably, with some process in the eclipse phase.

TABLE 1

EFFECT OF PRIOR EXPOSURE OF INFLUENZA PR_8 VIRUS TO AB-50 AND AB-3 FOR VARYING PERIODS ON HAEMAGGLUTININ PRODUCTION

Compound	Period of contact (hr.)	Mean haemagglutination titre
AB-50	0	128
(Concn. 1 mg/ml.)	0·5	128
	1·0	64
	2·0	64
	12·0	64
	24·0	64
Control (without the substance)		128
AB-3	0	4
	0·5	4
	1·0	0
	2·0	0
Control (without the substance)		256

TABLE 2

EFFECT OF AB-50 AND AB-3 ON HAEMAGGLUTININ PRODUCTION BY THE INFLUENZA PR_8 VIRUS IN EGGS WHEN ADMINISTERED BEFORE OR AFTER INFECTION

Time interval (hr)	Mean haemagglutination titre	
	Before infection	After infection
AB-50 (1 mg/egg)		
0·5	64	4
1·0	64	32
2·0	32	64
6·0	16	256
12·0	0	256
24·0	0	256
AB-3 (0·5 mg/egg)		
0·5	64	8
1·0	64	8
2·0	64	32
6·0	24	48
12·0	0	128
24·0	0	256
Virus control		256

AB-44 (2 mg/egg) produced a survival rate of 50% when administered at different time intervals up to 24 hr before the infection and showed maximum inhibitory effect (75% survivors) up to 12 hr in the post-infection period. No visible growth was observed on the membranes of the survivors. The effect of prior exposure of influenza PR_8 to AB-50 on haemagglutinin production has been studied (Table 1). At comparable levels the effects produced by AB-50 are less striking than those of AB-3.

While the two compounds, AB-3 and AB-50, exert their action when given within about 2 hr after infection (eclipse phase) their striking prophylactic activity is displayed when administered 12 hr or more earlier (Table 2).

Effect of B group vitamins on the growth inhibition of influenza PR_8 virus by AB-3 and AB-50

It has been demonstrated earlier that aneurine decreased the inhibitory activity of AB-3, when added 1 hr after inoculation and administration of AB-3, while the other members of the B group vitamins are devoid of any effect. The growth inhibitory effect of AB-50 is likewise decreased by the addition of aneurine. The results are presented in Table 3. Lowering of antistaphylococcal activity of AB-3 by aneurine, but not by pyridoxal, observed by Kurup and Narasimha Rao (1954), is thus reproduced with both

TABLE 3

EFFECT ON ANEURINE ON THE GROWTH INHIBITION OF INFLUENZA PR_8 VIRUS BY AB-3 AND AB-50

Compound	Time interval between administration of virus-compound and aneurine (1 mg/egg)	Mean haemagglutination titre of the virus recovered from eggs treated with aneurine
AB-50 (1 mg/egg)	0	32
	1 hr before	256
	1 hr after	128
Control (virus AB-50)		16
AB-3 (0·5 mg/egg)	0	32
	1 hr before	512
	1 hr after	32
Control (virus AB-3)		4
Virus controls		256

AB-3 and AB-50 in these antiviral effects. Like pyridoxal, glutamic acid, earlier reported to decrease antistaphylococcal activity of AB-3, as well as other amino acids failed to influence the anti-influenza activity of the compounds in eggs.

Since a qualitative correlation of the antibacterial and antiviral effects was manifested in earlier experiments, the two compounds have been assayed for their antibacterial and antifungal activities. The effects of the addition of the B group of vitamins and L-glutamic acid on their activities have been presently studied and the results are incorporated in Table 4. Only aneurine and pyridoxine at 50 μg/ml. level decreased the activities to half to one-fifth. The dependence of the bacterium on exogenous glutamic acid and freedom of the yeast from this limitation are apparently reflected in the decrease of bacterial inhibition in the presence of added glutamic acid, while the other amino acids exerted no effect.

TABLE 4

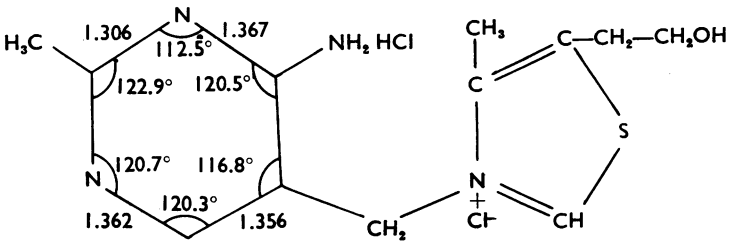
EFFECT OF ADDITION OF ANEURINE, PYRIDOXINE AND L-GLUTAMIC ACID ON THE ANTIMICROBIAL ACTIVITIES OF AB-3, AB-50 AND AB-52
(MIC values are the same for all the three compounds)

Vitamins and L-glutamic acid added (μg/ml.)	Minimal inhibitory concentration, MIC (μg/ml.)	
	M. aureus	*C. albicans*
None	10·0	10·0
Aneurine hydrochloride		
10	10·0	10·0
25	10·0	15·0
50	20·0	50·0
Pyridoxine hydrochloride		
10	10·0	10·0
25	10·0	15·0
50	10·0	20·0
Aneurine hydrochloride + pyridoxine hydrochloride (50+50)	10·0	10·0
L-Glutamic acid		
20	10·0	10·0
100	15·0	10·0
200	20·0	10·0

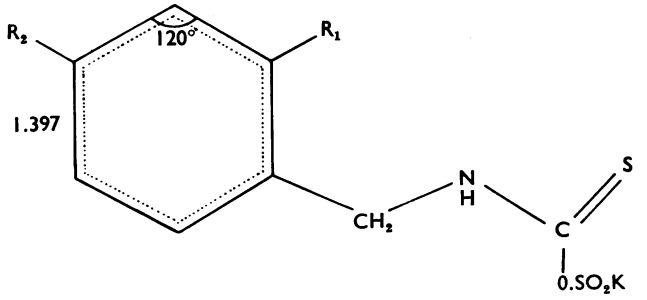
DISCUSSION

The observation that the virulence of vaccinia virus to eggs is greatly lowered, when exposed to AB-3 and AB-50, suggests interference with the formation of a mature virus component required to induce mortality in eggs. Support for this view comes from the findings that one of the minor viral antigens (fraction 2 in the scheme of Gispen's double diffusion analysis of pox virus antigens) disappears on exposure to AB-3 (Krishnamurthy, 1958). The data presented in Tables 1 and 2 suggest that the two compounds may exert activity by interference with the invasion, or, more probably, the eclipse phase of the virus. AB-44, which does not belong to this class of compounds, probably has a different mode of action.

The mechanism of action of AB-3 and the parent compound, benzyl isothiocyanate, is probably different from that of derivatives of isatin β-thiomesemicarbazone and hydroxybenzylbenzimidazole (Bauer & Sadler, 1960, 1961) which seem to involve hydrogen bond formation (O'Sullivan & Sadler, 1961). Steric, more than electrical, effects seem to influence their action (Nageswara Rao *et al.*, 1967). The specific antagonism of aneurine to these compounds in their antiviral, antibacterial and antifungal activities seems to raise the issue whether isosterism forms the basis of their action. The structural analogy of the compounds is apparent from Fig. 1. The pyrimidine moiety with the methylene bridge to the nitrogen and extending to the sulphur may be isosteric with the structural sequence of benzyl isothiocyanate and its derivatives. A methyl group in the ortho-position as in AB-50 for the amino group in the pyrimidine moiety may seem inconsequential, as both the groups possess similar molecular volume (Mautner, 1956), but the low activity of *p*-methyl compound (AB-52) and the inactivity of *o*, *p*-dimethyl compound (AB-58) cannot be readily explained on this basis. The crystal structure of aneurine hydrochloride with bond angles and inter-atomic distances elucidated by Kraut and Reed (1962) (Fig. 1) shows that the methyl and amino groups at positions 2 and 4 in the pyrimidine ring



Aneurine hydrochloride



($R_1 = R_2 = H$; AB—3)
($R_1 = CH_3$; $R_2 = H$; AB-50)
($R_1 = H$; $R_2 = CH_3$; AB-52)

Fig. 1. Isosterism of aneurine and potassium benzylaminothiomethanesulphonate derivatives, AB-3, AB-50 and AB-52.

bring it very close to a benzenoid one in the matter of bond angles and distances, causing an effective isosterism between the compounds and aneurine. Even in thiaminase systems, the benzenoid mimics the pyrimidine moiety (Kenten, 1957).

Counteraction by the addition of glutamic acid seems more complicated. As in the case of *Micrococcus pyogenes* var. *aureus*, the amino acid is required for optimum growth of influenza virus in chorioallantoic membrane tissue cultures (Veeraraghavan, 1962). It is possible that the amino acid preformed or synthesized may be rendered unavailable in optimum levels for the viral protein synthesis in presence of the inhibitors. Nevertheless, the interpretation of the antagonistic relationship between the inhibitors, aneurine and glutamic acid, in virus infected cells requires a more intimate knowledge of the influence of viral multiplication on the metabolism of the host tissue. The characteristics of the prophylactic action of the inhibitors and their effectiveness within about 3 hr following the infection seem to support the earlier views of the probable inhibition of the viral growth during the eclipse phase, thus suggesting that their probable site of action is intracellular and connected with the functions of co-carboxylase. In this context the observations of Bradley (1957) on the elevation of pyruvic oxidase activity to normal levels by the addition of co-carboxylase in mouse brain homogenates infected with neurotopic vaccinia, influenza NWS strain, and encephalomyocarditis viruses seem

significant. Further, following infections with herpes simplex, vaccinia, and swine influenza viruses, the glycolytic and pentose cycle enzymes apparently operate more actively, resulting in greater lactic acid levels in chorioallantoic membranes (Kun, Ayling & Siegel, 1960). That the pyruvate metabolism of the membranes infected with influenza A undergoes modifications has been described by Wielgosz (1957). These observations, no doubt, indicate the significance of co-carboxylase in the metabolism of the infected membranes and it is quite possible that the normal pathway at this focus is altered by infection and further influenced by the inhibitors. Efforts are now being made to obtain a clear insight into these aspects.

SUMMARY

1. The antiviral action of potassium benzylaminothiomethanesulphonate (AB-3) and of its *o*-methyl derivative, AB-50, against vaccinia virus in eggs apparently differs from that of AB-44. AB-50 acts best when administered at 2 to 3 hr after the infection. On the other hand, AB-44 maintains high activity as long as 12 hr after the infection and is curative in that it inhibits the multiplication of the virus. All the compounds show some prophylactic effect.

2. AB-3 and AB-50 possess marked prophylactic effect against influenza PR_8 virus when administered 12 to 24 hr before the infection.

3. AB-3 and AB-50 seem to exert their antiviral effects by interference during the eclipse phase.

4. While the action of AB-44 is not affected, that of AB-50, like AB-3, is antagonized by added aneurine.

5. The results indicate that apart from liberation of free isothiocyanate *in vivo*, isosterism of AB-3, AB-50 and their derivatives with aneurine is a basic factor in the expression of their activity.

The authors thank Dr. P. L. Puttaswamy and Mr. B. Hanumiah for their co-operation and the authorities of the Indian Institute of Science, Bangalore, for the award of a research fellowship to S.R.

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