ANTIVIRAL ACTIVITY OF RIFAMYCINS AND N-AMINOPIPERAZINES

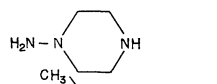
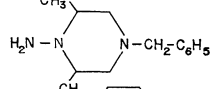
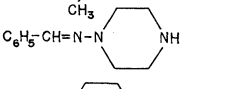
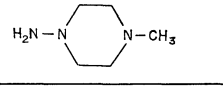
Sir:

Rifampicin (Table 2, compound 7), a semisynthetic antibiotic of the rifamycin family orally effective against tuberculosis and other bacterial infections, has been shown to inhibit the replication of pox viruses in cell cultures[1,2]. Studies on its mode of action and the isolation of resistant virus mutants indicate that the antibiotic acts on the formation or function of some viral protein[3~7]. However several other rifamycins possessing antibacterial activities similar to that of rifampicin were found not to have antiviral properties[2,8]. This suggested that vaccinia growth inhibition could be connected with the 4-methylpiperazinyl-1-iminomethyl side chain in position 3 of the rifamycin molecule. In fact 1-amino-4-methylpiperazine (Table 1, compound 4) itself as well as its benzylidene derivative were shown to inhibit, although at high concentration, vaccinia viruses; moreover it was shown[8] that they have a mechanism of action similar to that of rifampicin.

This suggested that other 1-aminopiperazines and their benzylidene derivatives might also produce interesting antiviral activity and that rifamycins obtained by condensing more active aminopiperazines with 3-formylrifamycin SV might be more effective inhibitors of viruses than rifampicin. A series of 1-aminopiperazines were then prepared and tested for their activity on the growth of vaccinia, herpes simplex, pseudorabies, Newcastle disease, fowl plague, Sindbis and WEE viruses. The products most active against vaccinia are shown in Table 1: these compounds (comp. 1, 2 and 3) were also active on Herpes simplex and pseudorabies viruses, which are both members of the herpes-virus group. The activity of 1-amino-4-methylpiperazine is listed for comparison. No activity was detected against the other viruses examined. The corresponding rifamycins (compounds 5 and 6) prepared[9] by condensing respectively compounds 1 and 2 with 3-formylrifamycin SV, were then examined. As predicted their antiviral activity (Table 2) was several times higher than that of rifampicin. However here an abnormal dose-response effect was observed since these compounds appear to be more active at concentrations of 1~2 μg/ml than at 20~50 μg/ml. The cause of this abnormal effect is now under investigation.

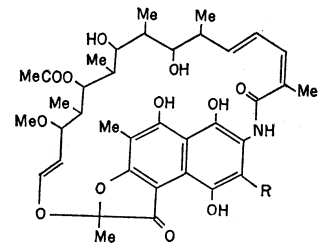
As in the case of rifampicin, we have obtained evidence[10] that the RNA polymerase activity extracted from the cytoplasm of infected cells was greatly reduced when the cells were treated with 20 μg/ml of compound 2 during infection. It is important to notice that the activity appears to be specific for viruses and that the host

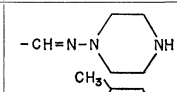
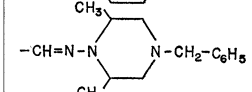
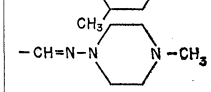
Table 1. Antiviral activity of 1 aminopiperazine

Product		Vaccinia			Herpes simplex	Pseudorabies
No.	Structure	Under agar	Under methylcellulose	Liquid medium	Under methylcellulose	Liquid medium
1		100	50	50	20	20
2		5~10	2	20	10	100
3		2~10	10	1	20	10~20
4		100~200		200	>400	>400

Figures represent drug concentrations in μg/ml which reduced to 10% the number of plaques (under agar or methylcellulose) or the virus titer (liquid medium after 48 hours of growth) with respect to controls. Vaccinia and pseudorabies viruses were grown on chick embryo fibroblasts, Herpes simplex virus type 2 on primary cultures of rabbit kidneys.

Table 2. Antiviral activity of rifamycins.



Product		Vaccinia			Herpes simplex	Pseudorabies
No.	Structure	Under agar	Under methylcellulose	Liquid medium	Under methylcellulose	Liquid medium
5		2～10	1	0.1～0.3	5～10	5
6		2～10	1～2	0.5～2	1～2	20
7	 (Rifampicin)	200	100	100	>400	>400

Conditions as described under Table 1.
Compounds 5 and 6 showed an anomalous dose response: Concentrations 10～20 times higher than that reported in the table and no effect on plaque formation or virus growth.

cells' functions are practically uneffected since after treatment with 100 μg/ml of the aminopiperazines the cells normally stained with neutral red after 4 days, allowed the normal growth of myxoviruses and arboviruses, and gave a normal response to interferon inducers[10].

It has been reported[11] that concentrations of 30 μg/ml of rifampicin substantially inhibit foci formations in cells infected with Rous Sarcomavirus. We have now found that in a similar experiment the number of foci was reduced to 15 % of the control by concentrations of 0.2 μg/ml of compound 5 and of 2 μg/ml of compound 3. Whether their action is on the virus replication or is specific for cell transformation has not yet been investigated.

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References

1) HELLER, E.; M. ARGMANN, H. LEVI & M. GOLDBLUM: Selective inhibition of vaccinia virus by the antibiotic rifampicin. Nature 222 : 273～274, 1969
2) SUBAK-SHARPE, J. H.; M. C. TIMBURY & J. F. WILLIAMS: Rifampicin inhibits the growth of some mammalian viruses. Nature 222 : 341～345, 1969
3) MCAUSLAN, B. R.: Rifampicin inhibition of of vaccinia virus replication. Biochim. Biophys. Res. Comm. 37 : 289～295, 1969
4) MOSS, B.; E. N. ROSENBLUM, E. KATZ & P. M. GRIMLEY: Rifampicin, a specific inhibitor of vaccinia virus assembly. Nature 224 : 1280～1284, 1969
5) NAGAYAMA, A.; B.G.T. POGO & S. DALES: Biogenesis of vaccinia, separation of early stages from maturation by means of rifampicin. Virology 40 : 1039～1051, 1970
6) COSTANZO, F.; L. FIUME, M. LA PLACA, A. MANNINI-PALENZONA, F. NOVELLO & F. STIRPE: Ribonucleic acid polymerase induced by vaccinia virus; lack of inhibition by rifampicin and α amanitin. J. Virology 5 : 266～269, 1970
7) KATZ, E. & B. MOSS: Formation of vaccinia virus structural polypeptide from a high molecular weight precursor: inhibition by rifampicin. Proc. Natl. Acad. Sci. 66 : 677～684, 1970

8) THIRY, L. & G. C. LANCINI: Inhibition of vaccinia virus growth by 1-methyl-4-aminopiperazine. Nature 227 : 1048～1050, 1970
9) MAGGI, N.; R. PALLANZA & P. SENSI: New derivatives of rifamycin SV. Antimicr. Agents & Chemoth. -1965 : 765～769, 1966
10) THIRY, L. & G. C. LANCINI: in preparation.
11) DIGGELMANN, H. & C. WEISSMANN: Rifampicin inhibits focus formation in chick fibroblasts infected with Rous sarcoma virus. Nature 224 : 1277～1279, 1969