AMPHOTERICIN INHIBITS CORONAVIRUS SD, SK AND A59 GROWTH

B.L. DeVald, J.C. Gerdes, J.S. Burks, and M.C. Kemp

Center for Neurological Diseases/Rocky Mountain Multiple Sclerosis Center, Veterans Administration Medical Center
University of Colorado School of Medicine
Denver, Colorado, U.S.A.

Fungizone (AMB) a clinical formulation of Amphotericin B has been widely used as an antifungal agent. The synthetically derived analogue Amphotericin B Methyl Ester (AME) has a similar antifungal activity.

The biological effects of polene macrolide antimicrobial agents (AMB, AME) depend on their high affinity for sterols in the cell membrane. Therefore, they have been shown to inhibit growth of certain enveloped viruses. Antiviral activity was not demonstrated against non-enveloped viruses (2).

In our viral isolation studies, we routinely collected human brain tissue at autopsy in cold sterile Hanks balance salt solution (HBSS) with double strength penicillin and streptomycin. To decrease the potential of fungal contamination, we began adding AMB at 25 mcg/ml (the manufacturers recommended concentration) to HBSS at the time of collection of brain at autopsy. We have never added AMB or AME to our tissue culture. Before we began adding AMB to HBSS, coronaviruses were isolated while working with human tissue in 2 of 4 autopsies. After adding AMB to the isolation protocol, 20 consecutive autopsies on MS patients were negative for viral isolation. The possibility of inhibition of coronavirus growth by AMB or AME was explored in this study.

Coronaviruses SD, SK and A59 (200 pfu/ml) were mixed with AMB or AME in equal volumes of media with or without fetal calf serum. The antifungal agents were diluted to a final concentrations of 1, 10, 25, 50 or 100 mcg/ml. All mixtures of virus and antifungal agents were assayed in triplicate. A 50% reduction in the number of

virus plaques by the antifungal agents was considered to be the minimal inhibitory concentration (MIC).

Using AMB and AME in the tissue culture system, inhibition of all three coronaviruses was noted when the cultures were incubated with serum free medium (Table 1). The addition of 2% fetal calf serum, eliminated inhibition of SK and A59. However, SD growth was still inhibited at MIC of 10 mcg/ml.

Since fungal contamination is an inherent problem associated with primary viral isolation studies using autopsy material, the choice and concentration of AMB or AME must be considered. As we have demonstrated, AME and AMB inhibit coronavirus SD, SK and A59 multiplication. Therefore, we have eliminated these agents from our autopsy tissue procurement procedure.

TABLE I
MINIMAL INHIBITORY CONCENTRATION

VIRUS	% SERUM	AMPHOTERICIN B	AMPHOTERICIN B METHYLESTER
A59	0	100mcg/ml	25mcg/ml
SD	0	1mcg/ml	1mcg/ml
SK	0	1mcg/ml	1mcg/ml
A59	2	No Inhibition	No Inhibition
SD	2	No Inhibition	10mcg/ml
SK	2	No Inhibition	No Inhibition
SD	10		No Inhibition

REFERENCES

1. Bonner, D.P., W. Mechliniski and C.P. Schaffner. 1972 Polyene Macrolide Derivatives I-III. J. Antibio. XXV: 256-262.
2. Stevens, N.M., C.G. Engle, P.B. Fisher, W. Mechliniski, and C.P. Schaffner. 1975. In Vitro Antiherpetic Activity of Water Soluble Amphotericin B Methyl Ester. Arch. Virol. 48:391-394.

This work was supported by the Kroc Foundation and the Veterans Administration.