

EVALUATION OF THE EFFICACY OF SELECTED ANTIVIRAL DRUGS IN JAPANESE ENCEPHALITIS VIRUS INFECTION

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OBJECTIVE : To develop an effective antiviral drug for the treatment of Japanese encephalitis.

BACKGROUND : Japanese encephalitis virus (JEV) is endemic in many parts of Southeast and East Asia with case fatality rates between 10 and 90 per cent. In addition, large epidemics have been reported in Taiwan, Thailand, Korea, and India since WW II. JEV infection is therefore a serious threat to local populations and military forces deployed anywhere in this region. Recent progress in rapid diagnosis of the subpopulation of JEV patients at highest risk makes the use of an effective antiviral drug more attractive. We are therefore screening likely candidate drugs for their in vitro and in vivo efficacy. Ribavirin has been shown to be an effective inhibitor of Dengue virus replication in vitro in studies performed at WRAIR. Conflicting reports exist in the literature as to its efficacy in treating JEV and other flavivirus infections. Isoprinosine is an antiviral drug with reported immunopotentiating properties, which has not yet been tested against flavivirus infections.

METHODS : Confluent LLC-MK 2 monolayers were treated with various concentrations of isoprinosine in regular growth media. After 24 hours, this media was removed and the cells were adsorbed with JEV at an MOI of 1.0. After one hr the virus was removed and the cells were washed with three changes of Hanks Balanced Salt Solution. The washing solution was then removed and replaced with growth media containing drug. Small samples were harvested twice a day for 7 days and held at -70°C until assay by plaque formation on LLC-MK2 cells at 35°C . Ribavirin treated cells were treated in the same manner.

IgG anti-dengue antibody capture radioimmunoassays were performed on supernatants of dengue hemorrhagic fever PBLs as described elsewhere in this report. Briefly, microtiter plates were sensitized with goat antihuman gamma chain, tissue culture supernatants were incubated in the microtiter plates, followed by dengue antigen and ^{125}I -labeled hyperimmune human anti-dengue IgG. The PBL were separated from patient plasma by Ficoll-Paque gradient centrifugation and washed seven times before incubation in tissue culture media. The values in Table 2 represent the amount of IgG anti-dengue synthesized during 72 hr of culture minus the amount of IgG anti-dengue present in the last 0 hr wash.

RESULTS : As a preliminary to animal studies isoprinosine has been tested in vitro for its ability to inhibit JEV replication in LLC-MK2 cells. As anticipated there was no significant inhibition of JEV replication in LLC-MK2 cell monolayers which were pretreated for 24 hr before infection and continuously during infection with a range of drug concentration from 0.1 -

300 ug/ml (Table 1). This result was as expected since isoprinosine has been reported to act as an immunopotentiator. Isoprinosine (100 ug/ml) also failed to cause any stimulation of flavivirus - specific IgG or IgM synthesis by dengue hemorrhagic fever patients peripheral blood lymphocytes (PBL) in culture as measured by IgG antibody capture radioimmunoassays (Table 2). This negative result could result from using already stimulated B cells or plasma cells in the assay. Presumably isoprinosine works at the level of T cells and would be ineffective when added to already committed B cells. Definitive in vivo studies are necessary before abandoning isoprinosine as a treatment for JEV infections.

Ribavirin, which has been previously shown to inhibit dengue replication with a minimal inhibitory concentration (MIC) of 10 ug/ml, was tested against JEV replication. Significant inhibition of JEV replication in LLC-Mk2 monolayers resulted, with a MIC of 30 ug/ml (Figure 1). Maximal virus titers were reduced from 10^7 pfu/ml to less than 4×10^4 pfu/ml in the presence of a high therapeutic dose (100 ug/ml). Error bars show one standard deviation although ribavirin significantly depresses JEV replication, it does not completely inhibit replication even at 100 ug/ml. Therefore JEV is more refractile to the effects of the drug than dengue virus.

Table 1. Virus replication in the presence of isoprinosine

DRUG CONCENTRATION ug/ml	TITER (pfu/ml)	
	DAY 0	DAY 2 (Peak titer)
0	9.7×10^3	2.0×10^7
0.1	5.7×10^3	1.0×10^7
1.0	4.2×10^3	1.2×10^7
10	7.2×10^3	2.2×10^7
30	9.3×10^3	1.2×10^7
100	7.2×10^3	1.6×10^7
300	1.1×10^3	1.8×10^7

Table 2. IgG anti-dengue antibody synthesized by PBL from dengue hemorrhagic fever patient D81-066.

	DAY 1 (DAY OF ADMISSION)	DAY 7	DAY 15
No. Isoprinosine	12,100	1,360	1,120
100 ug/ml Isoprinosine	11,300	2,730	570

Values recorded are (IgG anti-dengue antibody present at 72 hr). Minus (IgG anti-dengue antibody present at 0 hr).

Fig. 1. JEV Replication in the Presence of Ribavirin.

