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Short communication

Interferon, ribavirin, 6-azauridine and glycyrrhizin: antiviral compounds active against pathogenic flaviviruses

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Abstract

Ribavirin, interferon-α (IFN-α), 6-azauridine and glycyrrhizin were tested in vitro for their antiviral activities against 11 pathogenic flaviviruses belonging to principal antigenic complexes or individual serogroups of medical importance: dengue, Japanese encephalitis, mammalian tick-borne and yellow fever virus (YFV) groups. Antiviral activity was estimated by the reduction of the cytopathic effect of each flavivirus in Vero cells and by the reduction in virus titer. Cytotoxicity was evaluated by determining the inhibition of Trypan blue exclusion in confluent cell cultures and by the evaluation of the inhibitory effect on cell growth. The specificity of action of each tested compound was estimated by the selectivity index (CC_{50}/EC_{50}). IFN-α proved to be a selective and potent inhibitor of the replication of the 11 tested pathogenic flaviviruses. Ribavirin and 6-azauridine proved to be active on the replication of the 11 tested pathogenic flaviviruses at the concentrations which did not alter normal cell morphology, but they were not selective inhibitors when selectivity indices were evaluated with regard to the inhibition of cell growth because of their cytostatic effect. Glycyrrhizin inhibited the replication of flaviviruses at high non-cytotoxic concentrations. These antiflavivirus compounds should be further evaluated for their efficacy in the treatment of flavivirus infections in vivo.

Keywords: Flavivirus; Ribavirin; Interferon; 6-Azauridine; Glycyrrhizin; Antiviral

1. Introduction

Flaviviruses are mainly arthropod-transmitted viruses that belong to the Flaviviridae family. The genus *Flavivirus* includes more than 70 single-stranded positive RNA viruses sharing common antigenic determinants, and the group is divided into eight serosubgroups (antigenic complexes) and nine individual serotypes (Monath and Heinz, 1996). Flaviviruses are responsible for considerable morbidity and mortality and may cause severe encephalitic, haemorrhage, hepatic, and febrile illnesses in vertebrates, including humans. Viruses in this genus include the etiologic agents of yellow fever, dengue, Japanese encephalitis, tick-borne encephalitis, West Nile encephalitis, Murray Valley encephalitis and St. Louis encephalitis.

Even with a safe efficient vaccine against yellow fever virus (YFV) at hand, the incidence of the disease has dramatically increased in Africa in recent years, involving thousands of cases a year with a 50% death-rate (Monath, 1987).

Mosquito-borne dengue viruses (DENV) are responsible for an important public health problem caused, among other things, by the failure to maintain programs for controlling the mosquito vector, *Aedes aegypti*. It is estimated that up to 100 million cases of dengue and at least 500,000 cases of dengue haemorrhage fever with a 5% mortality rate, occur annually world-wide (Gubler, 1998; Monath and Heinz, 1996). It is estimated that DENV threatens up to 2 billion people in tropical areas and is continuously emerging throughout the world.

Japanese encephalitis virus (JEV), the third flavivirus of medical importance, is widely distributed in Asia. This mosquito-borne virus is the principal cause of viral encephalitis world-wide. Approximately 35,000 cases and 10,000 deaths are reported annually throughout Asia. The case/fatality rate is high (30%) and neuropsychiatric sequelae, which occur in 30% of survivors (Kalita and Misra, 1998; Misra et al., 1998), are particularly severe in children (Kumar et al., 1993).

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Other flaviviruses cause encephalitis with high mortality rates or neurological sequelae. Tick-borne encephalitis viruses (TBEV) belonging to the mammalian tick-borne virus group, are the most important group of arboviruses in Europe in terms of morbidity and mortality rate. The far eastern subtype of TBEV causes severe encephalitis among humans with a mortality of about 30% and neurologic sequelae in 30–60% of the survivors. The European strains are less virulent with a case-fatality rate of 1–2% but with long-lasting or permanent neuropsychiatric sequelae in some survivors (Heinz and Mandl, 1993; Dumpis et al., 1999). West Nile virus (WNV) is endemic principally in Africa, the Middle East and around the Mediterranean sea but this virus also causes some epidemics in other regions of the world. In 1996 an epidemic of 393 cases of West Nile meningoencephalitis, 17 of which with fatal outcome, occurred in Romania (Han et al., 1999). More recently, in August 1999, an outbreak of 77 cases of encephalitis caused by WNV with seven deaths was reported in New York City (Briese et al., 1999). St. Louis encephalitis virus in the United States and Murray Valley encephalitis virus in Australia are other flaviviruses also responsible for encephalitis with high case/mortality rates (Monath and Heinz, 1996). Omsk haemorrhage fever virus and Kyasanur Forest disease virus cause severe haemorrhage fever in the Omsk region in Russia and in India, respectively.

Despite the considerable impact of the pathogenic flaviviruses on human health, there is no effective antiviral therapy. However, several antiviral compounds such as ribavirin and interferon (IFN) have proved to be active in vitro against the replication of YFV, DENV and JEV (Neyts et al., 1996; Leyssen et al., 2000). In a preliminary work carried out in our laboratory, antiviral compounds were screened in vitro for their effects on various pathogenic flaviviruses. Besides ribavirin and interferon-α (IFN-α), two other compounds, 6-azauridine and glycyrrhizin, were selected and tested for their antiviral action against different flaviviruses. In the present study these four antiviral compounds were further tested for their inhibitory effect on the replication of 11 different flaviviruses in cell culture.

2. Materials and methods

2.1. Viruses

For this study we used dengue virus type 1 (DENV-1) (strain Hawaii), dengue virus type 2 (DENV-2) (strain NGC), and dengue virus type 4 (DENV-4) (strain YUNH), JEV (strain Nakayama), the strains 17D and FNV of YFV, WNV (strain E101), Wesselsbron virus (WESSV), Zika virus (ZIKV) (strain Dak Ar B 11514), Usutu virus (USUV) (strain Dak Ar D 19848) and Langat virus (LGTV). The strains of DENV-1, USUV, ZIKV and the strain FNV of YFV were provided by Dr. Jean-Pierre Digoutte of Institut Pasteur de Dakar, Senegal. The strains of DENV-2, DENV-4, JEV, WNV, LGTV and WESSV were provided by Dr. Vincent Deubel of Institut Pasteur de Paris, France. The nature and the purity of each virus were confirmed by genome amplification and sequencing (Scaramozzino et al., 2001). All the viruses of this study were handled under biosafety level 3 containment at the Centre de Recherches du Service de Santé des Armées (La Tronche, France). Each virus was adapted to Vero cells by serial passages. A stock virus was prepared in Vero cells infected at an appropriate multiplicity of infection (MOI) and incubated at 37 °C until the beginning of the cythopathic effect (CPE). Then the supernatant was harvested and clarified using low speed centrifugation. Virus titres were determined by the 50% tissue infective dose ($TCID_{50}$) method in Vero cell cultures (Reed and Muench, 1938; Crance et al., 1997).

2.2. Cells

Vero cells (ATCC CCL81) were grown at 37 °C in 5% CO_2 in 199 medium (M199) supplemented with 5% heat-inactivated foetal calf serum (FCS), 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Virus-infected cells were maintained at 37 °C in M199 supplemented with 2% FCS.

2.3. Antiviral compounds

Ribavirin, 6-azauridine and glycyrrhizin were purchased from Sigma-Aldrich Chimie SARL (L'Isle d'Abeau, France). Human recombinant IFN-α, IFN-α2b was purchased from Schering-Plough (Levallois-Perret, France).

2.4. Inhibition of virus-induced cytopathogenicity

Vero cells in 96-well tissue culture plates were used when confluent. Culture medium was removed and the cells were washed with M199. Then, 0.1 ml of diluted virus suspension and 0.1 ml of medium supplemented with 2% FCS containing an appropriate concentration of the antiviral compound were added. Eight concentrations were tested for each inhibitor. It was a two-fold dilution scheme for ribavirin, 6-azauridine and glycyrrhizin and a ten-fold dilution scheme for IFN-α. Eight wells were used for each concentration of the test compounds. Eight wells were used as virus controls (virus-infected non-drug-treated cells) and eight wells were used as cell controls (non-infected non-drug-treated cells). The culture plates were incubated at 37 °C in 5% CO_2 for 4 or 7 days until maximum CPE was obtained (4-day incubation with YFV (17D), YFV (FNV), WNV, LGTV, WESSV, ZIKV and USUV; 7-day incubation with DENV-1, DENV-2, DENV-4 and JEV). For a 7-day incubation, each medium containing the antiviral compound at the appropriate concentration was renewed at day 3 post-infection. After the appropriate duration of incubation the CPE of each virus was recorded. Antiviral activity was expressed as 50% effective concentration (EC_{50}),

i.e. the concentration of compound required to inhibit the cytopathic effect to 50% of the control value.

2.5. Cytotoxicity of the antiviral compounds

Vero cells grown to confluence in 24-well plates were exposed to different concentrations of the antiviral compounds (four wells per compound concentration) in maintenance medium for 4 or 7 days at 37 °C, in parallel with the virus-infected cell cultures. For each antiviral compound, four wells were used as controls (non-drug-treated cells). After 4 or 7 days of incubation, cytotoxicity was evaluated by the Trypan blue exclusion test as previously described (Crance et al., 1990). The concentration of antiviral compound that reduced the viability of Vero cells to 50% of the control was estimated as the 50% cytotoxic concentration (CC_{50}).

The inhibitory effect of the compounds on cellular proliferation was also studied: the cells were seeded at a rate of 2×10^4 cells per well in a volume of 1.0 ml into 24-well tissue plates and allowed to proliferate for 24 h in M199, containing 5% FCS. The next day, 1.0 ml of medium containing two-fold increasing concentrations of the test compounds were added (four wells/concentration). After 3 days of incubation at 37 °C in 5% CO_2, medium was aspirated from the wells and cells were removed from monolayer culture by incubation with 0.5 ml of trypsin–EDTA in normal saline for 5 min at 37 °C. After addition of 0.5 ml of FCS, 0.05 ml of 2.5% Trypan blue was added to the cell suspension. Cell number and viability were determined by counting the cells in presence of Trypan blue. The concentration of compound that reduced cell growth by 50% was estimated as the 50% cytotoxic concentration (CC_{50}).

2.6. Inhibitory effect of compounds on flavivirus yield in infected cells

When the Vero cells in 24-well tissue culture plates were confluent, the culture medium was removed, and then 2 ml of maintenance medium containing the antiviral compound at an appropriate concentration and 0.1 ml of diluted virus suspension were added (four wells per concentration). Five concentrations were tested for each inhibitor. It was a two-fold dilution scheme for ribavirin, 6-azauridine and glycyrrhizin and a ten-fold dilution scheme for IFN-α. For each virus and each compound, four wells were used as controls (virus-infected non-drug-treated cells). After a 37 °C incubation period ranging from 28 to 96 h (28 h with WESSV; 40 h with YFV (FNV) and YFV (17D); 50 h with WNV, LGTV and ZIKV; 68 h with JEV and DENV-4; 96 h with DENV-1 and DENV-2), when a maximum virus titre was reached in cell controls at the beginning of CPE appearance, the maintenance medium was removed, cells were washed and 1.0 ml of M199 was added. The virus was extracted by freezing and thawing. The homogenates were used for virus titre determination in cell culture.

2.7. Statistical analysis

Statistical analysis of the data was carried out using Student's *t*-test and one-way ANOVA.

3. Results

3.1. Selectivity of IFN-α, ribavirin, 6-azauridine and glycyrrhizin as inhibitors of different flaviviruses

The cytotoxicity of the compounds for Vero cells was determined either on confluent cells or on exponentially growing cells. The 50% cytotoxic concentration (CC_{50}) was both >10,000 IU ml^{-1} for IFN-α and >8000 and 58 μg ml^{-1}, respectively for ribavirin; >50 and 0.9 μg ml^{-1}, respectively for 6-azauridine; and >3000 and >2500 μg ml^{-1}, respectively for glycyrrhizin.

When selectivity indices were calculated as the ratio of the CC_{50} for cell viability in monolayer cell cultures to the average EC_{50} (CC_{50}/EC_{50}), recombinant IFN-α, ribavirin, 6-azauridine showed selectivity indices of ≥33 (Tables 1–3). Glycyrrhizin exhibited the lowest selectivity index, but this compound always inhibited the viral cytopathogenicity induced by the different flaviviruses at a concentration below the cytotoxicity threshold (Table 4). Recombinant IFN-α showed the highest selectivity index (>291) for all the pathogenic flaviviruses tested (Table 1). Ribavirin and 6-azauridine also proved to be effective as inhibitors of the viral cytopathogenicity induced by these different viruses: their selectivity indices were >55 and >33, respectively (Tables 2 and 3). Glycyrrhizin had a selectivity index >4 (Table 4).

When selectivity indices were calculated on the basis of the ratio of the 50% cytotoxic concentration (CC_{50}) for cell growth to the average EC_{50} (CC_{50}/EC_{50}), ribavirin and 6-azauridine exhibited lower selectivity indices because of their cytostatic effects (Tables 2 and 3). These compounds had a marked inhibitory effect on the growth of uninfected cells, although they did not affect normal cell morphology. Glycyrrhizin showed selectivity indices ≥4 (Table 4) and IFN-α proved to be the most potent inhibitor of the replication of all tested flaviviruses (Table 1). The selectivity index of IFN-α calculated for all flaviviruses was >291 (Table 1).

3.2. Inhibitory effects of IFN-α, ribavirin, 6-azauridine and glycyrrhizin on flavivirus yield in infected cells

Recombinant IFN-α, ribavirin, 6-azauridine and glycyrrhizin were further studied for their inhibitory effect on replication of the flaviviruses in Vero cells. For each compound the virus titre reduction was determined at different non-cytotoxic concentrations below the maximum tolerated concentration. The EC_{90} (90% effective concentration) was determined from the dose–response curves.

Table 1
Interferon-α: cytotoxicity, inhibition of virus-induced cytopathogenicity, inhibition of virus yield and specificity of action of the compound as inhibitor of different pathogenic flaviviruses

Virus	EC_{50}[a] (IU ml^{-1})	EC_{90}[b] (IU ml^{-1})	Selectivity index[c]	
			Based on CC_{50} for confluent cells[d]	Based on CC_{50} for growing cells[e]
DENV-1	15.8	1.0	>632	>632
DENV-2	16.9	200	>591	>591
DENV-4	10.6	30	>943	>943
JEV	4.8	10	>2083	>2083
WNV	5.9	10	>1694	>1694
USUV	3.9	10	>2564	>2564
LGTV	5.5	50	>1818	>1818
YFV (17D)	5.2	80	>1923	>1923
YFV (FNV)	7.8	60	>1282	>1282
WESSV	11.7	200	>854	>854
ZIKV	34.3	30	>291	>291

The CC_{50} values on confluent cells was >10,000 IU ml^{-1}. The CC_{50} values on exponentially growing cells was >10,000 IU ml^{-1}. The data represent average values for three experiments.

[a] EC_{50} is effective concentration required to reduce virus-induced cytopathogenicity by 50%.

[b] EC_{90} is 90% effective concentration for inhibition of virus yield.

[c] Selectivity index (CC_{50} divided by EC_{50}).

[d] Calculated from confluent cells. CC_{50} > 10,000 IU ml^{-1}.

[e] Calculated from exponentially growing cells. CC_{50} > 10,000 IU ml^{-1}.

Recombinant IFN-α proved to be effective as inhibitor of replication of all tested flaviviruses (Table 1). For DENV-2 and WESSV, the EC_{90} value was 200 IU ml^{-1}, which is a concentration more than 50 times lower than the cytotoxicity threshold. For the other flaviviruses, an IFN-α concentration of 100 IU ml^{-1} decreased virus yield by over 90%.

Ribavirin showed an antiviral activity higher than 90% at the concentration of 250 μg ml^{-1} (Table 2). At the concentration of 125 μg ml^{-1}, ribavirin still significantly inhibited (*t*-test; $P < 0.05$) the virus titre for each virus (data not shown).

The EC_{90} of 6-azauridine was <5 μg ml^{-1} for all the flaviviruses tested (Table 3). At the concentration of 2.5 μg ml^{-1} (i.e. more than 20 times lower than the cytotoxicity threshold in quiescent cell cultures) this compound still exhibited a significant inhibition ($\geq 0.7 \log_{10}$) of the replication of each flavivirus (*t*-test; $P < 0.05$) (data not shown).

The EC_{90} of glycyrrhizin was ≤633 μg ml^{-1} for all the flaviviruses tested (Table 4). This concentration was four times lower than the inhibitory concentration for cell growth.

Table 2
Ribavirin: cytotoxicity, inhibition of virus-induced cytopathogenicity, inhibition of virus yield and specificity of action of the compound as inhibitor of different pathogenic flaviviruses

Virus	EC_{50}[a] (μg ml^{-1})	EC_{90}[b] (μg ml^{-1})	Selectivity index[c]	
			Based on CC_{50} for confluent cells[d]	Based on CC_{50} for growing cells[e]
DENV-1	19.8	<62.5	>404	3
DENV-2	41.9	87	>190	1
DENV-4	36.5	112	>219	2
JEV	134.1	<62.5	>59	0.4
WNV	71.2	159	>112	1
USUV	62.6	79	>127	1
LGTV	33.9	<62.5	>235	2
YFV (17D)	42.4	174	>188	1
YFV (FNV)	48.2	68	>165	1
WESSV	91.7	160	>87	1
ZIKV	142.9	<62.5	>55	0.4

The data represent average values for three experiments.

[a] EC_{50} is effective concentration required to reduce virus-induced cytopathogenicity by 50%.

[b] EC_{90} is 90% effective concentration for inhibition of virus yield.

[c] Selectivity index (CC_{50} divided by EC_{50}).

[d] Calculated from confluent cells. CC_{50} > 8000 μg ml^{-1}.

[e] Calculated from exponentially growing cells. CC_{50} = 58 μg ml^{-1}.

Table 3
6-Azauridine: cytotoxicity, inhibition of virus-induced cytopathogenicity, inhibition of virus yield and specificity of action of the compound as inhibitor of different pathogenic flaviviruses

Virus	EC_{50}[a] ($\mu g\,ml^{-1}$)	EC_{90}[b] ($\mu g\,ml^{-1}$)	Selectivity index[c]	
			Based on CC_{50} for confluent cells[d]	Based on CC_{50} for growing cells[e]
DENV-1	0.5	3.3	>100	2
DENV-2	0.2	2.1	>250	5
DENV-4	0.1	<0.3	>500	9
JEV	0.5	0.8	>100	2
WNV	0.2	3.2	>250	5
USUV	0.1	0.3	>500	9
LGTV	0.2	<0.3	>250	5
YFV (17D)	0.2	1.3	>250	5
YFV (FNV)	0.2	0.9	>250	5
WESSV	1.3	2.5	>38	1
ZIKV	1.5	0.3	>33	1

The data represent average values for three experiments.
[a] EC_{50} is effective concentration required to reduce virus-induced cytopathogenicity by 50%.
[b] EC_{90} is 90% effective concentration for inhibition of virus yield.
[c] Selectivity index (CC_{50} divided by EC_{50}).
[d] Calculated from confluent cells. $CC_{50} > 50\,\mu g\,ml^{-1}$.
[e] Calculated from exponentially growing cells. $CC_{50} = 0.9\,\mu g\,ml^{-1}$.

Table 4
Glycyrrhizin: cytotoxicity, inhibition of virus-induced cytopathogenicity, inhibition of virus yield and specificity of action of the compound as inhibitor of different pathogenic flaviviruses

Virus	EC_{50}[a] ($\mu g\,ml^{-1}$)	EC_{90}[b] ($\mu g\,ml^{-1}$)	Selectivity index[c]	
			Based on CC_{50} for confluent cells[d]	Based on CC_{50} for growing cells[e]
DENV-1	450.0	316	>6	6
DENV-2	174.2	317	>6	5
DENV-4	632.7	416	>4	4
JEV	383.7	<156	>7	7
WNV	228.9	625	>13	11
USUV	293.4	315	>10	9
LGTV	465.3	343	>6	4
YFV (17D)	450.0	530	>6	6
YFV (FNV)	474.2	429	>6	5
WESSV	632.7	633	>4	4
ZIKV	383.7	<156	>7	7

The data represent average values for three experiments.
[a] EC_{50} is effective concentration required to reduce virus-induced cytopathogenicity by 50%.
[b] EC_{90} is 90% effective concentration for inhibition of virus yield.
[c] Selectivity index (CC_{50} divided by EC_{50}).
[d] Calculated from confluent cells. $CC_{50} > 3000\,\mu g\,ml^{-1}$.
[e] Calculated from exponentially growing cells. $CC_{50} = 2500\,\mu g\,ml^{-1}$.

Recombinant IFN-α, ribavirin, 6-azauridine and glycyrrhizin caused a concentration-dependent reduction in the virus yield with all tested flaviviruses (one-way ANOVA, $P < 0.05$).

4. Discussion

In this paper, we demonstrated that IFN-α, ribavirin, 6-azauridine and glycyrrhizin inhibited infection of Vero cells induced by 11 different pathogenic flaviviruses belonging to principal virus groups of medical importance: dengue, Japanese encephalitis, tick-borne encephalitis and YFV.

Based on the evaluation of its specificity of action (CC_{50}/EC_{50}), IFN-α was the most effective antiviral compound. Glycyrrhizin was only active at high non-cytotoxic concentrations. Ribavirin, and 6-azauridine showed an antiviral activity at the concentrations lower than the cytotoxic concentration in quiescent cell cultures. However, these compounds proved to be cytostatic at these concentrations. The selectivity indices of these antiviral compounds appeared to be moderately influenced by the strain of flavivirus tested.

Based on the inhibitory effect on virus yield in infected cells, IFN-α was effective against all the tested flaviviruses at relatively low concentrations. At 100 IU ml^{-1}, a concentration more than 100 times lower than the cytotoxicity threshold, IFN-α still significantly inhibited the titre of each virus tested in our study. The inhibitory effect of glycyrrhizin was observed at concentrations lower than those which inhibited cell growth. Ribavirin and 6-azauridine proved to be active against the 11 tested flaviviruses at concentrations which did not affect normal cell morphology but had a marked inhibitory effect on the growth of uninfected cells.

6-Azauridine was active against YFV replication in cell culture (Neyts et al., 1996). 6-Azauridine triacetate has already been used therapeutically in a wide range of diseases including severe forms of psoriasis (Deneau and Farber, 1975). This drug, which can be used at high doses (up to 200 mg kg^{-1}) (Crutcher and Moschella, 1975), should exhibit plasma concentrations higher than those enabling inhibition of flavivirus replication in vitro. The drug showed very low toxicity in animal experiments, and this was confirmed in man. Therefore, its potential usefulness in the treatment of flavivirus infections should be further evaluated.

In our study, glycyrrhizin showed a low selectivity index but it was a significantly potent inhibitor of the replication of all the flaviviruses tested. This drug has been used in Japan in the treatment of chronic hepatitis C (Ito et al., 1987; Van Rossum et al., 1999). Its use in the treatment of flavivirus infections may be considered.

Ribavirin exhibited an expected antiviral action. This broad-spectrum antiviral drug was previously shown to be inhibitory to YFV, DENV and WNV in cell cultures (Canonico et al., 1984; Huggins et al., 1984; Huggins, 1989; Neyts et al., 1996; Jordan et al., 2000). Trials of ribavirin in primates showed that this drug had a weak prophylactic effect on viremia in rhesus monkeys infected with DENV (Malinoski et al., 1990). In mice, ribavirin had no effect on survival after intracerebral inoculation with DENV, but treatment with ribavirin-2′,3′,5′-triacetate, a prodrug of ribavirin led to a significantly increased survival time and rate (Koff et al., 1983). Ribavirin has been approved for the treatment of both naive and relapsing hepatitis C virus in combination with IFN-α2b (Poynard et al., 1998; McHutchison et al., 1998). It has also been used intravenously at high doses in the treatment of subacute sclerosing panencephalitis caused by measles virus (Hosoya et al., 2001). Similarly to its use in the management of chronic hepatitis C, ribavirin might be used in combination with IFN-α for the treatment of flavivirus infections.

Human recombinant IFN-α proved to be much more selective and effective than the other three antiviral compounds. Our present results complement previous studies, which showed the antiviral action of interferon against JEV and DENV in vitro (Vithanomsat et al., 1984; Diamond et al., 2000). IFN-α was also shown to be active in experimental flavivirus infections. It was effective in the prophylactic treatment of St. Louis encephalitis virus infection in mice (Brooks and Phillpotts, 1999). Moreover, a beneficial effect of human recombinant IFN-α on the course of JEV infection has been reported (Harinasuta et al., 1985). Several studies have described the antiviral activity of molecules used as interferon inducers. The nuclease-resistant polyriboinosinic–polyribocytidilic acid complex was found to protect rhesus monkeys against lethal infection with a virulent YFV strain (Stephen et al., 1977). A fungal interferon inducer, 6-MFA, had a protective effect against JEV in bonnet macaques (Ghosh et al., 1990). Treatment of weanling mice and baby hamsters with carboxymethylacridanone prevented mortality caused by JEV infection (Taylor et al., 1980). The prophylactic efficacy of poly (IC) was demonstrated in a lethal Modoc virus infection in mice (Leyssen et al., 2001).

IFN-α is now being used for the treatment of chronic hepatitis C (Lau et al., 1998). Its therapeutic activities on HCV infection in man, the encouraging results in flavivirus-infected animal models and the results obtained in the present study on its inhibitory effect on 11 different flaviviruses in vitro suggest that IFN-α should also be further evaluated for its efficacy in the treatment of the flavivirus infections in man. Because IFN-α in combination with ribavirin was found to significantly improve the sustained biochemical and virological response rates compared with interferon alone in the treatment of chronic hepatitis C (Reichard et al., 1997), ribavirin might also be able to increase the efficacy of IFN-α in flavivirus.

In conclusion, ribavirin, IFN-α, 6-azauridine and glycyrrhizin proved to be active against 11 flaviviruses including those that are most pathogenic to man: YFV, DENV, JEV. These antiviral compounds have already been used in patients in the treatment of other diseases. They should be further considered for use, either alone or in combination, for the treatment of flavivirus infections.

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