ELSEVIER

Antiviral Research 55 (2002) 107–116

Antiviral Research

www.elsevier.com/locate/antiviral

Identification of active antiviral compounds against a New York isolate of West Nile virus

John D. Morrey [a,*], Donald F. Smee [a], Robert W. Sidwell [a], Christopher Tseng [b]

[a] *Department of Animal, Dairy, and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT 84322-4700, USA*

[b] *Virology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA*

Received 12 September 2001; accepted 14 January 2002

Abstract

The recent West Nile virus (WNV) outbreak in the United States has increased the need to identify effective therapies for this disease. A chemotherapeutic approach may be a reasonable strategy because the virus infection is typically not chronic and antiviral drugs have been identified to be effective in vitro against other flaviviruses. A panel of 34 substances was tested against infection of a recent New York isolate of WNV in Vero cells and active compounds were also evaluated in MA-104 cells. Some of these compounds were also evaluated in Vero cells against the 1937 Uganda isolate of the WNV. Six compounds were identified to be effective against virus-induced CPE with 50% effective concentrations (EC_{50}) less than 10 μg/ml and with a selectivity index (SI) of greater than 10. Known inhibitors of orotidine monophosphate decarboxylase and inosine monophosphate dehydrogenase involved in the synthesis of GTP, UTP, and TTP were most effective. The compounds 6-azauridine, 6-azauridine triacetate, cyclopententylcytosine (CPE-C), mycophenolic acid and pyrazofurin appeared to have the greatest activities against the New York isolate, followed by 2-thio-6-azauridine. Anti-WNV activity of 6-azauridine was confirmed by virus yield reduction assay when the assay was performed 2 days after initial infection in Vero cells. The neutral red assay mean EC_{50} of ribavirin was only 106 μg/ml with a mean SI of 9.4 against the New York isolate and only slightly more effective against the Uganda isolate. There were some differences in the drug sensitivities of the New York and Uganda isolates, but when comparisons were made by categorizing drugs according to their modes of action, similarities of activities between the two isolates were identified.

Keywords: West Nile virus; Ribavirin; Antiviral; Flavivirus; 6-Azauridine

1. Introduction

There has been a heightened interest in the West Nile virus (WNV) within the United States due to an outbreak in the New York City area in 1999 (CDC, 1999). WNV is a common arbovirus

* Corresponding author. Tel.: +1-435-797-2622; fax: +1-435-797-2766.

E-mail address: jmorrey@cc.usu.edu (J.D. Morrey).

0166-3542/02/$ - see front matter
PII: S0166-3542(02)00013-X

in sub-Saharian Africa. It occasionally causes epidemics in Mediterranean regions and southern Europe, as with the recent outbreak in Israel where 12 people died (Siegel-Itzkovich, 2000). Even though the virus infection is often asymptomatic or mildly symptomatic, a growing number of deaths involving the central nervous system (CNS) have been identified in elderly people in Israel, Algeria, and Romania (Zeller, 1999). The virus infection had never before been identified within the United States prior to 1999 and is considered to be one more example of an emerging virus due to increased population densities and accessible travel (Zorpette, 2000). In 1999 within the New York City area, seven people died from the virus infection, and 62 WNV-infected persons were identified with neurological symptoms (CDC, 2000a). Eight hundred and eighty-five WNV-infected dead birds were identified (CDC, 2000b). Serosurveys in the New York area after the 1999 outbreak indicated that $< 1\%$ of WNV-infected persons developed severe neurologic diseases (CDC, 2000b). To date, available information indicates that in the year 2000 within the United States, one person died, 12 infected persons have been identified, and 536 infected dead birds have been identified (CDC, 2000b). The data for the year 2000 may indicate a decline in incidence of infection; however, the presence of the virus has now spread from the New York City area to a total of six states (CDC, 2000b), suggesting a continued geographic spreading of the virus. This continued expansion of the virus and the possible serious prognosis underscores the need to identify effective antiviral therapies that might assist the immune system in the clearance of the virus and diminishing serious symptoms. In this report, 34 potential antiviral substances were evaluated in a Vero cell culture assay system using a recent New York isolate of the virus. Comparisons were also made with a 1937 Uganda isolate.

2. Materials and methods

2.1. Viruses and cells

Two strains of WNV were used, a Uganda strain B 956 isolated in 1937 (ATCC VR-82, American Type Culture Collection, Manasses, VA) and a New York isolate from homogenized crow brain dated 8/20/00 (Robert Lacniotti, CDC, Division of Vector-Borne Infectious Diseases, Ft. Collins, CO). Cells used were African green monkey kidney (Vero 76, ATCC CCL1587), and embryonic African green monkey kidney (MA104, BioWhitaker, Walkerville, MD) cells, which were grown in medium 199 with 5% fetal bovine serum (FBS), 0.1% $NaHCO_3$, and MEM with 9% FBS and 0.1% $NaHCO_3$, respectively. MEM with 1% FBS, 0.1% $NaHCO_3$, and 50 μg/ml gentamycin (Sigma, St. Louis, MO) were used to maintain Vero or MA-104 cells during antiviral experiments Virus stocks were prepared in MA104 cells and stored at -80 °C. The viruses were titered in subconfluent Vero cells in 96-well microtiter plates.

2.2. Compounds

The sources of test compounds were: (*S*)-9-(2,3-dihydroxypropyl) adenine ((*S*)-DHPA), pyrazofurin, ribavirin, tiazafurin, 3-deazaguanosine, ribamidine, selenazofurin, and 3-deazauridine from ICN Pharmaceuticals (Costa Mesa, CA), hypericin, 5-fluorouridine, 2-thiouracil, 5-azacytidine, actinomycin D, rhodamine, 6-bromotoyocamycin, rifamycin, dihydro-5-azacytidine, cyclopentenyluridine (CPE-U), formycin, and 3-deazaneplanocin A from the National Cancer Institute ((NCI), Bethesda, MD), β-methylene TAD from Dr Victor Marquez (NCI), cyclopentenylcytosine (CPE-C) from Dr John Driscoll (NCI), 2-thio-6-azauridine, 6-azauridine, and mycophenolic acid from Sigma Corp. (St. Louis, MO), 6-azauridine triacetate from Dr Roland Buelow (SangStat Corporation), rimantadine from DuPont, uridine 2′, 3′-dialdehyde, formycin B, enviroxime, streptonigrin, actidione, 3-*t*-butyl-1-adamantylthiourea, and suramin from the US Army Medical Research Institute for Infectious Diseases (USAMRIID).

2.3. Visual inhibition of viral cytopathic effect assay

This cytopathic effect (CPE) inhibitory assay was described elsewhere (Smee et al., 1988) with

the following modifications. Serial dilutions of test compounds were added to subconfluent Vero or MA-104 cells in 96-well microplates, after which dilutions of viral stocks were added to the cells, which were assayed to be 14 $CCID_{50}$ (50% cell culture infectious dose) of WNV strain B 956 or 5 $CCID_{50}$ of WNV New York isolate. The dosages used uninfected cells, infected cells with no drug, and uninfected drug-treated cells were used as controls. Duplicates of toxicity controls at each drug concentration and triplicates of test samples were performed. After 6 days post-infection, cells were visually scored for CPE. The 50% effective concentration (EC_{50}) and the 50% inhibitory cytotoxic concentration (IC_{50}) were calculated by regression analysis using the means of the CPE ratings at each concentration of the compound. A selectivity index (SI) was determined as the IC_{50}/EC_{50}.

2.4. Neutral red inhibition of CPE assay

Neutral red (NR) vital stain was used to verify the visual CPE assay and to provide a more quantitative result (Player et al., 1998). After visually reading the CPE, cells were incubated with NR dye for 2–3 h at 37 °C. Free dye was washed from the wells and the uptake dye was quantified using a microplate reader (Bio-Tek EL 1309, BioTek, Burlington, VT) at absorbance 540 and 405 nm. Absorbance values were expressed as percentages of controls, and EC_{50} and IC_{50} values were calculated by regression analysis. The CPE and NR assays were repeated at least two times in different experiments for compounds having anti-WNV activity. Only the visual CPE assay, and not the NR assay, could be performed using MA-104 cells, because the cell monolayer easily detached during NR assays at day 6, which was the earliest point at which CPE could be read.

2.5. Virus yield reduction assay

To delineate the actual antiviral effects of test compounds from the potential cytotoxic effects of the compounds, the infectious virus recovered from the antiviral assays was quantified using the virus yield reduction assay (VYR) (Smee et al., 1992). The test method as described above for visual inhibition of CPE assay was used; inhibition of CPE was read visually. The 96-well plate was then frozen at −80 °C and thawed, and the virus from the supernates was then assayed by using a series of 10-fold dilutions and assaying each in quadruplicate in a monolayer of Vero cells grown in 96-well microplates. Viral CPE was determined visually 6 days later after incubation at 37 °C. The virus titer vs. concentration of test compound was plotted to determine a 90% effective concentration (EC_{90}), the dose which reduced virus titer by 1 $\log_{10}$. The mean values were calculated from triplicate measurements. The VYR assay for 6-azauridine using the New York isolate was repeated two times to verify the results.

3. Results

A total of 34 compounds were screened for antiviral activity against the New York isolate of WNV using visual and NR CPE assays with Vero cells (Table 1). Six compounds were identified with an $EC_{50} \leqslant 10$ and a $SI \geqslant 10$ using the NR assay. Of these, 6-azauridine, 6-azauridine triacetate, cyclopentenylcytosine, mycophenolic acid and pyrazofurin appeared to have the greatest activities, followed by 2-thio-6-azauridine. Four out of the six active compounds were inhibitors of orotidine monophosphate (OMP) decarboxylase (De Clercq, 1993). One inhibitor of CTP synthetase (Marquez et al., 1988), cyclopentenylcytosine, was active; however, another CTP synthetase inhibitor, 3-deazauridine, was not active. Selenazofurin did not have effective concentrations below 10 μg/ml using Vero cells, but the toxic concentration was high enough to have a SI well above 10. Ribavirin, also an inosine monophosphate (IMP) dehydrogenase inhibitor, had marginal activity. An inhibitor of thymidylate synthetase and dihydrofolate reductase, an adenosine analogue and polyamines did not have

Table 1
Activity of compounds against New York and Uganda isolates of WNV using Vero cells

Compound	WNV isolate	Visual assay			NR assay		
		EC_{50}[a] (μg/ml)	IC_{50}[b] (μg/ml)	SI[c]	EC_{50} (μg/ml)	IC_{50} (μg/ml)	SI
Inhibitors of orotidine monophosphate decarboxylase							
6-Azauridine	New York	1.5[d]	201	134	1.2	157	131
6-Azauridine	Uganda	1.4	143	102	0.9	59	66
6-Azauridine triacetate	New York	3	67	22	1.3	80	62
6-Azauridine triacetate	Uganda	0.6	14	23	0.8	50	63
Pyrazofurin	New York	2.1	91	43	1.8	33	18
Pyrazofurin	Uganda	0.5	90	180	0.5	19	38
2-Thio-6-azauridine	New York	10	>1000	>100	8	>1000	>125
2-Thio-6-azauridine	Uganda	100	1000	10	78	761	10
Inhibitors of inosine monophosphate dehydrogenase							
3-Deazaguanosine	New York	>32	32	NC[e]	>30	30	NC
Mycophenolic acid	New York	2.7	>100	>37	1.4	>100	>71
Mycophenolic acid	Uganda	0.1	>100	>1000	0.12	>100	>833
Ribamidine	New York	1000	>1000	NC	1000	>1000	NC
Ribamidine	Uganda	320	1000	3.1	304	1000	3.2
Ribavirin	New York	178	>1000	>5.6	106	>1000	>9.4
Ribavirin	Uganda	41	>1000	>24	36	>1000	>28
Selenazofurin	New York	20	610	31	6.2	>1000	>161
Selenazofurin	Uganda	14	1000	71	10	>1000	>100
Tiazofurin	New York	308	>1000	>3.2	307	>1000	>3.2
β-Methylene TAD	New York	100	>1000	>10	125	>1000	>8
β-Methylene TAD	Uganda	22	>1000	>45	53	>1000	>19
Inhibitor of CTP synthetase							
3-Deazauridine	New York	>683	683	NC	>593	593	NC
3-Deazauridine	Uganda	320	800	2.5	40	320	8
Cyclopentenylcytosine	New York	0.90	320	356	<0.32	>32	>100
Cyclopentenylcytosine	Uganda	0.029	>100	>3449	0.046	>100	>2174
Polyanions							
Hypericin	New York	>10	10	NC	>50	50	NC
Suramin	New York	119	150	1.2	56	152	27
Suramin	Uganda	>80	80	1.0	20	127	6.3
Inhibitor of thymidylate synthetase and dihydrofolate reductase							
5-fluorouridine	New York	>0.32	0.32	NC	>0.82	0.82	NC

Table 1 *(continued)*

Compound	WNV isolate	Visual assay			NR assay		
		EC_{50}[a] (μg/ml)	IC_{50}[b] (μg/ml)	SI[c]	EC_{50} (μg/ml)	IC_{50} (μg/ml)	SI
Inhibitors of S-adenosylhomocysteine hyrolase							
(*S*)-DHPA	New York	>1000	>1000	NC	>1000	>1000	NC
(*S*)-DHPA	Uganda	>1000	>1000	NC	>1000	>1000	NC
3-Deazaneplanocin A	New York	>100	100	NC	>2.6	2.6	NC
Purine analogs							
6-Bromotoyocamycin	New York	>10	10	NC	>10.6	10.6	NC
Formycin B	New York	>25	25	NC	>43	43	NC
Pyrimidine analogs							
2-Thiouracil	New York	100	100	NC	100	>100	NC
5-Azacytidine	New York	>320	320	NC	>565	565	NC
Cyclopentyluracil	New York	1000	1000	NC	1000	>1000	NC
Dihydro-5-azacytidine	New York	>90	90	NC	>9.8	9.8	NC
Uridine 2′,3′-dialdehyde	New York	>250	250	NC	85	210	2.5

[a] 50% effective concentration.
[b] 50% cytotoxic concentration.
[c] Selectivity index (IC_{50}/EC_{50}).
[d] Average of two or more replicate experiments.
[e] Not calculatable.

appreciable anti-WNV activity. Miscellaneous other compounds did not have anti-WNV activities, namely, 3-*t*-butyl-1-adamantylthiourea, actidione cyclohexamide, actinomycin D, enviroxime, rhodamine, rifamycin, rimantadine, and streptonigrin (data not shown).

Differences in phosphorylation of nucleoside analogs in certain cell types may result in different anti-WNV activities (Smee and Huggins, 1999); therefore, the active compounds against the New York isolate identified using Vero cells were also assayed using MA-104 cells (Table 2). The compounds 6-azauridine, 6-azauridine triacetate, pyrazofurin, selenazofurin, and ribavirin all appeared to be more active in MA-104 cells against the New York isolate, however, the toxicity of some compounds also increased in MA-104 cells so that the SI was not much different between the use of the two cells lines with these compounds. The EC_{50} activities of mycophenolic acid and cyclopentenylcytosine, however, were only 3-fold different between using the two difference cell lines.

The WNV strain isolated in Uganda in 1937 was also evaluated against some of the same compounds tested against the recent New York isolate (Table 1). The greatest differences of drug activities between the two different viruses were mycophenolic acid and cyclopentenylcytosine. These two compounds were $>1\ \log_{10}$ more active against the Uganda isolate as compared to the New York isolate. Conversely, 2-thio-6-azauridine was $1\ \log_{10}$ less active against the Uganda isolate. The overall profile of antiviral activity against to two viruses was similar, i.e. three inhibitors of OMP decarboxylase, one inhibitor of IMP dehydrogenase, and one inhibitor of CTP synthetase (De Clercq, 1993) were active against the Uganda isolate (Table 1). Ribavirin, also an IMP dehydrogenase inhibitor, still had marginal activity in Vero cells. An inhibitor of thymidylate synthetase and dihydrofolate reductase, an adenosine analogue and polyamines did not have appreciable anti-Uganda WNV activity.

Follow-up VYR assays were performed using Vero cells to confirm the antiviral activity of 6-azauridine against both the New York and the 1937 Uganda isolates. The VYR assays were performed 2, 4 and 6 days after the addition of compounds and virus. At day 2 using the New York isolate of WNV, over $2\ \log_{10}$ reduction in virus titer occurred at doses of 3.2 μg/ml and higher (Fig. 1A). The EC_{90} was calculated to be 1.6 μg/ml, which was comparable to the EC_{50} values determined by visual CPE and NR assays performed at day 6 (Table 1). However at days 4

Table 2
Activity of compounds[a] against New York isolate of WNV using MA-104 cells

Compound	Visual assay		VYR	
	EC_{50}[b] (μg/ml)	IC_{50}[c] (μg/ml)	SI[d]	EC_{90} (μg/ml)
6-Azauridine	0.1[e]	25	250	0.3
6-Azauridine triacetate	0.5	14	26	ND[f]
Pyrazofurin	0.5	90	191	0.3
Selenazofurin	8.1	>1000	>123	ND
Ribavirin	5	>100	>20	2.2
Mycophenolic acid	0.9	>100	>111	0.3
Cyclopentenylcytosine	0.3	>100	>400	1.4

[a] Compounds shown to be active in Vero cells.
[b] 50% effective concentration.
[c] 50% cytotoxic concentration.
[d] Selectivity index (IC_{50}/EC_{50}).
[e] Average of two or more replicate experiments.
[f] Not done.

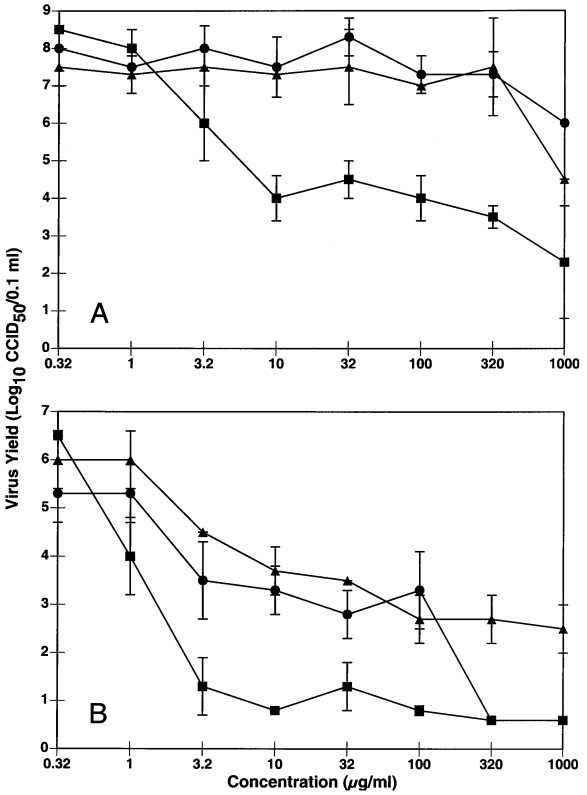

Fig. 1. Dose response curve of WNV titer of a New York isolate (A) and a 1937 Uganda isolate (B) at various days after treatment with 6-azauridine. (■) Day 2, (●) Day 4, (▲) Day 6 post-virus exposure.

and 6, the virus titers were not significantly reduced with any drug dosage except 1000 μg/ml (Fig. 1A). No CPE was observed at day 2. At day 4, partial CPE was observed (data not shown). At 6 days, 100% CPE (complete cell death) was observed for placebo-treated controls.

The VYR assay was also performed using 6-azauridine with the 1937 Uganda isolate. At day 2, the EC_{90} of 6-azauridine was 0.4 μg/ml (Fig. 1B) compared to the 1.4 μg/ml EC_{50} seen using the Uganda isolate (Table 1). At dosages greater than 10 μg/ml at day 2, the WNV titer was near or below the limits of detection so that a dose-responsive effect on virus titers could not be determined (Fig. 1B). Using the Uganda isolate, the VYR effect was again lessened when the virus was assayed on days 4 and 6 as compared to day 2. Antiviral activities could be detected at these days with the Uganda isolate, but not with the New York isolate at days 4 and 6. The average titers of the placebo control of the experiment using the New York isolate was 10^8 CCID/0.1 ml as compared with 10^6 CCID/0.1 ml for the 1937 Uganda isolate (Fig. 1).

4. Discussion

The compounds tested against the New York and Uganda WNV isolates were categorized in Table 1 according to possible mechanisms of action (De Clercq, 1993), which involve mostly cellular enzymes. One exception is the recent findings with ribavirin, which acted as a mutagen to cause mutational crisis with poliovirus and perhaps other RNA viruses (Crotty et al., 2000, 2001). The categorizations used in Table 1 revealed a pattern or profile that was somewhat similar for the two isolates of the WNV. Inhibitors of the OMP decarboxylase had the most members that exhibited significant antiviral activity. Some inhibitors of IMP dehydrogenase and CTP synthetase, whereas none of the other compounds displayed anti-WNV activity. One known inhibitor of CTP synthetase, cyclopentenylcytosine (Verschuur et al., 2000), effectively reduced CPE of both viruses; however, the other known CTP synthetase inhibitor, 3-deazauridine (Gao et al., 2000), was not inhibitory to the virus. Three assays were run with 3-deazauridine from two different sources to confirm its inactivity against WNV (data not shown). Differences in phosphorylation of nucleoside analogs in certain cell types may account for differences in anti-WNV activity. For example, 13-fold more ribavirin monophosphate is formed in 3T3 cells as compared to Vero cells (Smee and Huggins, 1999). Consequently, the low efficacy of ribavirin for WNV using Vero cells might be due to low phosphorylation of ribavirin.

To test this hypothesis that differences in cell lines may yield different results, visual CPE assays and VYR assays at 2 days post-infection were performed using MA-104 cells with the New York isolate (Table 2). The activity of ribavirin improved from 178 EC_{50} to 5 EC_{50} when using

MA-104 cells, which is consistent with the observation that 13-fold more ribavirin monophosphate is formed in some cells as compared to Vero cells (Smee and Huggins, 1999). The toxicity in MA-104 cells increased with some compounds, which is consistent with these compounds acting on cellular enzymes. Future studies can also include the use of exponentially growing cells to better characterize such compounds that probably act through cellular enzymes.

Compounds in the categories of inhibitors *S*-adenosylhomocysteine hyrolase, polyanions, inhibitors of thymidylate synthetase and dihydrofolate reductase, purine analogs and pyrimidine analogs did not selectively reduce the viral CPE (Table). None of the miscellaneous compounds outside of these category were significant WNV inhibitors. This profile of antiviral activity for WNV was similar to the activities detected for yellow fever virus (vaccine strain 17D) (Neyts et al., 1996). Inhibitors of OMP decarboxylase, IMP dehydrogenase, and CTP synthetase reduced yellow fever virus-induced CPE, but compounds in the other categories were not effective.

Two previous studies also found 6-azauridine to be active against nine different flaviviruses (Crance et al., 1998, 1999) including WNV. The studies also identified recombinant interferon-α and ribavirin to be active. Interferon was not evaluated in this study, but ribavirin, an IMP dehydrogenase inhibitor, was shown by CPE inhibition to have marginal activity against the two WNV isolates using Vero cells, but it was reasonably active in MA-104 cells. A recent report by Jordon et al. (2000), using a human neural cell-based assay, indicated only high concentrations of ribavirin inhibited virus and CPE. In this cited study, the EC_{50} was 60 μM (15 μg/ml), which was intermediate between the values obtained in Vero and MA-104 cells.

The putative active compounds against WNV have also been reported to be active against other viruses. The compound 6-azauridine has been found to have antiviral activity against a broad range of DNA and RNA viruses in vitro (Rada and Bragun, 1977; Smee et al., 1987). Antiviral activity in animal models, however, has been difficult to demonstrate with this compound (Klein et al., 1974; Steffenhagen et al., 1976; Smee et al., 1987). Cyclopentenylcytosine also has exhibited antiviral activity against a broad range of DNA and RNA viruses (Marquez et al., 1988; De Clercq et al., 1990), although antiviral activity in animal models has not been reported. Mycophenolic acid may have utility as a broad-spectrum antiviral agent or as immunomodulating agent in combination with other drugs to treat patients with human immunodeficiency virus (Capuis et al., 2000) or hepatitis C virus (Rostaing et al., 2000). Sindbis virus-resistant mutants, however, have been generated in vitro to mycophenolic acid (Scheidel et al., 1987). Pyrazofurin has broad-spectrum antiviral activity in vitro and in vivo, but associated toxicity has raised concerns with the drug (Wyde et al., 1989). Previous studies of compounds such as these with other viruses may be useful for the design of future treatment strategies of WNV.

Most people with WNV infection are asymptomatic or have nonspecific symptoms for which WNV testing would not be performed (CDC, 2000b). Probably only $< 0.1\%$ of WNV-infected persons developed severe neurologic disease in the 1999 New York City outbreak. Many WNV-infected people, therefore, may not be recognized for antiviral treatment until they develop a neurological infection. Since many antiviral drugs do not effectively cross the blood–brain barrier, treatment of a neurologic infection would be especially problematic. Ribavirin, for example, has had little effect on viral infections of the CNS. The compound was ineffective against infections in mice inoculated intracerebrally with Venezuelan equine encephalitis, Japanese encephalitis, yellow fever virus, herpes types 1 and 2, vaccinia, rabies, Semliki Forest, western equine encephalitis, or neurotropic influenza viruses (Sidwell, 1973; Stephen et al., 1980; Bussereau et al., 1988; Sidwell, 1996). Treatment routes used in one or more of the above infections included intraperitoneal, subcutaneous, per os, and intravenous inoculation. Neurological infections induced in guinea pigs and primates by Junin virus have also responded poorly to ribavirin therapy (Kenyon et al., 1986; Weissenbacher et al., 1986; McKee et

al., 1988). This inability to treat CNS infectious by ribavirin is attributed to failure of the drug to adequately pass the blood–brain barrier. Ribavirin's low lipid solubility would be a major factor in this failure. The partitian coefficient for ribavirin is −2.06, indicating an inability to affect passage through lipoid membranes (Smee et al., 1981; Sidwell, 1996).

One of the active compounds identified in this study, 6-azauridine, was evaluated for its ability to inhibit virus replication using the VYR assay in Vero cells at 2-, 4- and 6-days after initiation of treatment. At 2 days post-initiation, the VYR assay confirmed the antiviral effects of 6-azauridine. No CPE was expected to be observed at an early time point (day 2) with increasing amounts of CPE in subsequent days after initiation of the experiment. Consequently the best time to read CPE was on days 4 or 6. It was unexpected, however, to find high titers of virus using the VYR assay on days 4 or 6 when CPE was inhibited. If the assay was read on day 8, however, CPE was 100% with all concentrations of 6-azauridine tested (data not shown).

The six most active drugs or their derivatives: 6-azauridine, 6-azauridine triacetate, cyclopentenylcytosine, and mycophenolic acid, 2-thio-6-azauridine and pyrazofurin, should be evaluated further in cell culture systems and possibly in animals as candidate treatments for the WNV infection.

Acknowledgements

We thanks Robert Lacniotti (CDC, Division of Vector-Borne Infectious Diseases, Ft. Collins, CO) for supplying the New York isolate of WNV. Supported by Contract NO1-AI-65291 from the Virology Branch, NIAID, NIH.

References

Bussereau, F., Picard, M., Blancou, J., Sureau, P., 1988. Treatment of rabies in mice and foxes with antiviral compounds. Acta Virol. 32, 33–49.

Capuis, A.G., Paolo Rizzardi, G., D'Agostino, C., Attinger, A., Knabenhans, C., Fleury, S., Acha-Orbea, H., Pantaleo, G., 2000. Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. Nat. Med. 6, 762–768.

CDC, 1999. Outbreak of West Nile-like viral encephalitis—New York. MMWR 48, 845–849.

CDC, 2000a. Guidelines for surveillance, prevention, and control of West Nile infection—United States. MMWR 49, 25–28.

CDC, 2000b. Update: West Nile virus activity—Northeastern United States. MMWR 49, 820–822.

Crance, J.M., Grain, D., Gratier, D., Blancquaert, H., Rothlisberger, C., Guimet, J., Jouan, A., 1999. Selection of antiviral compounds active on several flaviviruses: clinical perspectives. Travaux Scientifiques des Chercheurs du Service de Sante des Armees 20, 35–36.

Crance, J.M., Gratier, D., Blancquaert, H., Rothlisberger, C., Bouvier, C., Jouan, A., 1998. Antiviral substances inhibiting in vitro replication of several flaviviruses. Travaux Scientifiques des Chercheurs du Service de Sante des Armees 19, 53–54.

Crotty, S., Cameron, C.E., Andino, R., 2001. RNA virus error catastrophe: direct molecular test by using ribavirin. Proc. Natl Acad. Sci. USA 98, 6895–6900.

Crotty, S., Maag, D., Arnold, J.J., Zhong, W., Lau, J.Y., Hong, Z., Andino, R., Cameron, C.E., 2000. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. Nat. Med. 6, 1375–1379.

De Clercq, E., 1993. Antiviral agents: characteristic activity spectrum depending on the molecular target with which they interact. Adv. Virus Res. 42, 1–55.

De Clercq, E., Bernaerts, R., Shealy, Y.F., Montgomery, J.A., 1990. Broad-spectrum antiviral activity of carbodine, the carbocyclic analogue of cytidine. Biochem. Pharmacol. 39, 319–325.

Gao, W.Y., Johns, D.G., Mitsuya, H., 2000. Potentiation of the anti-HIV activity of zalcitabine and lamivudine by a CTP synthase inhibitor, 3-deazauridine. Nucleosides Nucleotides Nucleic Acids 19, 371–377.

Jordan, I., et al., 2000. Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. J. Infect. Dis. 182, 1214–1217.

Kenyon, R.H., Canonico, P.G., Green, D.E., Peters, C.J., 1986. Effect of ribavirin and tributyl ribavirin on Argentine hemorrhagic fever (Junin virus) in guinea pigs. Antimicrob. Agents Chemother. 29, 521–523.

Klein, R.J., Friedman-Kein, A.E., Brady, E., 1974. Herpes simplex virus skin infection in hairless mice: treatment with antiviral compounds. Antimicrob. Agents Chemother. 5, 318–322.

Marquez, V.E., Lim, M., Treanor, S.P., Plowman, J., Markovac, A., Khan, M.S., Kaskar, B., Driscoll, J.S., 1988. Cyclopentenylcytosine. A carbocyclic nucleoside with antitumor and antiviral properties. J. Med. Chem. 31, 1687–1694.

McKee, K.T.J., Huggins, J.W., Trahan, C.J., Mahlandt, B.C., 1988. Ribavirin prophylaxis and therapy for experimental Argentine hemorrhagic fever. Antimicrob. Agents Chemother. 32, 1304–1309.

Neyts, J., Meerbach, A., McKenna, P., De Clercq, E., 1996. Use of the yellow fever virus vaccine strain 17D for the study of strategies for the treatment of yellow fever virus infections. Antiviral Res. 30, 125–132.

Player, M.R., Barnard, D.L., Torrence, P.L., 1998. Potent inhibition of respiratory syncitial virus replication using a 2-5 A-antisense chimera targeted to signals within the virus genomic RNA. Proc. Natl Acad. Sci. 95, 8874–8879.

Rada, B., Bragun, M., 1977. Antiviral action and selectivity of 6-azauridine. Annals NY Acad. Sci. 284, 410–417.

Rostaing, L., Isopet, J., Sandres, K., Cisterne, J.M., Puel, J., Durand, D., 2000. Changes in hepatitis C virus RNA viremia concentrations in long-term renal transplant patients after introduction of mycophenolate mofetil. Transplantation 69, 991–994.

Scheidel, L.M., Durbin, R.K., Stollar, V., 1987. Sindbis virus mutants resistant to mycophenolic acid and ribavirin. Virology 158, 1–7.

Sidwell, R.W., 1973. Effect of 1-β-D-ribafuranosyl-1,2,4-triazole-3-carboxamide (Virazole, ICN1229) on herpes and vaccinia keratitis and encephalitis in laboratory animals. Antimicrob. Agents Chemother. 3, 242–246.

Sidwell, R.W., 1996. Ribavirin: a review of antiviral efficacy. Antimicrob. Agents Chemother. 1, 219–255.

Siegel-Itzkovich, J., 2000. Twelve die of West Nile virus in Israel [news]. BMJ 321, 724.

Smee, D.F., Alaghamandan, H.A., Kini, G.D., Robins, R.K., 1988. Antiviral activity and mode of action of ribavirin 5′-sulfamate against Semliki Forest virus. Antiviral Res. 10, 253–262.

Smee, D.F., Huggins, J.W., 1999. Mode of action of ribavirin against cowpox and monkeypox viruses. Antiviral Res., 41.

Smee, D.F., McKernan, P.A., Nord, L.D., Willis, R.C., Petrie, C.R., Riley, T.M., Revankar, G.R., Robins, R.K., Smith, R.A., 1987. Novel Pyrazolo[3,4-D]pyrimidine nucleoside analog with broad-spectrum antiviral activity. Antimicrob. Agents Chemother. 31, 1535–1541.

Smee, D.F., Morris, J.L.B., Barnard, D.L., Van Aerschot, A., 1992. Selective inhibition of arthropod-borne and arenavirus in vitro by 3′-fluoro-3′-deoxyadenosine. Antiviral Res. 18, 151–162.

Smee, D.F., Sidwell, R.W., Clark, S.M., Barnett, B.B., Spendlove, R.S., 1981. Inhibition of bluetongue and Colorado tick fever orbiviruses by selected antiviral substances. Antimicrob. Agents Chemother. 20, 533–538.

Steffenhagen, K.A., Easterday, B.C., Galasso, G.J., 1976. Evaluation of 6-azauridine and 5-iododeoxyuridine in the treatment of experimental viral infections. J. Infect. Dis. 133, 603–612.

Stephen, E.L., Jones, D.E., Peters, C.J., Eddy, G.A., Loizeaux, P.S., Jahrling, P.B., 1980. Ribavirin treatment of toga-, arena-, and bunyavirus infections in subhuman primates and other laboratory animal species. In: Smith, R.A., Kirkpatrick, W. (Eds.), Ribavirin: a Broad Spectrum Antiviral Agent. Academic Press, New York, pp. 169–183.

Verschuur, A.C., Van Gennip, A.H., Leen, R., Meinsma, R., Voute, P.A., van Kuilenburg, A.B., 2000. In vitro inhibition of cytidine triphosphate synthetase activity by cyclopentenyl cytosine in paediatric acute lymphocytic leukemia. Br. J. Haematol. 110, 161–169.

Weissenbacher, M.C., Cabello, M.A., Merani, M.S., McCormic, J.B., Rodriguez, M., 1986. Therapeutic effect of the antiviral agent ribavirin in Junin virus infection of primates. J. Med. Virol. 20, 261–267.

Wyde, P.R., Gilbert, B.E., Ambrose, M.W., 1989. Comparison of the anti-respiratory syncytial virus activity and toxicity of papaverine hydrochloride and pyrazofurin in vitro and in vivo. Antiviral Res. 11, 15–26.

Zeller, H.G., 1999. West Nile virus: a migrating arbovirus of current interest. Med. Trop. (Mars) 59, 490–494.

Zorpette, G., 2000. Biohazards—emerging diseases. A plum of an Island. Sci. Am. 282, 22–23.