SELECTIVE ANTIVIRAL ACTIVITY OF THE ANTIBIOTIC 2′-AMINO-2′-DEOXYRIBOFURANOSYL ADENINE

FUMIAKI TAGUCHI, YOSHIKO IMATANI and DAIZO NAGAKI

School of Hygienic Sciences, Kitasato University
Sagamihara, Kanagawa-ken 228, Japan

AKIRA NAKAGAWA and SATOSHI ŌMURA

School of Pharmaceutical Sciences, Kitasato University
Minato-ku, Tokyo 108, Japan

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The effect of new anti-mycoplasmal antibiotic, 2′-amino-2′-deoxy-9-β-D-ribofuranosyl adenine (2-AA) on virus multiplication was investigated. The 2-AA inhibited only the multiplication of measles virus among the viruses tested; *i.e.*, herpes simplex virus, BK virus, vesicular stomatitis virus, measles virus and Echo virus. At a concentration of 5 μg/ml of 2-AA, the inhibition of measles virus replication was complete, *i.e.*, no infectious virus nor viral antigen detected. In contrast, 9-β-D-arabinofuranosyl adenine (50 μg/ml) was active to herpes simplex virus and BK virus, and was inactive to measles virus, vesicular stomatitis virus and Echo virus. Results described herein may suggest that 2-AA affects the late function (perhaps the translation step) of the replication of measles virus.

Arabinosylnucleosides, such as 1-β-D-arabinofuranosyl cytosine (Ara-C), 9-β-D-arabinofuranosyl adenine (Ara-A) and 1-β-D-arabinofuranosyl thymine (Ara-T), are selective inhibitors of herpes virus replication[1~3], and Ara-C and Ara-A have been used in chemotherapy of herpes virus infections[1,2]. The natural metabolite of *Actinomadura* sp. No. SA-4427, 2′-amino-2′-deoxy-9-β-D-ribofuranosyl adenine (2-AA) was found to show an inhibitory activity to mycoplasma and no effect on bacteria[4]. This paper describes the antiviral activity and mode of action of 2-AA.

Materials and Methods

Virus: The DNA containing viruses of herpes simplex virus (type 2, 196 strain, 10^7 plaque forming units (PFU)/ml) and BK virus (Gardner strain, 256 hemagglutinating units (HAU)) and the RNA containing viruses of measles virus (Edmonston strain, 10^6 PFU/ml), vesicular stomatitis virus (New Jersey strain, 10^8 PFU/ml) and Echo virus (type 7, 10^8 PFU/ml) were used. Herpes simplex virus (HSV), measles virus and vesicular stomatitis virus (VSV) were grown and assayed in cultures of a human embryonic lung HEL-R66 cell line[5], BK virus (BKV) was grown in HEL-R66 cells and assayed by hemagglutination (HA) test[6], and Echo virus (Echo) was grown and assayed in cultures of an African green monkey kidney Vero cell line. Infectivity of all viruses except BKV was expressed in term of plaque forming units (PFU) per ml and the amount of BKV was expressed in term of hemagglutinating units (HAU).

Cell culture: HEL-R66 and Vero cells were propagated in EAGLE's minimum essential medium (MEM) containing 60 μg/ml of kanamycin. Heat-inactivated calf serum was added to MEM at a concentration of 2% for HEL-R66 and 10% for Vero cells for the cell growth and 0.5% for HEL-R66 and 1% for Vero cells for maintenance of the cells after virus infection. Cell cultures were prepared by treating monolayer cultures with a solution of 0.25% trypsin and 0.1% pronase, washing dispersed-cells with HANKS' balanced salt solution and distributing 10^6 cells/2-oz bottle in 5 ml of the growth medium. Cells in culture were incubated at 37°C.

Drug: The stock solutions of 2-AA and Ara-A were prepared and diluted to appropriate concentrations with MEM (pH 7.2).

Cytotoxicity test: Monolayer cultures of HEL-R66 and Vero cells were fed for the first 2 day incubation at 37°C with the maintenance medium containing 2-fold dilutions of 2-AA or Ara-A, and refed with drug-freed medium for an additional 4 days. Morphology and cell degeneration of treated cultures were observed for 6 days. Non-toxic maximum concentrations of 2-AA and Ara-A were 7.5~10 μg/ml and 100 μg/ml, respectively.

Virus titration: Monolayer cultures of the cells were inoculated with 0.5 ml of 10-fold serial dilutions of virus samples and after virus adsorption for 60 minutes at room temperature, overlaid with 5 ml of the maintenance medium containing 0.5% methylcellulose. The cultures were incubated at 37°C for 4 days and numbers of plaques were counted.

Antiviral test: Bottle cultures of HEL-R66 or Vero cells were infected with 0.5 ml virus containing 10^4 PFU/ml (a multiplicity of infection was about 0.01 PFU/cell) or 100 HAU of BKV, washing 3 times with HANKS' solution after virus adsorption for 60 minutes at room temperature and fed with 5 ml of the maintenance medium containing 5 μg/ml of 2-AA or 50 μg/ml of Ara-A. When virus-inoculated control cultures showed cell degeneration of approximately 80% or more (in general, it was the 2nd or 3rd day after infection, but the 5th~7th day after BKV infection), the cultures were treated by one cycle of freezing and then thawing, and sonicated for one minute. The amounts of virus in the supernatant after low-speed centrifugation were determined by plaque titration or HA test.

Results

Antiviral Activity of 2-AA

As shown in Table 1, in the presence of Ara-A (50 μg/ml), maximum yields of RNA containing viruses, such as measles virus, VSV and Echo were not significantly changed, although those were somewhat lower than in the controls. The multiplication of DNA virus, such as HSV and BKV, was markedly influenced, and the final yield of HSV was reduced to less than 10 PFU/ml ($10^{7.8}$ PFU/ml in the control) and hemagglutinin production of BKV was reduced to less than 2 HA units (256~512 HA units in the control, data not shown in Table 1).

Table 1. Antiviral activity of 2-AA.

Drug	Virus yields, $\log_{10}$ PFU/ml			
	HSV	Measles	VSV	Echo
None	7.8	5.9	8.8	7.4
2-AA, 5 μg/ml	7.8	<1.0	7.5	6.2
Ara-A, 50 μg/ml	<1.0	5.0	7.1	6.5

The virus was harvested when 80% or more cells had degenerated, and the virus yields were determined.

On the other hand, the antiviral effect of 2-AA on viruses was significantly different from that of Ara-A. As results from triplicated experiments, only the multiplication of measles virus among the viruses tested was completely inhibited by 2-AA. No appearance of cytopathic effect (CPE) and no production of infectious virus was detected in measles virus inoculated-cultures in the presence of 2-AA (5 μg/ml). It is noteworthy that the selective effect of Ara-A and 2-AA was highly reproducible, and repeatedly shown in experiments in HEL-R66 cells and also in Vero cells (data not shown).

Mode of the Action of 2-AA

Preliminary tests were performed to determine the mode of action of 2-AA against measles virus. The effect of 2-AA on the virus multiplication were first determined by inhibitions of plaque formation and virus yield. Bottle cultures of HEL-R66 cells were inoculated with 200 PFU of measles virus. One half of the cultures received 10 μg/ml of cycloheximide (the remaining control group received only

the diluent) and were incubated at 37°C for one day. After washing of the cultures with HANKS' solution, half of two groups of the cultures which had been treated and non-treated with cycloheximide, received 5 μg/ml of 2-AA. For plaque counts, the cultures were overlaid with 0.5% methylcellulose-containing medium and for virus yields, the cultures were fed with the maintenance medium. The replication of the virus, as judged by two criteria of plaque formation and virus production, was completely inhibited by the addition of 2-AA to the cultures with or without the pretreatment with cycloheximide (Table 2).

Table 2. Mode of action of 2-AA.

Treatment with		Plaque counts per bottle	Virus yields PFU/ml
Cycloheximide	2-AA		
10 μg/ml	5 μg/ml	0, 0	<5
10 μg/ml	0	151, 155	$10^{5.7}$
0	5 μg/ml	0, 0	<5
0	0	195, 202	$10^{5.9}$

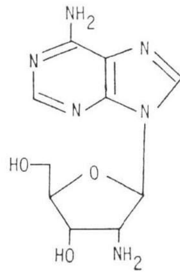
Bottle cultures of HEL-R 66 cells were inoculated with 200 PFU of measles virus. One half of the cultures received 10 μg/ml of cycloheximide (the remaining control cultures received only the medium) and were incubated at 37°C for one day. After washing of the cultures with HANKS' solution, half of the cultures which had been treated or non-treated with cycloheximide, received 5 μg/ml of 2-AA. For plaque counts, the cultures were overlaid with 0.5% methylcellulose containing medium and for virus yields, the cultures were fed with the maintenance medium.

Secondly, the influence of 2-AA on measles virus antigen production was determined by immunofluorescent microscopy. Monolayer cultures of HEL-R66 cells on cover-slips were infected with the virus at a multiplicity of infection of about 1 PFU/cell. One half of the cultures received 5 μg/ml of 2-AA and the remaining controls received only the medium. After 2 day incubation at 37°C, the cells on cover-slips were fixed with cold acetone and stained with fluorescein-labeled anti-measles virus immune serum (Toshiba Inst. Biol. Sci., Co.). No antigen was detected in the cells in the presence of 2-AA, although the antigen was clearly observed in whole syncytia in the cultures without 2-AA. In some instances, small and fine dot-like structures with very weak fluorescein were present in the cytoplasm of the cells in the presence of 2-AA, and the positive ratio (0.01%), number and size of the structures in the cell, and intensity of the immuno-fluorescein were not changed with prolonged incubation, although the cell morphology of infected cells was not different from that of mock-infected control cells (data not shown).

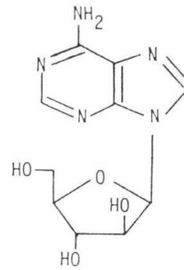
Discussion

It is well known that arabinosyl nucleosides, such as Ara-C, Ara-A and Ara-T are selective inhibitors of DNA virus replication, and Ara-C and Ara-A are used in chemotherapy of herpesvirus infections[1,2]. Antiviral activities of arabinosyl nucleosides and nucleotides have been extensively studied[7]. In this study, preliminary experiments were undertaken to determine the influence of the new antibiotic, 2-AA on virus replication. Although 2-AA and Ara-A are very similar in structure (Fig. 1), the antiviral effect of 2-AA on virus was found to be significantly different from that of Ara-A (Table 1). In the presence of 2-AA (5 μg/ml), measles virus replication was completely inhibited, and neither the production of the virus antigen nor the appearance of CPE, which is thought to be associated with the synthesis of cell-fusion factor, was detected in the cells in infected cultures, and furthermore in combination with pretreating the infected cells with cycloheximide (10 μg/ml), no virus could be recovered. These results, obtained by rather indirect methods, may suggest that 2-AA affects the late functions (perhaps the translation step) of the replication of measles virus rather than its early functions (perhaps the transcription). On the other hand, the reason for the ineffectiveness of 2-AA on the virus such as HSV and BKV remained unsolved. However, the observation indicates that 2-AA preferentially differentiates, through unknown processes, two RNA viruses such as measles

Fig. 1.



2′-Amino-2′-deoxy-9-β-D-ribofuranosyl adenine (2-AA)



9-β-D-Arabinofuranosyl adenine (Ara A)

virus and VSV, in which virus genome is negative-stranded RNA.

Our findings that the antibiotic, 2′-amino-2′-deoxy-9-β-D-ribofuranosyl adenine is a selective inhibitor of measles virus, although the viruses tested were limited, and has a selective anti-mycoplasmal activity, an observation consistent with a previous report[4], are very unique and new. It is our hope that 2-AA will be utilized as a preferential inhibitor in studies on negative-stranded RNA viruses.

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