ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1977, p. 475-481
Vol. 11, No. 3

Printed in U.S.A.

Effects of 2-Deoxy-D-Glucose and 3-Deazauridine Individually and in Combination on the Replication of Japanese B Encephalitis Virus

DANIEL R. WOODMAN* AND JIMMY C. WILLIAMS

Department of Microbiology, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

Received for publication 9 November 1976

We have tested the potencies of the competitors of glucose, 2-deoxy-D-glucose, and of uridine, 3-deazauridine, on the inhibition of Japanese B encephalitis virus multiplication in BHK-21 cell cultures. The relative effectiveness of the viral inhibitors were evaluated individually and in combination in relation to cytotoxicity as a measure of the selectivity of inhibition. When the drugs were administered individually, the antiviral activity was masked by the cytotoxic effect on the host. By combining the two drugs, it was possible to inhibit Japanese encephalitis virus production at noncytotoxic concentrations. The effects of 2-deoxy-D-glucose and 3-deazauridine on the growth inhibition of BHK-21 cells in cultures were only additive, while they were clearly synergistic on the inhibition of Japanese encephalitis virus production. Thus, it was possible to achieve an increased antiviral effect without a significant increase in cytotoxicity. Although the precise biochemical mechanism of the antiviral activity of these antimetabolites in combination is not known, our results indicate the potential value of this approach in viral chemotherapy.

A considerable amount of biochemical information has been gathered on the mode of action of antiviral drugs, but such information has not yet been reflected in improved chemotherapy of viral infections. In general, the conclusion has been drawn that viral infections do not respond well to chemotherapy, largely because viruses depend upon the synthetic mechanisms of the host cells. Therefore, any compound that effectively reduces the number of viral particles is likely to cause severe toxic side effects. Of the agents that have been tested on a variety of enveloped ribonucleic acid (RNA) viruses, the glucose competitor, 2-deoxy-D-glucose (2-DG), has probably been the most promising, largely because it interferes with the synthesis of viral glycoprotein (5, 7, 10). Another compound that possesses significant antiviral activity against RNA viruses is the antipyrimidine, 3-deazauridine (3-deazaUR). Although 3-deazaUR greatly restricts host as well as viral RNA synthesis at high concentration, this compound has antiviral activity at noncytotoxic levels (9, 11, 16).

Unfortunately, both 2-DG and 3-deazaUR exhibit severe cytotoxic side effects when administered in the high concentrations required for substantial antiviral activity. In this paper we describe preliminary studies in vitro in which we examined the feasibility of combining these two drugs to reduce the replication of Japanese B encephalitis virus (JEV) without greatly affecting the host cells. The relationship of the metabolic sites inhibited by the two agents under discussion are summarized in Fig. 1. Administration of 2-DG and its subsequent incorporation into host as well as viral glycoproteins causes toxic effects and a reduction in viral particles (for review, see reference 15). The antipyrimidine, 3-deazaUR, when phosphorylated to the triphosphate level, 3-deazauridine 5'-triphosphate (3-deazaUTP), exhibits strong competitive inhibition of the synthesis of cytidine 5'-triphosphate (CTP), thereby restricting host and viral RNA synthesis (11, 16). A consideration of the pathways suggested to us that 2-DG might potentiate the effect of 3-deazaUR and thus affect viral replication. This combination of drugs, at a low concentration, should be reasonably antiviral while not being cytotoxic. The effects of these combinations on antiviral activity and cytotoxicity were tested in the baby hamster kidney cell line (BHK-21) infected with JEV.

MATERIALS AND METHODS

Virus strain. The Nakayama strain of JEV (12) was used throughout this investigation. Virus seed

was prepared from the harvest of a single passage in brains of suckling NIH-Nmri CV mice and stored at −70°C until used.

Cell cultures and media. BHK-21 clone 13 cell cultures from the American Type Culture Collection provided an in vitro assay system for both the titration of JEV (1, 8) and cytotoxicity evaluations. Stock cell cultures were grown in Eagle minimum essential medium (2) with Earle balanced salt solution (EMEM), supplemented with 10% tryptose phosphate broth and 10% heat-inactivated dialyzed fetal calf serum ($EMEM_{90}DFCS_{10}$). The cells were subcultured and passed in EMEM with the substitution of various metabolites for glucose as shown in Table 1. The cells were infected as nonconfluent monolayers (40,000 to 60,000 viable BHK-21 cells per 1 ml of maintenance medium) in each well of 24 well-dishes (Costar, Cambridge, Mass.). The maintenance medium contained the components of growth medium except that the level of fetal calf serum was reduced to 1% ($EMEM_{99}DFCS_1$).

Cytotoxicity tests. The cytotoxicities of 2-DG and 3-deazaUR were determined for each modification of growth ($DFCS_{10}$) and maintenance ($DFCS_1$) media used in this study by viability staining (eosin Y exclusion) and by microscopic examination. Growth medium was used to test inhibitor cytotoxicity (Table 2). Although maintenance medium was used for the cultures that served as controls for virus yield reduction tests with either 2-DG or 3-deazaUR, differences in cytotoxicity attributable to the concentration of serum were negligible. The cytotoxic dose

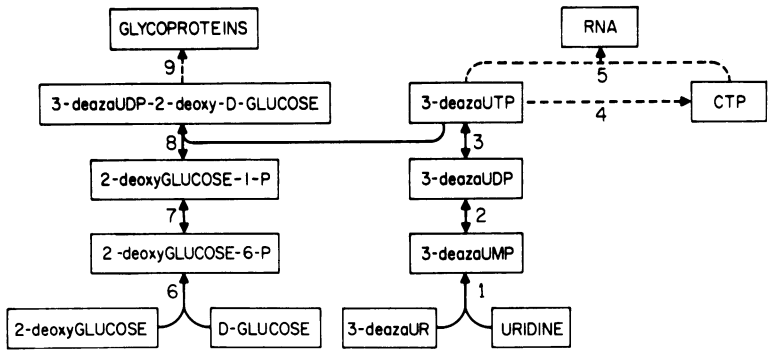

FIG. 1. *Simplified schematic map of the putative mechanism of action of the antipyrimidine 3-deazaUR in combination with 2-DG. Enzymes that phosphorylate the natural substrate (e.g., uridine and glucose) are known to phosphorylate also the substrate analogue (e.g., 3-deazaUR and 2-DG) to the next intermediate (15, 16). A synergistic action may be due to the inhibition of RNA synthesis and glycoprotein synthesis. Abbreviations: 1, uridine kinase (EC 2.7.1.48); 2, nucleoside monophosphate kinase (EC 2.7.4.4); 3, nucleoside diphosphate kinase (2.7.4.6); 4, cytidine 5'-triphosphate (CTP) synthetase (EC 6.3.4.2); 5, ribonucleic acid (RNA) nucleotidyltransferase (EC 2.7.7.6); 6, hexokinase (EC 2.7.1.1); 7, phosphoglucomutase (EC 2.7.5.1); 8, uridine 5'-diphosphate glucose (UDPG) pyrophosphorylase (EC 2.7.7.9); 9, UDPG-glycosyltransferase; UR, uridine; MP, monosphosphate; DP, diphosphate; TP, triphosphate (---), reactions inhibited by the respective activated antimetabolites.*

TABLE 1. *BHK-21 cell multiplication and JEV replication in modified EMEM containing increasing glucose levels*

Glucose (mg/liter)	Viable cell count[a] and JEV titer[b] in media							
	D-Galactose (900 mg/liter)[c]		D-Galactose (900 mg/liter), sodium pyruvate (550 mg/liter)[c]		Mannose (1,000 mg/liter)[c]		Mannose (1,000 mg/liter), sodium pyruvate (550 mg/liter)[c]	
	BHK-21[a]	JEV[b]	BHK-21[a]	JEV[b]	BHK-21[a]	JEV[b]	BHK-21[a]	JEV[b]
0	5.3	1.0	1.8	1.9	1.8	0.68	10.0	1.7
0.1	5.7	1.2	1.9	1.1	2.8	0.65	9.6	1.8
1	5.0	1.4	1.6	1.8	5.0	0.8	9.8	1.7
10	5.4	1.0	1.7	1.5	4.0	1.1	10.0	1.9
100	9.5	1.2	2.9	1.6	4.1	1.1	8.5	2.0
1000	10.7	1.1	6.8	1.7	4.5	1.0	10.0	2.0

[a] Viable cell count × 10^5 48 h after seeding 2 × 10^5 cells/ml of $EMEM_{90}DFCS_{10}$ in 1-ml wells.

[b] JEV titer × 10^6 PFU/ml 48 h after infection of BHK-21 cells at a multiplicity of infection of 10:1 in $EMEM_{99}DFCS_1$.

[c] Additional metabolites of EMEM.

TABLE 2. *Relative effectiveness of 2-DG and 3-deazaUR on the reduction of JEV titer and cytotoxic dose in EMEM and in modified EMEM without glucose*

Media	2-Deoxy-D-glucose			3-Deazauridine		
	MED_{50} (μM)[a]	Cytotoxic dose (μM)[b]	PER[c]	MED_{50}	Cytotoxic dose	PER
EMEM (glucose)	500	900	1.8	20	30	1.5
Modified EMEM (no glucose)						
D-(+)-Galactose (900 mg/liter)	500	800	1.6			
Sodium pyruvate (550 mg/liter)	500	1,500	3.0			
D-(+)-Galactose (900 mg/liter) and sodium pyruvate (550 mg/liter)	150	1,000	6.6			
Mannose (1,000 mg/liter)	300	2,400	8.0			
Mannose (1,000 mg/liter) and sodium pyruvate (550 mg/liter)	300	2,750	9.2	20	4	0.2

[a] Forty-eight-hour minimum effective dose (MED_{50}) based upon PFU reductions in maintenance medium.
[b] Forty-eight-hour cytotoxicity based upon viability determinations in growth medium.
[c] Physiologically effective range = cytotoxic dose/MED_{50}.

was considered to be that amount of compound, either alone or in combination, which reduced the viable cell count by 50% in growth media by 48 h.

Plaque assay. JEV plaque titrations were conducted in confluent monolayers of BHK-21 cell cultures. Serial 10-fold dilutions of JEV samples were prepared in pH 7.4 phosphate-buffered saline containing 0.75% bovine serum albumin fraction V (Calbiochem). Overlay medium contained a final concentration of 0.8% agarose (Seaken, Microbiological Associates, Inc.) in Gey's medium. Infected cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere for 96 h before neutral red staining.

Virus yield reduction tests. Cultures were inoculated with 10 plaque-forming units (PFU) of JEV per viable cell, absorbed for 90 min at 37°C, and then rinsed twice with pH 7.4 phosphate-buffered saline. The appropriate maintenance medium containing increasing concentrations of either 2-DG (Aldrich Chemical Co.) or 3-deazaUR (ICN Corp., Irvine, Calif.) was then added to the cell cultures as well as to control uninfected cells. The cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere for 48 h, and supernatant fluids were sampled at 12-h intervals. Viral titers expressed as PFU per cell were determined by plaque assays on BHK-21 cell cultures. The antiviral activity of a given concentration of either 2-DG or 3-deazaUR was determined as the percentage of control PFU obtained without either compound. The amount that reduced the number of PFU by 50% was defined as the minimal effective dose (MED_{50}) (3). Various combinations of 2-DG and 3-deazaUR were likewise evaluated for antiviral and cytotoxic effects.

RESULTS

Glucose requirement for cell multiplication and viral replication. When uninfected BHK-21 cells were subcultured with $EMEM_{90}DFCS_{10}$ either in the absence of glucose or in 0.1 to 1 mg of glucose/liter, the total number of cells doubled between 24 and 48 h. Significant growth after 48 h required at least 100 mg of glucose per liter. With a multiplicity of infection of 10 PFU, there was a definite requirement for glucose for optimal JEV replication. Viral assays for each glucose concentration at 24, 48, and 72 h indicated that JEV replication was always maximal at 48 h and the effect of the glucose level was also most evident at 48 h. Although there was some indication that JEV replicated in the absence of glucose or in the presence of small concentrations of glucose, maximum levels were reached only when the glucose concentration was either 100 or 1,000 mg/liter.

Effect of other carbon sources. Cell multiplication in $EMEM_{90}DFCS_{10}$ and JEV replication in $EMEM_{99}DFCS_1$ obtained with increasing glucose concentrations in the presence of various carbon sources are presented in Table 1. In the case of galactose, galactose plus sodium pyruvate, and mannose, the cell count approximately doubled as the concentration of glucose was increased. However, with a combination of mannose and sodium pyruvate the cell count was approximately the same at all concentrations of glucose. The titer of JEV, on the other hand, with the possible exception of the mannose medium, was not significantly stimulated by increasing the glucose concentration. Increasing serum levels from 1 to either 5 or 10% did not result in a significant change in JEV titers.

Antimetabolite effectiveness. The effects of 2-DG and 3-deazaUR on BHK-21 cells and JEV replication are summarized in Table 2. The data presented are the means of triplicate experiments. The cytotoxic dose was measured in $EMEM_{90}DFCS_{10}$ and the MED_{50} in $EMEM_{99}DFCS_1$. The potency of an inhibitor of viral replication can best be judged by the ratio of cytotoxic level to MED_{50}, expressing the physiologically effective range (PER), which is

a measure of the selectivity of inhibition. A large PER reflects relatively high viral inhibition with respect to cytotoxicity. In complete EMEM and in modified EMEM containing galactose, the PER with 2-DG was small (<2) because of its cytotoxicity. When glucose was replaced by pyruvate, galactose plus pyruvate, mannose, or mannose plus pyruvate, the PER increased to 3.0, 6.6, 8.0, and 9.2, respectively.

The effect of increasing the concentration of 2-DG on cell viability and JEV production are presented in greater detail in Fig. 2. The results can be summarized as follows. (i) The cytotoxicity concentrations of 2-DG were markedly different in the four media. When mannose, galactose, and pyruvate-containing media were compared to glucose, the BHK-21 cells were protected from the effects of 2-DG most effectively by mannose. Toxicity levels were significantly lower ($P < 0.05$) at 0.6, 1.2, and 2.4 mM 2-DG. (ii) The reduction in yield of JEV was not greatly different in the four media. Hence, these results suggest that the reduction in yield of JEV by 2-DG in glucose-, pyruvate-, and galactose-containing media may have been due in part to the cytotoxic effects of 2-DG on the BHK-21 cells. Mannose, on the other hand, undoubtedly protected the host cell from the effects of 2-DG, whereas the reduction in yield of JEV was not altered.

The cytotoxic effect of 3-deazaUR was 30-fold greater than that of 2-DG in glucose medium. Surprisingly, its cytotoxicity, in contrast to 2-DG, was much greater in mannose-sodium pyruvate medium. Thus, there was an almost 700-fold ratio in the toxicity of the two compounds in the mannose-pyruvate medium. The MED_{50} was approximately the same in the two media. The PER was low in the glucose medium, approximately the same as for 2-DG, and very low (0.2) in the mannose-pyruvate medium.

Reversal of 2-DG inhibition by metabolite replacement. The effect of replacing 2-DG (0.5 mM) with various metabolites at 6, 12, 24, and

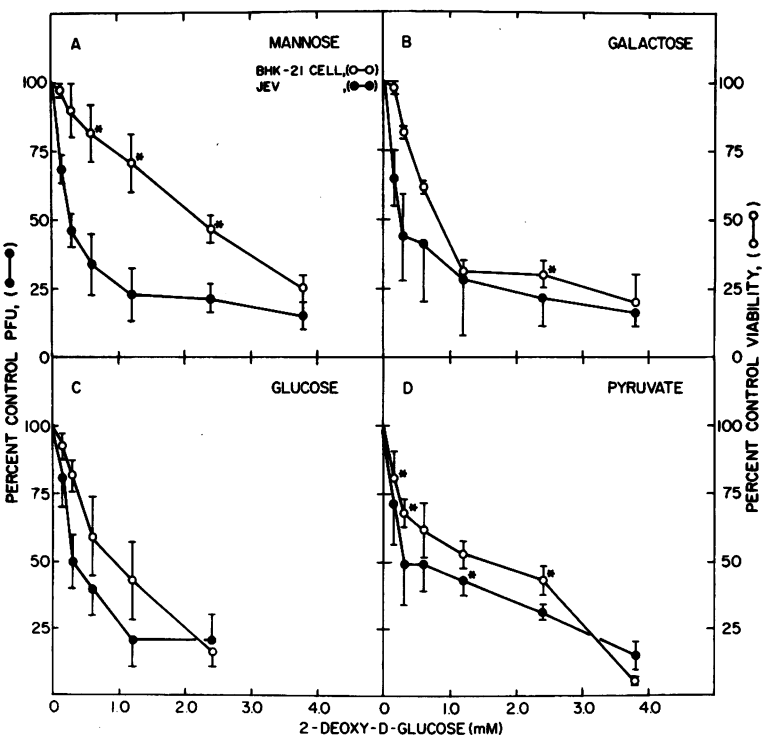

FIG. 2. *JEV yield reduction per infected BHK-21 cell at 48 h by 2-DG in 1 ml of modified maintenance EMEM. JEV titers in the presence of 2-DG were compared with PFU from controls consisting of infected cell cultures containing none of the antimetabolite. Total viable cell counts at 48 h from the uninfected wells containing increasing concentrations of 2-DG in modified maintenance media were compared to cell counts in control wells which contained no 2-DG. Metabolic concentrations were glucose (1 g/liter), galactose (900 mg/liter), pyruvate (550 mg/liter), or mannose (1 g/liter). Each point represents the mean ± standard error of the mean of four or more separate tests. Means that are significantly different from the glucose medium ($P < 0.05$) are indicated by an asterisk (*). (100% = 20 JEV/cell.)*

36 h after JEV infection of BHK-21 cells in $EMEM_{99}DFCS_1$ was most evident when metabolite replacement was accomplished at 24 h. Mannose most efficiently reversed viral inhibition by 2-DG, whereas differences in the effectiveness of galactose, pyruvate, and glucose were relatively small (Fig. 3). When replacement was made at 36 h (data not shown) comparable results were obtained, but the yield of JEV was reduced possibly because of the considerable amount of JEV removed with the medium change at 36 h. Results with infected control cultures containing no 2-DG indicated that any change of medium after 12 h reduced infectious virus production. In uninfected controls in which 2-DG medium was replaced with various carbon sources, glucose most effectively increased the number of viable BHK-21 cells at both 6-h (180%) and 24-h (100%) replacement. The 6-h mannose replacement was less effective (110%), whereas galactose was even poorer (85%).

Antiviral effect of 3-deazaUR and 2-DG in combination. To test the hypothesis that 3-deazaUR could potentiate the growth inhibitory effects of 2-DG on JEV, combination experiments were carried out. The effects of four different 3-deazaUR concentrations were tested alone and in the presence of three concentrations of 2-DG (Table 3). In every experiment the combination of the two drugs inhibited JEV replication to an extent greater than expected from purely additive effects while showing an inhibition of BHK-21 cell growth approximately the same or somewhat less than the additive effect. Hence, these two agents when used in combination display a considerable degree of synergism in JEV inhibition. Increasing concentrations of 3-deazaUR in the presence of a constant concentration of 2-DG brought about a significant decrease in the concentration of 3-deazaUR required to reduce the MED_{50} (Fig. 4).

The MED_{50} of the agents in combination in EMEM and in mannose-pyruvate medium indicates that 3-deazaUR was slightly more inhibitory of JEV replication in EMEM than in glu-

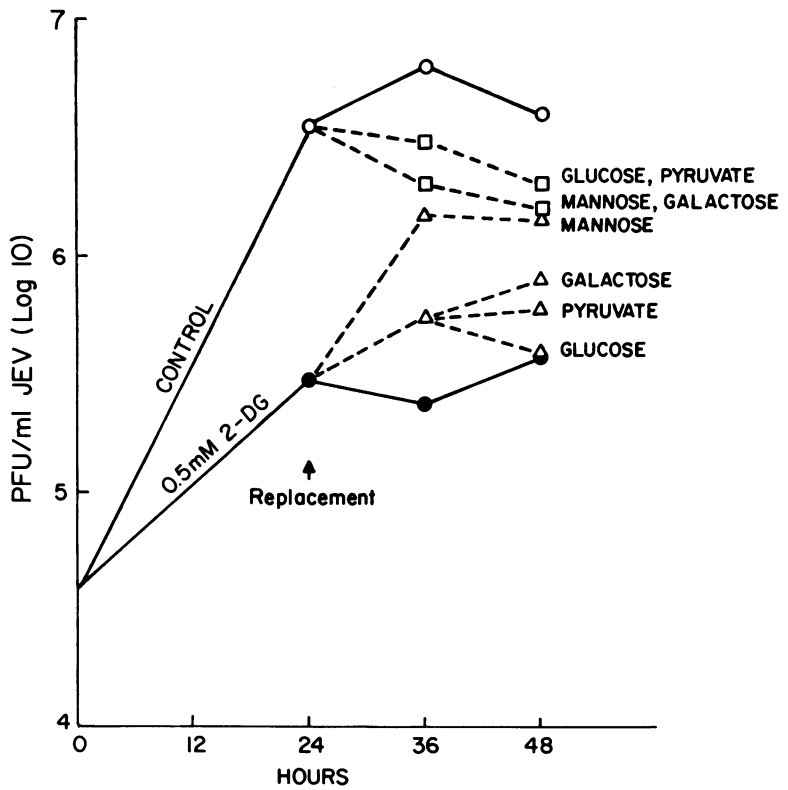

FIG. 3. *Effect of replacing 0.5 mM 2-DG with various metabolites 24 h after JEV infection of BHK-21 cell cultures maintained on EMEM. Metabolite concentrations in maintenance media were glucose (1 g/liter), sodium pyruvate (550 mg/liter), galactose (900 mg/liter), or mannose (1 g/liter). JEV titers in the presence of 0.5 mM 2-DG (●), with metabolite replacements containing no 2-DG (△), control without 2-DG (○), and control with metabolite replacement (□).*

TABLE 3. *Synergistic interaction of 2-DG and 3-deazaUR on JEV replication in glucose medium*

Agent	Concn (μM) of:		Count as % of untreated controls in presence of:			
	2-DG	3-deazaUR	2-DG (a)	3-deazaUR (b)	2-DG + 3-deazaUR	
					Predicted[a] (c)	Measured (d)
BHK-21	75	2.0	96	77	74	78
	150	2.0	85	77	65	68
	333	2.0	73	77	56	64
	75	4.0	96	73	70	74
	150	4.0	85	73	62	63
	75	20.0	96	55	53	55
	150	20.0	85	55	47	55
	150	40.0	85	40	34	46
JEV	75	2.0	78	62	48	31[b]
	150	2.0	54	62	33	20[b]
	333	2.0	43	62	27	18[b]
	75	4.0	78	58	45	20[b]
	150	4.0	54	58	31	11[b]
	75	20.0	78	54	42	20[b]
	150	20.0	54	54	29	11[b]
	150	40.0	54	34	18	5[b]

[a] Summation as defined by Webb (19), i.e., c = (a times b)/100. The letters refer to the numbers in the labeled columns.

[b] Significant potentiation, i.e., d < 0.7 times c.

cose-free medium (Fig. 4). Both lines in Fig. 4 indicate the enhanced antiviral activity demonstrated by the combination of noncytotoxic levels of 2-DG and 3-deazaUR in either EMEM or glucose-free medium. BHK-21 cell viability data computed for each compound, individually and in combination, indicated no significant cytotoxicity.

DISCUSSION

The production of viral glycoproteins and viral RNA via host-mediated mechanisms is a prerequisite for the subsequent assembly of the viral particle. The pivotal position of uridine 5'-triphosphate (UTP) and CTP as common precursors for RNA and the relative importance of UTP and glucose in glycosylation reactions was emphasized in Fig. 1. Multiple inhibition of sites immediately preceding RNA and glycoprotein synthesis would greatly facilitate the action of each competitive inhibitor. If the pool of competing natural substrate can be depleted via a judicious combination of antimetabolites, this should greatly enhance the effectiveness of the inhibitors. Clearly, the depletion of the CTP pool via the competitive action of 3-deazaUTP on CTP synthetase and the subsequent depletion of the UTP pools through the incorporation of 2-DG and 3-deazaUTP into the sugar nucleotides might potentiate the effect of 2-DG on JEV production. Our experiments were designed to test this prediction and our results have confirmed it for studies in vitro.

The uridine analogue 3-deazaUR and the antimetabolite 2-DG have both been shown to inhibit the production of infectious RNA viruses (5, 7, 9–11). The precise step of inhibition by 2-DG is not known; however, if the group B arboviruses are first assembled as immature forms containing two membrane glycoproteins, the incorporation of 2-DG might prevent assembly and/or subsequent maturation of JEV to the infectious form (6, 14, 17, 18). Recently, the nucleoside-diphosphate derivatives of 2-DG in BHK-21 cells have been identified as uridine

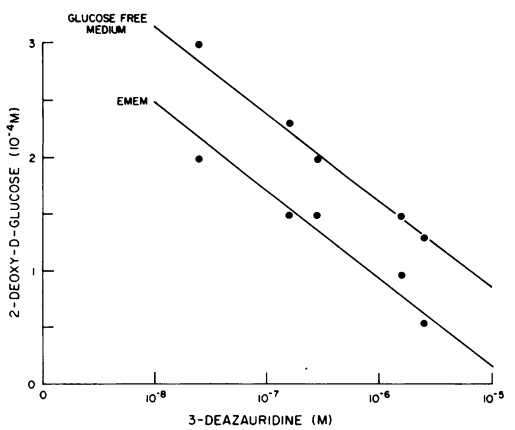

FIG. 4. *JEV yield reduction measured as the MED_{50} dose of various combinations of 3-deazaUR and 2-DG in BHK-21 cell cultures maintained in either EMEM or glucose-free medium.*

diphosphate-2-deoxy-D-glucose (UDP-2-DG) and guanidine diphosphate-2-deoxy-D-glucose (GDP-2-DG) (13). The discovery of the activated intermediates of ^{14}C-labeled 2-DG in BHK-21 cells indicates that the corresponding nucleoside diphosphate derivatives of UDP-2-DG and GDP-2-DG can be expected to contribute to the inhibition of the transglycosylation reactions during arboviral infections (4, 7). The viral inhibitory activity of 3-deazaUR, shown to occur during viral replication, was reversed by the naturally occurring pyrimidine nucleoside (9). Metabolic studies have shown that 3-deazaUR, phosphorylated by leukemia L-1210 cells, exerts its inhibitory activity by interfering with the activity of the CTP synthetase (11). Shannon et al. (16) showed that 3-deazaUR possessed a broad spectrum of antiviral activity against several important human RNA viruses. Although the precise biochemical mechanisms of these two drugs are not known, their combination could be expected to interfere with both glycoprotein and RNA synthesis in a synergistic manner.

Our studies of the inhibition of the production of JEV in BHK-21 cells by either 2-DG or 3-deazaUR alone or in combination were limited to determinations of infectivity. Although each of these compounds was shown to possess some antiviral activity against JEV at noncytotoxic concentrations, the levels of inhibition observed with combinations of 2-DG and 3-deazaUR were synergistic. Since it was possible to achieve this increased antiviral effect without any significant increase in cytotoxicity, our results demonstrate the potential feasibility of this approach to antiviral chemotherapy. Ours is the first attempt to study the combination of these two drugs against viral infections. Since JEV is an important human pathogen, and no effective treatment for the disease has been described, our studies should be extended to the animal model in order to evaluate the treatment potential for man. Indeed, our results indicate that this approach to antiviral chemotherapy might parallel the advances made in cancer chemotherapy.

ACKNOWLEDGMENTS

We wish to express our thanks to Robert Nye and Richard Grays for their excellent technical assistance. We also wish to thank Emilio Weiss for his constructive and helpful reviews of the manuscript.

This investigation was supported by the Navy Medical Research and Development Command, Research Task no. MPN03.12.5031.

LITERATURE CITED

1. Diercks, F. H., and W. McD. Hammon. 1958. Hamster kidney cell tissue cultures for propagation of Japanese B encephalitis virus. Proc. Soc. Exp. Biol. Med. 97:627-632.
2. Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. Science 130:432-437.
3. Ehrlich, J., B. J. Sloan, F. A. Miller, and H. E. Machamer. 1956. Searching for antiviral materials from microbial fermentations. Ann. N.Y. Acad. Sci. 130:5-16.
4. Froger, C., and P. Louisot. 1972. Glycoprotein biosynthesis in arbovirus infected cells. II. Study of microsomic mannosyl transferase activity. Int. J. Biochem. 3:613-622.
5. Gandhi, S. S., P. Stanley, J. M. Taylor, and D. O. White. 1972. Inhibition of influenza viral glycoprotein synthesis by sugars. Microbios 5:41-50.
6. Kaluza, G., M. F. G. Schmidt, and C. Scholtissek. 1973. Effect of 2-deoxy-D-glucose on the multiplication of Semliki Forest virus and reversal of the block by mannose. Virology 54:179-189.
7. Kaluza, G., C. Scholtissek, and R. Rott. 1972. inhibition of the multiplication of enveloped RNA viruses by glucosamine and 2-deoxy-D-glucose. J. Gen. Virol. 14:251-259.
8. Karabatsos, N., and S. M. Buckley. 1967. Susceptibility of the baby hamster kidney cell line (BHK-21) to infection with arboviruses. Am. J. Trop. Med. Hyg. 16:99-105.
9. Khare, G. P., R. W. Sidwell, J. H. Huffman, R. L. Tolman, and R. K. Robins. 1972. Inhibition of RNA virus replication *in vitro* by 3-deazacytidine and deazauridine. Proc. Soc. Exp. Biol. Med. 140:880-884.
10. Klenk, H. D., C. Scholtissek, and R. Rott. 1972. Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. Virology 49:723-734.
11. McPartland, R. P., M. C. Wang, A. Bloch, and H. Weinfeld. 1974. Cytidine 5'-triphosphate synthetase as a target for inhibition by the antitumor agent 3-deazauridine. Cancer Res. 34:3107-3111.
12. Mitamura, R. 1936. Uber das Virus der epidemischen Enzephalitis (Sommerenzephalitis) mit besonderer Berucksichtigung seiner immunologischen Eigenschaften und seiner Beziehung zu einem Virus-Stamm der Winterenzephalitis. Trans. Soc. Pathol. Jpn. 26:429-452.
13. Schmidt, M. F. G., R. T. Schwarz, and C. Scholtissek. 1974. Nucleoside-diphosphate derivatives of 2-deoxy-D-glucose in animal cells. Eur. J. Biochem. 49:237-247.
14. Scholtissek, C. 1973. Glycoprotein synthesis of enveloped viruses. Adv. Biosci. 11:193-204.
15. Scholtissek, C. 1975. Inhibition of the multiplication of enveloped viruses by glucose derivatives. Curr. Top. Microbiol. Immunol. 70:101-119.
16. Shannon, W. M., G. Arnett, and F. M. Schabel, Jr. 1972. 3-Deazauridine: inhibition of ribonucleic acid virus-induced cytopathogenic effects in vitro. Antimicrob. Agents Chemother. 2:159-163.
17. Shapiro, O., W. E. Brandt, and P. K. Russell. 1972. Change involving a viral membrane glycoprotein during morphogenesis of group B arboviruses. Virology 50:906-911.
18. Shapiro, D., K. A. Kos, and P. K. Russell. 1973. Japanese encephalitis virus glycoproteins. Virology 56:88-94.
19. Webb, J. L. 1963. Enzyme and metabolic inhibitors, vol. 1. Academic Press Inc., New York.