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EVALUATION OF THE ANTI-HERPESVIRUS DRUG COMBINATIONS: VIRAZOLE PLUS ARABINOFURANOSYLHYPOXANTHINE AND VIRAZOLE PLUS ARABINOFURANOSYLADENINE

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Combinations of Virazole plus arabinofuranosylhypoxanthine (ara-Hx) and Virazole plus arabinofuranosyladenine (ara-A) were investigated in KB or BHK cells infected with types 1 or 2 herpes viruses. Combinations of Virazole and ara-Hx exhibited significant synergy as evaluated graphically (isobolograms) or by fractional inhibitory concentration (FIC) indices. Optimal ratios for the combination were 1:1 to 1:10 for Virazole to ara-Hx. At these ratios, FIC indices in the range of 0.5–0.2 were commonly observed. Combinations of Virazole and ara-A were antagonistic when observed in the presence of pentostatin, an adenosine deaminase inhibitor. In the absence of pentostatin, the minimum inhibitory concentration (MIC) of ara-A and degree of synergy with Virazole were variable.

Virazole ara-A ara-Hx herpesvirus, types 1 and 2

INTRODUCTION

The search for antiviral drugs has resulted in the licensing of several for clinical use. The investigation of drug combinations and drugs whose mechanisms are specific for viruses has been stimulated by the lack of sensitivity of some viruses to existing drugs, development of drug resistance by viruses, drug toxicity and lack of virus specificity. As indicated by many investigators [1,3,6,10,12,19–21], combinations of drugs should be more effective or synergistic if the mode of action of the drugs is different. Another desired effect of combinations is virus inhibition at minimally toxic or non-toxic drug levels. The goal of combinations is to attain potent antiviral activity with a minimum of drug toxicity.

Combinations of antiviral compounds were first explored by Bauer [2], who observed synergy between isatin thiosemicarbazone and certain phenoxypyrimidines against vaccinia mouse encephalitis.

Since that original observation by Bauer, many combinations of drugs or drugs with

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interferon (IFN) or IFN inducers have been initiated. A review of these investigations revealed that many methodological problems existed. Regardless of the drug pair, results of combination studies depend on the virus–cell system and the particular parameter of inhibition which was observed. Also, the criterion for evaluation of effects was often not defined and inadequate numbers of observations were made. Recently, two groups [1,19–21] have adopted techniques previously developed for use in bacterial systems by Elion et al. [3], Loewe [6], and Sabath [12]. Each used a graphic technique (isobologram) for plotting the minimum inhibitory concentration (MIC) of each drug alone and in combinations or the fractional inhibitory concentrations (FIC). The FIC [3,10] is derived by the following formula: MIC drug in combination/MIC drug alone. Regarding the isobologram, all authors [1,3,6,12,19–21] agree that points (representing combination concentrations) falling below a line drawn between the MIC of drugs A and B represent synergism. Points near or on the line represent additive effects. There is no consensus in the interpretation of drug interaction when points fall above the line. As indicated in the methods section, we offer interpretation based on the concepts of Loewe [6].

Another useful technique [1,10] is that of summing the FIC to derive an FIC index. We have expanded the criteria for utilization of FIC indices in the identification of the type of drug interaction as indicated in the methods.

Traditionally, methods developed for antibacterial drug combinations [3,6,10,12] involved testing a checkerboard of drug concentrations; that is, for any one concentration of one drug several concentrations of the other were combined with it and the reverse situation for the other drug. Many of the early antiviral drug combination studies neglected consideration of this concept probably because of the extensive methodology involved. Unfortunately, when only a limited number of concentrations is used, it is very difficult to evaluate the effectiveness of the combinations. We have found that evaluation of several concentrations of selected fixed ratios of one drug to the other provides ample data for analyses of type of drug interaction.

In addition, we emphasize the importance of toxicity evaluations and determinations of antiviral indices which utilize maximum tolerated concentrations and minimum inhibitory concentrations as the therapeutic index does.

Since our study has in part been directed at better defining evaluation criteria, we have limited our current investigations to combinations of ara-A and its major metabolic product ara-Hx with Virazole, drugs readily available in our laboratory.

MATERIALS AND METHODS

Compounds

Ara-A (9-β-D-arabinofuranosyladenine), ara-Hx (9-β-D-arabinofuranosylhypoxanthine), and pentostatin [(*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydro(4,5-d)(1,3)diazepin-8-ol] were obtained from Warner Lambert/Parke Davis, Ann Arbor, MI.

Virazole (ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was received from ICN Pharmaceuticals, Covina, CA.

Cells and media

Two cell lines were used in these studies. KB cells (nasopharyngeal carcinoma) were supplied by Dr. William Shannon, (Southern Research Institute, Brimingham AL). The BHK-21 (baby hamster kidney) cells were from the cell culture laboratory*. Growth medium consisted of Eagle's minimum essential medium (MEM, with Hanks' balanced salts, Biofluids, Inc., Rockville, MD), 10% fetal bovine serum (FBS, Irvine Scientific, Irvine, CA), 1 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes, Sigma Chemical Company, St. Louis, MO), and 50 μg/ml gentamycin (Schering Corporation, NJ). For antiviral experiments 2.4×10^4 cells in 0.2 ml were dispensed into each well of microtissue culture plates (Linbro, Flow Laboratories, Rockville, MD). The medium used for drug experiments was 199 with Earle's balanced salts plus 5% FBS, gentamycin, Hepes, and 0.065% $NaHCO_3$.

Viruses

Type 1 herpes virus (HV-1), strain HF, was obtained from the ICN Nucleic Acid Research Institute, Irvine, CA. Type 2 herpes virus (HV-2), strain VF-2, was obtained from Howard Moss at Wright State Medical School, Dayton, OH.

Antiviral evaluations

Initially, active levels of each drug alone were determined. For each combination, a series of five fixed ratios (10:1, 5:1, 1:1, 1:5, 1:10) of one drug to the other were evaluated. Each microplate was utilized to test seven concentrations of a fixed ratio and each drug alone. In some experiments, a single concentration of one drug was observed with varying concentrations of the other drug.

In these experiments, serial two-fold dilutions (four cups per concentration) of drug or combinations from totally inhibitory to non-inhibitory concentrations were added to ~ 18–24 h monolayers in microplates [14]. Within 15–30 min 100–320 times the 50% infectious dose (ID_{50}) of virus was added. Two cups per concentration containing drug and media only were used for observation of drug cytotoxicity. Experimental controls included: cell controls (cells plus 0.2 ml medium) and virus controls (cells plus 0.1 ml medium and 0.1 ml medium with virus). Microplates were then wrapped with Saran Wrap (Dow Chemical Company, Midland, MI) and incubated at 37°C for 72 h. The

*These cells were produced with support from the National Cancer Institute, Biological Carcinogenesis Branch; Division of Cancer Cause and Prevention, under the auspices of the office of Naval Research and the Regents of the University of California.

cytopathic effect (CPE) was then observed microscopically and scored on a 0–4 basis with 4 representing total cell destruction. The concentration of drug which inhibits CPE by 50% is considered the minimum inhibitory concentration (MIC). When necessary, these concentrations were calculated by a linear regression technique.

Evaluation of virus inhibitory activities of the drug combinations

A graphic method of evaluating combinations [3,6,12] as illustrated in Fig. 1 was utilized. The method consists of plotting on an arithmetic scale the amount of drug A and B that alone or in various combinations produce the same effect (50% CPE inhibition or MIC). If the combinations are simply additive, the points will fall near or on a line drawn between the MIC of drugs A and B. If combinations are synergistic, the concentrations required to produce the desired effect will be lowered and points will fall below the line. The action is called indifference if the drugs do not interact or interfere with each other and the points fall above the line. Antagonism occurs when an MIC of either drug is greater in combination than it is alone. In this case the points fall beyond lines perpendicular to each MIC.

In addition to the graphic technique, a mathematical technique was used to evaluate the combination effectiveness. As originally utilized [1,3,10], fractional inhibitory concentration (FIC) indices were derived by the following formula:

$$\text{FIC index} = \frac{(\text{MIC of drug A in comb.})}{(\text{MIC of drug A alone})} + \frac{(\text{MIC of drug B in comb.})}{(\text{MIC of drug B alone})}$$

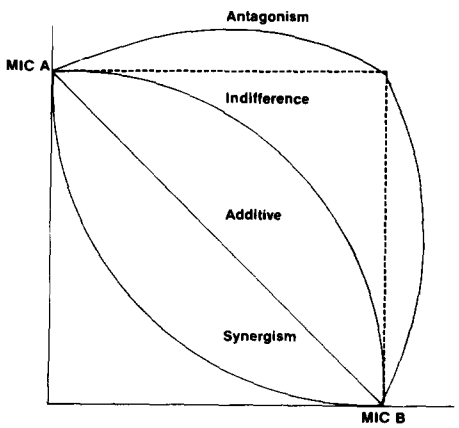


Fig. 1. Graphic evaluation of antiviral drug combinations.

We have expanded the interpretation of the indexes as follows:

FIC index <0.5	Significant synergism
FIC index 0.5–0.9	Suggestive of synergism
FIC index ~1	Effects are additive
FIC index 1.1–1.9	Indifference or partial antagonism
FIC index >2	Antagonism

Partial antagonism may occur if the amount of one drug in combination is increased over the MIC it exhibits alone. If the MIC of both drugs is increased, the result is clear antagonism.

Toxicity evaluation

Drug toxicity was evaluated by a viable cell count technique and by a neutral red spectrophotometric measure of cell mass/viability (dye uptake method).

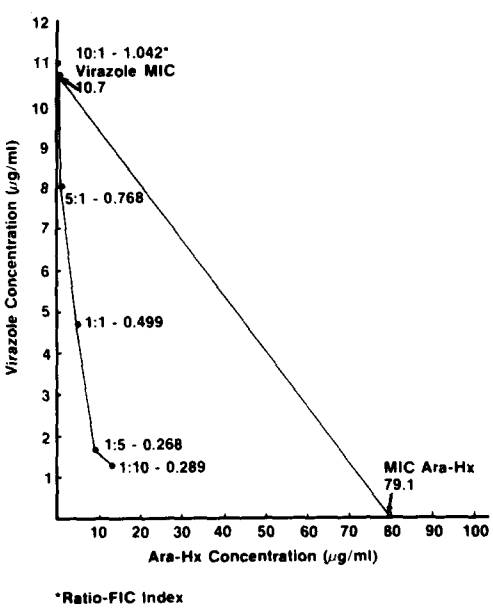
In the cell count method, varying drug concentrations were added 4 h after 10^5 KB cells/ml were seeded into 35 mm wells of multi-well plates (Linbro). Drug exposure was terminated at 72 h by replacement of medium with fresh drug-free medium. At 24, 72 and 120 h after seeding, cells were washed with saline, trypsinized, and viable cells were counted using trypan blue.

The other method allowed 18–24 h for establishment of cell monolayers in microplates before drugs were added. Cell mass and viability were evaluated before addition of drug and after 72 h drug exposure. For evaluation, cells were treated 30 min with medium containing 50 μg/ml neutral red, washed, lysed with 50% ethanol and 0.03 M NaH_2PO_4 and diluted 1 : 5. The absorbance was determined at 540 nm in a Gilford spectrophotometer. Pretreatment uptake values were substracted from 72 h values. The dye uptake of drug-treated cells was then compared to cell controls. The concentration which did not kill but reduced uptake by 80% was calculated by a linear regression technique.

RESULTS AND DISCUSSION

Evaluation of combinations of Virazole and ara-Hx

Virazole and ara-Hx were combined in the five standard fixed ratios and tested against HV-1 in KB cells. The mean values of three experiments are presented in Fig. 2. The most effective combinations were these 1:5 (1.7 + 8.5 μg/ml) and 1:10 (1.33 + 13.3 μg/ml) ratios of Virazole and ara-Hx. The MIC of Virazole alone was 10.7 μg/ml and that of ara-Hx alone 79.1 μg/ml. As indicated by the FIC indices, the 1:5 ratio is slightly better than the 1:10 ratio. In a different protocol, a standard amount of Virazole (8, 4, 2 or 1 μg/ml) was added and the amount of ara-Hx needed to produce 50% protection was



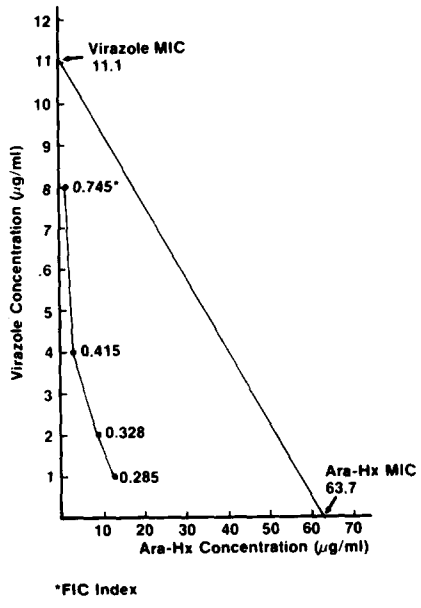


Fig. 2. Comparison of the MIC of Virazole and ara-Hx alone and in various fixed ratio combinations in KB cells.

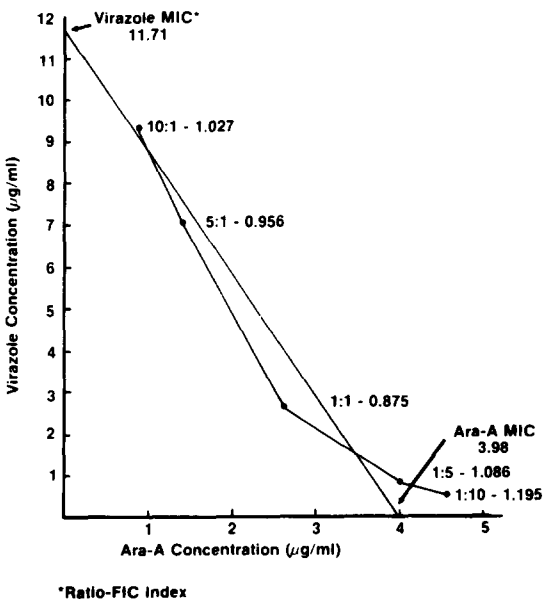
Fig. 3. Effect of a single Virazole concentration on the MIC of ara-Hx in KB cells.

determined (Fig. 3). In these experiments, Virazole and ara-Hx alone had MIC values of 11.1 and 63.7 μg/ml. The lowest FIC indices were observed when Virazole was used at 2 μg/ml (plus 9.4 μg/ml ara-Hx) or 1 μg/ml (plus 12.4 μg/ml ara-Hx). The ratios of drugs were 1 : 4.7 and 1 : 12.4.

Evaluation of combinations of Virazole and ara-A

In Virazole–ara-A combination experiments, when ara-A was quite active as in Fig. 4 with an MIC of ~4 μg/ml, there was little or no synergy and some antagonism was observed. In other experiments, where the ara-A MIC was higher, some synergy was observed. These results seemed to change with culture conditions, such as lot and source of serum and media. On the contrary, the MIC of Virazole and ara-Hx did not vary appreciably with culture conditions. It is possible that serum deaminase and various culture conditions affect the levels of cellular adenosine deaminase and the rate and/or degree of conversion of ara-A to ara-Hx. Since ara-Hx is less active and the major metabolic product of ara-A, these findings led us to believe that ara-Hx was acting differently from ara-A and that it was the active component in synergistic combinations.

We decided to use pentostatin to prevent the conversion of Ara-A to ara-Hx in an attempt to differentiate the activities of Ara-A and ara-Hx. Combinations of Virazole and ara-A exhibited antagonism as shown in Fig. 5. In KB cells, pentostatin reduced the MIC of Virazole from ~12 to ~6 μg/ml and that of ara-A from ~6 to ~0.6 μg/ml. Pento-



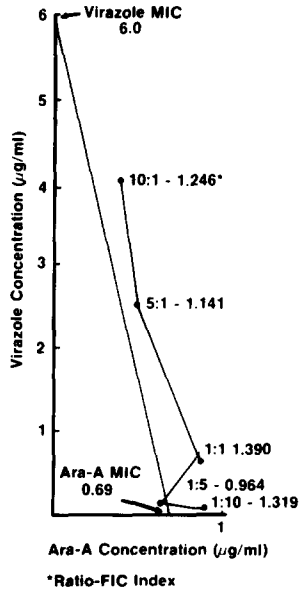


Fig. 4. Comparison of the MIC of Virazole and ara-A alone and in various combinations in KB cells under cultural conditions in which ara-A is highly active.

Fig. 5. Effect of adenosine deaminase inhibitor (ADI) on combinations of Virazole and ara-A in KB cells.

statin not only reduced the MIC of ara-A, but also increased its toxicity. In BHK cells, the effect of pentostatin on ara-A activity and toxicity was similar to that seen in KB cells. In contrast, pentostatin reduced the MIC of Virazole from ~14 to ~2 μg/ml with a concomitant increase in toxicity in BHK cells. These findings may suggest that some of the same deaminase enzymes are involved with the degradation and detoxification of both ara-A and Virazole. Pentostatin only slightly affected the MIC of ara-Hx in KB cells (~75–~65 μg/ml) or BHK cells (~15–~14 μg/ml). When combinations of Virazole and ara-Hx were tested in the presence of pentostatin FIC indices of 0.5–0.6 were observed at the 1 : 5 and 1 : 10 ratios of Virazole to ara-Hx. These experiments actually represent combinations of three drugs and many actions which cannot be defined may occur in the virus–host system.

Combinations of Virazole and ara-Hx in BHK cells

Since drug action and metabolism as well as virus replication may vary with the host cell system, we tested the usual fixed ratios against the same virus, but in a different cell line (BHK). As shown in Fig. 6, the drugs were nearly equal in effectiveness in this system. Synergism was demonstrated under these conditions with the optional ratios of Virazole to ara-Hx being 1 : 1 and 1 : 5.

Combination of Virazole and ara-Hx in BHK cells infected with HV-2

Using this virus (HV-2), Virazole was again more effective than ara-Hx. Even though the 1 : 5 ratio had the lowest FIC index, the 1 : 1 and 1 : 10 ratios were also quite effective (Fig. 7). These two latter studies illustrated that the synergy observed with Virazole and ara-Hx was not cell- or virus-dependent.

As indicated previously, drug combinations are expected to be synergistic if the drugs possess different mechanisms of action. Prior to initiating this study, combinations of Virazole plus ara-A and Virazole plus ara-Hx were both expected to exhibit synergism. As recently reviewed by Smith et al. [16], Virazole and ara-A have multifaceted but different mechanisms of action. Virazole 5′-monophosphate is a competitive inhibitor of inosine monophosphate (IMP) dehydrogenase [18] which results in depletion of cellular pools of guanosine monophosphate (GMP), thus inhibiting RNA synthesis [15]. This mechanism, thought to be responsible for toxicity associated with Virazole, is readily reversed by removal of the drug. Other mechanisms include Virazole 5′-triphosphate inhibition of influenza virus RNA polymerase [4] and the incorporation (as a guanosine analogue) of Virazole into the 5′ "cap" of certain viral messenger RNA (mRNA)

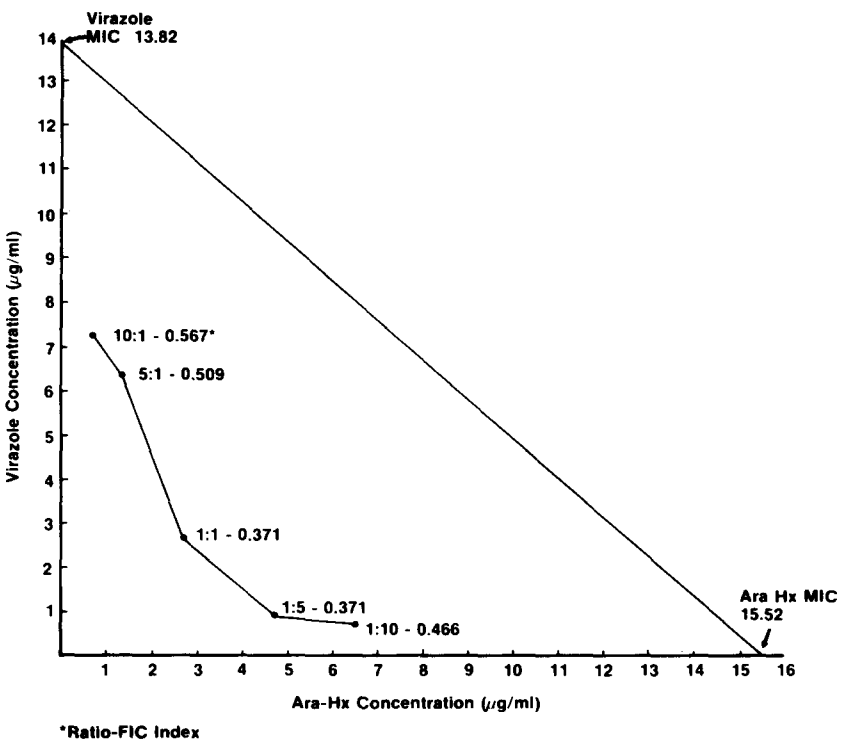


Fig. 6. Comparison of the MIC of Virazole and ara-Hx alone and in various fixed ratio combinations in BHK cells.

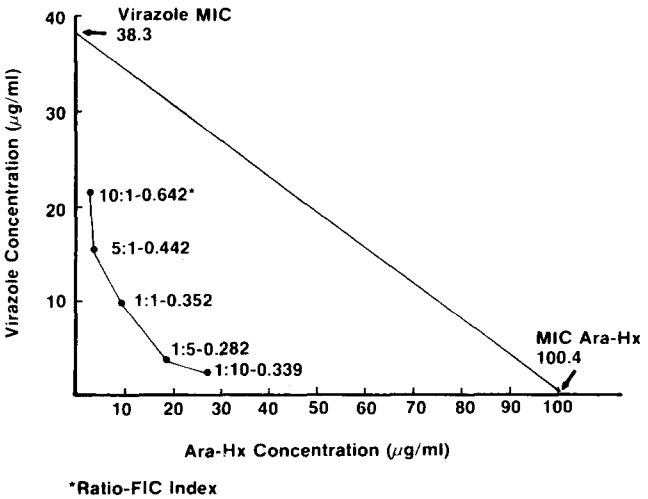


Fig. 7. Comparison of the MIC of Virazole and ara-Hx in various combinations in BHK cells infected with type 2 herpes virus.

strands [5]. In the light of the depletion of GMP pools and the incorporation into the mRNA of viruses, it is not surprising that Virazole has many effects on viral proteins. Inhibition of herpes virus may result from these effects on RNA synthesis or by mechanisms as yet unknown.

Metabolites of ara-A inhibit at least six enzymes [16]. Its mechanisms include: inhibition of cellular and herpes DNA synthesis [7–9,11], incorporation into DNA and RNA [9,11], DNA chain termination [8,11] and inhibition of viral mRNA polyadenylation [11]. The mechanism of action of ara-Hx is not clearly understood. It is both less active and less toxic than ara-A [13]. It does not have the strong inhibitory effect on cellular DNA synthesis that ara-A does [13]. Since ara-Hx 5′-triphosphate apparently can inhibit cellular DNA polymerases [8], it would not be surprising to find that it could inhibit herpes DNA polymerase. Other findings [17] suggest that ara-A and ara-Hx have similar anti-herpes mechanisms since both can be reversed by deoxyadenosine. Yet in this study, the Virazole–ara-Hx synergy is more pronounced than that seen with Virazole and ara-A, which may suggest that the anti-herpes mechanisms are somewhat different. Lack of antiviral synergism might result from combinations of two drugs having many actions if synergistic cellular toxicity occurred. This is not believed to be the situation with Virazole and ara-A, since cellular toxicity as observed microscopically was not appreciably changed in combinations of the two.

Toxicity evaluations

The effects of Virazole (5, 25, 125 μg/ml), ara-Hx (25, 125, 625 μg/ml) and a 1 : 5 ratio of Virazole to ara-Hx (1+5, 5+25, 25+125 μg/ml) on growth of KB cells was ob-

served (Figs. 8–10) by viable cell counts. Since no concentrations tested were lethal, we calculated the doses required to reduce growth by 80% on day 5 for comparison of effects. These were: Virazole 84.7 μg/ml, ara-Hx 471.21 μg/ml, and the 1 : 5 combination 20 + 100 μg/ml. The calculated degree of growth inhibition at the MIC (shown in Fig.2) of each drug and the 1 : 5 combinations were: Virazole 50.5%, ara-Hx 38.75%, and the combination 20.6%.

The effects of the drugs on an established monolayer culture were observed by the neutral red uptake technique. Once again, the 80% reduction concentration was calculated. These were: Virazole 183.7 μg/ml, ara-Hx ~1600 μg/ml, and the 1 : 5 combination 51.7 μg/ml + 258.5 μg/ml. Microscopic observation of ara-Hx-treated cells revealed some abnormal changes including swollen and enlarged cells. The cells remained capable of absorbing neutral red as indicated by marginal effect on uptake.

We utilized the toxicity data to determine if the antiviral index of the combination was different from that of the individual drugs. The formula for the antiviral index (AI) is:

$$\frac{\text{Maximum tolerated dose (MTD)}}{\text{Minimum inhibitory dose (MID)}}$$

The 80% concentrations per MIC were used to obtain estimates of AI (Table 1). Using the viable cell counts, the AI values for Virazole, ara-Hx, and the 1 : 5 combination were 7.85, 5.96 and 11.76, respectively. When the neutral red technique was used, the AI were 17.17, 20.23 and 30.41, respectively. In comparing these data for the viable cell count

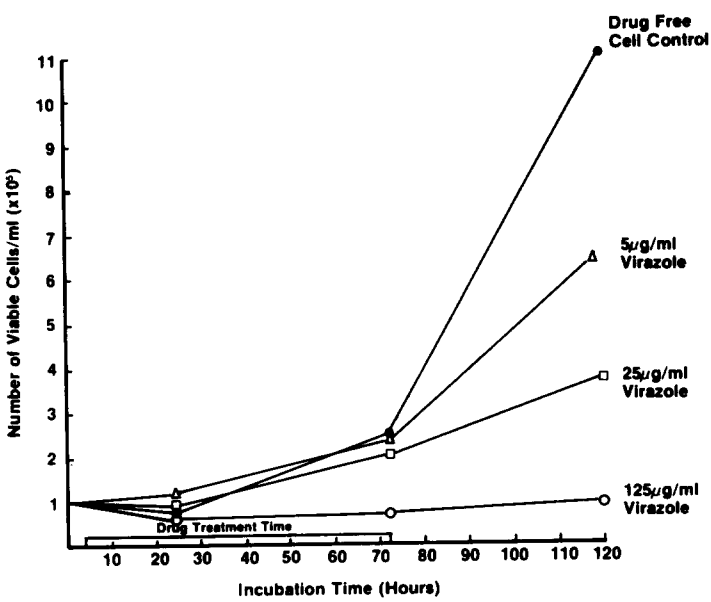


Fig. 8. Effect of Virazole on the growth of KB cells.

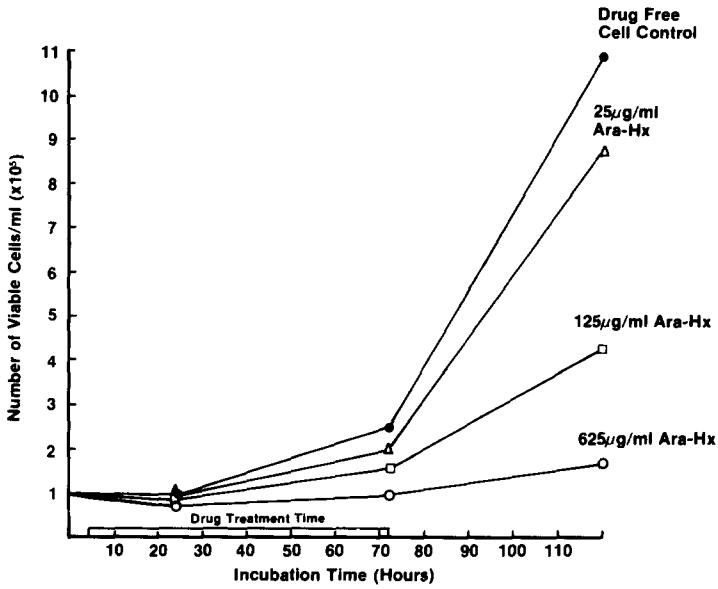


Fig. 9. Effect of ara-Hx on the growth of KB cells.

technique, the AI of the combination is 85.2% of the total of the individual drug AI values (7.85 + 5.96 = 13.81). For the dye uptake method, the AI of the combination is 81.3% of the total of the individual drugs (17.17 + 20.23 = 37.4).

As indicated, combinations of Virazole and ara-Hx exhibited both increased anti-

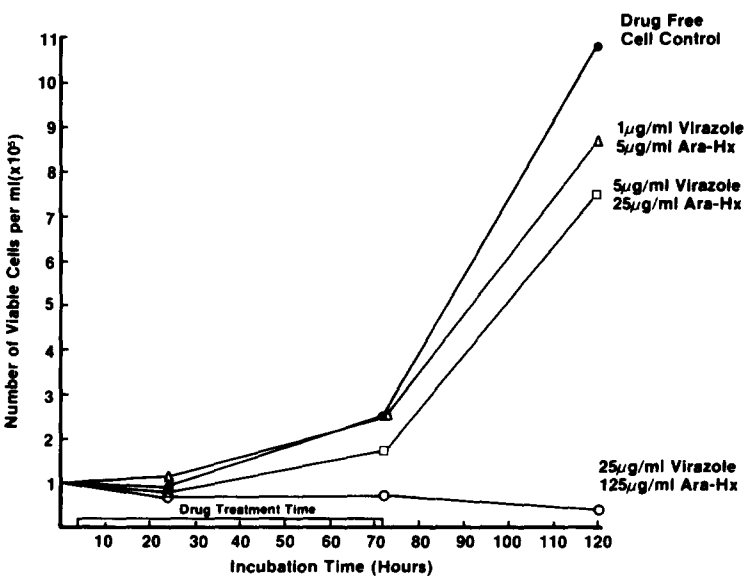


Fig. 10. Effect of a combination of Virazole and ara-Hx in a 1 : 5 ratio on the growth of KB cells.

TABLE 1

Antiviral indices of Virazole and ara-Hx alone and in a 1:5 Virazole to ara-Hx combination in KB cells

Experimental group	80% cytostatic concentration (μg/ml)	Minimum inhibitory concentration (μg/ml)	Antiviral index
Viable cell count technique			
Virazole	84.7	10.7	7.85
Ara-Hx	471.21	79.1	5.96
Virazole + ara-Hx	20.0	1.7	11.76
	100.0	8.5	
Neutral red dye uptake			
Virazole	183.7	10.7	17.17
Ara-Hx	∿1600.0	79.1	20.23
Virazole + ara-Hx	51.7	1.7	30.41
	258.5	8.5	

herpes activity and cytostatic effects in KB cells. Although there were increased cytostatic effects, the antiviral indices of the combination were higher than either drug alone. This indicates that the effective concentrations were appreciably lower than the cytostatic concentrations and that the margin of safety was increased.

SUMMARY AND COMMENTS

In these investigations, we have shown that evaluations of seven concentrations of five fixed ratios in five microplates provide ample data for evaluation of antiviral drug combinations. We have extended the criteria for utilizing FIC indices and recommend that these be used in addition to isobologram evaluation of drug combinations. It appears that the relative effectiveness of each drug strongly affects the optimal ratios and position of points in the isobolograms. Drugs that have similar MIC are likely to have optimal ratios of ~1 : 1. When optimal ratios are not equal, the drug with a weaker activity will generally be needed in greater quantities as was seen with Virazole and ara-Hx. In the isobolograms, the curves will be skewed to the axis of the more active drug as seen in Fig. 2. For example, in those experiments the Virazole MIC was 10.7 μg/ml and that of ara-Hx 79.1 μg/ml. The optimal ratios were in the 1 : 5–1 : 10 range with the points on the curve being nearer to the Virazole axis.

We have also utilized two techniques for assaying drug cytotoxic or cytostatic effects on cells and believe that toxicity/activity of combinations should be assayed by a therapeutic index type technique such as the antiviral index. No evaluation of drug combinations should be considered complete without toxicity evaluations.

We believe these methods are applicable to the evaluation of combinations of the many promising antiviral drugs developed in recent years. Unfortunately, in vitro investigations do not necessarily predicts effects in animal models or humans. Systemic administration of promising combinations will only be beneficial if the metabolism and distribution of the separate drugs result in significant concentrations of each being achieved in the target organs. Topical application of promising anti-herpes virus combinations presents an ideal opportunity for in vivo evaluation without the complications associated with systemic administrations.

REFERENCES

1 Ayisi, N.K., Gupta, S., Meldrum, J.B., Taneja, A.K. and Babiuk, L.A. (1980) Combination chemotherapy: interaction of 5-methoxymethyldeoxyuridine with adenine arabinoside, 5-ethyldeoxyuridine, 5-iododeoxyuridine and phosphonoacetic acid against herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. 17, 558–566.

2 Bauer, D.J. (1955) The antiviral and synergistic actions of isatin thiosemicarbazone and certain phenoxypyrimidines in vaccinia infection in mice. Br. J. Exp. Pathol. 36, 105–114.

3 Elion, G.B., Singer, S. and Hitchings, G.H. (1954) Antagonists of nucleic acid derivatives. VIII. Synergism in combinations of biochemically related antimetabolites. J. Biol. Chem. 208, 477–488.

4 Eriksson, B., Helgstrand, E., Johansson, N.G., Larsson, A., Misiorny, A. Noren, J.O., Philipson, L., Sternberg, K., Stening, G. Stridh, S. and Oberg, B. (1977) Inhibition of influenza virus ribonucleic acid polymerase by ribaviran triphosphate. Antimicrob. Agents Chemother. 11, 946–951.

5 Goswami, B., Borek, E., Sharma, O., Fujitaki, J. and Smith, R.A. (1979) The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. Biochem. Biophys. Res. Commun. 89, 830–836.

6 Loewe, S. (1953) The problem of synergism and antagonism of combined drugs. Arzneimittel-Forsch. 3, 285–290.

7 Muller, W.E.G., Zahn, R.K., Beyer, R. and Fallse, D. (1976) 9-β-D-Arabinofuranosyladenine as a tool to study herpes simplex virus DNA replication in vitro. Virology 76, 787–796.

8 Muller, W.E.G., Arendis, J., Maidhof, A., Zahn, R.K. and Geurtsen, W. (1981) Metabolism of 9-β-D-arabinofuranosylhypoxanthine and 9-β-D-arabinofuranosyladenine in L5178y mouse lymphoma cells. Chemotherapy 27, 53–60.

9 Pelling, J.C., Drach, J.C. and Shipman Jr., C. (1981) Internucleotide incorporation of arabinosyladenine into herpes simplex virus and mammalian cell DNA. Virology 109, 323–335.

10 Phillips, I. and Warren, C. (1976) Activity of sulfamethoxazole and trimethoprim against *Bacteriodes fragilis*. Antimicrob. Agents Chemother. 9, 736–740.

11 Rose, K.M. and Jacob, S.T. (1978) Selective inhibition of RNA polyadenylation by ara-ATP in vitro: a possible mechanism for antiviral action of ara-A. Biochem. Biophys. Res. Commun. 81, 1418–1424.

12 Sabath, L.D. (1968) Synergy of antibacterial substances by apparently known mechanisms. In: Antimicrobial Agents in Chemotherapy. Ed.: Hobby, G.L. (American Society for Microbiology, Bethesda) pp. 210–217.

13 Shipman, Jr., C., Smith, S.H., Carlson, R.H. and Drach, J.C. (1976) Antiviral activity of arabinosyladenine and arabinosylhypoxanthine in herpes simplex virus-infected KB cells: selective inhibition of viral deoxyribonucleic acid synthesis in synchronized suspension cultures. Antimicrob. Agenst Chemother. 9, 120–127.

14 Sidwell, R.W. and Huffman, J.H. (1971) Use of disposable microtissue culture plates for antiviral and interferon induction studies. Appl. Microbiol. 22, 797–806.
15 Smith, C.M., Fontenelle, L.J., Unger, N., Brox, L.W. and Henderson, J.F. (1974) Inhibitors of inosinate dehydrogenase activity in Ehrlich acites tumor cells in vitro. Biochem. Pharmacol. 23, 2727–2735.
16 Smith, R.A. Sidwell, R.W. and Robins, R.K. (1980) Antiviral mechanisms of action. Ann. Rev. Pharmacol. Toxicol. 20, 259–284.
17 Smith, S.H., Shipman, C. and Drach, J.C. (1978) Deoxyadenosine antagonism of the antiviral activity of 9-β-D-arabinofuranosyladenine and 9-β-D-arabinofuranosylhypoxanthine. Cancer Res. 38, 1916–1921.
18 Streeter, D.G., Witkowski, J.T., Khare, G.D., Sidwell, R.W., Bauer, R.J. Robins, R.K. and Simon, L.N. (1973) Mechanism of action of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole) a new broad spectrum antiviral agent. Proc. Natl. Acad. Sci. U.S.A. 70, 1174–1178.
19 Surjano, I. and Wigand, R. (1981) Combined inhibition of vaccinia virus multiplication by inhibitors of DNA synthesis. Chemotherapy 27, 179–187.
20 Wigand, R. and Hassinger, M. (1980) Combined antiviral effect of DNA inhibitors on herpes simplex virus multiplication. Med. Microbiol. Immunol. 168, 179–190.
21 Wirjawan, E. and Wigand, R. (1978) Combined antiviral effect of DNA inhibitors on adenovirus multiplication. Chemotherapy 24, 347–353.