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Review

Consensus symposium on combined antiviral therapy

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1. Introduction

During the last decade, considerable progress has been made in the development of antiviral agents, resulting in an increasing list of drugs approved by national regulatory authorities, such as the United States Food and Drug Administration, for the treatment of viral infections. The expanded efforts in drug screening and rational drug design in response to the human immunodeficiency virus (HIV) pandemic and the consequent occurrence of HIV-related opportunistic viral infections have greatly contributed to this progress in the field of antiviral therapy. Monotherapy with the array of currently available antiviral drugs has been shown to result in varying degrees of clinical benefit in a variety of viral infections, including herpesviruses, hepatitis viruses, respiratory viruses and HIV. However, these benefits are often limited by incomplete suppression of viral replication, or by the development of drug resistance, while drug toxicity may limit optimal dosing. These limitations of antiviral therapy have become especially clear in the treatment of HIV-1 infections. While the quest for novel and more potent drugs continues, combination treatment strategies are gaining increasing attention from the scientific community. In this respect, the development of antiviral treatment historically resembles the development of treatment strategies against other infectious diseases, e.g. tuberculosis and enterococcal endocarditis, as well as malignant diseases, e.g. childhood leukemia, which evolved from monotherapy to the current use of combination strategies as a result of drug resistance and limited efficacy of monotherapy.

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The most important rationale for combined antiviral treatment is that it may result in more effective and prolonged suppression of viral replication. Ideally, a combination of antiviral agents should have an additive or synergistic activity, should be active in a broad range of viral reservoirs by targeting different tissues and cell types, should be active in cells at different stages of activation, and should delay the development of drug resistance or suppress established resistant viral populations. Finally, the synergistic activity of combination therapy should allow for dose reductions of the toxic components of the combination, thereby limiting drug toxicity.

Several combinations of antiviral drugs have indeed been shown to be effective in vitro and have promising effects in vivo. While these observations encourage increasing efforts to pursue the development of combination strategies, there is a need to address issues regarding preclinical and clinical testing of combinations. For this reason, a consensus symposium was organized and convened by The Macrae Group (New York City, NY, USA) in Lisbon, Portugal on July 25–27, 1995 to discuss the state-of-the-art knowledge on combined antiviral therapy. Current insights in the role of in vitro analysis, animal models and pharmacologic considerations for selecting clinical combinations of antiviral agents, and the design of clinical trials for drug combinations were discussed. In addition, the current knowledge of in vitro and in vivo efficacy of drug combinations in viral infections, including HIV, herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV), respiratory viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV), were reported. This article provides a review of the information presented at this symposium, and the consensus reached in certain areas based on this information.

Members of the consensus panel were C.A.B. Boucher, Utrecht University, The Netherlands; G.J. Galasso, National Institutes of Health, USA; M.S. Hirsch, Harvard University, USA; E.R. Kern (co-chair), University of Alabama at Birmingham, USA; J.M.A. Lange, University of Amsterdam, the Netherlands; and D.D. Richman (co-chair), University of California San Diego, USA.

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2. Preclinical evaluation of drug combinations

2.1. In vitro testing of combinations

The importance of in vitro analyses for the selection of clinical drug combinations is widely

accepted. The main aim of in vitro testing is to assess whether a combination of drugs displays additive, synergistic or antagonistic activity in inhibiting viral replication. In addition, other rationales for combination therapy can be evaluated in vitro, including the activity in different cell types and stages of infection, the absence of cross resistance, and differences in the toxicity patterns of the respective drugs. However, in vitro testing has many limitations and is no substitute for carefully controlled trials in animal or human populations.

The results of in vitro analysis of drug combinations are dependent on a large number of variables, including the virus isolate used in the assay (e.g. laboratory or clinical isolate), the concentration of virus, the cell type, and the stage of infection of the cells (e.g. chronically infected or newly infected), as well as the endpoints used in the assay and the timepoints at which these endpoints are measured. Furthermore, the concentrations of the respective drugs used in the experiment will influence the outcome. When the concentrations of the respective drugs used in an experiment are very high, e.g. equalling IC_{99} values, it is difficult to compare the activities of two combinations, since effective suppression of viral replication will be observed with all combinations (Rusconi et al., 1994). For this reason, a range of concentrations of the respective drugs should be tested, while the analysis of drug-drug interactions also necessitates the assessment of dose-effect relationships of each drug alone. In addition, uninfected control cells should be exposed to the drugs in the same experiment in order to exclude the possibility that the antiviral effects observed are secondary to cellular drug toxicity, and to identify synergistic toxicity.

Over 20 methods are available to calculate whether drug combinations have additive, synergistic or antagonistic effects, indicating that the methodology of analysis of drug-interactions is also subject to variability (Greco et al., 1995). Drug-drug interactions concern the relationships of two or more drugs in combination, both quantitatively and qualitatively. Synergy is defined as a greater than expected effect, and antagonism as a less than expected effect. Two mathematical assumptions are used to calculate the expected effect, viz. Bliss independence and Loewe additivity (Greco et al., 1992). Bliss independence assumes that two drugs act independently, and that the combined effect is the sum of their fractional inhibitions. This is equated by $z = x + y(1 - x)$, in which z is the expected fractional inhibition of the combination, while x and y are the fractional inhibitions of the two respective drugs alone. Loewe additivity assumes a summation of the effects of drugs acting at the same site, according to the equation $(D)_1/(IC_x)_1 + (D)_2/(IC_x)_2 = 1$, where $(IC_x)_{1,2}$ and $(D)_{1,2}$ are the concentrations of drugs 1 and 2 alone and in combination, respectively, which result in x percent inhibition. From this equation, an iso-effective dose curve or isobologram can be constructed, which according to Loewe additivity will result in a straight line, while synergistic activity in vitro will yield a concave curve below the additive straight line. An antagonistic interaction will result in a convex curve above the straight line.

The various methods for analysis of drug-drug interactions use equations assuming either Bliss independence or Loewe additivity, and all require the assessment of dose-effect relationships of the respective drugs alone, as well as in combination. In some methods, statistical analysis can be applied to assess the significance of the observed drug-interaction. In addition to the ability of statistical analysis of the interactions observed, three-dimensional models have the advantage that analysis of the biological effects at all concentrations of the respective drugs is possible. Two-dimensional models require an experimental design of fixed ratios of the drugs, and will thus only reveal the specific drug-interaction at the ratio of drug-concentrations chosen by the investigator, while three-dimensional plots reveal the quantity of both synergistic and antagonistic interactions and identify the drug concentrations at which they are observed.

Even in the most ideal circumstances, in vitro systems remain crude surrogates for the complex biological system with regard to the pathogenesis of infection and pharmacology of drugs. For this reason, it is difficult to extrapolate in vitro dose-effect relationships to the in vivo situation, and synergy in vitro cannot automatically be trans-

lated to clinical benefit. Nevertheless, as exemplified by the predicted synergy of zidovudine (ZDV) and zalcitabine, didanosine or 2′-deoxy-3′-thiacytidine (3TC), in vitro analysis is useful in selecting clinical drug combinations (Johnson et al., 1991; Eron et al., 1992; Viner et al., 1993). The major value of in vitro analysis of drug combinations may be to exclude significant antagonistic interactions as exemplified by the observed in vitro interactions between ZDV and ribavirin (Vogt et al., 1986) and, to a lesser extent, synergistic toxicities.

2.2. Animal models

In comparison with in vitro analysis, the main advantage of the use of animal models for selecting clinical combinations is that animals constitute complete biologic and physiologic creatures, and thus more closely resemble the in vivo situation in humans. Synergistic or antagonistic efficacy can be analyzed, while synergistic toxicity can be evaluated more accurately than in in vitro assays. However, a number of disadvantages of animal models limit their value for selection of drug-combinations for clinical studies. There are differences in absorption, distribution, metabolism and excretion of drugs between humans and animals, especially small animals, such as rodents. Furthermore, in many animal models, surrogate viruses are used which often show relatively little homology with, and have different replication machineries than their human counterparts, while the disease they produce may also be somewhat different than in humans. For some models, the number of available non-rodent animals is relatively small, and may be insufficient for analyses of drug-combinations in view of the large number of animals required for these analyses. Finally, experiments using animal models are labor-intensive, time-consuming, and may be relatively hazardous to the health of the investigator and other personnel.

Despite these drawbacks, many animal models appear useful for testing antiviral drug combinations (Kern, 1990). Suitable animal-models for selection of drug-combinations may be available for CMV, HSV and influenza virus, while the usefulness of animal models for HBV, HCV and HIV seems doubtful because of the requirement of large numbers of woodchucks and non-human primates.

Human CMV does not infect animals, necessitating the use of surrogate viruses in animal models. The murine CMV model, as well as the rat and, to a lesser extent, the guinea pig have been demonstrated to be predictive for the efficacy of monotherapy in human studies (Kern, 1991). While the limited data available also suggest a predictive value of these models for combination therapy, more studies are needed. Sufficient numbers of suitable animals and patients with CMV disease are available for this purpose. Various combinations have been tested in CMV models, of which a combination of ganciclovir (GCV) and neutralizing antibodies confirmed promising synergistic effects in vitro (Rubin et al., 1989; Gehrz et al., 1992). Surprisingly, three-dimensional analysis of a combination of GCV and foscarnet showed antagonistic interactions in animal models, in contrast with the observed synergy in vitro and in humans (Freitas et al., 1989; Dieterich et al., 1992). Similarly, antagonistic interactions were observed between GCV and cidofovir.

Mice, guinea pigs and rabbits are the animal models used for the manifestations of HSV infection, including encephalitis, neonatal, ocular, genital and cutaneous infections. Similar to CMV, these models are very predictive for monotherapy in human studies. Although sufficient numbers of animals are available, the number of patients with manifestations of HSV infection which may require combination therapy, e.g. HSV encephalitis or neonatal herpes, is small. For this reason, the predictive value of these models for combination strategies may be difficult to assess. Of the variety of drug-combinations tested, combinations of acyclovir (ACV) with vidarabine or neutralizing antibodies appear most promising in animal models (Schinazi et al., 1982; Schinazi, 1986), and should be evaluated in herpes encephalitis and neonatal herpes infections in humans.

Animal models used for human HBV-infections are pekin ducks and woodchucks. To a certain extent, both models have been predictive for monotherapy. The advantages of duck HBV are

that a chronic infection is produced and the virus is transmitted vertically, resulting in the generation of a large number of infected animals after infection of one duck. Woodchuck is more similar to human HBV, and is a very suitable model with respect to the disease it produces. However, woodchucks have to be colony bred, and are only available in short supply, which limits their role in drug combination studies. The limited number of combination studies to date have all been performed in the duck-model (Niu et al., 1993; Wang et al., 1995).

Animal models for HIV include simian immunodeficiency virus (SIV) in macaques, feline immunodeficiency virus (FIV) in cats, and the murine retroviruses, as well as HIV in murine chimeras (e.g. Thy-Liv implants or peripheral blood lymphocytes in severe combined immunodeficient (SCID)-mice), transgenic mice expressing certain parts of the HIV genome, and simian-HIV chimeric viruses (SHIV) in monkeys (Luciw et al., 1995). Drug combinations tested in animal models include ZDV and interferon alpha (feline leukemia virus (FeLV), murine leukemia virus), and ZDV and interleukin-2 (FeLV) (Zeidner et al., 1990a; Zeidner et al., 1990b; Gardner, 1991). Murine retroviruses show little homology with HIV, have different regulatory proteins, and cause markedly different diseases. Although SIV and FIV seem suitable animal models for treatment of HIV (Gardner, 1991), their role in the analysis of combination therapy is limited by the relatively small number of animals available. Furthermore, the large number of HIV infected patients and the time-constraints in the quest for effective treatment argue against the use of animal models for selecting clinical drug combinations in HIV infection; rather, compounds that are safe should be tested directly in patients.

2.3. *Pharmacology of multiple drug regimens*

Pharmacologic issues can be divided into pharmacodynamics and pharmacokinetics. Pharmacodynamics deal with the relationship between the concentration of the active drug and the effect on its target, while pharmacokinetics address absorption, metabolism, transport, anabolism and excretion of the drug and its metabolites. Pharmacodynamic issues can be exemplified by the interaction between ZDV-triphosphate (ZDV-TP) and its target enzyme reverse transcriptase (RT). The competitive inhibitory activity of ZDV-TP on RT should be a function of the concentrations of ZDV-TP and the natural substrate thymidine-triphosphate (TTP), the inhibitor constant (K_i) and the substrate constant (K_m). However, according to pharmacodynamic principles describing this function, the effects of ZDV appear less than observed in vivo at physiologic and attainable concentrations of TTP and ZDV-TP, respectively, suggesting that enzyme inhibition does not account for the drug's activity. In contrast, pharmacodynamic modelling of chain termination as the mechanism of ZDV-TP activity would suggest a greater effect of ZDV than observed in vivo (Reardon, 1992). Assuming that the incorporation of nucleoside or the analogues is a stochastic process, the chances of correctly incorporating all approximately 2160 thymidine residues in the HIV genome are extremely small.

Although these observations illustrate the difficulties in extrapolating the rational use of drugs from enzyme to man, it is clear that the activity of ZDV, whatever the mechanism may be, is dependent on the concentrations of ZDV-TP and TTP. This pharmacodynamic principle can be used by combining ZDV with drugs that affect these concentrations. For example, dipyridamole blocks the transport of endogeneous thymidine into the cell without affecting the transport of ZDV, resulting in an augmented effect of ZDV in vitro (Betageri et al., 1990). Preliminary studies in humans have not revealed a pharmacokinetic interaction (Hendrix et al., 1994). However, to date, the toxicity pattern of dipyridamole has dampened the further pursuit of its clinical use. Alternatively, depletion of endogeneous deoxyadenosine-triphosphate concentrations and resulting enhancement of the inhibitory activity of dideoxyadenosine-triphosphates can be achieved by ribonucleotide reductase-inhibitors, such as hydroxyurea (Gao et al., 1994; Lori et al., 1994; Malley et al., 1994). This same principle can be used in enhancing the inhibitory effect of GCV-triphosphate against CMV DNA polymerase. The

ribonucleotide reductase-inhibitor 1110U81 (2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone) has been shown to enhance the inhibitory effects of GCV in vitro by reducing deoxyguanosine-triphosphate concentrations (Hamzeh et al., 1993). Interestingly, CMV replication in vitro is also inhibited by 1110U81 alone. In addition, a large array of inhibitors of endogeneous purine and pyrimidine biosynthesis have also been shown to inhibit CMV replication in vitro. These findings indicate that depletion of endogeneous deoxynucleosides is specifically detrimental to CMV replication, suggesting a unique need for a markedly augmented pool of endogenous deoxynucleosides for viral replication (Biron et al., 1985).

An important pharmacodynamic issue is that, over time, drug concentrations fluctuate continuously. In addressing the pharmacodynamic characteristics of a drug, one should thus not consider a constant exposure to a given drug concentration. Since the effects of ZDV are reversible, the potential influence of time-dependent fluctuation of ZDV-TP concentrations on the inhibitory activity on viral replication is important and needs to be addressed (Stretcher et al., 1994). Fluctuation of drug concentrations also needs to be considered in modelling combination strategies. When two drugs are given in combination, depending on their respective pharmacokinetic characteristics, both drug-concentrations will fluctuate producing independent pharmacodynamic effects. This fluctuation of the two drugs will not necessarily be in parallel. As has been described in the section on in vitro testing of drug combinations, three-dimensional methods of analysis can identify synergistic or antagonistic interactions between drugs, depending on the respective drug concentrations. Within a dosing-interval, fluctuations of the respective drug-concentrations may thus result in periods of synergistic activity, alternating with periods of antagonistic interactions.

Pharmacokinetic interactions between drugs may also be used in combination strategies, which again can be exemplified by treatment with ZDV. After absorption, ZDV is metabolized to ZDV-glucuronide, which together with non-glucuronidated ZDV is excreted. Only one isoform of glucuronosyl-transferase appears responsible for ZDV-glucuronidation (Rajaonarison et al., 1991). By blocking the glucuronidation, probenecid reduces the excretion of ZDV (De Miranda et al., 1989; Kornhauser et al., 1989; Hedaya et al., 1990). In addition, probenecid also inhibits renal excretion of ZDV. These interactions would enable dose-reduction of the drug and treatment of a larger number of patients with the same amount of drug, which would especially be of importance in the developing countries. In fact, probenecid was designed to allow for dose-reduction of penicillin at a time when this drug was still very expensive. Unfortunately, however, the clinical value of combined treatment with ZDV and probenecid is greatly limited by increased toxicity of the combination, especially probenecid-related skin rashes (Petty et al., 1990). In vitro and in vivo studies have shown that a number of other drugs, including vancomycin, chloramphenicol and fluconazol, potentially inhibit ZDV-glucuronidation at attainable plasma-concentrations by competing for the same glucuronosyl-transferase (Sim et al., 1991; Rajaonarison et al., 1992; Sahai et al., 1994). On the other hand, some drugs, including rifampicin, ganciclovir and phenobarbital, induce glucuronosyl-transferase activity, resulting in increased ZDV-glucuronidation and elimination (Haumont et al., 1990; Medina et al., 1992; Rajaonarison et al., 1992; Burger et al., 1993). The attainment of sufficient plasma-concentrations is an increasingly important issue of treatment of HIV-infection, especially with protease-inhibitors. Since most protease-inhibitors induce and are metabolized by the cytochrome p450 system, pharmacokinetic interactions in protease-inhibitor combinations and combinations of protease-inhibitors with other drugs that interact with this system should be investigated. This research will be facilitated by the identification of the particular enzyme of the p450 system responsible for the metabolism of the respective protease-inhibitors.

Intracellular pharmacokinetics of ZDV, i.e. the phosphorylation to ZDV-triphosphate, can also be affected by combinations with other drugs. For example, ribavirin inhibits the phosphorylation of ZDV in vitro, resulting in lower concentrations of

phosporylated ZDV, which explains the observed antagonizing effect of ribavirin on ZDV efficacy in vitro (Vogt et al., 1986).

3. Drug resistance

It is well established that treatment with most antiviral drugs selects for virus with reduced drug susceptibility, which is conferred by mutations in the viral target. The clinical significance of viral drug resistance has been difficult to address for a number of reasons. These include the presence of mixtures of virus populations with different drug susceptibilities in patients, which, in addition, may be distributed differently in various organs. Furthermore, development of resistance is often variable and progressive, and clinical endpoints are not clearcut or are not the direct consequence of viral replication. Finally, the development of resistance is correlated with other independent predictors of clinical drug failure, such as immunosuppression and virus load. Nevertheless, viral drug resistance appears to contribute to drug failure of most antiviral drugs.

For this reason, an important rationale of antiviral combination treatment would be to prevent or delay the emergence of drug resistant virus variants. The likelihood that resistance-conferring mutations in the viral genome occur is a function of viral replication, and consequently of the virus load. Therefore, drug-resistant virus variants emerge more rapidly in immunocompromised patients, as has been shown for GCV- and ACV-resistance in infection with herpesviruses (Ehrlich et al., 1989; Erice et al., 1989) and ZDV-resistance in HIV infection (Richman et al., 1990). High levels of viral replication and turnover are observed in many virus infections, including those with HBV, HCV and HIV. Moreover, mutation rates of, for example, single stranded RNA viruses, including HIV, influenza virus and HCV, are high (approximately 3×10^{-5} mutations per nucleotide for HIV-1 (Mansky and Temin, 1995)). Increasing evidence suggests that high mutation rates and rapid virus turnover may result in the presence of varying proportions of drug-resistant mutants in the viral population before treatment is started, which readily emerge upon the institution of drug pressure (Najéra et al., 1994; Coffin, 1995; Kozal et al., 1995; Nájera et al., 1995). Consequently, one of the rationales of combination therapy would be the activity against a broad range of these pre-existing mutants.

The selective pressure of an antiviral drug influences the emergence of drug-resistant virus, i.e. the more active a drug, the more rapid drug-resistant mutants will emerge. On the other hand, effective suppression of viral replication will decrease the opportunities of the virus to acquire resistance-conferring mutations, and thus delay the emergence of drug-resistant virus. Indeed, results of clinical studies in HIV infection of regimens with protease-inhibitors are emerging which suggest a delayed appearance of drug-resistant virus variants when larger and more sustained reductions of plasma HIV-1 RNA are achieved (Schapiro et al., 1995). Increasing antiviral activities thus seem to enhance the rate of resistance development, by increasing the selective pressure, up to a point at which suppression of viral replication is sufficient to diminish the likelihood of development of resistance-conferring mutations. Drug resistance will not develop when viral replication is completely suppressed, which is the ultimate goal of antiviral treatment.

However, identifying a treatment regimen which completely inhibits viral replication has not been possible to date. The aim of current antiviral chemotherapy strategies is therefore to sustain suppression of virus replication despite the development of resistance. This may be accomplished by several mechanisms, which must be exploited in designing antiviral combination strategies. First, a sustained effect of treatment in the light of resistance may be achieved by increasing plasma levels of a drug to levels that exceed the susceptibility of drug resistant virus. Treatment with high doses of nevirapine, a nonnucleoside inhibitor of HIV-1 reverse transcriptase, has indicated the potential use of this strategy (Havlir et al., 1995). Alternatively, an impairment of the replicative capacity of the virus secondary to the presence of resistance-conferring mutations may result in sustained antiviral activity. By this mechanism, the 3TC resistance-conferring M184V mutation in

HIV-1 reverse transcriptase may contribute to the observed sustained antiviral effects despite high levels of resistance (Schuurman et al., 1995). Similarly, impaired enzyme function of HIV-1 protease has been observed in vitro after introduction of resistance-conferring mutations (Ho et al., 1994). However, background mutations outside the substrate-binding regions, and even outside the open reading-frame may compensate for these impairments of growth in vivo (Anton et al., 1995; Lamarre et al., 1995).

Combined treatment with two drugs or more which are directed at the same viral target (convergent therapy) and have non-overlapping genotypic resistance patterns may result in sustained antiviral activity by interactions between resisistance-conferring mutations. Convergent combination therapy may constrain the evolutionary options for acquiring resistance to both drugs (Richman, 1993). Alternatively, a mutation conferring resistance to one drug may sensitize the virus to the other drug. For HIV infection, several examples of this mechanism have been generated in vitro (Larder, 1994). The virus may circumvent the suppressive interactions of mutations by choosing alternative pathways for resistance development. For example, during combined treatment with ZDV and nevirapine, the virus does not select for the Y181C RT mutation, which is the most commonly observed mutation during nevirapine-monotherapy and resensitizes ZDV-resistance in vitro, but selects for other nevirapine resistance-conferring mutations instead (Richman et al., 1994). However, the suppressive effect on ZDV resistance of the 3TC resistance-conferring M184V mutation in reverse transcriptase holds promise in vivo (Boucher et al., 1993; Tisdale et al., 1993; Larder et al., 1995). Similar convergent combination strategies should be explored using two potent HIV-1 protease-inhibitors with non-overlapping resistance patterns.

4. Current insights in combined antiviral therapy

4.1. Respiratory viruses

Respiratory virus infections potentially amenable to combined antiviral treatment are influenza, common colds and respiratory syncytial virus (RSV) infections causing bronchiolitis and pneumonia in young children. However, the relative lack of effective agents against these viruses has slowed the progress in pursuing combination strategies. Although controlled clinical trials of combination therapy have not been performed to date, in vitro and animal model studies, as well as limited clinical studies, suggest a potential benefit of combination treatment for respiratory viruses. The following sections summarize the current knowledge, based on these studies.

4.1.1. Influenza

Amantadine and rimantadine are highly effective as prophylactic or therapeutic agents against influenza A virus. The development of drug resistance against these agents, however, requires improved therapeutic options. Combinations of amantadine or rimantadine with ribavirin or interferon have shown additive or synergistic antiviral activity in vitro (Hayden, 1986). Interestingly, a synergistic activity of rimantadine and ribavirin was also observed against a rimantadine-resistant influenza A strain, while this combination did not show synergism against intrinsically rimantadine-resistant influenza B virus. Synergistic activity of amantadine or rimantadine and ribavirin was confirmed in murine models, showing enhanced antiviral activity and improved survival when the combination was given either parentally or by aerosol (Hayden, 1986). These observations indicate that the clinical use of combination therapy with rimantadine and ribavirin should be investigated, especially in hospitalized patients with influenza.

Dual combinations of rimantadine or ribavirin with the novel agents 2′-deoxy-fluoroguanosine (2-FDG) and the neuraminidase inhibitor GG167 have also been tested in vitro, and generally showed additive, rather than synergistic antiviral activity (Madren et al., 1995). Although a combination of ribavirin and 2-FDG shows synergistic activity, synergistic cytotoxicity was also observed, which appears to limit its clinical use.

4.1.2. Rhinoviruses

This class of viruses remains one of the greatest causes of morbidity and absence from work and school. An effective therapeutic regimen would therefore be most desireable. However, no effective treatment is yet available.

Several combinations have been shown to enhance antiviral effects in vitro, including interferon alpha or interferon beta with interferon gamma, enviroxime or capsid-binding agents, and capsid-binding agents with enviroxime. The combination of interferon alpha and enviroxime has been tested clinically, and showed no enhanced antiviral effects, which did not seem surprising since treatment with enviroxime alone showed no significant effects in experimental rhinovirus infection in humans (Higgins et al., 1988).

The pharmacological properties of the respective antiviral agents provide difficulties in combining them in terms of topical administration. In this respect, a novel soluble form of intracellular adhesion molecule-1 (sICAM-1) appears a promising candidate for topical combination treatment with interferon (Crump et al., 1994). It has been shown to inhibit viral replication in vitro, shows enhanced antiviral activity when combined with interferon and, similar to interferon, is highly soluble in aqueous solutions.

Since host-reponses contribute to disease pathogenesis of rhinovirus infections, and other acute respiratory virus infections, an alternative treatment strategy is to combine antiviral drugs with agents that intervene with inflammatory mediators of disease, which may result in symptomatic benefits. A clinical trial of combined treatment with oral naproxen and intranasally administered interferon alpha and the anticholinergic ipratropium in acute rhinovirus infection showed significant antiviral effects and increased symptomatic relief when compared to placebo and historical controls (Gwaltney, 1992). A number of inflammatory mediators have been identified for rhinovirus, RSV and influenza virus infections, which may act as targets for similar strategies.

4.1.3. Respiratory syncytial virus

This virus causes serious disease in newborns. Aerosolized ribavirin appears effective but is difficult to administer. A more convenient treatment regimen is therefore desired. A combination of ribavirin and immunoglobulins has been tested in vitro and showed minimally additive effects. However, significantly enhanced antiviral effects of intravenous immunoglobulins and aerosolized ribavirin were observed in the cotton rat model (Gruber et al., 1987). A clinical study in adult bone marrow transplant recipients with RSV pneumonia suggested an improved clinical outcome when this combination was started early in the clinical course of infection (Whimbey et al., 1994). Further study of this combination in hospitalized patients with serious RSV infections appears warranted.

4.2. Herpesviruses

This class of viruses causes a wide range of pathogenic manifestations and contains the viruses for which the greatest successes in antiviral therapy have been observed.

4.2.1. Herpes simplex virus

Manifestations of HSV infection which may require combined antiviral treatment are neonatal herpes and HSV encephalitis. However, clinical studies of combination therapy for these disease manifestations are difficult, in view of the limited numbers of patients available and the general practice of instituting approved monotherapy with ACV in case of suspected disease. Moreover, brain biopsies, which have been the gold standard in clinical trials of HSV encephalitis, are now rarely performed. However, detection of virus in cerebrospinal fluid by polymerase chain reaction appears to correlate well with brain biopsy results (Aurelius et al., 1991; Guffond et al., 1994; Lakeman and Whitley, 1995). Combinations which have shown promising antiviral effects in vitro and in animal models, and may be of clinical use in neonatal and CNS infections, include ACV with monoclonal antibodies or vidarabine (Schinazi et al., 1982; Schinazi, 1986).

Since acute or recurrent mucocutaneous infections in immunocompetent adults respond well to oral ACV, combination therapy does not appear

to be warranted for these manifestations of HSV infection. Furthermore, reports of drug resistance in this patient group are anecdotal. Development of drug resistance does, however, provide a clinical problem in immunocompromised HIV-infected patients receiving chronic treatment with ACV. Treatment of ACV-resistant HSV with novel agents, including topical trifluorothymidine, foscarnet or cidofovir, are currently being studied. Whether combination treatment of drug-resistant HSV, or combined prophylaxis to prevent drug resistance needs to be pursued is still open to question.

4.2.2. Varicella zoster virus

Chickenpox causes significant morbidity in immunocompromised children and young adults. Although some clinical benefit has been observed with ACV alone, the exploration of combined treatment may be worthwhile in these patients. Again, however, clinical studies of combinations are complicated by the limited numbers of available patients.

A number of drugs that have been approved or await approval for use in herpes zoster appear to have clinical benefit and influence zoster-associated pain and post-herpetic neuralgia. These include ACV, penciclovir and valacyclovir. In addition, preliminary results showed increased efficacy of the novel agent arabinofuranosyl-bromovinyluracil (BV-araU, sorivudine) in the treatment of herpes zoster in HIV-infected patients, when compared to ACV (Machida et al., 1994). Clinical trials of antiviral combinations in herpes zoster have not been performed to date.

Although formally not considered combined antiviral treatment, an alternative treatment strategy is to combine antiviral agents with corticosteroids or the newer generation analgesics. Promising results were obtained in a clinical study comparing a combination of ACV and prednisone with prednisone alone, ACV alone and placebo. This as yet unpublished study showed a faster resolution of acute neuritis and zoster-associated pain in patients receiving the combination.

4.2.3. Cytomegalovirus

Infections with CMV, in particular neonatal infections and CMV disease in immunocompromised patients, including transplant recipients and HIV-infected individuals, appear to be important targets for combined antiviral prophylaxis or treatment. Therapeutic and prophylactic options for CMV disease are available, including neutralizing antibodies, intravenous and oral GCV, and intravenous foscarnet. However, the benefits of prophylactic treatment are often limited and, especially in HIV-infected patients, relapses of disease occur frequently despite chronic active treatment.

Although CMV disease can affect multiple organs, including the lungs, gastrointestinal tract and central nervous system, the most important manifestation of CMV disease in HIV-infected patients is retinitis accounting for 85% of all CMV disease in this patient group (Gallant et al., 1992). Approved drugs for treatment of CMV retinitis are intravenous GCV and foscarnet, which are effective in controlling retinitis. However, despite chronic maintenance treatment with these drugs, relapses of disease almost invariably occur, which may be due to the progressive deterioration of the immune system, the development of drug resistance and/or the acquisition of borderline effective drug concentrations intraocularly (Studies of Ocular Complications of AIDS (SOCA) Research Group (SOCA, 1994). A combination of GCV and foscarnet shows additive to synergistic antiviral activity in vitro (Freitas et al., 1989; Manischewicz et al., 1990), while uncontrolled clinical case series suggested a prolongation of the progression-free interval in patients treated with this combination (Dieterich et al., 1992; Kuppermann et al., 1993; Weinberg et al., 1994). In view of these encouraging observations, a controlled trial comparing a combination of foscarnet and GCV with foscarnet alone and high dose GCV alone has been completed by the SOCA Research Group. A clinical study evaluating the combination of GCV and foscarnet in the treatment of CMV encephalitis may also be warranted, since anecdotal reports have shown little efficacy of treatment with either drug alone.

Combination therapy of GCV with neutralizing antibodies also appears encouraging in the treatment of CMV disease, and has shown efficacy in the treatment of CMV pneumonitis in allogeneic bonemarrow transplant recipients (Emanuel et al., 1988; Reed et al., 1988). A combination of GCV with hyperimmune serum has been tested in rat models of generalized CMV infection, meningo-encephalitis and pneumonitis (Stals et al., 1994). These studies showed a synergistic antiviral effect and improved clinical outome of the combination in generalized infection and pneumonitis, while beneficial effects in meningo-encephalitis were not observed with either combination treatment or treatment with either drug alone. Relative to polyclonal antibodies, monoclonal antibodies have the advantage that they are more potent and more standardizable, and have better defined functional properties and pharmacokinetics. MSL 109 is a monoclonal antibody directed at the gH glycoprotein of CMV, which has been shown to have potent neutralizing activity against CMV in vitro, including laboratory strains and patient isolates, and displays additive to synergistic antiviral activity when combined with GCV or foscarnet (Lakeman et al., 1991; Nokta et al., 1994). Uncontrolled phase I/II studies in CMV seropositive individuals and patients with CMV retinitis showed that MSL 109 is well tolerated, has favorable pharmacokinetics, and does not induce production of antibodies against the agent (Pollard and Nokta, 1992). Two randomized placebo-controlled clinical trials evaluating the efficacy of MSL 109 in combination with standard therapy in the treatment of CMV retinitis have been initiated. In one sudy (NIH ACTG 266), standard treatment is compared with combinations of standard treatment with two doses of MSL 109 with respect to the time to progression of newly diagnosed CMV retinitis. In the other study, initiated by the SOCA Research Group, the efficacy of combined therapy with GCV or foscarnet in the treatment of episodes of newly diagnosed or recently relapsing CMV retinitis is compared with standard therapy.

4.3. Hepatitis B virus (HBV)

Infection with HBV affects 5% of the world population, and is the ninth cause of death worldwide, due to development of cirrhosis and cancer of the liver. While the objectives of antiviral treatment include inhibition of viral replication and elimination of virus from the liver, the removal of viral antigens is especially important since cirrhosis of the liver is secondary to the immune response rather than to HBV itself.

The viral replication cycle of HBV is characterized by the formation of a covalently closed circular form of HBV DNA (cccDNA) after transfer of the virus into the nucleus, which acts as a template for RNA and mRNA production. This plasmid form of the virus is very stable, and, in the absence of active viral replication, can remain detectable in liver cells of patients and experimental animals for years after the acute infection. Furthermore, by mechanisms not yet understood, the intracellular pool of ccc HBV DNA is maintained even at low levels of replication, by preferential shunting of replicating viral genomes from the cytoplasm back to the nucleus. As a result of the stability of the intranuclear plasmid form of HBV DNA, an immediate return of viral replication is generally observed after termination of antiviral treatment. This may be prevented by more active and complete inhibition of viral replication, resulting in a reduction of the rate of replenishment of cccDNA below the rate of decay. Until recently, the efforts in exploring the potential use of combined antiviral treatment for this purpose were largely limited by a lack of effective antiviral agents.

The only approved drug for treatment of chronic HBV infection is interferon alpha, which is usually administered three times a week in a dosage of 5–10 million units for at least 6 months. Beneficial responses to interferon are generally limited to patients with HBV infections acquired in adulthood. In addition, females respond better than males, while better responses are also observed in case of high pretreatment transaminase levels, low pretreatment HBV DNA levels, active liver histology, absence of anti-delta antibodies, and absence of HIV infection (Brook et al., 1989).

Novel agents which are currently evaluated in clinical trials of chronic HBV infection include the

nucleoside analogues penciclovir and lamivudine (3TC). While both agents display effective inhibition of HBV replication in vitro as well as in the duck model (DHBV), lamivudine is 3–5-fold more potent in cell culture than penciclovir and, in fact, appears to be the most powerful agent against HBV available to date (Doong et al., 1991; Tyrrell et al., 1993; Ashman et al., 1994; Shaw et al., 1994). Human studies of lamivudine have shown 100% suppression of HBV DNA in virtually all patients treated with doses of 100 mg/day or more (Benhamou et al., 1995; Dienstag et al., 1995.

However, withdrawal of the drug resulted in a rebound of viral replication in a high percentage of patients. In a 3-month study of lamivudine, only 5 of 32 patients remained HBV DNA-negative upon cessation of the drug. To evaluate whether prolonged treatment with lamivudine will result in a lower relapse rate, studies of 24, 48 and 96 weeks of lamivudine treatment are ongoing. However, the observation of relapses in ducks after one year of treatment are not encouraging in this respect. Fortunately, drug resistance against lamivudine appears to develop slowly, despite the high turnover rate of the virus. Drug resistance has not been observed in ducks treated for over a year; moreover, these ducks show similar responses as previously untreated ducks after resumption of treatment. Similar observations were made in human studies to date. Interestingly, similar to HIV-1 RT, site-directed mutagenesis studies in DHBV have shown that a mutation in the conserved YMDD motif of hepadnavirus polymerase (M512V) confers high level resistance to lamivudine. However, these mutants are impaired because of the overlapping HBsAg reading frame for this region of the polymerase.

A combination of penciclovir and lamivudine has been tested in vitro, and showed synergistic efficacy without increases in toxicity. Further testing of this nucleoside-analogue combination in animal models and humans may be warranted.

In addition, even at relatively low concentrations, lamivudine markedly enhanced the antiviral activity of interferon alpha in vitro. Theoretically, a combination of effective inhibition of viral replication, e.g. by lamivudine, and the development of a strong immune response, particularly a Th1-type response, e.g. by interferon, potentially results in a clearance of persistently infected cells expressing viral antigens. Based on this theory, as well as the in vitro observations, a clinical study evaluating a combination of lamivudine and interferon has been initiated. In this randomized, placebo-controlled study, treatment with lamivudine alone and interferon alone is compared with the addition of interferon alpha to lamivudine after 8 weeks of treatment.

4.4. Hepatitis C virus (HCV)

Interferon alpha also forms the mainstay of treatment for HCV infection. However, responses to treatment with interferon alpha are variable, and sustained responses are observed in only 5–40% of patients (Davis et al., 1989; Di Bisceglie et al., 1989; Chayama et al., 1991; Varagona et al., 1992; Iino et al., 1993). A potential target of combined antiviral treatment is to improve responses in patients who respond poorly to treatment with interferon. A number of variables may aid in identifying such patients before the start of treatment, including liver histology, viral genotype, virus load and heterogeneity of quasispecies (Kanai et al., 1992; Yamada et al., 1992; Jouët et al., 1994). Good responses to interferon treatment are associated with viral genotypes other than type 1, low virus loads, homogeneous viral quasispecies, and absence of liver cirrhosis. In view of the latter three factors, early treatment may generally be more advantageous.

Progress in the field of combination therapy for HCV infection has been constrained by the lack of tissue culture systems or animal models for HCV infection, as well as a lack of effective agents. Monotherapy with ribavirin has been shown to result in improved transaminase levels during treatment, which immediately rebounded upon cessation of the drug (Reichard et al., 1991; Camps et al., 1993; Di Bisceglie et al., 1993; Koskinas et al., 1995). However, the mechanism of these biochemical effects remains unclear, since no appreciable effects on HCV RNA load and liver histology were observed. Nevertheless, a number of small studies evaluating the effects of

combination treatment with ribavirin and interferon alpha have suggested an enhanced benefit of the combination, which needs further evaluation in larger controlled studies (Brillanti et al., 1994). Alternative combination strategies which have shown some benefits in preliminary studies and may warrant further evaluation in larger studies include interferon alpha with thymosine alpha 1, granulocyte colony stimulating factor, or n-acetylcysteine (Beloqui et al., 1993; Wright et al., 1994). The latter agent is a precursor of gluthathione, and may prevent tissue injury by increasing gluthatione levels, which have been shown to be decreased during chronic HCV infection. Clearly, efforts in discovering more effective agents against HCV need to continue.

4.5. Human immunodeficiency virus

The potential role of combined antiviral treatment is most obvious in HIV infection. In recent years, limited beneficial effects of antiretroviral monotherapy and issues of drug resistance have encouraged increasing efforts to pursue the development of combination strategies. The design of potentially effective combination strategies has been enabled by the discovery of an increasing list of effective antiretroviral agents, including nucleoside analogue and nonnucleoside RT inhibitors, as well as the novel class of protease inhibitors. Several combinations have been studied and show promising results, while a number of dual and triple combination are currently undergoing clinical evaluation, including combinations of two or more RT inhibitors and combinations of RT inhibitors with protease inhibitors. In the following sections, the current knowledge on combination treatment of HIV infection is summarized.

4.6. Combinations of RT inhibitors

4.6.1. ZDV and zalcitabine or didanosine

Combinations of ZDV with zalcitabine (ddC) or didanosine (ddI) belong to the first combinations tested in HIV infection. Both combinations show synergistic activity in vitro (Dornsife et al., 1991; Johnson et al., 1991; Eron et al., 1992), and have non-overlapping toxicity patterns. Based on these encouraging observations, several studies evaluating these combinations were initiated (Meng et al., 1992; Collier et al., 1993). A recently completed study (BW34,225) compared both combinations with ZDV alone in previously untreated patients, and confirmed that treatment with either combination results in greater and more sustained effects on CD4 + lymphocyte counts and plasma HIV-1 RNA levels than treatment with ZDV alone (Schooley et al., 1995). Both combinations displayed similar antiviral effects, while neither combination influenced the rate of development of ZDV resistance. Although initial virus load was higher in symptomatic patients, the relative responses in virus load to combination treatment were similar in all disease stages. Furthermore, there were no differences in response to treatment depending on the biological phenotype of the virus. Although no differences in progression rates and survival were observed between ZDV monotherapy and the combination regimens, this study lacked power to detect such differences. Differences in progression rates were also not observed in the NIH ACTG 155 study, which compared a combination of ZDV and zalcitabine with either drug alone (Fischl et al., 1995). However, since all patients in the latter study were pretreated with ZDV for prolonged periods of time, the effects of combination treatment could not reliably be evaluated in this study. Furthermore, exposure to combination treatment within this study was limited by a rigid management of drug toxicity. Nevertheless, subgroup analysis within this study suggested a clinical benefit of the combination in patients with CD4 + lymphocyte counts higher than 150/mm^3, indicating that patients with less advanced disease may benefit more from combination treatment.

The results of two large clinical studies (NIH ACTG 175, European-Australian DELTA study), comparing both combinations with ZDV alone in pretreated as well as in previously untreated patients, were announced shortly after the Lisbon symposium (Hammer et al., 1995; Yeni, 1995). The NIH ACTG 175 study evaluated the safety and efficacy of nucleoside monotherapy (ZDV or

ddI) vs. combination therapy (ZDV/ddI or ZDV/ddC) in individuals with CD4 cell counts between 200 and 500/mm^3; 2467 patients were randomized, 1067 of whom were antiretroviral naive. The combination of ZDV and ddI was found to be superior to ZDV monotherapy with respect to the primary endpoint (50% CD4 cell count decline/AIDS/death) or the solely clinical endpoints of AIDS or death. This was true both in the antiretroviral-experienced and naive groups, although for the naive group, the purely clinical endpoint differences did not reach statistical significance. No significant differences were seen between ZDV/ddI and ddI monotherapy, however, with respect to these endpoints. Similar results were observed in the ACTG 175 populations treated with ZDV/ddC. The combination was superior to ZDV, but not to ddI, with respect to the primary aggregate endpoint (CD4 cell decline/AIDS/death) in the overall population, as well as in antiretroviral-experienced and naive populations. In antiretroviral naive patients, ZDV/ddC was also superior to ddI for the aggregate endpoint, but not for the solely clinical endpoints. In the antiretroviral-experienced group, the clinical endpoints were comparable for the ZDV/ddC and ZDV monotherapy groups.

In the DELTA study, ZDV monotherapy was compared with combination therapy (ZDV/ddI or ZDV/ddC) in patients with ARC or AIDS (CD4 count $> 50/mm^3$), or CD4 cell counts below 350/mm^3; 2131 randomised antiretroviral naive patients, and 1083 ZDV-experienced patients were prescribed trial therapy. In antiretroviral naive patients, both combinations were superior to ZDV monotherapy with respect to survival and progression to AIDS. When comparing both combinations, there was a suggestion that the AZT/ddI group fared better than the AZT/ddC group. In the ZDV-experienced population, no significant differences between monotherapy and combination therapy in terms of survival or disease progression were observed.

Overall, both studies support the benefit of combination nucleoside therapy over ZDV as initial therapy for individuals with intermediate stage HIV disease, but do not answer the question of whether combinations are preferable to ddI monotherapy in this situation. Of note, in the ACTG 175 study, reductions in viral load in ZDV-naive patients, as measured by plasma HIV-1 RNA, were greater in combination therapy recipients than in either ZDV or ddI monotherapy recipients (Katzenstein et al., 1995).

The combination of ZDV and didanosine has also been compared with a triple combination of ZDV, didanosine and nevirapine in pretreated patients (NIH ACTG 241). This study showed significantly better, although limited, virologic and immunologic responses for the three-drug combination. The limited effects of the triple combination may be explained by the extensive prior treatment of the study participants, with 66% of the subjects having received prior combination treatment with ZDV and didanosine. An ongoing randomized, placebo-controlled study in Europe, Canada and Australia will evaluate the efficacy of the same triple combination regimen in previously untreated patients, comparing it with dual combinations of ZDV and didanosine or nevirapine.

4.6.2. ZDV and 3TC

A promising regimen, which has received much recent attention, is the combination of ZDV and 3TC. Monotherapy with 3TC is remarkably well tolerated and results in a potent reduction of virus load and moderate increases in CD4+ lymphocytes (Pluda et al., 1995; Van Leeuwen et al., 1995), and was recommended for approval by the FDA advisory panel at its November 1995 meeting. However, these effects are of very limited duration due to a rapid emergence of drug resistance, which is conferred by a single M184V nucleotide change in the YMDD motif of HIV-1 RT (Schuurman et al., 1995). As mentioned previously, this mutation sensitizes ZDV-resistant virus in vitro, and it may also impair the replicative capacity of the virus (Boucher et al., 1993; Tisdale et al., 1993; Schuurman et al., 1995). These considerations, as well as a marked synergistic activity of ZDV and 3TC in vitro (Viner et al., 1993), have prompted widespread clinical testing of this combination. Both in pretreated and especially in previously untreated patients, these sudies show a remarkable improvement over ZDV monotherapy

with regard to the magnitude and durability of immunological and virological responses. A number of patients receiving this combination have sustained reductions in virus load and increases of CD4+ cell counts over one year of treatment. The M184V mutation invariably emerges within 8 weeks of treatment in ZDV-pretreated as well as in naive patients receiving combined treatment. Dual resistance against ZDV and 3TC has been observed, and may involve alternative ZDV resistance-conferring RT mutations. However, in previously untreated patients treated with a combination of ZDV and 3TC, the emergence of ZDV resistance is delayed (Larder et al., 1995). It remains unclear whether this delay is attributable to the marked reduction in virus load or to an interaction between the M184V RT mutation and ZDV resistance-conferring mutations.

An ongoing study evaluates the addition of 3TC or 3TC and loviride, a nonnucleoside RT inhibitor, to current treatment with ZDV alone or in combination with zalcitabine or didanosine (NUCB 3007). A study on the efficacy of combined ZDV and 3TC treatment in preventing vertical transmission is planned.

4.6.3. Other combinations

A combination of stavudine (d4T) and didanosine shows additive to synergistic antiviral activity in vitro. However, since both drugs induce peripheral neuropathy, synergistic toxicity is a major concern. An ongoing pilot study is evaluating the safety of this combination, and depending on the results will be followed by a larger controlled study. A trial comparing monotherapy with stavudine or didanosine with combinations of ZDV and either didanosine or stavudine in pretreated patients (NIH ACTG 290) is in progress, as well as a study comparing monotherapy with stavudine or ZDV with a combination of ZDV and stavudine in untreated patients (NIH ACTG 298).

4.7. Combinations of RT inhibitors and protease-inhibitors

In recent years, a number of inhibitors of HIV protease have been developed, including saquinavir, indinavir and ritonavir, which exhibit potent antiviral effects in vitro and in vivo. While RT inhibitors prevent new infections of cells, inhibition of protease prevents production of infectious virions by infected cells and is active in both acutely and chronically infected cells. Of several combinations of protease-inhibitors with nucleoside analogue RT inhibitors currently undergoing clinical evaluation, promising results of combinations involving saquinavir were discussed at the symposium.

Saquinavir exhibits synergistic antiviral activity in vitro when combined with ZDV or zalcitabine. A clinical study in previously untreated symptomatic patients with less than 300 CD4+ lymphocytes per mm^3 compared the immunologic and virologic effects of a combination of ZDV and saquinavir at 3 dosages with saquinavir alone and ZDV alone (V13330). In this study, both the magnitude and the duration of the response on CD4+ cell counts and HIV-1 RNA load were superior in patients receiving a combination of ZDV and saquinavir at a dosage of 600 mg per day. Individual responses varied considerably between patients, which may be secondary to differences in absorption of the drug; similar to other protease-inhibitors, oral bioavailability of saquinavir is low. Interestingly, combined treatment appeared to delay the development of ZDV resistance. No clear differences in the development of saquinavir resistance were observed between monotherapy and combination therapy. Within one year of treatment, virus with reduced susceptibility to saquinavir emerged in approximately 50% of patients, which is predominantly conferred by a L90M amino acid change in HIV-1 protease, occasionally in combination with a G48V change. Other mutations in the protease gene did not appear to significantly contribute to drug resistance. During saquinavir monotherapy, the appearance of resistance-conferring mutations was associated with a transient response to treatment, while patients with sustained immunologic and virologic responses did not develop resistance mutations. The rate of emergence of resistant virus was not dependent on initial CD4+ cell counts and HIV-1 RNA load.

In the NIH ACTG 229 study, combinations of saquinavir with either ZDV or zalcitabine were compared with a combination of all three drugs in patients previously treated with ZDV. While triple combination treatment resulted in superior virologic and immunologic responses when compared to both dual regimens, these effects were limited. However, similar to the NIH ACTG 241 study, the subjects participating in this study were heavily pretreated; the mean duration of prior ZDV treatment was approximately 2 years, while 38% of the subjects had also received prior treatment with zalcitabine. Significantly poorer responses to combination treatment with ZDV and zalcitabine, but not to the other combinations, were observed in patients who were previously treated with this combination. Furthermore, responses to the saquinavir/ZDV combination were much less pronounced in this study when compared to the previously described study, which evaluated the same combination in previously untreated patients. Studies evaluating triple combination treatment in therapy-naive patients are clearly needed. Ongoing combination studies include a study comparing combined saquinavir and zalcitabine treatment with treatment with either drug alone in patients who are intolerant to or fail on ZDV monotherapy. A multinational study is comparing ZDV monotherapy with combinations of ZDV and zalcitabine or saquinavir or both in patients with limited prior ZDV exposure.

An FDA advisory committee meeting in November, 1995, recommended that saquinavir should be put in a fast track for approval for use in combination with currently available nucleoside RT inhibitors. Approval for saquinavir as monotherapy was not recommended.

To date, studies evaluating combinations of two or more protease-inhibitors have not been initiated. However, a combination of two potent protease-inhibitors with largely non-overlapping genotypic resistance patterns, e.g. saquinavir and ritonavir or indinavir, warrants clinical investigation. A novel protease-inhibitor, which appears a good candidate for combination treatment with either RT inhibitors or other protease-inhibitors, is VX-478. This agent shows potent antiviral activity in vitro, and acts additively to synergistically in combination with ZDV and didanosine. Although it is 90% protein-bound, no marked reductions in antiviral activity were observed in the presence of 45% human plasma. Analysis of drug resistant virus selected in vitro revealed a unique genotypic resistance pattern involving mutations at codons 46, 47 and 50 of the protease gene. Interestingly, these mutations appeared to sensitize virus to other protease-inhibitors, including saquinavir and indinavir. Furthermore, passaging virus containing these mutations in the presence of saquinavir induced the acquisition of saquinavir resistance, but restored susceptibility to VX-478.

Similar interactions should encourage the exploration of protease-inhibitor combinations which constrain the options for the escape of protease. This is facilitated by the fact that protease is more amenable to structure-based design than other target substrates because of its size and the relative ease in gaining structural information by crystallography.

4.8. Other combinations

Interferon alpha is an immunomodulatory agent which augments the function of natural killer- and cytotoxic T cells. In addition, it exhibits antiviral activity in vitro, and anecdotal clinical reports suggest anti-HIV activity (Ho et al., 1985; Lane et al., 1990). Since interferon alpha has also been shown to act synergistically with ZDV in vitro (Johnson et al., 1990; Johnson et al., 1991), several studies evaluating treatment of HIV infection with this combination have been performed (Edlin et al., 1992; Frissen et al., 1994). However, no studies to date have convincingly demonstrated an additional benefit of the addition of interferon alpha to ZDV treatment. A combination of interferon with didanosine also does not appear to result in enhanced antiviral effects in vivo. Possible explanations for the apparent lack of additional activity of interferon include the acquisition of insufficient concentrations of the drug and the development of drug resistance. Alternatively, cellular resistance to the immunomodulatory effects may limit the activity, which may be prevented by intermittent therapy.

The possibilty of an enhanced effect in combination with other antiretrovirals cannot be excluded.

A recent study of intermittent courses of interleukin-2 (IL-2) in combination with continued current antiretroviral therapy in the treatment of HIV-1 infection showed marked sustained rises in CD4 + lymphocyte counts in patients with initial CD4 + cell counts above 200 cells per mm^3 (Kovacs et al., 1995). However, in contrast, increasing levels of viremia and declining CD4 + cell counts were observed in more immunosuppressed patients, which can be explained by increased viral replication through activation of T-cells or increased production of IL-6 and tumor necrosis factor (TNF), mediated by IL-2. In the relatively immunocompetent group of patients studied, increases of viral replication during IL-2 infusion, as measured by increases in plasma HIV-1 RNA load, were also observed but were transient, lasting only less than 1 week. It appeared that the outbursts of viremia in this group could be prevented by the concurrent administration of delavirdine, a potent nonnucleoside RT inhibitor, during IL-2 infusion. The role of IL-2 in the treatment of HIV infection needs further research, as this therapy is associated with considerable toxicity. Important questions in this respect are whether the induced CD4 + cells are functional, i.e. whether these increases result in long term suppression of viral replication and prevention of opportunistic disease. Moreover, future clinical trials need to elucidate further which patients benefit from treatment and which treatment regimens and routes of administration are optimal.

As previously discussed in the pharmacologic section, hydroxyurea potentially enhances the effect of didanosine by reducing the concentration of endogeneous deoxyadenosine-triphospates. Indeed, synergistic antiviral activity of combined hydroxyurea and didanosine, without enhanced cellular toxicity, has been observed in vitro, in a variety of cells, including resting and activated T cells and monocytes/macrophages (Gao et al., 1994; Lori et al., 1994; Malley et al., 1994). Based on these observations, a small uncontrolled study of this combination has been performed (Biron et al., 1995). Twelve asymptomatic, previously untreated patients were treated for a period of 3 months with didanosine 200 mg bid and hydroxyurea 500 mg bid. Marked virologic and immunologic responses were observed in all patients. Six subjects had undetectable plasma RNA levels after 3 months, while the other six showed an average decrease of 1.87 log. Similar findings were observed in the responses on PBMC load. CD4 + cell counts increased from an average 392 cells/mm^3 at baseline to 573 cells/mm^3 after 3 months of treatment. Several larger scale randomized controlled studies are planned to validate the encouraging results of the pilot study.

5. Discussion and consensus conclusions

Increasing evidence suggests the potential efficacy of combined antiviral therapy in a variety of viral infections for which currently approved treatment results only in limited clinical benefit. In HIV-1 infection, improved virological and immunological responses to several combination treatment regimens suggest that combination therapy may become the treatment of choice. In addition, a delay in the development of resistance to ZDV, representing one of the rationales of combination therapy, has been observed in some clinical studies of potent combination regimens. In the treatment of a variety of other viral infections, including infections with respiratory viruses, herpesviruses and hepatitis viruses, results of in vitro studies, animal studies and limited clinical studies suggest potential enhanced efficacy of combined antiviral treatment. These promising observations encourage continuing efforts to develop combination strategies. The list of antiviral agents which are approved or await approval by regulatory authorities increases, while the quest for novel potent antiviral agents continues. The number of possible combinations thus continues to expand.

A rational basis for the selection of clinical drug combinations is desirable, and should ideally include considerations of drug-drug interactions with respect to efficacy and toxicity, pharmacologic issues, and analysis of drug resistance patterns. The rational design of combination strategies is not restricted to convergent or divergent combinations of antiviral agents, but also

includes combinations of antiviral drugs and non-antiviral drugs, such as biological response modifiers.

5.1. What is the role of preclinical analysis in selecting clinical combinations?

As has been discussed, in vitro analyses of drug combinations are subject to a large number of variables, which pose difficulties in interpreting results of two drug combinations, let alone combinations of three or more drugs. While standardization of in vitro analyses would be desirable, reaching a consensus on this point will be equally difficult in view of the number of variables. In general, in vitro analysis should include the use of clinical virus isolates and physiologic host cells, as well as a range of drug and virus concentrations. Furthermore, activity should be tested in a range of cell types and at different stages of infection. Finally, the results should be reproducible and amenable to statistical analysis. Three-dimensional analysis of drug-drug interactions has the advantage of exploring drug-interactions at all concentration ratios of the drugs.

Even an ideal in vitro system is far removed from the in vivo situation. The more complex pathogenesis of infection in vivo and the pharmacology of drugs present difficulties in extrapolating in vitro dose-effect relationships to the clinical situation. Although promising efficacy of several combinations in vivo, e.g. ZDV and 3TC, had been predicted in vitro, other combinations which held promise in vitro have not fulfilled their promise in vivo to date, e.g. ZDV and interferon alpha. Alternatively, the activity of a combination in vitro may be less pronounced than the observed efficacy in vivo, as appears to be the case for the combination of ribavirin and immunoglobulins for RSV infection. The limitations of in vitro analysis are likely to be more pronounced when testing combinations of three or more drugs. Clinical trials will be needed to validate in vitro testing of all in vitro drug combination studies.

In view of complicated pharmacokinetics, it is often difficult to predict whether similar active drug concentrations will be attained in vivo as used in the in vitro experiments. Furthermore, these drug-concentrations may not be maintained at constant levels in vivo because of time-dependent fluctuations of drug concentrations. Pharmacokinetic studies should thus be included in clinical studies of combination therapy. In general, the doses selected for initial clinical combination regimens should be the same as used with monotherapy or should equal the maximum tolerated dose in combination. While dose reduction of the toxic components of a combination is one of the rationales of combination therapy, this should probably be restricted to combinations which display clear synergistic activity in vitro or have already shown benefit at higher doses.

While the use of in vitro analysis for the selection of effective clinical drug combinations is clearly limited, an unequivocally important role of in vitro testing is to rule out significant antagonistic interactions. For example, in vitro observations of clear antagonism between ZDV and ribavirin have rightly resulted in a reluctance to test this combination in vivo. To a limited extent, significant synergistic toxicity may likewise be excluded by in vitro analysis. Finally, drug resistance patterns observed in vitro may aid in selecting drug combinations.

Some of the limitations of in vitro analysis are overcome in animal models; however, while toxicity patterns and pharmacokinetic interactions can be evaluated relatively accurately, analysis of the efficacy of drug combinations requires a large number of animals. Limited availability of suitable animal models appears to preclude the usefulness of these models for the selection of clinical drug combinations for infections with HIV, HBV and HCV, which represent major targets of combination therapy. Suitable animal models seem to be available for CMV and HSV infections. However, the number of studies evaluating drug-combinations in these animal models is limited, and there are insufficient data to assess whether and which animal models are predictive for the efficacy of combination therapy in a human infection.

In summary, the most important roles of preclinical testing for selecting clinical drug combinations are to exclude significant antagonistic interactions or synergistic toxicity, and to analyze

drug resistance patterns. Although animal models may be helpful in selecting clinical combinations for CMV and HSV infections, the 'human model' appears the only practical model for the efficacy analysis of drug combinations for HIV, HBV and HCV infections. Time constraints in the quest for effective treatment of these infections and the large number of patients support timely evaluation of drug combinations in humans.

5.2. How should clinical combinations be evaluated?

The endpoints used in clinical studies and required for regulatory approval of drug combinations are subject to much controversy, especially in HIV infection. In general, the use of clinical endpoints is preferable for the analysis of the efficacy of a drug combination. Several viral infections are clearly amenable to analysis of clinical endpoints, e.g. infections with RSV or influenza in immunocompromised or hospitalized patients, CMV and other herpesvirus infections. However, in view of the long latent phase of HBV and HCV infections, clinical endpoints, which would include death or development of hepatoma, are not generally used nor required by regulatory authorities in clinical trials of these infections. Instead, clearance of virus associated with histological improvement of the liver and normalization of transaminase levels are generally used as endpoints in clinical studies of viral hepatitis.

In contrast, despite the long latent phase of HIV infection, clinical endpoints are still required by many regulatory authorities for the approval of antiretroviral drugs or combinations of drugs. However, analysis of clinical endpoints in HIV infection, which include disease progression and death, is only realistically possible in patients with late stage disease or pediatric HIV infection. In asymptomatic HIV-infected persons, representing the group of patients which would probably benefit most from treatment, the generation of sufficient clinical endpoints for analysis cannot be achieved within reasonable time. In addition, as exemplified by the Concorde study (Concorde Coordinating Committee, 1994), a long duration of a study increases the potential risk that all patients will eventually receive the same treatment regimen, which complicates the analysis. Increasing evidence indicates that low levels of HIV-1 RNA load are associated with a better prognosis, and that virus load is predictive for the clinical course of HIV-1 infection (Cao et al., 1995; Mellors et al., 1995; Pantaleo et al., 1995). Similar to viral hepatitis studies, virological markers, together with immunologic and safety parameters, should probably suffice as endpoints for regulatory purposes in clinical studies of early treatment in HIV-1 infection. Research should therefore include the definition of the magnitude and durability of immunologic and virologic responses which are associated with clinical benefit. This research should be performed in earlier stages of infection, since suppression of virus load may not translate into clinical benefit in late stage disease patients. The analyses of ongoing large clinical endpoint studies, e.g. NIH ACTG 175 and DELTA, will aid in elucidating correlations between the clinical course and virologic and immunologic responses to treatment. However, as time progresses, insight into this relationship will also be derived from ongoing and future studies using virological and immunological endpoints.

5.3. Which combinations are promising and warrant further evaluation?

5.3.1. Respiratory viruses

Based on potent synergistic activity in vitro and in animal models, it is recommended that the efficacy of combined rimantadine and intravenous ribavirin be evaluated in hospitalized patients with influenza. In addition, a combination of immunoglobulins and ribavirin may prove efficacious in hospitalized patients with serious RSV infections.

5.3.2. Herpesviruses

Promising antiviral effects in vitro and in animal studies warrant clinical evaluation of a combination of ACV and monoclonal antibodies in the treatment of neonatal HSV infections. Ongoing studies are evaluating the potential efficacy of combinations of ganciclovir and foscarnet and combinations of these drugs with monoclonal an-

tibodies in the treatment of HIV-related CMV retinitis.

5.3.3. Hepatitis B virus

Lamivudine is a very promising drug in the treatment of HBV infections. Long-term treatment with this drug may prove efficacious and is currently being studied. In addition, ongoing studies will evaluate the potential enhanced efficacy of lamivudine when combined with interferon alpha.

5.3.4. Hepatitis C virus

A lack of effective agents appears to currently preclude the development of combination strategies. Clinical studies evaluating the efficacy of interferon and ribavirin are ongoing. The viral serine protease may prove to be a potential target for novel potent agents.

5.3.5. Human immunodeficiency virus

Since both divergent and convergent combination strategies have theoretical merits, both strategies should be investigated. However, the choice of potentially effective combinations is limited by the number of drugs made available by pharmaceutical companies for clinical evaluation of combined strategies. More rapid progress in the quest for effective combination strategies may therefore require early or provisional approval of drugs for combination treatment, or active intervention of regulatory authorities in the design of combination treatment strategies.

Several nucleoside analogue combinations and combinations of nucleoside analogue RT inhibitors with protease-inhibitors have shown promising results. Three or more drug combinations may prove to have additional benefits, but should be evaluated in previously untreated patients, as well as in more experienced patients. Theoretically, combinations of potent protease-inhibitors with non-overlapping drug resistance patterns hold much promise and should be investigated clinically in the near future.

Addendum

Since this article was submitted, the reverse transcriptase inhibitors stavudine and 3TC, as well as the protease inhibitor saquinavir have been approved by the United States Food and Drug Administration for the treatment of HIV infection, the latter two only in combination with other approved nucleoside-analogues.

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