Antiviral Research, 17 (1992) 305–310

AVR 00544

Mini-Symposium – HIV Infections*

Challenges in the clinical development of antiretroviral drugs

Robert T. Schooley

University of Colorado Health Sciences Center, CO, U.S.A.

Introduction

Since the initial recognition of the acquired immune deficiency syndrome (AIDS) as a clinical entity in 1981, much progress has been made in the understanding of HIV pathogenesis, and in the clinical management of people with human immunodeficiency virus infection. With the delineation of human immunodeficiency virus type 1 (HIV-1) as the etiologic agent for the syndrome, a major effort was initiated toward the development of effective antiviral chemotherapy. After initial failures with agents such as HPA-23 and suramin, the development of zidovudine validated the concept that effective inhibition of retroviral replication could have a positive impact on clinical manifestations of HIV infection (Yarchoan et al., 1986, 1987; Fischl et al., 1987; Richmann et al., 1987). In parallel with the development of zidovudine there has been an explosive growth in understanding of the replicative cycle of HIV, and a concomitant proliferation in the number of potentially effective antiretroviral therapeutics. As the number of potential antiviral strategies increases, it will be increasingly important to make use of lessons from mistakes in prior antiretroviral drug development, and of basic knowledge of the pathogenesis of HIV infection to maximize the rate at which effective therapeutics can be demonstrated to be efficacious.

Potential pitfalls in the development of antiretroviral drugs

Since the initiation of clinical trials of suramin in the management of HIV infection, much has been learned about the complexities of moving from the

*Based on a presentation originally made at the Fourth International Conference on Antiviral Research held in New Orleans, Louisiana, U.S.A., April 21–26, 1991.

Correspondence to: R.T. Schooley, University of Colorado Health Sciences Center, CO, U.S.A.

TABLE 1

Potential pitfalls in antiretroviral drug development

(1)	Insufficient in vitro data (AL-721, compound Q, peptide T)
(2)	In vitro studies may not reflect in vivo activity (rsCD4, CD4-IgG)
(3)	Insufficient consideration to pharmacokinetics (dextran sulfate)
(4)	Insufficient preclinical toxicity data, or dose finding (zidovudine, ddI, ddC)

demonstration of antiretroviral activity in vitro to the demonstration of efficacy in the management of HIV infection (Table 1). A number of compounds with putative antiretroviral activity in vitro have entered clinical trials with suboptimal in vitro data. In the use of peptide T, for example, the in vitro antiretroviral activity demonstrated by one laboratory could not be duplicated in the hands of most other investigators. Receptor-based therapies have illustrated another difficulty in the extrapolation from in vitro data to in vivo efficacy. Several investigative groups sought to exploit the role of the CD4 molecule as the principal cellular ligand for HIV (Smith et al., 1987; Fisher et al., 1988; Hussey et al., 1988; Deen et al., 1988; Traunecker et al., 1988). Each of these groups demonstrated the ability of the extracellular portion of the CD4 molecule to inhibit HIV replication in vitro using the IIIB strain of the virus as the basis for the initiation of clinical trials. Several clinical trials of recombinant soluble CD4 were initiated. These studies demonstrated that the molecule could be administered intravenously, intramuscularly, or subcutaneously with little morbidity, and that serum levels could be achieved which were in the range of those required to inhibit HIV replication in vitro (Schooley et al., 1990; Kahn et al., 1990). Despite the maintenance of serum levels of up to 300 ng/ml for up to one month, little or no effect was demonstrated upon serum HIV p24 antigen levels, CD4 cell numbers, or plasma viremia. This apparent paradox between in vitro and in vivo activity led Daar and colleagues to examine the activity of rsCD4 against clinical isolates of HIV (Daar et al., 1990). These studies demonstrated that clinical isolates of HIV were roughly 100-fold less susceptible to inhibition by rsCD4 in vitro than strains of virus which had been passaged repetitively in vitro in T-cell lines. This difference in the susceptibility of clinical and laboratory isolates of HIV to rsCD4 was the first demonstration that in vitro passage of the virus could lead to artifacts in the apparent susceptibility of the virus to antiretroviral agents. The mechanism of the resistance of clinical isolates of HIV to rsCD4 compared to that of T-cell-passaged virus has not yet been delineated.

Insufficient attention to pharmacokinetic considerations have led to additional difficulties in clinical trials of antiretroviral compounds. Dextran sulfate and similar compounds exhibit antiviral activity against HIV in vitro at extremely low concentrations (De Clercq, 1986). On the basis of these data, the NIAID initiated an escalating dose tolerance trial of dextran sulfate in individuals with immunologically advanced HIV infection without prior pharmacokinetic studies (Abrams et al., 1989). Subsequently, it was demonstrated that poor gastrointestinal absorption of dextran sulfate led to

dose limiting toxicity of the drug long before potentially efficacious serum levels of the drug could be achieved (Abrams et al., 1989).

A final common mistake in the development of antiretroviral chemotherapy has arisen from the tendency to design clinical trials on the basis of the maximal tolerated dose of antiretroviral agents, as opposed to those which are required for maximal antiretroviral activity. In the case of zidovudine, the initial clinical trial for efficacy was launched on the basis of extremely limited tolerance data (Yarchoan et al., 1986). As a result of this strategy, although the subsequent randomized clinical trial demonstrated a decrease in HIV-related morbidity and mortality, the hematologic toxicity of zidovudine was greatly exaggerated (Richman et al., 1987). This situation was the result, in part, of the absence of an assay for in vivo antiviral activity prior to the time the phase II/III trial was initiated. The subsequent experiences with neurotoxicity from dideoxycytidine and pancreatitis associated with dideoyxinosine have underscored the importance of careful dose finding in phase I/II studies before the initiation of clinical trials with endpoints based on efficacy.

The impact of improved clinical management of HIV-infected individuals on clinical trial design

At the time of the initial placebo-controlled trial of zidovudine, the projected median survival for HIV-infected individuals after an initial bout of *pneumocystis carinii* pneumonia was 6 months. The recruitment of individuals with advanced HIV infection for this clinical trial resulted in an extremely high projected rate of HIV-related morbidity and mortality in study participants. Indeed, the clinical trial was terminated after a median period of enrolment of only four months when the Data Safety and Monitoring Board noted a 19-fold increase in mortality in study participants receiving zidovudine compared to placebo recipients (Fischl et al., 1987). After the completion of this study, however, zidovudine therapy was approved for HIV-infected individuals with a CD4 cell count of 200/mm^3 or less, or those with AIDS. With the recognition that prophylaxis for *pneumocystis carinii* pneumonia could further decrease HIV-related morbidity and mortality the prognosis for those infected with HIV infection improved further (Centers for Disease Control, 1989). As a result of these improvements in the life expectancy of individuals with advanced HIV infection, it became clear that subsequent trials with clinical endpoints would require a much larger study subject base and/or a longer period of clinical observation.

Subsequent clinical studies of zidovudine have focused on the ability of zidovudine to delay the clinical progression of HIV infection. These studies include two conducted by the NIAID-sponsored AIDS Clinical Trials Groups (ACTG) (Fischl et al., 1990; Volberding et al., 1990). In these studies the design focused primarily on the ability of zidovudine to delay progression from the HIV-infected asymptomatic state (ACTG 019), or from the condition of early clinical symptoms or signs of HIV infection (ACTG 016) to AIDS or advanced

TABLE 2

Clinical study	Study subject characteristics	Enrollment	Study duration	Study duration patient-months
BW 02	AIDS and advanced ARC	280	4 months	1100
ACTG 016	Early ARC	713	9 months	6400
ACTG 019	HIV infected, asymptomatic	3200	13 months	41600

AIDS-related complex. As illustrated in Table 2, the clinical event rate is a function of the severity of HIV infection prior to trial entry. In the case of each of these studies the number of enrollees and the duration of observation was much greater than that required by the initial placebo-controlled study with more advanced disease. It should be noted that each of these trials was initiated prior to the demonstration that prophylaxis for *pneumocystis carinii* pneumonia would delay the clinical progression of HIV infection. In each of these studies *pneumocystis carinii* pneumonia accounted for a large number of the trial endpoints. Thus, if either of these studies were repeated with the standard application of *pneumocystis carinii* pneumonia prophylaxis, it would be necessary to greatly expand the number of enrollees or the period of observation.

Alternatives to clinical endpoints in the design of antiretroviral trials

With the improved prognosis of HIV infection, it has become apparent that clinical trials with primarily clinical endpoints will require much greater numbers of study subjects and/or a longer period of observation. In that most potential study subjects are unwilling to stay on a single agent through several periods of disease progression until death, it is no longer possible to conduct clinical trials in which death is used as the primary endpoint. Indeed, the increased use of prophylaxis for other HIV-related opportunistic infections will render trials in which AIDS defining opportunistic infections are used as endpoints less and less practical. Thus, as it becomes more difficult to utilize purely clinical endpoints in trial design, there will be an increasing reliance on surrogate markers of efficacy. These surrogate markers will include, but will not be limited to, evidence that antiretroviral agents decrease viral replication, or restore (or delay decline of) immunologic function. An extensive effort has been underway to attempt to validate surrogate markers such as CD4 cell number, HIV serum p24 antigen, and other markers of viral replication as potential correlates of clinical endpoints. A major portioning of this effort which has been undertaken by the NIAID-supported Statistical Data and Analysis Center at the Harvard School of Public Health. Statisticians in this group have demonstrated that changes in CD4 cell number were predictive of clinical endpoints in the initial Burroughs-Wellcome-sponsored placebo-controlled trial of zidovudine. Indeed, changes in CD4 cell number actually appeared to underestimate the clinical response to zidovudine (Eric Schoenfeld,

Ph.D., Personal Communication), in that they appeared to account for only approximately one third of the clinical benefits associated with zidovudine. Although the factors which appeared to account for the additional benefits of zidovudine remain to be determined, the study validates the concept that increases in CD4 cell number correlated with clinical benefits in at least one study. At this time of writing it has not been possible to make similar statements for other potential surrogate markers such as serum HIV-1 p24 antigen, beta 2 microglobulin, or neopterin.

CD4 cell number changes are thus an excellent candidate surrogate marker for evaluation of promising antiretroviral therapies. Several potential drawbacks to this approach must, nonetheless, be elucidated. The peripheral blood CD4 cell component accounts for less than 5% of the total number of CD4 cells in the body given the presence of such cells in spleen, lymph nodes, and other organs. Thus, monitoring changes in the peripheral blood compartment assesses only a small subcomponent of CD4 cells overall.

CD4 cell number and function are not necessarily synonymous. Relevant considerations include whether CD4 cells of a given specificity have been selectively deleted by HIV. Thus a given individual might retain immunologically relevant *pneumocystis carinii* antigen-directed CD4 cells through a much lower overall CD4 cell number than another individual. CD4 cell number and function appear even less well correlated in neonates in that it has been demonstrated that neonates are at much greater risk for developing pneumocystis at a given CD4 cell number than adults who are HIV infected (Leibovitz et al., 1990). Finally, the correlation between CD4 cell numbers and clinical outcome has, to date, been demonstrated only for zidovudine. It does not necessarily follow that identical results will be achieved when other antiretroviral agents are examined, especially those with other mechanisms of action. In the case of immune based therapeutics, even more potential diversity is likely in terms of mechanism of action and possible surrogate markers of efficacy.

Despite these potential problems, it is highly likely that the increasing difficulty in performing trials with strictly clinical endpoints will mandate increasing reliance on such surrogate markers in the conduct of clinical trials. This will be a challenge to regulatory agencies, but, in many ways, it can be held that such endpoints are much closer to the underlying pathogenesis of HIV infection than are the extremely distant clinical endpoints which are so heavily influenced by opportunistic pathogen burden, and the level of sophistication of the clinical management of trial participants. If we are to avoid the pitfalls of the past, reliance on an understanding of the basic pathogenesis of HIV-1 infection in the design and conduct of clinical trials will be increasingly important.

References

Abrams, D.I., Kuno, S., Wong, R., Jeffords, K., et al. (1989) Oral dextran sulphate (UA001) in the treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Ann. Intern. Med. 110, 183–188.

Centers for Disease Control (1990) Guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for persons infected with human immunodeficiency virus. MMWR 38, 1–9.

Daar, E.S., Li, X.L., Moudgil, T. and Ho, D.D. (1990) High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc. Natl. Acad. Sci. USA 87, 6574–6578.

De Clercq, E. (1986) Chemotherapeutic approaches to the treatment of acquired immune deficiency syndrome (AIDS). J. Med. Chem. 29, 1561–1569.

Deen, K.C., McDougal, J.S., Inacker, R., et al. (1988) A soluble form of CD4 (T4) protein inhibits AIDS virus infection. Nature 331, 82–84.

Fischl, M.A., Richman, D.D., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., et al. (1987) The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N. Engl. J. Med. 185–191.

Fischl, M.A., Richman, D.D., Hansen, N., Collier, A.C., Carey, J.T., et al. (1990) The safety and efficacy of zidovudine (AZT) in the treatment of patients with mildly symptomatic HIV infection: a double-blind, placebo-controlled trial. Ann. Intern. Med. 112, 727–737.

Fisher, R.A., Bertonis, J.M., Meier, W., et al. (1988) HIV infection is blocked in vitro by recombinant soluble CD4. Nature 331, 76–78.

Hussey, R.E., Richardson, N.E., Kowalski, M., et al. (1988) A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. Nature 331, 78–81.

Kahn, J.O., Allan, J.D., Hodges, T.L., et al. (1990) The safety and tolerance of recombinant soluble CD4 (rsCD4) in subjects with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex: A Phase 1 study. Ann. Intern. Med. 112, 254–261.

Leibovitz, E., Rigaud, M., Pollack, H., et al. (1990) Pneumocystis carinii pneumonia in infants infected with the human immunodeficiency virus with more than 450 CD4 T lymphocytes per cubic millimeter. N. Engl. J. Med. 323, 531–533.

Richman, D.D., Fischl, M.A., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., et al. (1987) The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N. Engl. J. Med. 317, 192–197.

Schooley, R.T., Merigan, T.C., Gaul, P., Hirsch, M.S., et al. (1990) Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Ann. Intern. Med. 112, 247–253.

Smith, D.H., Byrn, R.A., Marsters, S.A., Gregory, T., et al. (1987) Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. Science 238, 1704–1707.

Traunecker, A., Luke, W. and Karjalainen, K. (1988) Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature 331, 84–86.

Volberding, P.A., Lagakos, S.W., Koch, M.A., Pettinelli, C., Myers, M.W., et al. (1990) Safety and efficacy of zidovudine in asymptomatic HIV infected individuals with less than 500 CD4+ cells/mm^3. N. Engl. J. Med. 322, 941–949.

Yarchoan, R., Weinhold, K., Lyerly, H.K., et al. (1986) Administration of 3′-azido-3′-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet 575–580.

Yarchoan, R., Brouwers, P., Spitzer, A.R., et al. (1987) Response of human immunodeficiency virus-associated neurological disease to 3′-azido-3′-deoxythymidine. Lancet 132–135.