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Review

Early nucleoside reverse transcriptase inhibitors for the treatment of HIV: A brief history of stavudine (D4T) and its comparison with other dideoxynucleosides

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ABSTRACT

The occasion of this 25th anniversary issue encouraged us to reminisce about the important history of the discovery of the dideoxynucleoside analogues for the treatment of HIV/AIDS and to chronicle our thoughts about a particular exciting and rewarding period of our scientific careers. Following the identification of the anti-HIV activity of zidovudine (AZT), we participated in the urgent quest to discover optimal treatments of HIV infection and AIDS. A number of previously synthesized nucleoside analogues were comparatively evaluated, and stavudine (D4T) emerged as a promising candidate for development. Following clinical evaluation, D4T became a mainstay of the initial antiretroviral combination therapy, prolonging and saving numerous lives. It has only recently been supplanted by better-tolerated treatments.

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Our story begins in the second half of the 1980s. At that time, we had made separate contributions to antiviral and nucleic acid research and participated in the discovery and development of several of the earlier nucleoside antiviral drugs: 5-iodo-2′-deoxyuridine (Prusoff, 1959), dihydroxypropyladenine (De Clercq et al., 1978) and valaciclovir (Colla et al., 1983), and ganciclovir (Martin et al., 1983). The beginning of the HIV epidemic occurred at the midpoints of our careers, and our research groups made the discovery of new treatments for HIV/AIDS a top priority. The key to progress was to develop or have access to surrogate and specific assays for measuring activity against retroviruses and HIV. It became clear that the group that first determined the anti-HIV activity of a particular nucleoside analogue would be awarded the patent rights to the use of that compound. During the 1950s and 1960s, a small group of pioneering chemists began to advance DNA chemistry and as a result synthesized a number of nucleoside analogues. Notable among these individuals were Jack Fox, Antonin Holý, Jerome Horwitz, Tai Shun Lin, John Moffatt, William Prusoff, Roland Robins, David Shugar, Tohru Ueda, and Helmuth Vorbrüggen. Thus, in the late 1980s many nucleosides analogues were available off the shelf or well characterized in the literature. In fact, the syntheses of azidothymidine (AZT) (Horwitz et al., 1964), 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T, stavudine) (Horwitz et al., 1966), and ddC (Horwitz et al., 1967) (Fig. 1) were first described about two decades earlier, and these molecules were prepared as potential anticancer agents.

Stemming from their interest in human retrovirus research, it was not surprising that researchers at the National Institutes of Health pioneered the development of an HIV assay. First, Mitsuya and Broder of the National Cancer Institute (NCI) were able to use the new assay to report on the anti-HIV activity of suramin (Mitsuya et al., 1984). This was quickly followed with the documentation of the inhibitory effects of AZT (Mitsuya et al., 1985) and dideoxynucleosides (ddI and ddC) (Mitsuya and Broder, 1986). Their pioneering work led to the successful clinical development of these three nucleoside drugs: AZT (zidovudine), ddI (didanosine), and ddC (zalcitabine) with respective dates of US approval of 1987, 1991 and 1992. AZT had been submitted to the NCI for blinded testing by scientists at Burroughs Wellcome, so that company was awarded the method-of-use patent subsequent to the discovery of its antiviral activity. Mitsuya and Broder directly tested ddI and ddC from samples that were purchased commercially (Prisbe and Martin, 1985), and thus those patents were issued to the NCI. Bristol-Myers obtained the license to ddI, and Hoffmann LaRoche carried out the development of ddC. The reported literature synthesis of ddI occurred after the publication of its activity (Webb et al., 1988). Furthermore, a novel synthetic approach for the preparation of dideoxynucleosides from L-glutamic acid was developed (Farina and Benigni, 1988). In addition, this procedure utilizing

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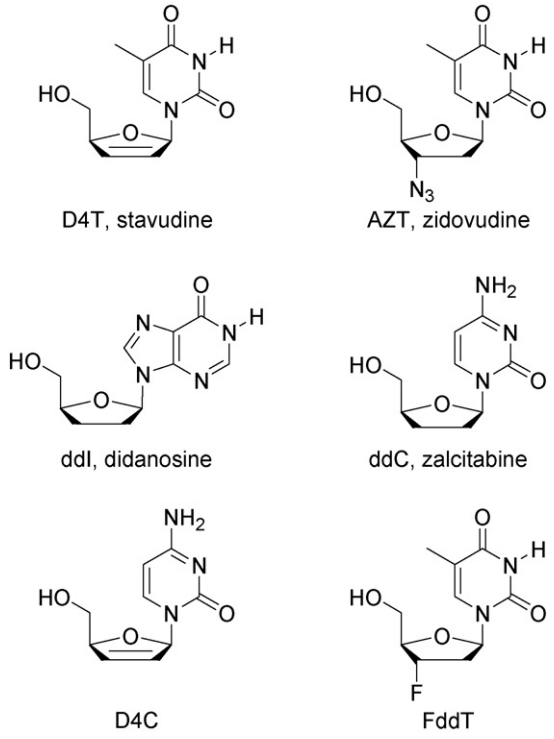

Fig. 1. Structures of anti-HIV dideoxynucleosides

the enantiomeric D-glutamic acid allowed for the identification of the anti-HIV activity of L-nucleosides (L-ddC) (Mansuri et al., 1991).

The preclinical evaluation of ddI was preceded by that of 2′,3′-dideoxyadenosine (ddA), however nephrotoxicity was observed. ddA is acid labile, so that oral administration leads to exposure to the acidic pH of the stomach and degradation to adenine. Adenine is further metabolized to 2,8-dihydroxyadenine which causes nephrotoxicity by crystallization in the kidney (Lindblad et al., 1973). In parallel to the preclinical evaluation of ddA, Arnold Fridland of St. Jude Children's Research Hospital was able to show that the mechanism of action of ddA involved metabolism to ddI by adenosine deaminase, so that all of the antiviral activity of ddA resides in ddI (Ahluwalia et al., 1987). The administration of ddI avoids the production of adenine and the resulting nephrotoxicity (McLaren et al., 1991). This key observation rescued the development program and ultimately resulted in the approval of ddI. Because ddI is also acid labile, its formulation was complicated by the need to prepare large buffered tablets to neutralize the stomach pH. Patients had considerable difficulty taking the medication in this form. This inconvenience plus poor tolerability, pancreatitis and peripheral neuropathy (Cooley et al., 1990; Lambert et al., 1990), limited the initial use of ddI monotherapy to a salvage therapy for patients who had failed AZT.

Eventually ddI found a somewhat broader use. A big advance was the approval in 2000 of an enteric coated form of ddI. As a result, ddI could be administered as a small capsule. The enteric coating protects ddI from the acid environment of the stomach and allows for absorption from the intestine to the blood stream. Additional clinical research also demonstrated efficacy in previously untreated patients (Saag et al., 2004; Berenguer et al., 2008). At peak, ddI was being taken by 64,800 patients in the United States in 2001. After that, the number of ddI treated individuals steadily declined to 18,000 today (Wolters Kluwer Health estimate). This decline is in spite of the fact that ddI is still listed as an alternative choice for first-line therapy by the United States Department of Health and Human Services' guidelines.

The market approval of ddC followed that of ddI and provided another treatment choice. However, this substance was found to cause significant toxicity, delaying its development and limiting its dosage and efficacy. The most concerning side effect was painful peripheral neuropathy that occurred in patients treated beyond 6 weeks (Yarchoan et al., 1988). This toxicity appeared to be dependent on the cumulative dose as it developed in all patients who completed the trial. Increasing this concern was that discontinuation of the drug did not resolve the neuropathy, which in fact often continued to progress. The delayed development of neuropathy has been suggested to be the result of the very potent inhibition of mitochondrial DNA polymerase by the active metabolite of ddC (Chen and Cheng, 1989). Because of its poor efficacy and side effect profile, ddC has never received significant use.

These initial therapies (AZT, ddI, and ddC) were approved as single agents, and this type of monotherapy was found to only temporarily slow the fatal disease progression. As techniques improved for growing HIV and assaying its drug susceptibility, it became clear that drug resistance emerged rapidly, requiring new treatment options. Also, these products were poorly tolerated requiring that therapy be initiated only after patients had experienced the advanced disease that would justify the risk–benefit profile. Among the toxicities of AZT was bone marrow suppression (Richman et al., 1987) such that it could not be used in combination with ganciclovir (Hochster et al., 1990), which was used to prevent blindness caused by cytomegalovirus retinitis in advanced AIDS patients. Patients were thus given the cruel choice of dying sooner or living longer without sight. Most chose to preserve their eyesight. For a durable treatment of HIV, more and safer therapeutic agents were needed to provide for combination therapy to fully suppress HIV to prevent the development of resistance.

Initially, the Prusoff group at Yale University (August et al., 1988; Lin et al., 1987a,b,c) and the De Clercq group at the Rega Institute of the University of Leuven (Baba et al., 1987; Balzarini et al., 1987) independently characterized the activity of D4T. A research team in Japan also reported similar results (Hamamoto et al., 1987). At Yale, Tai-Shun Lin synthesized D4T for evaluation. His sample was tested for anti-HIV activity by Raymond Schinazi of Emory University. Piet Herdewijn was the chemist who prepared D4T at the Rega. Because of the company's proximity to Yale in Connecticut, Bristol-Myers (Hitchcock and Martin) worked closely with Professor Prusoff's group to characterize a variety of nucleoside analogues from which D4T emerged as the preferred candidate for full development (Mansuri et al., 1989a,b). A collaboration agreement was also negotiated with the Rega Institute because it was unclear at the time which patent application would prevail. Yale was eventually awarded the patent.

As was the case with AZT and the dideoxynucleosides, the chemical substance of D4T was already described in the literature, therefore the patent application could not be for its chemical composition but rather for a method of use. Since the use was for treatment of HIV infection, the final patent (Lin and Prusoff, 1990) was only granted once the invention was reduced to practice by the demonstration of antiviral activity in human clinical studies. At that time, the demonstration of antiviral activity in cell culture was discounted from supporting the patents, because there was limited precedent for a predictive value against human disease.

At the beginning of this research, D4T was compared to other anti-HIV dideoxynucleoside analogues in a variety of evaluations to determine which candidate had a chemical and biological profile that would improve the chances of successful drug development. The first comparison was to D4C (Balzarini et al., 1986; Lin et al., 1987b). Synthetic approaches were developed to synthesize multigram quantities of D4T (Mansuri et al., 1989a,b) and D4C (Starrett

et al., 1990). Ultimately, D4C was not pursued into development because it appeared less selective than D4T (Herdewijn et al., 1987; Martin et al., 1990) and also demonstrated high acid lability which could make formulation for oral absorption difficult. In contrast, D4T exhibited good acid stability (Kawaguchi et al., 1989; Martin et al., 1990). An additional consideration was the lack of a predictive in vitro or animal model for the peripheral neuropathy seen with ddC, which made it impossible to exclude that another cytidine analogue such as D4C would exhibit a similar toxicity.

Once D4T was deemed to be preferable to D4C as a candidate for further development, the evaluation continued with a direct comparison to FddT (FLT; alovudine) (Sterzycki et al., 1989) using AZT as the control. An agreement for the rights to FLT had been secured by Bristol-Myers in a collaboration with Fritz Eckstein of the Max Planck Institute (Hartmann et al., 1988). The Rega Institute carried out an independent investigation on FLT (Balzarini et al., 1988), as did two other groups (Matthes et al., 1988; Bazin et al., 1989). The evaluation of thymidine analogues focused on the recognition that the bone marrow toxicity of AZT, which resulted in anemia and cytopenia, could be characterized in preclinical assays (Sommadossi and Carlisle, 1987).

The comparative studies demonstrated that while D4T was less potent against HIV in vitro than AZT and FLT, it had a far better safety profile in a variety of in vitro and animal studies (Mansuri et al., 1990). In measurements of the activity against HIV in cell culture, FLT was more potent than AZT, which in turn was more potent than D4T. Although all three compounds showed similar in vitro toxicity in human erythrocyte progenitor cells, D4T proved the least toxic to both mouse and human bone marrow progenitor cells. Importantly, D4T was less toxic in a 30-day mouse study. FLT showed more pronounced hematological toxicities than AZT, whereas D4T showed a different profile of hepatotoxicity. The conclusion was that FLT, although the most potent, was also the most toxic of the three thymidine analogues. At the higher doses of FLT, a significant incidence of lethality occurred during animal testing (Mansuri et al., 1990). D4T was therefore selected for clinical development. This decision was bolstered at the time by the finding that D4T remained active against AZT-resistant strains of HIV (Larder et al., 1989).

AZT was initially evaluated in patients at a dose of 250 mg given six times per day (Fischl et al., 1987). This was based on its short plasma half-life of 1 h. However, our research indicated that thymidine nucleosides, including AZT and D4T, could be dosed only twice a day and still have full efficacy. The higher initial dose of AZT was driven by a desire to keep the blood level above a particular threshold in order to maximize the effect against the virus, but the trade-off was poor long-term tolerability. The rationale for developing D4T as a twice-daily (BID) drug was based on animal pharmacokinetic studies plus a detailed understanding of the cellular pharmacology. The half-life of D4T in mice was only 17 min (Russell et al., 1989) which was not enough to justify the BID strategy. However, within the cell, dideoxynucleoside analogues are metabolized to the triphosphate, which is the active substance that inhibits the viral reverse transcriptase (Furman et al., 1986). Several labs independently confirmed that D4T triphosphate has better dose proportionality than AZT. This allows the concentration of D4T triphosphate to be controlled by the D4T dose, whereas increasing the dose of AZT only provides a limited increase in AZT triphosphate. In addition, D4T triphosphate was determined to exhibit an intracellular half-life of about 200 min, supporting twice-daily dosing (Balzarini et al., 1989; Ho and Hitchcock, 1989; Zhu et al., 1990). Also, in early human Phase I studies, the high oral bioavailability shown previously in animals was confirmed (Dudley et al., 1992). Because AZT is metabolized by glucuronidation and D4T is not (Russell et al., 1989), D4T achieves four-fold the exposure of the same dose of AZT (Martin et al., 1990).

Early in clinical development, a Phase II study of D4T was carried out in 152 patients, with BID doses of 0.1, 0.5 and 2.0 mg/kg (Petersen et al., 1995). These doses were selected to bracket the range of expected efficacy, based on data from preclinical models, especially the four-fold greater exposure compared to AZT. Although this clinical study predated the availability of viral load testing, the relatively crude p24 assay demonstrated that the lowest dose had limited potency, but the two higher levels showed more substantial virus inhibition. All dose levels appeared safe after 1 year of therapy. Phase III studies were therefore performed in a blinded fashion at the higher doses of 1 and 2 mg/kg. In a large compassionate-use protocol, the 2 mg/kg dose was discontinued because of a finding of greater side effects (Anderson et al., 1995). However, the clearest efficacy signal was also noted in the 2 mg/kg group (Spruance et al., 1997). Faced with this reality, in 1994 the FDA approved both dose levels (40 and 80 mg) to provide the possibility for dose adjustment. Two other capsule sizes were approved (30 and 60 mg) for a 25% dose reduction in patients who weighed less than 60 kg.

Beginning with the approval of the protease inhibitors saquinavir, ritonavir and indinavir in 1996, AIDS patients were treated for the first time with a combination therapy of three drugs, which resulted in fully suppressing the virus and providing durable HIV therapy without rapid development of resistance. Unfortunately, the drugs were dosed multiple times per day and had toxicities that reduced compliance. Patients needed to be highly compliant or resistance would develop. As a result, guidelines indicated that therapy should still be initiated only for advanced disease. The accepted three-drug therapy was either AZT or D4T plus lamivudine (3TC) and a protease inhibitor. At its peak in 1999, D4T was being taken as a part of a regimen by 154,000 of the 375,000 patients receiving therapy in the United States (Synovate data). With time, the use of the dideoxynucleoside analogues were found to be associated with side effects attributed to the inhibition of mitochondrial function (Chen et al., 1991; Brinkman et al., 1999), lipodystrophy, lactic acidosis, and peripheral neuropathy (Dieterich, 2003). Thus, D4T ultimately was shown to have significant toxicities including a 19% versus 3% incidence of lipodystrophy compared to tenofovir disoproxil fumarate after 3 years of therapy (Gallant et al., 2004). As a consequence, only about 7000 patients in the United States are still on a D4T regimen (IMS data). In contrast to the United States where neither the US DHHS nor the International AIDS Society treatment guidelines recommend D4T to be included in first-line combination regimens, its low manufacturing cost has allowed it to persist as the predominant first-line therapy in the developing world, where it is supplied by generic companies and paid for primarily with public funds. This low cost has allowed for the rapid expansion of patients having access to treatment in the developing world from less than 500,000 in 2003 to over 4 million at the end of 2008. The WHO/UNAIDS estimates that approximately 2 million of those patients are on a D4T based regimen. HIV patients worldwide who are still benefiting from D4T 15 years after its approval, together with those who are currently being treated with ddI or AZT, are a testament to the enormous life-saving effects of the dideoxynucleosides developed during the early days of antiretroviral therapy.

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