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Mini Review

Antiretroviral activity of stavudine (2′,3′-didehydro-3′-deoxythymidine, D4T)

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Abstract

Stavudine, 2′,3′-didehydro-3′-deoxythymidine (D4T), is a potent inhibitor of HIV-1 reverse transcriptase in vitro. In clinical studies, stavudine has excellent oral bioavailability in excess of 80%. The dose-limiting toxicity is peripheral neuropathy, which occurred in 15% of stavudine versus 6% of zidovudine-treated patients for 80 weeks in a randomized, blinded, phase III trial. Stavudine-treated groups have experienced significant increases in mean CD4 cell counts and decreases in both mean serum p24 antigen levels and infectious HIV titers in peripheral blood mononuclear cells. In subjects with prior zidovudine treatment, the duration of these responses is limited; CD4 counts and serum p24 antigen levels return to baseline after approximately 6 months. The effect of stavudine on clinical outcome and survival has not yet been established in comparative trials. Stavudine offers an additional therapeutic option to those individuals who are refractory to or intolerant of other available antiretrovirals.

Keywords: Stavudine; Antiretroviral activity; HIV

1. Introduction

Stavudine, 2′,3′-didehydro-3′-deoxythymidine, has recently been approved by the Food and Drug Administration for the treatment of HIV-1 infection in adults who demonstrate clinical progression or immunologic deterioration while receiving zidovudine, didanosine, or zalcitabine or who are intolerant of these drugs. Formerly called

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D4T, stavudine is a synthetic pyrimidine nucleoside analog that is a potent inhibitor of HIV replication in vitro in human cells at concentrations similar to zidovudine. Antiretroviral activity in humans has been demonstrated in several phase I and II clinical trials. The final results of an ongoing, multicenter, double-blind, phase III comparative trial of stavudine versus continued zidovudine therapy in HIV-infected adults with < 500 CD4 cells/mm^3 will be available in 1995. A large parallel track (expanded access) program has evaluated two dosing regimens of stavudine in individuals with advanced HIV disease. In this article, we will first summarize the preclinical information on stavudine, and then review the clinical experience and antiretroviral activity of stavudine in clinical trials conducted to date.

2. Intracellular metabolism and activation

Stavudine rapidly enters cells by non-facilitated diffusion (August et al., 1991). In a variety of cell systems, stavudine is sequentially phosphorylated to the 5′-mono, di-, and triphosphate forms. The 5′-triphosphate form is the inhibitor of reverse transcriptase. Activation to the 5′-monophosphate form by cellular thymidine kinase is the rate-limiting metabolic step (Ho and Hitchcock, 1989). Stavudine monophosphate does not accumulate and the ratio of mono- to di- to triphosphate is relatively constant, ranging from 1:1:1 to 1:1:3 (Balzarini et al., 1989a). This is in contrast to zidovudine, the monophosphate form of which accumulates to high levels because of rate-limiting metabolism to the diphosphate form. The intracellular concentration of stavudine triphosphate increases in proportion to the extracellular concentration of the parent drug. In comparison, intracellular zidovudine triphosphate levels show a poor concentration–response relationship to the extracellular zidovudine concentration (Martin et al., 1990; Balzarini et al., 1989a). Once formed, the triphosphates of both stavudine and zidovudine have intracellular half-lives of approximately 3.5 h (Ho and Hitchcock, 1989).

3. Mechanism of action

Stavudine is a thymidine nucleoside analog in which the 3′-hydroxyl group is replaced by a double bond between the 2′- and 3′-carbons of the pentose ring (Fig. 1). The synthesis of stavudine was first reported by Horwitz et al. (1966). Like the other

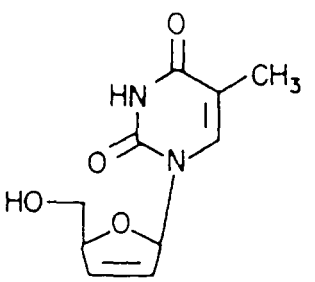

Fig. 1. Structure of stavudine.

Table 1
In vitro activity of stavudine and zidovudine [a]

Cell	50% Inhibitory concentration (IC_{50}) (μM)					
	Stavudine			Zidovudine		
	Virus	Cell	TI [b]	Virus	Cell	TI
MT-4 [c]	0.01	1.2	120	0.006	3.5	580
MT-4 [d]	0.009	119	13,000	0.005		
ATH8 [e]	4.1	110	27	2.4	40–45	17–19
CEM [f]	0.15	90	600	0.1	29	290
Tall 1 [g]	0.5			0.005		
PBMC [h]	0.009–0.04	70	> 1,750	0.002–0.009	200	> 200,000
M/M [i]	0.05			0.1		
M/M [j]	0.3			0.2		
MT-4 [k]	0.05	19	380	0.003	4.8	1600

[a] Adapted from Hitchcock, 1991.
[b] TI, therapeutic index.
[c] Baba et al., 1987.
[d] Hamamoto et al., 1987.
[e] Balzarini et al., 1987; Herdewijn et al., 1987.
[f] Mansuri et al., 1989.
[g] Inoue et al., 1989.
[h] Lin et al., 1987; Chu et al., 1989. PBMC, peripheral blood mononuclear cells.
[i] Perno et al., 1989. Monocyte/macrophage with HTLV-III_B.
[j] Perno et al., 1989. Monocyte/macrophage with HTLV-III_{Ba-L}.
[k] Balzarini et al., 1989b.

dideoxynucleoside compounds, stavudine-5′-triphosphate is believed to inhibit retroviral replication by competing with endogenous deoxythymidine triphosphate as substrate for HIV reverse transcriptase and by blocking DNA chain elongation after incorporation into viral DNA (Yarchoan et al., 1989). The latter DNA chain terminating effect is due to the absence of a 3′-OH group in stavudine for a subsequent 3′–5′-phosphodiester linkage. Whether competitive inhibition or chain termination is the predominant intracellular mechanism of action is unknown.

4. In vitro antiviral activity

The activity of stavudine against HIV-1 was discovered at nearly the same time by groups in the United States (Lin et al., 1987), Belgium (Baba et al., 1987), and Japan (Hamamoto et al., 1987). Subsequently, the in vitro activity of stavudine against HIV-1 has been confirmed in a number of cell systems (Table 1). In most systems, including primary human peripheral blood mononuclear cells, stavudine is comparable to zidovudine in antiviral potency with a wide range of 50% virus inhibitory concentrations (IC_{50}) from 0.009 to 4.1 μM. One investigator reported stavudine to be 100-fold less potent than zidovudine against HIV-1 (Inoue et al., 1989). Stavudine was equally effective against HIV-1 and HIV-2 in MT-4 cells, but was 20-fold less potent than zidovudine against these viruses (Balzarini et al., 1989b).

Stavudine has inhibitory activity against Moloney murine leukemia virus (Lin et al., 1987), Friend murine leukemia virus (Sidwell et al., 1992), and simian immunodeficiency virus (Tsai et al., 1990). But unlike zidovudine, stavudine has no significant activity against hepatitis B virus (Yokota et al., 1991) or enteric bacteria (Hitchcock, 1991).

5. In vitro resistance

In general, HIV-1 isolates that are resistant to zidovudine have not shown cross-resistance to stavudine. In the original report of zidovudine resistance by Larder et al. (1989), 5 clinical isolates exhibiting 200- to 400-fold zidovudine resistance were tested for susceptibility to stavudine. None of these isolates were cross-resistant to stavudine (Larder et al., 1989; Larder et al., 1990; Richman et al., 1990). In another study by Rooke et al. (1991), one pair of sequential clinical isolates from a patient receiving zidovudine was found to have a 10-fold increase in IC_{50} to zidovudine and a similar increase in IC_{50} to stavudine. Another zidovudine-resistant strain had a stavudine IC_{50} of 8.8 μM, but no pretherapy isolate was available for comparison (Rooke et al., 1991). It is unclear whether these two examples represent specific cross-resistance between zidovudine and stavudine or are the result of highly cytopathic isolates. Lin et al. (1994) failed to show any cross-resistance between stavudine and zidovudine using several zidovudine-resistant clinical isolates. Cross-resistance has also not been observed between stavudine and recombinant viruses encoding resistance to didanosine, zalcitabine, 2′,3′-dideoxy-5-fluoro-3′-thiacytidine, 2′,3′-dideoxy-3′thiacytidine, or non-nucleoside reverse transcriptase inhibitors (Lacey and Larder, 1994; Mellors et al., 1992; Mellors et al., 1993).

Two groups have recently reported the in vitro selection of stavudine-resistant variants of HIV-1. Gu et al. (1994) isolated a variant that exhibited 30-fold resistance to stavudine (IC_{50} = 19 μM) by serial passage of HIV-1 in an increasing concentration of stavudine in MT-4 cells. This virus encoded a mutation at codon 50 (Ile to Thr), which was shown by site-specific mutagenesis to confer stavudine resistance. The recombinant virus was not cross-resistant to zidovudine or zalcitabine (Gu et al., 1994). Lacey and Larder (1994) isolated a second mutant strain of HIV-1 that exhibited 7-fold resistance to stavudine (IC_{50} 3.4 μM) by serial passage of HIV-1 in MT-4 cells. This virus encoded a two nucleotide change at codon 75 (GTA to ACA), altering the predicted valine to threonine. This mutation conferred cross-resistance to didanosine, zalcitabine, and 2′,3′-didehydro-2′,3′-dideoxycytosine (Lacey and Larder, 1994). Of note is that stavudine-resistant virus could not be isolated by this group when the RTMC strain that encodes zidovudine resistance mutation at codons 67, 70, 215, and 219 was used.

6. In vitro toxicity

The cytotoxicity of stavudine has been compared to zidovudine in colony-forming assays of human or murine bone marrow by several investigators with differing results

(Sommadossi et al., 1990; Mansuri et al., 1989; Mansuri et al., 1990; Inoue et al., 1989; Gogu et al., 1989). In some studies, stavudine was 20- to 100-fold less toxic than zidovudine; whereas in others, the cytotoxic drug concentrations were similar. In an in vitro assay of neuronal toxicity, stavudine demonstrated toxicity at a lower concentration than zidovudine. Both didanosine and zalcitabine were more toxic than stavudine in this model (Sommadossi and Xie, 1992). Additionally, the inhibition of mitochondrial DNA synthesis by stavudine occurred at concentrations lower than those that inhibit replication of cells (Chen et al., 1991). Other agents that produce mitochondrial depletion in vitro include didanosine and zalcitabine, which have shown dose-limiting peripheral neuropathy in clinical trials.

The interaction of the triphosphates of stavudine and zidovudine with cellular DNA polymerases have been studied by several investigators. In general, stavudine triphosphate (TP) inhibits α-polymerase only at high concentration and is not irreversibly incorporated into the synthesized DNA (Huang et al., 1992). Both stavudine and zidovudine triphosphates partially inhibit β-polymerase (Matthes et al., 1987; Ono et al., 1989). Matthes et al. (1987) reported the IC_{50} of stavudine-TP for HIV-1 reverse transcriptase to be 0.03 μM and the IC_{50} for β-polymerase to be 1.0 μM. The comparative values for zidovudine-TP were 0.05 and 31.0 μM, respectively. γ-Polymerase is sensitive to inhibition by stavudine-TP at lower concentration than zidovudine-TP (Ono et al., 1989). The inhibition of γ-polymerase by the triphosphates of dideoxynucleosides and mitochondrial toxicity has been proposed as a possible mechanism for drug-induced peripheral neuropathy in HIV-infected patients (Chen et al., 1991).

7. In vitro drug combination studies

Zidovudine-monophosphate inhibits the phosphorylation of stavudine to the active triphosphate form when high concentrations compete as substrate for thymidine kinase (Ho and Hitchcock, 1989). The combination of the two drugs resulted in only 50, 6, and 3% of the control levels of mono-, di-, and triphosphates of stavudine. Other in vitro virologic studies have suggested that at different concentration ratios, the two drugs may be additive or synergistic (Sørensen et al., 1993). The combination of stavudine and zidovudine has not been evaluated in clinical trials.

The combination of stavudine and didanosine in vitro results in synergistic anti-HIV activity without evidence of additive toxicity (Brankovan et al., 1989). Combination therapy with stavudine and didanosine is currently being evaluated in pilot clinical trials to determine the safety and antiviral activity of concurrent administration.

8. Pharmacokinetics

Stavudine is stable at gastric pH and is rapidly absorbed after oral administration. Oral bioavailability was $\geqslant 80\%$ in several studies of HIV-infected individuals given both oral and intravenous doses (Dudley et al., 1992). Peak plasma concentration (C_{max})

occurred ⩽ 1 h after oral dosing and was linearly correlated with dose across the 0.03–4 mg/kg dose range. The mean peak concentrations were 0.70–4.2 μg/ml following single doses equivalent to 1–12 mg/kg/day administered in 2, 3, or 4 daily doses (Dudley et al., 1992; Bristol-Myers Squibb, 1994a). Administration of stavudine with a standardized high-fat meal resulted in a reduction of the peak plasma concentration and a delay in reaching C_{max}. However, the area under the plasma concentration–time curve (AUC) after dosing with a meal was unchanged from administration under fasting conditions (Bristol-Myers Squibb, 1994a).

Stavudine is distributed widely throughout total body water after intravenous administration. The volume of distribution is independent of dose and does not correlate with body weight. Cerebrospinal fluid (CSF) levels of stavudine measured in 3 patients after oral doses of 1.3, 3, and 4 mg/kg and were 0.08, 0.20, and 0.48 μg/ml at 0.5, 1.75, and 5 h postdose, respectively, indicating distribution to the CSF (Dudley et al., 1992; Bristol-Myers Squibb, 1994a). Unfortunately, concurrent plasma levels were not available for comparison. Additional studies of the pharmacokinetics of stavudine in the CSF are needed.

The mean terminal elimination half-life ($T_{1/2}$) after single oral doses (0.03–4 mg/kg) is 1.44 ± 0.30 h. Plasma clearance is independent of dose and body weight, and renal elimination accounts for approximately 40% of the overall clearance (Bristol-Myers Squibb, 1994a). The pharmacokinetic properties at steady state are similar to those after single doses of stavudine with no evidence of accumulation (Dudley et al., 1992). The apparent oral clearance of stavudine is decreased and the $T_{1/2}$ is prolonged in individuals with renal insufficiency. Stavudine dosage should be reduced by 50% in individuals with creatinine clearance of less than 50 ml/min. Pharmacokinetic data for individuals with severe reduction in renal function (CL_{cr} < 10 ml/min) or those with hepatic insufficiency are not yet available (Bristol-Myers Squibb, 1994a).

9. Overview of clinical trials

Three phase I and two phase II clinical trials of stavudine involving 272 HIV-infected individuals with CD4 lymphocyte counts of ⩽ 500/mm^3 have been completed (summarized in Table 2). The first two phase I studies (BMS 002 and 003) were dose-ranging trials designed to evaluate stavudine doses of 0.5–12 mg/kg/day, given orally in two, three, or four daily doses, in symptomatic HIV-infected individuals. In the dose-escalation phase of the studies, the maximum tolerated dose was determined to be 2 mg/kg/day, with the major dose-limiting toxicities being peripheral neuropathy and increased hepatic transaminases (Browne et al., 1993; Murray et al., 1994). The minimum effective dose that resulted in either a 50% decline in p24 antigen level or a 50 cell/mm^3 increase in CD4 lymphocytes was 1 mg/kg/day (Murray et al., 1994). New or recurrent opportunistic infections occurred in 5% (BMS 002) and 36% (BMS 003) of patients enrolled in these trials. A third phase I study (BMS 004/005) enrolled 23 individuals with advanced HIV-1 infection (median CD4 lymphocyte count of 55/mm^3) and hematologic intolerance of zidovudine. Stavudine doses of 0.5 or 1 mg/kg/day were evaluated for safety and activity. Stavudine was well tolerated in this

Table 2
Phase I and II clinical trials of stavudine

Toxicity [a]					Markers of antiviral activity			Toxicity	
Phase	(*n*)	Dose range [b] (mg/kg/day)	Baseline CD4 [c]	Week [d]	CD4 response [e]	p24 response [f]	PBMC titer [g]	Neuropathy	Other adverse events [h]
I [i]	41	0.5–12	192	10		13/18	ND	20	4 hepatic
I [j]	41	0.5–12	171	10	20%	13/18	ND	22	4 hepatic
I	23	0.5–1.0	55 [k]	18	29%	6/9	ND		
II [l]	152	0.1–2.0	250 [k]	10	15.8%	11/26	−77%	27	

[a] Number of cases.
[b] Oral dosing.
[c] Mean CD4 cell count at study entry.
[d] Duration of stavudine therapy when viral marker response was measured.
[e] Percent of individuals with a 25 cell or 25% increase in CD4 count sustained for $\geq$ 4 weeks.
[f] Number of responders (50% reduction from baseline sustained for 4 weeks) over the number of patients with detectable p24 antigen at baseline.
[g] Percent change from baseline for individuals in 2.0-mg/kg/day dose group; ND, not determined.
[h] Dose-limiting events.
[i] Browne et al., 1993.
[j] Murray et al., 1994.
[k] Median value.
[l] Petersen et al., 1994.

group of patients and was not associated with myelosuppression or anemia (Skowron et al., 1992).

A phase II trial (BMS 006) of stavudine enrolled 152 HIV-infected individuals into a multicenter, open-label, randomized study of 3 doses: 0.1, 0.5, and 2.0 mg/kg/day. Most patients (73%) had taken zidovudine previously with a mean duration of 69 weeks of zidovudine therapy. Twenty-two (14%) patients enrolled developed opportunistic infections or died during the study period. The incidence of a new AIDS-defining event or death were significantly associated with the baseline CD4 count (Petersen et al., 1994). Another smaller (17 patients), phase II trial (BMS 009) of similar design as BMS 006 has been completed. The clinical outcomes of patients in this study have not been reported.

The pivotal study of stavudine using clinical endpoints is an ongoing, phase III, multicenter, randomized, double-blind trial of stavudine (40 mg twice a day) vs continued zidovudine (200 mg 3 times a day) in 822 individuals with at least 24 weeks of prior zidovudine and CD4 cell counts between 50 and 500 cells/mm^3. The median duration of prior zidovudine therapy was 85 weeks (range 24–246 weeks). Among the first 359 subjects enrolled in this study, approximately 40% of the participants were asymptomatic at study entry, and 40% were p24-antigen-positive using an immune complex dissociated method.

A large parallel track program (expanded access) has provided stavudine to 13,383 individuals with advanced HIV disease for whom no other treatment options were available. The parallel track program evaluated two doses of stavudine (40 mg twice a

day vs 20 mg twice a day for persons weighing > 60 kg) in a double-blind randomized fashion. An interim analysis performed 14 months after the initiation of the program revealed no significant difference in survival at 40 weeks between the two dose groups (Bristol-Myers Squibb, 1994a).

10. CD4 responses

In the phase I and II trials of stavudine, increases in CD4 lymphocyte counts were noted at all dose levels. In general, CD4 responses occurred by 4–8 weeks after initiation of therapy, although some individuals showed a response as late as 6 months after initiation of stavudine. In the phase I trials, approximately 20% of participants had a 25 cell/mm^3 or 25% increase in CD4 lymphocyte count which was sustained for at least 4 weeks (defined as a 25:25 response). In the phase II trials, a significant dose response was noted: 6% of individuals in the lowest dose group (0.1 mg/kg/day) had a 25:25 response compared with 25% of individuals in the highest dose group (2.0 mg/kg/day; $P = 0.04$). Additionally, patients who had received prior therapy with zidovudine had lower CD4 response rates and a trend toward shorter duration of responses as compared to zidovudine naive individuals. Interim analysis of the ongoing phase III trial of stavudine vs continued zidovudine revealed a 25:25 CD4 response in 23% of the stavudine-treated patients compared with 7% of the zidovudine patients ($P = 0.0001$). After 12 weeks, the mean change in CD4 cell count from baseline was +22 cells/mm^3 among stavudine-treated patients as compared to −22 cell/mm^3 for those continued on zidovudine. The stavudine-treated group had CD4 cell counts that averaged 30–50 cells higher than the zidovudine-treated patients throughout the follow-up period of greater than 80 weeks. As shown in Fig. 2, however, the mean CD4 cell

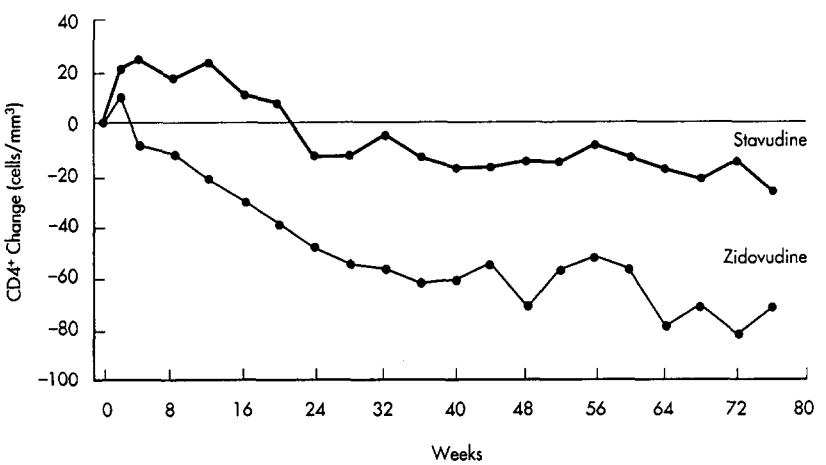

Fig. 2. Mean change in absolute CD4 cell count in patients receiving stavudine vs continued zidovudine in the phase III BMS 019 trial (interim analysis of 359 patients). Differences in mean CD4 cell counts between the treatment groups were statistically significant ($P < 0.0001$).

count in the stavudine group returned to baseline levels by 24 weeks (Bristol-Myers Squibb, 1994a; Dunkle et al., 1994).

11. Adverse effects

The most frequent significant toxicity observed in patients receiving stavudine is peripheral neuropathy, which was the primary dose-limiting event in all initial studies (Browne et al., 1993; Murray et al., 1994). The onset of neuropathy occurred between 1 and 66 weeks after the initiation of stavudine therapy. Peripheral neuropathy has been reported at all dose levels from 0.1 to 12.0 mg/kg/day. The frequency of this event, however, was significantly higher at doses of 2.0 mg/kg/day or greater and with a unit dose exceeding 1 mg/kg. In the phase I studies, dose-limiting peripheral neuropathy occurred in 23% of individuals receiving 0.5–1.0 mg/kg/day, 53% of those receiving 2.0 mg/kg/day, and 67% at doses of 4.0 to 12.0 mg/kg/day (Bristol-Myers Squibb, 1994a). A prior history of neuropathy or the presence of neurologic symptoms, such as parasthesias at the time of initiation of stavudine, were strongly associated with the development of peripheral neuropathy (Browne et al., 1993; Murray et al., 1994). The incidence of neuropathy requiring dose modification reported from the interim analysis of the ongoing phase III clinical trial was 15% in stavudine-treated patients and 6% of those receiving zidovudine (Bristol-Myers Squibb, 1994a).

The symptoms of peripheral neuropathy resolve with prompt interruption of stavudine in most individuals (Petersen et al., 1994). The median duration of symptoms after interruption of stavudine was 2 weeks, and ranged from less than 1 week to several months. Approximately one-half of the patients with peripheral neuropathy in the phase I and II trials were rechallenged and tolerated prolonged further treatment with a reduced dose of stavudine (median treatment duration of 32 weeks, generally at one-half of the original dose).

Asymptomatic increases in hepatic transaminases occurred in 11% of patients in the initial dose escalation trials of stavudine (Browne et al., 1993; Murray et al., 1994). In patients receiving doses of 0.5 and 2.0 mg/kg/day in the phase II trial, modest elevations of SGOT (grade 1–2) were noted, but these did not require dose modification (Petersen et al., 1994). The incidence of elevations in hepatic transaminases requiring dose modification (grade 3–4) in the phase III trial is similar for both stavudine and zidovudine, occurring in approximately 10% in each group (Dunkle et al., 1994). Pancreatitis has been reported in fewer than 1% of stavudine-treated patients and was not related to dose in the parallel track study.

12. Anti-HIV activity

12.1. p24 antigen response

Most of the available data regarding the anti-HIV activity of stavudine consist of serum p24 antigen responses, which were evaluated in all clinical studies. A serum p24

antigen response, defined as a 50% or greater reduction from baseline levels sustained for at least 4 weeks, was demonstrated in 59–83% of the p24-positive individuals in the 3 phase I stavudine trials (Browne et al., 1993; Murray et al., 1994; Bristol-Myers Squibb, unpublished data). Fig. 3 shows the combined p24 antigen responses in patients enrolled in the phase I studies. A dose-dependent response is evident with doses $\geqslant 1$ mg/kg/day demonstrating superior efficacy over the 0.5-mg/kg/day dose.

Seventeen percent of patients enrolled in the initial phase II trial (BMS 006) were p24 antigen-positive at baseline. Of these, 42% had a sustained decline in p24 antigen level. As with the CD4 responses, p24 responses were dose-dependent with 12.5, 40, and 62% of patients responding in the 0.1, 0.5, and 2.0 mg/kg/day groups, respectively. The p24 response rate was independent of baseline CD4 count and prior treatment with zidovudine, although the number of zidovudine naive individuals was small (5 of 26). The duration of p24 response was also directly related to dose; the 2.0 mg/kg/day dose was superior to the lower doses. In the 2.0 mg/kg/day group, median p24 levels remained at least 50% below baseline throughout the first 48 weeks after study enrollment. In comparison, the median p24 antigen level returned to baseline after 18 weeks in the group receiving 0.5 mg/kg/day, and a sustained decrease was not evident at any point in the group receiving 0.1 mg/kg/day. Detectable p24 antigen developed in 17 of 122 individuals (13.9%) who were initially negative (p24 < 30 pg/ml) during stavudine treatment. The occurrence of new p24 antigenemia was distributed equally among the 3 dose groups.

In the phase III study of stavudine vs continued zidovudine, the mean decrease in acid dissociated serum p24 antigen (Abbott Laboratories, Chicago, IL) in the stavudine group was maximal at 8 weeks, but returned to baseline by 24 weeks (Fig. 4).

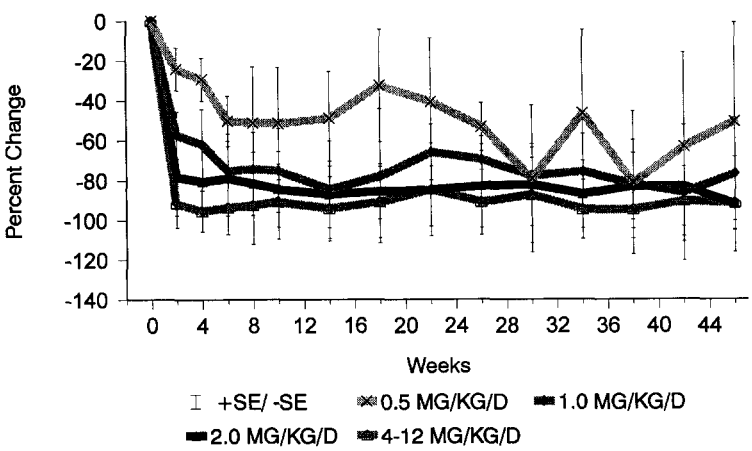

Fig. 3. Percent change in p24 antigen-combined data from all phase I studies of stavudine (BMS 002, 003, and 004/005). The data shown are the mean percent change ± S.E. (bars) (Browne et al., 1993; Murray et al., 1994; Bristol-Myers Squibb, unpublished data).

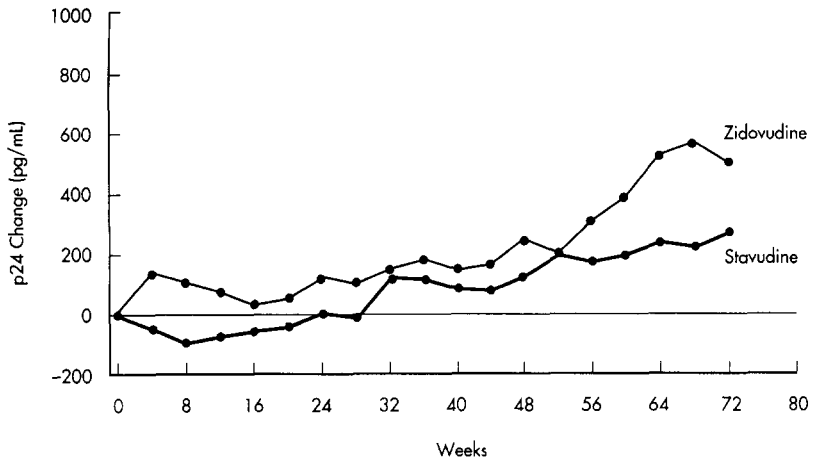

Fig. 4. Mean change in acid dissociated serum p24 antigen (pg/ml) in patients receiving stavudine vs continued zidovudine in the phase III BMS 019 trial (interim analysis of 359 patients). $P = 0.09$ for the difference between the treatment groups at 72 weeks (Bristol-Myers Squibb, 1994b; Dunkle et al., 1994).

12.2. Infectious HIV titer in PBMC

The infectious titer of HIV in peripheral blood mononuclear cells (PBMC) was determined in a subset of individuals receiving stavudine at 0.1, 0.5 and 2.0 mg/kg/day in the phase II study, BMS 006. Mean PBMC titers after 10 weeks of stavudine therapy declined significantly from baseline among individuals receiving 2.0 mg/kg/day, but not in those receiving the lower doses (Table 3). Fifty percent of individuals receiving 2.0 mg/kg/day had a $\geqslant 1$ $\log_{10}$ decrease in median HIV titer in PBMC at week 10, as compared to 15% in the 0.1 mg/kg/day dose group. At week 26, no significant differences between dose groups were present in PBMC HIV titers compared with baseline. However, the percent decrease in mean PBMC HIV titer in the 2.0 mg/kg/day group was 77% ($P = 0.11$), indicating a trend toward an improved duration of response at this dose. After 18 months of stavudine therapy, no significant difference in PBMC HIV titer from baseline was observed at any dose level.

Table 3
Peripheral blood mononuclear cell (PBMC) HIV titers after 10 weeks of stavudine therapy

Stavudine dose (mg/kg/day)	*n*	Baseline (mean titer) IU/10^6 PBMC [a]	Week 10 (mean titer) IU/10^6 PBMC	% change from baseline [b]	*P*-value
0.1	14	22.7	32.3	+42	0.44
0.5	13	31.3	27.1	−12	0.88
2.0	15	34.5	8.0	−77	0.007

[a] IU, infectious units.
[b] Comparing change among dose groups, $P = 0.02$.

13. HIV-1 RNA in PBMC

A branched DNA (bDNA) assay (Quantiplex HIV-RNA Assay, Chiron Corporation, Emeryville, CA) was used to measure the HIV-1 RNA level directly in PBMC in a subset of individuals enrolled in the BMS 006, phase II stavudine trial. This quantitative technique was performed on PBMC samples obtained at baseline, and after 10, 26, and 52 weeks of stavudine therapy. The baseline HIV RNA levels in PBMCs ranged from 6200 to 32,500 genome equivalents/10^7 cells, which correlated with the titer of infectious HIV recovered from PBMCs. After 10 weeks of stavudine therapy, the PBMC HIV RNA level decreased to below baseline in 0 of 3, 4 of 5, and 2 of 3 individuals receiving stavudine doses of 0.1, 0.5, and 2.0 mg/kg/day, respectively (Anderson et al., 1994).

14. In vivo resistance

Stavudine susceptibility of HIV isolates from 11 individuals before and after 18 months of therapy in phase II studies has been studied using a standardized assay (Lin et al., 1994). The mean stavudine IC_{50} of pretherapy isolates was 0.23 μM (range 0.02–0.59 μM). After 18 months of treatment, the mean IC_{50} rose less than 2-fold to 0.41 μM (range 0.01–0.87 μM). The magnitude of change in IC_{50} was $<$ 2-fold in 7 of 11 paired isolates. Two pairs had a 3- to 4-fold increase in IC_{50}, and the remaining two pairs showed increases in IC_{50} of 8- and 12-fold. DNA sequencing of multiple clones of RT derived from post-therapy isolates failed to identify mutations capable of transferring the resistance phenotype to recombinant clones. The mutation at codon 50 (Thr to Ile) observed in a stavudine-resistant isolate selected in vitro was not detected in any of the post-therapy isolates. The codon 75 mutation (Val to Thr) reported by Lacey and Larder (1994) was found in one isolate that remained susceptible to stavudine (Lin et al., 1994).

15. Conclusions

Stavudine has demonstrated definite antiviral activity in clinical trials at the approved dose of 1.0 mg/kg/day. At this dose, stavudine is generally well tolerated during prolonged therapy. The primary toxicities are peripheral neuropathy and elevation of hepatic transaminases. The current data support the use of stavudine in individuals who are intolerant of zidovudine, didanosine and zalcitabine or in patients in whom therapy with these drugs fails to control disease progression. The ongoing phase III trial of stavudine vs continued zidovudine will determine whether stavudine therapy has a significant impact on disease progression and survival. The use of stavudine for initial therapy has not been assessed in the clinical trials performed to date. Additional trials of stavudine in comparison with other antiretrovirals as initial therapy and in combination regimens are needed to clarify its role in the management of HIV infection.

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