Neurophysiological evaluation of zidovudine in asymptomatic HIV-1 infection: a longitudinal placebo-controlled study

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Abstract

The effect of early antiretroviral medication with zidovudine on neurophysiological functions was evaluated in subjects with asymptomatic HIV-1 infection. Patients were recruited participants of a larger double-blind randomised placebo-controlled treatment trial with zidovudine (Concorde). The main outcome measures included: quantitative electroencephalography (QEEG), auditory event-related potentials (AEP) and pattern-reversal visual evoked potentials (PRVEP), as well as standard clinical, virological and immunological markers. No significant impairment and no difference between treatment groups was found in visual P100 latency and auditory long-latency P3 responses which is in agreement with the absence of neurological and neuropsychological impairment over the study period. Significant treatment effects were revealed by quantitative electroencephalography (QEEG). While the placebo group showed a significant increase in delta and theta slow frequency QEEG activity over the study period, slow wave amplitude remained unchanged in the zidovudine group after a mean follow-up period of 28 months. In summary, the data provide evidence for a low level neuropathological process in asymptomatic HIV-1 infection which can be effectively suppressed by antiretroviral medication.

Keywords: Central nervous system; Zidovudine; Azidothymidine AZT; Placebo-controlled trial; Double-blind; Quantitative electroencephalography; Evoked potentials; Human immunodeficiency virus 1 (HIV-1)

1. Introduction

Despite the development of new anti-HIV-1 drugs (Johnston and Hoth, 1993) zidovudine still remains the major antiretroviral drug available for clinical use. Furthermore, zidovudine is currently the main drug available for treatment of primary HIV-1-associated central nervous system (CNS) disorders, in particular HIV-1-associated dementia (Portegies and Brew, 1991). Benefit from zidovudine in advanced neurological conditions is well documented (Pizzo et al., 1988; Brew et al., 1992; Sidtis et al., 1993; Thomas and Borg, 1994) and observations from the Netherlands suggested that the prevalence of HIV-1-associated dementia declined after introduction of zidovudine (Portegies et al., 1989). Nevertheless, little direct evidence exists for a prophylactic role of antiretrovirals in delaying onset or reducing the severity of central nervous system (CNS) involvement. In order to address this question we carried out a prospective placebo-controlled study in patients with asymptomatic HIV 1 infection using standardized clinical, neuropsychological, psychiatric and neurophysiological assessments. Due to the low incidence of neurological symptoms in asymptomatic disease (McArthur et al., 1989; Riccio et al., 1993), quantitative neuropsychological assessments were expected to be particularly useful in monitoring subtle CNS changes (Parisi et al., 1989). This report will document findings obtained using computerized quantitative electroencephalography (QEEG) and evoked potential recordings. Findings of the detailed neuropsychological and psychiatric assessments are reported elsewhere (Riccio et al., in preparation).

2. Methods

2.1. Patient population

Subjects were recruited from amongst HIV-seropositive homosexual or bisexual men enrolled in the Anglo-French
double-blind placebo controlled trial (Concorde Coordinating Committee, 1994) with the antiretroviral drug zidovudine (3'-azidothymidine, AZT, retrovir). Subjects were recruited from three London genitourinary clinics within the Riverside Health District. Injecting drug users, or subjects with a major current psychiatric or neurological disorder, with pre-existing cognitive impairment of other known cause or with active syphilis were excluded from the study. Informed written consent was obtained from all subjects. The study was approved by the local ethics committee.

2.2. Study design and follow-up

The study was designed as a placebo-controlled trial with double blind assessments. Patients entered into the Concorde trial were randomly assigned to either the deferred (placebo) or immediate (zidovudine) treatment groups. All patients received the study medication (placebo or 1000 mg zidovudine in 4 capsules) during their regular clinic appointments with the trial physicians. In order to avoid interference with the main trial no pre-treatment assessments were possible for this study. The first neuropsychiatric and neurophysiological assessment was within 3 months after recruitment into the Concorde trial. New assessments were made every 6 months. The last assessment was made between 19 and 32 months following recruitment with a mean time on trial of 28 months for both treatment groups. A full follow-up over the whole study period was made for 27 patients (11 on placebo and 16 on zidovudine).

2.3. Statistical analysis

All analyses were carried out on intention to treat. The analysis involved 27 patients (11 on placebo, 16 on zidovudine) for whom follow-up assessments were made at the first visit (3 months) and last visit (28 months). These data were analysed using a repeated-measures design. Continuous variables were tested for normality of distribution and, if necessary, were logarithmically transformed. Dependent variables were compared between treatment groups using repeated-measures analysis of variance. Between subjects factor was treatment (placebo, zidovudine) and time was the within-subjects factor (assessments at 3 and 28 months, respectively). A significant interaction effect of treatment and time was regarded as evidence for a differential treatment effect over the study period. Categorical data were analysed using Pearson’s $\chi^2$ statistics except for 2 × 2 tables with expected cell size of less than 5 where Fisher’s exact probability test was used. To determine the relationship between changes in neurophysiological measures and changes in laboratory markers and clinical status between the visits multiple regression analyses were computed. Change scores of QEEG amplitudes, AEP P3 latency and PRVEP P100 latency were used as dependent variables in each analysis. Independent variables were treatment with zidovudine, change in CD4 and CD8 counts, and progression to CDC stage IV.

2.4. Patient assessments

All assessments, interviews, neuropsychological testing, EEG and evoked potential recordings were performed on the same day. A semi-structured interview collected data about medical and psychiatric history, family history, current subjective symptoms, medication, alcohol and drug use. The neurological examination followed a standardized form and was carried out by the treating physicians. All clinical, laboratory and treatment data were collected by the Medical Research Council Clinical Trials Centre and were supplied for the purpose of the present analysis. Based on scores in the neuropsychological tests a global performance rating was obtained from an independent blinded clinical neuropsychologist indicating normal, borderline normal and abnormal performance for each subject at study entry and end.

2.5. Quantitative EEG (QEEG)

The electroencephalogram (EEG) was recorded from 28 scalp derivations referenced to linked earlobe electrodes. Surface tin electrodes were applied according to the international 10–20 system and included eight additional scalp derivations. Signal bandpass was 1.0–40.0 Hz and the digital sampling interval per channel was 5 ms. The raw data were stored on optical discs for review and further analysis. Methods are reported in more detail elsewhere (Baldeweg et al., 1993a,b). EEG was recorded in two blocks for approximately 7 min under resting conditions during which the subjects were asked to keep their eyes closed and subsequently opened.

Editing

Prior to processing of the computerized EEG data all recordings were reviewed by one investigator blinded to the clinical data of the patient according to a standard protocol using both standard bipolar and unipolar EEG montages. Epochs that showed EEG signs of drowsiness, eye movements, blinks, muscle activity or other artifacts were excluded from further analysis. EEG averages for 25 subjects were computed for whom at least 1 min of artifact free EEG in the resting condition with eyes open could be retained after editing.

EEG data processing

The Fast Fourier Transform (FFT), using the Cooley-Tukey algorithm was applied to each 2.56 s epoch and the square root of the absolute power coefficients in the delta (1.0–4.0 Hz), theta (4.0–8.0 Hz), alpha (8.0–12.0 Hz), and beta (12.0–17.0 Hz) frequency ranges were computed for each epoch. EEG amplitudes were averaged over their artifact free epochs within the four EEG bands for all
electrode sites. In order to achieve a reasonable data reduction and to reduce the number of statistical comparisons between the groups (Oken and Chiappa, 1986) the EEG amplitudes were averaged within each frequency band across all electrode derivations with the exception of those electrodes most vulnerable to ocular and muscle artifacts.

2.6. Pattern-reversal visual evoked potentials (PRVEP)

PRVEP were recorded under full-field monocular stimulation using a high-contrast checkboard pattern (check size \(15 \times 15\text{mm}, 0.85^\circ\), overall visual angle of \(17^\circ\)). Latency and amplitude the major positive peak (P100) was measured at the central occipital derivation.

2.7. Auditory event-related potential (AEP)

The auditory discrimination task required the subject to press a button with both right and left thumbs whenever a higher-pitched tone (1500 Hz) randomly embedded in a series of frequent lower-pitched tones (1000 Hz, overall probability 80%) occurred. The tones were presented binurally for 50 ms with a rise and fall time of 5 ms and an intensity of 80 dB SPL. The interstimulus interval was 1 s. Signal bandpass was set to 0.3–40.0 Hz, the digital sampling interval per channel was 2 ms and the length of the sampling epoch was 650 ms including a 50 ms pre-stimulus baseline. The recording was continued until the system had collected 32 artifact free EEG responses to the higher-pitched tones. Two separate recordings were made. Baseline-to-peak amplitudes and latencies were scored using the tangential method (Goodin et al., 1978) at the frontal midline derivation Fz for N2 and at the parietal midline derivation Pz for P3.

3. Results

3.1. Characteristics of the treatment groups

The treatment groups were comparable with regard to demographical characteristics. Mean age was 36.8 years (range 26–46) for the placebo group and 38.1 years (range 25–51) for the zidovudine group. Most subjects were employed (9/11 and 15/16 for placebo and zidovudine groups, respectively). Estimated pre-morbid intelligence scores according to the National Adult Reading Test (Nelson, 1982) were 105.3 (SD 9.6) and 102.7 (SD 10.2) for placebo and zidovudine groups, respectively. No significant neurological history or developmental problems were reported. Two subjects in the zidovudine group had a previous alcohol problem. There was no difference between groups in past and current alcohol and illicit drug use at baseline and study end. None of the subjects were taking psychotropic medication at baseline, and one subject in each group was on antidepressants at study end.

Changes in the trial medication were recorded for 3 subjects during the time of the study. For two subjects in the zidovudine group a dose reduction from 1000 mg to 500 mg per day was prescribed. Three subjects in the placebo group received open treatment with 500 mg zidovudine per day at weeks 48, 88 and 128 on trial, respectively.

3.2. Clinical characteristics

At study entry all subjects had asymptomatic HIV-1 infection according to the CDC Classification (Centers for Disease Control, 1987). During the course of the study 21 patients remained completely asymptomatic (6 on placebo; 15 on zidovudine) and 7 progressed to symptomatic disease (CDC IV A, C2 and E; 5 on placebo and 2 on zidovudine; Fisher’s exact: n.s.). Among the latter group two subjects in the placebo group developed AIDS (Kaposi sarcoma, CDC IV D).

3.3. Neurological and psychiatric symptoms

None of the participants had neurological abnormalities throughout the study period. Complaints such as headache, drowsiness, insomnia, anxiety, depression and other symptoms at any time during the study were reported for 10 patients (3 on placebo, 7 on zidovudine). The commonest symptom was headache reported in 7 patients (3 on placebo, 4 on zidovudine). All of these symptoms were mild and...
Table 2

<table>
<thead>
<tr>
<th></th>
<th>3 months Placebo</th>
<th>Zidovudine</th>
<th>28 months Placebo</th>
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<tr>
<td>PRVEP</td>
<td></td>
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<td>P100 latency, ms</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>left eye</td>
<td>114 (6)</td>
<td>111 (10)</td>
<td>114 (4)</td>
<td>114 (9)</td>
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<tr>
<td>right eye</td>
<td>112 (6)</td>
<td>114 (10)</td>
<td>114 (5)</td>
<td>113 (10)</td>
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<tr>
<td>AEP</td>
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<td>N2</td>
<td></td>
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<tr>
<td>Latency (ms)</td>
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<td>252 (24)</td>
<td>240 (23)</td>
<td>257 (28)</td>
</tr>
<tr>
<td>Amplitude (μV)</td>
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<td>-6.2 (4.3)</td>
<td>-4.6 (6.6)</td>
<td>-5.3 (3.8)</td>
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<tr>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>372 (23)</td>
<td>373 (29)</td>
<td>371 (25)</td>
<td>372 (30)</td>
</tr>
<tr>
<td>Amplitude (μV) $</td>
<td>12.7 (3.9)</td>
<td>8.8 (4.7)</td>
<td>14.6 (3.9)</td>
<td>12.7 (6.1)</td>
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Table 3

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<tr>
<th>EEG band amplitude (μV)</th>
<th>3 months Placebo</th>
<th>Zidovudine</th>
<th>28 months Placebo</th>
<th>Zidovudine</th>
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<tr>
<td>Delta $</td>
<td>10.5 (3.4)</td>
<td>10.0 (2.7)</td>
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<td>10.4 (1.7)</td>
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<tr>
<td>Theta $</td>
<td>9.4 (3.0)</td>
<td>9.6 (3.4)</td>
<td>11.5 (3.8)</td>
<td>9.8 (3.4)</td>
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<tr>
<td>Alpha $</td>
<td>12.7 (5.2)</td>
<td>13.3 (5.9)</td>
<td>14.9 (3.6)</td>
<td>14.9 (3.4)</td>
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<td>Beta</td>
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<td>4.9 (1.1)</td>
<td>6.0 (1.6)</td>
<td>5.4 (1.6)</td>
</tr>
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Transient and did not require further intervention. None of these symptoms, however, were present when subjects attended the neurophysiological and neuropsychological assessments.

3.4. Laboratory markers

In the zidovudine treated group CD4 cell counts remained relatively unchanged over the study period (Table 1), in contrast to the placebo group showing a decline in CD4 cell counts at follow-up (interaction effect treatment and time: $p < 0.05$). CD8 cell counts were markedly increased in the zidovudine group at follow-up (interaction effect treatment and time: $p < 0.01$). The CD4/CD8 ratio declined in both groups (time effect: $p < 0.01$). No significant changes in β2-microglobulin, p24 antibody and p24 antigen between treatment groups as well as over time were observed. The clear increase in mean corpuscular volume in the zidovudine treated group ($p < 0.01$) suggests compliance with study medication.

3.5. Neuropsychological rating

Global performance was rated abnormal for two subjects at the begin of the study (1/11 and 1/16, for placebo and zidovudine, respectively) and three subjects at study end (2/11 and 1/16 for placebo and zidovudine, respectively). The change score based on the difference in the rating between the two visits indicated improvement in four subjects on zidovudine and deterioration for one subject on placebo who had progressed to CDC stage IV.

3.6. Neurophysiological markers

**Pattern-reversal visual evoked potentials (PRVEP)**

No significant differences between the treatment groups were found for the P100 peak latency (Table 2). No statistically significant prolongation of P100 latency between visits and no abnormally delayed P100 latencies were found when compared with an HIV-1 seronegative control group.

**Auditory event-related potential (AEP)**

No significant impairment in N2 and P3 long-latency AEP waves at follow-up (tbr2) and no difference between treatment groups was found. The significant increase in P3 amplitude in both groups at 28 months is possibly related to practice effects. No abnormal N2 and P3 latency delays were detected when compared with age-normalised control values (Baldeweg et al., 1993a).

**Quantitative EEG (QEEG)**

None of the subjects had any focal or diffuse EEG abnormalities, neither at study begin nor end. The quantitative
The EEG analysis (QEEG) revealed a significant treatment effect for the slow frequency QEEG amplitudes (Table 3). While the placebo group showed a significant increase in delta and theta activity over the study period, QEEG slow wave amplitude remained relatively unchanged in the zidovudine group after a mean follow-up period of 28 months (interaction effect treatment and time: \( p < 0.05 \) for both delta and theta bands). The mean topographical distribution across the scalp for theta amplitudes in both groups is shown in Fig. 1.

A multiple regression analysis indicated that for both delta and theta amplitudes treatment with zidovudine was the only significant predictor variable (delta: \( \beta = 0.44, t = 2.36, p < 0.05 \), and theta: \( \beta = 0.43, t = 2.31, p < 0.05 \)). Increased alpha amplitude at 28 months was associated with progression to CDC stage IV ( \( \beta = 0.46, t = 2.51, p < 0.05 \)). QEEG amplitude was not correlated with changes in CD4 and CD8 counts.

4. Discussion

This prospective, placebo-controlled study provided evidence that despite the absence of significant cognitive and neurological impairment, changes in electrocortical activity can be detected which could in turn be suppressed by antiretroviral medication with zidovudine.

Although the zidovudine effects on systemic HIV-1 infection and T cell subsets were qualitatively similar to the results of the main Concorde study (Concorde Coordinating Committee, 1994), it appeared that the effect on CD4 counts was slightly larger than observed in the main trial. In order to test the representativeness of the sample with regard to immunological status a subsequent comparison was made between the study sample and the Concorde subsample which was recruited within the local health district (\( n = 194 \)). However, no significant differences in CD4 counts at pre-treatment baseline, study entry and end were found (Riccio et al., 1995).

The absence of significant neurological impairment and neurophysiological deficits as measured by visual and auditory evoked potentials in our study is generally in accordance with findings from larger cohort studies and reflects the low level of neurological and cognitive impairment in asymptomatic HIV-1 infection (McArthur et al., 1989; Riccio et al., 1993). Although abnormalities in evoked potentials have been reported previously (Smith et al., 1988; Jakobsen et al., 1989; Goodin et al., 1990; Ollo et al., 1991), further investigations did not confirm these findings for asymptomatic subjects in general (McAllister et al., 1992; Goodwin et al., 1990; Jabbari et al., 1993; Baldeweg et al., 1993a). Only a subgroup of asymptomatic individuals with low immune competence may be affected (Malessa et al., 1989; Bocculli et al., 1993). Delays in long-latency N2 and P3 event-related potentials occur in HIV-1 infected subjects with symptomatic HIV-1 infection (Goodin et al., 1990, Goodwin et al., 1990). The P3 delay is correlated with reduced information processing speed and psychomotor slowing (Messenheimer et al., 1992; Baldeweg et al., 1993a). Similarly reduction of N2/P3 amplitude reflects cognitive decline in HIV-1 infection

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**Fig. 1.** Topographical distribution of mean QEEG amplitude in the theta frequency band in the placebo group (left side) and zidovudine groups (right side) at study entry (3 months) and end (28 months). The front of the maps is upwards and the view is from the top of the head. The difference between two map isolines corresponds to 0.5 \( \mu \text{V} \) amplitude difference.
The lack of both latency and amplitude impairment in long-latency AEP waves in this study is in agreement with the absence of neuropsychological impairment in the majority of patients over a mean follow-up time of more than 2 years.

The differential change in slow wave QEEG activity in both study groups was the only evidence for subtle CNS changes in subjects with asymptomatic infection and the only long-term treatment effect of zidovudine in this study. The QEEG findings were not related to EEG abnormalities as seen in conventional EEG of subjects with symptomatic HIV-1 infection, such as intermittent theta and delta slowing, often with bilateral anterior dominance (Gabuzda et al., 1988; Tinuper et al., 1990). The preliminary evidence suggests that these subclinical QEEG changes occurred in the majority of subjects on placebo, irrespective of whether they had progressed clinically or not. Similarly, Jabbari et al. (1993) reported three patients who developed abnormal EEGs with diffuse slowing at follow-up despite remaining clinically asymptomatic with CD4 counts above 400 cells/μl. Computerised quantitative EEG (QEEG), despite its limitation for clinical use, facilitates the detection of slow wave activity that is not easily detectable by visual inspection (Nuwer, 1988). Although EEG changes are not diagnostically specific, quantitative EEG has been shown to detect the first signs of developing neurological involvement in HIV-1 infection (Parisi et al., 1989).

It is however possible that other factors account for the EEG findings. Zidovudine itself may have altered the EEG. Although such a drug effect cannot be ruled out, no differences in any of the EEG frequency bands were detectable in the baseline data after subjects had taken 1000 mg zidovudine per day for an average of 3 months. In addition, activation of other pathogens than HIV may have played a role. However, opportunistic infections of the brain are generally more common in more severely immunosuppressed patients than the subjects in this study, the vast majority of whom had CD4 lymphocyte counts above 200 cells/μl. All subjects remained clinically free of neurological symptoms during the study and a further follow-up period of one year. Furthermore, no evidence of focal brain lesions was found in a collaborative magnetic resonance imaging study (Hall-Craggs et al., 1995) in 47 Concorde participants (13 from this study) with very similar immunological status as in this study.

Neuropathological findings in HIV-1 infected asymptomatic subjects who died before developing AIDS are characterised by predominantly T cell lymphocytic leptomeningitis which is not seen in advanced disease (Bell et al., 1993). Giant cell encephalitis and microglial nodules are uncommon. Polymorph reactivity is either negative for HIV-1 or the amount of detectable virus is very low (Bell et al., 1993). Massive spread of HIV-1 into non-lymphoid tissues, including the CNS occurs only with breakdown of the immune system and progression to AIDS (Donaldson et al., 1994). Because of the very low viral load it is possible that the chronic immunological activity associated with viral clearance (review in Tyor and Johnson, 1992) made a larger contribution to the neuropathological changes underlying the QEEG findings than direct HIV-1 neurotoxicity. Upregulation of cytokines, the main messengers of immunoreactive cells, has indeed been implicated in HIV-1-related neuropathology (Tyror et al., 1992; Wesselingh et al., 1993). Experimental application of cytokines directly increases fast and slow electrocortical activity (Carolen et al., 1993). In addition, cytokines, such as tumour necrosis factor, impair subcortical white matter, lesioning of which can increase slow wave delta activity (Gloor et al., 1977).

Our findings lend support to recent evidence from two large cohort studies showing that cognitive impairment may indeed occur in a group of HIV-seropositive individuals during the asymptomatic stages of infection. Subjects with low educational status were found at higher risk for cognitive impairment than subjects with higher education level and HIV-1 seronegative controls (Satz et al., 1993; Maj et al., 1994). This finding has been interpreted as evidence for a “cerebral reserve capacity” (Satz et al., 1993), which can compensate for gradual CNS alterations. A large “cerebral reserve capacity” associated with high educational status and pre-morbid intelligence in the majority of subjects in this study could have prevented cognitive decline despite evidence for low grade pathological changes in the placebo group.

The preliminary evidence from this study suggests that the zidovudine effect on QEEG was not entirely due to its effect on immune status. The primary action of zidovudine in the brain is to reduce the spread of HIV-1 infection and the release of viral neurotoxins by inhibiting reverse transcriptase (Geleziunas et al., 1992). It is conceivable that the favourable CSF penetration of zidovudine (Burger et al., 1993) and the high dose used in the Concorde study (1000mg) resulted in effective suppression of HIV-1 in the CNS thereby preventing detectable QEEG changes in the zidovudine group.

Due to the small sample size caution is needed in interpreting these results. Despite its shortcomings, the study provides a controlled assessment of CNS effects of zidovudine in early HIV-1 disease with the longest follow-up reported so far. Moreover, the findings are supported by recent data from a large natural history cohort study (Baldeweg et al., 1995), showing that long-term medication with zidovudine suppressed slow wave EEG and reduced neurocognitive impairment in subjects with symptomatic HIV-1 infection and AIDS.

Neurological and psychiatric complications of HIV-1 infection still contribute significantly to the clinical picture of AIDS. With improvements in management of opportunistic infections in AIDS the prevention of direct HIV-related CNS damage becomes even more important. Future anti-HIV drugs should therefore be tested thoroughly for CNS efficacy.
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