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Recombinant human interferon beta ser protects against zidovudine-induced genetic damage in AIDS patients

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Summary

This study was conducted to evaluate the in vivo genotoxicity of zidovudine (ZDV) in patients with Acquired Immune Deficiency Syndrome (AIDS). Patients with this disease who were non-smokers and on ZDV (1200 mg/day) as their only medication for 4 weeks to 7 months were studied. Patients with AIDS who had not received ZDV served as a negative control. Whole blood cultures were initiated by conventional methods with PHA 1:50 dilution. In addition, for each culture there was an untreated control and a recombinant interferon-β (rIFN-β)-treated culture. The IFN-treated cultures were exposed to 10, 100, 1000, or 10000 units of rIFN-β for the entire incubation period. The cells were harvested at 72 h and stained with a fluorescence plus Giemsa method which permits the determination of the number of division cycles a cell has completed. One hundred metaphases from first division cells were scored from each culture for chromosome aberrations that were mainly from the chromatid-type, i.e. chromatid, chromosome, and isochromatid breaks. The frequency of breaks in the ZDV and no ZDV group was 8.29 ± 2.65 and 0.5 ± 0.29 per 100 cells respectively ($P<0.05$). Cultures from ZDV patients that were incubated with 100 and 1000 units of rIFN-β, however, showed a frequency of 1.3 ± 0.71 and 1.9 ± 1.08 respectively, which was significantly lower than observed in the cultures not exposed to IFN ($P< 0.05$). At the highest dose of rIFN-β utilized no aberrations were detected. It would appear, therefore, that ZDV treatment results in a significant increase in chromosomal aberrations and that cul-

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tivation of cells with rIFN-β significantly decreased the appearance of these aberrations in vitro.

ZDV; Interferon beta; Chromosomal damage; AIDS

Introduction

AIDS patients are known to develop immunologic abnormalities resulting from HIV infection. This leads to a quantitative and functional deficiency of T4 lymphocytes, besides affecting other components of the immune system (Masur et al., 1981; Bowen et al., 1985; Lane and Fauci, 1985; Bonavida et al., 1986; Nokta and Pollard, 1989, 1990). ZDV is the current drug of choice for treatment of HIV-infected patients (Fischl et al., 1987) and acts by inhibiting HIV replication. In randomized double-blind placebo-controlled trials, AIDS patients, those with ARC, and asymptomatic patients with less than 500 CD4 cells receiving ZDV or placebo had prolonged survival, fewer chances of developing opportunistic infections (Fischl et al., 1987; Volberding et al., 1990) and decreased risk of disease progression if they received ZDV as compared to placebo groups. Controlled clinical trials are currently underway to use ZDV in asymptomatic HIV carriers, early ARC and pediatric patients.

Combining ZDV with other retroviral or immunomodulatory drugs has been shown to have at least additive effects on HIV replication, in vitro. Clinical studies of ZDV and rIFN-β as well as other combination therapies are in progress (Miles et al., 1990). ZDV treatment is not, however, without side effects. In placebo-controlled studies, anemia, neutropenia, headache and nausea have been the most common side effects reported, more frequently in recipients of ZDV (Richman et al., 1987; Gill et al., 1987). Little is known about the genotoxic effects from prolonged exposure to ZDV. Agents that produce genotoxicity could possibly lead to somatic cell lesions resulting in cancer and/or a germinal cell lesion that could even be transmitted vertically. This would not seem to pose a serious problem for AIDS patients, however, it could be significant for others, i.e. asymptomatic HIV seropositives and/or normal individuals receiving prophylactic therapy. There is evidence that ZDV is mutagenic at high doses (4–5 mg/ml) using the L 5178 y/tk$^{+/-}$ mouse lymphoma assay (Burroughs Wellcome). In an in vitro cytogenetic study performed in cultured human lymphocytes, ZDV was reported to induce chromosomal aberrations only in high doses (3 μg/ml and higher), i.e. 10-fold higher than the therapeutic serum levels (Burroughs Wellcome).

The present study was performed to evaluate the in vivo cytogenetic damage induced by ZDV in AIDS patients. It was also designed to study the peripheral blood mononuclear cells of ZDV-exposed individuals cultured in vitro with different doses of rIFN-β, as the combination of the two agents is currently under therapeutic investigation.

Materials and Methods

Subjects

A 5-ml sample of peripheral blood was drawn by venipuncture from 11 AIDS patients (CDC classification stage 4C). Seven of them were receiving ZDV therapy (1200 mg/day) as their sole medication for periods of 4 weeks to seven months, which is sufficient to show the subchronic and chronic effects of the drug in case it has the potential to cause genetic damage. The other 4 AIDS patients were not receiving ZDV therapy. All of the patients were non-smokers and none of them suffered or had history of malignancy nor were under or had history of radio or chemotherapy. Their ages varied from 19–39, the mean age was 32.6 years and their T4 cell count was $172 \pm 75/mm^3$. Blood from normal individuals served as controls (non-smokers, laboratory technicians, ages 23–37).

Cell culture and cytogenetic analysis

For cytogenetic analysis, human peripheral blood cells were used. They are a suitable target in which to look for cumulative damage resulting from long periods of exposure to mutagenic agents (Nowell, 1965). Whole blood cultures were established for each experiment. 0.5 ml of blood was added to 9.5 ml RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 1% glutamine, 1% heparin, 1% penicillin and streptomycin, and 2 phytohemagglutinin (PHA) purchased from Burroughs Wellcome. For each study subject each experiment consisted of an untreated control and rIFN-β (donated by Triton Biosciences) experimental groups. Experimental group cultures received 10, 100, 1000, or 10000 units of rIFN-β 24 h before initiation and during the whole incubation period. Duplicate cultures were set for each experimental datum point. The protocol for detection of chromosomal aberrations, using the bromodeoxyuridine (Brdu) labeling technique was a modification of that previously described (Shafik et al., 1988). Briefly 7 h after culture initiation, 20 μM Brdu was added to each culture. At 71 h colcemid (0.2 μg/ml) was added for 1 h. Cultures were then harvested at 72 h. Slides were prepared by air drying, stained with 5 μg/ml Hoechst 33258, exposed to black light for 30 min at 55°C, and then stained with 5% Gurr Giemsa for 5 min according to the technique of Goto et al. (1977).

One hundred metaphase spreads from the first division cells were scored blindly from each culture for chromosome aberrations. This staining procedure allows one to distinguish between first, second, and third division cycles. To assess cellular proliferation, one thousand mitoses were screened for the number of cells in each division cycle. The mitotic index (MI) for each treatment was also determined. The MI was low among AIDS patients whether or not on ZDV therapy. The reported cases in this communication had relatively better mitotic indices that enabled us to score 100 metaphases.

Statistical analysis

Since chromosomal aberration frequencies do not follow a normal distribution, arc sin transformations were carried out on the observed data prior to analysis using Wilcoxon's Rank Sum Test (Brown et al., 1977):

$$\text{arc sin} = \sqrt{\frac{x + 0.25}{n + 0.50}}$$

where x is the number of abnormal cells observed for each culture and n is the number of cells scored (Whorton, 1985).

However, statistical analyses with and without transformation gave similar values, thus the P-values reported are derived from the original data.

Results

The frequency of ZDV-induced chromosomal aberrations in patients with AIDS

Because there is an increasing number of HIV-infected individuals and health care workers who are otherwise healthy but using ZDV as chemoprophylaxis against HIV, it was decided to examine the genotoxicity of ZDV after prolonged administration of the drug; therefore the frequency of chromosomal aberrations in lymphocytes from AIDS patients who were maintained on ZDV for at least 1 month was studied. The chromosome aberrations scored were of the chromatid-type aberrations, i.e. chromosome, chromatid, or isochromatid breaks (gaps were not included). The frequency of chromosome aberrations in AIDS patients is shown in Table 1 and Fig. 1. There was a 16-fold increase ($P<0.05$) in the frequency of aberrant cells in the ZDV-treated group compared to the group not receiving ZDV. These data suggest that ZDV has the ability to induce genetic dam-

TABLE 1

The effect of rIFN-β on the frequency of zidovudine-induced chromosomal aberrations

Zidovudine[a]	rIFN-β[b] (U/ml)	Frequency of chromosomal breaks	Reversal of ZDV effect (%)
–[c]	–	0.5 ± 0.29	
+[d]	–	8.3 ± 1.97	
+	10^1	2.0 ± 1.69	81
+	10^2	1.3 ± 0.71	90
+	10^3	1.9 ± 1.08	82
+	10^4	0.6 ± 0.57	99

[a]200 mg, administered every 4 h.

[b]Was added in vitro to peripheral blood cultures from AIDS patients on AZT therapy.

[c]AIDS patients not on AZT – mean of 4 patients ± the standard deviation (SD).

[d]AIDS patients on AZT – mean of 7 patients ± SD.

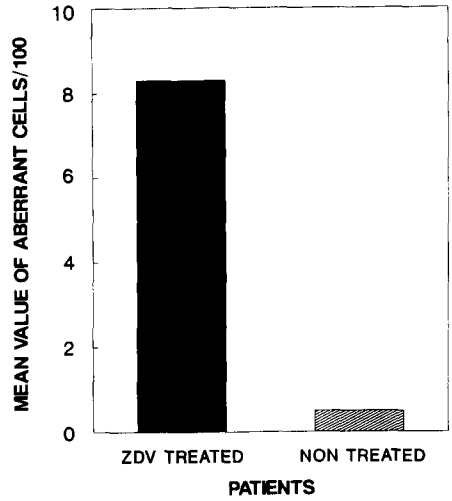

Fig. 1. Frequency of chromosome aberration in AIDS patients. Mean value of aberrant cells per 100 metaphase spreads. Mean values from 7 ZDV-treated (■) and 4 ZDV-nontreated (▨) AIDS patients. The data presented were pooled from 4 independent experiments with identical conditions and fulfilling the criteria for scoring.

age at therapeutic levels of dosing, which could produce a potential increase in carcinogenic risk associated with its prolonged intake.

Effect of rIFN-β on the frequency of ZDV-induced genetic damage

rIFN-β is currently being examined for treatment of AIDS in combination with ZDV. Thus it seemed to be important to determine the effect of rIFN-β on ZDV-induced genetic damage, particularly whether it could enhance or inhibit the changes observed. Therefore, rIFN was added to the cultures at different doses. The frequency of aberrant cells in the presence of rIFN-β is shown in Table 1 and Fig. 2. The percentage of aberrant cells dropped by 81% ($P<0.05$) for the 10 U/ml, 90% for the 100 U/ml ($P<0.05$), 82% for the 1000 U/ml ($P<0.05$), and 99% for the 10000 U/ml co-cultures ($P<0.05$). When rIFN-β was added at the above doses to normal blood cultured from normal individuals, no genetic damage was observed (data not shown). These data suggest that there may be a potential role for rIFN-β as a protective agent against ZDV-induced genetic damage and potentially against damage induced by other genotoxic therapeutic agents although they were not examined in the present study.

Discussion

These data suggest that ZDV at therapeutic levels appeared to induce genetic damage in the form of chromosome aberrations in peripheral blood lymphocytes

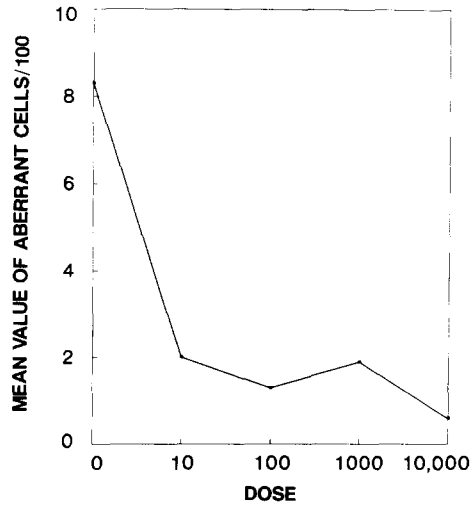

Fig. 2. Effect of rIFN-β on the ZDV-induced genetic damage. Mean value of aberrant cells per 100 metaphase spreads. Cultures were maintained in presence or absence of increasing doses of rIFN-β.

from AIDS patients. This appears to be the initial study on the clastogenic activity of ZDV in vivo in humans. In an in vivo cytogenetic study conducted in rats given single intravenous injection of ZDV, no chromosomal aberrations were noted despite plasma levels that were highly elevated after dosing (Burroughs Wellcome, unpublished observations). The reason for the discrepancy could possibly be due to the duration of exposure to ZDV, particularly in that it is known to have a rapid rate of elimination (Blum et al., 1988). In the in vivo survival study, rats were acutely exposed, while in our study the subjects were maintained for at least 4 weeks on ZDV before their cells were tested for chromosomal damage. Alternatively these differences could be due to different capacities of repair mechanisms between species.

Since drug combination therapy is under study, different doses of rIFN-β were added to the cultures. As shown in Table 1 and Fig. 1 the frequency of aberrant cells dropped significantly at all the doses of interferon tested. The in vivo administration of rIFN-β in combination therapy regimens thus might have a potentially protective effect against ZDV-induced chromosomal genotoxicity. The mechanism for the protective effect exerted by the interferon is presently unclear.

It could be speculated that the ZDV-damaged cells in the presence of rIFN-β had either a prolonged time before the S-phase to repair the damage (cell cycle delay), or potent repair enzymes were released in the presence of interferon that were capable of repairing the damage. Examination of cells undergoing first, second, and third division cycles in the absence or presence of interferon did not reveal any cell cycle delays (data not shown). Thus cell cycle delay may not explain the speculated mechanism by which IFN apparently prevented chromosomal aberrations. Recently, Suzuki and Suzuki (1988) reported on the suppression of UV-induced mutagenicity by natural human interferon alpha, in a completely in vitro system,

utilizing a human transformed cell line (RSA) and a xeroderma pigmentosa-derived fibroblast cell line (XPIKY). These results also suggest that human IFN-α seemed to have antimutagenic activity that could possibly be mediated via an error-free repair mechanism. Studies are now underway to evaluate the relationship between interferon and the induction of repair enzymes.

The potential carcinogenic risk of ZDV has been recently substantiated by Dr. David Barry, Burroughs Wellcome Company (Research Triangle Park, NC; personal communication) describing 2 non-metastasizing vaginal squamous cell carcinomas in rats receiving ZDV for 20 months. Moreover the development of non-Hodgkin lymphoma in patients with severe HIV infection on long-term ZDV therapy has been documented (Pluda et al., 1990). Although the benefits of the drug outweigh the potential risk of increased carcinogenicity in untreated HIV infection, the potential hazard of the drug will have to be taken into consideration, particularly in HIV patients who are asymptomatic with normal hematological and immunological parameters with less than the previously acceptable standard clinical indication for treatment. Moreover, whether or not it should be used as chemoprophylaxis in otherwise healthy HIV-seronegative individuals, for prolonged periods of time, in, for example, needle stick recipients must be considered.

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