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Research Article

Confirmation of the Chromosome Damaging Effects of Lamivudine in In Vitro Human Peripheral Blood Lymphocytes

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The aim of this study was to investigate genotoxic effects of lamivudine (an analogue of cytidine) using in vitro sister chromatid exchange (SCE), chromosome aberration (CA), and micronucleus (MN) tests in human peripheral lymphocytes. The cells were treated with 75, 100, 125, and 150 μg/ml concentrations of lamivudine (roughly 30–60 times higher than plasma levels achieved in patients receiving this drug) for two (24- and 48-hr) treatment periods. Lamivudine induced SCEs at the highest concentration (150 μg/ml) in the 24-hr treatment, and at 125 and 150 μg/ml in the 48-hr treatment, when compared to the solvent control. During both treatment periods, structural chromosome aberrations were significantly increased at 100, 125, and 150 μg/ml lamivudine concentrations. However, the increases of SCEs (22%) and CAs (50%) were weak. In addition, lamivudine reduced both the proliferation index (PI) and the mitotic index (MI) significantly at all concentrations for the two treatment periods. The MI was reduced by lamivudine in a dose-dependent manner during both the 24- and 48-hr treatment periods. In contrast, the PI was reduced by lamivudine only during the 48-hr treatment period. A weak but significant increase in MN formation was observed following lamivudine treatment at 100, 125, and 150 μg/ml for 48 hr, but no significant increase in micronuclei were observed following 24-hr treatment. In conclusion, lamivudine has a weak genotoxic effect at elevated doses on human peripheral lymphocytes. Environ. Mol. Mutagen. 49:328–333, 2008.

Key words: lamivudine; human peripheral lymphocytes; sister chromatid exchange; chromosome aberration; micronucleus

INTRODUCTION

Nucleoside analogues were first tested as antiviral agents 2 decades ago and are currently used in the treatment of several viral infections, including human immunodeficiency virus type-1 (HIV-1), hepatitis B virus (HBV), and adenovirus infection [Wutzler and Thust, 2001; Olivero, 2007]. Those nucleoside analogues used in the treatment of HIV-1 are now commonly referred to as nucleoside reverse transcriptase inhibitors (NRTIs). Lamivudine (3TC, the negative enantiomer of 2′-deoxy-3′-thiacytidine), an analogue of cytidine, is a dideoxynucleoside frequently combined with other antiviral agents for treatment of HIV-1 and HBV. The "dideoxy-type" NRTIs are analogues of normal nucleosides that lack the 3′-OH of the deoxyribose sugar, and are therefore unable to extend the nascent DNA chain by forming a 5′ to 3′-phosphodiester bond with the proceeding nucleic acid [Kakuda, 2000; Olivero, 2007]. NRTIs inhibit virus replication through their insertion into proviral DNA by the viral reverse transcriptase, causing DNA chain termination [Kakuda, 2000]. The use of nucleoside analogues in antiviral therapy is essentially a strategic decision requiring careful weighing of risk and benefits. Because mammalian DNA polymerases have some, albeit lower, affinity for NRTIs, these drugs can be incorporated into host cell nuclear and mitochondrial DNA, and lead to persistent damage if not resolved successfully [Olivero et al., 1997, 1999; Meng et al., 2007]. This unrepaired DNA damage

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can yield point mutations and large deletions [Olivero, 2007].

Recent studies of the genotoxic effects of lamivudine suggest weak cytogenetic responses to this drug; summarized in Physicians' Desk Reference [2000], Wutzler and Thust [2001], Von Tungeln et al. [2002], and Torres et al. [2007]. Lamivudine had a weak clastogenic effect in human peripheral blood lymphocytes in vitro, and induced mutations at the TK locus in the L5178Y*TK*$^{+-}$ mouse lymphoma cell line. Torres et al. [2007] further reported that, in human TK6 lymphoblastoid cells, dose-related increases in HPRT and TK mutant frequencies were found following 3 days of exposure to lamivudine (33, 100, or 300 μM). In contrast, lamivudine treatments did not induce micronucleus (MN) formation in rat bone marrow [Physicians' Desk Reference, 2000] or cause increases in micronucleated polychromatic erythrocyte frequency in exposed B6CF1*TK*-heterozygous mice [Von Tungeln et al., 2002]. Furthermore, there was no reported evidence for carcinogenic potential in lifetime cancer studies of lamivudine in mice and rats at exposures of 10 or 58 times, respectively, the recommended clinical dose in humans.

In general, there is a paucity of data and a lack of detail available in the literature for studies that have assessed the chromosomal damaging effects of lamivudine in vitro in human cells, including lymphoid cells. Many of the results related to the genotoxicity testing of lamivudine were acquired by the manufacturer for drug approval, and the findings summarized briefly in the Physicians' Desk Reference [2000] have not been published as original data. Moreover, the clastogenic potential of lamivudine has yet to be evaluated using test methods that measure the frequencies of sister chromatid exchange (SCE) and MN formation in human peripheral lymphocytes in vitro. The purpose of this study is to extend and confirm the limited evidence of cytogenetic responses to lamivudine [Physicians' Desk Reference, 2000] by investigating whether or not this antiviral drug has clastogenic effects on human periperal lymphocytes in vitro, as determined by SCE, chromosome aberration (CA), and MN tests.

MATERIALS AND METHODS

Test Samples and Chemicals

Human peripheral blood samples were taken from four (n = 4) healthy 24- to 25-year-old volunteers (two males, two females, all non smokers). All donors had no previous exposure to lamivudine.

The test substance 3TC (lamivudine) (2′,3′-dideoxy-3′-thiacytidine) was obtained from GlaxoSmithKline (Istanbul, Turkey) (trade name, zeffix). The chemical structure and the formula of lamivudine are shown in Figure 1. This compound was dissolved in dimethylsulphoxide (DMSO, purity 99%), supplied by Merck (Hohenbrunn, Germany). Mitomycin-C was used as a positive control (MMC, Kyowa, Hakko, Japan, CAS registry number: 50-07-7) and was dissolved in sterile double-distilled water. The 5-bromodeoxyuridine (B-5002), colchicine (C-9754), and cytochalasin B (C-6762) were purchased from Sigma (St. Louis, MO). Giemsa and all other chemicals were purchased from Merck (Darmstadt, Germany). All test solutions were freshly prepared prior to each experiment.

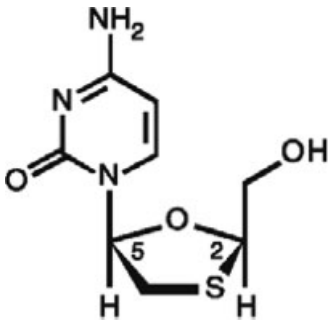

Fig. 1. The chemical name of lamivudine is 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one (CAS registry number: 134678-17-4). It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3 g/mol.

Cell Culture for SCE and CA Tests and Preparations

In this study, human peripheral lymphocytes were treated with a commercial formulation of lamivudine. Fresh blood from volunteer donors was collected and transferred to culture tubes, which contain chromosome medium B (Biochrom F-5023; Berlin, Germany), and was used immediately for the determination of the genotoxicity of lamivudine.

The methods used for the SCE and CA tests were as described previously, with minor modifications [Evans, 1984; Perry and Thompson, 1984]. This study was performed according to the IPCS guidelines [Albertini et al., 2000]. Lymphocyte cultures were prepared by adding 0.2 ml of whole blood from a healthy donor to 2.5 ml of chromosome medium B, supplemented with 5-bromodeoxyuridine (final concentration 10 μg/ml). The cultures were incubated at 37°C for 72 hours. In this study, test concentrations were chosen according to the cytotoxicity of lamivudine. To determine which lamivudine concentrations should be used in the experiments, a pilot study (50–200 μg/ml) was done first (exposure time was 24 hr) and the mitotic index (MI) was determined. We found that 150 μg/ml lamivudine was an approximate LD_{50} according to the cytotoxicity. Lamivudine exhibited high cytotoxic effects at concentrations greater than 150 μg/ml. Also, lamivudine decreased the ratio of dividing cells (i.e., the MI) at a concentration of 150 μg/ml. Therefore, 150 μg/ml was chosen as the highest concentration of lamivudine. The MI of cells exposed to 50 μg/ml lamivudine was not significantly different than the control. The test concentrations were chosen as 75, 100, 125, and 150 μg/ml (327–654 μM lamivudine, roughly 30 to 60 times higher than plasma levels achieved in patients receiving this drug) based on the concentration that resulted in 50% (LD_{50}) cell survivals (150 μg/ml). Lamivudine was dissolved in DMSO (based on the active ingredient) under sterile conditions. Thus, DMSO (8 μl/ml) was used as solvent control. A positive control (0.25 μg/ml MMC) was established in parallel. The cells were treated with 75, 100, 125, and 150 μg/ml concentrations of lamivudine for 24 hours (lamivudine was added 48 hr after initiating the culture) and 48 hours (lamivudine was added 24 hr after initiating the culture). To arrest the cells in metaphase, the cells were exposed to 0.06 μg/ml colchicine 2 hr before harvesting. To collect the cells, the cultures were centrifuged (1,200 rpm, 15 min), treated with hypotonic solution (0.4% KCl) for 13 min at 37°C, and then fixed three times in a cold solution consisting of methanol (glacial acetic acid [3:1 v/v]) for 20 min at room temperature. Finally, the centrifuged cells were dropped onto clean slides and air-dried slides were stained following standard methods (i.e., with 5% Giemsa in Sorensen Buffer [pH 6.8] for

TABLE I. Effect of Lamivudine on SCE and PI in Human Peripheral Lymphocytes During 24-hr and 48-hr Treatment Periods

Test substance	Time (hr)	Doses (μg/ml)	Minimum–maximum SCE	SCE/cell ± SE	M1	M2	M3	PI ± SE
DMSO	24	8 μl	2–15	7.07 ± 0.18	53	132	215	2.45 ± 0.06
MMC	24	0.25	15–46	26.61 ± 1.90	204	149	47	1.61 ± 0.06
Lamivudine	24	75	2–18	8.03 ± 0.59	225	140	35	1.53 ± 0.11[a]
Lamivudine	24	100	2–18	8.42 ± 0.60	205	158	40	1.60 ± 0.13[a]
Lamivudine	24	125	3–18	8.53 ± 0.89	203	164	34	1.58 ± 0.05[b]
Lamivudine	24	150	3–17	9.08 ± 0.58[c]	202	145	26	1.54 ± 0.07[b]
DMSO	48	8 μl	2–12	7.39 ± 0.17	70	146	186	2.35 ± 0.07
MMC	48	0.25	35–68	50.78 ± 2.65	196	179	27	1.59 ± 0.09
Lamivudine	48	75	2–17	8.72 ± 0.66	137	199	64	1.83 ± 0.02[b]
Lamivudine	48	100	2–18	8.85 ± 0.67	150	203	47	1.75 ± 0.02[b]
Lamivudine	48	125	3–18	8.67 ± 0.16[a]	176	198	26	1.63 ± 0.07[b]
Lamivudine	48	150	3–19	9.02 ± 0.09[b]	179	201	20	1.60 ± 0.09[b]

Statistical significance of treatment versus solvent control.
[a]$P < 0.01$.
[b]$P < 0.001$.
[c]$P < 0.05$.

15 min for CA analysis, and with a modified fluorescence plus Giemsa method [FPG] for SCE analysis) [Speit and Haupter, 1985]. For FPG staining, 1-day-old slides were covered with Sorensen Buffer (pH 6.8) and irradiated with UV light (254 nm) for 30 min. After irradiation, the slides were incubated in 1× SSC (150 mM NaCI, 15 mM sodium citrate) at 58–60°C for 60 min and then stained with Giemsa (%5 in Sorensen Buffer) for 20 min. Slides were scored under blind code.

Scoring of SCEs was carried out according to the ICPEMC guidelines [Carrano and Natarajan, 1988]. Twenty-five well-differentiated second division metaphase cells were analyzed per donor (a total of 100 sec division metaphase for each donor) for SCE scoring at 1,000× magnification. The results were used to determine the mean number of SCE (SCE/cell). One hundred well-spread metaphases per donor (a total 400 metaphase per concentration) were examined at 1,000× magnification for the occurrence of different types of CA. Thus, structural and numerical CAs could be obtained. However, only structural chromosomal aberrations were scored to evaluate genotoxicity. Percentages of cells with structural chromosomal aberrations were calculated for each donor separately. CAs were classified according to the ISCN as shown by Mitelman [1995] and Paz-y-Miño et al. [2002] and then evaluated as chromatid-type (breaks, sister unions, and exchanges) and chromosome-type (breaks, dicentrics, rings, and fragments) aberrations. Gaps were not considered as CAs as recommended by Mace et al. [1978]. Polyploid cells were recorded, but not considered for the evaluation of mutagenicity. In addition, a total of 400 cells (100 cells per donor) were scored to determine the proliferation index (PI), which was calculated according to the following formula: PI = 1 × M1 + 2 × M2 + 3 × M3/100, where M1, M2, and M3 represent the number of cells at the first, second, and third metaphases, respectively. In addition, the number of dividing cells (i.e., cells in metaphase) were counted in a total of 12,000 cells (3,000 cells per donor) per concentration to calculate MI. MI was calculated as: MI = 100 × cells in metaphase/3,000.

Cell Culture for Micronuclei Test and Preparation

For the MN test, whole-blood cultures were set up as described for the SCE and CA assay except for 5-bromodeoxyuridine [Fenech and Morley, 1985]. Peripheral lymphocytes were incubated at 37°C for 68 hr. The cells were exposed to lamivudine at concentrations of 75, 100, 125, and 150 μg/ml for 24 and 48 hr (lamivudine was added 44 and 20 hr after initiating the culture, respectively). Cytochalasin B (Sigma) (final concentration of 6 μg/ml) was added after 44 hr of incubation to arrest cytokinesis and obtain binucleated cells. At the end of the incubation period, cultures were harvested by centrifugation and slides were prepared according to the method described by Rothfuss et al. [2000]. Obtained cells were first treated with hypotonic solution (0.4% KCI) for 10 min at 37°C, followed by a first fixation (glacial acetic acid: methanol: 0.9% NaCI; 1:5:6 v/v respectively) for 20 min at room temperature. A second fixation was performed with methanol: glacial acetic acid (3:1) solution two times. Finally, slides were air-dried and stained with 5% Giemsa in Sorensen Buffer (pH 6.8) for 13 min.

The criteria used for binuclear cell and MN evaluation were those suggested by Titenko-Holland et al. [1997]. To determine MN formation, 1,000 binuclear cells were analyzed for each donor giving a total of 4,000 binuclear cells.

Statistical Significance

All of the subjects (i.e., the four donors; $n = 4$), were used as the experimental unit (n) for all statistical analyses. Results are expressed as the mean ± S.E. (standard error). The significance of differences between the percentage of cells with structural CA, mean SCE, PI, MI, and percentage of the micronucleated binuclear cells in treated cultures and their controls were determined using the Students' *t*-test. Dose–response relationships were determined from the correlation and regression coefficients for the percentage of structural CA, mean SCE, percentage of binuclear cells with micronuclei, PI, and MI.

RESULTS

Four different concentrations (75, 100, 125, and 150 μg/ml) and five different parameters (SCE, CA, MN, PI, MI) were evaluated in two different exposure periods (24 and 48 hr) to determine the cytotoxic and clastogenic effects of lamivudine, on human peripheral lymphocytes in vitro.

The effect of lamivudine on SCE and cell proliferation is summarized in Table I. During the 24-hr treatment, lamivudine increased SCEs in a statistically significant fashion at 150 μg/ml. After a 48-hr treatment with lamivudine, SCEs were significantly increased at 125 and 150 μg/ml. The observed increases in SCEs of ~22% were rather weak, but were statistically significant. Lamivudine significantly decreased the PI at all concentrations and treatment periods when compared to solvent controls. The decrease in PI during the 48-hr treatment period occurred in a dose dependent manner ($r = -0.978$, $P < 0.05$).

TABLE II. Effect of Lamivudine on CA and MI in Human Peripheral Lymphocytes During 24-hr and 48-hr Treatment Periods

Test substance	Time (hr)	Doses (μg/ml)	Structural CA		% Cells with structural CA ± SE	MI ± SE
			Chromatid type	Chromosome type		
DMSO	24	8 μl	28	5	8.25 ± 0.48	5.77 ± 0.10
MMC	24	0.25	59	45	26.00 ± 1.00	4.76 ± 1.10
Lamivudine	24	75	24	8	8.00 ± 0.82	3.84 ± 0.04[a]
Lamivudine	24	100	41	12	13.25 ± 1.25[b]	3.70 ± 0.04[a]
Lamivudine	24	125	42	8	12.50 ± 1.19[b]	3.56 ± 0.04[a]
Lamivudine	24	150	48	6	13.50 ± 1.44[b]	3.45 ± 0.03[a]
DMSO	48	8 μl	20	4	6.00 ± 0.82	5.22 ± 0.10
MMC	48	0.25	96	79	43.75 ± 1.31	3.19 ± 0.09
Lamivudine	48	75	32	4	9.00 ± 1.08	3.85 ± 0.20[c]
Lamivudine	48	100	35	11	11.50 ± 0.65[c]	3.58 ± 0.11[a]
Lamivudine	48	125	37	8	11.25 ± 0.95[b]	3.47 ± 0.10[a]
Lamivudine	48	150	35	11	11.50 ± 1.44[b]	3.27 ± 0.11[a]

Statistical significance of treatment versus solvent control.
[a]$P < 0.001$.
[b]$P < 0.05$.
[c]$P < 0.01$.

Both lamivudine (at all concentrations) and positive control (MMC) caused similar decreases in PI during the 24-hr treatment; in the 48-hr treatment, only the highest concentration of lamivudine (150 μg/ml) caused a decrease in PI similar to that of the positive control (MMC).

As shown in Table II, lamivudine caused both chromatid and chromosome type aberrations. During both the 24-hr and 48-hr treatments, structural chromosome abnormalities were significantly increased at 100, 125, and 150 μg/ml with respect to the solvent control. However, the changes in CAs were also relatively weak with only about a 50% increase over background observed in the treated cultures during both 24- and 48-hr treatment times. The background levels of aberrations in our laboratory (about 6–8%) are higher than those reported in many laboratories, but since the slides were scored under blind code, we can be confident in the weak increase in aberrations seen here, especially because they confirm the report in the PDR, and there is also an increase in micronuclei in vitro. Our experimental design is such that cells in their first, second, and third metaphase from the beginning of treatment are scored. Classically, aberrations are scored at the first metaphase to avoid loss of damaged cells during mitosis, but prolonged treatment or later sampling times are often more effective in detecting nucleoside analogues. During a 24-hr treatment, lamivudine decreased the MI in a significant fashion for all concentrations with respect to for solvent control and positive control. After a 48-hr lamivudine treatment, MI was significantly decreased at all concentrations, as compared to solvent control. The positive control and two highest doses of lamivudine (125 and 150 μg/ml) caused similar decreases in MI for this treatment period. These decreases were dose-dependent ($r = -0.997$, $P < 0.01$ and $r = -0.987$, $P < 0.05$ for 24 and 48 hr, respectively).

TABLE III. Effect of Lamivudine on Micronucleus Formation in Human Peripheral Lymphocytes During 24-hr and 48-hr Treatment Periods

Test substance	Time (hr)	Doses (μg/ml)	Percent micronucleated binucleated cells ± SE
DMSO	24	8 μl	0.73 ± 0.09
MMC	24	0.25	3.05 ± 0.25
Lamivudine	24	75	0.75 ± 0.06
Lamivudine	24	100	0.83 ± 0.08
Lamivudine	24	125	0.83 ± 0.08
Lamivudine	24	150	0.98 ± 0.11
DMSO	48	8 μl	0.75 ± 0.05
MMC	48	0.25	7.18 ± 0.29
Lamivudine	48	75	0.95 ± 0.24
Lamivudine	48	100	1.20 ± 0.28[a]
Lamivudine	48	125	1.00 ± 0.06[a]
Lamivudine	48	150	1.08 ± 0.03[b]

Statistical significance of treatment versus solvent control.
[a]$P < 0.05$.
[b]$P < 0.001$.

The results of the MN tests are given in Table III. A 24-hr lamivudine treatment did not induce a statistically significant increase in MN formation as compared with the solvent control. However, a 48-hr lamivudine treatment significantly increased MN formation at all concentrations except the low-dose level of 75 μg/ml when compared with solvent control. The increases in micronuclei were fairly weak (less than onefold) after the 48-hr lamivudine treatment period.

DISCUSSION

The focus of this study was the possible chromosome damaging effects resulting from in vitro treatment of human peripheral blood lymphocytes with lamivudine, a

nucleoside analogue frequently used in the treatment of HIV-1 and HBV. The frequencies of SCEs have been found to be increased in animal and human cells following exposure to many mutagens. The effects of lamivudine on SCEs have not been previously studied. Results of the current work demonstrated that lamivudine was a rather weak inducer of SCEs at all concentrations and treatment periods. Previous studies have demonstrated that other antiviral nucleoside analogues also induce SCEs [Perry and Evans, 1975; Cassiman et al., 1983; de Clercq and Cassiman et al., 1986; Gonzalez and Larripa, 1994; Thust et al., 1996; Albertini et al., 2000].

CAs are the result of chromosome-level DNA damage. Moreover, since the mechanism of CA formation is similar in different tissues, levels of abnormal lymphocytes are thought to indicate the level of damage in cancer-prone tissue and, therefore, cancer risk [Albertini et al., 2000; Bonassi et al., 2000, 2004, 2005, 2007]. In the current study, lamivudine was a relatively weak inducer of CAs at all concentrations and treatment periods. Few studies related to the clastogenic effects of lamivudine are available in the literature, and it is not possible to compare the findings of the current work to an earlier brief report of lamivudine induced CAs in human lymphocytes because the data have only been summarized [Physicians' Desk Reference, 2000; Wutzler and Thust, 2001]. However, other nucleoside analogues have been shown to have clastogenic effects [Gonzales and Lapira 1994; Schilling et al., 1995; Thust et al., 1996].

MN can be formed from acentric chromosomal fragments or whole chromosomes left behind during mitotic cellular division. Both clastogenic and aneugenic effects can be determined with the MN test [Kirsch-Volders et al., 1997; Norppa and Falck, 2003]. Furthermore, an increased MN frequency in peripheral blood lymphocytes implies cancer risk in humans in vivo [Bonassi et al., 2005, 2007]. In the current work, MN formation was not significantly increased at all concentration of lamivudine following a 24-hr treatment, but significantly increased at all concentrations except the lowest following a 48-hr treatment. While the induction of micronuclei by in vitro lamivudine treatment has not been previously reported, the present study showed that this antiviral drug was a fairly weak inducer of micronuclei in cultured human lymphocytes after the 48-hr lamivudine treatment period.

The underlying mechanisms by which lamivudine weakly enhances DNA damage is obscure. Nevertheless, lamivudine may induce rather weak genetic damage by altering intracellular nucleotide pools. It is well known that faithful DNA replication and repair requires a subtle balance of intracellular nucleotide levels, and disturbance of such a balance may cause many forms of genetic alterations, from point mutation to oncogenic transformation [Kuntz et al., 1994]. Numerous authors have reported that exposure to NRTIs alters the composition of the nucleoside pools. Darnowski and Goulette [1994] concluded that alterations in the concentration of either thymidine-triphosphate TTP or AZT-TP may reduce the TTP/AZT-TP ratio and may favor AZT incorporation into DNA. It has been speculated that such changes could contribute to the weak increased lamivudine-DNA incorporation. In addition to this, Poirier et al. [2004] reported that in monkeys, 3TC was incorporated into the DNA from multiple fetal organs taken at birth after administration of human-equivalent protocols to pregnant dams during gestation. In human infants, 3TC-DNA incorporation has been documented in cord blood from infants exposed in utero to lamivudine. The biological consequences of lamivudine-induced damage to DNA depend on the site of lamivudine-DNA incorporation; unremoved damage results in deletions and point mutation as well as several events related to clastogenicity, including MN, CAs and SCEs [Poirier et al., 2004; Olivero, 2007].

In our experiments, both PI and MI decreased at all dose levels of lamivudine (75, 100, 125, and 150 μg/ml) during both 24- and 48-hr treatment periods. Mitosis and DNA replication are impeded by some nucleoside analogues [Gonzales and Lapira, 1994; Thust et al., 1996]. Based on the results of this study, it can be concluded that impairment of cell-cycle progression, a consequence of DNA damage, reflects an inability of the cell to accomplish basic biological processes.

CONCLUSION

In a study of the potential chromosome damaging effects of lamivudine in cultured human peripheral blood lymphocytes, we found that this frequently used nucleoside analogue was a relatively weak inducer of SCE, CA, and MN at doses exceeding those used in clinical practice. In addition, lamivudine decreased PI (48-hr treatment period) and MI (24-hr and 48-hr treatment periods) in a dose-dependent manner. While the current work confirms and extends the earlier limited report in the Physician's Desk Reference [2000] of the in vitro clastogenicity of lamivudine, the findings of various cytogenetic responses at high-dose in vitro treatments may not be relevant to low-dose clinical treatments with lamivudine.

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