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Inhibitory effect of folinic acid on radiation-induced micronuclei and chromosomal aberrations in V79 cells [1]

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Abstract

Folinic acid (FA), clinically called leucovorin, has been widely used as a nutrient supplement in dietary intake and is capable of inhibiting cytotoxicity and chromosomal damage induced by chemicals. However, data on its antigenotoxic effect on radiation-induced chromosomal damage are limited. The present study was, therefore, performed to investigate the effect of FA on radiation-induced (X-rays and UV radiation) micronuclei (MN) and structural chromosomal aberrations (SCA) concurrently in V79 Chinese hamster lung cells. Exponentially growing cells were exposed to five doses of X-rays (1–12 Gy) and UV radiation (50–800 $\mu J \times 10^2/cm^2$) and post-treated with 5 or 50 μg FA/ml of culture medium for 16 h. The slides were analyzed for the presence of MN and SCA using standard procedures. The results showed that X-ray treatment alone produced dose-related cytotoxicity as measured by nuclear division index (NDI) and mitotic index (MI). X-rays produced a clear dose-related clastogenicity as measured by percent of micronucleated binucleated cells (MNBN) (5–79%) and percent of aberrant cells (11–92%). FA at 5 μg/ml slightly decreased X-ray induced chromosomal damage in both assays; however, the inhibition was significant (12–46% of MNBN, 14–48% in aberrant cells) only when X-ray-treated cultures were post-treated with 50 μg FA/ml. Post-treatment of FA had no effect on X-ray induced cytotoxicity as measured by NDI and MI. A similar a dose-related increase in % MNBN (0.5–10.3%) and percent aberrant cells (6–35%) was produced by UV radiation treatment alone. There were significant percentages of MNBN and aberrant cell inhibitions at both 5 and 50 μg/ml in both assays. As in the case of X-ray-treated cells, there was a clear dose-related cytotoxicity in UV-treated cells alone. No reduction in NDI or MI was found when UV-exposed cells were post-treated with 5 or 50 μg of FA. These data demonstrate the beneficial effect of FA in decreasing radiation-induced chromosomal damage.

Keywords: Folinic acid (leucovorin); X-ray; UV radiation; Micronucleus; Chromosomal aberration; Anti-mutagen; V79 cell

1. Introduction

Folinic acid (FA), chemically known as 5,10-methenyl tetrahydrofolate, is an ubiquitous member of biological folate pools, usually comprising between 10–25% of total cellular folates (Cossins, 1984; Horne et al., 1989). It is widely used as a

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rescue agent for methotrexate toxicity and in combination with 5-fluorouracil. FA has been used for over 30 years in cancer chemotherapy along with methotrexate. Recent studies suggest that a therapeutic advantage with reduced toxicity can be achieved by the administration of high doses of methotrexate in malignant disease. The cytotoxic effect of methotrexate can be reversed by the administration of folinic acid which bypasses the major site of methotrexate action, the binding of dihydrofolate reductase, preventing the formation of tetrahydrofolic acid. The Food and Drug Administration (FDA, 1993) now allows claim that FA in early pregnancy can reduce the threat of certain birth defects. Folate deficiency acts synergistically with mutagens/carcinogens/γ-irradiation to magnify genetic damage (Branda and Blickensderfer, 1993; Libbus et al., 1990). FA also has been used as an antigenotoxic agent to prevent chromosome damage in bone marrow cells of mice receiving methotrexate (Pienkowska and Koziorowska, 1986). Although several other studies also indicate that FA acts as an antigenotoxic agent against different chemicals, limited knowledge is available as to its antigenotoxic effect on radiation-induced chromosomal damage.

The in vitro structural chromosomal aberration (SCA) test has been used often for screening genotoxicity of chemicals (Galloway et al., 1994). This assay, which involves metaphase analysis, requires considerable experience and skill to produce high quality metaphase preparations. Furthermore, the identification and classification of SCAs is subjective and often time consuming. For example, a gap observed by one scorer may be classified as break by another scorer and these judgements indeed will have significant impact in classifying a test agent as positive or negative in the SCA assay. The micronucleus (MN) assay is now used more often than the SCA assay because of its simplicity and objectivity. The MN are believed to be formed from chromosome fragments or entire chromosomes which lag behind during cell division. The MN assay can thus detect genotoxic agents that cause chromosome breaks (clastogens) or affect spindle fiber function (aneugens).

Although there are a number of studies on the induction of MN and SCAs by physical agents such as X-rays and UV radiation for genotoxicity assessment, limited information is available on the relationship between these two cytogenetic endpoints under concurrent conditions. The aim of this work was to determine the radioprotective capacity of FA, by measuring the reduction of radiation-induced (X-rays and UV radiation) chromosomal damage as measured by MN and SCAs concurrently in V79 Chinese hamster lung fibroblasts.

2. Materials and methods

2.1. Cell line

V79 Chinese hamster lung fibroblast cell line was obtained from Dr. C.C. Chang (Michigan State University, East Lansing, MI). This cell line, after arrival, was tested for viability and mycoplasma contamination. Cells were grown exponentially in 75 cm^2 Falcon tissue culture flasks each with 15 ml Eagle's Minimum Essential Medium (MEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U penicillin/ml medium and 100 μg streptomycin/ml medium (Gibco). Cells were subcultured by treatment with trypsin-EDTA solution (Gibco) in phosphate-buffered saline (PBS) and were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. Irradiation source and chemicals

A scanray X-ray source (Model Torrex 120 D, Harbor City, CA) was used for X-ray treatment at a dose rate of 1.47 Gy/min and the quality of X-rays was according to the specifications indicated in the operating manual. The dose selection for X-ray was based on previous studies (Keshava et al., 1995; Krishna et al., 1992). The cells were exposed to 1, 2, 4, 8 or 12 Gy at 25 ± 2°C.

Stratalinker™ UV crosslinker 2400 (Stratagene, La Jolla, CA) with a wave length of 254 nm was used as a source of UV irradiation. Irradiation was done at 25 ± 2°C with doses of 50, 100, 200, 400 or 800 $\mu J \times 10^2/cm^2$. The dose selection for UV radiation was based on earlier reports (Fenech and Neville, 1992; Keshava et al., 1995).

Folinic acid (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water at a concentra-

tion of 2 mg/ml as a stock solution and diluted with distilled water prior to use. The dose selection for FA (5 or 50 μg/ml) was based on studies conducted by Burres and Cass (1987), Cohen et al. (1986) and Theiss et al. (1989).

Cytochalasin B (CYB, Sigma) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 2 mg/ml, stored at −20°C, and diluted with PBS immediately prior to use. A concentration of 3 μg CYB/ml culture medium was used. To arrest cells in metaphase, colcemid (Gibco) was prepared in Hanks Balanced Salt Solution with phenol red at 10 μg/ml and a concentration of 0.025 μg/ml culture medium was used.

2.3. Micronucleus assay

Approximately one million cells were seeded in each of 6 well tissue culture dishes with 5 ml culture medium. The cells were allowed to attach and grow for approx. 20 to 24 h. Duplicate cultures were used for each treatment group.

The MN assay was performed according to standard procedures (Krishna et al., 1989). Briefly, FA and CYB were added following exposure to X-rays/UV radiation. After 16 h of post-treatment incubation with FA and CYB, the media were removed, flasks rinsed with PBS and cells dislodged with trypsin-EDTA at 37°C were collected and centrifuged at 1000 rpm for 5 min. The supernatant was removed and the pellet resuspended in the remaining solution. Cells were treated with 5 ml hypotonic solution (0.075 M KCl, dropwise) at 37°C for 3 to 5 min and recentrifuged. The supernatant was removed, pellet resuspended, and two drops of cell suspension per slide were carefully dropped onto prelabeled clean dry slides angled at 45° and air-dried for 5 min. The slides were then dipped in absolute methanol once to fix the cells and air-dried. Carl Zeiss (Oberkochen, Germany) microscope was used for scoring. For cell cycle analysis, 400 cells per treatment group were scored for the presence of one, two, or more than two nuclei and nuclear division index (NDI) was calculated NDI = $[1N + (2 \times 2N) + (4 \times > 2N)]/400$ cells scored. In this assay, the nuclei continue to divide while the cells do not. The number of mitotic cycles that cells have passed through can be determined by quantitating the number of nuclei present within each cell. The NDI represents an average of the cell cycling effects in each treatment. For MN analysis, 1000 binucleated cells per treatment group were analyzed.

The criteria for MN scoring in binucleated cells were similar to those reported by Countryman and Heddle (1976) and Roberts et al. (1986). Briefly, the diameter of the MN must be no larger than one-third the main nuclei. The MN must be non-refractile, thus excluding small stain particles. The color of MN must be the same as or brighter than the main nuclei, and MN must be located within the cytoplasm but not in contact with the main nuclei.

2.4. Structural chromosomal aberration analysis

The SCA assay was also performed using procedures described earlier (Galloway et al., 1994). Briefly, 16 h post-treatment of FA, 0.025 μg of colcemid/ml culture medium was added to each flask to arrest metaphase cells. Cells were harvested 2 h later by trypsinization and collected into 15 ml conical tubes. Five ml of hypotonic solution (0.075 M KCl) was added dropwise to each tube and incubated for 15 min at 37°C. Approximately five drops of freshly prepared fixative (methanol/acetic acid, 3:1) was added and spun for 5 min at 1000 rpm. The supernatant was decanted and resuspended with the remaining solution. Then, 5 ml of fixative was added, kept for 20 min at 4°C, and spun for 5 min at 1000 rpm. This step was repeated twice. Two to four drops of cell suspension were dropped onto a cold, wet slide. Following air drying at room temperature for a day, cells were stained with 10% Gurr's Giemsa in Gurr's phosphate buffer (pH 6.8) and 100 metaphase cells were analyzed for SCA in each treatment. The types of SCAs were classified according to standard cytogenetic procedures. The following types of SCAs were recorded: chromatid gaps, chromatid breaks, chromatid deletions, fragments, minutes, triradials, quadriradials, complex rearrangements, chromosome gaps, chromosome breaks, acentric fragments, double minutes, dicentrics and rings. For cytotoxicity, mitotic/metaphase index (MI) was calculated based on the number of metaphase cells present in a total of 1000 cells per treatment group. All slides were blind coded and scored.

2.5. Statistical analysis

The MN and SCA data were analyzed by χ^2 test. A sequential linear dose-trend test was also performed to compare the test groups with their respective controls. Correlation co-efficients (r) were calculated for both MN and SCA, NDI and MI, as well as for percent inhibition of both MN and SCA at 5 and 50 μg of FA/ml of culture medium.

3. Results

3.1. X-ray-induced chromosome damage

In the MN assay the cell cycle kinetic data indicated that with increase in X-ray dose the frequencies of mononucleated cells increased and the binucleated cells decreased (Table 1). However, the multinucleated cells (> 2N) remained approximately the same for all doses of X-rays and control (Table 1). The maximum number of binucleated cells in the cytokinesis blocked method was found in the control (70%) and the minimum (16.25%) in the highest X-ray-dose tested. These data clearly indicate a cell cycle delay in V79 cells induced by X-rays. The NDI decreased from 1.74 in control to 1.21 in the highest dose ($r = -0.99$). The percent micronucleated binucleated (MNBN) cells increased as the dose increased (Table 1). It ranged from 0.7% in the control to 78.8% in the highest dose tested. Also, cells with $\geq$ 2MN increased dramatically with increase in dose. In the SCA assay, MI decreased as X-ray dose increased (Table 2). The decrease was from 7.9 to 4.6 in the control and 12 Gy of X-ray-treated cultures, respectively, indicating a clear cytotoxicity induced by X-rays ($r = -0.87$). The most common types of aberrations were dicentrics, chromatid breaks and triradials. The percent aberrant cells also increased with the dose of X-rays and in the highest dose (12 Gy), 92% of cells showed one or more aberrations. There were approximately equal number of chromosome and chromatid type aberrations.

3.2. UV radiation-induced chromosome damage

In the MN assay, results of the cell cycle kinetics indicated the number of mononucleated cells increased significantly as the dose of UV radiation increased. The binucleated and multinucleated cells decreased with an increase in UV dose. The NDI also decreased from 1.99 in the control to 1.10 in the highest dose tested (Table 3). These results clearly indicate the cytotoxic effect of UV radiation on cell cycle ($r = -0.87$). The MNBN cells increased in a dose-dependent manner with a correlation coefficient of $r = 0.99$. There was a 6.8 to 20.6 fold increase in MNBN in various doses tested in a dose-dependent manner (Table 3). The results of chromosomal aberration assay showed that the MI decreased as the dose increased ($r = -0.90$) (Table 4). No metaphase cells were available at the last two doses (400 and 800 $\mu J \times 10^2/cm^2$) tested, indicating that there was a significant cell cycle delay and/or cytotoxicity. The aberrations/100 metaphase cells also increased significantly from 8 in controls to 47 at 200 $\mu J \times 10^2/cm^2$ tested. The percent of aberrant cells also showed dose-dependent increase ($r = 0.99$). Minutes, chromatid breaks and double minutes were more frequent than other types of aberrations scored.

3.3. Inhibition of radiation-induced chromosome damage by FA

FA had a minimal effect on X-ray induced cytotoxicity. The proportions of mono-, bi- and multinucleated cells remained similar in both 5 and 50 μg of FA/ml treated and non-treated cultures (Table 1). Therefore, the NDI also remained similar to their respective controls of X-ray-treated cultures. However, there was a significant ($p \leq 0.05$) decrease in percent MNBN cells for 50 μg of FA at all doses of X-rays tested. The percent inhibition ranged from 11.8 to 46.3% (Table 1, Fig. 1a). As the X-ray dose increased, the percent inhibition decreased. Significant reduction of X-ray-induced damage by FA at 5 μg occurred only at 4 and 12 Gy. The percent MNBN inhibition ranged from 0.94 to 16.6% when post-treated with 5 μg of FA; however, the inhibition of MNBN increased when the X-ray-treated cells were incubated with 50 μg of FA (Fig. 1a). These data indicate that FA added at higher doses (50 μg/ml) can inhibit/reverse the damage caused by X-irradiation. In the SCA assay, the MI did not decrease when cultures were post-treated with either 5 or 50 μg of FA (Table 2). However, the %

Table 1
Micronuclei induced by X-rays and their inhibition by folinic acid in V79 cells

Dose			Cell cycle kinetics/400 cells				MNBN cells/1000 BN cells					
X-ray (Gy)	+	FA (μg/ml)	1N	2N	> 2N	NDI [a]	1MN	2MN	> 2MN	Total	% MNBN cells	% MNBN inhibition
0	+	0	115	280	5	1.74	6	0	1	7	0.7	
0	+	5	112	286	2	1.73	3	0	0	3	0.3	57.14
0	+	50	121	277	2	1.71	1	0	0	1	0.1	85.71
1	+	0	109	280	11	1.78	39	10	5	54	5.4	
1	+	5	113	281	6	1.75	43	2	0	45	4.5	16.66
1	+	50	129	267	4	1.70	29	0	0	29 **	2.9 **	46.29
2	+	0	159	234	7	1.64	93	10	3	106	10.6	
2	+	5	152	242	6	1.65	98	4	3	105	10.5	0.94
2	+	50	147	250	3	1.65	67	6	2	75 *	7.5 *	24.24
4	+	0	170	218	12	1.64	206	43	12	261	26.1	
4	+	5	187	209	4	1.55	175	32	10	217 *	21.7 *	16.85
4	+	50	181	215	4	1.57	162	18	4	184 **	18.4 **	29.50
8	+	0	271	118	11	1.38	338	127	50	515	51.5	
8	+	5	291	101	8	1.31	321	133	34	488	48.8	6.00
8	+	50	277	118	5	1.33	296	98	20	414 **	41.4 **	19.60
12	+	0	329	65	6	1.21	316	269	203	788	78.8	
12	+	5	322	66	12	1.26	348	240	125	713 *	71.3 *	9.50
12	+	50	324	69	7	1.23	365	211	119	695 **	69.5 **	11.80

[a] Nuclear division index (NDI) = $[1N + (2 \times 2N) + (4 \times > 2N)]/400$ cells scored; N, number of nuclei; BN, binucleated cells; MNBN, micronucleated binucleated cells.
* $p < 0.05$; ** $p < 0.01$, significant values with their respective X-ray doses.

Table 2
Chromosomal aberrations induced by X-rays and their inhibition by folinic acid in V79 cells

Dose			Mitotic index [a]	Aberrations per 100 metaphase cells [b]														Aberrations/100 metaphase cells (without gaps)	Abs [c] (%)	Abs [c] inhibition (%)
X-ray (Gy)		CFA (μg/ml)		Chromatid type								Chromosome type								
				tg	tb	td	f	m	tr	qr	cr	sg	sb	af	dm	d	r			
0	+	0	7.9	1	0	1	0	0	0	0	0	0	0	0	1	1	0	3	3	
0	+	5	7.6	1	0	0	1	0	0	0	0	0	0	0	1	0	0	2	2	33.33
0	+	50	7.5	1	0	0	0	0	0	1	0	0	0	0	0	4	0	5	2	33.33
1	+	0	6.3	1	2	1	1	2	0	1	0	1	0	0	3	4	0	14	11	
1	+	5	6.6	0	5	0	0	3	0	0	0	0	1	0	1	2	0	12	10	9.09
1	+	50	6.1	2	1	2	1	1	1	0	0	1	0	1	1	3	0	11	9	18.18
2	+	0	6.0	1	4	2	1	1	0	2	0	0	0	0	3	12	1	26	21	
2	+	5	5.9	2	3	1	1	1	1	0	0	0	2	2	6	2	0	19	14	33.33
2	+	50	5.9	1	3	0	0	1	1	0	0	1	0	1	2	6	0	14	11 *	47.61
4	+	0	5.5	2	6	2	1	7	2	1	1	0	4	3	17	15	3	62	44	
4	+	5	5.5	2	4	1	1	4	5	0	1	0	2	5	11	12	2	48	32	27.27
4	+	50	6.1	2	3	1	0	1	7	0	0	0	1	1	9	12	6	41	31 *	29.54
8	+	0	4.9	3	15	1	4	12	8	6	9	2	3	3	24	35	6	126	72	
8	+	5	4.8	8	13	6	8	7	17	7	4	0	3	13	20	28	3	129	61	15.27
8	+	50	4.2	2	14	4	1	10	11	23	5	1	1	7	16	29	5	106	57 *	20.83
12	+	0	4.6	5	44	2	5	40	44	11	9	4	11	3	40	48	15	272	92	
12	+	5	3.3	1	44	5	17	31	39	16	20	2	9	41	26	53	7	308	90	2.17
12	+	50	3.2	3	13	6	3	18	20	10	0	3	0	12	13	47	3	145	79 *	14.13

[a] Mitotic index, number of metaphase cells × 100/1000 cells scored.
[b] tg, chromatid gap; tb, chromatid break; td, chromatid deletion; f, fragment; m, minute; tr, triradial; qr, quadriradial; cr, complex rearrangement; sg, chromosome gap; sb, chromosome break; af, acentric fragment; dm, double minute; d, dicentric; r, ring.
[c] Abs, aberrant cells.
* $p < 0.05$ significant values with their respective X-ray doses.

Table 3
Micronuclei induced by UV radiation and their inhibition by folinic acid in V79 cells

Dose			Cell cycle kinetics/400 cells				MNBN cells/1000 BN cells					
UV radiation ($\mu J \times 10^2/cm^2$)	+	FA (μg/ml)	1N	2N	> 2N	NDI [a]	1MN	2MN	> 2MN	Total	% MNBN cells	% MNBN inhibition
0	+	0	56	317	27	1.99	5	0	0	5	0.5	
0	+	5	36	319	45	2.14	4	0	0	4	0.4	20.00
0	+	50	32	325	43	2.14	4	0	0	4	0.4	20.00
50	+	0	57	330	13	1.92	22	7	5	34	3.4	
50	+	5	53	329	18	1.96	17	6	2	25	2.5	26.47
50	+	50	50	336	14	1.95	18	5	0	23	2.3	32.35
100	+	0	131	245	24	1.72	26	9	3	38	3.8	
100	+	5	150	242	8	1.67	18	8	5	31	3.1	18.42
100	+	50	155	236	9	1.70	18	6	5	29	2.9	23.68
200	+	0	266	132	2	1.35	34	6	6	46	4.6	
200	+	5	298	94	8	1.30	29	5	2	36	3.6	21.74
200	+	50	284	107	9	1.34	21	5	4	30	3.0	34.78
400	+	0	342	56	2	1.16	52	11	27	90	9.0	
400	+	5	359	39	2	1.11	51	14	10	75	7.5	16.66
400	+	50	361	36	3	1.11	45	9	13	67 *	6.7 *	25.55
800	+	0	367	30	3	1.10	55	16	32	103	10.3	
800	+	5	375	24	1	1.07	38	21	24	83	8.3	19.42
800	+	50	375	23	2	1.07	40	17	18	75 *	7.5 *	27.18

[a] Nuclear division index (NDI) = $[1N + (2 \times 2N) + (4 \times > 2N)]/400$ cells scored; N, number of nuclei; BN, binucleated cells; MNBN, micronucleated binucleated cells.
* $p < 0.05$, significant values with their respective UV-radiation doses.

Table 4
Chromosomal aberrations induced by UV radiation and their inhibition by folinic acid in V79 cells

Dose			Mitotic index [a]	Aberrations per 100 metaphase cells [b]														Aberrations/100 metaphase cells (without gaps)	Abs [c] (%)	Abs [c] inhibition (%)
UV radiation	+	FA		Chromatid type								Chromosome type								
($\mu J \times 10^2/cm^2$)		($\mu g/ml$)		tg	tb	td	f	m	tr	qr	cr	sg	sb	af	dm	d	r			
0	+	0	16.3	1	1	0	0	4	0	0	1	0	0	0	0	1	1	8	6	
0	+	5	19.3	0	0	0	1	2	0	0	0	0	1	0	0	0	1	5	5	16.66
0	+	50	19.3	0	1	0	0	2	0	0	0	0	0	0	2	1	0	6	5	16.66
50	+	0	18.8	3	3	1	1	8	1	0	0	1	0	0	9	0	0	23	15	
50	+	5	18.1	0	0	0	0	4	0	1	0	2	0	0	3	1	0	9	7	53.33
50	+	50	18.3	2	0	0	0	3	0	0	0	2	0	0	1	0	1	5	5 *	66.66
100	+	0	14.0	1	4	1	0	14	2	1	0	5	1	0	2	4	0	29	23	
100	+	5	14.3	1	2	0	0	4	1	0	1	1	0	0	3	0	0	11	10 * *	56.52
100	+	50	13.5	1	0	0	0	7	1	1	0	1	0	0	1	0	1	11	8 * *	65.21
200	+	0	6.7	7	9	1	3	15	4	4	0	7	2	2	3	2	2	47	35	
200	+	5	6.5	7	8	0	2	11	2	2	0	2	1	0	5	1	0	32	28	20.00
200	+	50	6.9	3	6	0	2	12	3	3	0	2	0	0	3	0	0	29	19 * *	45.71
400	+	0	– [d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
400	+	5	– [d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
400	+	50	– [d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
800	+	0	– [d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
800	+	5	– [d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
800	+	50	– [d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Mitotic index, number of metaphase cells × 100/1000 cells scored.
[b] tg, chromatid gap; tb, chromatid break; td, chromatid deletion; f, fragment; m, minute; tr, triradial; qr, quadriradial; cr, complex rearrangement; sg, chromosome gap; sb, chromosome break; af, acentric fragment; dm, double minute; d, dicentric; r, ring.
[c] Abs, aberrant cells.
[d] No metaphase cells available for scoring.
* $p < 0.05$; * * $p < 0.01$, significant values with their respective UV-radiation doses.

aberrant cells significantly decreased at 50 μg of FA in all doses tested except 1 Gy. A non-statistically significant, numerical decrease in aberrant cells was found with the 5 μg FA post-treatment. The aberrations/100 metaphase cells decreased by 50% both at 2 and 12 Gy with 50 μg of FA. The percent inhibition of aberrant cells ranged from 2.17 to 33.3% in 5 μg treated cells; however, this increased to 47.6% when 50 μg of FA was administered (Fig. 1b). A negative dose correlation was noted for percent inhibition of X-ray-induced chromosomal aberrations following treatment with 5μg ($r = 0.69$) or 50 μg of FA ($r = -0.60$).

The results of FA post-treatment of UV-exposed

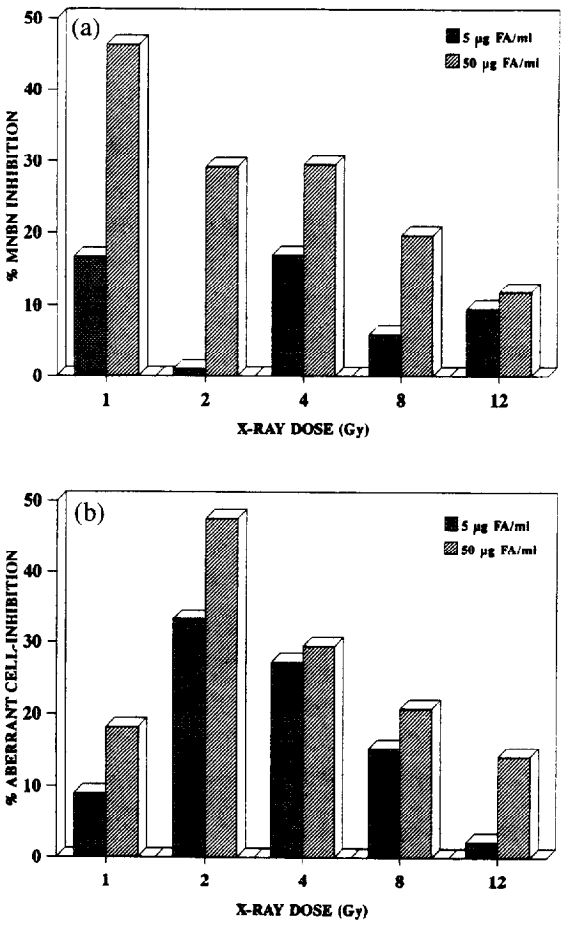

Fig. 1. Inhibitory effect of folinic acid on X-ray-induced chromosomal damage in V79 Chinese hamster lung cells: (a) Percent inhibition of micronucleated binucleated cells (% MNBN); (b) Percent inhibition of aberrant cells.

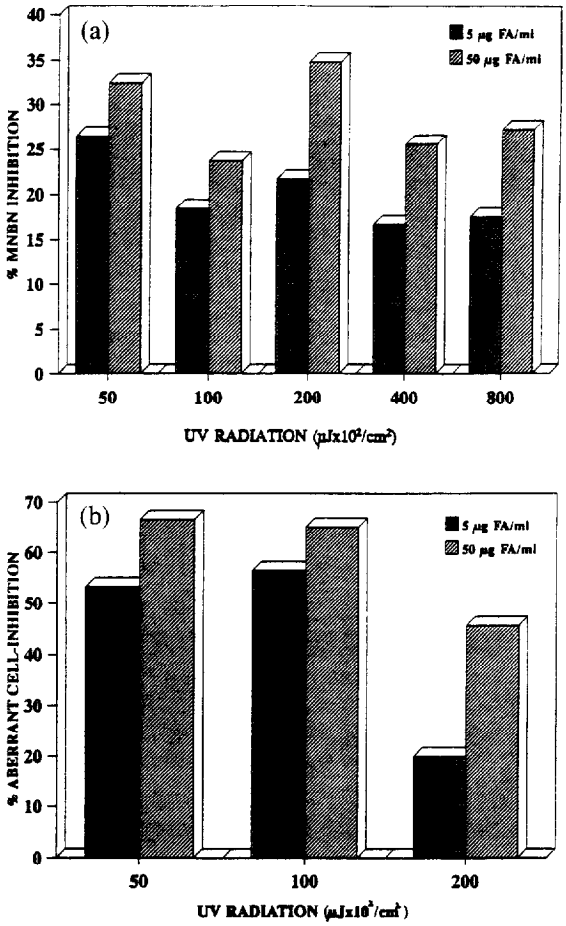

Fig. 2. Inhibitory effect of folinic acid on UV radiation-induced chromosomal damage in V79 Chinese hamster lung cells: (a) Percent inhibition of micronucleated binucleated cells (% MNBN); (b) Percent inhibition of aberrant cells.

V79 cells indicated that, as in the case of X-rays, FA had minimal effect on cytotoxicity. The NDI remained approximately the same when compared to their respective controls of UV-treated groups. Percent MNBN was not reduced significantly when the cell cultures were treated with 5 μg of FA (Table 3). At 50 μg of FA/ml of culture tested, percent MNBN decreased significantly ($p \leq 0.05$) at 400 and 800 $\mu J \times 10^2/cm^2$. The percent MNBN inhibition for 5 μg of FA ranged from 16.6–26.4% (Table 3, Fig. 2a) and percent inhibition for 50 μg ranged from 23.6–34.7%. In the SCA assay, there was no significant effect on MI in cells treated with either 5 or 50 μg/ml of FA. However, the aberrations/100

metaphase cells decreased substantially in all doses. The percent aberrant cells was reduced significantly in all doses of UV radiation when 50 μg of FA was administered; however, only 100 $\mu J \times 10^2/cm^2$ irradiation – post-treated with 5 μg of FA showed significant reduction (Table 4, Fig. 2b). The calculated percent inhibition of aberrant cells indicated a decrease of aberrations from 20–56.5% in 5 μg of FA and 45.7–66.6% inhibition in 50 μg of FA treatment groups.

4. Discussion

In earlier studies, we showed that X-rays and UV radiation caused significant clastogenicity as measured by % MNBN and % aberrant cells and also cytotoxicity as assessed by NDI and MI. Also, the two cytogenetic end points (% MNBN and % aberrant cells) correlated well (Keshava et al., 1995). In this study, the main objective was to investigate the inhibitory effect of FA as an antigenotoxic agent on radiation-induced chromosomal damage.

It is well known that X-rays produce DNA double-strand breaks (DSBs), DNA single-strand breaks (SSBs), base damages and DNA-protein cross-links (Natarajan, 1984) and that DSBs are the main lesions responsible for chromosome aberrations (Bender et al., 1974). Our results indicate that there was a significant increase in the number of MNBN and SCAs with increase in dose. There was also a dose related cell cycle delay. These results are in agreement with those of Littlefield et al. (1989) who have shown that acentric chromosome fragments-to-micronucleus transmission probabilities vary as a function of X-ray dose in human lymphocytes. It is also known that exposure of cells to ionizing radiation results in cell cycle delay which could be due to G_1 block, G_2 arrest or an S phase delay. S phase delay occurs following relatively high doses (> 5 Gy), and G_1 arrest may be absent in many cell lines. However, G_2 arrest is seen in all eukaryotes. The mechanism underlying may involve suppression of cyclin B1 mRNA and/or protein, which play a significant role in cell cycle (Maity et al., 1994).

In this study, the effect of FA on the number of DSBs was not demonstrated. However, the extent of radiation-induced (X-rays and UV radiation) chromosome damage decreased when cells were treated with FA immediately after irradiation. Also, FA can reduce X-ray induced damage by approx. 25–50% when administered at 50 μg of FA; however, only slight inhibition was found with 5 μg/ml indicating that there was not enough FA to be effective at 5 μg/ml. One possible explanation for the suppression of X-ray-induced chromosome aberrations may be a decrease in the number of SSBs before their conversion into DSBs. Libbus et al. (1990) have shown that folate deficiency in Chinese hamster ovary cells was associated with a 22.5% frequency of damaged mitoses compared to 2.3% in folate-replete cells. Breaks, gaps and fragments accounted for about one-half of the observed aberrations.

Folate deficiency alone caused DNA strand breaks equivalent to 26 cGy of irradiation (Branda and Blickensderfer, 1993). Following 400 cGy of irradiation, folate-deficient cells exhibited strand breaks equivalent to a dose of 710 cGy. These observations indicate that folate deficiency acts synergistically with mutagens/carcinogens to magnify genetic damage (Branda and Blickensderfer, 1993). In our study, although there was no folate deficiency, X-ray induced damage was reversed by the addition of folate which may indirectly influence the synthesis and/or repair of DNA after damage. When there is damage, a certain amount of DNA repair occurs within the cell using the nucleotide pool present in the cell. However, excessive breaks caused by radiation might deplete all of the nucleotide pool leading to a deficiency of repair process. When supplemented with FA, it can be hypothesized that folinic acid may help in the synthesis of a new thymidylate pool. Dihydrofolate reductase (DHFR) enzyme is known to catalyze the reduction of dihydrofolate to tetrahydrofolate, a cofactor required for synthesis of thymidylate used to synthesize DNA. Inhibition of DHFR ultimately leads to the depletion of folate cofactor involved in purine biosynthesis, thus decreasing DNA synthesis and/or repair subsequently leading to cell death. Because folate compounds are required by the mammalian cells for de novo production of purines and pyrimidines, the chromosomal aberrations associated with folate deficiency probably result from decreased DNA synthesis and failure of repair systems. The exact mechanism of inhibition of chromosome aberration by FA induced by radiation has not

been completely defined. However, it can be attributed, at least in part, to a reduction in the thymidylate pool or its availability for DNA repair. Similar chromosomal abnormalities – single- and double-strand DNA breaks, DNA degradation, chromosomal aberration and rearrangements – are associated with imbalances in the cellular nucleotide pools caused by drugs. It should be mentioned that the numerical scores on the aberrations observed were based on a single fixation time at 18 h post-treatment. The data could be different with a different fixation time. It is also possible that at higher doses of X-rays, because of severe cytotoxicity, cells which had already reached metaphase stage (probably less damaged) were scored.

UV radiation is one of the most common sources of skin cancer (Strange, 1995). UV radiation induces strand breaks, pyrimidine dimers and sometimes both inter- and intra-strand cross-links. Generally, the clastogenicity induced by UV radiation is S-dependent and thus results from a different mechanism than ionizing radiation. Most biological effects induced by short wave UV (254 nm) irradiation have been attributed to pyrimidine dimers. These include cell killing, induction of chromosomal aberrations, sister chromatid exchanges in eukaryotic cells, point mutations in prokaryotic and eukaryotic cells and tumors in fish (Natarajan et al., 1980; Van Zeeland et al., 1980).

Results of FA post-treatment to UV-exposed V79 cells indicate inhibition of MNBN and SCA by FA. These data indicate that FA acted effectively in terms of inhibition of chromosome damage caused by UV radiation. The main DNA lesion produced by UV radiation is pyrimidine dimers which are repaired by excision repair (Cleaver, 1968). DSBs are generated from UV-induced DNA lesions in the S phase (Bender, 1979). DSBs are thought to be repaired by post-replication repair in the G_2 phase and that unrepaired DSBs result in breakage-type chromosome aberrations (Hartley-Asp et al., 1980; Kihlman et al., 1982). It is a well known fact that UV causes cyclobutane pyrimidine dimers and these dimers kill cells by blocking DNA replication and transcription and if the DNA polymerase bypasses the dimer, a mutation is produced at the site of lesion. 5,10-methenyl tetrahydrofolate is a part of photolyase (DNA repair enzyme for UV damage) which serves as a light harvesting antenna in plants and certain bacteria. Although its exact mechanism of action is not known in mammalian cells, it may act in a similar manner in the repair process of UV induced DNA damage. It has been shown that thymidylate starvation increases the susceptibility of cells to damage by ultraviolet light and is associated with an increased frequency of chromosome aberrations such as dicentrics, rings, quadriradial exchanges and micronuclei as well as aneuploidy (Libbus et al., 1990).

In conclusion, the present study has demonstrated that FA has an anticlastogenic activity against X-ray and UV-induced chromosomal damage primarily at 50 μg/ml. It will be of interest to further investigate the exact mechanism of action of FA in reducing radiation-induced chromosomal damage.

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