Effects of Inhibitors of Nucleic Acid and Protein Synthesis on Ethyl Methanesulfonate-Induced Sister Chromatid Exchanges in Chinese Hamster Cells

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ABSTRACT. DNA synthesis inhibitors enhanced and protein synthesis inhibitors reduced the frequency of sister chromatid exchanges induced by the alkylating agent, ethyl methanesulfonate in Chinese hamster cells. The synergism and antagonism mainly occurred when cells were exposed to these agents during the first half of the S phase.

Our purpose was to examine the effects of various inhibitors of nucleic acid and protein synthesis on ethyl methanesulfonate (EMS)-induced sister chromatid exchange (SCE) frequency in Chinese hamster cells. Although there are many studies on the SCE-inducing ability of mutagenic agents such as alkylating chemicals, interaction between the alkylating agent and other kinds of inhibitors on SCE frequency have not been studied except for caffeine. Such studies could be important for detecting the potential synergistic actions of two mutagenic agents on mutagenesis and carcinogenesis.

Results of our investigation of the combined effects of EMS and a number of inhibitors on SCE formation are reported here. Several combinations displayed either synergism or antagonism.

MATERIALS AND METHODS

Cultures of Chinese hamster fibroblasts (clone DON D-6) were the generous gift of Dr. Kato of the National Institute of Genetics, Mishima, Japan. The cells were grown in stationary cultures at 37°C with Eagle's minimal essential medium supplemented with 10% fetal calf serum (GIBCO, heat-inactivated at 56°C, 30 min), nonessential amino acid, penicillin and streptomycin. The duration in hours of the phases of the cell cycle in this medium containing 10^{-5} M BrdU was: G₁, 3.8; S 8.7; G₂, 3.5; M, 0.5. The generation time (T_G) was 16.5 h. Exponentially growing cells were treated with BrdU at 10^{-5} M for two cell cycles. Colcemid (0.05 µg/ml) was added to the medium for 1.5 h before harvest. Cells were treated with 0.075 M KCl for 10 min then fixed with methanol:acetic acid (3:1). Air-dried prep-

Abbreviations used: bred, bredinin; 6-MP, 6-mercaptopurine; Ara-C, 1-β-D-arabinofuranosylcytosine; 5-FU, 5-fluorouracil; 5-FUdR, 5-fluorodeoxyuridine; BLM, bleomycin; AMD, actinomycin D; PM, puromycin; CH, cycloheximide; MMC, mitomycin C; EMS, ethylmethanesulfonate; BrdU, 5-bromodeoxyuridine; SCEs, sister chromatid exchanges.

arations were processed with the fluorescent plus Giemsa (FPG) technique of Wolff and Perry (9). More than 50 cells were scored for SCEs after each treatment. The source of agents used in this study was the same as in previous studies (7, 8).

RESULTS

Tables 1 and 2 summarize the effects of individual inhibitors in the presence or absence of EMS after a 16 h incubation. Nucleic acid antagonists in the absence of EMS profoundly affected the frequency of SCEs, the effect increasing with the concentration of the agent. In contrast inhibitors of protein synthesis, puromycin (PM) and cycloheximide (CH) had no significant effect. Several agents had dramatic effect on the frequency of induced SCEs in the presence of EMS. Bredinin, hydroxyurea (HU) and 5-fluorodeoxyuridine (5-FUdR) acted synergistically whereas PM and CH acted antagonistically. Other combinations were neither synergistic nor antagonistic.

Table 3 and 4 show the influence of the S phase on the interactions of EMS, FUdR, and CH tested with various drug combinations at 6.5, 9.5 and 12.5 h prior to harvest (the late, middle and early S phase respectively). In each case, chromosome preparations were made after a 3 h-incubation.

In Table 5, cells were treated with CH for 1, 2 or 3 h followed by incubation with EMS for 12.5 h prior to harvest. In one case, cells were treated with CH for 2 h, then after suspension for 2 h in the basal medium then were incubated with EMS for 12.5 h. The interference effect occurred even when treatment was given 1 h immediately before S. On the other hand, treatment with CH for 2 h in the early stage at G₁ had no effect on the frequency of EMS-induced SCEs (Table 5).

DISCUSSION

There are a number of possible explanations for the results in Tables 1 and 2. Several authors have proposed a relationship between the formation of SCEs and DNA repair (2, 3, 4, 6, 10). Since long-lived DNA breaks and gaps induced by EMS would normally be subject to post-replication repair, some of the lesions could serve as the origins of SCEs and chromosome aberrations. In the presence of DNA inhibitors the repair process might be suppressed and recombination favored. Bredinin, HU and FUdR could cause such suppression by depleting DNA precursor pools. Precursor depletion would act primarily at the replicating points and secondarily at the repair sites. One speculation is that a considerable portion of the EMS-induced lesions occur at sites of semiconservative DNA synthesis, so that abnormalities at the replication forks enhance the frequency of EMS-induced SCEs. This explanation is nevertheless inadequate because the other DNA inhibitors listed in Table 1 (for example 6-MP) did not display synergism. Moreover, protein inhibitors such as PM and CH reduced the frequencies of EMS-induced SCEs. Protein synthesis may therefore play an important role in the production of SCEs.

The influence of the cell cycle phase on interactions between EMS and the inhibitors listed in Tables 1 and 2 was tested with a modification of the procedure described by Benedict *et al.* (1).

In cells exposed to 10⁻³ M EMS 16 h prior to harvest and each of the inhibitors 3.5 h before harvest (corresponding to the G₂ phase), additive effects occurred in all

TABLE 1. EFFECTS OF NUCLEIC ACID SYNTHESIS INHIBITORS AND ANTITUMOR AGENTS ON THE FREQUENCY OF EMS-INDUCED SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER CELLS

Treatment		(Mol.)	SCEs per Cell (Mean $\pm$ S.D.)		
			Observed (A)	Expected (B)	(A/B) $\times$ 100
BrdU	10^{-5}		4.73 $\pm$ 1.12	—	—
EMS	10^{-3}		22.92 $\pm$ 4.46	—	—
	5×10^{-4}		13.84 $\pm$ 2.16	—	—
	10^{-4}		8.48 $\pm$ 1.76	—	—
EMS	10^{-3} + bred	10^{-5}	30.84 $\pm$ 4.57 ^a (7.13)	25.32	121.8
		10^{-6}	22.69 $\pm$ 4.54 (5.42)	23.61	96.1
//	5-FUdR	10^{-5}	31.95 $\pm$ 3.39 ^a (7.48)	25.67	124.5
//		10^{-6}	23.08 $\pm$ 2.62 (6.22)	24.41	94.6
//	Ara-C	10^{-6}	25.75 $\pm$ 2.99 (7.59)	25.78	99.9
//		10^{-7}	22.33 $\pm$ 2.85 (5.58)	23.77	93.9
//	AMD	10^{-8}	32.89 $\pm$ 5.03 (15.1)	33.28	98.8
//	BLM	10^{-7}	25.30 $\pm$ 3.57 (6.20)	24.39	103.7
EMS	5×10^{-4} + HU	5×10^{-5}	22.13 $\pm$ 4.17 ^a (7.13)	16.24	136.3
		10^{-5}	17.85 $\pm$ 2.52 (5.84)	14.95	119.4
	6-MP	10^{-6}	16.48 $\pm$ 2.49 (7.59)	16.70	98.7
		10^{-7}	16.52 $\pm$ 2.79 (5.65)	14.76	111.9
	5-FU	10^{-5}	17.64 $\pm$ 2.68 (6.57)	15.68	112.5
		10^{-6}	13.92 $\pm$ 2.68 (4.60)	13.71	101.5
	MMC	10^{-7}	52.29 $\pm$ 6.56 (41.4)	50.54	103.5
		10^{-8}	21.05 $\pm$ 3.62 (10.2)	19.35	108.8

TABLE 2. EFFECTS OF PROTEIN SYNTHESIS INHIBITORS ON THE FREQUENCY OF EMS-INDUCED SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER CELLS

Treatment		(Mol.)	SCEs per Cell (Mean $\pm$ S.D.)		
			Observed (A)	Expected (B)	(A/B) $\times$ 100
BrdU	10^{-5}		4.73 $\pm$ 1.12	—	—
EMS	5×10^{-4}		13.84 $\pm$ 2.16	—	—
EMS	5×10^{-4} + PM	5×10^{-6}	11.27 $\pm$ 2.19 ^b (4.92)	14.03	80.3
		10^{-6}	13.80 $\pm$ 2.55 (5.16)	14.27	96.7
//	+ CH	10^{-7}	10.24 $\pm$ 1.98 ^b (6.22)	15.33	66.8
		10^{-8}	14.46 $\pm$ 2.62 (6.28)	15.39	93.9

DON D-6 cells were pretreated with 10^{-5} M BrdU for 16 h. The cells then were incubated with various concentrations of inhibitors for 16 h only or in combination with EMS. Each value in parentheses represents the frequency of SCEs induced by the inhibitor alone. For the combined effect, the expected SCE values were calculated as in Ray *et al.* (5).

^a The observed value, which was significantly higher than the expected one ($P \leq 0.05$) represents the synergistic effect.

^b The significantly lower value represents the antagonistic effect.

TABLE 3. EFFECTS OF FUdR ON THE FREQUENCY OF EMS-INDUCED SCEs AT DIFFERENT TIMES IN THE S PHASE OF THE SECOND REPLICATION CYCLE

Treatment				SCEs per Cell (Mean $\pm$ S.D.)
BrdU				5.34 $\pm$ 1.78
EMS	early S			12.68 $\pm$ 2.73
	middle S			12.54 $\pm$ 2.23
	late S			8.80 $\pm$ 2.11
FUdR	early S			6.40 $\pm$ 1.48
	middle S			6.29 $\pm$ 1.53
	late S			5.51 $\pm$ 1.38
EMS	early S + FUdR	early S		16.14 $\pm$ 2.96 ^a
		middle S		12.71 $\pm$ 2.66
		late S		11.60 $\pm$ 2.42
	middle S + FUdR	early S		13.83 $\pm$ 2.57
		middle S		13.03 $\pm$ 2.42
		late S		9.46 $\pm$ 2.11
	late S + FUdR	early S		9.60 $\pm$ 2.35
		middle S		10.00 $\pm$ 1.99
		late S		9.41 $\pm$ 1.67

TABLE 4. EFFECTS OF CH ON THE FREQUENCY OF EMS-INDUCED SCEs AT DIFFERENT TIMES IN THE S PHASE OF THE SECOND REPLICATION CYCLE

Treatment				SCEs per Cell (Mean $\pm$ S.D.)
BrdU				5.34 $\pm$ 1.78
EMS	early S			12.68 $\pm$ 2.73
	middle S			12.54 $\pm$ 2.23
	late S			8.80 $\pm$ 2.11
CH	early S			6.06 $\pm$ 1.30
	middle S			5.77 $\pm$ 1.44
	late S			6.00 $\pm$ 1.21
EMS	early S + CH	early S		9.86 $\pm$ 1.72 ^b
		middle S		9.66 $\pm$ 1.24 ^b
		late S		12.46 $\pm$ 2.13
	middle S + CH	early S		9.80 $\pm$ 1.64 ^b
		middle S		9.34 $\pm$ 1.28 ^b
		late S		11.43 $\pm$ 2.44
	late S + CH	early S		8.31 $\pm$ 1.53
		middle S		8.66 $\pm$ 2.29
		late S		8.83 $\pm$ 1.85

DON D-6 cells were first treated with 10^{-5} M BrdU for 16 h after which the cells were exposed to 10^{-8} M EMS only or to EMS plus 2×10^{-5} M FUdR or 10^{-7} M CH 12.5–9.5 h, 9.5–6.5 h, and 6.5–3.5 h before harvest. Thus cells were treated with these agents at approximately the early, middle or late S phase of the second replication cycle in BrdU.

^a synergistic effect, ^b antagonistic effect

TABLE 5. EFFECTS OF CH FOLLOWED DURING THE G₁ PHASE OF THE SECOND REPLICATION CYCLE ON THE FREQUENCY OF EMS-INDUCED SCEs

Treatment (Mol.)		SCEs per Cell (Mean ± S.D.)
BrdU	10 ⁻⁵	6.00 ± 1.29
EMS	10 ⁻³	17.43 ± 2.97
CH	10 ⁻⁶ 2 h pulse 2 h chase before S	6.23 ± 1.43
	3 h pulse before S	5.07 ± 1.36
	2 //	5.80 ± 2.11
	1 //	5.80 ± 1.69
EMS plus CH	2 h pulse 2 h chase before S	17.64 ± 2.79
	3 h pulse before S	14.04 ± 3.25 ^b
	2 //	14.00 ± 2.86 ^b
	1 //	15.00 ± 2.91 ^b

DON D-6 cells were first treated with 10⁻⁵ M BrdU for 16 h, then with 10⁻⁶ M CH for 1, 2, or 3 h before treatment with 10⁻³ M EMS for 12.5 h. In one experiment, cells were treated with CH for 2 h then chased 2 h before treatment with EMS as shown above. Therefore treatment with CH approximately took place at the G₁ phase of the second replication cycle in BrdU.

^b antagonistic effect

cases. Interactions during G₂ were therefore neither synergistic nor antagonistic; such effects might have occurred with treatment during the S or G₁ phases.

As shown in Table 3, a synergistic effect between FUDR and EMS occurs only in cells exposed simultaneously to the inhibitor during the early S phase. Other phase combinations showed no synergistic action. This indicates that disturbance of replication rather than repair is the important factor in synergism. Indeed, the reduction in SCE frequency when FUDR is administered after EMS clearly indicates that repair is not the factor in synergism.

The characteristic response of CH was to reduce the formation of EMS-induced SCEs (Table 4). Interference occurred when CH was added during or before the middle S but not during the late S phase. A similar relationship was observed with combinations of EMS and PM (unpublished data).

Treatment with CH before the S phase also caused a significant decrease in the frequency of EMS-induced SCEs during the S and G₂ phases (Table 5). Apparently the protein synthesized during the late G₁ or early S phase has a role in regulating SCE frequency. Presumably, this protein is not associated with DNA replication. The principal question arising from these studies is what is the specific nature of the role played by protein synthesis in the mechanism of SCE formation.

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