6-Thioguanine (6 TG) Resistant Mutation, Chromosomal Aberrations and Sister Chromatid Exchanges (SCE's) in V 79 Cells

III. Concluding remarks

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It is one of the widely accepted view that one of the basic cause of malignancy is alteration in genetic material. Various test systems have been putforwarded to evaluate the DNA damaging potentialities of carcinogens (Bacterial—Ames *et al.* 1975, Kada *et al.* 1984; Eukaryotic chromosomal aberrations—Grant 1978, Ishidate *et al.* 1981, Brewen 1982; Sister chromatid exchanges (SCE's)—Wolff *et al.* 1977, Wolff 1978, Carrano *et al.* 1978, '79, Hsu *et al.* 1979; Point mutation assay in mammalian cells—Chu and Malling 1968, Kao and Puck 1968, Bradley *et al.* 1981, Kuroda *et al.* 1985). The first mammalian cell lines to be used in mutagenesis studies were derived from Chinese hamster (V79) cells (Chu and Malling 1968) and ovary (CHO) cells (Kao and Puck 1968). The drug resistant mutants which confer resistance to specific drugs provide very useful endpoints for various types of genetic changes occurring within a cell. A large number of well characterized drug resistant genetic selection systems have been developed (Arlett 1977, Carver *et al.* 1980, Clive *et al.* 1972, Clive *et al.* 1979, Gupta and Goldstein 1981, Gupta and Siminovitch 1980, O'Neill *et al.* 1977, Singh and Gupta 1982, Gupta and Singh 1982). Some of the genetic markers which have proved useful in mutagen screening studies are those in which the genetic alterations affect the purine and pyrimidine salvage pathway enzymes e.g. resistance to 6-thioguanine (TG^r) 8-azaguanine (AG^r) results from the loss or deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) (Beaudet *et al.* 1973, Caskey and Kruch 1979, Leinwand *et al.* 1978, Strauss and Albertini 1979). The gene for HGPRT is located on the X-chromosome of human and rodent cells. The enzyme consists of 2–4 protein sub-units and catalyzes the salvage of hypoxanthine and guanine by conversion of the purines to the corresponding nucleoside-5-monophosphates on reaction with phosphoribosyl pyrophosphate (Caskey and Kruch 1979, Chu 1971b). The enzyme also catalyzes the conversion of the purine analogs, 6-mercaptopurine (MP), 6-thioguanine (TG) and 8-azaguanine (AG) into their lethal nucleoside-5-monophosphates (Caskey and Kruch 1979, Chu 1971b). Thus mutant which inactivates the gene product (HGPRT) grows in medium containing purine analogs and non-mutant cells with normal HGPRT activity are killed. Mutants survive due to their ability to synthesize the purine by a *de novo* pathway. HGPRT locus has been used for detecting a wide variety of mutagens (base substitution—Caskey and Kruch 1979, DeMars 1974, Fox *et al.* 1976, Theill and Strauss 1977; frameshift—Huberman and Sachs 1976; deletion—Change *et al.* 1978, Chu 1971a, Huberman and Sachs 1976, Thacker *et al.* 1978, van Zeeland and Simons 1976; chromosomal rearrangements—Cox and Masson 1978; structural or regulatory genes—Caskey and Kruch 1979, Kadouri *et al.* 1978). Bradley *et al.* (1981) suggested that if a compound induces mutation frequency 3 times higher than the spontaneous, the compound should be classified as mutagenic.

Perry and Evans (1975) observed that most of the mutagens and carcinogens produced both SCE's and chromosomal changes. However, few studies (Furukawa *et al.* 1978, Abe and Sasaki 1977) observed enhanced SCE's but not chromosomal aberrations. Kihlman (1975), Abe

and Sasaki (1977) found that some of the potent clastogens do not induce SCE's. Carrano *et al.* (1978) observed a linear relationship between induced SCE's and mutation. However, a comparative study involving all of these effects and mutagens of different mode of action is lacking. The present study was undertaken with a aim to know the interrelationship between these end points of genotoxicity testing in the same cell system and under identical conditions of mutagen treatment and cultivation.

Material and methods

See part I and II.

Table 1. HGPRT gene locus mutation frequency, chromosomal aberrations, sister chromatid exchanges and their correlation coefficient value (r) due to Safrole treatment

Treatment ($\mu\text{g/ml}$)	Mutation frequency/ 10^6 survivors	Aberrant metaphase (%) (after 24 hr recovery)	'r' value between Aberrant metaphase (%) and mutation frequency	SCE's per cell Mean $\pm$ S.D.	'r' value between mutation frequency and SCE's
Control		2		7.38 $\pm$ 3.08	
50	0.33	10.0		6.60 $\pm$ 2.66	
100	0.10	11.0		7.38 $\pm$ 2.81	
200	0.13	13.0		7.96 $\pm$ 3.13	
300	0.48	9.0		8.02 $\pm$ 3.13	
			-0.84 (N.S.)		0.06 (N.S.)

N.S.: Non significant.

Table 2. HGPRT gene locus mutation frequency, chromosomal aberrations, sister chromatid exchanges and their correlation coefficient values due to ICR-170 treatment

Treatment ($\mu\text{g/ml}$)	Mutation frequency/ 10^6 survivors	Aberrant metaphase (%) (after 24 hr recovery)	'r' value between Aberrant metaphase (%) and mutation frequency	SCE's per cell Mean $\pm$ S.D.	'r' value between mutation frequency and SCE's
Control	0	3.00		6.12 $\pm$ 2.37	
0.1	3.65	9.5		9.30 $\pm$ 2.97	
0.3	15.22	10.5		11.36 $\pm$ 3.50	
1.0	47.91	15.5		15.88 $\pm$ 5.11	
			0.99*		0.99*

* Significant at 0.05 probability.

Results and discussion

It is apparent (Table 1) that Safrole appears to be weak mutagen but induced considerably high percentage of chromosomal aberrations. However the effect was not linear. The correlation coefficient (r) between mutation frequency and chromosomal aberrations is -0.84 and non significant. SCE's per cell was little increased but not statistically significant. The 'r' value between mutation frequency and SCE's is 0.06 (non significant). Contrary to this, ICR-170 treatment caused linear elevation of mutation frequency, chromosomal aberrations and SCE's (Table 2). The 'r' value between mutation, chromosomal aberrations and mutation,

sister chromatid exchanges is 0.99 (significant at 0.05 probability). It suggests that interrelationship between these end points is entirely specific to mutagens and their mode of action. The higher magnitude of mutation, chromosomal aberrations, SCE's due to ICR-170 treatment in compare to slightly enhanced values of mutation, SCE's but considerably higher value of chromosomal aberrations lead us to presume that due to specific action these biological end points may or may not share the identical primary DNA lesions for their manifestation. Carrano *et al.* (1979) have also expressed the similar view that each agent produces a spectrum of lesions and that for a given chemical some lesions are more readily converted to one form of damage than another, and many types of lesions may probably culminate in an SCE. Earlier, it was presumed that efficiency of inducing SCE's is directly correlated with the potentiality of inducing point mutations (Wolff *et al.* 1977, Wolff 1978, Carrano *et al.* 1978). However, detailed study led to the conclusion that "not every agent which induced SCE's is necessarily mutagenic" (Carrano and Thompson 1982). Natarajan *et al.* (1984), Kaina (1985) reported that the lesions causing gene mutation are different from those causing chromosome breaks or SCE's. Natarajan *et al.* (1984) also suggested that for monofunctional alkylating agents cell killing, chromosomal aberrations and SCE's are correlated with each other and may share at least some common DNA lesions. Thus, it seems very difficult to draw universal generalized view because it has been observed that X-rays and Bleomycin are poor inducers of point mutation and SCE's but potent inducers of chromosomal aberrations. Inhibitors of poly (ADP-ribose) polymerase are potent inducers of SCE's but do not induce mutation or chromosomal aberrations (Natarajan *et al.* 1981, '83). The correlation between mutation, chromosomal aberrations and SCE's is unclear. Both positive and negative correlations have been reported (Bradley *et al.* 1979, Carrano *et al.* 1978). Jostes Jr. (1981) has rightly suggested that the correlation between SCE, mutation and chromosomal aberrations can be positive or negative depending on the physical or chemical agent.

Thus from the present and other studies, it seems necessary to have further detailed study involving chemicals of widely variable mode of actions to establish clear interrelationship between these biological end points and specificity of mutagen. It will be of great significance to identify the particular DNA lesion(s) which may be responsible for a particular mutagenic event viz. mutation or chromosomal aberration or SCE's.

Summary

The widely accepted assays (HGPRT gene locus mutation, chromosomal aberrations and sister chromatid exchanges) for the evaluation of genotoxicity of environmental chemicals in mammalian cell systems, were comparatively studied due to Safrrole and ICR-170 treatments to know the interrelationship between them. The correlation co-efficient between mutation frequency-chromosomal aberrations and mutation frequency-SCE's due to Safrrole was insignificant contrary to ICR-170 treatment. Thus it is deduced that the correlation between these biological end points may be positive or negative depending upon the nature of physical or chemical agents.

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