PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 269–274 (1978)

Sister Chromatid Exchange Induced by Promutagens/Carcinogens in Chinese Hamster Cells Cultured in Diffusion Chambers in Mice[1] (40186)

S. R. SIRIANNI AND C. C. HUANG

Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, New York 14263

Metaphase chromosomes of cells which have undergone two cell cycles in 5-bromodeoxyuridine (BUdR) containing medium can be stained differentially by the fluorochrome Hoechst 33258 (1) and by Giemsa after special pretreatment (2). Applying this technique, sister chromatid exchanges (SCE) can easily be seen as a distinct reciprocal exchange of segments at homologous loci between two chromatids and therefore can be used for quantitative analysis. Several studies have suggested the induction of SCE be used as an indicator for screening certain classes of suspected environmental mutagens or carcinogens (3–14).

It is well known that most carcinogens and many mutagens require metabolic activation (15). Thus, SCE studies *in vitro* may underestimate potential mutagenic and possibly carcinogenic compounds under test. *In vitro* assays can be made more sensitive by the addition of liver microsome extracts to the cultures, these extracts can convert otherwise inactive substances into active agents (16–18). Since there are chemicals, however, that require activation by enzymes not present in liver extracts, either lost in preparation of the extract or found in organs other than the liver, this *in vitro* test would still prove insensitive to many chemicals. A transfer of the SCE test from *in vitro* to *in vivo* conditions circumvents these problems and therefore improves its validity as a test system for mutagenic and carcinogenic agents active in mammals and man.

Previously, we have described a modified host-mediated system which includes implantation of diffusion chambers (DC) containing human or Chinese hamster cells into the peritoneal cavity of mice. The effects of chromosome aberrations and SCE on the target cells in DC after injecting the hosts with the indirect mutagen cyclophosphamide (Cy) were studied (19–22). The present paper describes our further study on the effect of a group of seven promutagens/carcinogens including Cy and three nonmutagens/noncarcinogens on the frequency of SCE in Chinese hamster V-79 cells cultured in DC in mice.

Materials and Methods. Chinese hamster cell line V-79 was used, it was obtained from Dr. C. C. Chang, Michigan State University, East Lansing, Michigan. The cells were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum without antibiotics. The modal chromosome number of V-79 cells was 21. The seven promutagens/carcinogens tested were: Cy (Mead Johnson Laboratories, Evansville, Indiana), 1-(pyridyl-3)-3,3-dimethyltriazene (PyDT, gracious gift from Dr. R. Preussman, Freiburg, Germany), dimethylnitrosamine (DMN, Aldrich Biochemicals, Milwaukee, Wisconsin), diethylnitrosamine (DEN), benzo(α)pyrene (BP), 7,12-dimethylbenz(α)-anthracene (DMBA) and 3-methylcholanthrene (MCA, all from Eastman Kodak Co., Rochester, NY). The three nonmutagens/noncarcinogens used as negative controls were: pyrene (Py), anthracene (An, also from Eastman Kodak Co.) and perylene (Pr, from K & K Laboratories, Long Island City, NY). Cy, PyDT, DMN and DEN were dissolved in sterile water while all the other chemicals were dissolved in dimethyl sulfoxide (DMSO) at the appropriate concentrations.

The technique of DC culture was used as described elsewhere (19–22). A dry-sterilized DC was filled with 0.33 ml of a suspension containing approximately 10^5 cells. Each DC was then implanted into the peritoneal cavity of a 6 to 8-week old C3H/St mouse under ether anesthesia. Three days after DC implantation the mice received 6 hourly injections (ip) of 0.2 ml of BUdR solution (¼ ×

[1] Supported by Grant No. ES-01290 from the National Institute of Environmental Health Sciences and the Environmental Protection Agency. We are grateful to Dr. R. Preussman for his gift of triazene.

0037-9727/78/1582-0269$01.00/0

FIG. 2. (a) Metaphase chromosomes from the Chinese hamster cell line V-79 cultured in DC in a mouse without treatment of chemicals; it has 6 SCE's. (b) A metaphase from the Chinese hamster cell line V-79 in DC in a mouse treated with Cyclophosphamide (Cy) at 15 μg/g of bw. It has 36 SCE's.

ine and hexabarbital (24). Rat liver DEN diethylase activity has also been shown to be enhanced by various inducers of drug metabolizing enzymes (29). Thus, the possibility exists that Aroclor 1254 treatment may cause an enhancement of this and other enzymes involved in DEN metabolism. This induction may then allow adequate amounts of active agents to reach the V-79 target cells, resulting in increased formation of SCE, a phenomenon not observed in the absence of Aroclor 1254 injection.

SCE analyses of the two carcinogens DMN and DEN have been studied in cultured Chinese hamster cells (16) and human lymphocytes (14). No increase in SCE frequency was observed in both systems. However, a significant increase was seen in Chinese hamster cell cultures when liver microsomes were included in the test of these two compounds (16). Similar to the results observed in this study, the rate of SCE increase by DEN was much less than that of DMN. Bauknecht *et al.* (11) studied the effect of SCE in mouse bone marrow cells in vivo after injections of DMN and DEN. They also found a significant SCE increased induced by DMN but not DEN.

PyDT is an effective SCE inducer (Table I, Fig. 1). To our knowledge, the effect of this indirect carcinogen on SCE in mammalian cells either *in vitro* or *in vivo* has not been investigated. Bauknecht *et al.* (11) studied SCE induction by another triazene dimethylphenytriazene in mouse bone marrow cells *in vivo.* An increase incidence of SCE was also observed but only at a relatively high dose.

Effect of SCE by the carcinogenic hydrocarbons, BP, DMBA, and MCA have also been investigated before in cultured Chinese hamster cells (13), in human lymphocytes (14) and in Chinese hamster bone marrow cells *in vivo* (12). In the Chinese hamster cells *in vitro,* Popescu *et al.* (13) found no increase in SCE frequency using these agents. When the metabolizing feeder layer cells were added to this system, all the agents induced a large increase in SCE. BP and DMBA also induced high frequencies of SCE in Chinese hamster bone marrow cells *in vivo* (12). Unlike Chinese hamster cells *in vitro,* BP treatment of human lymphocytes caused a significant SCE increase (14). This may be due to the presence of activating enzymes in the

human lymphocytes. The absence of SCE induction by Pr and Py has also been reported elsewhere (13).

In vivo SCE analyses have been reported in mice, rats, Chinese hamsters, *Microtus agrestis,* rabbits and man (5, 8–12, 30, 31). In general the frequencies of "spontaneous" SCE *in vivo* were less than those *in vitro.* Similarly, the induced SCE frequencies by various agents in mammalian cells *in vivo* were also less than *in vitro* systems (3–14, 16–18). In the present study, the mean "spontaneous" SCE in V-79 cells in DC in mice was 5.5 which was higher than the average value found in Chinese hamster bone marrow cells *in vivo* (12) but lower than CHO cells *in vitro* (32). V-79 cells from DC in mice injected with any one of the chemicals used in this study generally had less than 30 SCE per metaphase. Rarely was there a metaphase scored with a greater value (Fig. 2). While *in vitro* systems the induced SCE values were much higher (3–7, 13, 14, 16–18).

In recent years, various short-term assay systems for predicting mutagenicity or carcinogenicity of chemicals have been developed. The modified host-mediated assay described here offers another practical and useful approach for screening certain classes of environmental mutagens or carcinogens. In evaluating this system, we have also used chromosome aberrations and gene mutations at the loci of 8-azaguanine and ouabain resistance as indicators for mutagenicity of a given compound under test. A dose-dependent increase of chromosome aberrations in the target cells (human or V-79 cells) and dose-dependent increase in mutation frequencies (V-79 cells) were observed (19, 20; Furukawa and Sirianni, unpublished data). This system takes into account host activation or deactivation of a chemical. It is sensitive and economical in time and cost. However, simplification of the procedure especially the BUdR administrations, is needed. The use of BUdR tablets instead of repeated injections as described by Allen *et al.* (33) could be adapted to this system.

We have used both human and V-79 cells in DC in mice (19–22). In certain respects, V-79 cells are more advantageous than human cells. The rapid proliferating nature of V-79 cells in DC in mice and the relatively high percentage (86%) of metaphases with differentially stained chromatids after only 4 hourly BUdR injections to the hosts make V-79 cells ideal for *in vivo* SCE analysis (22). For human target cells, 12 or 13 injections with BUdR at the same concentration was needed (21). Furthermore, point mutations in V-79 cells *in vitro* have been extensively studied (34, 35). Thus, the same cells cultured in DC in mice provide an ideal and convenient system for studying induction of gene mutations *in vivo* (Furukawa and Sirianni, unpublished data).

Summary. A system for *in vivo* analysis of sister chromatid exchange (SCE) was described. It involved culturing Chinese hamster V-79 cells in diffusion chambers (DC) in mice. Treatment of the hosts with repeated injections of 5-bromodeoxyuridine permitted the demonstration of differentially stained metaphases in the V-79 target cells after using the fluorescence plus Giemsa technique. Thus, it was possible to determine the number of SCE's under *in vivo* conditions. Induction of SCE in V-79 cells *in vivo* were studied following treatment with seven known promutagens/carcinogens: Cyclophosphamide, 1-(pyridyl-3)-3,3-dimethyltriazene, dimethylnitrosamine, diethylnitrosamine, benzo(α)pyrene, 7,12-dimethylbenz(α)anthracene, 3-methylcholanthrene and three nonmutagens/noncarcinogens: perylene, pyrene, and anthracene. With the exception of DEN, all the promutagens/carcinogens caused a significant increase in the frequency of SCE. DEN was effective at the highest dose studied only after pretreatment with Aroclor 1254, a known mixed-function oxidase inducer. SCE analyses of the nonmutagens/noncarcinogens resulted in no increase of SCE frequency as compared to the controls. These results indicate that SCE analyses in V-79 cells in DC in mice may provide a convenient, sensitive, and economical host-mediated assay system for detecting certain classes of environmental mutagens or carcinogens.

1. Latt, S. A., Proc. Nat. Acad. Sci. **70,** 3395 (1973).
2. Perry, P., Wolff, S., Nature (London) **251,** 156 (1974).
3. Latt, S. A., Proc. Nat. Acad. Sci. **71,** 3162 (1973).
4. Kato, H., Exp. Cell Res. **85,** 239 (1974).
5. Perry, P., and Evans, H. J., Nature (London) **258,** 121 (1975).

6. Solomon, E., and Bobrow, M., Mutation Res. **30,** 273 (1975).
7. Kato, H., and Shimada, H., Mutation Res. **28,** 459 (1975).
8. Vogel, W., and Bauknecht, T., Nature (London) **260,** 448 (1976).
9. Allen, J. W., and Latt, S. A., Nature (London) **260,** 449 (1976).
10. Stetka, D. G., and Wolff, S., Mutation Res. **41,** 333 (1976).
11. Bauknecht, T., Vogel, W., Bayer, U., and Wild, D., Hum. Genet. **35,** 229 (1977).
12. Bayer, U., and Bauknecht, T., Experientia (Basel) **33,** 25 (1977).
13. Popescu, N. C., Turnbull, D., and DiPaolo, J. A., J. Nat. Cancer Inst. **59,** 289 (1977).
14. Craig-Holmes, A. P., and Shaw, M. W., Mutation Res. **46,** 375 (1977).
15. Miller, J. A., Cancer Res. **30,** 559 (1970).
16. Natarajan, A. T., Tates, A. D., Van Buul, P. P. W., Meijers, M., and DeVogel, N., Mutation Res. **37,** 83 (1976).
17. Stetka, D. G., and Wolff, S., Mutation Res. **41,** 343 (1976).
18. Takehisa, S., and Wolff, S., Mutation Res. **45,** 263 (1977).
19. Furukawa, M., and Huang, C. C., Proc. Soc. Exp. Biol. Med. **153,** 536 (1976).
20. Huang, C. C., Environ. Res. **13,** 267 (1977).
21. Huang, C. C., and Furukawa, M., Exp. Cell Res., in press.
22. Furukawa, M., and Huang, C. C., Mutation Res., in press.
23. Alvares, A. P., Bickers, D. R., and Kappas, A., Proc. Nat. Acad. Sci. **70,** 1321 (1973).
24. Bickers, D. R., Harber, L. C., Kappas, A., and Alvares, A. P., Res. Commun. Chem. Pathol. Pharmacol. **3,** 505 (1972).
25. Czygan, P., Greim, H., Garro, A., Hutterer, F., Schaffner, F., Popper, H., Rosenthal, O., and Cooper, D. Y., Cancer Res. **33,** 2983 (1973).
26. Popper, H., Czygan, P., Greim, H., Schaffner, F., and Garro, A. J., Proc. Soc. Exp. Biol. Med. **142,** 727 (1973).
27. de Serres, F. J., Mutation Res. **41,** 43 (1976).
28. Malling, H. V., and Frantz, C. N., Mutation Res. **25,** 179 (1974).
29. Magour, S., and Nievel, J. G., Biochem. J. **123,** 8p (1971).
30. Schneider, E. L., Chaillet, J. R. and Tice, R. R., Exp. Cell Res., **100,** 396 (1976).
31. Pera, F., and Mattias, P., Chromosoma **57,** 13 (1976).
32. Kato, H., Int. Rev. Cytol., **49,** 55 (1977).
33. Allen, J. W., Shuler, C. F., Mendes, R. W., and Latt, S. A., Cytogenet. Cell Genet. **18,** 231 (1977).
34. Chu, E. H. Y., *in* "Chemical Mutagens: Principles and Methods for their Detection" (A. Hollaender, ed.), Vol. 2, p. 411, Plenum Press, New York (1971).
35. Arlett, C. F., Turnbull, D., Harcourt, S. A., Lehman, A. R., and Collella, C. M., Mutation Res. **33,** 261 (1975).

Received October 4, 1977. P.S.E.B.M. 1978, Vol. 158.