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Sister chromatid exchanges (SCE) in human lymphocyte were used to demonstrate the cytological effects of 2 agents : chlorambucil (CBC) and γ-rays. These two agents appeared to have more effects on the larger than the smaller chromosomes, the exchanges taking place predominantly in the long arms. Multiple SEC occurred as a result of CBC treatment. Results suggest that CBC and γ-rays causes SCE randomly on non-specific base pairs.

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การทดสอบผลของสารที่มีต่อเซลล์ของสารสองชนิดคือ chlorambucil (CBC) และ γ-rays โดยใช้การดู sister chromatid exchanges (SCE) ใน lymphocyte ของมนุษย์ พบว่าสารสองชนิดนี้มีผลต่อโครโมโซมตัวใหญ่มากกว่าตัวเล็ก SEC ที่เกิดขึ้นจะพบมากในแขนด้านยาว (long arm), chlorambucil มีผลทำให้เกิด SEC แบบ multiple นอกจากนี้การเกิด SCE เนื่องจากสารสองชนิดนี้เป็นแบบ random และไม่เกิดจำเพาะกับเบส (base) คู่ใด ๆ

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Since sister chromatid exchange (SCE) was first demonstrated autoradiographically by Taylor[1], the technique has been widely used with great improvements. Recently, a new technique has been developed to demonstrate sister chromatids by using a 5-bromodeoxyuridine (BrdU) labelled cell, stained with fluorescent dye or Giemsa.[2] This technique is advantageous in that it is simple and permits the detection of very small chromatid fragment exchanges. With this technique, several mutagenic, carcinogenic and non-carcinogenic agents were tested for their effects upon the induction and increase of SCE frequency in mammalian cells.[2-7] The frequency of SCE induced by antineoplastic and antibiotic drugs such as mitomycin, anthramycin and chlorambucil were also increased in human lymphocytes and Indian muntjac skin fibroblasts.[8-10]

Aldicarb, a highly toxic carbamate ester, has been extensively used for insect and nematode control and the negative result of genotoxicity has been assayed by the micronucleus test and Ames test, but SCE can be induced by this agent.[11] Physical agents such as γ-rays and ultraviolet can also induce a high frequency of SCE in human lymphocyte.[12,13] These agents can induce SCE at a concentration well below those required to cause an appreciable increase in chromosome aberraton.[14] Further interests concern not only the sensitivity but the elucidation of cytotoxicity of chemical agents and a reliable method for the detection of mutagens. Occurrence of SCE in any region of chromosome indicates the effect of any specific agents. The purpose of this investigation is to study the effect of two agents: chlorambucil, an antineoplastic drug and γ - radiation, by studying the groups and sites of SCE occurring in different parts of the long arms of chromosomes in vitro.

Materials and methods

Cell culture

Human leukocyte cultures used throughout this study were obtained from 10 randomly selected male medical students, aged 20 to 22 years. The male subjects were used in order to avoid bias from hormonal variations. Leukocytes were cultured in chromosome medium 1A (Lyophilized) with phytohermagglutinin (GIBCO). The culturing was a simple dilution of the chromosome medium 1A (Lyophilized) with 5ml of the chromosome diluent in a Lightton tube. Ten drops of whole blood (hypodermic needle # 22) were used in each culture. The 5-bromodeoxyuridine (BrdU) at a concentration of 0.09 mM was added and gently but well shaken. The tubes were wrapped tightly in aluminum foil to avoid light and kept for 96 hours in an incubator at 37°c. From each subject, four cultures were prepared, for control, with the diluent, with diluent plus chlorambucil, and plus irradiation.

Chemical agent

Chlorambucil (CBC), donated by BURROUGHS WELLCOME & CO;, BANGKOK, THAILAND, we made into a solution with 1 part ethanol and 4 parts of phosphate buffered saline. Only freshly prepared CBC solutions were used. The concentration of CBC in culture medium is 2 μg/ml. The diluent (1 part ethanol and 4 parts phosphate buffered saline) was also tested for its possible effects on inducing SCE.

Irradiation

Theratron 80 Cobalt unit (manufactured by Atomic Energy of Canada Limited) was used. The exposure rate was at 83.01 rad/min in air (measured with a Farmer dosimeter) at field size of 10 × 10, SSC 80 cm. in air. Each 5 ml culture was irradiated in a Lightton tube with a single total dose of 200 rad.

All treatments (CBC and γ-rays) were given to the cell cultures at 72 hours of age (second G_1 phase). Then the cultures were returned to the incubator and kept for a further 96 hours.

Harvesting of cells

A drop of colchicine solution (0.005%) was added to the medium 1 hr. before harvesting. The hypotonic treatment (0.075 M.KCl) and fixation (methanol : glacial, 3:1) were performed. In order to obtain a good metaphase, chromosome preparations were made by dissolving the pellet in the fixation agent (Mathanol: glacial, 6:1) and dropped to clean, dry slide from a height of 3 ft., then allowed to air dry.

Giemsa staining of sister chromatid

The technique for Giemsa staining followed the method described by Goto et al.[15] The air-dried slides were immersed in distilled water and then treated with 10^{-4} M 33258 Hoechst solution for 10 minutes, rinsed in distilled water and mounted with a cover slip in 0.16M sodium phosphate-0.04M sodium citrate at pH7. The slides were then exposed to sunlight for 15-20 minutes before the coverslip was removed and the slide dipped in distilled water, stained with 4% Giemsa in Sorensen buffer (pH 6.8), and allowed to dry at room temperature. All slides were blinded to avoid bias. The metaphase of 45-46 chromosomes with and without SCE were considered. Any gross chromosomal aberration was ignored. In each sample, only 50 metaphase plates were scored. The site of SCE in each chromosome was observed while the group of the chromosome was also considered.

Results

A total of 50 metaphase plates were observed in each sample. The number of cells with and without SCE were scored in order to compare the effect of CBC and γ-rays. The figures are tabulated in Table 1. The average number of cells with SCE was significantly higher that without SCE in all samples, especially with CBC treatment ($p < 0.001$). Chromosome groups were also considered. In control and diluent treated samples. the average percentage of groups showing SCE were nearly equal and all groups were affected. The comparison between CBC and γ-rays trated samples showed that larger chromosomes (A, B and C) were affected more than the smaller (D, E,F and G) after CBC treatment. The difference was statistically siginificant ($p < 0.05$, based on Sheffe' test. Moreover CBC treatment induced SCE in several chromosomes each with multiple sites of exchanges (Figure 1). On the other hand, in (γ-rays) treated samples although larger groups of chromosome were significantly affected than the smaller ($p < 0.05$), the frequency of SEC site in each chromosome was lower than with CBC treatment. Thus in γ-rays treatment each chromosome showed only 1-2 sites in the long arm (Figure 2).

The frequencies of SCE in both arms of a chromosome and at the centromere were also observed (Table 2). The frequency in the long arm was significantly higher than in the short arm and the centromere.

In order to determine whether the distribution of the site of SCE was random or not, the long arm was divided into three equal parts : telomeric (T), middle (M) and centromeric (c) regions.[3] The average sites of SCE in one metaphase plate were 9.36, 9.08 and 9.02 in T, M and C regions respectively for CBC treatment and 1.28, 1.94 and 1.39 respectively for γ-rays treated samples (Table 3). The differences between these chromosomal were not statistically significant using z-test ($p < 0.001$). Thus the data seem to indicate that CBC and γ-rays induced SCE in a random fashion.

Table 1 The average number of cells with and without SCE and average percentage of groups showing SCE.

Treatment	No. of cells with SCE	No. of cells without SCE	Average percentage of group of chromosome showing SCE.						
			A	B	C	D	E	F	G
CONTROL	48.50	1.50	20.20	18.00	10.86	6.56	2.60	0.50	0.50
DILUENT (DIL)	47.40	2.60	15.44	12.75	10.17	4.75	2.31	0.65	0.15
CBC + DIL	50	-	63.33	55.50	62.80	41.6	35.00	16.50	10.00
IRRADIATED (γ-ray)	48.90	1.10	25.67	22.85	13.86	9.10	4.47	0.80	0.75

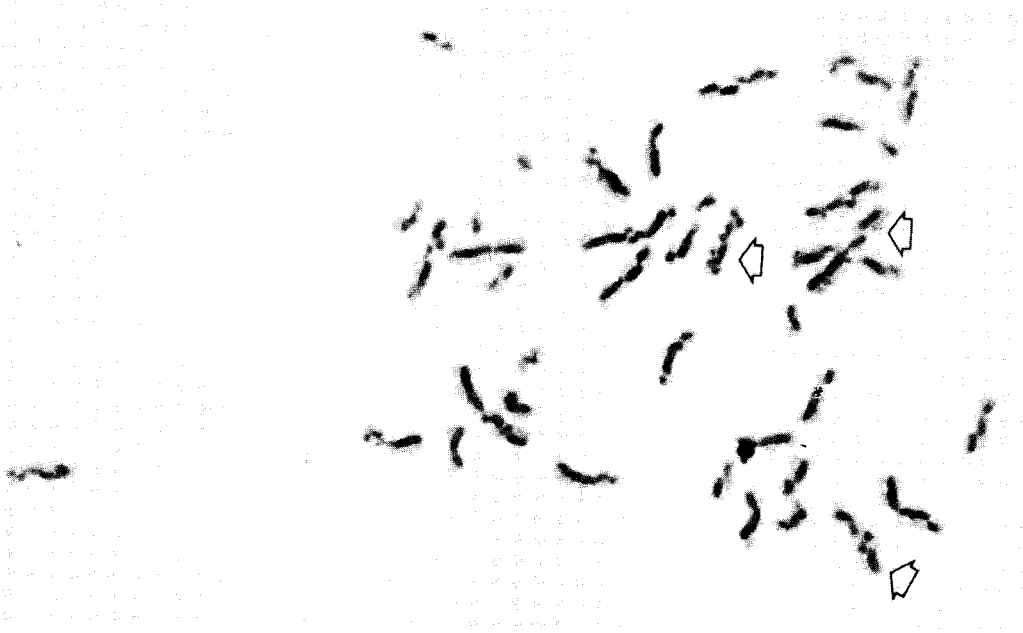

Figure 1 Miltiple sister chromatid exchange occured in a chromosome (arrows) affer treatment with chlorambucil in concentration 2 mg/ml, (× 100)

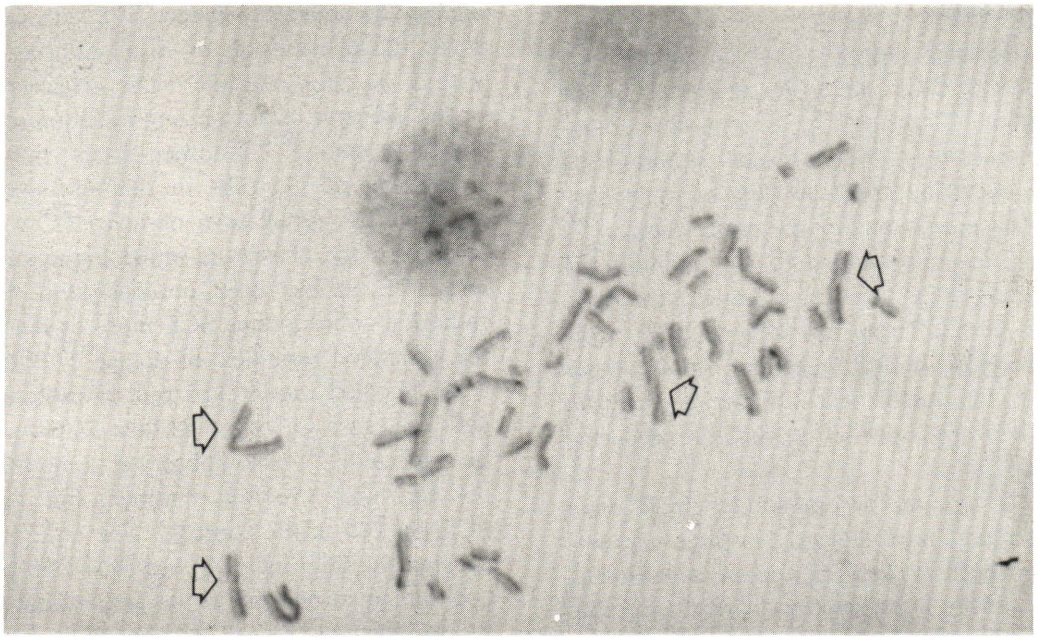

Figure 2 Site of sister chromated exchange occured in arm of a chromosome (arrows), after irradiation with 200 R. of gamma rays. SCE is produced only one site in the arm (× 100).

Table 2 Average number of chromosome showing SCE sites on the long arm, short arm and centromere per one metaphase plate in each treatment.

Treatment	Long arm	Short arm	Centromere
CBC	26.94	4.98	0.58
Irradiated (γ-rays)	4.70	0.83	0.48

Table 3 Average number of distribution of SCE sites on the long arm of chromosome per one metaphase with CBC and γ-rays treatments.

Treatment	Long arm		
	Telomeric (T)	Middle (M)	Centromeric (C)
CBC		9.08	9.02
Irradiation (γ-rays)	1.38	1.94	1.39

Discussion

Results showed that the number of cells with SCE were nearly equal in all samples. This may have been due to of several factors. For instance, spontaneous SCE occurring about 15-18% of metaphase may have been one of the major factors.[16] BrdU, an agent added to the medium for detecting SCE, was also known to cause SCE. Kim[17] reported 25% of SCE after three cycles in BrdU medium. On the other hand, the metabolism of the erythrocyte was known to have an influence in producing SCE.[18]

In human chromosomes, SCE have been found predominantly in large chromosomes both in control and in experiment. This can be interpreted as partly being due to the larger amount of DNA content in a larger chromosome. Moreover, SCE found in a larger chromosome could be the direct results of the CBC treatment. CBC was known as a bifunctional alkylating agent with a high efficiency in inducing SCE. Many authors have postulated that the high effeciencies of alkylating agents in inducing SCE are inportant factors in forming different DNA adducts.[19-22] It is believed that an alkylating agent has many efficiencies for inducing SCE at the same time. Thus SCE in a chromosome can be induced at multiple sites. However, other factors may be involved in causing SCE. Incorporation of BrdU into DNA seems to enhance the sensitivity of bases to be damaged by a alkylating agent and especially CBC. It has been found that there was a slight tendency toward synergism between MMC and BrdU ant it was observed in cells lebelled with BrdU and trated with MMC.[23] Other factors such as mitogen, an agent for stimulating culture growth, and some influent enzymes involved in both the detoxification and activation of chemical carcinogen[24] may enhance the potency of CBC and hence increase its abilities to induce SCE. In Contrast, the distribution of SCE induced by γ-rays was not multiple. The effects due to the property of an ionizing radiation were therefore poor.[25] Another factor in the irradiation of G_1 cells in that chromatid exchange has rarely been observed.[26] With respect to the effects of ionizing radiation and SCE, it has been demonstrated that radiation could induce SCE more or less at the same level regardless of dosage.[27] Wolff et all showed that if 150 rad of radiation was given to a G_1 cell, 0.68 false SCE could be expected.[28] They observed 2.56 SCE per cell after 150 rad treatment but 1.56 SCE per cell in the control. The radiation causing the SCE increase was thus 0.60 per cell, which turned out to be approximately the number of false SCE predictable from the aberration data. Therefore, it appears that γ-irradiation of a G_1 chromsome does not induce the persistent lesion leading to an increase in SCE. However if the dose was higher than 200 rad, a marked increase in the frequency of SCE was observed.[29] It seems that the ionizing radiation effect in inducing SCE occured in a stepwise manner.

On the distribution of SCE, we used the long arm of chromosomes for researching this phemomenon. The average value of all chromosomes was used to determine the effect of both CBC and γ-rays. With respect to the CBC effect, the guanine base is the site of alkylation by this substance, so the distribution of G-C base pairs are not clustered in the long arm. From the study of Crossen et al.,[30] using BrdU, they found that the distribution of exchanges concentrated in the centromeric region which was enriched with A-T base pairs. On this basis it can be envisaged that in the long arm of a human chromosome the distribution of G-C base pairs was scattered while A-T base pairs were concentrated around the

centromeric region. With respect to the effect of γ-rays, the distributions of SCE can be regarded as random and similar to that of other ionizing radiations causing chromosome aberrations.

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อ้างอิง

1. Taylor JH. Sister chromatid exchanges in Tritium labelled chromosome. Genetic 1958; 43 : 519-529
2. Abe S, Sasaki M. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J Natl Cancer Inst 1977 Jun; 58(6) : 1635-1641
3. Roman CS, Bobrow M. The sites of radiation induced-breakage in human lymphocyte chromosome, determined by quinacrine fluoresence. Mutat Res 1973 Jun; 18 : 325-331
4. Raffetto G, Parodi S, Faggin P, Maconi A. Relationship between cytotoxicity and induction of sister-chromatid exchanges in mouse foetal cells exposed to several doses of carcinogenic and non carcinogenic chemicals. Mutat Res 1979 Dec; 63(2) : 335-343
5. Allen JW, Latt SA. Analysis of sister chromatid exchange formation in vivo in mouse spermatogonia as a new test system for environmental mutagens. Nature (London) 1976 Apr 1; 260(5550) : 449-451
6. Stetka DG, Wolff S. Sister chromatid exchange as an assay for genetic damage induced by mutagen-carcinogen, I. "In vivo" test for compounds requiring-metabolic activation. Mutat Res 1976 Dec; 41(2-3) : 333-342
7. Popescu NC, Turnbull D, Dipaolo JD. Sister chromatid exchange and chromosome aberration analysis with the uses of several carcinogens and non carcinogens J Natl Cancer Inst 1977 Jul; 59(1) : 289-292
8. Latt SA. Sister chromatid exchanges, indices of human chromosome damage and repair : detection by fluoresecence and induction by mitomycin C. Proc Nat Acad Sci USA 1974 Aug; 71(8) : 3162-3166
9. Solomon E, Bobrow M. Sister chromaid exchanges : a sensitive assay of agents damaging human chromosomes. Mutat Res 1975 Nov; 30(2): 273-278
10. Ved Brat S, Verma RS, Dosik H. Anthramycin-induced sister-chromatic exchange and caffeine potentiation in the chromosomes of Indian Muntjac, Mutat Res 1979; 63 : 325-334
11. CID MG, Mators E. Induction of sister chromatid exchanges in cultured human lymphocytes by Aldicarb, a carbamate pesticide. Mutat Res 1984; 175-179
12. Marin G, Prescott DM. The frequency of sister chromatid exchanges following exposure to varying doses of H^3 - Thymidine or X-rays. J Cell Bio 1964 May; 21 : 159-167
13. Bianchi HO, Bianchi MS, Larramendy

M. Kinetics of human lymphocyte division and chromosomal radiosensitivity. Mutat Res 1979 Dec; 63(2) : 317-324

14. Latt SA, Loveday KS. Characterization of sister chromatid exchange induction by 8-methoxypsoralen plus near UV light. Cytogenet Cell Genet 1978; 21(4) : 184-200
15. Goto K, Akematsu T, Shimazu H. Sugiyama T. Simple differential Giemsa staining of sister chromatics after treatment with photosensitive dyes and exposure to light and the mecnanism of staining. Chromosoma (Berl) 1975 Dec 10; 53(3) : 223-230
16. Latt SA, Allen JW. Stetten G. In vivo and in vitro analyses of chromosome structure, raplication and repair using BrdU-33258 Hoechst techniques. In : Brinkley BR, Porter KK, eds. Proceeding of the First International Congress of Cell Biology. New York : Rockefeller University Press, 1977. 520-527
17. Kim HA. Chromatidaustausch and heterochromatin-veranderungen menschlicher chromosomen nach DUdR-Markierung. Nachweis mit Benzimidazolfluorochrom und Giemsafarbstoff Humangenetik 1974; 25(3) : 179-188 (Eng. Abstr.)
18. Norppa H, Vainio H, Sorsa M. Metabolic activation of styrene by erythrocyte detected as increased sister chromatic exchanges in carcinogen exposures by sister chromatid exchange. Nature (London) 1975 Nov 13; 258(5531) : 121-125
19. Heflich RH, Beranek DT, Kodell RL, Morris SM. Induction of mutations and sister-chromatid exchanges in Chinese hamster ovary cells by ethylating agents; relationship to specidic DHA adducts. Mutat Res 1982 Nov; 106(1) : 147-161
20. Vogel E, Natarajan AT. The relation between reaction kinetics and mutagenic action of monofunctional alkylating agents in higher eukarryotic systems : interspecific comparison, In : de Serres FJ, Hollaender A, eds. Vol 7. New York : Plenum, 1983. 295-338
21. Duncan AM, Evans HJ. Molecular lesions involved in the induction of sister-chromatid exchanges. Mutat Res 1982 Dec; 105(6) : 423-427
22. Natarajan AT, Simons JWIM, Vogel EW, Van Zeeland AA. Relationship between cell killing, chromosomal aberrations, sister-chromatid exchanges and point mutations induced by monofunction alkylating agents in Chinese hamster cells, a correlation with different ethylation products in DNA. Mution Res 1984 Aug; 128(1) : 31-40
23. Kato H, Shimada H. Sister chromatid exchanges induced by mitomycin C: a new method of detecting DNA damage at chromosomal level. Mutat Res 1975 Jun; 28(3) : 459-404
24. Whitelock JP, Cooper HL, Gelboin HV. Aryl hydrocarbon (benzopyrene) hydroxylase is stimulated in human lymphocytes by mitogens and benz (a) anthracene. Science 1972 Aug 18; 177(4040) : 618-619
25. Uggla AH, Natarajan AT. X-rays induced SCEs and chromosomal aberration in CHO cell : influence of nitrogen and air during irradiation in different stages of the cell cycle. Mutat Res 1983 Nov; 122(2); 193-200
26. Antoine JL, Dutrillaux B. Chromosomal sequences of irradiation of human lymphocyte during S-phase, with special reference to chromatid exchanges. Mutat Res 1984 Nov; 129 (2) : 173-179
27. Ardito G, Lamberti L, Piccotti F, Poggio G. Analysis of chromosome

aberrations after exposure to ionizing radiation in "in vitro" cultures of human lymphocyte by means of incorporation. Boll Suc Ital Biol Sper 1980 Apr; 56(7) : 719-724

28. Molff S, Bodycote J, Painter RB. Sister chromatid exchange induced in Chinese hamster cell by UV irradiation of different stages of the cell cycle : the necessity for cells to pass chrough S. Mutat Res 1974 Oct; 25(1) : 73-81

29. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposures by sister chromatid exchange. Nature (London) 1975 Nov 13; 258(5531) : 121-125

30. Crossen PE, Drets ME, Arrighi FE, Johnston DA. Analysis of the frequency and distribution of sister chromatid exchanges in cultured human tymphocytes. Hum Gener 1977 Mar 14; 35(5) : 345-352

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