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Study on the Sister Chromatid Exchange Inducibility in Chinese Hamster Don Cell by Metal Compounds in Work Environment

Kwang Seok Seo and Chong Sam Lee*

Dept. of Environmental Engineering, Kyungki Jr. College, Incheon, Korea.

** Dept. of Biology, College of Natural Sciences, Sungshin Women's Univ., Seoul, Korea.*

作業環境中의 金屬化合物이 Chinese Hamster Don 細胞의 姉妹染色分體交換 誘發性에 미치는 硏究

徐光錫 · 李鍾三*

京畿專門大學 環境工業科

* 誠信女子大學校 自然科學大學 生物學科

국문요약

산업장이나 생활환경에서 접하기 쉬운 수용성 염화물을 중심으로 19개 원소 24종의 금속화합물이 Chinese Hamster Don 세포에 있어서의 sister chromatid exchange(SCE) 출현빈도에 미치는 영향을 조사하였다.

Chinese Hamster Don 세포에 대한 자매염색분체 교환출현빈도의 증가가 CrO_3, K_2CrO_4, $K_2Cr_2O_7$, $MnCl_2$, K_2SeO_3, CH_3HgCl ($p<0.01$), $CoCl_2$, Na_2HAsO_4, $HgCl_2$ ($p<0.05$) 9종의 금속화합물에서 나타났으며, dose-response relationships이 현저한 금속화합물은 6가 크롬화합물과 K_2SeO_3이었다.

Keywords : Chinese hamster Don cell, sister chromatid exchange, metal compounds

I. Introduction

The increasingly wide use of metal compounds due to the rapid development of the heavy chemical industry has led to the destruction of the work environment and an ecosystem.[1,2] Severe social problems of industrial hygiene and environmental pollution have been well suggested by Sunderman[3] and Norseth[4]; the former reported that workers in the production of chromic acid were likely to run the risk of relatively higher lung cancer and the latter did that those exposed to the dust of cadmium oxides more suffered from the prostate cancer.[5,6,7]

The recent improvement of molecular biology and genetic engineering contributes to the development of detection of mutagenicity used for a varietal bacteria, which in turn allows more detection of toxic matters which cause the mutation of bacteria.[8,9,10] As experimental findings show that most of mutational substances detected through these methods give rise to a cancer for higher animals, these are used easily as a group screening test prior to the experiment of cancer development in samples like cattle. An organism of higher animals is not appropriate to be used directly for the human body only of the basis of findings from bacteria, since it has a strong defence function against toxic materials with mutational or carcinogenic substance.

To see the influence on the organism for toxic substance,[11,12] therefore, experimental subjects may be generally classified into four experimental stages; organism, individual tissue, organic cell, and extract of cell. Among them an experiment with cells is useful to observe the proliferation of cells, change of chromosome, mutagenicity or carcinogenic substances, or biological changes.[13] Espe-

cially, a cell-used sister chromatid exchange (SCE) is widely used to detect mutations, as it is a phenomenon resulting from a series of cutting and reunion of DNA.

The purpose of this study is to measure the SCE inducibility of metal compounds which can be easily obtained in work environment in Don cell separated from a Chinese Hamster and to discover genetic mechanism in organism of metal compounds by determining the correlation between the increasing of frequency of SCEs and the concentration of metal compounds, which also may contribute to the prevention of environmental pollution and occupational diseases.

II. Materials and Methods

1. Metal compounds

Metal compounds used for the experiment are same as those used by Seo and Lee.[2]

2. Cells

The cells separated from the healthy lung of a Chinese Hamster Don cells (Flow making) were used for sister chromatid exchange (SCE) inducibility test.

3. Medium

An Eagle's MEM medium (Nitsui making) containing L-glutamine(0.292 g/*l*), 10% $NaHCO_3$ (12 m*l*/*l*), and 10% cattle embryo serum was used.

4. Culture and preservation of cell

Don cells separated from the healthy lung of a Chinese Hamster were scattered thinly with trypsin-EDTA flow to cells which had proliferated enough of the wall of square-shaped culture bottle, suspended in Eagle's MEM medium to which 7% cattle embryo serum was added (Wako making) so that cultured at 37℃ for 3 days until their density would be 5×10^5 cells/m*l*. To preserve cells, cells reaching to a logarithmic proliferative phase had been suspended in a medium to which 10% glycerin and 15% cattle embryo serum (Gibco making) were added so that their density would be 1×10^6 cells/m*l*, bottled into ampoules by 5 m*l*, and frozen at the temperature of -80℃.

5. SCE test

Fig. 1 showed the procedure of SCE inducibility test. Namely, 5 m*l* of culture fluid of Don cell (3×10^5 cells/m*l*) reaching to a logarithmic proliferative phase through preculture was transferred to a petri dish and cultured in a incubator of 5% CO_2 at 37℃ for 24hrs.

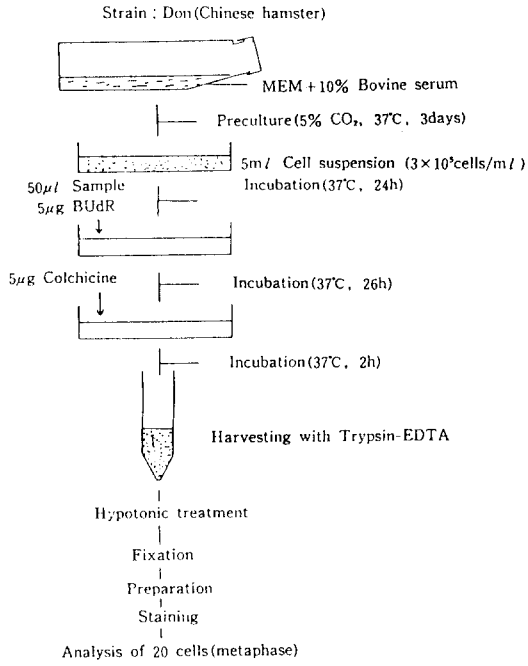

Fig. 1. The schematic procedure for sister chromatid exchange.

After 0.05 m*l* solution of metal compounds which had been diluted to have a given final centration and 0.05 m*l* of 5-bromodioxyuridine (BUdR, Wako making, 100 μg/m*l*)were added and mixed, the mixture had been cultured during 26hrs in a dark box. Then another 0.05 m*l* solution of colchicine (Wako making, 100 μg/m*l*) was added and cultured again at 37℃ for 2 hrs.

To the control, 0.05 m*l* of ethanol or distilled water used as a solvent was added instead of metal compounds. After culture, the culture fluid was transferred to a centrifugal tude and cells separated with trypsin-EDTA fluid were added and stirred, and then the mixture was separated in the centrifuge (Sakuma #2000) at 1,000 rpm for 5 minutes to remove the supernatant. 1 m*l* of 0.075M KCl of preserved fluid was added to the deposited cells and mixed, and then left at the room temperature for 7 minutes. And 10 m*l* of fixed fluid (methanol : acetic acid = 3 : 1) was added to it and mixed slowly, and then left for 10 minutes; then, it was separated by

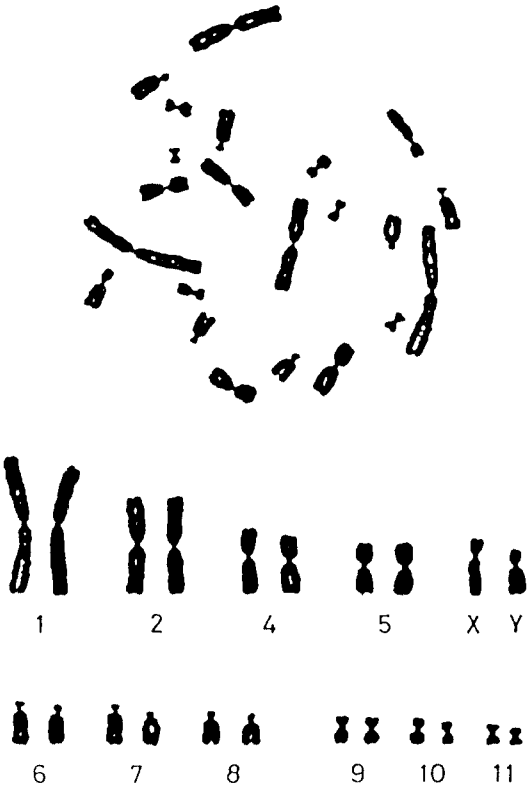

Fig. 2. Karyo type of Don cell.

centrifugation at 1,000 rpm for 5 minutes to get the precipitation, to which again 10 m*l* of fixed fluid was added : the precipitation was separated at 1000 rpm in the centrifuge for 5 minutes to get another deposit : and these procedure had been repeated two times. To the cell deposit obtained appropriate amount of fixed fluid was added and mixed, and two or three drops of this mixture were dropped on a slide glass, which would be dried to be a sample of chromosome.

For differential staining of sister chromatid exchange, a method which was derived by Kanda and Kato[14] from a fluorescence plus giemsa (FPG) developed by Perry and Wolff[15] was employed. Namely, the sample of chromosome had been stained with a 1/15 M Sorensen phosphate buffer solution (pH 6.8) containing Hoechst 33258 (Wako, 1 μg/m*l*) twenty minutes, then washed with a Sorensen phosphate buffer solution, and covered with a cover glass.

The slide glass was put 30 cm below a UV lamp

Fig. 3. Induced SCEs in Don cell.
→ : sister chromatid exchange(SCE)

(H400 or SAN-39 of Iwasaki) and irradiated in UV with a fan cooling it. Then, the cover glass was removed ; it was stained using 3% giemsa (1/15 M phosphate buffer solution) for 15 minutes and washed to distilled water ; it was sealed with Canada balsam through xylol to be a permanent sample.

20 of the second metaphases per a material were randomly selected and the induced number of SCE was counted by optical microscope (×1000). The number of SCEs appeared on 20 metaphase was averaged to get an average number of SCE per cell according to the method of Sketka and Wolff.[16]

III. Results

Typical nuclear type of Chinese Hamster Don cells used for experiments is shown in Fig. 2. The number of chromosomes is 2n=22, or 2 diploid same as in the Chinese Hamster.

There is in Fig. 3 an example of divided stained second metaphase according to a method which was derived by Kanda and Kato[14] from a fluorescence plus giemsa (FPG) developed by Perry and Wolff.[17] When a cell is divided in the presence of 5-bromodioxyuridine (BUdR), the same system of thymidine which is a nucleic acid base, no chromatid in the first metaphase would not be stained partially because the newly formed DNA chain is substituted with BUdR, while that in the second could be stained because it would be divided into one chromatid one of whose DNA chains was substituted with BUdR and another chromatid both of whose DNA

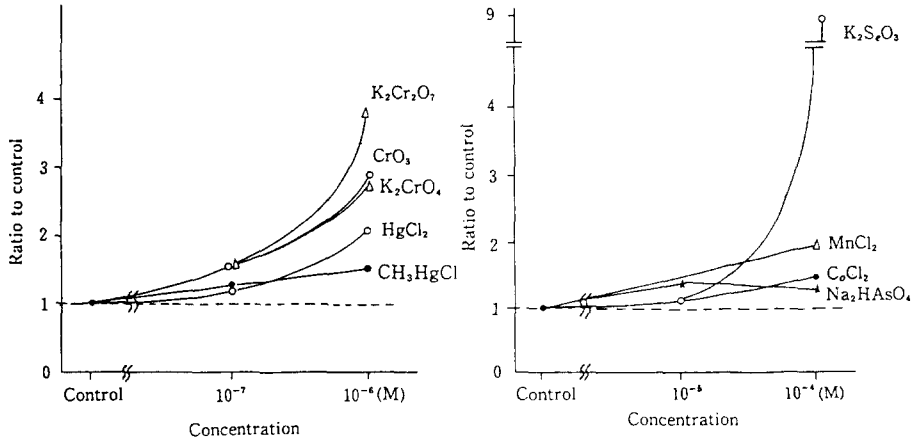

Fig. 4. Dose response curve of SCEs induced by metal compounds.
(-------):control

chains were substituted with BUdR. The former would be stained dark and the latter weak that the strong and weak staining of sister chromatids would appear inversely at a place where SCE occurred. It can be seen that there have been 8 SCEs in Fig. 3.

Table 1 shows the number of SCEs induced by metal compounds. The average number of SCEs in the control was between 6.3 and 11.4, being different in each experiment. To compare with them all together, average number of SCEs induced by the experimental result by each metal compound was expressed as proportion to the number of SCEs in the control, whether the proliferation of Don cells would be inhibited or not by the various metal compounds also was marked qualitatively by observing using a microscope when making a sample of chromosome. Namely, the proliferation a little bit less than that of the control group, though the shape of cell was as fibriform as in the control group, was marked+ and the poor proliferation where circular cells appeared much was marked++.

Since the staining would not take place unless the second division ensues after the treatment of the various metal compounds, no detection of SCE in the concentration with strong toxicity was possible. And, though the number of SCE was counted and averaged from 20 metaphases per one sample are, the toxicity of $CoCl_2$ 10^{-4} M and K_2SeO_3 10^{-4} M was so strong that their metaphases were less than 20 and thus SCEs induced for 4 and 7 metaphases respectively were counted and averaged.

The SCE inducibility frequencies (the average SCE number per cell) obtained from tests for various metal compounds per each concentration and the number of SCE in the control have been compared using a t-test. As shown in Table 1, the concentration, whose significant difference was approved wihin 1% of risk of 3 metal compounds like C_rO_3, $K_2C_rO_4$ and $K_2C_rO_7$ were 10^{-7}M, 10^{-6} and 10^{-4}M respectively. And CH3HgCl 10^{-6} M, $MnCl_2$ and K_2SeO_3 were 10^{-4} M. Within 5% of risk, the significant concentration of metal compounds like $CoCl_2$ and Na_2HAsO_4 were 10^{-4} M, $HgCl_2$ and CH_3HgCl were 10^{-6} M and 10^{-7}M respectively. And remaining 15 metal compounds showed more than 10% significant level.

The dose-response curve of 9 metal compounds, which significant difference was approved within 5% of risk. $K_2Cr_2O_7$, CrO_3, K_2CrO_4, $HgCl_2$, CH_3HgCl, K_2SeO_3, $MnCl_2$, $CoCl_2$, and Na_2HAsO_4. were shown Fig. 4.

As shown in Fig. 4, among the 9 metal compounds $K_2Cr_2O_7$, CrO_3, K_2SeO_3, and K_2CrO_4 were shown very high dose-responses, while those of $HgCl_2$, CH_3HgCl, $MnCl_2$, $CoCl_2$, and Na_2HAsO_4 were very low levels.

IV. Discussion

The SCE inducibility was first observed by Taylor[5] who confirmed the thymidine of DNA expressed in ^3H using by autoradiography, and, later, a clear, simple FPG method developed by Wolff and Perry[17] revealed the fact that the SCE phenomena

Table 1. Inducibility of SCEs in Don Cells treated with metal compounds

Metal compound	Concentration(M)	Induced SCEs/cell	Ratio to control	Growth inhibition
$CrCl_3$	0	6.3		
	10^{-4}	6.2	1.0	
	10^{-3}	7.7	1.2	+
	10^{-2}	N.D	-	++
$Pb(CH_3COO)_2$	0	6.3		
	10^{-4}	5.8	0.9	
	10^{-3}	6.6	1.1	
	10^{-2}	N.D.	-	++
$MnCl_2$	0	6.3		
	10^{-4}	12.7	2.0*	+
	10^{-3}	N.D.	-	++
$Cr_2(SO_4)_3$	0	6.3		
	10^{-4}	5.9	0.9	
	10^{-3}	N.D.	-	++
$NiCl_2$	0	6.3		
	10^{-4}	5.5	0.9	
	10^{-3}	N.D.	-	++
$CuCl_2$	0	6.3		
	10^{-4}	7.0	1.1	
	10^{-3}	N.D.	-	++
$CoCl_2$	0	11.4		
	10^{-5}	12.3	1.1	
	10^{-4}	17.0	1.5	+
	10^{-3}	N.D.	-	++
$ZnSO_4$	0	11.4		
	10^{-5}	12.8	1.1	
	10^{-4}	14.6	1.3	
$CdCl_3$	0	7.2		
	10^{-6}	8.6	1.2	
	10^{-5}	8.7	1.2	+
	10^{-4}	N.D	-	++
$SnCl_2$	0	7.2		
	10^{-6}	6.5	0.9	
	10^{-5}	7.3	1.0	+
	10^{-4}	N.D.	-	++
$BaCl_2$	0	7.2		
	10^{-6}	7.2	1.0	
	10^{-5}	6.8	0.9	
	10^{-4}	7.9	1.1	
$LaCl_3$	0	7.2		
	10^{-6}	6.5	0.9	
	10^{-5}	7.0	1.0	+
	10^{-4}	N.D	-	++
$CeCl_3$	0	7.2		
	10^{-6}	5.8	0.8	
	10^{-5}	6.3	0.9	
	10^{-4}	6.3	0.9	
K_2SeO_3	0	7.2		
	10^{-6}	7.4	1.0	
	10^{-5}	8.2	1.1	
	10^{-4}	62.3	9.0**	+
Na_3HAsO_4	0	7.2		
	10^{-6}	6.7	0.9	
	10^{-5}	9.9	1.4	

	10^{-4}	9.6	1.3*	
LiCl	0	8.2		
	10^{-6}	7.4	0.9	
	10^{-5}	8.4	1.0	
	10^{-4}	8.3	1.0	
$AlCl_3$	0	8.2		
	10^{-6}	6.2	1.0	
	10^{-5}	8.5	1.1	
	10^{-4}	N.D.	-	+
$NaVO_3$	0	8.2		
	10^{-6}	7.6	0.9	
	10^{-5}	8.1	1.0	+
	10^{-4}	N.D.	-	++
Na_2MoO_4	0	8.2		
	10^{-6}	7.8	0.9	
	10^{-5}	8.5	1.0	
	10^{-4}	8.0	1.0	
CrO_3	0	6.8		
	10^{-7}	10.9	1.6**	
	10^{-6}	20.0	2.9**	
	10^{-5}	N.D.	-	++
K_2CrO_4	0	6.8		
	10^{-7}	11.0	1.6**	
	10^{-6}	19.1	2.8**	
	10^{-5}	N.D.	-	++
$K_2Cr_2O_7$	0	6.8		
	10^{-7}	10.8	1.6**	
	10^{-6}	25.9	3.8**	
	10^{-5}	N.D.	-	++
$HgCl_2$	0	6.8		
	10^{-7}	8.3	1.2	
	10^{-6}	14.4	2.1*	+
	10^{-5}	N.D.	-	++
CH_3HgCl	0	6.8		
	10^{-7}	8.8	1.3*	
	10^{-6}	10.5	1.5**	+
	10^{-4}	N.D.	-	++

*Significant ($p<0.05$),
**Significant($p<0.01$)

would be caused at least by a series of cutting and reunion of DNA chain. Since then, the SCE inducibility to more mutagenicity substances has been examined and observed response sensitively comparing to chromosome abnomality inducibility[18] and now is regarded as one of the most recommendable detection of mutagenicity substances. Bradley et al.[18] also suggested that SCE was related with carcinogenesis and mutagenicity and further that it was a sensitive and quantitative test to check any genetic disorder supposedly due to the damage of DNA.

The number of SCEs induced by metal compounds in this experiment were shown between 6.3 and 11.4 per cell in the control with slightly different value for each experiment. It is higher than 2.3 for Don-6 cells reported by Kato[19] or 3.9 by Ohno et al.[20] for Don-6 cells. Kato[19] and Davidson et al.[21] pointed that the elements on the number of SCEs induced spontaneously were the BUdR density, visible ray, and kinds of and culture conditions of experimental cells and also said that they might be different depending on strain or substrain,[16,22,23] as reported that 8.5 (range 2~14) in one example or otherwise 3.8 (range 0~10) in substrain isolated from Don cell that SCE number induced at average al-

though same cell was 4.6 (range 1~10).

As shown in Table 1, the increase of the number of SCEs by 10^{-7} M was observed in three Cr^{+6} compounds like $K_2Cr_2O_7$, CrO_3 and K_2CrO_4 but no increase in Cr^{3+}compounds like $CrCl_3$, and $Cr_2(SO_4)_3$, even by the high concentration of 10^{-4} M. This result was similiar to the reports by Macrae et al.[24] who examined the number of SCEs induced by mammal cells. Ohno et al.[20] however, reported an increase of the number of SCEs by $CrCl_3$, though very insignificant, in Don cells. And Nakamuro et al.,[1] reported chromosome abnomality inducibility of Cr $(CH_3COO)_3$, $Cr(NO_3)_3$, and $CrCl_3$, in the study of chromosome abnomality and mutagenicity of Cr^{3+} and Cr^{6+} using Don cells.[25] The conclusion on its action to the chromosome of Cr^{6+} has to be reviewed further.

For the compounds like Cr^{6+}, Se, Mn, Hg, Co, and As, the frequency of SCEs increased ($p<0.05$), but only Cr^{6+} and Se showed distinct dose-responses (Fig. 4). The positive reaction of Cr^{6+} is lower to compare with that of strong SCE induced materials like alkali products as suggested by Ohno et al.[20] In this point, metal compounds are thought to belong to a group with the relatively lower inducibility of SCE. Majone and Levis[26] also compared and reviewed the inducibility of chromosome abnomality of Cr^{6+} compound and SCE and suggested that the Cr^{6+} compound would be a compound whose SCE inducibility was lower comparing to the inducibility of chromosome abnomality such as caffein and bleomycin.[27]

Table 1, however, was shown significant increased dose-response by resulting in much inducibility of SCE that was treated with 10^{-4} M K_2SeO_3. But the second metaphase showing toxicity in this concentration was very few that there was no more than 7 metaphase. Thus, it seems to be owing to the limiting concentration of SCE detection, and, if the time of culture had been extended, the second metaphase could have taken place more. It was not known if the significant dose-response by K_2SeO_3 might occur to other compounds or not until the measurement at the limiting concentration of SCE detection had been made (Fig. 4).

The metal compounds used for the study are chemicals soluble in a medium, but most of what was proved to be carcinogenic in experiments for animals are insoluble. Costa and Mollenahur[28,29] and Costa et al[30] reported the influence of insoluble Ni compounds using culture cells. So, a further study of the group screening of toxicity of insoluble compounds, for example, as the use of reproduction function of cells,[31] is required.

Summary

Among soluble chlorides frequently detected in working or living places 24 metal compounds of 10 elements were studied to evaluate their effect on the frequency of sister chromatid exchange (SCE) in Chinese Hamster Don cells.

The increases of the frequency of sister chromatid exchange in Chinese Hamster Don cells were found in 9 metal compounds such as CrO_3, K_2CrO_4, $K_2Cr_2O_7$, $MnCl_2$, K_2SeO_3, CH_3HgCl ($p<0.01$), $CoCl_2$, Na_2HAsO_4, and $HgCl_2$ ($p<0.05$). but Remarkable dose-response relationships were found in hexavalent chromium compound and K_2SeO_3.

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