Effect of 2, 3, 4, 7, 8-Pentachlorodibenzofuran and Its Analogues on Induction of Sister Chrornatid Exchanges in Cultured Human Lymphocytes

NAGAYAMA, Junya
Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University

NAGAYAMA, Mayumi
Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University

HARAGUCHI, Koichi
Daiichi College of Pharmaceutical Sciences

KUROKI, Hiroaki
Daiichi College of Pharmaceutical Sciences

他

https://doi.org/10.15017/7323437

出版情報：福岡醫學雜誌. 86 (5), pp.184-189, 1995-05-25. 福岡医学会
バージョン：
権利関係：

Fukuoka Acta Med. 86(5) : 184－189, 1995

Effect of 2, 3, 4, 7, 8-Pentachlorodibenzofuran and Its Analogues on Induction of Sister Chromatid Exchanges in Cultured Human Lymphocytes

Junya NAGAYAMA and Mayumi NAGAYAMA

Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University 90, Fukuoka 812-82

Koichi HARAGUCHI, Hiroaki KUROKI and Yoshito MASUDA

Daiichi College of Pharmaceutical Sciences, Fukuoka 815

Abstract We have been already contaminated with various chemicals including highly toxic organochlorine compounds such as 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2, 3, 4, 7, 8-pentachlorodibenzofuran (PenCDF) and 3, 4, 5, 3', 4'-pentachlorobiphenyl (Co-PenCB). In this study, in order to evaluate the genotoxicity of the three chemicals, we have examined their effects on the induction of sister chromatid exchanges (SCEs), which has been frequently utilized as an indicator of biological and genetic damage due to exposure to carcinogens or mutagens, in cultured human lymphocytes in the absence or presence of 7, 8-benzoflavone (ANF) and the following results were obtained. 1) TCDD, PenCDF and Co-PenCB significantly increased the frequency of SCEs with almost the same dose-dependent manner in terms of the concentration of TCDD toxic equivalent. 2) 8×10^{-5} MANF significantly enhanced the frequency of SCEs and the simultaneous treatment of ANF and either of TCDD, PenCDF or Co-PenCB seemed to exert an additive effect as SCEs inducer. 3) TCDD, PenCDF and Co-PenCB were considered to be very potent inducers of SCEs, because their 50% effective concentration in SCEs enhancement were only 5 to 10 times higher than the level of the adipose tissue in healthy Japanese, namely, 70ppt as TCDD.

Consequently, the respective TCDD toxic equivalency factors of 0.5 and 0.2 for PenCDF and Co-PenCB seemed to be reasonable so far as the induction of SCEs was employed as an indicator of the genotoxic potency. The three chemicals are regarded as highly genotoxic ones and one of the most important problems which should be solved is further comprehensive genotoxicity and health consequences of the mixed contamination of these chemicals to our descendants.

Introduction

Recently, cytogenetic changes such as the induction of micronucleated cells (MNs) and sister chromatid exchanges (SCEs) have frequently been utilized as indicators of biological damage due to exposure to different carcinogens or mutagens. These two cytogenetic changes are considered to occur as results of different mechanisms to DNA or chromosome damage. MNs have been considered to be the result of chromosome fragments or whole chromosomes lagging behind the genome at cell division. On the other hand, SCEs are formed during the S phase after an initial change in the form of DNA base damage[23], when quadriradials as mitotic chiasmas are a consequence of mitotic crossing over[22].

Many studies indicate that we have been already contaminated with numerous chemicals which have been intentionally or accidentally made by man and some of those are highly toxic organochlorine compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co - PCBs)

[5)11)]. In this study, we have investigated the induction of SCEs in mitogen-stimulated lymphocytes by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic congener among PCDDs, 2, 3, 4, 7, 8 - pentachlorodibenzofuran (PenCDF), the most toxic congener among PCDFs and 3, 4, 5, 3', 4'-pentachlorobiphenyl (Co-PenCB), the most toxic congener among Co-PCBs, in order to elucidate their genotoxicity.

Materials and Methods

1. Chemicals

TCDD and PenCDF were synthesized by Dr. Y. Masuda and his colleagues and Co-PenCB was purchased from Cambridge Isotope Laboratories, Woburn, USA. Their purities were over 99% by gas chromatography. The sources of chemicals used in this study were as follows: demecolcin, 7, 8-benzoflavone (ANF) and hoechst 33258 from Wako, Osaka, 5-bromo-2'-deoxyuridine (BUdR) from Sigma, St. Louis, phytohemagglutinin M (PHA) from Difco, Detroit and penicillin, streptomycin and RPMI 1640 from Gibco, New York. All other chemicals and reagents were of the highest quality commercially available.

2. Chemical treatment and lymphocyte culture

We assumed that the total concentration of PCDDs, PCDFs and Co-PCBs in the adipose tissue of Japanese people was 70ppt as TCDD toxic equivalent value on fat weight basis and that the toxic equivalency factors (TEFs, relative toxic potency to TCDD) were 0.5 for PenCDF and 0.2 for Co-PenCB. Their effects on the induction of SCEs in peripheral human lymphocytes were examined at doses of about 5, 25 and 50 times higher concentrations than 70ppt as TCDD. Therefore, human lymphocytes in whole-blood cultures were treated with TCDD at 364, 1,470 and 2,940ppt, with PenCDF at 784, 3,948 and 7,896ppt and with Co-PenCB at 1,750, 8,750 and 17,500ppt. These test chemicals were added at the beginning of the culture time and left until harvesting.

Heparinized peripheral blood samples were obtained by venipuncture from healthy females (mean age: 37 years old). Lymphocyte cultures (5.0ml) were initiated from whole blood (0.3ml) aliquoted into culture tubes containing RPMI 1640 medium (4.5ml) supplemented with 15% fetal calf serum, 100 units/ml penicillin and 100μg/ml streptomycin, in either the presence or absence of 8×10^{-5} MANF. Hundred μM BUdR and 0.15ml PHA (a final concentration; 3%) were added at culture initiation. The whole blood cultures were then incubated at 37°C in 5 % CO_2 and 100% humidity. Seventy hrs later and 3 hrs before fixation, 2×10^{-7} M demecolcin was added. The cells were then collected by centrifugation, exposed to 0.075MKCl hypotonic solution for 15 min at 37°C and fixed 3 times in methanol: acetic acid (3: 1 v/v) at room temperature.

3. Slide preparation and scoring

Samples for microscopic observation were obtained by carefully dropping the lymphocyte suspensions from Pasteur pipettes onto precleaned wet slides and dried in vapor of the water. The slides were stained for SCEs analyses using a little modification of the fluorescence (Hoechst 33258) plus Giemsa (FPG) technique[3)]. Slides were coded and scored blind under a magnification of 1,000 fold. SCEs were analyzed in 30 to 64 metaphases for each point and scored.

4. Statistical analysis

The results were expressed as the average number $\pm$S. E. of SCEs per metaphase. Data were statistically analyzed by Student's *t*-test.

Results

1. Frequency of SCEs induced by TCDD, PenCDF and Co-PenCB in the absence of ANF

The experimental results are shown in Fig. 1. The frequency of SCEs/cell in the control culture was 10.5 ± 0.4, and the three chemicals significantly enhanced the SCEs formation even at the lowest concentration, namely, about 5

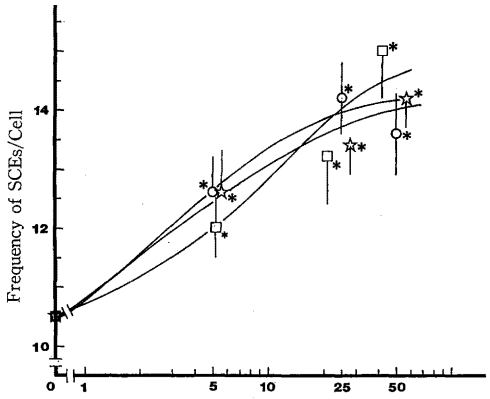

Fig. 1 Effect of TCDD (□), PenCDF (☆) or Co-PenCB (○) on the induction of SCEs in human lymphocytes of whole-blood cultures
＊: $P<0.05$, ＊: $P<0.01$

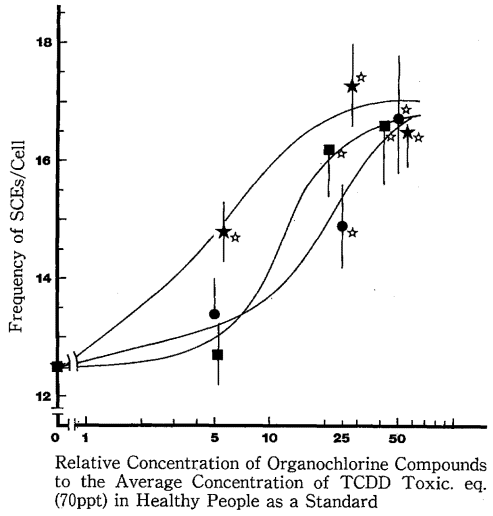

Fig. 2 Effect of simultaneous treatment of both TCDD (■), PenCDF (★) or Co-PenCB (●) and ANF (8×10^{-5}M) on the induction of SCEs in human lymphocytes of whole-blood cultures
☆: $P<0.01$

times more than the average Japanese level. The greatest SCEs induction was observed at about 25 or 50 times over the mean level in Japanese and those were 15.0±0.8SCEs/cell for TCDD, 14.2±0.5SCEs/cell for PenCDF and 14.2±0.6SCEs/cell for Co-PenCB. In the whole-blood culture without ANF, the freqency of SCEs in human lymphocytes seemed to increase in almost the same proportion to the concentration of TCDD, PenCDF and Co-PenCB in terms of TCDD toxic equivalent level. Therefore, our assumption that TEF values are 0.5 for PenCDF and 0.2 for Co-PenCB is considered to be acceptable.

Based on these findings, we recalculate the frequency of SCEs/cell values induced by the three chemicals at the same concentration in the toxic equivalent of TCDD and the result is shown in Fig. 3. The frequency of SCEs in the control culture was 10.5±0.4/cell, enhanced in proportion to their concentration and at the highest one it was 14.2±0.4/cell. Fifty % effective concentration (EC_{50}) for the induction of SCEs in the lymphocytes cultured without ANF appeared to be only 5.3 times higher concentration than the average one in Japanese, namely,

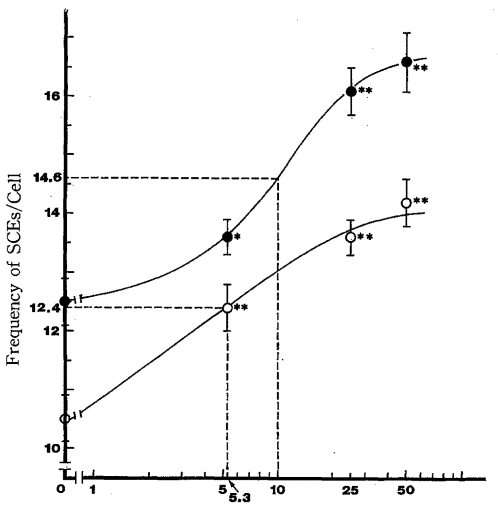

Fig. 3 Means in effects of the three organochlorine compounds (TCDD, PenCDF and Co-PenCB: ○) and these chemicals plus ANF (●) on the induction of SCEs in human lymphocytes of whole-blood cultures
＊: $P<0.05$, ＊＊: $P<0.01$

70ppt as TCDD.

2. Frequency of SCEs induced by TCDD, PenCDF and Co-PenCB in the presence of ANF

The results are indicated in Fig. 2. The frequency of SCEs in the control culture was 12.5±0.4/cell, PenCDF significantly induced the SCEs formation even at the lowest concentration and the other two chemicals from about 25 times over the mean Japanese level. The greatest SCEs formation was seen at about 25 or 50 times more than the mean level in Japanese and those were 16.6±1.0SCEs/cell for TCDD, 17.3±0.7SCEs/cell for PenCDF and 16.8±1.0SCEs/cell for Co-PenCB. In general, the three chemicals enhanced the frequency of SCEs in the lymphocytes of whole-blood cultures in the presence of ANF with almost the same dose-dependent manner in terms of TCDD toxic equivalent. Therfore, our assumption concerning TEF values for PenCDF and Co-PenCB seems to be reasonable not only in the absence of ANF but also in its presence.

Based on the findings mentioned above, we reevaluated the frequency of SCEs at each concentration, adding up the SCEs/cell of the three chemicals at the same concentration in TCDD toxic equivalent and the result is indicated in Fig. 3. The frequency of SCEs in the control culture was 12.5±0.4/cell, increased significantly in proportion to their concentration and at the greatest concentration it was 16.6±0.5/cell. Their EC_{50} for the induction of SCEs in the human lymphocytes cultured in the presence of ANF seemed to be 10 times higher concentration than the average one in Japanese people, namely, 70ppt as TCDD.

Discussion

The development of TEFs for PCDDs and related compounds has been necessitated by the identification of complex mixtures of these chemicals in almost every compartment of the global ecosystem. PenCDF is the second toxic congener among PCDDs, PCDFs and Co-PCBs and its proposed TEF value is 0.5[20]. Co-PenCB is the most toxic PCB congener and the range of its proposed TEF value is 0.1 to 0.4[2)4)20]. In this study, we assumed that TEF values for PenCDF and Co-PenCB were 0.5 and 0.2, respectively and this assumption seemed to be reasonable because TCDD, PenCDF and Co-PenCB enhanced the frequency of SCEs in almost the same dose-dependent manner in terms of the TCDD toxic equivalent concentration, as indicated in Figs 1 and 2.

PCDDs, PCDFs and PCBs did not bind covalently to DNA, did not affect the frequency of SCEs and were negative in other short-term genotoxicity tests[8)24]. Contrary to the findings mentioned above, the present study and other recent investigations indicated PCDDs, PCDFs and PCBs significantly increased the frequency of SCEs in cultured human lymphocytes[14)15)17)21] and that of micronucleated cells in vitro[15)16], and cytogenetic analysis of peripheral blood lymphocytes demonstrated that accidental or occupational exposure to PCBs and/or PCDFs resulted in an enhanced frequency of SCEs[6)9].

The present study also indicated that TCDD, PenCDF and Co-PenCB seemed very potent inducers of SCEs, because, as shown in Fig. 3, their EC_{50} values of SCEs induction were only 5.3 and 10 times higher concentration than that in healty Japanese, that is, 70ppt as TCDD in the absence and presence of ANF, respectively. ANF greatly enhanced the sensitivity of SCEs assay in detecting effects of cigarette smoking and accidental exposure to a mixture of PCBs and PCDFs in peripheral lymphocytes[9)10]. As indicated in Fig. 3, ANF significantly enhanced the frequency of SCEs in the control culture and the simultaneous treatment of ANF and either of the three chemicals was considered to be additive in SCEs induction. This kind of effect has been seen in our former study on the induction of micronucleated cells with the same chemicals[16].

Several, however, of these chemicals are carcinogenic in animal models, where they have

been classified as tumor promoters rather than initiators[1)7)18)19)]. Taking account of recent findings described above and the results of this study, we had better consider that these halogenated aromatics are carcinogenic partly as promoters and partly as initiators, because they are probably potent genotoxic compounds and sometimes seem to act through mechanisms involving direct genetic damage.

Human breast milk has been also contaminated with PCDDs, PCDFs and Co-PCBs[11)12)] and daily intakes of these chemicals in breast-feeding babies of healthy mothers have been estimated to be about 100 to 200 pg/kg/day as TCDD equivalent values (TEQs)[13)]. These TEQs values are about 100 to 200 times greater than the acceptable daily intake (ADI) value, namely, 1pg / kg / day. Therefore, we should give due attention to the comprehensive genotoxicity and possible health consequences due to PCDDs, PCDFs and related chemicals in the breast milk to breast-feeding babies.

References

1) Abernethy OJ, Greenlee WF, Hubrand JC and Boreiko CJ: 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) promotes the transformation of C3H/10T1/2 cells. Carcinogenesis 6: 651-653, 1985.

2) Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, Safe SH, Schlatter C, Wern F, Younes M and Yrjanheikki E: Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28: 1049-1067, 1994.

3) Goto K, Maeda, S, Kano Y and Sugiyama T: Factors involved in the differential Giemsa staining of sister chromatids. Chromosoma 66: 351-359, 1978.

4) Hanberg A, Wern F, Asplund L, Hanglund E and Safe S: Swedish dioxin survey: determination of 2, 3, 7, 8-TCDD toxic equivalent factors for some polychlorinated biphenyls and naphthalenes using biological tests. Chemosphere 20: 1161-1164, 1990.

5) Hirakawa H, Iida T, Matsueda T, Tokiwa H, Nagata T and Nagayama J: Concentrations and distribution of PCDDs, PCDFs and coplanar PCBs in various human tissues. Organohalo. Comp. 10: 93-96, 1992.

6) Kalina I, Sram RJ, Konecna H and Ondrussekova A: Cytogenetic analysis of peripheral blood lymphocytes in workers occupationally exposed to polychlorinated biphenyls. Teratogenesis Carcinog. Mutagen. 10: 21-29, 1990.

7) Kavanagh TJ, Rubinstein C, Liu PL, Chang C-C, Trosko JE and Sleight SD: Failure to induce mutations in Chinese hamster V79 cells and WB rats liver cells by polybrominated biphenyls, Firemaster BP-6, 2, 2', 4, 4', 5, 5'-hexabromobiphenyl, 3, 3', 4, 4', 5, 5'-hexabromobiphenyl and 3, 3', 4, 4'-tetrabromobiphenyl. Toxicol. Appl. Pharmacol. 79, 91-98, 1985.

8) Kociba R: Banbury Report 18, In Campbell HA and Poland A (eds): Evaluation of the carcinogenic and mutagenic potencial of 2, 3, 7, 8-TCDD and other chlorinated dioxins. pp. 73-84, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y., 1984.

9) Lundgren K, Collman GW, Wang-Wuu S, Tiernan T, Taylor M, Thompson CL and Lucier GW: Cytogenetic and chemical detection of human exposure to polyhalogenated aromatic hydrocarbons. Environ. Mol. Mutagen. 11: 1-11, 1988.

10) Lundgren K and Lucier GW: Differential enhancement of sister chromatid exchange frequencies by α-naphthoflavone in cultured lymphocytes from smokers and non-smokers. Mutation Res. 143: 131-135, 1985.

11) Masuda Y: Polychlorinated dibenzo-*p*-dioxins and related compound pollution in human tissues. Toxicol. Forum 10: 566-574, 1987.

12) Matsueda T, Iida T, Hirakawa H, Fukamachi K, Tokiwa H and Nagayama J: Comparison of concentrations of PCDDs, PCDFs and coplanar PCBs in breast milk of Yusho patients and normal controls. Organohalo. Comp. 9: 143-146, 1992.

13) Matsueda T, Iida T, Hirakawa H, Fukamachi K, Tokiwa H and Nagayama J: Toxic evaluation of PCDDs, PCDFs and coplanar PCBs in breast-fed babies of Yusho and healthy mothers. Chemosphere 27: 187-194, 1993.

14) Nagayama J, Nagayama M, Iida T, Hirakawa H, Matsueda T and Masuda Y: Effects of highly toxic organochlorine compounds retained in human body on induction of sister chromatid exchanges in cultured human lymphocytes. Chemosphere 29, 2349-2354, 1994.

15) Nagayama J, Nagayama M and Masuda Y: Genotoxicity of highly toxic organochlorine congeners in cultured human lymphocytes. Organohalo. Comp. 10: 165-168, 1992.

16) Nagayama J, Nagayama M and Masuda Y: Frequency of micronuclei induced in cultured lymphocytes by highly toxic orgnochlorine congeners.

Fukuoka Acta Med. 84: 189-194, 1993.

17) Nagayama J, Nagayama M, Wada K, Iida T, Hirakawa H, Matsueda T and Masuda Y: The effect of organochlorine compounds on the induction of sister chromatid exchanges in cultured human lymphocytes. Fukuoka Acta Med. 82: 221-227, 1991.

18) Pitot HC, Goldsworthy T, Campbell HA and Poland A: Quantitative evaluation of the promotion by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res. 40: 3616-3620, 1980.

19) Poland A, Palen D and Glover E: Tumour promotion by TCDD in skin of HRS/J hairless mice. Nature (London) 300: 271-273, 1982.

20) Safe S: Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds : environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Toxicology 21: 51-88, 1990.

21) Sargent L, Roloff B and Meisner L: In vitro chromosome damage due to PCB interactions. Mutation Res. 224: 79-88, 1989.

22) Therman E and Kuhn EM: Mitotic crossing over and segregation in man. Hum. Genet. 59: 93-100, 1981.

23) Uggla AH and Natarajan AT: X-ray-induced SCEs and chromosomal aberrations in CHO cells. Influence of nitrogen and air during irradiation in different stages of the cell cycle. Mutation Res. 122: 193-200, 1983.

24) Wasson JD, Huff JE and Lopriano NA: A review on the genetic toxicology of chlorinated dibenzo-*p*-dioxin. Mutation Res. 47: 141-160, 1977.

(Received for publication March 27, 1995)

(和文抄録)

2, 3, 4, 7, 8-五塩化ダイベンゾフランとその同族体による ヒトリンパ球培養細胞の姉妹染色分体交換誘発性

九州大学医療技術短期大学部環境科学研究室
長山淳哉・長山真弓
第一薬科大学物理分析学・環境化学研究室
原口浩一・黒木広明・増田義人

カネミ油症患者は言うに及ばず，健常者もきわめて毒性の高い2, 3, 7, 8-四塩化ダイオキシン(TCDD)，2, 3, 4, 7, 8-五塩化ダイベンゾフラン(PenCDF)や3, 4, 5, 3', 4'-五塩化ビフェニール (Co-PenCB) により汚染されている．この研究では，これら3種類の化学物質のS依存型遺伝毒性を評価するために，ヒトリンパ球培養細胞の姉妹染色分体交換 (SCEs) 誘発性について研究した．この際，SCEs誘発実験系の感度を高めることが示唆されている7, 8-ベンゾフラボン (ANF) が共存する場合のSCEs誘発性についても検討した．そして，次のような結果が得られた．1) TCDD毒性当量による濃度において，TCDD, PenCDFおよびCo-PenCBはほぼ同様の量・反応関係により有意なSCEs誘発性を示した．2) ANF(8×10^{-5} M)は有意にSCEs頻度を高め，ANFとTCDD, PenCDFあるいはCo-PenCBが共存する場合のSCEs誘発性は相加的であった．3) これら3種類の有機塩素化学物質のSCEs誘発の50%有効濃度が健常者の脂肪組織中濃度(TCDDとして約70 ppt)の5〜10倍というきわめて低いレベルにあった．

以上のような研究結果により，これらの有機塩素化学物質はいずれも強力なSCEs誘発性があり，また，S依存型遺伝毒性物質であると考えられる．従って，遺伝子や染色体の傷害に基づく疾病や子孫への影響などについて，一層の研究が望まれると同時に，そのような悪影響の予防法に関する研究も重要と考えられる．