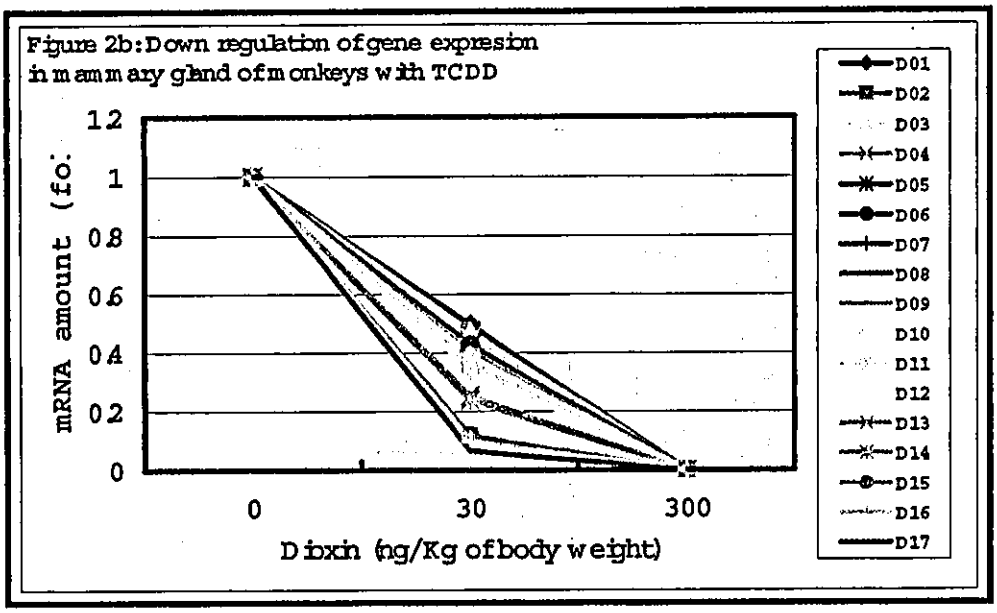

Acknowledgment

This study was supported by Health Labour Sciences Research Grants for Research on Food and Chemical Safety from the Ministry of Health Labour and Welfare of Japan, and MEXT Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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EFFECTS OF PARA-NONYLPHENOL ON MATRIX METALLOPROTEINASE SECRETION BY HUMAN LEUKEMIA CELLS

Shunichiro Kubota

Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Tokyo 153-8902, Japan

Introduction

Environmental chemicals disturb endocrine functions of various organs in humans[1-3]. Chemicals mimic steroid hormones through interactions with the estrogen receptor. A number of environmental chemicals which can bind to the estrogen receptor induce expression of various genes[4,5]. Alkylphenols which are widely used as surfactants in plastics are degraded to para-nonylphenol. Para-nonylphenol binds to the estrogen receptor and induces estrogen-dependent gene expression[5]. Human peripheral mononuclear cells and leukemia cells express the estrogen receptor[6,7].

The turnover of the extracellular matrix is critical for normal development, wound healing, inflammation, and cancer metastasis. The extracellular matrix is hydrolyzed by matrix metalloproteinases (MMP)[8,9]. MMPs play pivotal roles in regulation of angiogenesis, cell proliferation, differentiation, and cell death. Abnormal proteolysis due to up-regulation or down-regulation of MMPs may lead to abnormal angiogenesis, cell proliferation, differentiation, and cell death. MMPs are classified into four types according to their substrate specificity, collagenases, stromelysins, elastases, and gelatinases[9]. Collagenases exist as three distinct molecules, the fibroblast type (MMP-1, collagenase-1)[10], the neutrophil type (MMP-8)[11], and collagenase-3 (MMP-13)[12]. They all degrade type I, II, and III collagens as well as type VII and X collagens, and alpha-casein. MMP-8 effectively degrade type I and II collagens, and are stored in granules of human peripheral blood PMN[13]. In the present study the author studied the effect of para-nonylphenol on secretion of alpha-casein-degrading MMPs by human leukemia Jurkat cells in vitro.

Materials and Methods

Cell culture media (RPMI 1640), alpha-casein, anti-MMP-8 antibody and doxycycline were obtained from Sigma (Tokyo, Japan). Para-nonylphenol, obtained from Kanto Chemicals Co., Ltd. (Tokyo, Japan) was diluted with dimethyl sulfoxide or RPMI 1640 media to a final concentration.

Jurkat cells were grown in RPMI 1640 media containing 10% fetal bovine serum. 10^6 cells in 1ml RPMI 1640 media in 24 well plates were incubated for 24 h. Dimethyl sulfoxide (0.2%) was added to culture media, as a control. The serum free conditioned medium (1ml) was harvested for zymography.

Forty-five µl out of 1 ml serum free conditioned medium was used for alpha-casein substrate zymography as described[15].

At 24 h incubation after adding para-nonylphenol, or para-nonylphenol with doxycycline (50 µM) in serum free RPMI 1640 media, cells were harvested, and centrifuged at 1,500 rpm for 10 min. Cell pellets and the supernatant were used for western blotting, and zymography, respectively.

After SDS gel electrophoresis, western blotting was performed using the specific anti-MMP-8 antibody and ECL chemiluminescence system (Amersham-Pharmacia, Tokyo, Japan).

Results and Discussion

The author investigated effects of para-nonylphenol on alpha-casein degrading MMP secretion by human leukemia Jurkat cells using casein-substrated zymography. Jurkat cells secrete small amount of alpha-casein degrading MMPs. As shown in Figure 1 (left panel), at 24 h incubation time para-nonylphenol (0, 0.1, 1, 5, 10, and 50 μM) dose-dependently induced the secretion of alpha-casein degrading MMPs (MW; between 40-80 kDa). As doxycycline is considered to inhibit MMP-8 activities specifically, we next studied whether the activities were inhibited by doxycycline. Jurkat cells were pretreated with 50 μM doxycycline. The casein zymography was performed. As shown in Figure 1 (right panel), the activities of casein-degrading MMPs were completely inhibited, indicating that the casein-degrading MMPs may be MMP-8.

To confirm that the casein-degrading MMP was MMP-8 we performed western blotting using the specific anti-MMP-8 antibody. As shown in Figure 2, the signals of the bands (MW; between 40-80 kDa) were detected using the ECL chemiluminescence system. The results suggest that MMP induced by para-nonylphenol was MMP-8.

In the present study the author has shown for the first time that para-nonylphenol induced the secretion of MMP-8 by human leukemia Jurkat cells. MMP-8, human neutrophil collagenase has been considered as a polymorphonuclear neutrophil-specific MMP. MMP-8 is stored in granules in cells and released from cells upon activation[15]. The role of MMP-8 was shown in chronic bronchiectasis[16], cystic fibrosis[17], and rheumatoid arthritis[18]. The expression of MMP-8 was reported to be regulated by TNF-alpha and interleukin-1[19,20]. MMP-8 is also expressed in various cell types such as fibroblasts and endothelial cells other than polymorphonuclear neutrophil.

The molecular size of MMP-8 varies between 40 kDa and 80 kDa. The variance may reflect a different degree of glycosylation. In the present study we also observed several bands between 40-80 kDa molecular size. Although we have not confirmed whether these several bands may also reflect a different degree of glycosylation of MMP-8, it is considered that several bands reflected a different degree of glycosylation of MMP-8.

Doxycycline is an antibiotics and inhibits MMP-8 activity, but not MMP-1 activity[21,22]. The mechanism of inhibition is not well understood. It was shown that long term doxycycline treatment reduced MMP-8 serum levels in reactive arthritis[23]. In the present study the casein-degrading activities were completely inhibited with doxycycline treatment, indicating that the casein-degrading MMP induced by para-nonylphenol may be MMP-8.

As MMP-8 is involved in turnover of extracellular matrix proteins, especially collagens, induction of MMP-8 secretion induced by para-nonylphenol may affect normal turnover of collagnes. Further, as MMP-8 is also involved in pathological conditions such as rheumatoid arthritis and cystic fibrosis, induction of MMP-8 secretion induced by para-nonylphenol may affect pathological conditions.

Conclusions

In conclusion, the author has shown for the first time that para-nonylphenol induced MMP-8 activity.

Acknowledgment

This study was supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health and Welfare of Japan.

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Figure 1 Effects of para-nonylphenol on casein-degrading proteinase secretion by Jurkat cells

Effects of para-nonylphenol (0, 0.1, 1, 5, 10, and 50 μM) on casein-degrading proteinase secretion by Jurkat cells were analyzed using casein zymography. Serum free conditioned media were collected 24 h after the addition of para-nonylphenol. A typical zymography is shown in the left panel. The effect of doxycycline was studied. Doxycycline (50 μM) was added before the addition of para-nonylphenol (50 μM), and serum free conditioned media were collected 24 h after the addition of para-nonylphenol. The result is shown in the right panel.

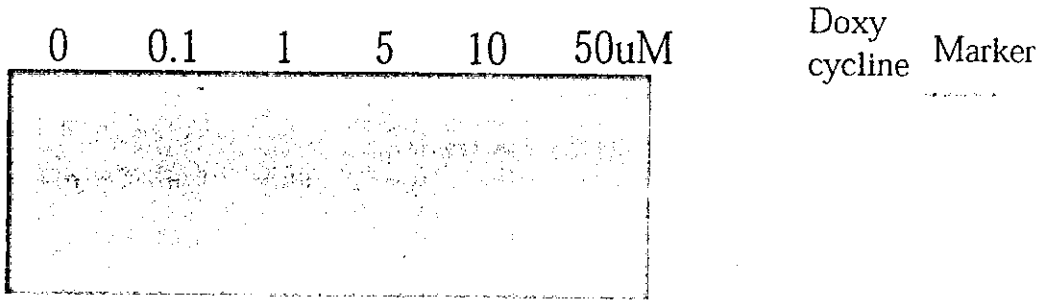
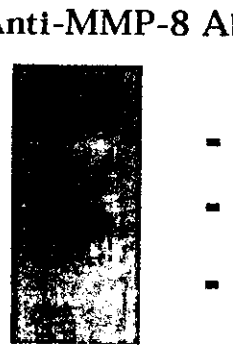


Figure 2 Western blot analysis using the specific anti-MMP-8 antibody

Western blotting using the specific anti-MMP-8 antibody was performed. The signals of the bands (MW; between 40-80 kDa) were detected using the ECL chemiluminescence system.



Endocrine Disrupters

環境ホルモン学会

Research

正式名　日本内分泌撹乱化学物質学会

第 6 回研究発表会
要旨集

The 6th Annual Meeting of Japan Society of Endocrine Disrupters Research

PROGRAM and ABSTRACTS

2-3, December, 2003
SENDAI INTERNATIONAL CENTER
Organized by Japan Society of Endocrine Disrupters Research

日時／2003年12月 2 日(火), 3 日(水)
会場／仙台国際センター
主催／環境ホルモン学会（正式名 日本内分泌撹乱化学物質学会）

PE-23

焼却場などの周辺大気環境とサルモデルによるモニタリング方法の構築

Establishment of a monitoring method of atmospheric environment around a public incinerator etc. using monkey model

浅岡一雄[1]、飯田景子[1]、渡辺邦夫[1]、鈴木樹理[1]、脇田真清[1]、西村剛[1]、
郷田浩志[2]、安田峯生[3]、久保田俊一郎[4]
Kazuo Asaoka[1], Hiroko Iida[1], Kunio Watanabe[1], Juri Suzuki[1], Masumi Wakita[1], Takeshi Nishimura[1], Hiroshi Goda[2], Mineo Yasuda[3], Shunichiro Kubota[4]

[1] Primate Research Institute, Kyoto University, Kanrin 41-2, Inuyma, Aichi 484-8506, Japan, (asaoka@pri.kyoto-u.ac.jp)
[2] Towa Kagaku Co., Ltd., 6-5 Funairi-machi, Naka-ku, Hiroshima 730-0841, Japan
[3] Faculty of Health Sciences, Hiroshima International University, Hiroshima 724-0695, Japan
[4] Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan

For the monitoring of air pollution in environment around a public incinerator etc., the estimation model of human affection must be included. Remarkable pollutant from the public incinerator is controlled legally for especially 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) because of variety of effects, including immunological dysfunctions, chloracne, teratogenecity, and carcinogenesis, as well as developmental and reproductive dysfunctions. We studied the model using macaque monkeys for the estimation of human affection. **Model evaluation:** the properties of the genes expressed in monkey were analyzed in comparison with that of human. From the mRNA of monkey embryo, the about 3,000 clones of their cDNA were collected and the nucleotide sequences were determined. There were 94% and 96% resemblance of the nucleotide sequence in a gene structure and in a gene frequency between the gene expressed in monkey and that in human, respectively. **Control levels:** the natural content of TCDD in the monkeys was measured for the control level of the model because of the stability of TCDD environmentally and biologically. Every 10 ml of individual blood was enough for the measurement of TCDD using Mass-spectrometer. **Monitoring:** the effect of TCDD on gene expression in monkeys was studied. TCDD (0, 30ng/kg, and 300ng/kg) was subcutaneously administered to female rhesus monkeys. At 49 days after administration, the gene expression in various organs was analyzed with a microarray and RT-PCR. 30/kg and 300ng/kg TCDD significantly increased the gene expression in mammary gland, skin, kidney, pancreas, liver and brain against that in non-treated control. **Teratogenecity:** some monkeys with 300ng/kg TCDD bore babys with abnormal tooth development. **Summary:** these results show the establishment of the monitoring method of atmospheric environment using monkey model.

PD-38

Effect of 2,3,7,8-TCDD on gene expression in tissues in Rhesus monkeys

Shunichiro Kubota[1], Toshio Ihara[2], Yoshiro Nishida[2], Toshio Fukusato[3], Nobuo Murata[4], Motoyoshi Nomizu[5], Hiroko Iida[6] and Kazuo Asaoka[6]

[1]Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan, [2]Shin Nippon Biomedical Laboratories, Ltd., 2438 Miyanoura, Yoshida-cho, Kagoshima 891-1394, Japan, [3]Department of Pathology, Teikyo University School of Medicine, Tokyo 192-0395, Japan, [4]Department of Surgery, Mizonokuchi Hospital, Teikyo University School of Medicine, Kanagawa Prefecture 213-8567, Japan, [5]Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan, [6]Primate Research Institute, Kyoto University, Kanrin 41-2, Inuyma, Aichi 484-8506, Japan

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is one of the most toxic dioxins which are environmentally and biologically stable. Exposure to dioxins results in a wide variety of effects, including immunological dysfunctions, chloracne, teratogenicity, and carcinogenesis, as well as developmental and reproductive dysfunctions. Assessment of TCDD exposure on human health requires more information concerning gene expression induced by TCDD in non-human primates. Although considerable amount of information is available from studies in rodents, not much is known of the gene expression induced by TCDD in non-human primates. In this study TCDD (0, 30ng/kg, and 300ng /kg) was subcutaneously administrated to female rhesus monkeys. At 49 days after administaration, gene expression in various organs was analyzed using a microarray. In general, increased gene expression was observed in the mammary gland, skin, kidney, pancreas, liver and brain. Both 30 and 300ng/kg TCDD significantly induced CYP1A1 gene expression. Because CYP1A1 gene induction was most prominent in mammary gland, expression of 100 genes was analyzed in mammary gland. Various induction patterns were observed depending on the dose of TCDD, and the 100 genes were classified into eight groups. One of the genes induced by TCDD was apoptosis-related gene. We are currently identifying other genes affected by TCDD. In summary, both 30 and 300ng/kg TCDD induced various gene expression in mammary gland.

Fifth AFMC International Medicinal Chemistry Symposium

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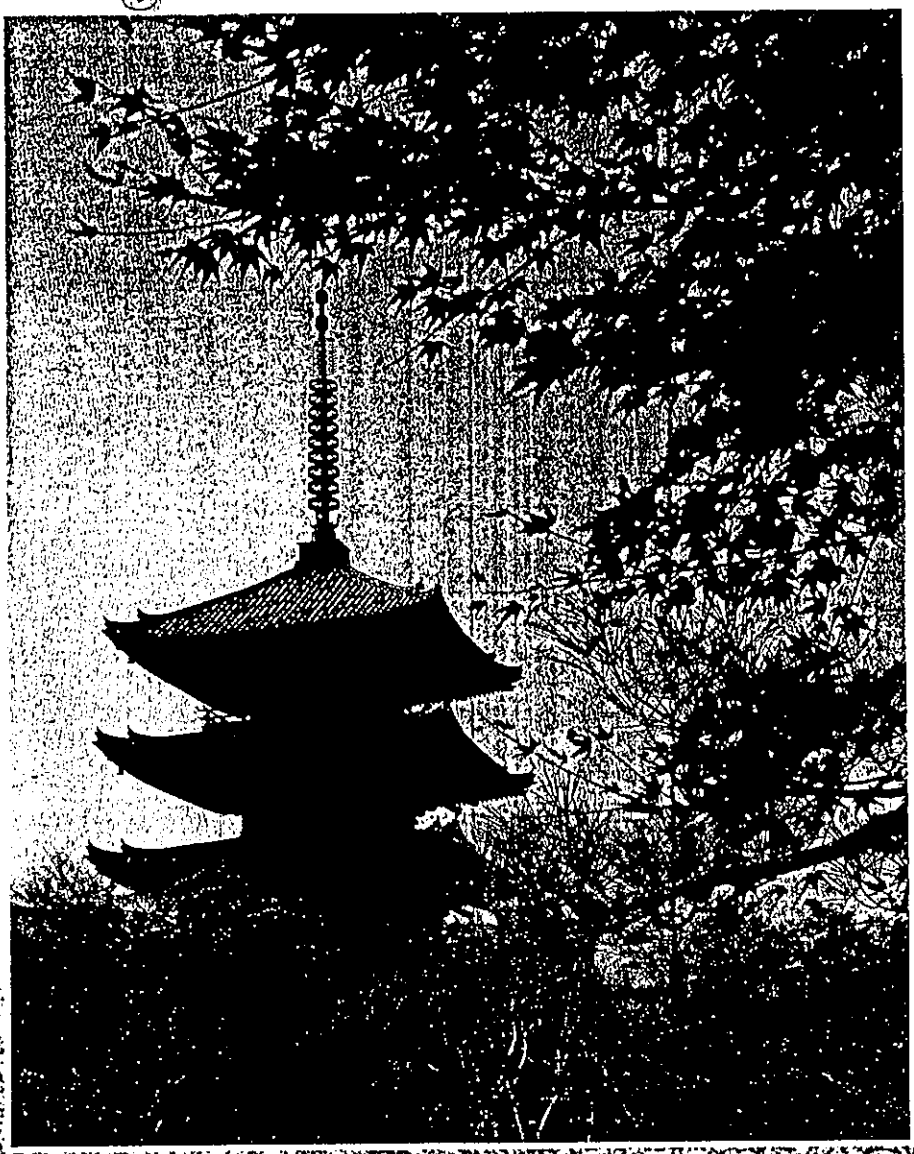
Program & Abstracts



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Medicinal Chemistry Symposium (AIMECS 03)

B-62

Quantitative prediction of transporter-mediated drug-drug interaction

Shitara, Y[1], Hirano, M[2,3], Sato, H[1] and Sugiyama, Y[2]

[1] Showa University, Tokyo, Japan, [2] The University of Tokyo, Tokyo, Japan, [3] Kowa Co. Ltd., Tokyo, Japan

Recent studies revealed the importance of transporters in the drug elimination in the liver. As these transporters exhibit a broad substrate specificity with a degree of overlap, the membrane permeability of drugs via transporters can be affected by concomitantly administered drugs which are also substrates or inhibitors of the same transporters, leading to a transporter-mediated drug-drug interaction (DDI). We have already clarified that a clinically relevant DDI between cerivastatin (CER), an HMG-CoA reductase inhibitor, and cyclosporin A (CsA), an immunosuppresant, was, at least partly, caused by transporter-mediated hepatic uptake. In this study, we have estimated the inhibitory effect of CsA on the uptake of CER into isolated rat hepatocytes and correlated with the effect of CsA on the *in vivo* disposition of CER in rats. *In vitro* studies using isolated rat hepatocytes revealed CsA inhibited the uptake of CER with a K_i value of 0.2 μM. This result quantitatively explained the decrease of *in vivo* total body clearance (CL_{tot}) of CER when coadministered with CsA by a physiologically-based pharmacokinetic (PB/PK) analysis. The present study suggests the risk of transporter-mediated DDI and the importance of *in vitro* uptake study using isolated hepatocytes and/or transporter-expression system for the development of new drugs with a low risk of such DDI.

Ref) Shitara Y et al. J. Pharmacol. Exp. Ther. 304: 610-6, 2003

B-63

Gene expression resemblance between *Macaca* monkey and human, and its application for environmental toxicogenomics

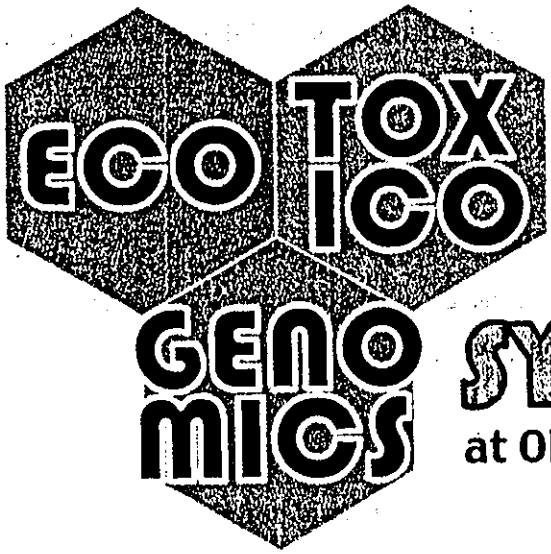
Kazuo Asaoka[1], Hiroko Iida[1], Yoshiro Kamanaka[1], Takeshi Nishimura[1], Masumi Wakita[1], Juri Suzuki[1], Kunio Watanabe[1], Yu-ichiro Ishikawa[2], Seiichi Kawashima[2], Chisato Mori[3], Mineo Yasuda[4], Shunichiro Kubota[5]

[1] Primate Research Institute, Kyoto University, Aichi 484-8506, Japan (Corresponding to K. Asaoka, asaoka@pri.kyoto-u.ac.jp), [2] Tokyo Metropolitan Institute of Medical Science, Tokyo 113-8613, Japan, [3] Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan, [4] Faculty of Health Sciences, Hiroshima International University, Hiroshima 724-0695, Japan, [5] Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan

Among the experimental animals, *Macaca* monkey has advantages for human models because of the most resemblance in physiological properties. But the limited information on gene sequences of *Macaca* monkeys especially in embryo, the analyses based on mRNA have to be started. The full-length cDNA were converted from mRNA expressed in the monkey embryo and transfected with vector to *E. coli*. The clones were selected randomly from about 3,000 plaques and their nucleotide sequences were determined. The resemblance of the genes expressed in between monkeys and humans were estimated about 94 % and 96 % based on the structure and the gene frequencies, respectively.

Because of their close resemblance in expressed genes, monkey model as toxicogenomics could expect to clear the events with environmental chemicals on humans. In this study, monkeys were administered with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or methylcholanthrene as Ah ligands. The chemicals were detected to be transported from the mother placenta to many tissues of the embryos, and to be changed the gene expression in the tissues both embryo and mother, for example in the mammary gland, uterus, liver, brain, and etc. It was analyzed using RT-PCR and DNA microarray. Some gene levels were changed depending on an amount exposed with Ah ligand to monkeys. Those genes may be candidate genes to detect the TCDD affection in primates. They were set on own DNA microarray to develop a detecting tool for affection with environmental chemicals. (This study was supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health and Welfare of Japan, and MEXT Grant-in-Aid for Scientific Research and 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan)

PROGRAM



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SYMPOSIUM
at Okazaki Conference Center
October 6 and 7, 2003

Phylogenic Properties and Dioxin Related Disruption of Genes Expressed in Macaque Monkeys

Kazuo Asaoka, Kunio Watanabe
asaoka@pri.kyoto-u.ac.jp
Primate Research Institute, Kyoto University

The National Bioresource Project by the Ministry of Education, Culture, Sports, Science and Technology of Japan has been promoted from 2002 including macaque monkeys. For experimental animals, macaque monkey has advantages for human models because of the most resemblance in physiological properties. But information on gene sequences is limited in the monkeys especially in embryo which is important in ecotoxicogenomics. Their analyses based on mRNA have to be started.

Full-length cDNA was converted from mRNA expressed in the monkey embryo and transfected with vector to *E. coli*. Their clones were selected randomly from about 3,000 plaques and their nucleotide sequences were determined. A resemblance of the genes expressed in between monkeys and humans was estimated about 94 % and 96 % based on the structure and the gene frequencies, respectively. Both values of the resemblance in rats and mice versus humans were reported about 80%. The monkey genes are 4 times closer to human genes than rat and mouse genes in phylogenic resemblance of the genes expressed.

Monkeys were administered with TCDD (2,3,7,8-tetrachlorodibenzo *p*-dioxin) or MC (Methylcholanthrene). The TCDD was detected to be transported from the mother placenta to many tissues of the embryos, and to be changed the gene expression in the tissues of embryo and mother, for example in mammary gland, fat, liver, uterus, brain, and etc. It was analyzed using RT-PCR and DNA microarray. Some gene levels were changed up or down depending on an amount exposed with the chemicals to monkeys. Those genes may be candidate genes to detect the ecotoxitic affection in primates including humans. They were set on own DNA microarray to develop a detecting tool for the gene affection with ecotoxitic chemicals.

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The 5th International Workshop on Advanced Genomics (June 26-27, 2003) Yokohama Japan
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Abstracts

P-53

Comprehensive analysis of gene expression in monkey tissues and development of DNA microarray for the detection of environmental chemical affection

ASAOKA, Kazuo

Kazuo Asaoka#1, Hiroko Iida#1, Juri Suzuki#1, Takeshi Nishimura#1, Masumi Wakita#1, Mitsuyoshi Ohba#2, Hiroshi Okamura#2, Shunichiro Kubota#3

#1 Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan (Corresponding to K. Asaoka, asaoka@pri.kyoto-u.ac.jp)

#2 Technical Research Laboratory, Toyo Kohan Co., LTD, Kudamatsu, Yamaguchi 744-8611, Japan

#3 Graduate School of Arts and Sciences, The University of Tokyo, Meguro, Tokyo 153-8902, Japan

Because of species specificity, monkey model could expect to clear the events with environmental chemicals on humans, especially with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) which is the most toxic environmental chemical. Among the tissues of monkeys administered with TCDD, the gene expression disorder was detected in the highest in the mammary gland. One hundred genes in the monkey tissues were analyzed with the microarray using the RNA from the tissues. In the mammary gland, the expression of 16 genes were increased and of 17 genes were decreased depending on an amount exposed with TCDD to monkey. Those 33 genes may be candidate genes to detect the TCDD affection of mammary gland in primates. Their oligo DNA was designed and set on a microarray based on GENE SILICON (trademark application) to develop a detecting tool for an affection with environmental chemicals.

The Pharmaceutical Society of Japan

第124年会
日本薬学会

2004 Osaka

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29【P2】II-376

環境化学物質の組織に与える影響と癌疾患の検査用マイクロアレイの開発

○浅岡 一雄[1], 飯田 景子[1], 釜中 慶朗[1], 渡辺 邦夫[1], 鈴木 樹理[1], 西村 剛[1], 脇田 真清[1], 大場 光芳[2], 岡村 浩[2], 久保田 俊一郎[3]([1]京都大学霊長類研究所,[2]東洋鋼鈑株式会社技術研究所,[3]東京大学大学院総合文化研究科)

【目的】近年に増加している乳癌などの疾患は環境化学物質による影響が懸念されている。そこで我々はヒトに最も近い遺伝子をもつサルを用いて、組織に発現する遺伝子へのダイオキシンの影響を検討し、検出用マイクロアレイの開発を行った。ヒト乳部の正常および乳癌に発現する遺伝子の相違を検討した。

【方法】マカクサルに0, 30, 300ng/kgのダイオキシンを投与し組織への移行を定量した。各種組織からmRNAを抽出してRT-PCRおよびマイクロアレイにより発現遺伝子を定量した。検出用マイクロアレイは設計したオリゴDNAをジーンスライド?(東洋鋼鈑製)に共有結合して開発した。ヒトの正常および乳癌mRNAを得て、開発したマイクロアレイを用いて遺伝子を検出した。

【結果および考察】投与されたダイオキシンは多くの組織へ移行が認められた。なかでも乳部への移行は最も大きかった。組織に発現している遺伝子においても乳部の変動が最も大きかった。マイクロアレイを用いて網羅的に遺伝子の変動を調べたところ約100個に変動が検出された。このうちダイオキシンの投与量に依存して変動が増加する遺伝子を16個と変動が減少する遺伝子を17個を認めた。

ダイオキシンの影響によって乳部に変動する遺伝子からオリゴプローブを設計した。ジーンスライドに共有結合させて開発したマイクロアレイはヒトの正常および乳癌mRNAに発現する遺伝子の相違を検出できた。

このことは、ダイオキシンなどの環境化学物質が多く取り込まれた乳部では発現遺伝子に大きな撹乱が生じており、ヒトの乳癌においても同様の遺伝子に変動することを示している。開発したマイクロアレイはサルおよびヒトの環境疾患および乳部検査において有用な方法を提供する。

第26回 日本分子生物学会年会

プログラム・講演要旨集

会　期　2003年12月10日（水）～13日（土）

会　場　神戸ポートアイランド

The 26th Annual Meeting of the Molecular Biology Society of Japan

Program and Abstracts

Kobe Port Island

December 10-13, 2003 Kobe, Japan

第26回 日本分子生物学会年会 組織委員会

（連絡先）（財）日本学会事務センター大阪事務所内

〒560-0082 豊中市新千里東町1丁目4－2

千里ライフサイエンスセンタービル14階

TEL (06) 6873-2301 FAX (06) 6873-2300

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2PA-079～084

2PA-079

転写因子結合部位のクラスタとヒトプロモータ配列の相関
○村上 勝彦, 小島 俊夫, 榊 佳之
(理化学研究所横浜研究所ゲノム科学総合研究センター)
Correlation between clusters of transcription factor binding sites and human promoter sequences
○Katsuhiko Murakami, Toshio Kojima, Yoshiyuki Sakaki
(RIKEN GSC)

遺伝子の発現制御にはDNA上の転写因子結合部位（シス配列）が重要な役割を果たしている。DNA上での転写制御部位を見つけるためには，既知のシス配列の集合で作られたPWM（position weight matrix）を使ってサーチすることで候補を予測できる。しかし，擬陽性が多いのでこれらを除くため，同じ発現制御を受けていると思われる複数の遺伝子のプロモータ配列間で保存度を調べたり，シス配列がクラスタになっているかを調べたりすることが行われる。本研究では，シス配列のクラスタに着目し，どのような条件で取り出されたクラスタならば特徴的といえるかを調べた。PWMを用いて予測された転写因子結合部位（PTFBS）を通してプロモータ配列の特徴を取り出すために，PTFBSのクラスタとプロモータの相関を各PWMで調べた。ヒト20番，21番，22番染色体のRefSeqおよび転写開始点データベースDBTSSの情報からプロモータ配列と非プロモータ配列を集め，非プロモータ配列よりプロモータ配列に有意にクラスタが見られるPWMを同定した。その結果，転写因子のデータベースTRANSFACにあるPWMのうち，クラスタとプロモータの相関がみられたのは半分以下であった。さらにCpGアイランドなどの組成バイアスとの関連をみるため，CpGアイランドの情報を除いたPTFBSクラスタとプロモータの偏相関係数を計算し，バイアスを除いても有意にプロモータにクラスタのみられるPWMを同定した。これらのPWMに対応する転写因子が，多くの遺伝子の制御に使われていることが示唆される。

2PA-080

受容体とリガンド遺伝子のプロモーター配列解析
渡辺 和彦, 名取 勇人, ○杉山 友康
(東京工科大・バイオニクス)
Analysis of the promoter sequence of receptors and ligands
KAZUHIKO WATANABE, HAYATO NATORI, ○TOMOYASU SUGIYAMA
(Tokyo Univ. Tech., Sch. Bionics)

細胞情報伝達において細胞膜の受容体とそのリガンドは重要である。近年のゲノムやcDNA配列解析によって，これら遺伝子を含む，ほぼ全てのヒト遺伝子配列が明らかにされた。しかし細胞膜の受容体がどれで，どの遺伝子がそのリガンドをコードするかについては十分に解明されていない。結合実験やタンパク質構造析等からリガンドを同定することが試みられているが，容易ではない。一方*in silico*的手法でペアを予測することができれば有用である。しかしそのような手法は報告されていない。そこでわれわれは次のような仮説をたてて，*in silico*解析による受容体とそのリガンドのペアの推定の可能性を探ることにした。時間的・空間的に受容体とリガンドが発現してペアリングするためには，何らかの規則があってもよい。ゲノム配列には受容体とそのリガンドのペアに関する情報が記載されているかもしれない。この仮説検証のために，受容体とリガンドのプロモーター配列解析を行うことにした。

ヒト配列データは公共データベースから入手した。配列解析にはDual-CPU構成のPCを使用した。既知受容体遺伝子として155種類を選択し，そのリガンド遺伝子をNCBIのRefSeq記載情報や文献情報を検索してペアとした。受容体によっては複数個のリガンドも許容して，プロモーターの配列解析を試みた。本大会では解析結果を示し，本手法の応用の可能性を報告する。

2PA-081

睡眠期マウス大脳皮質における網羅的遺伝子発現解析
○高山 貴至[1], 田仲 裕介[1], 八木 健[1,2]
([1]阪大・生命機能・時空生物・心生物, [2]CREST)
The exhaustive gene expression analysis in the mouse cerebral cortex at sleep phase.
○Takashi Takayama[1], Yusuke Tanaka[1], Takeshi Yagi[1,2]
([1]Osaka Univ., Graduate school of Frontier Biosci., Integrated Biol., KOKORO biol., [2]CREST)

睡眠は高等動物へと進化するに伴って確立されてきた活動であり，この時期に脳の再生や覚醒期で経験した記憶の固着が行われることが示唆されている。これらのことは，睡眠期での神経回路網再編成を示唆するものの，解析についてはほとんど行われていない。そこで本研究では，睡眠期特異的に発現，もしくは発現が増加する遺伝子を同定することにより，睡眠期での神経回路網再編成の分子メカニズム解明へのアプローチを行った。

睡眠期と覚醒期のマウスを判別する方法は先行論文に従い，睡眠期及び覚醒期からそれぞれ4個体の大脳皮質をサンプリングした。この大脳皮質よりmRNAを抽出し，プロトコールに従ってDNAチップ（affymetrix社製）を用いて発現解析を行った。DNAチップ上には12488個のプローブが搭載されており，うち遺伝子名が明らかなものは6042個（48.4%）であり，ESTは6446個（51.6%）である。DNAチップの解析ソフトmicro suiteによる解析の結果，睡眠期，覚醒期を問わず発現していると判定されたものは12488個のうち4491個（36%）であり，反対に発現していないと判定されたものは5011個（40.1%）であった。覚醒期4サンプルにおいて全く発現が検出されなかったプローブ中で，睡眠期において発現が複数サンプルで認められたものは67個（0.5%）であった。現在，DNAチップのアノテーションデータをもとに，これら67個のプローブに対応する遺伝子に対してmRNAの定量PCRを行い，睡眠期で発現が増加しているものの特定を行っている。また，増加が確認された遺伝子については，遺伝子構造を明らかにするとともに，*in situ* hybridization法による脳領域での発現特異性についても睡眠期で解析する予定である。本大会ではこれらの結果について紹介したい。

2PA-082

マカクサルの発現遺伝子マイクロアレイを用いたダイオキシン影響解析
○浅岡 一雄[1], 飯田 寛子[1], 釜中 慶朗[1], 鈴木 樹理[1], 西村 剛[1], 脇田 真清[1], 渡辺 邦夫[1], 石川 雄一郎[2], 森 千里[3], 安田 峯生[4], 久保田 俊一郎[5]
([1]京都大・霊長類研究所, [2]東京都臨床医学総合研究所, [3]千葉大・大学院医学研究院, [4]広島国際大・保健医療学部, [5]東京大・大学院総合文化研究科)
Analysis of Dioxin Effects on *Macaca* Monkeys using the Microarray of Their Expression Genes
○Kazuo Asaoka[1], Hiroko Iida[1], Yoshiro Kamanaka[1], Juri Suzuki[1], Takeshi Nishimura[1], Masumi Wakita[1], Kunio Watanabe[1], Yu-ichiro Ishikawa[2], Chisato Mori[3], Mineo Yasuda[4], Shunichiro Kubota[5]
([1]Kyoto Univ., Primate Research Inst., [2]Tokyo Metro. Inst. Medical Sci., [3]Chiba Univ., Gradu. School Med., [4]Hiroshima International Univ., Health Sci., [5]Univ. Tokyo, Gradu. School Arts Sci.)

サルはヒトに類似するとして，なかでも中型で数kgの体型をもつマカクサルは実験に多く用いられている。しかしサルにおけるゲノムワイドな分子生物学的知見は限られたものであり更に研究が必要である。今回，マカクサルに発現する遺伝子を網羅的に集積し塩基配列を解析した。サルの臓器よりmRNAを抽出してオリゴキャップ法による完全長cDNAライブラリーを作製した。インサートサイズは平均1.7Kbであった。約3千個のクローンについて塩基配列を解析してBlast検索ののちデータベース化した。完全長率は63.9%であった。遺伝子の配列比較からサルとヒトの類似度を算出した。サルとヒトの遺伝子を較べると分子構造では94%，発現頻度では96%，平均して95%の類似度であった。ラット，マウスなどとヒト間における発現遺伝子については約80%の類似度と報告されており，発現遺伝子においてサルは極めてヒトに類似していた。以上より，ヒトのモデル動物としてサルを用いる実験の有用性が明確となった。

集積した発現遺伝子クローンを用いてDNAマイクロアレイの開発を進め，ダイオキシン影響解析を行った。ダイオキシンはマカクザルに対して30ng-300ng/kg体重で単回投与した。投与49日目の臓器において分子の発現変動が検出された。CYP1A1の発現量増加は乳腺，皮膚，胃壁で数倍に増加し，膵臓，肝臓，脳において微増し，心臓，肺，甲状腺，卵巣では検出されなかった。発現変動はダイオキシンの臓器移行量に並行して増加していた。ダイオキシン移行の大きい乳腺においては，ダイオキシンの投与量に応じて16個の遺伝子に発現量の増加が検出され，17個の遺伝子に投与量に応じて発現量の減少が検出された。発現遺伝子においてサルがヒトと極めて類似していることを考慮すると，サルで見られたダイオキシン影響はヒトにおいても同様の発現プロファイルで生じる可能性が高いと外挿される。

2PA-083

マイクロアレイを用いた毒性評価系における培養細胞と個体間の比較
○栗原 渉[1,2], 濱崎 美久[1,2], 佐々木 仁[1,2], 宇津木 孝彦[2], 藤森 文啓[1], 村上 康文[1,2]
([1]東京理科大・基礎工・生物工, [2]東京理科大・ゲノム創薬研究センター)
Comparative analysis of toxicity to culture cell and tissue with the use of microarray
○Wataru Kurihara[1,2], Miku Hamasaki[1,2], Hitoshi Sasaki[1,2], Takahiko Utsugi[2], Fumihiro Fujimori[2], Yasufumi Murakami[1,2]
([1]Tokyo Univ. of Science, Fac. Indust. Sci. Tech., Dept. Biol. Sci. Tech., [2]Tokyo Univ. of Science, GDRC)

近年，薬剤の作用機序の解析や毒性の評価を行うための新しい手法として，マイクロアレイを用いた手法が研究・開発されつつある。これまでの研究で一定の評価は得られているものの，それぞれの実験系に於いて使用しているマイクロアレイ，薬剤の種類，及び投与対象群が異なっているため，各実験結果の単純な比較を行うことができず，また他の研究者がデータを利用する上ではやや利便性を欠いていた。そのような背景の中，米国や日本では，データの統一性を図り，大規模なデータベースを構築することを目的とした国家プロジェクト，いわゆるトキシコゲノミクス・プロジェクトを立ち上げており，現在進行中である。しかしこのような大規模の薬剤投与に関するフィンガープリント・データベースを構築できたとしても，生物種が異なった場合や，投与対象が培養細胞と個体で異なった場合における，データの互換性に関する問題が残っており，これを解決するための研究は殆ど行われていない。

そこで今回私たちは，培養細胞に薬剤を投与した場合と個体に投与した場合に発現パターンにどのような点で相関があり，またどのような点に相違があるのかを調べるための実験を行うことにした。具体的にはまず，マウスの個体，初代肝培養細胞，肝ガン細胞の3つの系を用意し，それらに対して肝毒性を示す薬剤を幾つか投与した。そしてこれらの発現パターンを，NIA 22k cDNAマイクロアレイによって解析することで，それぞれの投与系における相違・相関する点を探ることにした。また予備的な実験として，それぞれの投与系同士の薬剤投与前における発現パターンの相関を調べたところ，個体と培養系では大きく異なることが解った。そこでこのような違いと薬剤投与による発現パターンの違いについて，何らかの関連性を見出すことも目指したいと考えている。

2PA-084

マイクロアレイデータを集合的に取り扱うことによる内分泌撹乱化学物質の長期的影響の解析
○深田 秀樹[1], 足達 哲也[2], 大森 万里子[1,3,4], 長田 久夫[5], 森 千里[3,6]
([1]千葉大・院医・SRL環境健康医学, [2]大阪府大・先端研・生資セ, [3]千葉大・院医・環境生命医学, [4]千葉大・院医・生殖機能病態学, [5]千葉大・医附属病院・産婦人科, [6]科技団・CREST)
Analysis of the long-term effects of endocrine disruptors using microarray data as a set
○Hideki Fukata[1], Tetsuya Adachi[2], Mariko Omori[1,3,4], Hisao Osada[5], Chisato Mori[1,3,6]
([1]Chiba Univ., Grad. Sch. Med., Dept. Env. Med. Sci. (SRL), [2]Osaka Pref. Univ., Res. Inst. Advanced Sci. Tech., Center R&D Bioresources, [3]Chiba Univ., Grad. Sch. Med., Dept. Bioenv. Med., [4]Chiba Univ., Grad. Sch. Med., Dept. Reprod. Med., [5]Chiba Univ. Hosp., Ob/Gy, [6]JST, CREST)

内分泌撹乱化学物質（EDs）の長期的影響を調べる目的で，マウス新生仔に生後1日目から5日目までゲニステイン，ビスフェノールA，ポジティブコントロールとしてジエチルスチルベストロール（DES）を投与し，4週齢時あるは12週齢時の精巣，精巣上体・脾臓での遺伝子発現レベルの変化をDNAマイクロアレイで網羅的に解析した。DNAマイクロアレイは広く日常的に用いられるようになり，また，データ解析ソフトも多数開発されており，さまざまな解析が簡単にできるようになってきた。アレイデータから意味のある知見を導き出すのはまだ容易ではない。我々は得られたデータを集合的に取り扱う，すなわち，個々の遺伝子の変化を追うのではなく遺伝子集団の動きを追うことにより新たな知見を得ることができたので，ここに報告する。いわゆるハウスキーピングジーンの発現レベルもEDsの影響を受けて変動していたので，アレイデータの標準化は，発現レベルが比較的高く，投与したEDsの影響が低かった遺伝子群（G-CSFRやRASオンコジーンファミリー遺伝子等）を内部基準とした。解析の結果，(1) EDsは新生児期に一過的投与しただけであるが精巣への影響は12週齢時でも見られた。(2) EDsは，精子形成に関連していると考えられる遺伝子群（4週齢時の精巣では発現しておらず12週齢時ではじめて発現が確認される遺伝子群で，EDsの直接的な影響を受けていない）の発現にも多大な影響を及ぼしていた。(3) 作用が強いとされる内分泌撹乱化学物質ほど，遺伝子発現の点からも精巣の機能成熟を阻害していることがわかった。(4) EDsの影響は精巣のみならず，12週齢時の脾臓における遺伝子の発現レベルにも影響を及ぼしていた。

In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects tooth development in rhesus monkeys

Iku Yasuda[1], Yasuda Mineo[2], Sumida Hiroshi[2], Arima Akihiro[3], Ihara Toshio[3], Kubota Shunichiro[4], Asaoka Kazuo[5], Takasuga Takumi[6], Tsuga Kazuhiro[1], Akagawa Yasumasa[1]

[1]Hiroshima University, Hiroshima
[2]Hiroshima International University, Hiroshima
[3]Shin Nippon Biomedical Laboratories, Ltd., Kagoshima
[4]University of Tokyo, Tokyo
[5]Primate Research Institute, Kyoto University, Inuyama
[6]Shimadzu Techno-Research Inc., Kyoto

Introduction

The current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg TEQ/kg/day in Japan. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.* reported that a single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the offspring[1]. The maternal body burden at this dose was measured to be 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. An uncertainty factor of 10 was applied to this value, and the human TDI was established. However, due to great differences in the biological half life of TCDD between human and rodents, the validity of this calculation is questioned. To obtain more reliable LOAEL in the second generation, we initiated a long-term study in rhesus monkeys in 1999.

In rodents, teeth are known to be targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[2,3]. In humans also tooth abnormalities were reported among populations exposed to dioxins[4]. In our monkey experiment, some young were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate young exposed to TCDD *in utero* and lactationally. By macroscopic observation we found some tooth abnormalities among died young exposed to TCDD[5]. This prompted us to examine surviving young by radiography. This is an interim report of our findings in these young.

Methods and Materials

Animals: Adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijin, China) were used. Details of breeding conditions were given elsewhere[6]. Female monkeys were allowed to cohabit with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period was disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups each consisting of approximately 20 animals and allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PD0).

Administration of TCDD: TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy and lactation until day 90 after birth.

Measurement of TCDD in maternal serum: Approximately 20 ml of blood was taken from the femoral vein of the dams on day 80 of pregnancy, and centrifuged. The obtained serum was subjected to high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) by the method of Patterson *et al.*[7]

Observation of teeth of the young: Stillborn and postnatally died young were autopsied, and the upper and lower jaws were dissected for detailed observation. Surviving young were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

Pregnancy outcome and postnatal development of the young: Table 1 summarizes the pregnancy outcome and postnatal mortality of the young. Abortions, stillborns, and postnatal deaths occurred fairly frequently even in the control group. To increase the number of surviving young in the 300 ng/kg, we added 9 dams to the group approximately 2 years after the initiation of the experiment. However, only two young survived more than a year due to a high incidence of abortions. No significant differences were noted in the gestation length and birth weight among the control and TCDD-treated groups, indicating the body burden of TCDD at 300 ng/kg did not affected general growth of the young.