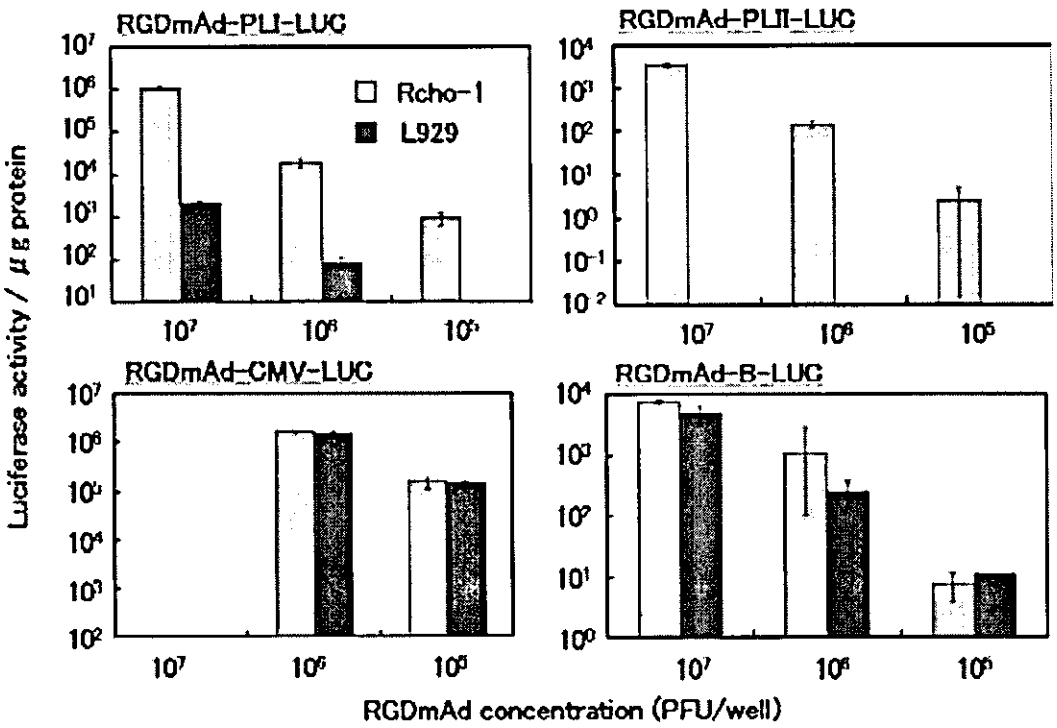

Fig. 2. Transgene expression in Rcho-1 and L929 cells after infection of RGD mAd. Rcho-1 and L929 cells were infected by each RGDmAd (10^5, 10^6, 10^7 PFU / well). After 48hr, luciferase reporter gene activities were measured from extracts of Rcho-1 and L929 cells infected. Data represent means±S.D. of triplicate cultures.

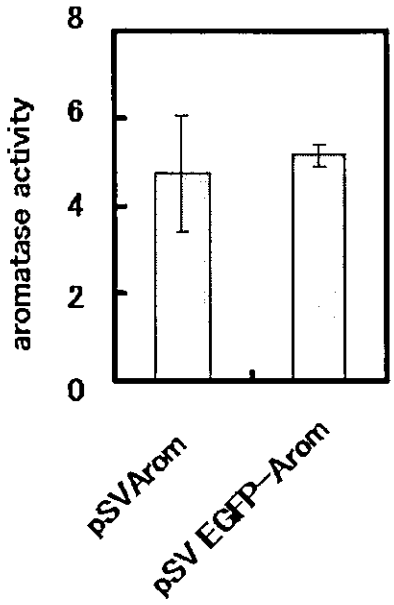

Fig. 3. Aromatase activity in L929 cells transfected by constructs. Aromatase activities were measured from extracts of L929 cells transiently transfected by each construct. Data represent means±S.D. of triplicate samples.

Table. I. Effect of PPARγ expression by RGDmAD in placenta on the lethality of PPARγ-null mice.

Mouse No	Total	WT(+/+)	PPARγ (+/-)	PPARγ (-/-)
1	5	0	5	0
2	6	3	3	0
3	7	2	5	0

別添４

研究成果の刊行物に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakanishi T, Itoh N, Tanaka K	Effects of organotin compounds on endocrine functions of human placental cells.	Toxicol. Appl. Pharmacol.	197	231-232	2004

The estrogen receptor (ER) is a nuclear protein that contains a hormone- and a DNA-binding domain and exerts its effects by binding to *cis*-acting element in the regulatory region of target genes. Therefore, estrogen or estrogen mimics (estrogenic compounds) is retained in target cells by affinity binding to specific estrogen receptor.

Our previous results demonstrated that the expression of medaka choriogenin was activated by estrogenic chemicals, while it was inhibited by anti-estrogenic compounds. At present, In order to characterize the regulatory elements of the choriogenin genes, the upstream region of the genes was cloned and sequenced. ERE (Estrogen Responsive Elements) sequences were identified in the regulatory region of the choriogenin L and choriogenin H genes. They are located between −340 and −200 from the transcription initiation site. To assess *cis*-binding requirements to ER to its epecific DNA elements of the choriogenin, ER binding to the ERE region of the medaka choriogenin was examined by using gel mobility shift assay. The levels of DNA-protein complexes were significantly increased in the reaction mixture composed of labeled oligonucleotides containing EREs and the liver extracts from the estrogen-treated medaka males. Also the binding complex of purified ER-alpha and choriogenin ERE could be formed. From the above results, choriogenin mRNA expression proved to be induced and promoted by forming the ER-ERE complex. Also these results indicate that the medaka choriogenin gene is ertrogen-regulated directly and can be a suitable biomarker for environmental estrogens.

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APPLICABILITY OF IN VITRO ASSAYS FOR SCREENING OF ENDOCRINE DISRUPTORS: RELATIONSHIPS BETWEEN IN VITRO AND IN VIVO SCREENING ASSAYS

M. Nakai, M. Takeyoshi, S. Noda, Y. Akahori, Y. Minobe, Y. Yakabe, and K. Yamasaki, *Chemicals Evaluation and Research Institute, Japan (CERI)*

In these years, several in vitro and in vivo assays for screening of endocrine disruptors have been developed. To screen the mechanism-specific endocrine disrupting potencies of numerous chemicals in a short term at a low price, it is indispensable to establish the reliable in vitro assays predicting the biological effects of chemicals with high accuracy. However, there is little research that clearly demonstrates the concordance of potencies between in vitro and in vivo screening methods based on receptor-mediated mechanism. Bindings of chemicals to estrogen receptor (ER) followed by gene transcriptional regulation are known as one of the key modes of actions that may disrupt the endocrine system. Therefore, in this study, we focused on the ER-mediated endocrine disrupting mechanism of chemicals. In order to evaluate the applicability of in vitro assays for screening of endocrine disruptors, ER-binding and ER-mediated transcriptional assays were compared to rat uterotrophic assay on 38 chemicals, respectively. Since rat uterine weight is increased depending on estrogenic activity of chemical exposed, uterotrophic assay is a typical in vivo screening assay detecting ER-mediated effect of chemical. Relative binding affinity (RBA), which was a percent ratio of IC50 values of E2 and chemicals, was employed for comparison of the receptor-binding potency among chemicals. Transcriptional activity of chemical was designated by a 10 percent-potent concentration (PC10), which defined as a concentration of test chemical activating at 10% of maximal transcriptional activity by E2. The lowest effective dose (LED) was utilized for quantitative analysis of estrogenic activity of chemical in a three-day uterotrophic assay on immature rat. RBA values showed good correlation with LED values and the concordance between ER-binding and uterotrophic assays was 87%. PC10 also exhibited excellent relationship with uterotrophic assay and the concordance between them was 89%. These results suggested that in vitro ER-binding and ER-mediated transcriptional assays were applicable to screen the endocrine disruptors, which act mediated by ER-binding.

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DI(2-ETHYLHEXYL)PHTHALATE MAY REDUCE MOUSE FERTILITY VIA PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α (PPARα)

T. Nakajima,[1] Y. Hata,[2] Y. Ito,[1] M. Omura,[3] H. Sone,[4] C. Toyama,[4] F.J. Gonzalez,[5] and T. Aoyama[2], [1]*Nagoya University Graduate School of Medicine, Nagoya, Japan*, [2]*Shinshu University School of Medicine*, [3]*Kyushu University Graduate School of Medical Sciences*, [4]*National Institute for Environmental Studies*, [5]*National Cancer Institutes, USA*

Di(2-ethylhexyl)phthalate (DEHP)-induced reduction of fertility and its role of PPARá, were assessed using wild-type and PPARá-null mice. All mice were given 0 or 0.05% DEHP-containing diets ad libitum throughout the experiment. Male and female pups (F_0) on the same dose and of the same genetic strain were mated after dosing at 0 or 0.05% DEHP for 4 weeks, and their pups (F_1) were further mated at maturity in the same manner as the F_0 generation. The resulting pups were designated as the F_2 generation. DEHP treatment induced the expression of hepatic peroxisomal enzymes in both mature males and females of the wild type, but not in their fetuses and pups, suggesting that the dose activates PPARá function only in mice fed DEHP-containing diets. No morphological abnormality was found in the genital organs from mature males and females of both genotypes under microscopic observations. Nevertheless, in the wild-type mice, DEHP exposure caused a reduction in the numbers of pups born and those that survived for 16 weeks per pair in both F1 and F2 generations. In another experiment involving 0, 0.01 and 0.05% DEHP exposure of both genotype mice, it was found that a reduction in the numbers of pups born was due to an increase in the resorption of fetuses; a reduction in those of surviving pups resulted from an increase in the mortality rates of newborn pups. Exposure to 0.05% DEHP also decreased body weight of pups only in wild-type mice. In contrast, PPARá-null mice were refractory to the adverse effects of DEHP observed in the wild-type mice in all generations. These results suggest that the reduction of fertility without the maternal or paternal genital organ toxicity found in DEHP-exposed wild-type mice is partly caused by a signal triggering energy overconsumption via PPARá in the maternal body.

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EFFECTS OF ORGANOTIN COMPOUNDS ON ENDOCRINE FUNCTIONS OF HUMAN PLACENTAL CELLS

Tsuyoshi Nakanishi, Norio Itoh, and Keiichi Tanaka, *Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan*

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), are known to induce an irreversible sexual abnormality of female neogastropod snails, which is termed "imposex". Human exposure to these organotin compounds may result from the consumption of organotin-contaminated meat and fish products. However, in humans, it remains unclear whether organotin compounds disturb the sexual development and reproduction. The principal hormones, estrogens and human chorionic gonadotropin (hCG) are produced by the placenta during pregnancy. These hormones are essential to several important events in establishment and maintenance of preg-

nancy. Thus such endocrine functions of placenta might be at high risk due to the developmental and reproductive toxicity of environmental contaminants which have endocrine-disrupting effects. Previously, we reported that TBT and TPT enhance hCG secretion and aromatase activity in human choriocarcinoma cells (J. Clin. Endocrinol. Metab. 87, 2830, 2002). In the present study, in order to extend the knowledge on correlation between the structure of organotin compounds and their endocrine-disrupting effects, we assessed effects of their various alkyl derivatives on placental hCG secretion and aromatase activity of human choriocarcinoma Jar cell, suggesting that the potency of disrupting effects induced by organotin compounds has relationships to both the number and the length of their side chain with regularity.

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INFLUENCE OF PHYTOESTROGENS ON AROMATASE ACTIVITY AND ESTROGEN-DEPENDENT CELL GROWTH

Y. Weidemann,[1] M. Schulze,[1] J. Schulze,[2] and C.-P. Siegers[1], [1]*Institute of Experimental and Clinical Pharmacology and Toxicology, University of Luebeck, Ratzeburger Allee 160, D-23538 Luebeck,* [2]*Office of the Dean, Faculty of Medicine, Johann Wolfgang Goethe-University Frankfurt, Theodor Stern Kai 7, D-60590 Frankfurt/Main, Germany*

Approximately 60% of all mammary carcinoma cells, the most common female cancer, grow in an estrogen-dependent fashion. Estrogen can be synthesized by tumour cells themselves from precursors with aromatase being the key enzyme involved. Consequently, estrogen positive mammary carcinomas are treated with anti-estrogens to slow further tumour growth. Epidemiological evidence indicate a correlation between breast cancer risk and the consumption of specific nutrients rich in phyto estrogens like soy beans. Estrogen formation is affected by a variety of environmental chemicals, prominent among them plasticizers like 2-ethylhexyl phthalate, and herbicides. Plant ingredients affecting estrogen formation are summarily called phytoestrogens, and have been detected e.g. in leguminoses like peas and soy beans. Isoflavones, coumestans and lignans are estrogenic or antiestrogenic, in a concentration dependent fashion. Other plants are poorly investigated, estrogenic or antiestrogenic activity has been detected in a variety of species including mulberry trees, cactus flowers or tobacco. Therefore it seems reasonable to assume that phytoestrogens are present in many plants and will be active at relevant concentrations. No studies have yet been conducted investigating aromatase effects from medical plants.

Besides direct interaction with estrogen formation or estrogen receptor binding, breast cancer growth may be affected by interference with intracellular estrogen signal transduction or nuclear activity interference; for genistein, a soy bean phytoestrogen, these effects have been postulated. We will present preliminary results for plant ingredient effects on aromatase activity as well as a summary of known and possible interactions between aromatase activity and plant extracts from food and medicinal sources.

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ALTERED GENE EXPRESSION OF NITRIC OXIDE SYNTHASE IN THE RAT PLACENTA EXPOSED TO 3,3′,4,4′, 5-PENTACHLOROBIPHENYL

K. Hayasaka,[1] Y. Sakurada,[1] M. Shirota,[2] M. Murakami,[1] and K. Shirota[1], [1]*Azabu University, Sagamihara,* [2]*Hatano Research Institute, FDSC, Hadano, Japan*

We have found that exposure to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) induces CYP1A1 in the fetal capillary endothelial cells in the labyrinth zone of the rat placenta. The induction of CYP1A1 indicates that PCB126 exposes fetal endothelial cells and binds to aryl hydrohydrocarbon receptor in these cells. However, the effect of PCB 126 on the placental function has not been evaluated. Among many important factors for the placental development and function, nitric oxide generated by its synthase (NOS) might play a role in the maintenance of the placental function. In the present study, we examined the gene expression of two isoforms of NOS (iNOS and eNOS) to asses the effects of PCB126 on the rat placenta. The pregnant Sprague-Dawley rats were given a single oral dose of 100 μg PCB 126/kg body wt or an equivalent volume of corn oil (control) on gestational day (GD) 18 and their placentas were collected on GD 20. The placental specimens were used for quantitative analysis of the gene expression of eNOS, iNOS and CYP1A1 by real-time PCR. The number of the placenta with live fetus did not significantly differ between control and PCB-exposed groups. Quantitative RT-PCR analysis showed significantly decreased expression of eNOS and obvious expression of CYP1A1 in the PCB-exposed placenta. Also the level of the expression of iNOS in the PCB-exposed rats was lower than that of controls. These results indicate that PCB126 alters gene expression of NOS in the rat placenta.

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IN VITRO ASSAYS FOR SCREENING OF ENDOCRINE DISRUPTORS: RELATIONSHIPS BETWEEN HUMAN ESTROGEN RECEPTOR BINDING ASSAY AND REPORTER GENE ASSAY ON 948 CHEMICALS

Y. Suzuki,[1] M. Nakai,[1] M. Takeyoshi,[1] Y. Akahori,[1] T. Tokunaga,[2] Y. Yakabe,[1] and Y. Shimohigashi[2], [1]*Chemicals Evaluation and Research Institute, Japan (CERI),* [2]*Kyushu University, Japan*

Bindings of chemicals to estrogen receptor (ER) followed by gene transcriptional regulation are known as one of the key modes of actions that may disrupt the endocrine system. Both in vitro receptor binding and gene transcriptional assays are simple and valuable to screen such endocrine disrupting chemicals from numerous chemicals produced. To utilize each in vitro assay practically, it is important to confirm the relationships between assays and evaluate the validity of each assay. In this study, ER-binding affinities and ER-mediated transcriptional activities of approximately 948 chemicals were measured and the relationships between the two assays were evaluated. The test chemicals were selected to encompass diverse chemical structures from commercially available reagents with molecular weight of 100 to 600, excluding inorganic compounds. Competitive binding assay was performed using recombinant human ER alpha ligand binding domain expressed in *E. coli* and tritium-labeled 17beta-estradiol (E2) as a tracer. Relative binding affinity (RBA), which was a percent ratio of IC50 values of E2 and test chemicals, was employed for comparison of the receptor-binding potency among chemicals. HeLa cell stably co-transfected with receptor expression and reporter plasmids was used for reporter gene assay. Transcriptional activity of chemical was designated by a half-potent concentration (PC50), which defines as a concentration of test chemical activating at 50% of maximal transcriptional activity by E2. As the results, 168 chemicals showed both ER-binding and ER-mediated transcriptional potencies in these assays, and RBA and PC50 values in most of these chemicals exhibited good relationships between two in vitro assays. Some outliers from this correlation were observed and they included characteristic chemicals, which had ester bond in their molecules or presumably behaved as antagonists based on their structures.