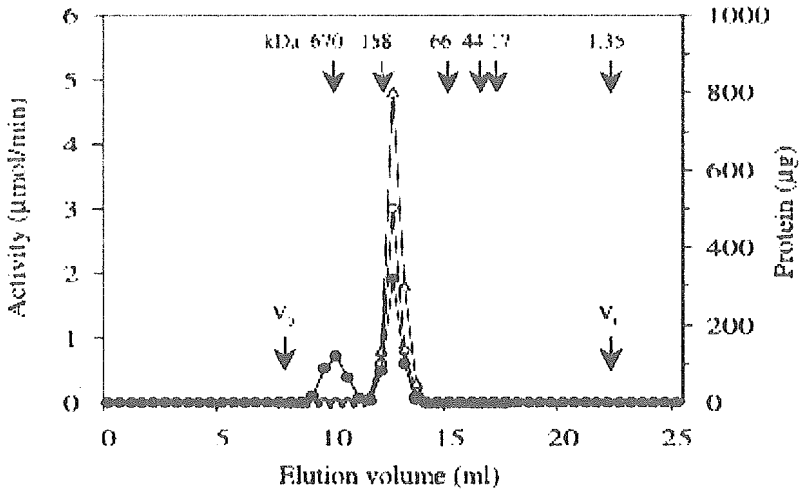

Fig. 3. Analytical gel-filtration chromatography of the purified OPRT OMPDC on a Superose 12 FPLC column equilibrated with a buffer containing 50 mM Tris HCl, pH 8.0, 150 mM KCl, 2.5 mM DTT, and 1 mM PMSF. Fractions (0.5 ml) were collected and then assayed for the co-eluting enzyme activities. The proteins were also measured for their elution position and activity profile. Molecular mass markers, void volume (V_0), and total eluting volume (V_t) are indicated with arrows. The symbols used are the same as in Fig. 1.

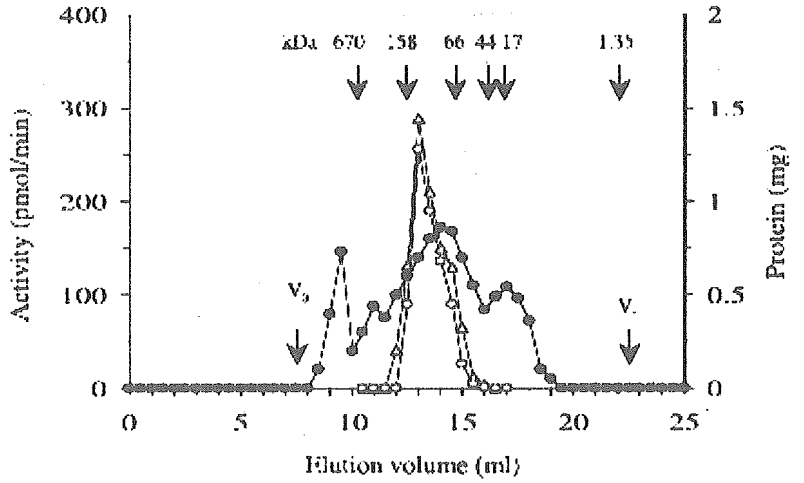

Fig. 4. Chromatographic profiles of OPRT and OMPDC activities from *P. berghei* crude extract on a Superose 12 FPLC column. Molecular mass markers, void volume (V_0), and total eluting volume (V_t) are indicated with arrows. The symbols used are similar as in Fig. 1.

担当者　大関一男

著者名:　Lehmann L, Metzler M.

論文題名：Bisphenol A and its methylated congeners inhibit growth and interfere with microtubules in human fibroblasts in vitro

出典:　Chem Biol Interact. 2004 Apr 15;147(3):273-85

チェック項目:

1．対象生物　( )ラット ( ) マウス、 ( ) 人、 (x) その他__

2．影響の標的臓器　( ) 神経系、 ( ) 免疫系、 ( ) 生殖系、 ( ) その他

3．影響の種類　(x) 細胞、 ( ) 組織、 ( ) 個体、 ( ) その他 Micronucleus assay, cell proliferation assay

4．曝露方法　( ) 経口、 ( ) 埋め込み、その他 cell culture

5．曝露時期　( ) 胚･胎児、 ( ) 周産期、 ( ) 出生後、 ( ) 成熟動物、 (x) 細胞

6．曝露濃度　用量段階 (５０ ～ 450 μM ；９種)

7．観察された影響の種類と濃度：

(200μM,　AG01522C fibroblast 異数性異常)

8．観察時期　( ) 出生前、 ( ) 出生後、 ( ) 思春期、 ( ) 成熟期

9．論文中に低用量影響への関心　( )あり、(x)なし

10．試験の信頼性について下記項目でチェックする。

GLP に準拠　　( )はい、(x)いいえ　論文中に「GLP に準拠」の記述の有無

ガイドラインへの準拠　( )はい：ガイドラインの名称＿＿＿＿＿＿＿＿、(x)いいえ

論文の概要:

human AG01522C fibroblast を使って異数性異常を調べた。初期生殖細胞分裂の抑制、kinetochore-positive micronuclei の誘発などから、異数性異常を引き起こす DES や 17β—エストラジオールと比べ、ビスフェノール A は小核形成は誘発しなかったが、G2 phase での AG01522C セルの増殖を阻害した、おそらく G1 phase も同様である。BPA 処理した後の蛍光顕微鏡で cytoplasmic microtubule complex (CMTC)の構造異常が観察され、それには BPA の濃度が高いほど異常が多くみられた。成長阻害、microtubule の抑制などのメカニズムは不明だが、AG01522C セルの CTMC に見られる ring, loop の生成はビスフェノール A 類似体、特にフェノール系水酸基のオルソ位にメチル基がある場合などで観察された。しかしながら、ビスフェノール A とは対照的に、これらの類似構造体で　DES で観察される　初期生殖細胞分裂の抑制、kinetochore-positive micronuclei が観察された。

添付資料

Figure 1、Figure 4

評価者のコメント：

以前、パトリシアハントらが報告したもとの類似のものと思われる。ハントらによる報告はそれ以降　追加のものはなく、信頼性がどうか疑問が残る。これはどうなのか、興味のあるところである。

Bisphenol A Exposure Causes Meiotic Aneuploidy in the Female Mouse", P. A. Hunt, K. E. Koehler, M. Susiarjo, C. A. Hodges, A. Ilagen, R. C. Voigt, S. Thomas, B. F. Thomas, and T. J. Hassold, *Current Biology* (2003), 13:546-553

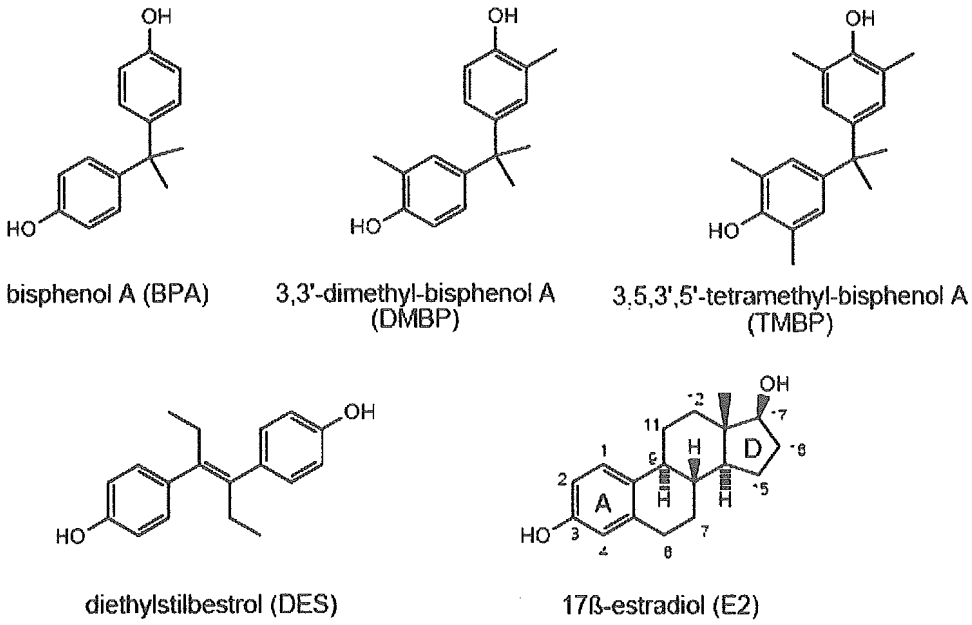

Fig. 1. Chemical structures of tested compounds.

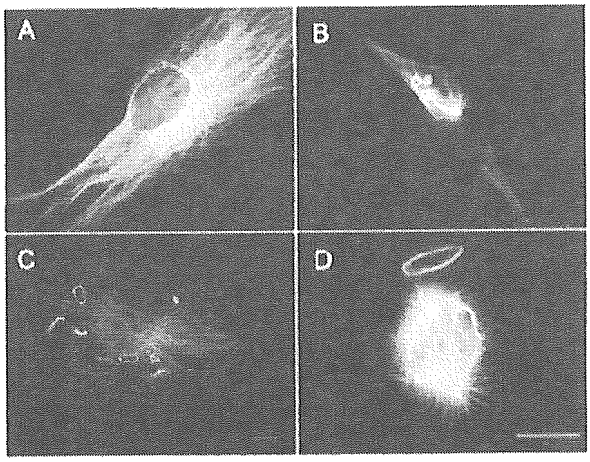

Fig. 4. Micrographs of the fluorescent-labelled microtubules of AG01522C cells treated with 400 μM BPA for 12 h. (A) untreated interphase cells; (B) and (C) treated interphase cells; (D) treated cell in metaphase; bar, 10 μm.

担当者 大関一男

著者名: Li H, Ruan XZ, Powis SH, Fernando R, Mon WY, Wheeler DC, Moorhead JF, Varghese Z

論文題名: EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism

出典: Kidney Int. 2005 Mar;67(3):867-74

チェック項目:

1．対象生物 ( )ラット ( ) マウス、 ( ) 人、 (x) その他 human kidney cell
2．影響の標的臓器 ( ) 神経系、( ) 免疫系、( ) 生殖系、(x) その他 human kidney cell
3．影響の種類 ( ) 細胞、( ) 組織、( ) 個体、 (X) その他_NF－κB転写因子の活性
4．曝露方法 ( ) 経口、( ) 埋め込み、その他_____
5．曝露時期 ( ) 胚･胎児、( ) 周産期、( ) 出生後、( ) 成熟動物、 (X) 細胞
6．曝露濃度 用量段階 (10 μM、100 μM)
7．観察された影響の種類と濃度：
(10μM)
8．観察時期 ( ) 出生前、( ) 出生後、( ) 思春期、( ) 成熟期
9．論文中に低用量影響への関心 ( )あり、(x)なし
10. 試験の信頼性について下記項目でチェックする。
GLPに準拠 ( )はい、(x)いいえ 論文中に「GLPに準拠」の記述の有無
ガイドラインへの準拠 ( )はい：ガイドラインの名称_____、(x)いいえ

論文の概要:

10 μmol/L, 100 μmol/L においてEPA(eicosapentanoic acid), DHA(decosahexanoic acid)は、lioppolysaccharide(LPS)を誘発するNF－κB転写因子の活性化、monocyte chemoattractant protein-1(MCP-1)発現を抑制減少させた。一方、HK-2 cellでのPPAR-γmRNAや蛋白活性を増加させた。100 μmol/のBADGE(BPA diglycidyl ether)の存在下では、EPA、DHAにより誘発されるPPAR-γmRNAの活性化が消失、HK-2 cellでのEPA、DHAによるLPS誘発 NF－κB転写因子の活性抑制効果を消失が観察された。

EPA、DHAはHK-2 cellでのPPAR-γ依存pathwayを通してNF－κB転写因子のLPS活性をダウンレギュレートする。EPA、DHAによるPPAR-γ活性はfish oilの効用のメカニズムに寄与するものと思われる

添付資料

Figure 1, 2, 3, 4, 5.

評価者のコメント:

本件はビスフェノールAでなく、フェノールのOH部分にエピクロロヒドリンがついたBADGEを使い、EPA、DHAの効果を調査したもの。

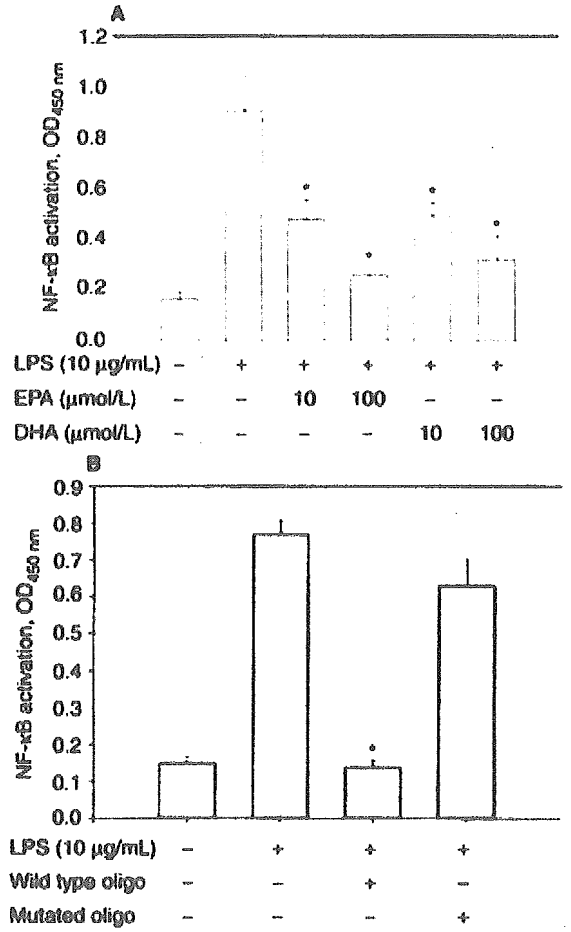

Fig. 1. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on nuclear factor-kappaB (NF-κB) activity in lipopolysaccharide (LPS)-stimulated human kidney-2 (HK-2) cells. (*A*) HK-2 cells were pretreated in keratinocyte serum-free media (K-SFM) without supplement but containing different concentrations of EPA, DHA for 23 hours, and then incubated for another 1 hour in the presence or absence of 10 μg/mL LPS. Consensus nuclear extracts were prepared and assayed using the Trans-AM enzyme-linked immunosorbent assay (ELISA) system as described in the Methods section. (*B*) The wild-type and mutated oligonucleotides were provided as a competitor for NF-κB binding in order to monitor the specificity of the assay. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. LPS induction group. OD is optical density.

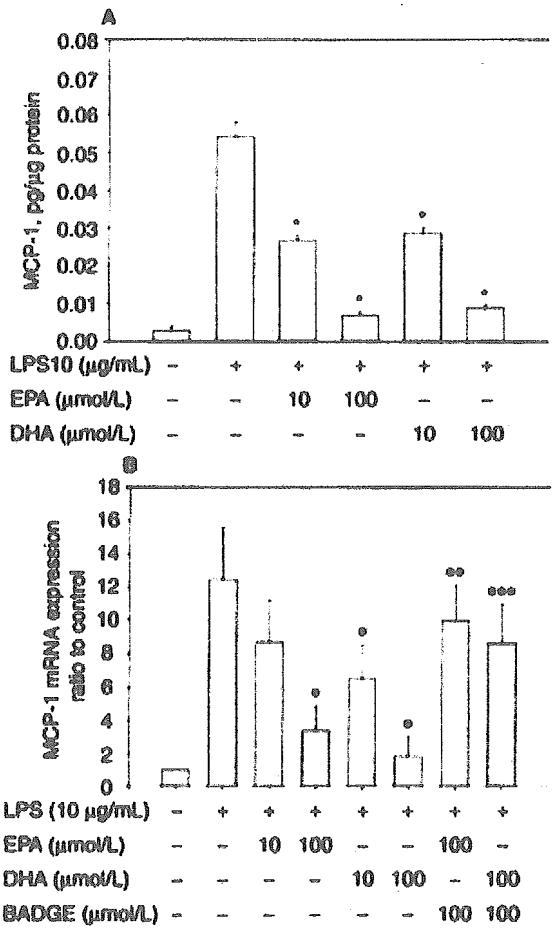

Fig. 2. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on monocyte chemoattractant protein-1 (MCP-1) protein levels and mRNA expression in lipopolysaccharide (LPS)-stimulated human kidney-2 (HK-2) cells. (*A*) HK-2 were incubated in keratinocyte serum-free media (K-SFM) without supplement but containing different concentrations of EPA and DHA in the presence or absence of 10 μg/mL LPS for 24 hours. Supernatants were collected and assayed for MCP-1 as described in the Methods section. Results are expressed as means ± SD of four independent experiments. (*B*) HK-2 cells were cultured in K-SFM containing 10 μg/mL LPS with different concentrations of EPA and DHA in the absence or presence of 100 μmol/L bisphenol A diglycidyl ether (BADGE) for 24 hours. MCP-1 mRNA was determined following the ΔCt protocol for real-time reverse transcription-polymerase chain reaction (RT-PCR) as described in the Methods section. β actin served as the housekeeper gene. $^{*}P < 0.05$ vs. LPS induction control; $^{**}P < 0.05$ vs. LPS induction group; $^{\circ\circ}P < 0.05$ vs EPA 100 plus LPS; $^{\circ\circ\circ}P < 0.05$ vs DHA 100 plus LPS.

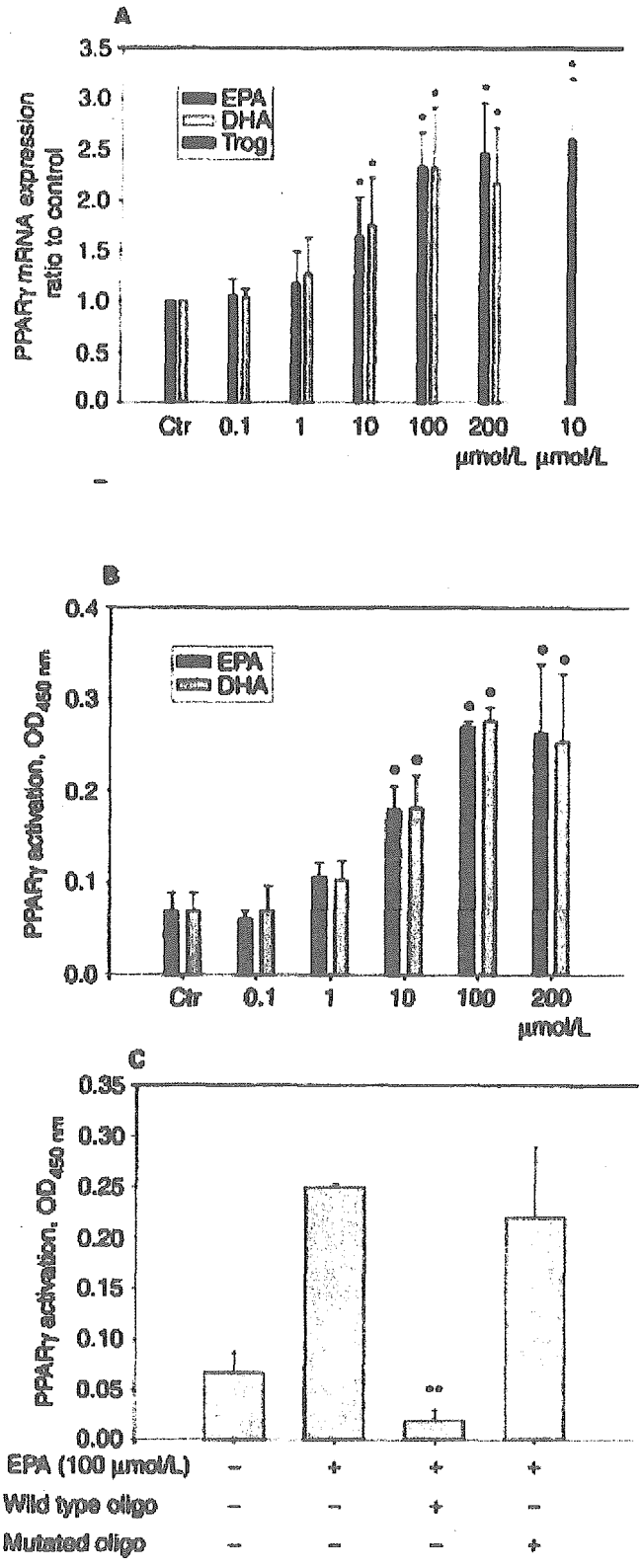

Fig. 3. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mRNA expression and activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) in human kidney-2 (HK-2) cells. (*A*) HK-2 cells were incubated in keratinocyte serum-free media (K-SFM) without supplement (Ctr) but containing different concentrations of EPA and DHA for 24 hours, and troglitazone (Trog) (10 μmol/L) was used as a positive control. PPAR-γ mRNA was determined following the ΔCt protocol for real-time reverse transcription-polymerase chain reaction (RT-PCR) as described in the Methods section. β actin served as the housekeeper gene. (*B*) Nuclear extracts

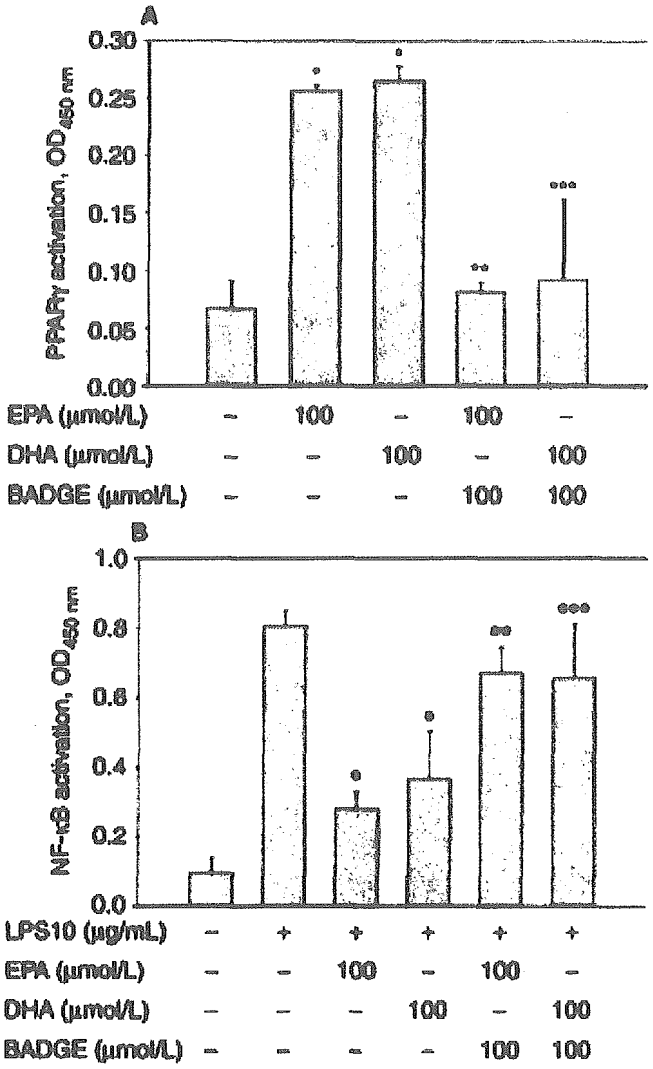

Fig. 4. Effects of the bisphenol A diglycidyl ether (BADGE) on the activity of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and nuclear factor-kappaB (NF-κB) in eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA)-treated human kidney-2 (HK-2) cells. HK-2 cells were incubated in keratinocyte serum-free media (K-SFM) without supplement but containing 100 μmol/L of EPA, DHA, and BADGE for 24 hours (*A*) or for 23 hours and then incubated for another 1 hours in the presence or absence of 10μg/mL lipopolysaccharide (LPS) (*B*). Nuclear extracts were prepared for measurements of PPAR-γ or NF-κB activation, respectively. Nuclear extracts were assayed as described in the Methods section. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. vehicle control; $^{**}P < 0.05$ vs. EPA alone; $^{***}P < 0.05$ vs. DHA alone; $^{\circ}P < 0.05$ vs. LPS induction group; $^{\circ\circ}P < 0.05$ vs. EPA plus LPS; $^{\circ\circ\circ}P < 0.05$ vs. DHA plus LPS. OD is optical density.

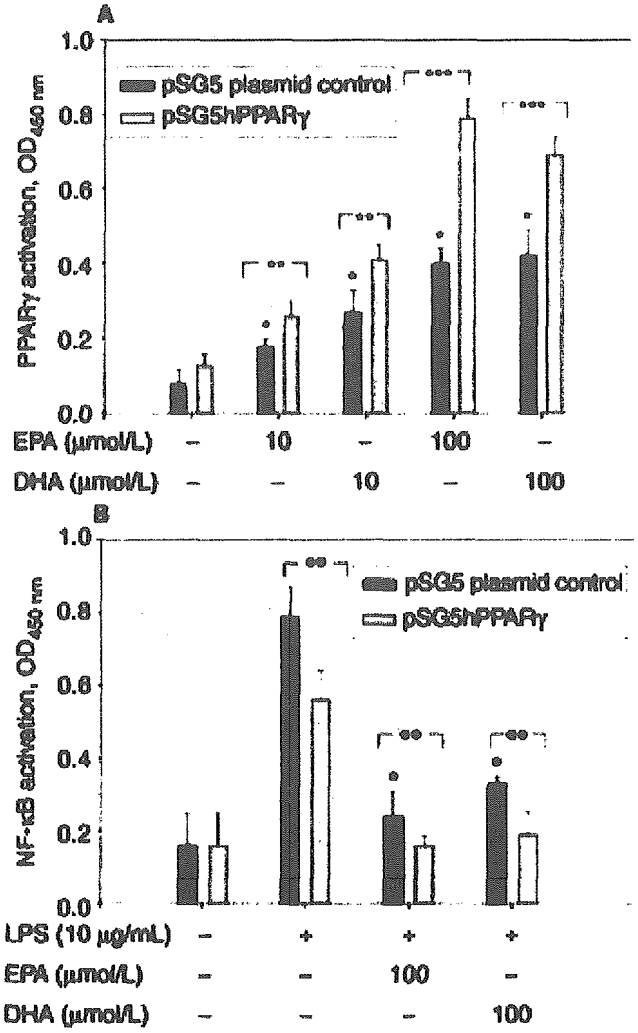

Fig. 5. Effects of over-expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ) on activation of PPAR-γ and lipopolysaccharide (LPS)-induced nuclear factor-kappaB (NF-κB) activation in human kidney-2 (HK-2) cells. HK-2 cells were transiently transfected with pSG5 plasmid control (■) or pSG5hPPAR-γ (□) using electroporation as described in the Methods section. Both transiently transfected HK-2 cells were incubated in keratinocyte serum-free media (K-SFM) without supplement but containing different concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 24 hours. Nuclear extracts were prepared for measurement of PPAR-γ activation (*A*). Both transiently transfected HK-2 cells were pretreated in K-SFM without supplement but containing 100 μmol/L of EPA and DHA for 23 hours and then incubated for another 1 hour in the presence or absence of 10 μg/mL LPS. Nuclear extracts were prepared for measurement of NF-κB activation (*B*). Nuclear extracts were assayed using the TransAM enzyme-linked immunosorbent assay (ELISA) system as described in the Methods section. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. corresponding vehicle control; $^{**}P < 0.05$ vs. pSG5 plasmid control; $^{***}P < 0.001$ vs. pSG5 plasmid control; $^{\#}P < 0.05$ vs. corresponding LPS induction group; $^{\#\#}P < 0.05$ vs. pSG5 plasmid control. OD is optical density.

担当者 大関一男

著者名: Masuno H, Iwanami J, Kidani T, Sakayama K, Honda K

論文題名 : Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway

出典: Toxicol Sci. 2005 Apr;84(2):319-27. Epub 2005 Jan 19

チェック項目:

1．対象生物 ( )ラット ( ) マウス、 ( ) 人、 (x) その他 Lipid staining in cell

2．影響の標的臓器 ( ) 神経系、 ( ) 免疫系、 ( ) 生殖系、 (x) その他 脳

3．影響の種類 ( ) 細胞、 (x) 組織、 ( ) 個体、 ( ) その他_____

4．曝露方法 ( ) 経口、 ( ) 埋め込み、その他 cell culture

5．曝露時期 ( ) 胚･胎児、 ( ) 周産期、 ( ) 出生後、 (x) 成熟動物、 ( ) 細胞

6．曝露濃度 用量段階 (4, 20, 40, 80 μM)

7．観察された影響の種類と濃度：

(20 μM)

8．観察時期 ( ) 出生前、 ( ) 出生後、 ( ) 思春期、 ( ) 成熟期

9．論文中に低用量影響への関心 ( )あり、(x)なし

10. 試験の信頼性について下記項目でチェックする。

GLP に準拠 ( )はい、(x)いいえ 論文中に「GLP に準拠」の記述の有無

ガイドラインへの準拠 ( )はい：ガイドラインの名称_____、(x)いいえ

論文の概要:

ビスフェノール A が脂肪生成に関与するのかを調べる目的で、3T3-L1 細胞と 6 日間処理。培地では triacylglycerol が増加、Oil Red O-staining 細胞の比率増加、lipoprotein lipase, adipocye-specific fatty acid binding protein(aP2) mRNA などの増加が見られた。このことより、3T3-L1 細胞を脂肪細胞に最終分化することをビスフェノール A が促進したことが理解される。PI 3-kinase の inhibitor である LY294002 は、triacylglycerol accumulation や LPL 及び aP2 mRNA 発現において BPA により増加された効果を完全にブロックした。Western block analysis から、 ビスフェノール A が PI 3-kinase、Akt kinase pathway を通して作用し、triacylglycerol accumulation や LPL 及び aP2 mRNA 発現をしたものと思われる。さらに、類似構造物質による影響も調査。８つの類似構造物質は triacylglycerol accumulation がビスフェノール A の 73-93%で、4-nonyl phenol, 4-tert-octylphenol は脂肪細胞への最終分化には寄与しなかった。

添付資料

Figure 1、3, 4, 5, 6

評価者のコメント：

用量相関等のデータはない。特になし。

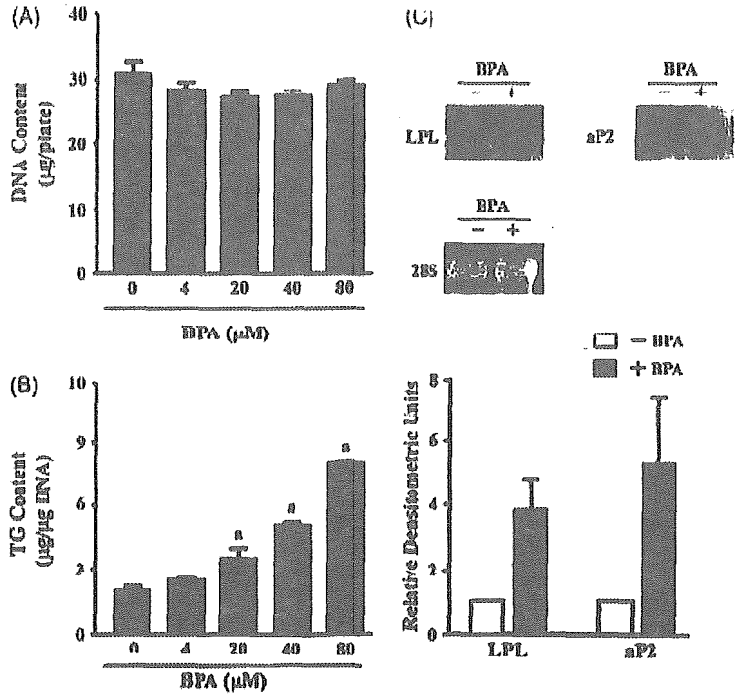

FIG. 1. Effect of BPA on the DNA and TG contents and expression of LPL and aP2 mRNAs. (A and B) Following the hormonal induction of differentiation, 3T3-L1 cells were treated for six days with BPA at the indicated concentrations. Cells were harvested and sonicated briefly at 0°C. The DNA and TG contents in aliquots of the homogenate were measured. Values given are the mean ± SD for four plates. $^{a}p < 0.01$ (compared with the value obtained from the untreated cultures). (C) Following the hormonal induction of differentiation, 3T3-L1 cells were treated for six days without or with 80 μM BPA. Total RNA was extracted from cells. RNA samples from two plates were combined for each treatment. Twenty μg of RNA was loaded to each lane. Expression of LPL and aP2 mRNAs was analyzed by Northern blot. Relative densitometric units were determined using the analysis software. Values given for LPL mRNA are the mean ± SD for four experiments. Values given for aP2 mRNA are the mean ± SD for seven experiments. 28S shows ethidium bromide-stained rRNA.

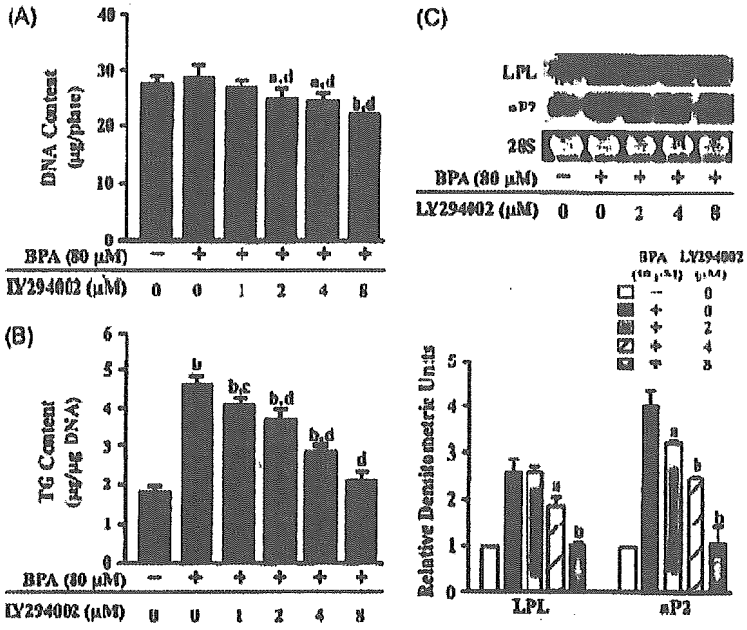

FIG. 3. Effect of LY294002 on the DNA and TG contents and expression of LPL and aP2 mRNAs. (A and B) Following the hormonal induction of differentiation, 3T3-L1 cells were treated for six days with 80 μM BPA in the presence of LY294002 at the indicated concentrations. Cells were harvested and sonicated briefly at 0°C. The DNA and TG contents in aliquots of the homogenate were measured. Values given are the mean ± SD for four plates. $^{a}p < 0.05$; $^{b}p < 0.01$ (compared with the value obtained from the untreated cultures). $^{c}p < 0.05$; $^{d}p < 0.01$ (compared with the value obtained from the cultures treated with BPA alone). (C) Total RNA was extracted from cells. RNA samples from two plates were combined for each treatment. Twenty μg of RNA was loaded to each lane. Expression of LPL and aP2 mRNAs was analyzed by Northern blot. Relative densitometric units were determined using the analysis software. Values given are the mean ± SD for three experiments. $^{a}p < 0.05$; $^{b}p < 0.01$ (compared with the value obtained from the cultures treated with BPA alone). 28S shows ethidium bromide-stained rRNA.

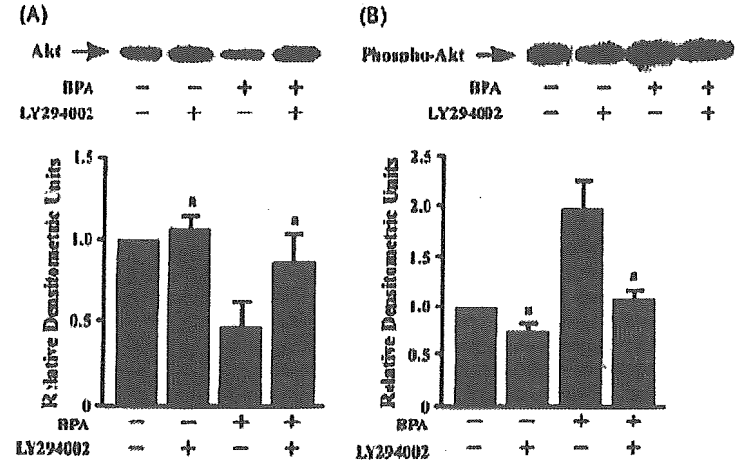

FIG. 4. Effect of BPA on phosphorylation of Akt. Following the hormonal induction of differentiation, 3T3-L1 cells were treated for six days without or with 80 μM BPA in the absence or presence of 8 μM LY294002. The proteins (10 μg of protein/lane) in the cell lysates were separated by SDS-PAGE. Expression of Akt (A) and phospho-Akt (B) was analyzed by Western blot. Relative densitometric units were determined using the analysis software. Values given are the mean ± SD for three experiments. $^{a}p < 0.05$ (compared with the value obtained from the cultures treated with BPA alone).

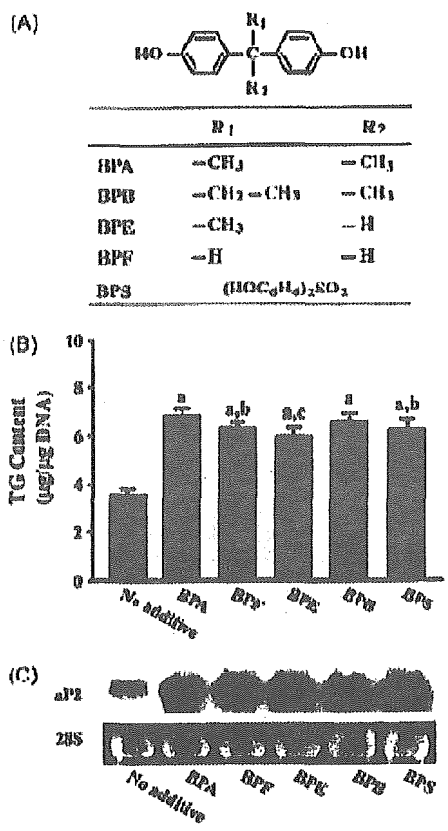

FIG. 5. Effect of BPA-related chemicals with different substituents at the central carbon atom on the TG content and expression of aP2 mRNA. (A) Structures of substituents at the central carbon atom and the whole structure of BPS are shown. (B) Following the hormonal induction of differentiation, 3T3-L1 cells were treated for six days with 80 μM BPA-related chemical. Cells were harvested and sonicated briefly at 0°C. The DNA and TG contents in aliquots of the homogenate were measured. No chemicals caused changes in the DNA content of the cultures (data not shown). Values given are the mean ± SD for four plates. [a]$p < 0.01$ (compared with the value obtained from the untreated cultures). [b]$p < 0.02$; [c]$p < 0.01$ (compared with the value obtained from the cultures treated with BPA alone). (C) Total RNA was extracted from cells. RNA samples from two plates were combined for each treatment. Twenty μg of RNA was loaded to each lane. Expression of aP2 mRNA was analyzed by Northern blot. 28S shows ethidium bromide-stained rRNA. The representative of two independent experiments is shown. BPA, bisphenol A; BPF, bisphenol F; BPE, bisphenol E; BPB, bisphenol B; BPS, bisphenol S.

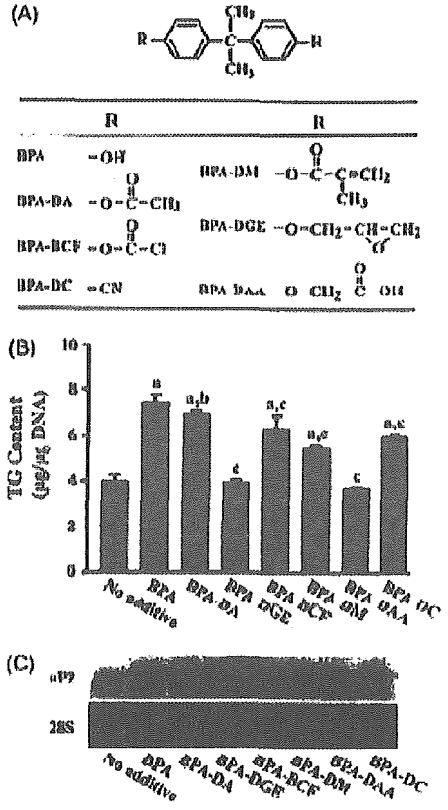

FIG. 6. Effect of BPA-related chemicals with different substituents at the phenolic hydroxyl groups on the TG content and expression of aP2 mRNA. (A) Structures of substituents at the phenolic hydroxyl groups are shown. (B) Following the hormonal induction of differentiation, 3T3-L1 cells were treated for six days with 80 μM BPA-related chemical. Cells were harvested and sonicated briefly at 0°C. The DNA and TG contents in aliquots of the homogenate were measured. No chemicals caused changes in the DNA content of the cultures (data not shown). Values given are the mean ± SD for four plates. [a]$p < 0.01$ (compared with the value obtained from the untreated cultures). [b]$p < 0.05$; [c]$p < 0.01$ (compared with the value obtained from the cultures treated with BPA alone). (C) Total RNA was extracted from cells. RNA samples from two plates were combined for each treatment. Twenty μg of RNA was loaded to each lane. Expression of aP2 mRNA was analyzed by Northern blot. 28S shows ethidium bromide-stained rRNA. The representative of two independent experiments is shown. BPA, bisphenol A; BPA-DA, bisphenol A diacetate; BPA-DGE, bisphenol A diglycidyl ether; BPA-BCF, bisphenol A bis(chloroformate); BPA-DM, bisphenol A dimethacrylate; BPA-DAA, bisphenol A *O,O*-diacetic acid; BPA-DC, bisphenol A dicyanate.

担当者 大関一男

著者名: Mu X, Rider CV, Hwang GS, Hoy H, LeBlanc GA

論文題名 : Covert signal disruption: anti-ecdysteroidal activity of bisphenol A involves cross talk between signaling pathways

出典: Environ Toxicol Chem. 2005 Jan;24(1):146-52

チェック項目:

1．対象生物 ( )ラット ( ) マウス、 ( ) 人、 (x) その他 ミジンコ
2．影響の標的臓器 ( ) 神経系、 ( ) 免疫系、 ( ) 生殖系、 (x) その他 脳
3．影響の種類 ( ) 細胞、 (x) 組織、 ( ) 個体、 ( ) その他
4．曝露方法 ( ) 経口、 ( ) 埋め込み、その他 水中での飼育
5．曝露時期 (X) 胚･胎児、 ( ) 周産期、 ( ) 出生後、 ( ) 成熟動物、 ( ) 細胞
6．曝露濃度 用量段階 (1 ppm - 20 ppm)
7．観察された影響の種類と濃度：
(9 ppm ; Intermolt duration)
8．観察時期 (x) 出生前、 ( ) 出生後、 ( ) 思春期、 ( ) 成熟期
9．論文中に低用量影響への関心 ( )あり、(x)なし
10. 試験の信頼性について下記項目でチェックする。
GLP に準拠 ( )はい、(x)いいえ 論文中に「GLP に準拠」の記述の有無
ガイドラインへの準拠 ( )はい：ガイドラインの名称、(x)いいえ

論文の概要:

無脊椎動物での内分泌かく乱による毒性メカニズムを調べため、2つのエクジステロイド依存性の生理プロセスをみた。ビスフェノールAはintermolt periodが長くし、胚成長阻害を誘発するアンチーエクジステロイド活性が認められる。これは、endogeneousなエクジステロイド活性の低下によるものでなく、エクジステロイド受容体のアンタゴニズムによるものである。ビスフェノールAはjuvenoidホルモン活性を誘発することはないが、crustacean juvenoid ホルモン methyl farnesoate活性を誘発する。ミジンコの生殖機能への影響として母体への毒性があるオーダーより一桁低い暴露レベルでの濃度―応答関係が観察された。これより、ミジンコ等の降格動物に対し、慢性毒性があり、それはエクジステロイド/juveniod制御系を阻害することによるものと思われる。しかしながら、慢性毒性を誘発する濃度は環境中で検出される濃度よりはるかに高く、これによる環境影響はないものと推定される

添付資料

Figure 1, Figure 3, Figure 5, Table 1.

評価者のコメント：

低投与作用関連の報告に属するものではなく、特にコメントなし。

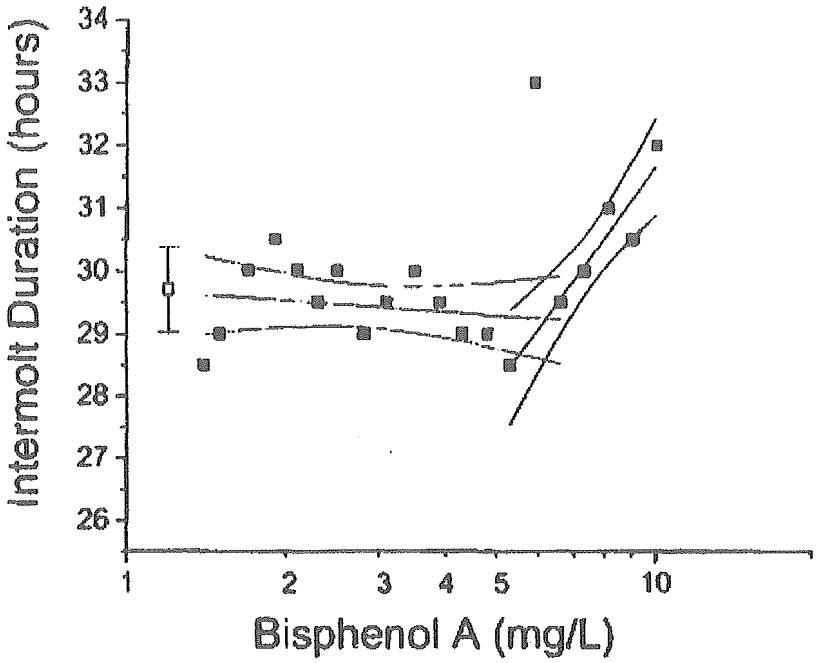

Fig. 1. Intermolt duration between birth and the first molt of neonatal daphnids exposed to concentrations of bisphenol A. Each data point represents an individual daphnid. The data were fit to a two-segmented line with associated 95% confidence intervals. Mean (± standard deviation) performance of 10 unexposed (control) daphnids is depicted by the open square.

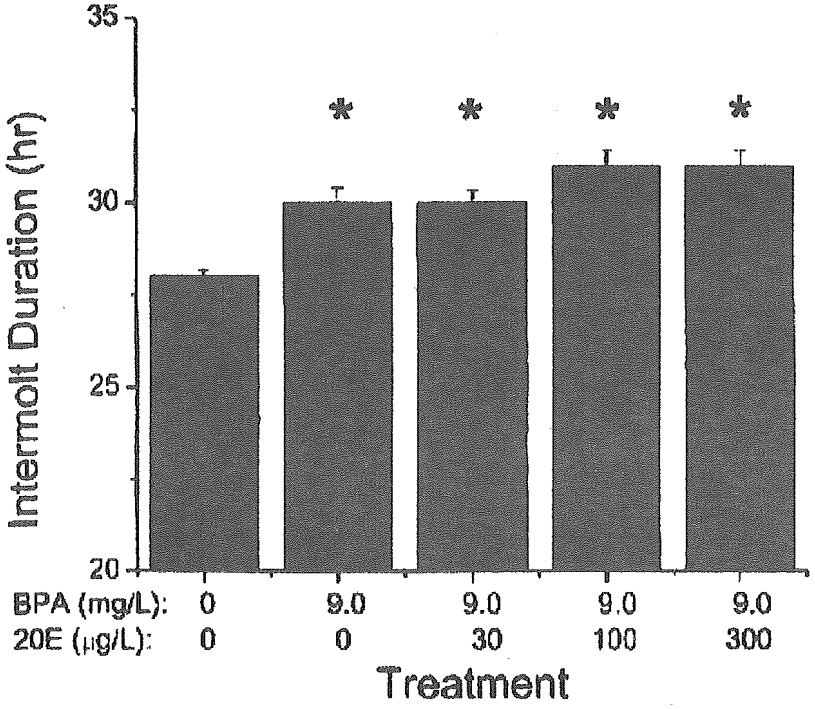

Fig. 3. Influence of 20-hydroxyecdysone (20E) exposure on delayed molting caused by bisphenol A (BPA). Neonatal daphnids (<1 h old) were individually exposed to 9.0 mg/L bisphenol A alone or in combination with concentrations of 20E. The duration of the first intermolt period was measured as described in the *Methods*. Data are presented as mean and standard deviation ($n = 8$). An asterisk indicates a significant difference from the control at $p \leq 0.05$ (analysis of variance, Dunnett's t test).

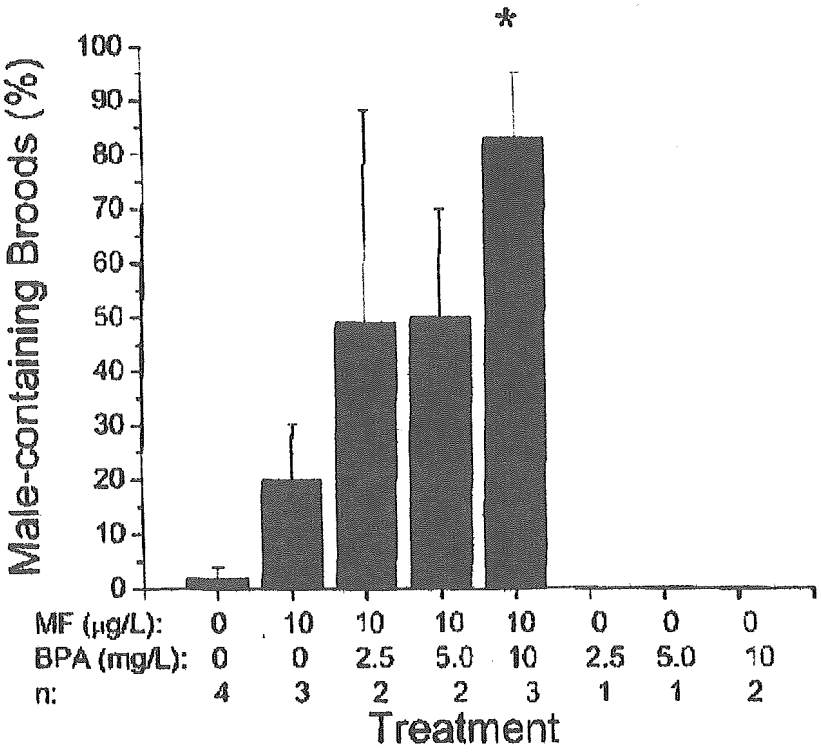

Fig. 5. Incidence of male-sex determination among offspring produced by maternal daphnids exposed to methyl farnesoate (MF), bisphenol A (BPA), or combinations thereof. Maternal daphnids (7–10 per treatment) were exposed to the chemicals, as indicated, through a complete period of oocyte/embryo maturation. Resulting neonates were sexed upon release from the maternal daphnids and the percentage broods that contained male offspring were determined for each treatment. The n denotes the number of times the experiment was repeated. Data are presented as the mean and standard error of the replicate experiments. An asterisk denotes a significant difference ($p \leq 0.05$, analysis of variance following arcsin transformation) from untreated daphnids and daphnids treated with MF alone (Student's t test).

Table 1. Susceptibility of variously aged embryos to the developmental toxicity of bisphenol A. Age denotes the length of time that embryos were allowed to develop in the brood chambers of maternal organisms following transfer from the ovaries. Embryos were removed at the designated time and allowed to continue development ex vivo while exposed to the indicated concentration of bisphenol A. The incidence of developmental abnormalities was assessed upon completion of development among the respective control embryos (~72 h). Each treatment consisted of 19 to 36 embryos

Experiment	Embryo age (h)	Bisphenol A (mg/L)	Developmental abnormalities (%)
1	6.5	0	0
		2.5	56
2	8.0	0	0
		10.0	35
3	8.0	0	0
		5.0	17
		10.0	21
4	12	0	0
		5.0	0
		10.0	0
5	18	0	0
		20.0	54

caused by a mechanism of toxicity distinct from that responsible for acute toxicity [26].

担当者 大西 純一

著者名: Nishizawa H, Morita M, Sugimoto M, Imanishi S, Manabe N.

論文題名: Effects of in utero exposure to bisphenol a on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos.

出典: J Reprod Dev. 2005 Jun;51(3):315-24. Epub 2005 Feb 28.

チェック項目:

1. 対象生物 ( )ラット、 (x) マウス、 ( ) 人、 ( ) その他_____
2. 影響の標的臓器 (x) 神経系、 ( ) 免疫系、 ( ) 生殖系、 ( ) その他_____
3. 影響の種類 ( ) 細胞、 ( ) 組織、 ( ) 個体、 (x) その他 mRNA レベル
4. 曝露方法 (x) 経口、 ( ) 埋め込み、その他_____
5. 曝露時期 (x) 胚・胎児、 ( ) 周産期、 ( ) 出生後、 ( ) 成熟動物、 ( ) 細胞
6. 曝露濃度 用量段階 (0.02, 2, 200, 20000 μg/kg/日)
7. 観察された影響の種類と濃度: ( )
8. 観察時期 (x) 出生前、 ( ) 出生後、 ( ) 思春期、 ( ) 成熟期
9. 論文中に低用量影響への関心 (x)あり、( )なし
10. 試験の信頼性について下記項目でチェックする。

GLP に準拠 ( )はい、(x)いいえ 論文中に「GLP に準拠」の記述の有無

ガイドラインへの準拠 ( )はい:ガイドラインの名称_____、(x)いいえ

論文の概要:

BPA の評価のために、胚発生におけるアリルハイドロカーボン受容体 (AhR) の mRNA 発現レベルを調べた。マウス胎児に BPA を交尾後 6.5-13.5 日あるいは 6.5-17.5 日にばく露した。その結果、極低濃度(0.02 μg/kg、環境ばく露の 1/100)において大脳、小脳、生殖腺 (精巣、卵巣) での AhR の mRNA 発現が、交尾 14.5 日と 18.5 日の雌雄胎児で明らかに増加した。2, 200, 20000 μg/kg でも AhR の mRNA が増加した。BPA ばく露後 14.5 日胎児の生殖腺の AhR mRNA レベルが増加し、二相性 (U 字) の用量反応曲線を示したが、18.5 日では逆 U 字型の用量反応曲線を得た。BPA ばく露により、RAR(レチノイン酸受容体) α と RXR(レチノイド X 受容体) α の mRNA 発現レベルが 14.5 日と 18.5 日の雌雄胎児の大脳、小脳、生殖腺で増加した。極低濃度(0.02 μg/kg) の BPA ばく露により、14.5 日と 18.5 日の雌雄胎児の小脳、14.5 日の雌胎児の生殖腺の RAR α の mRNA 発現を増加させ、14.5 日の雌雄胎児の大脳、小脳の RXR α の mRNA 発現を著しく増加させた。この知見は、極低濃度の BPA 子宮ばく露はマウス胎児において、AhR、RAR α、RXR の mRNA 発現を upregulate し、受容体依存性の情報伝達系をかく乱することを確認した。そしてまた、それは胎児での生体異物代謝とレチノイド情報伝達系への BPA の毒性影響評価に寄与することが出来る。

添付資料

Fig. 1, 2, 3

評価者のコメント:

AhR、RAR α、RXR の mRNA レベル変化を調べて BPA の代謝への影響と胚形成受容体への影響を明らかにしようとしている。著者等は、「RXR とチロイドホルモン受容体はヘテロダイマーを形成しており、BPA は恐らく RXR 経由のレチノイド情報伝達系のかく乱を通してチロイドホルモン受容体を介した転写を妨害していると思われる」と述べている。また、マウス胎児において、BPA ばく露は XMEs(CYP1A1, CYP1A2, CYP1B1 等)の mRNA レベルを直接コントロールし、そして内因性エストロゲン代謝を触媒している AhR の mRNA レベルを増加させることが既に知られている。そして、著者等は「BPA ばく露は XMEs 経由のエストロゲン代謝をかく乱し、その結果としてエストロゲン依存性情報伝達系がかく乱される」と推測している。

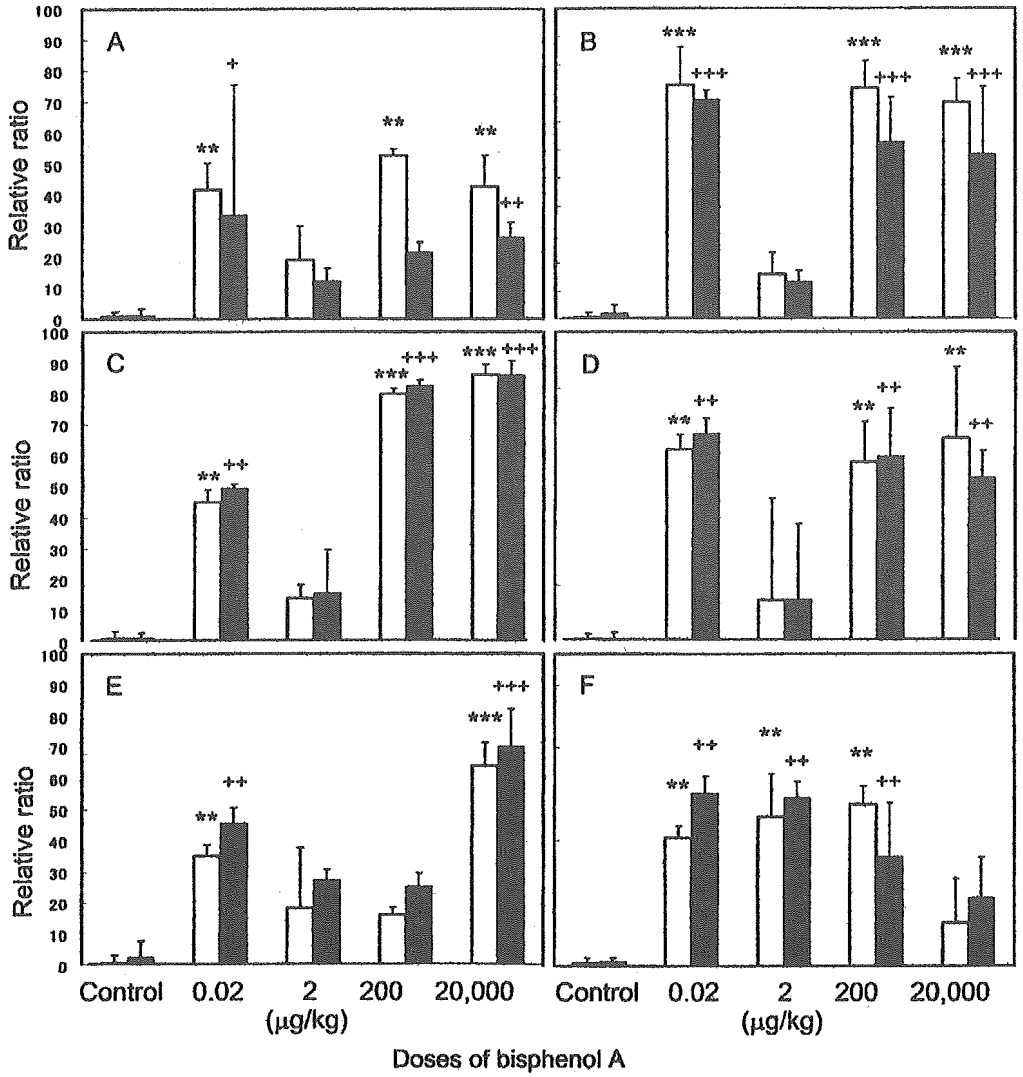

Fig. 1. Changes in expression levels of AhR mRNA in the cerebra (A and B), cerebebella (C and D), and gonads (testes and ovaries: E and F, respectively) of 14.5- (A, C, and E) and 18.5-dpc-embryos (B, D, and F). Open and closed bars represent males and females, respectively. Each value is the mean ± SEM. ** and ***: P<0.01 and 0.001, respectively, vs each male vehicle control. +, ++ and +++: P<0.05, 0.01, and 0.001, respectively, vs each female vehicle control.

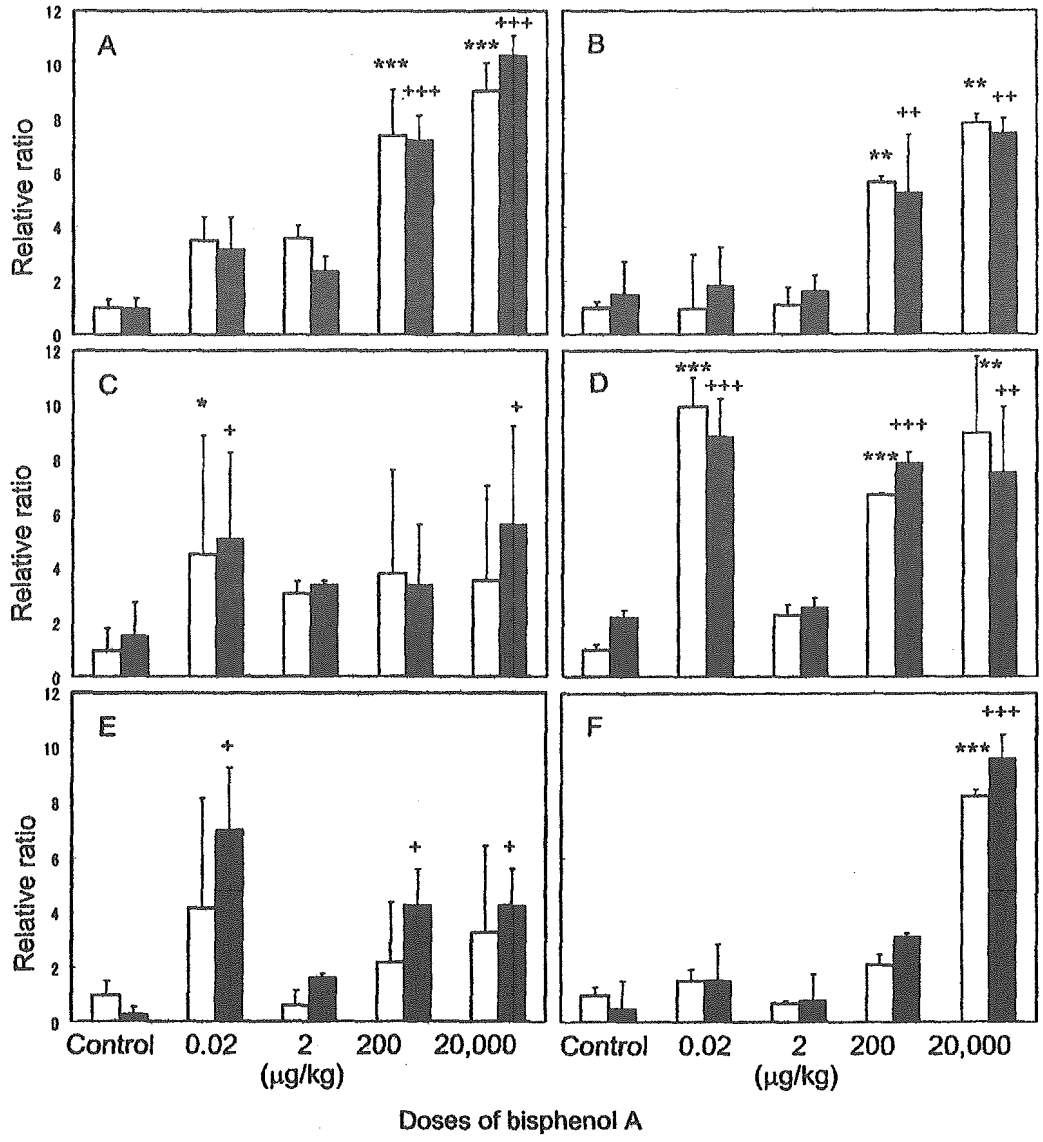

Fig. 2. Changes in expression levels of RARα mRNA in the cerebra (A and B), cerebebella (C and D), and gonads (testes and ovaries: E and F, respectively) of 14.5- (A, C, and E) and 18.5-dpc-embryos (B, D, and F). Open and closed bars represent males and females, respectively. Each value is the mean ± SEM. *, ** and ***: $P<0.05$, 0.01, and 0.001, respectively, vs each male vehicle control. +, ++ and +++: $P<0.05$, 0.01, and 0.001, respectively, vs each female vehicle control.