

MEDLINE®

Effect of Porcine Placenta Extract from Subcritical Water Extraction on Photodamage in Human Keratinocytes.

Abstract

The objective of this study was to evaluate the photoprotective effects of porcine placenta extract (PPE) on ultraviolet B (UVB)-induced oxidative stress in human keratinocytes (HaCaT) to evaluate its functional activities as a skin food ingredient. PPE prepared by subcritical water extraction was termed SPE, and subsequently digested by enzymes to prepare E-SPE. Increased intracellular reactive oxygen species (ROS) levels (192.0%) induced by UVB were decreased by SPE and E-SPE. SPE had more effective ROS scavenging activity than E-SPE treatment. UVB treatment increased expression of tissue inhibitor of metalloproteinase 1 (TIMP-1), and this elevated expression was decreased by E-SPE treatment. High-dose treatment with E-SPE (50 and 100 µg/mL) reduced TIMP-1 expression levels of UVB-C (control) to 33.5 and 34.6%, respectively. In contrast, at low SPE doses (1 and 10 µg/mL), the treatment slightly decreased TIMP-1 expression levels to 73.3% and 71.3% of UVB-C, respectively. In conclusion, the present study demonstrated the protective effect of SPE and E-SPE against UVB damage in keratinocytes via ROS scavenging, down-regulating MMP-2 expression and up-regulating TIMP-1 expression. This highlights the potential for SPE as an ingredient in the preparation of functional food against photoaging.

Citation

Effect of Porcine Placenta Extract from Subcritical Water Extraction on Photodamage in Human Keratinocytes.

**Park Y, Han BK, Choi HS, Hong YH, Jung EY, Suh HJ -
Korean J Food Sci Anim Resour - January 1, 2015; 35 (2);
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Korean journal for food science of animal resources

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Park Y, Han BK, Choi HS, Hong YH, Jung EY, Suh HJ

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MEDLINE®

Effects of Porcine Placenta Extract Ingestion on Ultraviolet B-induced Skin Damage in Hairless Mice.

Abstract

The aim of our study was to evaluate the potential benefits of an oral supplement containing porcine placenta extract (PPE) on skin parameters related to cutaneous physiology and aging. PPEs were administered orally to hairless mice for 12 wk. The effects of oral PPE administration on skin water-holding capacity and Transepidermal Water Loss (TEWL) were similar to those of oral collagen (HYCPU2) administered as a positive control. Magnified photographs and replica images showed a reduction in UVB-induced wrinkle formation after collagen and PPE treatments. PPE treatments ameliorated the thicker skin surface that results from UVB exposure, based on a histological examination of skin tissue. The groups that were orally administered PPE (0.05%, OL; 0.1%, OH group) showed significantly reduced Matrix Metalloproteinase-2 (MMP-2) mRNA expression levels compared with the UVB control (Con), by 33.5% and 35.2%, respectively. The mRNA expression of another collagen-degrading protein, MMP-9, was also significantly lower in the groups that received oral administration of PPE (especially in the OH group) than in the control group. Additionally, oral administration of PPE significantly upregulated tissue inhibitor of metalloproteinase-1 (TIMP-1) and -2 mRNA expression levels compared with expression levels in the control group ($p < 0.05$). This indicates that orally administered PPE activated the expression of Timp-1 and -2, inhibitors of MMP, which is responsible for collagen degradation in skin. Taken together, we propose that long-term oral administration of PPE might have a beneficial effect with respect to skin photo-aging.

Citation

Effects of Porcine Placenta Extract Ingestion on Ultraviolet B-induced Skin Damage in Hairless Mice.

Hong KB, Park Y, Kim JH, Kim JM, Suh HJ - Korean J Food Sci Anim Resour - January 1, 2015; 35 (3); 413-20

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Full Source Title

Korean journal for food science of animal resources

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Author Affiliation

Authors

Hong KB, Park Y, Kim JH, Kim JM, Suh HJ

MEDLINE®

Enhanced Anti-inflammatory Effects of γ -irradiated Pig Placenta Extracts.

Abstract

Porcine placenta extract (PPE) is known to possess anti-inflammatory properties owing to its high concentration of bioactive substances. However, the need to eliminate blood-borne infectious agents while maintaining biological efficacy raises concerns about the optimal method for sterilizing PPE. Therefore, the objective of this study was to compare the effects of the standard pressurized heat (autoclaving) method of sterilization with γ -irradiation on the anti-inflammatory effects of PPE. The anti-inflammatory actions of these two preparations of PPE were evaluated by measuring their inhibitory effects on the production of NO, the expression of iNOS protein, and the expression of iNOS, COX2, TNF- α , IL-1 β , and IL-6 mRNA in lipopolysaccharide-stimulated RAW 264.7 cells. Compared with autoclaved PPE, γ -irradiated PPE showed significantly greater inhibition of NO production and iNOS protein expression, and produced a greater reduction in the expression of iNOS, COX2, TNF- α , IL-1 β , and IL-6 mRNA. These results provide evidence that the sterilization process is crucial in determining the biological activity of PPE, especially its anti-inflammatory activity. Collectively, our data suggest that γ -irradiated PPE acts at the transcriptional level to effectively and potently suppresses the production of NO and the expression of pro-inflammatory cytokines.

Citation

Enhanced Anti-inflammatory Effects of γ -irradiated Pig Placenta Extracts.

**Kim KC, Heo JH, Yoon JK, Jang Y, Kim YK, Kim CK, Oh YK,
Kim YB - Korean J Food Sci Anim Resour - January 1, 2015;
35 (3); 293-8**

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Authors

**Kim KC, Heo JH, Yoon JK, Jang Y, Kim YK, Kim CK, Oh
YK, Kim YB**

MEDLINE®

Efficacy and safety of human placenta extract in alleviating climacteric symptoms: prospective, randomized, double-blind, placebo-controlled trial.

Abstract

To assess the efficacy and safety of human placenta extract in the relief of climacteric symptoms.

A prospective, randomized, double-blind, placebo-controlled trial was performed on 108 women with menopausal symptoms. Human placenta extract or placebo was administered to the women for 4 weeks. Climacteric symptoms were assessed with the Kupperman Index (KMI).

Both groups showed a significant reduction in the KMI score at the end of treatment. However, the decrease in the KMI score was significantly greater in the product group than in the placebo group (-12.30 +/- 10.44 vs -7.15 +/- 9.11, P = 0.012) after 4 weeks of treatment. The level of lipid profiles and liver function tests demonstrated no significant changes before and after treatment in both groups.

Human placenta extract reduced climacteric symptoms more than the placebo. The safety evaluation showed a good safety and tolerability profile in the placenta extract group. The results of the present study suggest that human placenta extract can be an alternative therapy in women with menopausal symptoms.

Citation

Efficacy and safety of human placenta extract in alleviating climacteric symptoms: prospective, randomized, double-

blind, placebo-controlled trial.

Lee YK, Chung HH, Kang SB - J. Obstet. Gynaecol. Res. -

December 1, 2009; 35 (6); 1096-101

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Author Affiliation

Authors

Lee YK, Chung HH, Kang SB

MeSH Terms (9)

- **Climacteric /blood /drug effects ***
- **Double-Blind Method**
- **Female**
- **Humans**
- **Middle Aged**
- **Placenta /chemistry ***
- **Pregnancy**
- **Prospective Studies**
- **Tissue Extracts /administration & dosage * /adverse effects**

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MEDLINE®

Protective effect of placenta extracts against nitrite-induced oxidative stress in human erythrocytes.

Abstract

Aqueous-saline human placenta extract (HPE) is known to possess antioxidant activity due to the high concentration of bioactive substances. This fact allows its application in clinical practice in order to treat oxidation-induced diseases. Extract antioxidant activity is mainly conditioned by proteins. Freezing of extracts has been shown to lead to their antioxidant activity increasing due to protein conformation changes. Different biological models are widely used in order to evaluate efficacy of novel antioxidants and mechanisms of their action. One such model appears to be erythrocytes under nitrite-induced oxidative stress. Nitrite is known to be able to penetrate erythrocyte membrane and to oxidize hemoglobin. In order to investigate whether HPE is able to decrease nitrite-induced oxidative injuries and to evaluate the protein contribution to this process, spectrophotometric and electron spin resonance (ESR) assays were used. Experimental data have revealed that antioxidant activity of extracts and of some of their fractions correlates with methemoglobin concentration lowering. Preliminary erythrocyte incubation with an extract fraction of 12 kDa allows preservation of the structural-dynamic cytosol state the closest to the control.

Citation

Protective effect of placenta extracts against nitrite-induced oxidative stress in human erythrocytes.

Rozanova S, Cherkashina Y, Repina S, Rozanova K, Nardid O - Cell. Mol. Biol. Lett. - June 1, 2012; 17 (2); 240-8

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Author Affiliation

Authors

**Rozanova S, Cherkashina Y, Repina S, Rozanova K,
Nardid O**

MeSH Terms (10)

- **Electron Spin Resonance Spectroscopy**
- **Erythrocytes /metabolism ***
- **Female**
- **Humans**
- **Methemoglobin /metabolism**
- **Nitrites /toxicity**
- **Oxidative Stress /drug effects ***
- **Placenta * /chemistry /metabolism**
- **Pregnancy**
- **Spectrophotometry**

MEDLINE®

Cow placenta extract promotes murine hair growth through enhancing the insulin - like growth factor-1.

Abstract

Hair loss is seen as an irreversible process. Most research concentrates on how to elongate the anagen, reduce the negative factors of obstructing hair growth and improve the hair number and size.

In our experiment, we tried to prove that the cow placenta extract can promote hair growth by elongating hair shaft and increasing hair follicle number.

Cow placenta extract (CPE), water and minoxidil applied separately on the back of depilated B57CL/6 mice for the case, negative and positive control respectively. We checked the proliferation of cells which are resident in hair sheath, and the expression of a few growth factors which stimulate hair growth.

Result shows that placenta extract more efficiently accelerates cell division and growth factor expression, by raising the insulin-like growth factor (IGF-1) mRNA and protein level to increase HF size and hair length.

The extract is not a purified product; so, it is less effective than minoxidil, which is approved by the US FDA for the treatment of male pattern baldness. If refinement is done, the placenta extract would be a good candidate medicine for hair loss.

Citation

Cow placenta extract promotes murine hair growth through enhancing the insulin - like growth factor-1.

Zhang D, Lijuan G, Jingjie L, Zheng L, Wang C, Wang Z, Liu L, Mira L, Sung C - Indian J Dermatol - January 1, 2011; 56 (1); 14-8

MEDLINE is the source for the citation and abstract for this record

Full Source Title

Indian journal of dermatology

NLM Citation ID

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Language

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Author Affiliation

Authors

Zhang D, Lijuan G, Jingjie L, Zheng L, Wang C, Wang Z, Liu L, Mira L, Sung C

MEDLINE®

Antioxidative collagen-derived peptides in human-placenta extract.

Abstract

Human-placenta extract (PLx), has been widely used in clinical and cosmetic fields, and is known to possess marked antioxidant activity. We have attempted to identify the antioxidative components in PLx. Initially, we purified PLx using Sephadex G-10 column. The first eluted peak which had optical density at 280nm and exhibited approximately 20 per cent of antioxidant activity of the applied PLx, did not contain uracil, tyrosine, phenylalanine, or tryptophan which are the previously identified antioxidants from PLx. This fraction was further purified by reverse-phase high performance liquid chromatography. All eluted peaks containing antioxidant activity exhibited optical density at 280nm. Six separate fractions of eluent having antioxidant activity were analyzed by an amino acid sequencer, and each turned out to contain Glycine(G)-XY amino acid repeats, which appear to be derived from collagen. These results suggest that peptides produced from collagen are also antioxidative components of PLx.

Citation

Antioxidative collagen-derived peptides in human-placenta extract.

Togashi S, Takahashi N, Iwama M, Watanabe S, Tamagawa K, Fukui T - Placenta - July 1, 2002; 23 (6); 497-502

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Full Source Title

Placenta

NLM Citation ID

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Author Affiliation

Authors

**Togashi S, Takahashi N, Iwama M, Watanabe S,
Tamagawa K, Fukui T**

MeSH Terms (11)

- **Adult**
- **Antioxidants /analysis ***
- **Chromatography, Gel**
- **Chromatography, High Pressure Liquid**
- **Collagen /analysis ***
- **Female**
- **Humans**
- **Peptides /analysis ***
- **Placenta /chemistry ***
- **Pregnancy**
- **Tissue Extracts /analysis ***

MEDLINE®

Effect of mesenchymal stem cells and extracts derived from the placenta on trophoblast invasion and immune responses.

Abstract

Tightly regulated trophoblast invasion and immunomodulation at the feto-maternal interface is important during implantation and fetal development. Although trophoblasts as a pregnancy-specific cell has been reported to be a key factor capable of regulating certain events during implantation, however, its regulatory mechanisms are still unclear. In this study, we analyzed the effects of chorionic plate-derived mesenchymal stem cells (CP-MSCs) and human placenta extract (hPE) isolated from human normal placentas on trophoblasts invasion and immune responses. We investigated the effects of CP-MSCs, hPE treatment, and their combination on trophoblasts invasion and on T-cells suppression through human leukocyte antigen-G (HLA-G) expression. Trophoblasts invasion was significantly increased by co-culture of CP-MSCs or by hPE treatment ($P < 0.05$), and enhanced by the combination of CP-MSCs and hPE treatment ($P < 0.05$). The proliferation of T-cells was decreased by co-culture of CP-MSCs and hPE treatment, whereas the population of regulatory T-cells was increased ($P < 0.05$). Also, the dynamics alterations of multiple cytokines were observed in the culture supernatants of trophoblasts and T-cells depending on CP-MSCs co-culture and hPE treatment. Interestingly, the concentration of soluble HLA-G was increased by CP-MSCs co-culture, by hPE treatment and by combination of them on trophoblasts and activated T-cells ($P < 0.05$). These findings suggested that CP-MSCs and hPE can regulate trophoblasts invasion and T-cell by alteration of

HLA-G expression. These results will provide understandings of trophoblasts invasion and the immunological network at the feto-maternal interface during pregnancy and contribute to the foundation of a new treatment strategy for pregnancy disorders.

Citation

Effect of mesenchymal stem cells and extracts derived from the placenta on trophoblast invasion and immune responses.

Choi JH, Jung J, Na KH, Cho KJ, Yoon TK, Kim GJ - Stem Cells Dev. - January 15, 2014; 23 (2); 132-45

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Full Source Title

Stem cells and development

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Language

eng

Author Affiliation

Authors

Choi JH, Jung J, Na KH, Cho KJ, Yoon TK, Kim GJ

MeSH Terms (13)

- **Cell Movement**
- **Cell Proliferation**
- **Cells, Cultured**
- **Coculture Techniques**
- **Embryo Implantation /immunology**
- **Female**
- **HLA-G Antigens /blood**

- **Humans**
- **Mesenchymal Stromal Cells /immunology ***
- **Placenta /cytology /metabolism ***
- **Pregnancy**
- **T-Lymphocytes, Regulatory /immunology ***
- **Trophoblasts /cytology * /immunology ***

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MEDLINE®

L-tryptophan as an antioxidant in human placenta extract.

Abstract

An antioxidant was purified from human placenta extract (HPE) by using gel filtration, liquid-liquid extraction, silicagel column chromatography, and HPLC. The purified antioxidant was identified to be L-tryptophan (L-Trp). L-Trp showed higher inhibitory activity than mannitol and DMSO on the Fenton reaction-induced degradation of 2-deoxy-D-ribose. L-Trp also had much higher inhibitory activity on the cytochrome P-450-dependent lipid peroxidation than the previously identified antioxidants of HPE, L-phenylalanine, L-tyrosine and uracil. On the other hand, the inhibitory effect of L-Trp on the Fenton reaction-induced protein oxidation was smaller than that of uracil. These results suggest that L-Trp is a main antioxidant of HPE of which the effect is based on the suppression of lipid peroxidation in the oxidative stress status.

Citation

L-tryptophan as an antioxidant in human placenta extract.
Watanabe S, Togashi S, Takahashi N, Fukui T - J. Nutr. Sci. Vitaminol. - February 1, 2002; 48 (1); 36-9
MEDLINE is the source for the citation and abstract for this record

Full Source Title

Journal of nutritional science and vitaminology

NLM Citation ID

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Author Affiliation

Authors

Watanabe S, Togashi S, Takahashi N, Fukui T

MeSH Terms (9)

- **Analysis of Variance**
- **Antioxidants /analysis ***
- **Chromatography, Gel**
- **Chromatography, High Pressure Liquid**
- **Deoxyribose /metabolism**
- **Humans**
- **Lipid Peroxidation /drug effects**
- **Placental Extracts /chemistry ***
- **Tryptophan /analysis ***

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MEDLINE®

Analysis of free and bound NADPH in aqueous extract of human placenta used as wound healer.

Abstract

NADPH is an important biomolecule involved in cellular regeneration. The distribution of free and bound NADPH in aqueous extract of human placenta used as a potent wound healer has been analyzed. Quantification from fluorescence and immuno-affinity chromatography indicates that 75.1±2.2% of NADPH present in the extract exists as free nucleotide or bound to very small peptides or amino acids whereas the rest remains bound to large peptides. Inability to dissociate the bound form of the nucleotide from the large peptides using urea or guanidium hydrochloride indicates that the binding is covalent. Identification of a fragmented mass of m/z 382.94 (nicotinamide+sugar+phosphate) from the NADPH-peptide conjugates supported the intactness of the nicotinamide moiety. Glutathione reductase assay indicated that 95.2±3.5% of the total NADPH pool of the extract can act as cosubstrate of the enzyme. This indicates that while a major fraction of free NADPH of the extract is easily available for cellular processes, the rest can also function locally where the conjugated peptides are deposited.

Citation

Analysis of free and bound NADPH in aqueous extract of human placenta used as wound healer.

De D, Chakraborty PD, Bhattacharyya D - J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. - August 15, 2009; 877 (24); 2435-42

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Full Source Title

Journal of chromatography. B, Analytical technologies in the biomedical and life sciences

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Author Affiliation

Authors

De D, Chakraborty PD, Bhattacharyya D

MeSH Terms (8)

- **Chromatography, Affinity /methods**
- **Female**
- **Humans**
- **Molecular Weight**
- **NADP /analysis * /metabolism**
- **Peptides /chemistry**
- **Placenta /chemistry * /metabolism**
- **Wound Healing ***

MEDLINE®

Culture and in vitro hepatogenic differentiation of placenta-derived stem cells, using placental extract as an alternative to serum.

Abstract

Translational research using adult stem cells derived from various tissues has been highlighted in cell-based therapy. However, there are many limitations to using conventional culture systems of adult stem cells for clinical applicability, including limited combinations of cytokines and use of nutrients derived from animals. Here, we have investigated the effects of placental extract (PE) for culture of placenta-derived stem cells (PDSCs) as well as their potential for hepatogenic differentiation.

Placental extract, extracted using water-soluble methods, was used as a supplement for culture of PDSCs. Cell viability was determined using the MTT assay, and cytokine assay was performed using Luminex assay kit. Gene expression, indocyanine green (ICG) up-take, PAS (Periodic Acid-Schiff) staining and urea production were also analysed.

The placental extract contained several types of cytokine and chemokine essential for maintenance and differentiation of stem cells. Expression of stemness markers in PDSCs cultured with PE is no different from that of PDSCs cultured with foetal bovine serum (FBS). After hepatogenic differentiation, expression patterns for hepatocyte-specific markers in PDSCs cultured with PE were consistent and potential for hepatogenic differentiation of PDSCs cultured with PE was similar to that of PDSCs cultured with FBS, as shown by PAS staining and urea production assays.

Our findings revealed that placental extract could be used as a new component for culture of adult stem cells, as well as for development of human-based medium, in translational research for regenerative medicine.

Citation

Culture and in vitro hepatogenic differentiation of placenta-derived stem cells, using placental extract as an alternative to serum.

Shin KS, Lee HJ, Jung J, Cha DH, Kim GJ - Cell Prolif. - October 1, 2010; 43 (5); 435-44

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Cell proliferation

NLM Citation ID

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Language

eng

Author Affiliation

Authors

Shin KS, Lee HJ, Jung J, Cha DH, Kim GJ

MeSH Terms (16)

- **Adult**
- **Cell Differentiation ***
- **Cell Line**
- **Cell Proliferation**
- **Cell Survival**
- **Cells, Cultured**
- **Cytokines /isolation & purification**

- **Female**
- **Hepatocytes /cytology ***
- **Humans**
- **Placenta /cytology ***
- **Placental Extracts /chemistry /metabolism ***
- **Pregnancy**
- **Proteins /isolation & purification /metabolism**
- **Stem Cells /cytology ***
- **Trophoblasts /cytology**

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MEDLINE®

Culture and in vitro hepatogenic differentiation of placenta-derived stem cells, using placental extract as an alternative to serum.

Abstract

Translational research using adult stem cells derived from various tissues has been highlighted in cell-based therapy. However, there are many limitations to using conventional culture systems of adult stem cells for clinical applicability, including limited combinations of cytokines and use of nutrients derived from animals. Here, we have investigated the effects of placental extract (PE) for culture of placenta-derived stem cells (PDSCs) as well as their potential for hepatogenic differentiation.

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Our findings revealed that placental extract could be used as a new component for culture of adult stem cells, as well as for development of human-based medium, in translational research for regenerative medicine.

Citation

Culture and in vitro hepatogenic differentiation of placenta-derived stem cells, using placental extract as an alternative to serum.

Shin KS, Lee HJ, Jung J, Cha DH, Kim GJ - Cell Prolif. - October 1, 2010; 43 (5); 435-44

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Cell proliferation

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Language

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Author Affiliation

Authors

Shin KS, Lee HJ, Jung J, Cha DH, Kim GJ

MeSH Terms (16)

- **Adult**
- **Cell Differentiation ***
- **Cell Line**
- **Cell Proliferation**
- **Cell Survival**
- **Cells, Cultured**
- **Cytokines /isolation & purification**

- **Female**
- **Hepatocytes /cytology ***
- **Humans**
- **Placenta /cytology ***
- **Placental Extracts /chemistry /metabolism ***
- **Pregnancy**
- **Proteins /isolation & purification /metabolism**
- **Stem Cells /cytology ***
- **Trophoblasts /cytology**

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ARTÍCULO

Anti-oxidative and anti-inflammatory activities of placental extracts in benzo[a]pyrene-exposed rats

S.Y. Park, S. Phark, M. Lee, J.Y. Lim y D. Sul

Placenta, 2010-10-01, Volúmen 31, Número 10, Pages 873-879, Copyright © 2010 Elsevier Ltd

Abstract

Objective

Placental extracts (PE) have been used for years as a folk remedy in Asian countries. PE mediates alleviation of menopausal symptoms, wound healing, liver regeneration and anti-inflammatory responses. In this study, we evaluated the protective effects of PE on rats exposed to benzo[a]pyrene (BaP).

Methods

The composition of amino acids, sugars and fatty acids in PE was analyzed. The effect of PE on DNA damage was determined by Comet assay, and oxidative damage was determined by measuring the activity of superoxide dismutase and the levels of lipid peroxidation. The effect of PE on cytokines and immunoglobulin levels was determined by western blot analysis.

Results

Exposure of rats to BaP significantly increased the Olive Tailmoments compared to controls, while pre-treatment with PE composed of diverse amino acids, monosaccharides and fatty acids significantly decreased the Olive Tailmoments induced by BaP. In addition, oxidative stress induced by BaP was attenuated by pre-treatment with PE. Furthermore, PE pre-treatment significantly decreased the levels of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6.

Conclusion

Pre-treatment of rats with PE significantly attenuates oxidative damage and immunotoxicity induced by BaP. These findings suggest the further studies regarding the protective effects of PE against environmental toxicants in humans.

1 Introduction

Placental extracts (PE) have been used for years as a wound healer and a cosmetic in many countries including India [1]. It is enriched in enzymes, nucleic acids, vitamins, amino acids, steroids, fatty acids, and minerals [2]. PE mediates immunotrophic, anti-oxidative, and anti-inflammatory responses. The major anti-oxidant components in PE are uracil, tyrosine, phenylalanine, and tryptophan. About 59% of the anti-oxidative effects of PE can be attributed to these components [3]. Peptides produced from collagen have also been reported to be anti-oxidative components of PE [4]. PE is widely marketed in Asian countries for its wound healing, immunotropic and anti-inflammatory activities [2].

Benzo[a]pyrene (BaP) is a well-known carcinogen classified into a Group 1 polycyclic aromatic hydrocarbon (PAH) by the International Agency for Research on Cancer (IARC) [5]. It is present in food, the workplace, and the environment. Grilled or smoked meat is the major source of dietary BaP intake [5]. BaP undergoes a metabolic activation to form reactive intermediates such as BaP-quinones. These intermediates are known to be involved in generating reactive oxygen species (ROS) and are associated with oxidative alteration of DNA, proteins, and anti-oxidant enzymes [6]. These reactive intermediates have mutagenic and carcinogenic effects in biological systems [7].

In the present study, we evaluated the protective effects of PE in rats exposed to BaP. To determine if PE protects lymphocytes from BaP-induced DNA damage, rats were exposed to PE-only, BaP-only or BaP + PE, and lymphocytes were then separated from the whole blood of rats and assayed using a Comet assay. We also measured levels of superoxide dismutase (SOD), malondialdehyde (MDA), and carbonyl to evaluate the possible anti-oxidant effect of PE against BaP in rat plasma. Furthermore, we evaluated the anti-inflammatory effect of PE treatment by examining the levels of immunoglobulins and pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6.

2 Materials and methods

2.1 Chemicals

BaP, benzyl alcohol, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Human PE was provided by Melsmon Pharmaceutical Co., Ltd. (Tokyo, Japan). PE (2 ml) contains 100 mg of constituents and 0.03 ml of benzyl alcohol.

2.2 High performance liquid chromatography (HPLC) analysis of amino acids

The HPLC equipment used in the present study was the Shimadzu System (Shimadzu, Kyoto, Japan), which included Shimadzu LC-10ADVP pumps and a Shimadzu RF-10AXL fluorescence detector. Shim-pack Amino–Na columns (6.0 mm \times 100 mm) were used with guard columns (Shim-pack ISC-30Na, Shimadzu). The column temperature was set at 60 °C by using a Shimadzu CTO-10ACVP column oven. The mobile phase was Mobile Phase Kit-Na, gradient elution (A: sodium citrate buffer, B: sodium citrate buffer containing boric acid, C: sodium

hydroxide solution). The mobile phase was filtered under vacuum through a 0.45 μm membrane filter and degassed before use. The analysis was carried out at a flow rate of 0.4–0.6 ml/min with the detection wavelength set at excitation 254 nm and emission 450 nm, and the injection volume set at 20 μl . The mixture of amino acids type H used as the standard was diluted to 0.1 mol/ml in sodium citrate solution. Fifty microliters of PE was diluted 20 times with the sodium citrate solution for the HPLC analysis.

2.3 Monosaccharide composition analysis

The amount of monosaccharides was measured by using a Bio-LC (DX-300, Dionex Corp., Sunnyvale, CA, USA) equipped with a CarboPac PA1 column (4 \times 250 mm, Dionex) with a guard column (4 \times 50 mm) followed by the method published elsewhere [8]. Monosaccharides were separated with 16 mM NaOH at a flow rate of 1 ml/min.

2.4 Lipids extraction and analysis of fatty acids by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS)

Total lipids were extracted using the monophasic neutral organic solvent system containing 0.01% butylated hydroxytoluene [9]. Fatty acid methyl esters (FAME) were prepared by heating at 60 °C for 30 min with boron trifluoride (BF_3 , Sigma Chemical Co.). FAME were analyzed by GC using a Hewlett–Packard 6890N series gas–liquid chromatography (Agilent Technologies, Wilmington, NC, USA) equipped with a capillary column (30 m \times 0.25 mm) coated with a film of DB-23 (J & W Scientific, Folsom, CA, USA). FAME were dissolved in hexane, injected onto the column, and separated by employing a temperature program from 150 to 250 °C at 2 °C/min, with both the injector and the flame ionization detector temperature set at 250 °C. Helium (ultra pure grade) was used as the carrier gas at a flow rate of 1 ml/min and a split ratio of 50:1 ratio mode. The gas chromatographic identification of fatty acids was verified by GC/MS on an Agilent 6890 GC equipped with an HP-5 column (Agilent Technology). The GC was interfaced to a high-resolution Agilent 5973 mass spectrometer. The obtained mass spectral data were analyzed using an Agilent G1034 mass spectrometry chemstation. Fatty acids were identified according to their elemental composition, the mass of their molecular ions, and the fragmentation patterns compared to those obtained from authentic standards.

2.5 Experimental design

Male Sprague–Dawley rats (173 \pm 5 g) were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). They were housed under standard laboratory conditions (Temperature 24 \pm 2 °C; humidity 50 \pm 10%, 12-h day/night cycles). Animals were allowed to acclimatize to the facility for 1 week, and were provided standard chow diet and drinking water *ab libitum*. Eighty rats (6 weeks old, 5 per group) were divided among the following four groups: 1) a control group (vehicle only), 2) a PE exposure group (20 μl \times 3 times/week for 2 weeks, intramuscular injection), 3) a BaP exposure group (200 mg/kg body weight, intraperitoneal injection) and 4) a PE group (20 μl \times 3 times/week for 2 weeks, intramuscular injection followed by BaP at

200 mg/kg body weight, intraperitoneal injection). The rats in the non-PE-treated groups and PE-treated groups were injected with either diluted benzyl alcohol 20 μ l (0.03 ml of benzyl alcohol diluted in 2 ml of a saline solution) or 20 μ l of PE, respectively. After 2 weeks of injections, BaP in corn oil or corn oil only was injected into the BaP-treated groups and the BaP-untreated groups, respectively. Rats were then sacrificed 1, 2, 4, or 8 days after BaP injection according to the toxicokinetic results of BaP [10].

2.6 Blood sample preparation

Blood samples (EDTA-treated whole blood) were collected from each rat by cardiac puncture. For the Comet assay, lymphocytes were prepared by removing red blood cells from the whole blood (3–5 ml) via centrifugation with a Ficoll-Plaque solution. For other experiments, whole blood was centrifuged at 4000 rpm at 4 °C for 20 min and the resulting plasma was aliquoted and stored at –70 °C until use.

2.7 Comet assay

The comet assays were performed as described previously [11]. For each treatment group, two slides were prepared and each 50–100 randomly chosen cells (total 100–200 cells) were scored manually. The parameter, Olive Tailmoment $[(\text{Tail.mean} - \text{Head.mean}) \times \text{Tail\%DNA}/100]$, was automatically calculated using the Komet 4.0 image analysis system (Kinetic Imaging; Liverpool, UK).

2.8 Determination of oxidative damage

The activity of the anti-oxidant enzyme SOD was measured according to the instructions provided with the kit (Cayman Chemical Company; Ann Arbor, MI, USA). MDA was measured using a thiobarbituric acid test and an HPLC method [12]. The standard curve was made from 1,1,3,3-tetraethoxypropane (TEP) because the acidic hydrolysis of TEP yields stoichiometric amounts of MDA [13].

The level of protein oxidation was evaluated by western blot analysis using anti-dinitrophenylhydrazine antibody. Ten μ g of proteins was separated electrophoretically and transferred onto polyvinylidene difluoride (PVDF) membrane. Thereafter, the membrane was incubated for 1 h with anti-dinitrophenylhydrazine antibody (1:4000; Invitrogen; Carlsbad, CA, USA), and developed with enhanced chemiluminescence reagents (Amersham Biosciences; Piscataway, NJ, USA). Densitometry was then performed using a Bio-Rad (Hercules, CA, USA) 700 flatbed scanner and Molecular Analyst software (Bio-Rad).

2.9 Protein determination, electrophoresis, and immunoblotting

The plasma was mixed with Laemmli buffer and boiled in a boiling water bath for 10 min. The proteins were separated electrophoretically and transferred to PVDF membranes. The following antibodies were used for immunodetection: horseradish peroxidase (HRP) conjugated goat anti-rat IgG1 (1 μ g/ml; Immunology Consultants Laboratory, Newberg, OR, USA), HRP

conjugated goat anti-rat IgG2a, goat anti-rat TNF- α (1 μ g/ml; R&D systems, Minneapolis, MN, USA), goat anti-rat IL-1 β (1 μ g/ml; R&D systems), goat anti-rat IL-6 (1 μ g/ml; R&D systems), goat anti-mouse α -tubulin (clone DM1A; 1:20,000; Sigma–Aldrich). Goat anti-rat and goat anti-mouse secondary antibodies conjugated to horseradish peroxidase (1:1000; Promega; Madison, WI, USA) followed by enhanced chemiluminescence reagents (Amersham Biosciences) were used to detect the target proteins. Immunoreactive bands were imaged using a ChemiDoc XRS system (Bio-Rad). Densitometry of these bands was performed using Quantity One software (Bio-Rad) and values were normalized using α -tubulin as an internal loading control.

2.10 Data analysis

Data in text and figures are expressed as mean \pm SD. Two-group comparisons were evaluated with one-way ANOVA test. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Placental extract contains diverse amino acids, sugars and lipids

3.1.1 Amino acids

The quantities of amino acids in PE were calculated based on the HPLC analysis data. The primary amino acids eluted sequentially from the Shim-pack Amino–Na columns reacted with the continuously added OPA reagent (*o*-phthalaldehyde), and were converted to fluorescent derivatives. As shown in [Table 1 \(tbl1\)](#), glutamic acid, glycine and aspartic acids were the major amino acids included in PE, whereas tyrosine and phenylalanine were also detected with concentrations of 0.133 ± 0.005 , 0.142 ± 0.004 mg/ml, respectively.

Table 1

Amino acid contents in placental extracts.

Amino acids	Contents (mg/ml)	Amino acids	Contents (mg/ml)
Aspartic acid	0.495 ± 0.008	Methionine	0.055 ± 0.002
Threonine	0.232 ± 0.004	Isoleucine	0.130 ± 0.001
Serine	0.283 ± 0.007	Leucine	0.483 ± 0.014
Glutamic acid	0.965 ± 0.017	Tyrosine	0.133 ± 0.005
Proline	0.275 ± 0.005	Phenylalanine	0.142 ± 0.004
Glycine	0.468 ± 0.007	Histidine	0.057 ± 0.002
Alanine	0.362 ± 0.009	Lysine	0.312 ± 0.004
Valine	0.205 ± 0.008	Arginine	0.370 ± 0.010

3.1.2 Monosaccharides

Sugars in PE were separated and detected by using a Bio-LC DX-300 system equipped with a CarboPac PA1 column. N-Acetylneuraminic acid, an N-acyl derivative of amino sugar derivatives, was detected to have the largest amount in PE. N-glycolylneuraminic acid, glucosamine, galactosamine, and glucose were also detected in PE ([Table 2 \(tbl2\)](#)).

Table 2

Sugar contents in placental extracts.

Sugars	Contents (g/100 g)
Glucose	0.01 ± 0.000
Galactosamine	0.05 ± 0.015
Glucosamine	0.10 ± 0.012
N-acetylneuraminic acid	0.23 ± 0.029
N-glycolylneuraminic acid	0.02 ± 0.006

3.1.3 Fatty acids

The major saturated fatty acid of the total extractable lipids in PE was 18:0 with smaller amounts of 16:0, while 18:1 (9) was the largest unsaturated fatty acids present in PE ([Table 3 \(tbl3\)](#)). 16:1 (9) and 18:1 (9) were the monoenoic monounsaturated fatty acids, and 18:2 (9,12), 20:4 (5,8,11,14), and 22:6 (4,7,10,13,16,19) were polyunsaturated fatty acids found in PE. Among them, 20:4 (5,8,11,14) and 22:6 (4,7,10,13,16,19) were highly unsaturated fatty acids of the omega-3 type in PE. Total SFA occupied 78.51 ± 1.240%, whereas total USFA occupied 8.95 ± 0.686% of total extractable lipids in PE. The ratio of SFA/USFA was 8.77.

Table 3

Fatty acid composition from total extractable lipids of placental extracts.

Fatty acid	RRT	Relative amount (%)
14:0	0.333	8.24 ± 3.510
16:0	0.549	11.77 ± 1.392
16:1 (9)	0.601	0.69 ± 0.277
18:0	1.000	54.47 ± 2.018
18:1 (9)	1.086	3.27 ± 2.079
18:2 (9,12)	1.224	0.68 ± 0.113

Fatty acid	RRT	Relative amount (%)
20:0	1.452	0.64 ± 0.009
20:1 (9)	1.487	1.30 ± 0.566
20:4 (5,8,11,14)	1.652	1.53 ± 0.662
22:0	1.858	1.50 ± 0.619
22:6 (4,7,10,13,16,19)	2.450	1.48 ± 0.615
26:0	2.883	1.24 ± 0.552
28:0	3.443	0.64 ± 0.196
Other ^a (tblfn1)		12.54 ± 0.931
SFA		78.51 ± 1.240
USFA		8.95 ± 0.686
SFA/USFA		8.77

a Unidentified fatty acids.

3.2 Placental extract attenuates DNA damage induced by BaP in rats

To evaluate the protective effect of PE against DNA damage induced by BaP in rats, lymphocytes prepared from whole blood were subjected to the Comet assay. The Olive Tailmoments of PE-treated rats in the 1-day group were decreased compared to control groups, whereas those in the 2-, 4-, and 8-day groups were not significantly different from control groups ([Fig. 1 \(fig1\)](#)). In contrast, exposure to BaP significantly increased the Olive Tailmoments compared to control rats and PE-treated rats in all groups. Conversely, rats pre-treated with PE for 2 weeks before BaP exposure exhibited a significant decrease in the Olive Tailmoments in all groups compared to BaP-only treated rats.

Fig. 1

Protective effect of placental extracts (PE) on DNA damage induced by BaP in rats. The effect of PE on DNA damage induced by benzo[a]pyrene (BaP) was evaluated using a Comet assay to measure single-strand DNA

breaks at the single-cell level. BaP treatment increased the Olive Tailmoments above control levels, while pre-treatment with PE prior to BaP exposure significantly decreased the Olive Tailmoments compared to BaP treatment alone. All data represent the mean \pm SD of five different rats. ^a $p < 0.05$ compared to the control rats, ^b $p < 0.05$ compared to PE-treated rats, ^c $p < 0.05$ compared to BaP-treated rats, ^d $p < 0.05$ compared to PE + BaP-treated rats.

3.3 Placental extract attenuates BaP-induced oxidative damage in rats

SOD, lipid peroxidation, and protein oxidation were evaluated to determine if PE has an anti-oxidative protective effect. The levels of SOD in PE-only treated rats were not significantly different from those in controls. However, the levels of SOD in BaP-exposed rats in the 1-, 2-, 4-, and 8-day groups were significantly increased compared to controls and PE-only treated rats ([Fig. 2 \(fig2\) A](#)). Conversely, exposure to PE prior to BaP significantly attenuated the activity of SOD in the 1-, 2-, and 8-day groups compared to BaP-only treated rats.

Fig. 2

The anti-oxidant effect of PE against BaP-induced oxidative stress. Plasma collected from rats exposed to BaP with or without PE pre-treatment was evaluated for oxidative stress markers. (A) The detoxification enzyme, SOD, was measured using commercially available kits. The BaP-induced elevation of SOD was

significantly decreased by pre-treatment with PE in the 1-, 2-, and 8-day groups. (B) The levels of lipid peroxidation were evaluated by measuring the amounts of malondialdehyde using a high performance liquid chromatography method. (C) The levels of protein oxidation were evaluated by western blot analysis using an anti-dinitrophenylhydrazine antibody. All data represent the mean \pm SD of five different rats. ^a $p < 0.05$ compared to the control rats, ^b $p < 0.05$ compared to PE-treated rats, ^c $p < 0.05$ compared to BaP-treated rats, ^d $p < 0.05$ compared to PE + BaP-treated rats.

The anti-oxidant effect of PE was also evaluated by measuring the amount of MDA produced in rat plasma using a thiobarbituric acid test. As shown in Fig. 2 (fig2) B, the production of MDA in control rats was not significantly different among the 1-, 2-, 4-, and 8-day groups. Treatment with PE alone did not increase the production of MDA, either. However, exposure to BaP in the 1- and 2-day groups significantly increased the levels of MDA compared to the control rats, whereas exposure of rats in the 4- and 8-day groups to BaP did not affect the production of MDA compared to the control rats. Conversely, pre-treatment with PE significantly decreased the levels of MDA in the 1- and 2-day groups compared to BaP-only treated rats.

Next, we evaluated the effect of PE on protein oxidation induced by BaP (Fig. 2 (fig2) C) by western blot analysis using anti-dinitrophenylhydrazine antibody. The level of immunoreactivity to the anti-dinitrophenylhydrazine antibody did not change significantly in any of the groups compared to the control, regardless of whether the rats were treated with PE or exposed to BaP.

3.4 Placental extract does not increase the levels of immunoglobulins in rats exposed to BaP

The levels of the two most abundant immunoglobulins in the plasma, IgG1 and IgG2a, were measured in rat plasma by western blot analysis (Fig. 3 (fig3)). Treatment with PE alone significantly increased the levels of IgG1 only in the 2-day group, whereas the immune boosting effect of PE was not observed in other groups of rats pre-treated with PE. However, exposure to BaP did not significantly affect the level of IgG1. Pre-treatment with PE before exposure to BaP did not have a significant effect on the levels of IgG1. In addition, the level of IgG2a was not significantly different among the control, PE-only treated, BaP-only treated, and PE + BaP-treated rats.

Fig. 3

The effect of placental extracts (PE) on immunoglobulin levels in rats exposed to benzo[a]pyrene (BaP). (A) Western blot analysis of immunoglobulins in plasma collected from rats exposed to BaP for 1, 2, 4, or 8 days with or without PE pre-treatment using anti-IgG1 and IgG2a antibodies. An equal amount of total protein was loaded into each lane. (B, C) Graphs showing changes in the levels of IgG1 and IgG2a in plasma from rats exposed to BaP with or without PE. Densitometric values were normalized using α -tubulin as an internal control. Values are expressed as a percentage of control rats in each group. All data represent the mean \pm SD of five different rats. ^a $p < 0.05$ compared to the control rats, ^b $p < 0.05$ compared to PE-treated rats, ^c $p < 0.05$ compared to BaP-treated rats, ^d $p < 0.05$ compared to PE + BaP-treated rats.

3.5 PE attenuate inflammation induced by BaP in rats

The effects of PE on the levels of BaP-induced pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6, were examined by western blot analysis (Fig. 4 (fig4)). Treatment of rats with PE-only did not increase the expression levels of TNF- α in any of the groups. BaP exposure did not significantly increase the levels of TNF- β compared to control rats in the 1-day group, either. However, in the 2-, 4-, and 8-day groups, BaP exposure significantly increased the levels of TNF- α compared to control rats. The increased levels of TNF- α in rats in the 2- and 4-day groups were not attenuated by pre-treatment with PE prior to BaP. The only significant decrease in TNF- α levels occurred in the 8-day rats pre-treated with PE prior to BaP exposure.

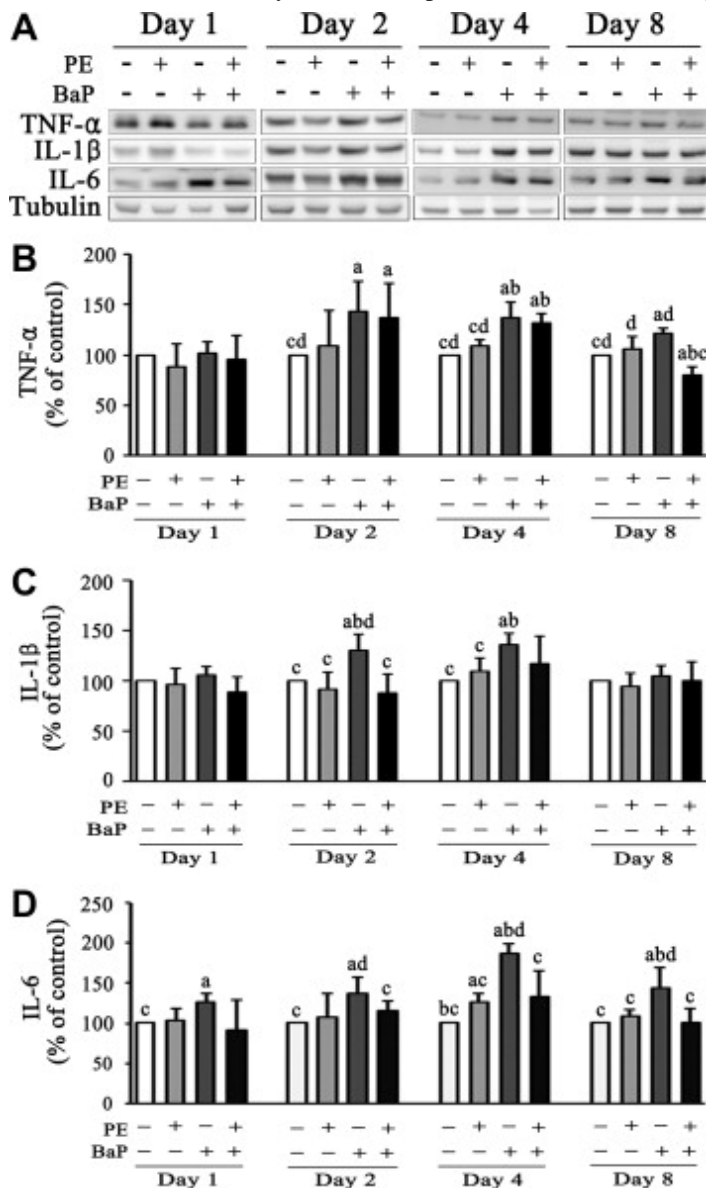


Fig. 4

The effect of placental extracts (PE) on pro-inflammatory cytokine levels in rats exposed to benzo[a]pyrene (BaP). (A) Western blot analysis of pro-inflammatory cytokines in plasma from rats exposed to BaP for 1, 2, 4, or 8 days with or without PE using anti-TNF- α , IL-1 β , and IL-6 antibodies. An equal amount of total protein was loaded into each lane. (B–D) Graphs showing changes in the levels of TNF- α , IL-1 β , and IL-6 in plasma from rats exposed to BaP with or without PE. Densitometric values were normalized using tubulin as an internal control. Values are expressed as a percentage of control rats. All data represent the mean \pm SD of five different rats. ^a $p < 0.05$ compared to the control rats, ^b $p < 0.05$ compared to PE-treated rats, ^c $p < 0.05$ compared to BaP-treated rats, ^d $p < 0.05$ compared to PE + BaP-treated rats.

Treatment of rats with PE-only did not have a significant effect on the levels of IL-1 β in all groups. Rats exposed to BaP for 2 or 4 days showed a significant increase in their levels of IL-1 β compared to control rats, but rats exposed to BaP for 1 or 8 days showed no significant changes in the levels of IL-1 β . The increased levels of IL-1 β in rats exposed to BaP for 2 days were significantly attenuated by pre-treatment with PE, but the levels exposed to BaP for 4 days were not attenuated by pre-treatment with PE.

PE treatment itself did not have any effect on the expression levels of IL-6 in the all groups. BaP treatment of rats from all groups resulted in a significant increase in the levels of IL-6 in plasma. The increased level of IL-6 in rats exposed to BaP for 1 day was attenuated by pre-treatment with PE prior to BaP, but the effect was not statistically significant. However, the levels of IL-6 in rats exposed to BaP for 2, 4 or 8 days were significantly decreased by pre-treatment with PE.

4 Discussion

Human placenta has been used for years as a folk remedy in Asian countries. PE, which is the hydrolysate of human placenta, was approved for treatment of liver cirrhosis in Japan due to its positive effects on liver regeneration [14 15]. Currently, PE is widely used in Korea for alleviation of fatigue, skin whitening, liver function, and menopausal symptoms [16]. Recent studies have reported the isolation of immunomodulatory peptide from bovine placenta water-soluble extract [17]. The isolation of a peptide having homology with human fibronectin type III was also reported as a principle for the wound healing properties of human placenta [18]. In addition, the anti-oxidative effects of PE can be attributed to amino acids such as tyrosine, phenylalanine and tryptophan [3]. Our sample analysis also indicated the presence of diverse amino acids, monosaccharides, and fatty acids in PE, suggesting that it had possible anti-oxidant, immunomodulating effects. For example, glutamic acids [19] and derivatives of aspartic acid and glutamic acid [20] identified in PE exhibited anti-oxidant activities that occurred via reductions in lipid peroxides or acting as free radical scavengers. In addition, N-acetylneuraminic acid [21], glucosamine [22], and glucose [23], which were identified as components of PE, were also reported to exhibit anti-oxidant or anti-inflammatory properties in various biological systems. Furthermore, nucleotides have been reported to have anti-oxidant effects in Fe/Cu-H₂O₂ systems that occur via acting as metal-ion chelators [24]. Diverse fatty acids, omega-3 fatty acids in particular, were also reported to have strong anti-oxidant and anti-inflammatory properties. For example, a recent clinical trial reported that omega-3 fatty acids significantly reduced oxidative stress in neonates when compared to standard lipids [25]. Moreover, omega-3 fatty acids decreased the expression of pro-inflammatory markers and increased the expression of anti-inflammatory markers in mice with *Pseudomonas aeruginosa* lung infection [26]. Taken together, these reports indicate that the anti-oxidant and anti-inflammatory effects of PE against BaP observed in the present study might be exerted by components of PE, including amino acids, monosaccharides and fatty acids. The major strength of this study is that it identifies the effect of PE on oxidative stress induced by the environmental contaminant, BaP. Most studies of PE that have been conducted to date have primarily focused on its anti-inflammatory effects. However, we demonstrated that the anti-oxidant effects of PE occur via recovery of DNA damage. In addition, the effects of PE on anti-oxidant enzymes such as SOD and lipid peroxidation confirmed the anti-oxidant effect of PE. Even though additional studies must be conducted to identify the detailed mechanisms by which PE recovers DNA damage, the anti-oxidant effect of PE could be an important basic concept to evaluate its biological activities.

BaP is one of the most carcinogenic PAHs. The metabolic activation of BaP by cytochrome P450

isoenzymes produces a variety of mutagenic or carcinogenic electrophiles. Particularly, benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE), which is a metabolite of BaP, binds covalently to DNA, RNA, and proteins [27]. BPDE-DNA adducts have been shown to initiate proto-oncogenic *ras* [28] and may induce various cancers such as breast cancers [29]. BaP-quinones are also one of the BaP-metabolites produced by cytochrome P450 isoenzymes [30]. BaP-quinones are highly active and easily undergo one electron redox cycling resulting in the formation of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals [31] that have been associated with cell damage and apoptosis [32]. The DNA damage and oxidative stress induced by BaP in this study could therefore have been induced by metabolites of BaP such as BPDE or BaP-quinones rather than being a direct effect of BaP.

BaP-induced DNA damage has been reported in many different experimental systems including human hepatoma cell line HepG2 [33], Zebra mussels (*Dreissena polymorpha*) [34], and the human prostate carcinoma cell line DU145 [35]. It has been also reported that exposure of rats to BaP can induce a high level of DNA damage in peripheral lymphocytes based on Comet assays [36]. These results are in agreement with ours, suggesting that BaP induces DNA damage in rats.

PAHs including BaP have been proposed to be immunotoxic based on human monitoring. Coke oven workers in Poland who are chronically exposed to PAHs show marked depression of serum IgG and IgA levels compared to other workers [37]. However, Winker et al. reported that serum IgG levels of the PAH-exposed group are not significantly lower than those of control [38]. In our study, exposure of rats to BaP for 1, 2, 4 or 8 days did not suppress the expression of immunoglobulins such as IgG1 and IgG2a.

Pro-inflammatory cytokines such as IL-1 β and IFN- γ have been implicated in the inflammatory effects in BaP-exposed cells [39]. Increased levels of IL-6 have also been reported in rats exposed to BaP [40]. However, conflicting results regarding cytokine TNF- α have been reported. Treatment of primary human macrophages with BaP increased the level of TNF- α significantly [41]. In contrast, TNF- α secretion was unchanged in the murine macrophage cell line RAW 264.7 treated with BaP [42]. These contradictory results may be due to species or cell type-specific differences. In this study, exposure of rats to BaP resulted in a significant increase in the levels of pro-inflammatory cytokines.

One of the most important roles of the placenta is to protect the embryo(s) from oxidative stress [43]. Therefore, PE is known to have anti-oxidative properties [44]. The major anti-oxidant components in PE are uracil, tyrosine, phenylalanine, and tryptophan. About 59% of the anti-oxidative effects of PE can be explained by those components [3]. Togashi et al. reported that peptides produced from collagen are also anti-oxidative components of PE [4]. In this study, PE significantly decreased BaP-induced oxidative stress which was evaluated by measuring the levels of SOD and lipid peroxidation [4].

Exposure of PE significantly attenuated carrageenin and prostaglandin E1-induced edema in rats

and this effect was almost the same as in the anti-inflammatory drug-treated groups, suggesting the anti-inflammatory properties [45]. In this study, PE pre-treatment significantly decreased the levels of TNF- α , IL-1 β , and IL-6 in rats exposed to BaP. These results suggest that PE attenuates the levels of pro-inflammatory cytokines and decreases the inflammation induced by BaP. To the best of our knowledge, this is the first report to show that PE decreases the expression of pro-inflammatory cytokines induced by BaP.

In this study, we evaluated the protective effects of PE on rats exposed to BaP. Exposure of rats to BaP significantly increased the levels of Olive Tailmoments above control levels, while pre-treatment with PE prior to BaP exposure significantly decreased the Olive Tailmoments, suggesting that PE protects against DNA damage induced by BaP. In addition, oxidative stress induced by BaP was attenuated by pre-treatment with PE, as examined by measuring the levels of SOD and lipid peroxidation. Furthermore, PE pre-treatment significantly decreased the levels of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6. Taken together, these results suggest that pre-treatment of rats with PE can significantly attenuate the oxidative damage and inflammation induced by BaP. However, the protective effects of PE against environmental toxicants in humans need to be elucidated further.

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Abstract

Citation

Biological and enzymic activities of placenta extract.

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MeSH Terms (6)

- Enzymes *
- Female
- Humans
- Placenta *
- Placental Extracts *

- **Pregnancy**

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Assessment of the total effective xenoestrogen burden in extracts of human placentas.

Abstract

We have standardized a method to assess the total effective xenoestrogen burden (TEXB) in human placentas by the extraction and separation by high-performance liquid chromatography of two fractions containing lipophilic xenoestrogens (alpha) and endogenous hormones (beta), followed by assessing their estrogenicity in MCF-7 breast cancer cell-based E-Screen and Yeast Estrogen Screen (YES) bioassays. The means of TEXB alpha concentrations (in estradiol equivalent (Eeq) units) were 1.32 and 0.77 Eeq pM g(-1) placenta in the E-Screen and YES, respectively; TEXB beta concentrations were 6.97 and 11.56 Eeq pM g(-1) placenta, respectively. The interclass correlation coefficient was low and a fair level of agreement was observed after kappa test correction. According to the E-Screen and YES, TEXB alpha was \geq LOD in 70.0 and 55.0% of the placentas and 92.5 and 82.5% in beta, respectively. Although both bioassays can be recommended for assessing TEXB, there is greater experience with the use of the E-Screen for estrogenic assessment after extensive extraction of complex human matrices.

Citation

Assessment of the total effective xenoestrogen burden in extracts of human placentas.

Lopez-Espinosa MJ, Silva E, Granada A, Molina-Molina JM, Fernandez MF, Aguilar-Garduño C, Olea-Serrano F, Kortenkamp A, Olea N - Biomarkers - August 1, 2009; 14 (5); 271-7

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MeSH Terms (11)

- **Adult**
- **Biological Assay /standards ***
- **Cell Extracts /chemistry ***
- **Cell Line, Tumor**
- **Chromatography, High Pressure Liquid**
- **Estrogens /analysis ***
- **Female**
- **Humans**
- **Placenta /chemistry ***
- **Pregnancy**
- **Xenobiotics**

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Effects of human placenta extracts on tissue cultures.

Abstract

Citation

Effects of human placenta extracts on tissue cultures.

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MeSH Terms (7)

- Female
- Immunologic Tests *
- Placenta *
- Placental Extracts *

- **Pregnancy**
- **Research Design ***
- **Tissue Culture Techniques /drug effects ***

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ARTÍCULO

Progesterone receptors and progesterone concentration in serum and porcine maternal placental extracts

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Pregnancy in the pig is characterized by rapid development and endocrinological changes involving the conceptus and the uterine environment. Porcine placenta is epitheliochorial, folded, non-decidual, non-invasive and diffuse. Progesterone receptor (PGR) is expressed as two isoforms, PGRA and PGRB, which have been shown to have different functional activities.

Objectives: To investigate a) progesterone concentration in serum and maternal placental extracts (HoPM), b) PGR, PGRA and PGRB expressions in porcine placenta of different gestational periods (16–17, 30–35 and 63–78 days of gestation).

Methods: The porcine females from slaughterhouses (n=12) of 16–17, 30–35 and 63–78 days of gestation were used. PGR, PGRA and PGRB were visualized by immunohistochemistry using monoclonal antibodies. Progesterone (P₄) concentration was measured by chemiluminescence in maternal placental extracts and serum.

Results: Progesterone (P₄) concentration in maternal placental extracts was $\bar{x} = 1.54$ ng/ml at 16-17 days, $\bar{x} = 1.02$ ng/ml at 30-35 days, and $\bar{x} = 1.38$ ng/ml at 63-78 days, whereas that in serum was $\bar{x} = 36.66$ ng/ml at 16-17 days, $\bar{x} = 24.16$ ng/ml at 30-35 days; and $\bar{x} = 27.9$ ng/ml 63-78 days. Total progesterone receptor was expressed at 16-17 days of gestation in uterine glands and maternal stroma, in coincidence with the expression of the PGRA. At 30-35 and 63-78 days of gestation PGR and PGRA were expressed in maternal stroma, while uterine glands and epithelium were negative. No expression of PGRB was observed in the periods of gestation and structures studied.

Conclusions: Progesterone levels remain constant through pregnancy in HoPM and serum with tissue expression of PGRA. The results suggest that progesterone acts via PGRA in the periods of swine gestation studied.

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