of differentiation, wereformed by hanging drop, and were used in assessment of the developmental toxicities of compounds including penicillin G, isoniazid,aspirin, indomethacin, dexamethasone, and retinoic acid. Section images of EBswereobtained from a phase contrast microscope and area of EBs was analyzed byprogram measuring image area.

Results: The size of EBs wasdose-dependently reduced by pharmaceutical compounds. The ID_{50}value of EB, 50% inhibition of growth in EBs, occurs inlogarithmic graph. Decrease in EB's size caused decline in beating ratio duringcardiac differentiation. Also, expressions of *Nanog, Isl1, Ryr2* and *cTn1*were down-regulated in a dose-dependent manner.

Conclusions: Exposure to toxicantsresults in increased cell death and inhibition of cardiac differentiation. EBsize-based toxicological screening is a novel drug screening system and isuseful tool to evaluate various embryo-toxic chemicals in a short time.

References

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P-180

The influenceof progesterone on intracellular calcium signaling of differentiated mESCs intocardiomyocytes

Eui-Bae Jeung*, Young-Kwon Choi, Hee Young Kang

Laboratory of Veterinary Biochemistry and Molecular Biology, Chungbuk National University, Cheongju, Chungbuk, 362-763, Republic of Korea

Introduction: Mouse embryonic bodies (mEBs) are required for early development of mouseembryonic stem cells (mESCs). These mEBs were spontaneously differentiated intocardiomyocytes. We investigated the effects of progesterone on calciumregulation during differentiation of mESCs. Calcium (Ca^{2+}) releasefrom sarcoplasmic reticulum (SR) regulates the smooth or skeletal musclecontraction. Also, the cardiac L-type Ca^{2+} channel plays a key rolein excitation-contraction coupling of cardiomyocytes.

Materials and Methods: The mESCs were performed hanging-drops for 4 days and were suspended indifferentiation medium without LIF for an additional 2 days. 6 days-old mEBswere attached onto 6 well culture plates and differentiated intocardiomyocytes. We analyzed mRNA expressions for the cardiomyogenesis,contraction and calcium channel-related genes.

Results: We observed time-dependently increased beating-rate and the highestbeating-rate (95.08%) of mESCs (E14) was peaked at the differentiated 12 days. However, beating rate (67.56%) in progesterone treated mEBs is more decreased. Expressionin mRNA levels of cardiac markers such as *Tbx20,Isl1, cTn1* and *RyR2* areincreased, and Troponin Ⅰprotein was found inbeating mEBs viaimmunocytochemistry. Next, we observed expression of calcium/contractionregulating genes such as *TRPV2, SERCA2a,RyR2, Calmodulin2* and *MLCK3* incardiomyogenesis of differentiated or progesterone-treated mESCs.

Conclusions:. These resultsmean that progesterone has influences on cardiac differentiation and contractionof cardiomyocytes through regulating intercellular calcium ion.

P-181

Mouse as In vivo model : Impact of the EDCs on placenta transporter

Eui-Bae Jeung*, Jae-Hwan Lee

Laboratory of Veterinary Biochemistry and Molecular Biology, Chungbuk National University, Cheongju, Chungbuk, 362-763, Republic of Korea

Introduction: Cation,oxygen, carbon dioxide and glucose are essential factors in fetal growth. Thesemolecules are transferred by specific receptors located on cell membrane orcytoplasm in placenta. Cation (ex> calcium, copper, iron, etc.) transfergenes are regulated by principally reproduction-related hormones. Duringpregnancy, expression of these receptors is controlled by the nutritionalstatus of the maternal and fetal. EDCs is similar structures to steroidhormones or endogenous hormones related to reproduction. These substancesdisturb action of reproduction-related hormones (ex> estrogen, progesterone)by interacting with their receptors, or affecting the expression oftransporting genes for cations.

Materials and Methods: Weused well-known EDCs and applied different doses of octyl-phenol (OP; 50 mg/kg/day), and bisphenol A (BPA; 50 mg/kg/day) in pregnancy mice for GD11.5~16.5. Ethinyl estradiol (EE; 0.2 mg/kg/day), which activates estrogen receptors, was used as apositive control. ICI 182 780(70 ug/kg)were used with estrogen antagonist. Transcriptionof calcium transporting genes, copper transporting genes, and iron transportinggenes was quantified by qRT-PCR.

Results: EDCs havebeen known to interfere with the endocrine system through various mechanisms.Treatment with OP, BPA in a mouse placenta affected expression of calciumtransporting genes (PMCA1, TRPV6), copper transporting genes (CTR1, ATP7A), andiron transporting genes (IREG1, HEPH) like positive control, EE. When EDCs weretreated by concentration, expression of the gene could be determined. Toclarify the effects of EDCs mediated by estrogen receptor, ICI 182 780 wastreated as an estrogen receptor antagonist. A control group was treated withEE,