Low-Dose of Benzo(a)pyrene, a Cigarette Smoke-Associated Hydrocarbon, Inhibits Myogenic Differentiation in Human Skeletal Muscle Stem Cells

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Disclosures:

Introduction: Recent epidemiological studies have observed that prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) may adversely affected fetal growth and birth outcomes including intrauterine growth retardation, low birth weight, length and reduction of head circumferences in the U.S., Poland and the Czech Republic. The previous human studies in Europe and U.S. have reported that transplacental exposure to benzo(a)pyrene (BaP), a member of PAHs existing in cigarette smoke, is associated with detrimental birth outcomes and fetal development [1]. Additionally, neonates born to smoking mothers have a significant reduction in birth weight and peripheral muscle area with longitudinal ultrasonographic examinations. Based on these epidemiological and animal studies, BaP and its epoxide metabolite elicited the adverse birth outcome and fetal development. However, the mechanisms of low birth weight induced by BaP and its epoxide metabolite are still unclear. We hypothesize that lower birth weight occurs owing to impeding skeletal myogenic differentiation by BaP. We investigated the cellular and molecular mechanism of BaP and benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) on myogenic differentiation in human skeletal muscle-derived stem/progenitor cells (HSMPCs).

Methods: Primary human skeletal muscle stem/progenitor cell culture: A total 10 human skeletal muscle biopsies (~ 0.2 g) (mean age, 64 years; range, 34 to 81 years, both male and female) were obtained during orthopedic surgery with institutional ethical committee approval and informed consent from the patient at the National Taiwan University Hospital, Taipei, Taiwan. Primary muscle cultures were performed as described previously with a modification. In brief, the muscles were minced into a slurry and enzymatically digested by Ham’s F10 medium containing 0.5% type XI collagenase, 2.4 IU/ml dispase solution and 0.2% trypsin-EDTA. The filtrate was spun at 3500 rpm for 5 min to sediment the dissociated cells. The pellet re-suspended in growth medium (GM) [Ham’s F-10 supplemented with 20% FBS, 5 ng/mL basic fibroblast growth factor, and 1% penicillin-streptomycin] was pass through a 70 μm cell strainer. The cells with positive desmin (myogenic marker) staining were used for identification of myogenic differentiation. Myogenic differentiation and BaP and BPDE treatment: HSMPCs were placed in a differentiation medium (DM) consisting of an equal mixture of two serum-free media (Nutrient Mixture F-12K Ham medium and MCDB201) along with 2% horse serum to induce differentiation with or without BaP and BPDE (0.25 and 0.5 μM) treatment. After 4 days of treatment, myoblast differentiation was determined morphologically by analysis of multinucleated myotube formation. Cells were analyzed morphologically by hematoxylin and eosin (H&E) staining. The expressions of myogenic differentiation markers and related signaling proteins were determined by Western blotting.

Results: As shown in Figure 1, BaP at submicromolar concentrations (0.25 and 0.5 μM) significantly repressed HSMPCs myogenic differentiation, including creatine kinase activity and myotube formation with no decrease in cell viability during HSMPCs myogenic differentiation. We also observed that BaP epoxide metabolite BPDE markedly inhibited myogenic differentiation and had greater potency than BaP. We further investigated the role of aryl hydrocarbon receptor (AhR) in both BaP- and BPDE-inhibited myogenesis in HSMPCs and the possible mechanism. As expected, BaP- and BPDE-induced inhibition in muscle-specific protein expressions [myogenin and myosin heavy chain (MHC)] and phosphorylated Akt (p-Akt) protein expressions were reversed by an AhR antagonist (α-naphthoflavone, α-NF). We further found that NF-kB plays a negative role in the HSMPCs myogenesis. As shown in Figure 2, both BaP and BPDE induced the phosphorylations of NF-κB-p65 and Akt and the protein expression of MHC during myogenesis, which could be reversed by α-NF and NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC).

Discussion: It has been known that BaP and BPDE are the activated ligands of aryl hydrocarbon receptor (AhR). Previous evidence suggested that NF-κB plays a negative role in the muscle-derived stem cell differentiation [2]. The PI3K/Akt signaling pathway is also known to trigger myogenin during myogenic differentiation [3]. These findings suggest that the submicromolar concentrations of BaP and its metabolite BPDE significantly inhibit myogenic differentiation in HSMPCs, and identify a possible mechanism of action via AhR/NF-κB-p65/Akt signaling pathway.

Significance: The findings of this study suggest that benzo(a)pyrene contained in diet or cigarette smoke may contribute to the lower birth weight of neonates reported in mothers with elevated benzo(a)pyrene intake.

Acknowledgments: This study was supported by grants from the Taiwan National Science Council (NSC101-2314-B-002-118-MY2) and the Kaohsiung Medical University (KMUER-020). The authors declare no actual or potential competing financial interests.

ORS 2014 Annual Meeting
Poster No: 1406