**RISK FACTORS_BASIC SCIENCE**

**PS-BP09-2 DEVELOPMENT OF LONG-ACTING HUMAN ADRENOMEDULLIN FC FUSION PROTEINS**

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**Background:** Human adrenomedullin (hAM) is a hypotensive peptide hormone with strong anti-inflammatory effects. AM consists of 52 amino acid residues with an amidated C-terminus and a ring structure formed by intramolecular disulfide bonds. Both of these structures are necessary for binding to receptors and to exert their biological functions. AM has also shown therapeutic effects in various animal disease models, including inflammatory bowel disease, ischemic heart disease, sepsis, and stroke. However, the short half-life of native AM in the blood required continuous administration for treatment. To solve this, we have developed four human IgG1 and IgG4 Fe fusion proteins containing full-length human AM or AM(6–52).

**Methods:** Recombinant Fe-AM derivatives were generated using mammalian cells. After that, we tested the biological activities of Fe-fusion AMs (Fe-AMs) to stimulate the intracellular accumulation of cAMP in HEK-293 cells stably expressing the AM1 receptor. Next, we compared IgG1-AM (6–52) and IgG4-AM (6–52) concentrations in the peripheral blood and tissues of rats after subcutaneous injection. In addition, the antihypertensive effect of Fe-AM was examined on spontaneously hypertensive rats (SHRs) which were given high salt diets.

**Results:** We have developed four Fe-AMs, which are long-acting AM derivatives in mammalian cells. Fe-AMs produced in mammalian cells do not require refolding or amidation. Indeed, Fe-AMs could be measured by mAM assay, which recognizes the amidated form, indicating that Fe-AMs produced in mammalian cells were amidated. Fe-AMs, generated in a mammalian cell production system can therefore easily be produced in large amounts with few purification steps. The Fe-AMs stimulated intracellular cAMP production in cultured cells stably expressing the AM type I receptor in vitro. Fe-AM (6–52) induced higher cAMP levels for the receptor than Fe-AM. The pharmacokinetic study was performed using N-terminal deficient AM derivatives, which have strong receptor binding ability. Sufficient concentrations of IgG1-AM (6–52) and IgG4-AM (6–52) were observed in blood 14 days after a single subcutaneous administration. Tissue transfer to the kidney and small intestine was observed after administration of IgG1-AM (6–52) or IgG4-AM (6–52) to rats. In addition, the single subcutaneous administration of IgG4-AM (6–52) suppressed the increase in blood pressure in SHRs which were loaded with high salt diets.

**Conclusion:** Fe-AM produced from mammalian cells is easily prepared and seems to be an effective new therapeutic agent for hypertension.

**PS-BP09-3 CIGARETTE SMOKE EXTRACT INDUCES DNA DAMAGE AND TRIGGERS AN INNATE IMMUNE RESPONSE**

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**Objective:** Elevated blood pressure and tobacco smoking are causes of preventable mortality. The effects of tobacco smoking on blood pressure are complex and chronic effects are inconsistent. We have previously reported increased DNA damage in peripheral mononuclear cells of smokers compared to non-smokers. In this study, we investigated the effects of cigarette smoke extract (CSE) on DNA damage and the physiological influence of DNA damage in endothelial cells.

**Design and method:** The effect of CSE extracted from tobacco smoke was examined in human umbilical vein endothelial cells (HUVECs). CSE was added to HUVECs and the formation of DNA damage was quantified by fluorescent immunostaining. The mRNA expression of inflammatory cytokines was quantified by real-time RT PCR.

**Results:** CSE increased double-strand breaks in HUVECs by 72 hours. CSE also increased oxidative DNA damage both in the nucleus and mitochondria, and decreased mitochondria membrane potential. Mitochondrial dysfunction partially induced mitochondrial outer membrane permeabilization, resulting in the partial activation of caspase-3 and nuclear translocation of caspase-activated deoxyribonuclease, which fragments DNA in the nucleus. It was suggested that this is one of the mechanisms of nuclear DNA double-strand breaks. CSE induced the accumulation of nuclear and mitochondrial DNA in the cytosol. We further examined whether accumulated cytotoxic DNA activated cytotoxic DNA-sensing pathways. The production of cGAMP, a second messenger in cGAS (a DNA-sensing receptor) signaling, was increased by CSE, followed by nuclear translocation of NF-κB and increased mRNA expression of IL-6. The increase in IL-6 expression was suppressed by si-cGAS.

**Conclusions:** Our study revealed that continuous exposure to CSE induces DNA damages not only in the nucleus but also in the mitochondria, which leads to cytotoxic DNA accumulation, and evokes chronic inflammation via the cGAS pathway.

**PS-BP09-4 ARTERIAL SITES AND SEX DIFFERENCES IN ENHANCING VASORELAXATION RESPONSE BY PERIVASCULAR ADIPOSE TISSUE IN METABOLIC SYNDROME RATS**

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**Objective:** Regulation of arterial tone by perivascular adipose tissue (PV AT) differs with sex. We have demonstrated that PVAT compensates vascular tone when vascular dysfunction occurs in mesenteric arteries of male SHRSP. Z-Leprfa/ IzmDmcr (SPZF) rats, but such compensation function disappears in the late stage of metabolic syndrome (MetS). In con-trast, the favorable effects of PVAT remain in female SPZF even after the effects disappear in age-matched males.

**Design and method:** Therefore, in this study, we investigated whether the sex differences in PVAT response are observed in another arterial sites in SPZF and in another MetS model, SHR/NDmcr-cp (CP) rats as well. Renal arteries were isolated from male and female SPZF and CP rats at 23 weeks of age. Ring preparations with and without PVAT were made. Vasodilation and mRNA transcript levels in PVAT were examined using organ bath methods and quantitative real-time PCR, respectively.

**Results, and Conclusions:** In renal arteries without PVAT of SPZF and CP rats, acetylcholine-induced relaxations were smaller in female than in male. The presence of PVAT increased the relaxations in CP rats only. In contrast, sodium nitroprusside-induced relaxations, nevertheless presence or absence of PVAT, were...