Benzo[a]pyrene-Induced Vascular Endothelial Adhesion Molecule Expression Can Be Disrupted By Selective Flavonoid Treatment

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ABSTRACT

Adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), play a critical role in the initiation of vascular diseases such as atherosclerosis. Exposure to the polycyclic aromatic hydrocarbon, benzo[a]pyrene (B[a]P) has been shown to correlate with the increased risk of cardiopulmonary diseases. Increased intake of dietary flavonoids may decrease the risk of developing these diseases. The goals of this research were to investigate the effects of B[a]P on endothelial cell adhesion and if flavonoids could protect against this endothelial pathology. Primary human umbilical vein endothelial cells (HUVECs) were pretreated overnight with vehicle or beta-naphthoflavone (β-NF) to induce aryl hydrocarbon receptor (AhR) and AhR-regulated metabolism. Since ICAM-1 is necessary for the adhesion and migration of inflammatory leukocytes such as macrophages across the endothelium, macrophage adhesion was measured. Cells pretreated with β-NF and treated with B[a]P were able to induce adhesion of fluorescently labeled macrophages to the activated endothelium, a physiological representation of ICAM-1 up-regulation. Various flavonoids were incubated with β-NF overnight, followed by treatment with B[a]P. Only flavonoids that contained a C-ring hydroxyl substitution and a B-ring double bond were able to protect against ICAM-1 induction by B[a]P measured by flow cytometry. This study suggests that B[a]P is able to increase endothelial cell adhesiveness by increasing ICAM-1, but only when activated by AhR-dependent enzymes and that this effect can be protected against by pretreatment with selective flavonoids.

RESULTS

B[a]P Increases ICAM-1 Expression When Pre-Treated with β-NF

HUVECs were pre-treated with vehicle (DMSO) or β-NF to induce the AhR regulated metabolizing enzymes. Cells were then treated with either B[a]P or fluorothene (Fl) for 24 hours. Whole cell lysate was extracted and ICAM-1 was measured by immunoblotting. * p<0.05 by One-Way ANOVA followed by Tukey post-hoc test.

B[a]P-Induced ICAM-1 Signals Through the MEK-p38 MAPK-AP-1 Pathway

HUVECs were pre-treated with β-NF (overnight), followed by the inhibitors SB203580 (p38) and PD98059 (MEK) for 1 h. The cells were then treated with B[a]P (24 h, 10µM). Cells were labeled with anti-human ICAM-1 and AlexaFluor 488 antibodies and contained with propidium iodide to measure cell viability. Positively labeled cells were analyzed by the University of Kentucky Flow Cytometry Core Facility. Bars represent mean ICAM-1 induction from B[a]P. * p<0.05 by One-Way ANOVA followed by Tukey, post-hoc.

B[a]P-Induced ICAM-1 Signals Through Caveolae

HUVECs were pre-treated with DMSO or β-NF overnight and caveolae-associated proteins were measured by Promega P450-Glo Assay System which measures luminescence after conversion of Luciferin-CEF to luciferin by the enzyme. Values were normalized to cell number by protein measurements. * p<0.05 by T-test.

CONCLUSIONS

- PAHs such as B[a]P are able to increase endothelial cell adhesiveness by increasing ICAM-1, but only when activated by AhR-dependent enzymes.
- B[a]P increases ICAM-1 through the redox sensitive MEK-p38 MAPK-AP-1 pathway and requires functional caveole.
- Plant based flavonoids with a 4’-B-ring hydroxyl group and a C-ring double bond at the 2-3 position can be used as models for pharmacological interventions.
- This study suggests that diet serves as a protective mechanism against cardiovascular injury caused by organic air pollutants and can be used as models for pharmacological interventions.

Supported by grants from NIEHS/NIH (P42ES07385), AHA Pre-doctoral Fellowship (0615216B), and the University of Kentucky AES.