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

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ABSTRACT

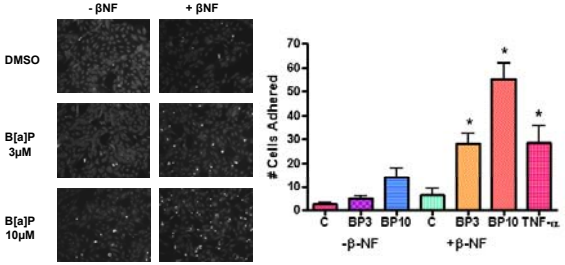
Adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), play a critical role in the initiation of vascular diseases such as atherosclerosis. Exposure to the polycyclic aromatic hydrocarbon, benzo[a]pyrene (B[a]P) has been shown to correlate with the increased risk of cardiopulmonary diseases. 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) regulated metabolizing enzymes. Cells were then treated with B[a]P for 24 hours. B[a]P increased ICAM-1 protein expression only when pre-treated with β-NF, suggesting B[a]P requires AhR to be converted into the active pro-inflammatory compound. 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.

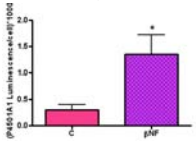
This study suggests that B[a]P is able to increase endothelial cell adhesiveness by increasing ICAM-1, but only when activated by AhR-dependent enzymes and that this effect can be protected against by pre-treatment with selective flavonoids.

RESULTS

B[a]P Increases Macrophage Adhesion After β-NF Pretreatment

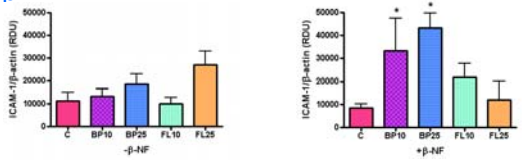


Human THP-1 monocytes were activated with TNF-α and loaded with the fluorescent probe calcein (Molecular Probes, Carlsbad, CA). Human umbilical vein endothelial cells (HUVECs) were pre-treated with β-NF or DMSO and then treated with B[a]P for 24 hours. Monocytes were added to treated endothelial cell monolayers and incubated (30 min), allowing for monocyte adhesion. Unbound monocytes were washed away and the monolayer was fixed with 1% glutaraldehyde. Attached fluorescent monocytes were counted using a fluorescent microscope (Olympus IX70). * p<0.05 by One-Way ANOVA, Tukey.



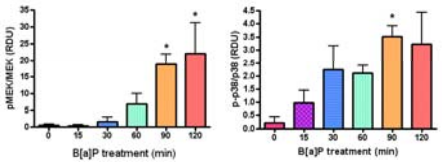
HUVECs were treated with DMSO or β-NF overnight and cytochrome P450 1A1 activity was measured by Promega P450-Glo Assay System which measures luminescence after conversion of Luciferin-CEE to luciferin by the enzyme. Values were normalized to cell number by protein measurements. * p<0.05 by T-test.

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



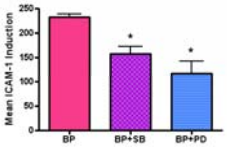
HUVECs were pre-treated with vehicle (DMSO) or β-NF to induce the AhR regulated metabolizing enzymes. Cells were then treated with either B[a]P or fluoranthene (FL) for 24 hours. Whole cell lysate was extracted and ICAM-1 was measured by immunoblotting. * p<0.05 by One-Way ANOVA followed by Tukey post-hoc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

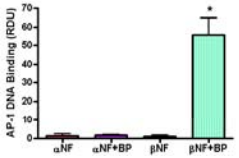
B[a]P-Induced ICAM-1 Signals Through the MEK-p38 MAPK-AP-1 Pathway



HUVECs were pre-treated with β-NF then treated with B[a]P for various time points. Whole cell lysate was extracted and phospho-MEK, MEK, phospho-p38, and p38 were measured by immunoblotting. * p<0.05 by One-Way ANOVA, Tukey post-hoc.

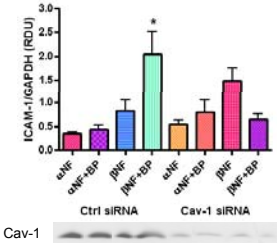
HUVECs were pre-treated with β-NF (overnight), followed by the inhibitors SB203580 (p38) and PD98059 (MEK) for 1 h. The cells were then treated with B[a]P (24 h, 10μM). Cells were labeled with anti-human ICAM-1 and AlexaFluor 488 antibodies and costained with propidium iodide to measure cell viability. 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.





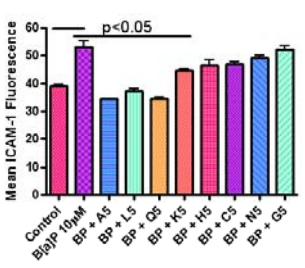
HUVECs were pre-treated with αNF or β-NF then treated with either DMSO or B[a]P for 1 h. Nuclear protein was extracted and AP-1 DNA binding was measured using the Pierce LightShift Chemiluminescent EMSA Kit. * p<0.05 by One-Way ANOVA followed by Tukey.

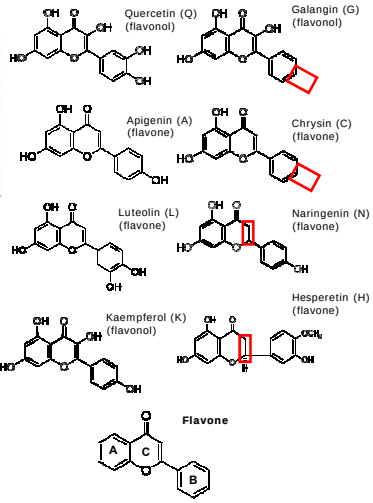
B[a]P-Induced ICAM-1 Signals Through Caveolae



HUVECs were treated with control or caveolin-1 siRNA using the GeneSilencer Transfection Kit (Genlantis). After 24 hours, cells were pretreated with α-NF or β-NF and then treated with DMSO or benzo[a]pyrene. Whole cell lysate was probed for ICAM-1, GAPDH, and Caveolin-1 by immunoblot analysis. Bars represent mean ± SEM of at least three independent experiments analyzed by Two-Way ANOVA. The bands below are representative blots of caveolin-1.

Flavonoids Decrease B[a]P-Induced ICAM-1 Expression

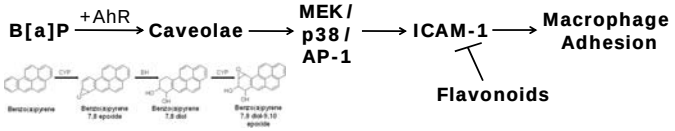




HUVECs were pre-treated with β-NF and flavonoids, followed by a 24 hour treatment with DMSO or B[a]P. Cells were labeled with mouse anti-human ICAM-1 and AlexaFluor 488 antibodies and costained with propidium iodide to measure cell viability. Positively labeled cells were analyzed by the University of Kentucky Flow Cytometry Core Facility (Becton-Dickinson FacsCalibur).

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 caveolae.
- Plant based flavonoids with a 4'-B-ring hydroxyl group and a C-ring double bond at the 2-3 position protect against ICAM-1 up-regulation by B[a]P.
- 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.



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