GENDER-DEPENDENT MECHANISMS OF ALPHA-TOCOPHEROL PROTECTION FROM BENZO[A]PYRENE EXPOSURE IN RATS

Honors Thesis of Caitlin A. Crimp

Mentor: Debbie J. Mustacich, Ph.D.

Abstract

Polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BP), are environmental pollutants linked to increased disease susceptibilities. Alpha-Tocopherol (αT) supplementation decreases BP-DNA adducts in smokers, particularly women; but the mechanism is unknown. To test the hypothesis that αT protection from BP exposure is gender-dependent, male and female rats received 7 daily subcutaneous (SQ) injections of αT (100 mg αT/ kg body wt) or vehicle, followed by a single ip injection of BP (20 mg/kg, spiked with ^{3}H-BP) on day 9. Urine and bile were collected pre- and post-BP. Plasma and tissues were collected 5 or 24 hr post-BP. αT supplementation increased αT levels in females greater than males. Compared to vehicle, αT supplementation increased total urinary and biliary excretion of BP and/or BP metabolites more than 2.5-fold in females, but decreased total BP and/or BP metabolite excretion in males ($p<0.05$). SQ αT prevented BP-induced increases in urine 8-isoprostanes (males) and decreased tissue malondialdehyde levels in a tissue- and gender-dependent manner. These data are the first to suggest that αT protection from BP exposure is gender-dependent and occurs by both antioxidant and non-antioxidant mechanisms. Further elucidation of the mechanism(s) of αT protection against PAHs may lead to the development of novel protective strategies for occupational PAH exposures.

Introduction

- PAHs are environmental toxins produced by incomplete combustion processes.
- High occupational exposures occur: road paving, roofing, second-hand smoke (bars, casinos), forest fires, smoke-houses.
- High level PAH exposure is linked to increased risk of several cancers: lung, skin, and scrotal cancer.
- Benzo[a]pyrene (BP) is often used as a model compound for PAH exposure studies as it is present in almost all PAH mixtures.
- αT supplementation decreases BP-DNA adduct levels in smokers.
  - Effects were greater in women than men (Mooney et al., 2005).
- SQ αT increases expression of enzymes and transport proteins involved in BP detoxification and excretion (Mustacich et al., 2006).

Hypothesis

We hypothesized that:

Elevated levels of αT decrease BP-induced damage by two synergistic mechanisms:

(1) increased antioxidant protection against oxidative stress-induced damage

(2) modulation of BP metabolism and/or excretion.

In addition, we hypothesized that this protection would be greater in female rats compared to males.

Results

Fig 1. αT supplementation increases tissue αT levels in BP exposed rats.

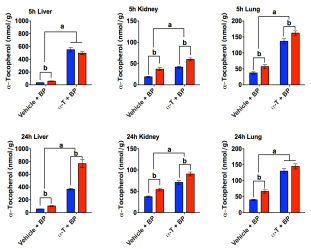


Fig 2. αT supplementation increases plasma αT levels in BP exposed rats.

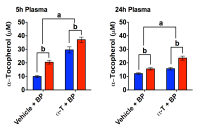


Fig 3. αT supplementation increases bile αT levels in BP exposed rats.

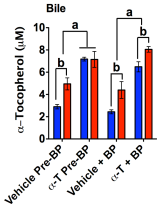


Fig 4. αT supplementation alters tissue MDA levels in a gender-dependent manner 5 and 24h post-BP.

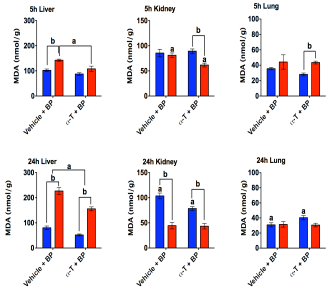


Fig 5. αT supplementation prevents BP-induced elevation of urine 8-IsoPFG2α levels 24h post-BP.

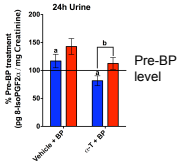


Fig 6. αT supplementation decreases hepatic glutathione in males, but not females 24h post-BP.

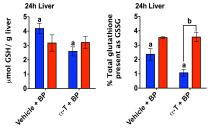
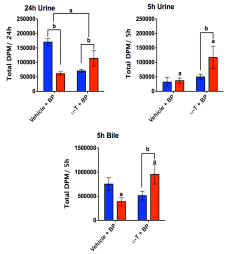


Fig 7. αT supplementation alters bile and urine excretion of BP/BP metabolites 5 and 24h post-BP.



a = $p < 0.05$ between treatment groups b = $p < 0.05$ between genders RED = Females Blue = Males

Methods & Study Design

Animal Studies. Male and female Sprague Dawley rats (n=5-8/gender/treatment) were given 7 daily subcutaneous (SQ) injections of either RRR-αT (100 mg/kg body wt.) or vehicle (saline). On day 9, rats received an intraperitoneal injection of BP (20 mg BP/kg body wt, spiked with ^{3}H-BP) dissolved in tocopherol-stripped corn oil. Urine and bile were collected pre- and post-BP exposure (bile only collected from 5h rats). 5 or 24 h post BP injections, rats were euthanized with sodium pentobarbital (80 mg/kg) and tissue and blood were collected. Plasma was obtained by centrifugation, and tissue and plasma were stored at -80˚C until analysis.

Measurement of Alpha-Tocopherol. Plasma and tissue αT concentrations were determined by a modification of the method described by Podda *et al.* (1996) and measured using HPLC with fluorescence detection and quantification by comparison to standard curves generated with authentic compounds.

Measurement of Total Radioactivity. Urine and bile total radioactivity were measured by liquid scintillation counting (LSC).

Measurement of 8IsoPGF2α and Creatinine. Urine 8-IsoPGF2α was extracted using the method described by Taylor *et al.* (2006), measured by enzyme immunoassay (Cayman Chemical), and normalized to creatinine (Jaffe reaction, Assay Designs).

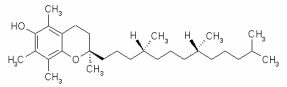
Measurement of Malondialdehyde. Tissue malondialdehyde concentrations were determined by a modification of Lykkesfeldt (2001) and measured using HPLC/fluorescence.

Measurement of Reduced and Oxidized Glutathione. Liver reduced and oxidized glutathione concentrations were determined as described by Farris and Reed (1987).

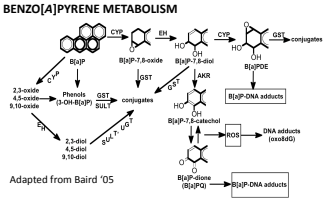
Statistical analysis. Data was log transformed to equalize variances between groups and then analyzed by two–way ANOVA (Prism, Graphpad, La Jolla, CA). Post hoc tests using Bonferroni Comparisons were performed when overall group effects were found to be significant. Data are expressed as mean ± SE and comparisons with p values < 0.05 were considered significant.

Structures & Pathways

ALPHA-TOCOPHEROL



BENZO[A]PYRENE METABOLISM



Adapted from Baird '05

Summary

αT supplementation in BP-treated rats:

- **Increases αT tissue, plasma, bile levels**
  - **Females greater than males**
- **Prevents BP-induced elevation of urinary 8-IsoPGF2α**
  - **Males greater than females**
- **Decreases tissue MDA levels in a gender-, tissue-, and time-dependent manner**
- **Decreases hepatic glutathione**
  - **Males only**
- **Alters BP/BP metabolite excretion in urine and bile**
  - **Increased excretion in females, but not males**

Conclusions

αT supplementation:

- **Alters BP exposure outcomes by both antioxidant and non-antioxidant mechanisms**
- **Alters BP exposure outcomes in a gender-dependent manner**

Elucidation of mechanisms of αT protection against PAHs may lead to development of novel protective strategies for occupational PAH exposures.

Acknowledgements

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