# Toxicity of Arsenic in Vascular Endothelial Cells and Effects of Prenatal Arsenic Exposure on Placental Integrity


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## Introduction

- Arsenic is an abundant toxin in ground water and soil around areas with extractive industries.
- Human epidemiological studies show increased miscarriage rates in high arsenic exposure areas.
- Vascular defects are often the basis for placentation defects linked to miscarriage.
- Arsenic exposure to cultured endothelial cells causes oxidative stress reactions by generating reactive oxygen species (ROS).
- **Overall goal**: To define the cellular & molecular mechanisms for how arsenic toxicity causes endothelial dysfunction leading to miscarriage.
- **Hypothesis:** Arsenic toxicity causes VEGF-mediated endothelial dysfunction that alters placental vasculogenesis thereby predisposing the embryo to spontaneous abortion.

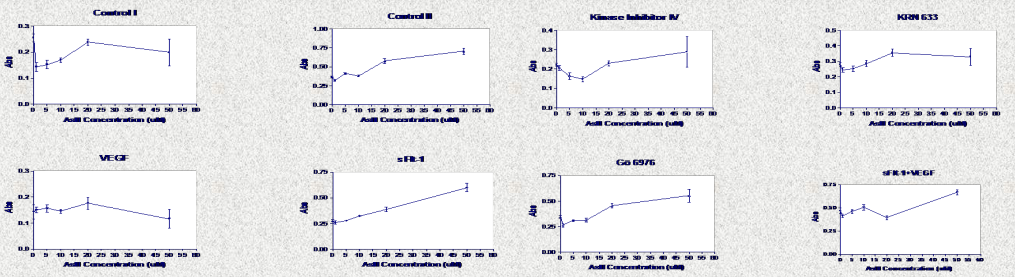



**Figure 1-** *BrdU cell proliferation data for AsIII-exposed BAE.* BAE were exposed to increasing AsIII concentrations as well as agonists (VEGF) and antagonists (Flt-1, KRN, KIV, Go6976) to the VEGF regulatory system for 24 hours. We hypothesized that cell proliferation would diminish with increasing AsIII concentration. The two AsIII treatments show contradictory results making interpretation of the other treatments equivocal. The error was likely due to variance in cell densities. Future experiments will repeat this assay using Human Microvascular Endothelial Cells (HMVEC) as well as BAE.

## RESULTS & CONCLUSIONS

- AsIII exposure decreased BAE viability. This could result from increased apoptosis, decreased proliferation or a combination of both.
- BAE proliferation and apoptosis assays (not shown) were equivocal due to technical problems. These experiments are being repeated in both BAE and HMVEC.
- DHE assays, an indicator of oxidative stress, were equivocal consistent with technical problems in other assays. Previous experiments have shown an increase in oxidative stress from AsIII exposures in BAE.
- AsIII exposure to BAE on coverslips show an increase in stress fiber formation that was reversed with the VEGF receptor (R1 & R2) inhibitor KRN. These results suggest that the AsIII mediated endothelial dysfunction, evidenced by stress fiber formation, is regulated by the VEGF system.
- Placental integrity experiments were conducted using the VEVO 770 with high-contrast microbubbles. High-resolution ultrasound reveal excellent resolution of the embryonic and maternal tissues. However, the microbubble assays could not be repeated due to lack of the necessary software for the VEVO. Future experiments will use vascular resin casting and ink injections in lieu of the microbubbles.

## Experimental Approach & Methods

- Cell culture
  - Bovine Aortic Endothelial (BAE) cells were grown in DMEM media with 10% FBS. For BrdU, MTS, and DHE assays, the cells were seeded in 96 well plates at a density of $2x10^4$ cells per well. For the stress fiber assay, poly-D-lysine coated coverslips (0.1mg/ml) were seeded at a density of $2x10^5$ cells. Cells were grown for three days to confulency. On the third day, the cells were serum starved for 24 hours (2% FBS/DMEM) and then exposed to increasing concentrations of AsIII. For the stress fiber assay, BAE were double stained with phalloidin and DAPI for fluorescence microscopy.
- Mouse model
  - FVB/N females (6 wks old) were mated with studs and a vaginal plug was considered successful. Each plugged mouse was transferred to either the AsIII treatment group (37.5 ppm AsIII/$dH_2O$) or the control treatment group ($dH_2O$) for 12.5 days. The mice were then anesthetized using isoflurane, and the placenta was imaged using the VEVO 770 High Performance Ultrasound. The mice were then injected with Microbubbles (Visual Sonics Corporation) via the tail vein and then the microbubble contrasted tissues were photographed.


This projects was supported by NIH (COBRE) RR017670 and NIH (STEER) 1R25ES-016247-01 grants.

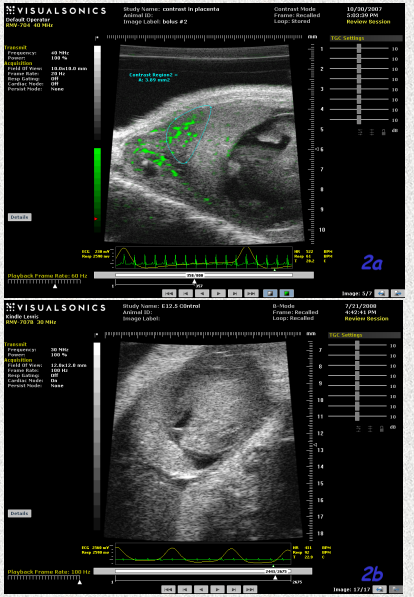
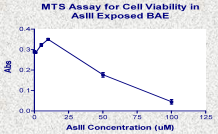




**Figure 2-** *Assessment of placental integrity-* High resolution ultrasound, obtained with the VEVO 770, can be used to assess placental integrity by injection (tail vein) with high-contrast-microbubbles. The 3 micron microbubbles remain on the maternal side of the placenta unless the barrier is compromised. Figure 2a shows an ultrasound from an E14.5 control (water only, no AsIII) mouse obtained with the VEVO 770. Microbubbles from VS Corp. were injected through the tail vein into the maternal circulation resulting on an accumulation on the maternal side. The microbubbles appear green in the maternal placenta (left), the embryonic placenta is virtually free in microbubbles implying good placental integrity. Figure 2b shows an E12.5 control mouse embryo and placenta. As we did not have the necessary software, we were unable to repeat experiments with the microbubbles. However, we were able to obtain a clear picture of the maternal and embryonic placenta at E12.5.

**Figure 3-** *MTS assay for viability of AsIII exposed BAE.* Cells were exposed to AsIII for 24 hours, and then assayed for viability. As shown in the figure, cell viability decreases as AsIII concentration increases. Though it seems to increase at first, viability drops off sharply at 20 uM AsIII.



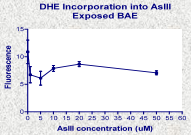




**Figure 4-** *DHE incorporation into AsIII-exposed BAE.* Cells were exposed to DHE for 30 min. The cells were then exposed to AsIII in PBS for one hour. We hypothesized that AsIII would increase oxidation within the cells with increasing concentration. The data show a decreasing trend. However, after multiple trials, the data were not consistent, indicating a problem with the plate reader. Future experiments will repeat the assay with HMVEC, using a different plate reader.

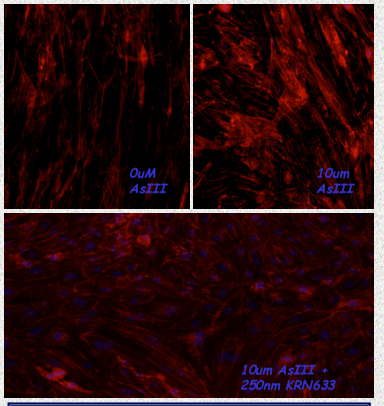



**Figure 5-** *Stress fiber formation in AsIII exposed BAE.* BAE were exposed to AsIII and 250 nm KRN 633 for 24 hours. The cells were then fixed (4% PF/PBS) and stained with phalloidin/DAPI to label stress fibers and nuclei. There is a marked increase in the number of stress fibers in 10uM AsIII treated cells relative to the control (0 uM AsIII). However, the cells treated with 10uM AsIII and KRN633 do not show this increase in stress fibers. As KRN 633 is an inhibitor in the VEGF pathway, this indicates that AsIII utilized the VEGF pathway to cause an increase in the stress fibers. Future experiments will be conducted on HMVEC with V-cadherin immunocytochemistry used to assess junctional integrity.