Myeloperoxidase (MPO) is produced by a subset of immune cells to stimulate the breakdown of hydrogen peroxide and the formation of the powerful oxidant hypochlorous acid (HOCL) at sites of inflammation in the body. HOCL, the active component of bleach, helps the body battle infections by triggering deleterious modifications of proteins and DNA of engulfed pathogens. However, during chronic diseases with associated inflammation, like rheumatoid arthritis, atherosclerosis, and certain cancers, elevated circulating MPO levels cause collateral damage to the host. As such, there is a need for developing safe ways to abrogate these unwanted MPO effects.

The goal of our research was to conduct in vivo safety screening of benzoic acid hydrazine (BAH) analogs, recently shown to inhibit MPO in vitro. Here, we use the zebrafish as our model for vertebrate development and organ function due to their ability to produce large numbers of rapidly developing, visibly transparent offspring with each weekly mating.

Figure, Wilkins. The vertical axis depicts percentage of MPO-inhibitor treated embryos with circulatory abnormalities at 4 days post-fertilization. Each bar depicts a different concentration (see bottom), while each grouping along the horizontal axis depicts values for a different drug. A: shows the tails of the representative embryos with normal appearance after treatment with 20μM (top) and pooled blood cells at 150μM dose (bottom) of NaN₃ after 2 days of treatment. B: shows the heart region of representative embryos with normal appearance after no treatment (top) and pericardial edema after treatment with 150μM 4-NBAH (bottom).
These properties allow us to closely monitor the effects of treatments before, during, and after organ development within the first five days after fertilization. In addition, zebrafish share a remarkable degree of genetic similarity to humans, having similar genes to at least 70 percent of all human protein-coding genes. Thus, the data generated from this study is likely predictive of potential adverse effects in humans.

We screened nine BAH analogs (2-ABAH, 4-ABAH, 3-DMABAH, NaN3, 4-FBAH, BAH, 4-NBAH, 4-TFMBAH, and isoniazid) for effects detrimental to proper organ development. Initially, 6 concentrations of each analog were added to groups of 40 embryos six hours post-fertilization, at which time gastrulation had just begun. In a second assay, we measured toxic effects on cardiac function by adding the compounds to embryos at 2 days post-fertilization, after the heart was developed. In both techniques wild-type (AB) and pigmentation mutant (Casper) embryos were observed for 5 days and grown under normal conditions, except that 0.4% DMSO was added to aid in chemical absorption.

If any, the primary defects observed after treatment during early development were pericardial edema and pooling of blood cells in the tail region, hereafter referred to as circulatory abnormalities (see Figure). Our studies found treatment with 300μM of NaN3, 4-NBAH, and 4-TFMBAH caused circulatory abnormalities in all embryos, with these defects appearing less frequently as dosage was lowered. Both NaN3 and 4-NBAH have an estimated EC50 of 150-300μM, while the estimated EC50 of 4-TFMBAH is 120-150μM. For all other chemicals, fewer than 25% were affected with doses up to 300μM. In our assay for effects on cardiac function, we found circulatory abnormalities only with NaN3 and 4-TFMBAH treatments at doses of 80-300μM (not shown).

Our results suggest that 2-ABAH, 4-ABAH, 3-DMABAH, 4-FBAH, BAH, and isoniazid cause little to no developmental or organ toxicity when administered at dosages of 300μM and below, thus making them ideal candidates for further in vivo testing of MPO-inhibitory function.

Our immediate future plans include LC50 determination for the above drugs and screening a new panel of 60 small molecule MPO inhibitors. Additionally, we will use transgenic zebrafish lines with either fluorescent neutrophils or blood vessels to study the impact of MPO inhibition on normal inflammation and circulation, respectively.

**Statement of Research Advisor:**

Andrew’s toxicity analysis of these compounds is part of a drug discovery process that allows us to reduce the number of potential drugs prior to further animal testing, thus lessening the number of mice or other species required. Discovery of new therapeutics for detrimental effects of the inflammatory process may prove useful in treatment of patients with rheumatoid arthritis, cancer, and cardiovascular disease.

- Jennifer Panizzi, Anatomy, Physiology, and Pharmacology

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**Depth Estimation with a Plenoptic Camera**

*William Roberts*

The Advanced Flow Diagnostics Laboratory (AFDL) at Auburn University has recently built a novel device called a plenoptic camera. This device is constructed like a conventional DSLR camera with an array of microlenses mounted in front of the sensor chip. Light passes through the microlenses in a way that allows the camera to capture both the location and angle at which light rays impact the image sensor. Because of this, the photographer captures the complete four-dimensional light field with a single exposure and can change the viewing perspective using image processing after the image has been taken. It is then possible to