Chemical and molecular chaperones and their importance to dogfish (*Squalus acanthias*) hemoglobin-oxygen affinity following temperature and osmotic stress

Suzanne Currie[1], Ashra Kolhatkar[1], Nathan S.B. Walker[1] and A. Kurt Gamperl[2]
[1]Department of Biology, Mount Allison University, Sackville, NB, Canada E4L 1G7
[2]Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF, Canada A1C 5S7

In most cells, proteins are protected from cellular stress by both chemical and molecular chaperones – organic compounds that stabilize protein folding. Using the dogfish red blood cell as a model, our goal was to determine if these chaperones are required to preserve cell function in the face of high temperature or osmotic stress. We show that hemoglobin-oxygen affinity, a measure of red blood cell function, is not dependent on the presence of chemical or molecular chaperones.

Elasmobranchs are marine osmoconformers, and use the organic osmolytes trimethylamine oxide (TMAO) and urea to maintain the osmotic concentration of their internal fluids similar to that of their environment. TMAO is also known to rescue proteins experiencing temperature-induced folding defects, and thus functions as a *chemical* chaperone[5]. The heat shock proteins (HSPs) are *molecular* chaperones that also prevent protein denaturation. Recently, we demonstrated that acute heat stress does not induce HSP70 expression in red blood cells of the spiny dogfish shark if physiological levels of TMAO are present[2], but that hypo-osmotic stress only results in an induction of HSP70 in cells with TMAO. Furthermore, we showed that cells experiencing hypo-osmotic stress have significantly more oxidative damage despite the protection afforded by both TMAO and HSPs. Thus, the goal of this work was to determine if cellular function is compromised following: 1) heat stress when cells are protected by TMAO but not HSPs, and 2) hypo-osmotic stress when cells have both TMAO and HSPs but appear to incur some degree of cellular damage. To this end and given that the main function of vertebrate red blood cells is to carry and deliver oxygen, we used hemoglobin-oxygen affinity as an indicator of cell function in dogfish red blood cells.

We sampled whole blood from pithed spiny dogfish in a procedure approved by the MDIBL IACUC. Blood was washed in elasmobranch saline, resuspended at a hematocrit of 15% in saline with and without TMAO and stored at 4°C for 24-48 h[1]. Red blood cells were then placed in a shaking water bath at 13°C for 1 h, and then subjected to either a 1 h heat shock at 24°C or a hypo-osmotic shock (at 50% NaCl concentration). We constructed oxygen dissociation curves[3] using the Tucker method[4] and a Cameron OM 200 oxygen meter prior to the stress at 13°C (control) and 2 and 20 h following the initiation of heat or hypo-osmotic stress. Using Hill plots, we calculated the PO_2 at which hemoglobin was 50% saturated with oxygen (P_{50}) to determine if hemoglobin-oxygen affinity is affected by the stress (heat or hypo-osmotic) and TMAO.

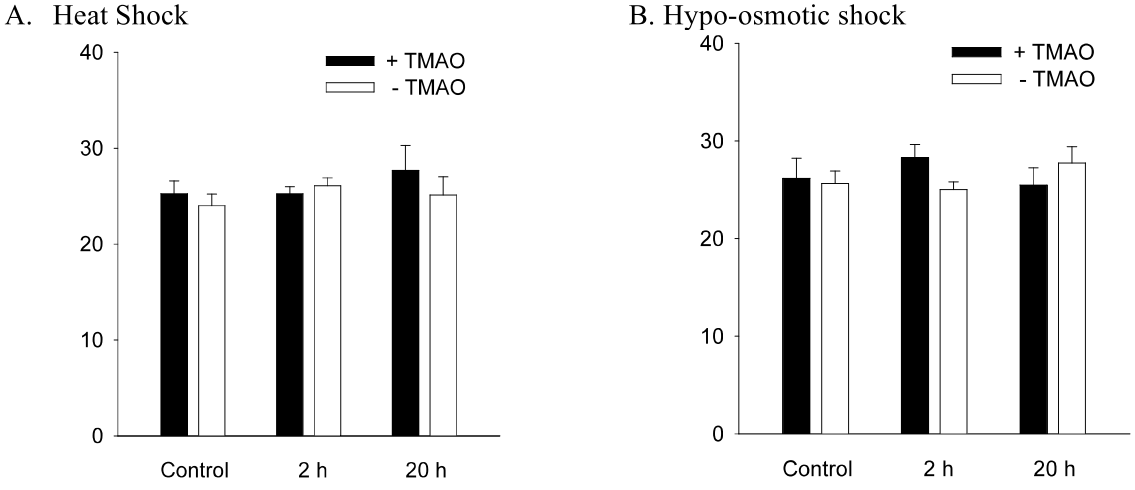

Figure 1. P_{50} values (mean ± SEM) for dogfish red blood cells incubated with (dark bars) and without (light bars) TMAO, before (control) and 2 and 20 h following a 1 h heat shock (A) or 2 and 20 h into a hypo-osmotic stress (B). N = 6 for both experiments. Two-way repeated measures ANOVAs revealed no significant differences between groups in both the heat shock (time: p = 0.881; TMAO: p = 0.645) and hypo-osmotic (time: p = 0.984; TMAO: p = 0.933) experiments.

Our results to date indicate that neither heat nor hypo-osmotic stress significantly affect how tightly oxygen is bound to hemoglobin in dogfish red blood cells (Figure 1). Furthermore, the presence or absence of exogenous TMAO in these cells does not impact P_{50}. These data suggest that hemoglobin - oxygen affinity is preserved in the face of potentially damaging stressors regardless of the presence of chemical or molecular chaperones.

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1. **Caldwell, S, Rummer, JL and Brauner, CJ.** Blood sampling techniques and storage duration: Effects on the presence and magnitude of the red blood cell β-adrenergic response in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A* 144:188-195, 2006.
2. **Currie, S and Robertson, C.** Cellular and molecular chaperones in the red blood cells of the spiny dogfish, *Squalus acanthias*. *Bull. Mt. Desert Isl. Biol. Lab.* 49:58-59, 2010.
3. **Gollock, MJ, Currie, S, Petersen, LH and Gamperl, AK.** Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. *J. Exp. Biol.* 209: 2961-2970, 2006.
4. **Tucker, VA.** Method for oxygen content and dissociation curves on microlitre blood samples. *J. Appl. Physiol.* 23:410-414, 1967.
5. **Welch, WJ and Brown, CR.** Influence of chemical and molecular chaperones on protein folding. *Cell Stress Chap.* 1:109-115, 1996.