EFFECT OF MEMBRANE-BOUND CARBONIC ANHYDRASE (CA) INHIBITION ON BICARBONATE EXCRETION IN THE SHARK, SQUALUS ACANTHIAS

Erik R. Swenson[1], Brian C. Taschner[2], and Thomas H. Maren[2]
[1]Department of Medicine, VA Medical Center and University of Washington, Seattle, WA, 98108
[2]Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL 32610

We reported last year (Swenson et al., Bull. MDIBL. 34:94,1995) on the effects in the elasmobranch shark of a high molecular weight polymer linked to a carbonic anhydrase inhibitor; polyoxyethylene-aminobenzolamide. Its high molecular weight (3700) and water solubility limit its distribution to extracellular space and restricts its inhibition to CA on cell surfaces. Indeed, we found no uptake by red cells, gill or muscle of the shark. An intravenous dose of 50 mg/kg had no effect on arterial PO_2, pH or PCO_2 in the normal fish but slowed the rate of gill bicarbonate excretion following an intravenous load of $NaHCO_3$. However, the effect was less than that of benzolamide, whose uptake into gill inhibits both intracellular and plasma membrane-bound CA. These results were interpreted as either submaximal inhibition of gill surface membrane-bound CA or independent additive roles for CA isozymes in gill HCO_3^- excretion. To distinguish between these possibilities a dose response study was undertaken. Squalus acanthias (wt. range 1.8-2.2 kg) were studied 12-16 hr after transfer into small Plexiglas tanks and placement of a dorsal artery catheter. A metabolic alkalosis was induced by a 1 hr infusion of 1 M $NaHCO_3$ (9 mmol/kg). At the start of the $NaHCO_3$ infusion 25, 100 or 200 mg/kg of polymer-linked inhibitor was given intravenously over 5 min. Arterial blood was sampled hourly for pH, total CO_2 and PO_2.

The table shows the time course of plasma HCO_3^- in mM with polymer inhibitor and compares it to the rapid normal (control) rate and to the suppressed rate of normalization by total gill CA inhibition with benzolamide (Swenson and Maren, Am J Physiol 253:R450,1987). Despite a four fold increase in the dose, there was no further statistically significant slowing in the rate of bicarbonate clearance. The effect of the lower dose of 25 mg/kg did not differ from the higher doses. These results suggest that bicarbonate excretion by the gill requires CA activity at both the plasma membrane and in the cytosol since the polymer inhibitor clearly causes HCO_3^- retention but less than benzolamide. In this regard, the gill appears similar to the mammalian kidney. Several studies involving CA II deficient mice and humans as well as selective inhibition of CA IV point to an additive role for both intracellular CA II and brush border membrane-bound CA IV activity in normal proximal tubular HCO_3^- reabsorption (Brechue, Kinne-Saffran, Kinne, and Maren. Biochim Biophys Acta 1066:201,1991; Sly, Whyte, Krupin, Sundaram. Pediatr Res 19:1033, 1985; and Hsu, Moeckel, and Lai. J Am Soc Nephrol 6:701A,1995).

Time hr	Control	Benzolamide 2 mg/kg	Polymer-linked inhibitor 25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
			plasma HCO_3^- (mM)			
0	4.8 (0.3)	5.1 (0.3)	5.1 (0.3)	5.0 (0.4)	4.9 (0.3)	5.2 (0.5)
1	35 (1.5)	37 (1.4)	33 (1.4)	37 (1.5)	38 (1.7)	39 (1.6)
2	14 (0.9)	29 (0.8)	22 (1.0)*	24 (1.4)*	22 (1.3)*	26 (1.5)*
3	10 (0.6)	24 (0.5)	19 (1.0)*	17 (0.8)*	17 (0.7)*	21 (1.0)*
4	8 (0.5)	21 (0.6)	16 (0.6)*	15 (0.7)*	12 (0.4)*	16 (0.8)*
n =	(8)	(7)	(5)	(5)	(6)	(3)

values are means $\pm$ (SD), * $p < 0.05$ vs. control and benzolamide

Supported by NIH grant HL-45571(ERS) and Univ. of Florida sponsored research grant (THM).