EFFECT OF GILL MEMBRANE-BOUND CARBONIC ANHYDRASE INHIBITION ON BRANCHIAL BICARBONATE EXCRETION IN THE DOGFISH SHARK, SQUALUS ACANTHIAS

Erik R. Swenson[1], Lincoln Lippincott[2], Thomas H. Maren[2],
[1]Department of Medicine, VA Medical Center and University of Washington School of Medicine, Seattle, WA, 98108, USA;
[2]Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL 32610, USA.

Gills of marine fish contain carbonic anhydrase (CA) (Maren, Physiol. Rev. 47: 595-781, 1967) but little is known of the isozyme types and subcellular distribution of the enzyme (cytosolic vs. plasma membrane-bound). Our previous studies have documented involvement of elasmobranch branchial CA in rapid correction of metabolic alkalosis and respiratory acidosis (Swenson and Maren, Am. J. Physiol. 253:R450-R458, 1987 and Swenson and Claiborne, Bull. M.D.I.B.L. 26:5-8, 1986). In these studies we used benzolamide (MW = 320) to selectively inhibit gill CA without significantly inhibiting red cell CA. Because benzolamide is taken up by the gill it inhibits both cytosolic and membrane-bound CA. To explore the role of plasma membrane-bound CA requires a compound unable to reach cytosolic CA. We now report the use of a newly synthesized polymer inhibitor, polyoxyethylene-aminobenzolamide (kindly supplied by Dr. Curtis Conroy, Dept of Pharmacology and Therapeutics, University of Florida) which by virtue of its high molecular weight (MW=3600) and water solubility should remain extracellular and thus restrict its inhibition to enzyme on cell surfaces.

Spiny dogfish, Squalus acanthias (males, weight range 1.8 - 2.2 kg) were studied 12 to 16 hours after transfer into small Plexiglas tanks and placement of a caudal artery catheter as described previously (Swenson and Maren, 1987). A metabolic alkalosis was induced in 5 fish by a one hour constant infusion of 1 M sodium bicarbonate (9 mEq/kg). At the start of the bicarbonate infusion, 50 mg/kg of polymer inhibitor was given iv over 5 min. Arterial blood was sampled hourly. In several fish, red cell (n=3), kidney (n=2), gill (n=2) and muscle (n=2) tissue were taken for measurement of the polymer-inhibitor four hours after drug administration. To study in vitro red cell uptake of polymer inhibitor, 20 ml of washed dogfish erythrocytes was suspended and continuously agitated in 20 ml of elasmobranch Ringer's solution containing 300 ug/ml of polymer inhibitor at 14 °C. At 30 min intervals, 5 ml of RBC solution was removed, centrifuged to remove supernatant and then washed three times with 10 ml Ringer's.

Arterial blood samples were analyzed for pH and PO_2 with a blood gas analyzer (Cameron Instruments) that was maintained and calibrated at 14° C. Total plasma CO_2 concentration was measured using a microgasometer (Kopp-Natelson). Plasma and tissue polymer-inhibitor concentrations were measured by the inhibition of a known amount of dog red cell CA and compared to a standard curve (Maren et al., J. Pharmacol. Expl. Therap. 130:389, 1960). Red cell and tissue samples were heated to ~ 80-90° C to denature all CA activity and the supernatants were assayed inhibitor concentrations after centrifugation at 1000g for 10 min. . Inhibition constants for the polymer inhibitor against red cell and gill CA were determined by the method of Maren et al. (1960).

Figure 1 shows the time course of plasma HCO_3^- normalization (mean $\pm$ SD) 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, 1987). At this single dose of polymer inhibitor there appears equivalence

with benzolamide at two hours (one hour after completion of the bicarbonate infusion), but not in the subsequent two hours ($p < 0.05$, unpaired t-test), where the rate of bicarbonate normalization is faster, although slower than control.

Figure 2 shows the time course of plasma polymer inhibitor clearance in three fish (mean ± SD). These data when analyzed by semilog plot show a drug concentration at injection of ~ 600 ug/ml, yielding a volume of distribution of ~ 8%. It is cleared with a plasma half life of 90 min.

Figure 1

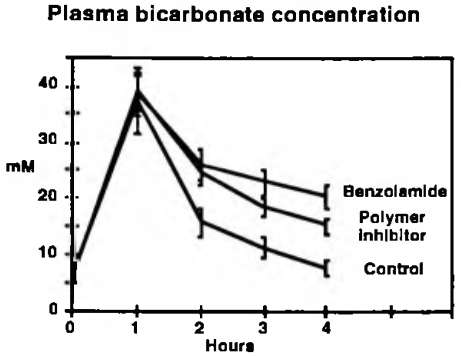
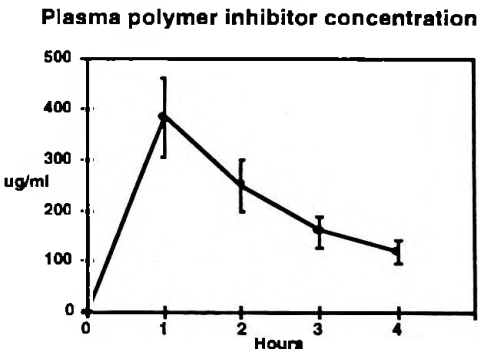


Figure 2



In vitro and in vivo red cell uptake of the polymer inhibitor was undetectable at all time points up to 4 hours in our assay system whose lower limit of detection is ~ 5 ug/ml or 1.2 uM. Gill and muscle tissue drug concentrations at 4 hours were also undetectable. At 4 hours kidneys had a concentration of ~ 12 ug/ml or 3 uM. The polymer inhibitor inhibits both shark gill and red cell CA with an I_{50} of 170 nM. From these inhibition data, we calculate that at two hours there was approximately 99.8 % inhibition of gill membrane-bound CA. The respective I_{50} values for benzolamide against gill and red cell CA are 12 and 30 nM.

These results demonstrate that the polymer inhibitor is a suitable compound for selective cell plasma membrane-bound CA inhibition in vivo. It has a volume of distribution intermediate between the vascular volume and extracellular space of the elasmobranch and shows no significant uptake into a variety of tissues either in vivo or in vitro. The kidney is an apparent exception, although urinary excretion likely accounts for detectable drug in this organ. The effect of the polymer inhibitor on branchial bicarbonate excretion in metabolic alkalosis suggests an important if not dominant role of membrane-bound CA in this process, since there were no differences between benzolamide and the polymer inhibitor at one and two hours. Declining concentrations of the ten-fold weaker polymer inhibitor leading to loss of effective inhibition may explain the differences between benzolamide and the polymer inhibitor at the later time points. Ultimately, dose response experiments will be necessary to uncover fully the roles of cytosolic and membrane-bound CA isozymes in branchial acid-base regulation.

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