EFFECTS OF A HIGH MOLECULAR WEIGHT CARBONIC ANHYDRASE (CA) INHIBITOR, F3500, ON RESPIRATORY ACIDOSIS IN THE SHARK, *SQUALUS ACANTHIAS*

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We report further investigation of a newly synthesized high molecular weight carbonic anhydrase (CA) inhibitor, F3500 (Conroy et al., Bioorganic Chem 24:262, 1996) in elasmobranch acid-base balance. F3500 is a polymer of polyoxyethylene bis acetic acid linked to the potent CA inhibitor aminobenzolamide. By virtue of its large molecular weight (MW = 3500), excellent water solubility, and high inhibitory constants (K_I at 15 degrees C: 0.07 μM and 1.5 μM vs mammalian cytosolic CA II and membrane-bound CA IV respectively, and an I_{50} of 0.17 μM against dogfish red cell and gill CA activity) this non-toxic compound remains extracellular and does not permeate fish or mammalian cell membranes (Swenson et al., Bull MDIBL 34:94, 1995 and Conroy et al., ibid.). Thus F3500 is an ideal compound to study the role of cell surface membrane-bound CA in the absence of intracellular CA inhibition. Our previous dose response studies of this compound on the branchial elimination of bicarbonate in metabolic alkalosis in the elasmobranch have demonstrated that gill cell surface membrane-bound CA is involved in this process (Swenson et al., ibid. and Swenson et al., Bull MDIBL 35:47, 1996) but that there is also a contribution of cytosolic CA, since benzolamide, a low molecular weight CA inhibitor (MW = 320) with access to intracellular CA results in even slower elimination of bicarbonate than does F3500 (Swenson and Maren, Am J Physiol 253:R450, 1987).

The compensation to respiratory acidosis in the elasmobranch is also a CA dependent process involving either the formation of bicarbonate at the gill or its uptake from seawater (Swenson and Claiborne, Bull MDIBL 25:77, 1985). In that study we used benzolamide, and so it was not possible to determine whether membrane-bound CA either at the apical or basolateral side was involved. In the present study we used F3500 to selectively inhibit cell surface membrane-bound CA and hypothesized that, as in bicarbonate elimination in metabolic alkalosis, loss of membrane-bound CA activity would slow the rate of pH recovery in acute respiratory acidosis but to a lesser extent than with combined intracellular and membrane-bound CA inhibition.

Dogfish were caught and transferred to large submerged cubicles under the lab docks. The fish were then brought to temporary holding tanks where they were allowed to acclimate to their new surroundings. Anywhere from 3-5 days following capture, the fish were transported to small, running seawater plexiglass tanks just large enough to accommodate them without allowing them to swim around. A catheter was introduced into the caudal artery in order to facilitate arterial blood withdrawal and drug infusion. The tanks were then covered with a black plastic bag to eliminate any external stimuli that may excite the fish.

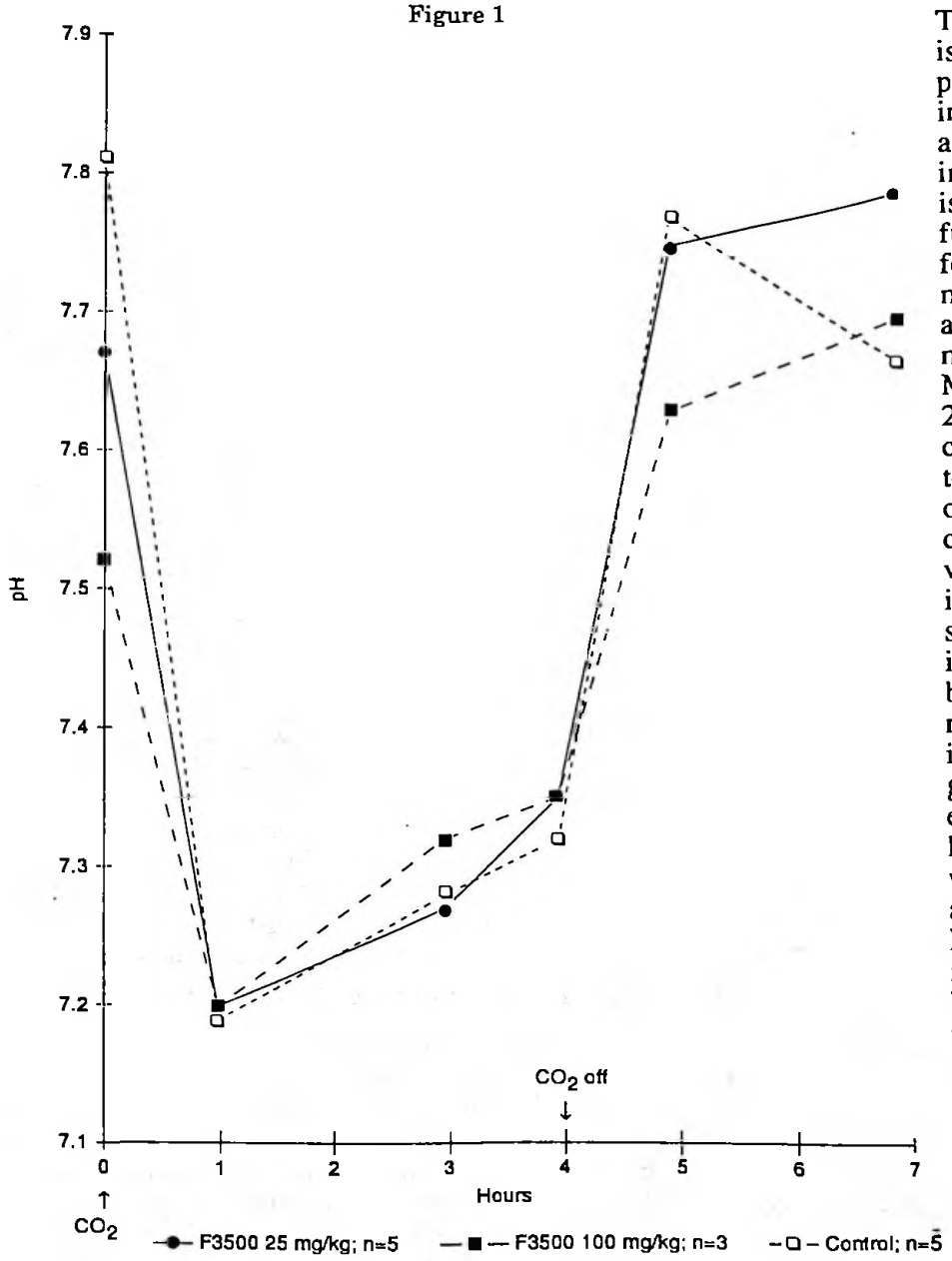
Following a 15-18 hour recovery period, several control arterial blood samples were taken for pH and tCO_2 measurement. At this time (time=0) a given dose of the inhibitor dissolved in 2cc dogfish Ringer's was injected. In addition, the running seawater was turned off and 1% CO_2 was introduced to the tank via an aerating stone. The CO_2 produced a state of respiratory acidosis in the shark. Arterial blood was sampled at 1, 3 and 4 hours. At t=4, the CO_2 was turned off, and the running seawater was turned on. Subsequently, arterial blood samples were taken at t=5 and 7 hours. Each blood sample was analyzed for pH via a standard pH electrode

and the plasma for tCO_2 via a Kopp-Natelson microgasometer. The same procedure applies to the control group except the fish were not given any inhibitor at t=0. In all, 13 fish were studied: control (n=5), 25 mg/kg F3500 (n=5) and 100 mg/kg F3500 (n=3).

The half-time decay of F3500 from plasma is 90 minutes (Swenson et al. 1995, ibid), which would yield a value of 13.4 μM at three hours with a dose of 25 mg/kg. Extrapolating this to the highest dose (100mg/kg) would give 53.6 μM at the 3 hour time point. The K_I for F3500 is 1.5 μM at 15 degrees C for the mammalian membrane-bound CA IV. Assuming no difference between the elasmobranch and mammal membrane-bound isozymes, the fractional inhibition (*i*) of gill membrane-bound CA can be calculated as follows:

$$i = I_f / (I_f + K_I) = 53.6 / 55.1.$$

Figure 1

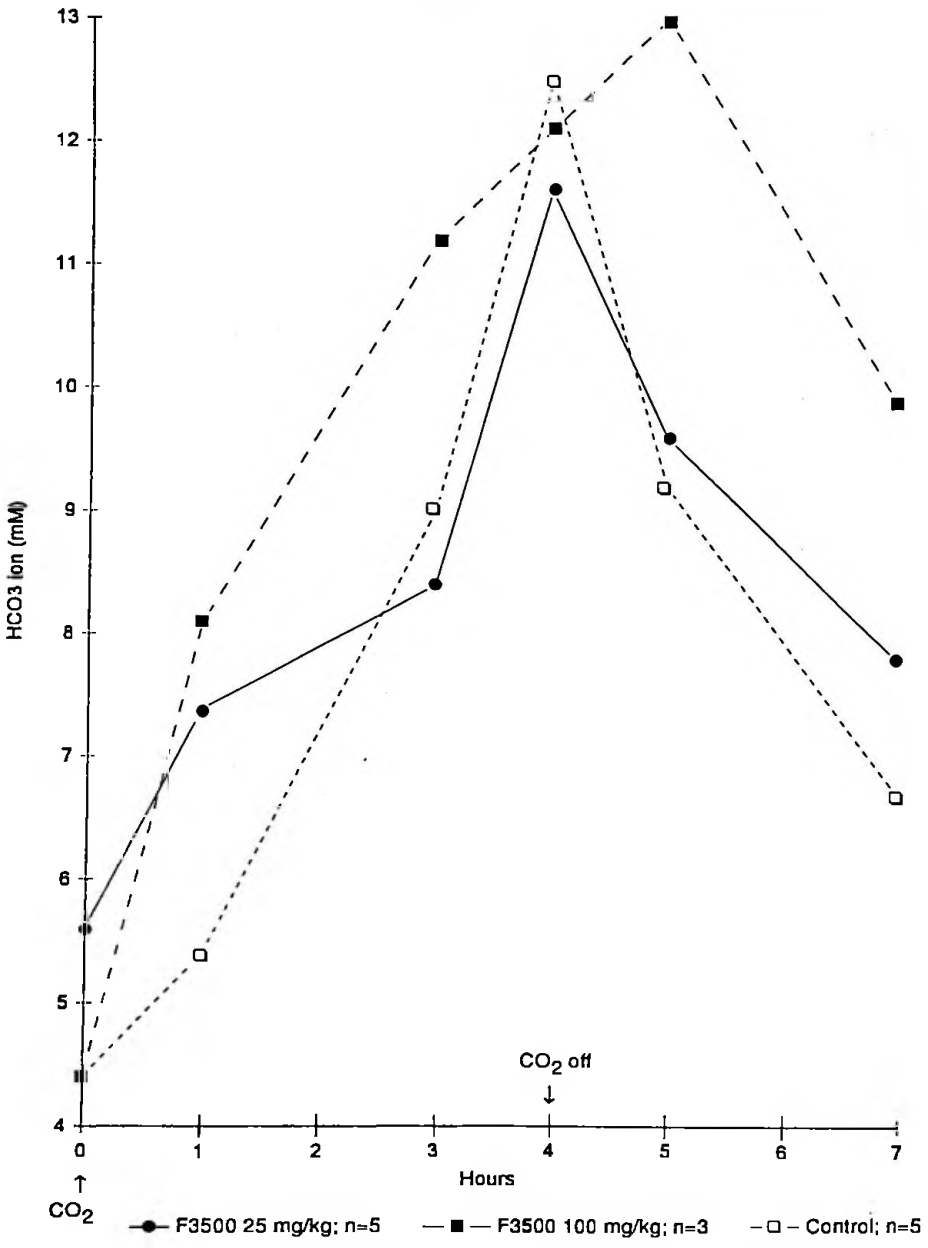


The degree of inhibition is 97%. We have shown previously that 98.5 % inhibition (of all gill CA activity), if enzyme and inhibitor are in contact, is sufficient to produce a full pharmacological effect with the low molecular weight sulfonamides such as benzolamide (Swenson and Maren, Am J Physiol 253:R450, 1987). Because there is an almost tenfold lower activity of mammalian CA IV compared with high activity mammalian cytosolic CA II, and assuming a similar (but not yet investigated) difference between cytosolic and membrane-bound CA isozymes in the shark gill, 97% inhibition is effectively complete inhibition. Supporting this, we showed last year that a two fold higher dose of F3500 (200 mg/kg) had no further effect on bicarbonate excretion in metabolic alkalosis than 100 mg/kg (Swenson et al., 1996, ibid.).

Figures 1-3 show the time course of changes in arterial pH, HCO_3,

and PCO_3^- during a four hour period of hypercapnic seawater exposure and the resolution of this respiratory acidosis following return to normal seawater. Figure 1 shows 1 % CO_2 caused a profound respiratory acidosis with a rise in PCO_2 (Fig. 3) from 3 to 13-18 mm Hg and a 0.4 unit fall in pH. Compensation to this acidosis with an elevation in plasma bicarbonate was only partially effective in correcting the pH and was not affected by F3500 in the two doses tested. Furthermore, with return to normal seawater at four hours, there were no statistically significant differences between control and drug-treated fish in the rate of HCO_3^- excretion (Fig. 2) in this period of post hypercapnic metabolic alkalosis. Although a trend towards a reduction in the 4-7 hours bicarbonate excretion in the fish given 100 mg/kg is suggested, the small number of fish (n=3) studied prevent any definitive conclusion. Our previous studies document slower HCO_3^- elimination when fish are given HCO_3^- exogenously. Although this may be the case in post hypercapnic metabolic alkalosis as well, the design of the present experiments was not adequate to test the question due to rapid clearance of the drug and insufficient concentrations in the later period (beyond four hours).

Figure 2



We interpret the new finding of no effect of F3500 in respiratory acidosis on the rate of branchial bicarbonate formation, but possibly slower bicarbonate elimination in metabolic alkalosis as follows. Membrane-bound CA located at the apical aspect (seawater) of the branchial epithelium is involved in new HCO_3^- formation (or uptake from seawater) in compensation of respiratory acidosis. In contrast, the process of bicarbonate excretion requires basolateral membrane-bound CA. Since F3500 is a large hydrophilic molecule and was only given intravascularly, it is unlikely that it could reach the apical side to affect events in respiratory acidosis. It is unknown whether F3500 is excreted by the gill; even so, it would be

quickly diluted to insignificant concentrations in the high volumes of seawater ventilating the gills. In further studies we must establish a dose response curve reaching 500 mg/kg (to yield > 99.5% inhibition) to rule in or out a basolateral membrane bound CA role in defense against respiratory acidosis. It will also be critical to administer sufficient F3500 into the seawater to yield similarly high concentrations at the apical side.

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Figure 3