Title: THE EFFECTS OF FLUORIDE ION ON THE ISCHEMIC AND NON-ISCHEMIC CANINE KIDNEY

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Introduction. Although it is well established that the concentration of fluoride (up to 25 uM) released from the metabolism of enflurane is not toxic to normal kidneys (1), it is unclear whether this concentration of fluoride is damaging to ischemic kidneys. We have used the isolated perfused kidney (IPK) to study the effect of fluoride on renal function in normal and ischemic canine kidneys. The isolated perfused kidney model has many advantages compared to in vivo studies including: 1. separation of the kidney from extra-renal influences such as neuronal, cardiovascular, and hormonal; 2. precise control of experimental conditions such as perfusion pressure, blood gases, temperature and concentration of fluoride, and; 3. avoidance of complications due to anesthetic administration to the whole animal.

Methods. Adult mongrel dogs (15-25 kg) were anesthetised with sodium pentothal and then maintained with halothane and oxygen. A laparotomy was performed and the left kidney was isolated including it's ureter, renal artery and vein. In the non-ischemic group, after mannitol and heparin treatment, the kidneys were immediately removed, flushed through the artery with a cold solution, and placed on the IPK system. The kidneys in the ischemic group, after mannitol and heparin treatment, were left in the abdomen for 30 minutes after clamping of the ureter, renal artery and renal vein, and then removed and treated like the non-ischemic kidneys.

The IPK circuit consisted of a Water's cassette, roller pump, membrane oxygenator and bubble trap, all connected in series by 1/4" tygon tubing. The perfusate was 600 ml of a bovine serum albumin (5gm%) containing solution (modified Krebs-Hansleit), with creatinine (2 mg%) added. The measured pO2 was greater than 450 mm Hg. In separate experiments, fluoride was added to the perfusate to obtain concentrations of either 0, 25, 50, or 100 uM. At least four kidneys were done in each of these four groups. The perfusion pressure, measured with a Statham transducer, was maintained at 100 mm Hg by adjusting the speed of the roller pump and the temperature was maintained at 37°C. Renal artery flow was read directly from the pre-calibrated roller pump. Each kidney was perfused for 120 minutes.

The ureter was cannulated and urine was continuously collected in 10 minute aliquots. These samples were analyzed for creatinine and sodium concentrations and from this data creatinine clearance and sodium reabsorption calculated. At the end of the experiment, a cortical tissue sample was taken for determination of ATP concentration. Results reported are mean values + SEM. Statistical analysis was performed using the paired t-test and significance was noted for $p < .05$.

Results. Fluoride at all concentrations studied had little effect on creatinine clearence or renal perfusate flow in the non-ischemic kidneys. 100 uM fluoride, however, caused a five-fold statistically significant increase in urine output (control = 6.00±3.25 vs. 100 uM fluoride = 32.58±10.22 ul/min/gm). Fig. 1 shows the effects of fluoride on the reabsorption of sodium and reveals a significant decrease in sodium reabsorption which was time and dose dependent in kidneys treated with both 50 and 100 uM fluoride. In the presence of 25 uM fluoride, sodium reabsorption was not significantly different from the control. Cortical ATP concentrations were significantly less in kidneys perfused with 100 uM fluoride compared to controls. (control =.880±.035 vs. 100uM =.572±.059 nmoles/gm wet weight)

In contrast, the effect of fluoride on the ischemic kidney was much more pronounced. Fluoride (25uM) caused a large and significant decrease in the ability of the ischemic kidney to reabsorb sodium (Fig. 2). Ischemic kidneys also had significantly reduced concentrations of ATP when exposed to 25 uM fluoride (ischemic control=.305±.082 vs. ischemic control plus 25 uM fluoride=.232±.058 nmoles/gm wet weight) This concentration of fluoride had no significant effect on the creatinine clearance or the urine output of ischemic kidneys.

Discussion. Using the isolated perfused kidney, we have shown that fluoride is toxic to normal kidneys at concentrations of 50 and 100 uM (reduced sodium reabsorption), which agrees with previous studies in animals and humans. While 25 uM fluoride caused no apparent effect in the non-ischemic kidneys, our data suggests that kidneys that have undergone 30 minutes of warm ischemia are affected adversely by this concentration of the anion, having reduced ability to reabsorb sodium. This may reflect a direct effect of fluoride on the proximal tubules and may in part be mediated by a suppression of ATP formation. Anesthetic agents that release concentrations of fluoride that are not injurious to normal kidneys may exacerbate damage in kidneys exposed to an ischemic insult.

This study was supported in part by an ASA Research Starter Grant.

References:

1. Cousins MJ, et. al.: Metabolism and Renal Effects of Enflurane in Man. Anesthesiology 44: 44-53, 1976

Figure 1

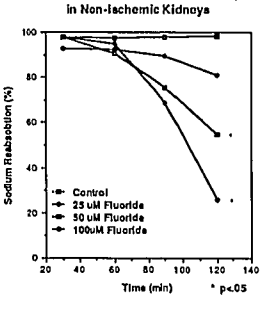

Figure 2