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INO-4995, A CANDIDATE CYSTIC FIBROSIS THERAPY, DECREASES NASAL POTENTIAL DIFFERENCE IN A MOUSE CF MODEL

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We have previously shown that INO-4995, a myo-inositol 3,4,5,6 tetrakisphosphate analog of a naturally occurring intracellular inositol signaling molecule, demonstrates the ability to modify the abnormal ion transport in cystic fibrosis (CF) airway epithelia, with a resulting hydration of the airway surface liquid. This compound acts intracellularly and bypasses the genetic defect in CF and is currently being developed as a therapy for CF. INO-4995 is an ion channel modulator affecting both chloride and sodium ion channels. In *in-vitro* experiments, 20 μ M of INO-4995 added to CF epithelial cells for 2 hours inhibited basal I_{sc} approximately 50% when measured 24-48 hours after addition. The effect could be observed as early as 30 min. after drug addition.

When the concentration was lowered approximately 10-fold to 2.5 μ M, and added to cells 1x/day for 3 days, a similar electrophysiological response was observed indicating that there may be a depotting or prolonged effect of the compound. In either the acute or the multiple day treatments, no effect on I_{sc} in normal epithelial cells was observed at similar concentrations. We demonstrate now, similar *in-vivo* activity in the mouse nasal potential difference (NPD) model. FABP (fatty acid binding protein) CFTR- mice were used in these studies. In the FABP/CFTR- mice, the gastrointestinal defect is corrected with the gut-specific promoter, however, the nasal electrophysiology is similar to that of the non-corrected CFTR- mice. Mouse NPD was tested 3 days prior to the addition of INO-4995 to measure baseline NPD. The NPD was measured again 1 day after the last dose with drug. INO-4995 was tested at a dose range of 0.02-20 μ g and was given either as a single dose (acute) or dosed once per day over 4 days. Preincubation with an acute dose of INO-4995 reduced the elevated baseline NPD at two (0.02 and 0.2 μ g) of the three doses tested, with NPD baseline values dropping 5-10 mV. Treatment with INO-4995 at the 0.2 μ g dose reduced the baseline elevated NPD by about 9 mV ($p < 0.05$, paired t test). Washout experiments after acute dosing indicate the potential difference measurements return to baseline levels after 10 days. In the cumulative dosing regimen, 16 animals were dosed with 0.02 μ g over 4 days and PD measurements taken 24 hours after the last dose. The average pretreatment PD was 20 mV and after treatment, 13 mV, with 13 of the 16 mice responding. The treatment response was more consistent in the multiple dosing regimen. A small cohort of animals was washed out after receiving drug and in these animals, NPD returned to baseline values. We are now assessing the pharmacokinetics and bioavailability of the compound and the histology of the nasal epithelium after intranasal drug treatment. Previous safety studies in animals have indicated no deleterious effects of INO-4995 when administered systemically at large doses. These data will help define the clinical dosing regimen for the planned proof of principal NPD trial in CF patients. Supported in part by a CFFT grant.