

Department of Clinical Laboratory Sciences

Graduate Masters Theses

**A Regulation Of Lung  $\alpha$ -Epithelial Sodium Channels And Glucocorticoid Receptors By Novel Synthetic Glucocorticoids.**

Babara Henson, MS. May 2007.

**ABSTRACT:**

Lung development in humans is a complex maturational process starting in early fetal life and continuing until after birth. In fullterm newborns, clearance of fetal lung fluid is predominately coupled to  $\text{Na}^+$  transport through epithelial  $\text{Na}^+$  channels (ENaC), located in the distal lung epithelium. However, clearance of fetal lung fluid is greatly retarded in premature neonates, mainly due to lung immaturity. Thus, a preterm infant accumulates excess lung fluid resulting in severe respiratory distress syndrome (RDS). ENaC is a multimeric channel consisting of three subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ). Mice knockout studies have shown that the  $\alpha$ -subunit is critical for efficient ENaC function in the newborn lung. Extensive studies have previously suggested that ENaC expression can be regulated by glucocorticoids (GC). GC appears to have, besides the stimulation of surfactant, additional effects on pulmonary maturation. In particular, antenatal GC therapy of pregnant women at risk of delivering prematurely accelerates lung development and function in the preterm neonate. The origins of this practice came in 1972 from the pioneering work of Liggins and Howie, who showed a significant reduction in the incidence of RDS in preterm babies whose mothers had received antenatal GC. However, when antenatal GC treatment is not accessible, postnatal GC treatment is utilized in premature neonates. Early postnatal treatment of premature infants with GC, dexamethasone (dex), has been shown to reduce the incidence of lung injury in preterm infants with RDS. Despite the obvious benefits of postnatal GC, their use is discouraged due to severe side effects (e.g. neurological and developmental delays). During development the presence of GC are essential for fetal lung maturation. Responsiveness to GC treatment is mediated by specific glucocorticoid receptors (GCR). A previous study demonstrated that GCR-deficient mice (GCR  $-/-$ ) die shortly after birth from respiratory failure. GCR act as a transcription regulator via DNA binding to nuclear glucocorticoid response elements (GREs) on target genes. After binding to the GCR, GC can regulate gene expression via two major pathways termed "transactivation" and "transrepression". Transactivation involves binding of the GC-GCR complex to the nuclear GRE and results in stimulating transcription of certain genes. Transrepression involves protein-protein interactions of the GC-GCR complex with other transcription factors that do not bind to the nuclear GRE. The binding of the GCR with these other transcription factors (e.g. AP-1, NF $\kappa$ B) can either repress or induce gene transcription. Dex, interestingly displays both transactivation and transrepressive properties in a cell specific manner. Recently the development of synthetic GC ligands (ZK-57740 and ZK-077945), have been introduced by Schering AG. Although these compounds are structurally similar to dex, they are only selective for transactivation, and display minimal transrepressive properties. To confirm the transactivation properties of the ZK compounds, LPS-induced nitric oxide synthase (iNOS) protein levels were assessed using

alveolar macrophages (NR8383). LPS-induced iNOS activity showed an almost complete inhibition by dex, in contrast only a partial reduction of iNOS in the presence of either ZK-57740 or ZK-077945. In the present study, we evaluated the effect of these ZK compounds on  $\alpha$ -ENaC and GCR protein expression compared to dex in cultured mouse lung epithelial (MLE-12) cells. Cultured MLE-12 cells consistently express low endogenous levels of  $\alpha$ -ENaC protein, but interestingly an abundance of GCR protein expression. Exposure of MLE-12 cells to either dex, ZK-57740 or ZK-077945 (10nM) resulted in an increase in  $\alpha$ -ENaC protein compared to untreated cells. Treatment of MLE-12 cells with dex for up to 72 hours greatly decreased endogenous GCR protein compared to untreated cells; but GCR protein levels remained abundant following treatment with ZK-57740 and slightly decreased in the presence of ZK-077945. Our findings suggest that ZK-57740 and ZK-077945 are indeed selective for transactivation when compared to dex. Synthetic GC, selective for transactivation, may have potential therapeutic value in instances when the transrepressive properties of dex are undesirable.