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Immune, inflammatory and infectious consequences of estrogen in cystic fibrosis women

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Introduction

Cystic fibrosis (CF), a multi-system genetic disease with predominantly pulmonary manifestations, occurs due to defective chloride secretion at epithelial surfaces. The consequential sodium hyper-absorption leads to mucus hypersecretion, compromised mucociliary clearance and pathogenic colonization by, for instance, *Staphylococcus aureus* and/or *Pseudomonas aeruginosa*. This sequence of events leads to recurrent infection and persistent chronic neutrophilic inflammation causing airway damage, irreversible bronchiectasis, respiratory failure and death.

The CF gender gap

Gender variation has long been recognized in CF: females have worse survival rates, poorer pulmonary function and earlier bacterial colonization (1-4). First reported in the early seventies, these observations appeared to lack explanation and have been controversial. However, published studies from the Canadian, Italian and United Kingdom registries support the existence of true gender disparities in CF survival (1, 4, 5). Although our understanding of CF pathophysiology and treatment interventions in CF improved hugely into the early 1990s leading to a doubling of the median survival age, notwithstanding such improvement, females continued to show poorer median survival, further evidence for genuine gender differences prompting ‘gender’ to be included in a CF survivorship model (2, 6).

It has been argued that individual centre practice should be reviewed if gender differences persist as lung function and nutrition are equivalent during childhood. However, the work upon which this recommendation is based was from a single centre that assessed pre-pubertal adolescents rather than adults, the group where genuine gender differences have been reported to exist (7). Interestingly, Italian registry data reported the absence of a gender gap in pre-pubertal children with CF (<16 years) but could not exclude gender differences if the cohort was followed into adulthood (8). Using a case mix adjustment method to overcome

problems comparing survival data from multiple sites, female gender was shown to be associated with an increased risk of death strengthening the argument for the existence of a true 'gender gap' (9). Data related to compliance suggests that age rather than gender are important factors for treatment adherence (10). A recent re-assessment of gender survival statistics including data from the Irish registry data have illustrated that whilst both genders have benefitted from exponential improvements to CF survival over time that female statistics remain inferior.

Estrogen

During the course of a menstrual cycle, secretion of the major circulating estrogen, 17β-estradiol (E_2), follows a cyclic, biphasic pattern with highest concentrations immediately preceding ovulation, thereafter levels fall rapidly. Female gender differences in CF may be related to hormones such as estrogen with emerging inflammatory, immune and microbiological data linked to E_2. In view of its physiological role, fluctuating concentrations, and ability to modulate cellular functions, responses and gene expression E_2 represents an attractive avenue for investigation in terms of the gender differences observed in CF disease. This prompted our investigation of its role in the CF gender gap (11, 12).

The immune and inflammatory consequences of estrogen exposure in the CF airway

The adverse clinical phenotype in females with CF is initially noted at puberty where E_2 concentrations gradually rise and secondary sexual characteristics develop. This is unsurprising as prior work illustrates that estrogens dehydrate the airway surface liquid (ASL) by increasing ENaC expression (13) while the severity of *P. aeruginosa* pneumonia worsens in the presence of E_2 through enhancement of Th17 inflammation and depletion of lactoferrin (14). *In vivo* nasal potential differences (NPDs) vary with menstrual cycling; such data are suggestive of direct associations between systemic hormonal concentration, disease manifestations and innate immune function (15, 16) and provide evidence of the hormonal influence on both inflammation and immune function. Investigating toll-like receptor (TLR) agonist induced IL-8 release in CF airway epithelial cells we found E_2 could dose-dependently inhibit this effect which is controlled by NF-κB. The estrogen receptor (ER) isoform mediating this response was ERβ, which, incidentally is also the predominant ER within the CF airway. The mechanism involved increased the expression of secretory leucoprotease inhibitor (SLPI), an NF-κB inhibitor. SLPI knockdown abrogated the

inhibitory effect of E_2 (17). Together these data suggest a hypo-responsive innate immune response in the CF lung during times of high E_2 exposure. This would be deleterious during times of exacerbation when robust, rapid innate immune responses are required. In tandem with a diminished ASL height and impaired antimicrobial defences we suggest this explains, in part, why females with CF do worse.

The microbial endocrine effects of estrogen in cystic fibrosis

Females acquire and convert to the more aggressive mucoid phenotype of *P. aeruginosa* ahead of males leading to poorer clinical outcomes, again without explanation. *P. aeruginosa* converts to mucoidy through alginate production. Alginate biosynthesis is controlled by the *mucA-algT* operon which encode the sigma factor AlgT and its inhibitor MucA, and the *algD* operon and remotely located *algC* gene which encode the alginate biosynthetic machinery. Mutations in *mucA* are a major cause of mucoid conversion. Defective MucA fails to inhibit AlgT allowing it to promote transcription of genes within the *algD* operon. We showed that E_2 selected for mutations in *mucA*, through increases in H_2O_2 and catalase inhibition (18). To further probe the link between E_2, infective exacerbations and mucoidy in CF females, we conducted a prospective clinical study and detected a significant relationship between *in vivo* E_2 concentrations and exacerbations with the majority occurring during the proliferative phase of the menstrual cycle, a stage of the highest *in vivo* E_2 concentrations (18). Utilizing Irish registry data, we discovered that those on the oral contraceptive pill (OCP) trended toward requiring reduced numbers of antibiotic courses however proper assessment of this potential therapy would require a well powered double blind placebo controlled trial.

Future directions and therapeutic prospects

Our group has demonstrated the multifaceted effects of high circulating E_2 states in the female CF airway. First, its anti-inflammatory effects through an ERβ-mediated upregulation of SLPI, causing a TLR hyporesponsiveness to a range of bacterial agonists and an inhibition of IL-8 release (17). Second, its microbial endocrine effects in the promotion of mucoid conversion of *P. aeruginosa* by selection for *mucA* mutations. Thirdly, we have confirmed its association with infective exacerbations and report initial observations of decreased antibiotic requirements in CF females using the OCP (18). These findings provide the basis for several interesting concepts to consider in the context of the reported gender

differences in CF. With respect to E_2–mediated TLR hyporesponsiveness when taken together with the compromised ASL known to occur in high circulating E_2 states (16), this could create an environment for a particular two week time period each month for CF females where they will be prone to both acquisition of infection and a subsequent compromised response to it (17). As a consequence, the context and timing of administration of an anti-inflammatory agent in CF requires careful thought particularly in the context of females during high circulating E_2 states.

Whilst ERβ specific agonists may represent a potential anti-inflammatory candidate to emerge from our work its development for safe clinical use remains a pharmacological challenge. Whilst E_2 was shown to influence SLPI expression, its effects on other antimicrobials have yet to be clearly established in a CF setting. Most morbidity and mortality in CF is attributed to the uncontrolled chronic inflammatory environment within the CF lung associated with an exuberant influx of inflammatory cells. It is plausible to suggest, in addition to IL-8, that other cytokines may be influenced by E_2 and its effects on neutrophils, monocytes, their receptors and response to pathogens would also be worth future exploration.

The interaction between E_2 and *P. aeruginosa* advances the field of microbial endocrinology which to date has solely described effects of the stress (adrenaline, noradrenaline) but not sex hormones (estrogen, testosterone) on microorganisms. Our findings in relation to *P. aeruginosa* may be extrapolated beyond CF to other clinical states involving this organism for instance immunocompromised hosts, non-CF bronchiectatics in the premenopausal population or patients with immunodeficiency syndromes. Further work may also be performed to assess the effects of E_2 on other organisms such as *Staphylococcus* or *Burkholderia spp*, both important in CF. The role of fungi continues to emerge in CF and the impact of E_2 on fungi such as *Aspergillus* or *Candida spp.* are areas for future study.

Perspectives

The *in vivo* link between systemic E_2 and infective exacerbations in CF females provide novel insight into female CF disease. Whether the majority or a specific subset of "hormone responsive" CF females exist that illustrate a disease course of recurrent exacerbations linked with hormonal variation remains to be established. It could be that a group of CF females have disease that fluctuates over the course of a menstrual cycle comparable to that observed in "catamenial asthmatics". Identification of such women would

be crucial in permitting intervention to modulate endogenous E_2 levels and theoretically lessen the risk of exacerbations. Avenues to modify endogenous E_2 concentrations include the use of antiestrogens such as Tamoxifen. Whilst *in vitro* data hasshown positice effects on ASL (16), clinical use of this agent remains a remote possibility owing to its negative effect on growth and unacceptable side effect profile in the CF setting. A safer option would be the use of the OCP to modulate endogenous E_2 concentrations. We observed lower requirements for antibiotics in CF females using the OCP compared to those that were not. It is not clear whether these benefits are a property of exogenous estrogen or that of the much lower endogenous E_2 concentrations that are observed in those taking the pill. Our initial observations with OCP use in CF warrant a formal double blind placebo controlled clinical study to assess the estrogen-based OCP as a potential treatment option for CF exacerbations before it can be routinely recommended. Future work should assess different CF cohorts and account for the various OCP subtypes and E_2 concentrations contained within them. In addition to recording infection frequency and antibiotic usage, such studies if possible should obtain NPDs and measure ASL. Recording lung function data would also be important. Overall, E_2 impacts upon the inflammatory, immune and infectious process within the CF female airway and at least in part accounts for the gender dichotomy observed in CF disease that until now remained unexplained.

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