Identification of causative factors in aetiology of delirium.

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I. Background

NUMEROUS immune signaling mechanisms have been hypothesised to cause delirium [1]. α_2 adrenoceptor agonism modulates some of these immune signals, as detected in vitro by transcriptomics of quiescent[ref] and activated[ref] cells, cytokine measurements[ref] and assays of cellular function[ref]. in vivo data from humans under immune activation are not available. If, as suggested by the background evidence supporting the A2B trial, α_2-adrenoceptor agonists modulate the immune processes that lead to delirium, then the A2B trial constitutes a unique opportunity to infer a causative role for specific mediators in the pathogenesis of delirium.

II. Hypothesis

Modifiable immune signals in peripheral blood cause delirium. Specifically:

A. Inflammatory signals are modified by α_2 agonism and associated with

1) reduction in delirium or:

2) reduction in duration of mechanical ventilation:

III. Study design

A. Generate hypothesis

We will systematically review published studies of (a) delirium and (b) immune effects of α_2 agonists on gene expression and protein production and release in humans and mice. We will perform a short series of in vitro experiments to provide additional gene expression data from dex and clonidine-treated immune cells from circulating populations from 3 volunteers (neutrophils, monocytes, T-lymphocytes, B-lymphocytes) under quiescent and stimulated (LPS) conditions at therapeutically-relevant doses. In this phase gene expression will be assayed using CAGE to provide the maximum regulatory signaling information. Signals will be systematically collapsed onto human gene names using publicly available annotation and orthology data, as in our previous work []. We will use a circular crossvalidation algorithm to perform a data-driven evaluation and collation of existing data. Associated cytokines will be systematically identified using annotated pathway data (KEGG, Reactome, Wikipathways). We anticipate that this will yield a shortlist of 500-1000 genes and cytokines. From these we will select the subset of targets that have also been previously associated with delirium.

B. Detect biological effect and compare to hypothesis using training and test set

In order to detect the biological effect of treatment we will obtain samples at 48-72h after beginning of treatment from all consenting patients in the A2B study (see trial protocol). In the subgroup of patients with successful adherence to protocol in whom there was also evidence of both delirium and sepsis, assays will be performed. For each gene or cytokine in the shortlist of hypothesised α_2 agonist-responsive causative factors in delirium, we will quantify evidence for causality by combining (a) statistical evidence for a difference between treatment and control groups and (b) statistical evidence for an association with outcome. After correction for multiple comparisons we will identify causative factors in delirium.

C. Training-test hypothesis

We will select a training set of 70% of transcriptome profiles and decompose these transcriptomes to unique profiles. Within those profiles we will detect α_2 agonist-responsive delirium-associated factors. We will test significant associations in the remainder as a test set.

References

[1] K. Schroder *et al.*, "Conservation and divergence in toll-like receptor 4-regulated gene expression in primary human versus mouse macrophages," *Proceedings of the National Academy of Sciences*, vol. 109, no. 16, p. E944E953, 2012. [Online]. Available: http://www.pnas.org/content/109/16/E944.abstract