Clinical therapeutic benefit of antidepressants is predicted by the in vitro effect of antidepressants on mineralocorticoid receptor function.

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ABSTRACT
There is now evidence that major depression is associated with inflammatory activation and that antidepressants may exert its therapeutic benefit by inhibiting inflammation. Accordingly, we have shown that in vitro antidepressants modulate the effect of glucocorticoid receptor (GR) function on inhibition of inflammation, and have suggested that these effects could be relevant for mechanism of action of antidepressants. In order to clarify whether the effect of antidepressants on corticosteroid receptor function is indeed relevant to psychopathology, we now assessed corticosteroid receptor function in an in-patient sample with depression and related this to prospectively determined severe treatment resistance. We evaluated the in vitro effect of the tricyclic antidepressant, clomipramine, on corticosterone receptor function and the cytokine profile in 22 moderate-severely treatment-resistant depressed patients and in 22 healthy controls. Diluted whole blood cells were incubated for 24h in the presence or absence of clomipramine 10 μM. Corticosterone receptor function was measured by dexamethasone (10-8M) and prednisolone (10-7M) inhibition of hypoxia-inducible factors (HIF) stimulated 21 day cultures. The results show that nonresponse to treatment was predicted by a functional mineralocorticosteroid receptor, lower levels of MCP-1 (CCL2) and a lack of effect of clomipramine on the inhibition of inflammation induced by LPS. Furthermore, patients who responded to antidepressants had higher levels of cortisol, IL-6, and MCP-1 (CCL2) when compared to controls. Nonresponders, on the other hand, had additional immunological impairment as shown by further alterations of pro-inflammatory cytokines like TNF, IL-4 and VEGF than those who responded to antidepressants. These data suggest that prospectively determined treatment resistance is associated with further immunological impairment and by the in vitro effect of antidepressants on mineralocorticoid receptor function.

AIM
To examine the effect of antidepressants on GR function in human whole blood in relation to treatment response.

METHODS

- **Subjects:** Twenty-two treatment-resistant depressed patients (TRD) and 21 volunteers were recruited to participate in this study. Exclusion criteria for patients were history of hyperthermia, to corticosteroids or steroid use, heavy smoking, viral illnesses during the preceding 2 weeks, pregnancy or lactating women, alcohol dependence, and significant physical illnesses (e.g. severe allergies, autoimmune diseases, hypertension, malignancy, haemorrhage, pulmonary, renal, hepatic, gastrointestinal, or neurological disease depressive disorder or an organic aetiology were excluded). For practical and ethical reasons, it was not possible to withdraw the antidepressants and assess the patients in a drug-free state; however, a switch in medication was avoided for at least 14 days before conducting the experimental procedure. Blood samples were collected once in 10 ml sodium-heparin tubes and immediately processed as below. The study was approved by the Research Clinical Committee of the Institute of Psychiatry, King’s College, and all subjects gave their written, informed consent.

- **Methods:** Glucocorticoid function was measured by glucocorticoid inhibition of LPS-stimulated IL-6 levels. Whole blood was diluted with supplemented RPMI-1640 medium. All solutions were prepared in pyrogen-free sterile saline (NaCl 0.9%) in order to achieve final concentrations in the cultures of: 20 ng/ml for LPS, 10 μM for CMI, 100 μM and 1000 μM for DEX, 0.1 μM and 1 μM for PRED. A total of 50 μl of diluted blood (in RPMI-1640 medium with L-glutamine supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin) was added onto 48-well cell culture plates (Falcon, 3×10^5) LPS, CMI and glucocorticoids were subsequently added to the wells. Samples were incubated for 24 h in a humidified atmosphere containing 5% CO2. After the incubation, plates were centrifuged (1000 rpm, 20 min, 4°C) and supernatant carefully collected and kept at -40°C until analysis.

- **Cortisol:** Cortisol was carried out using a commercially available Immulite kit for the fully automated IMMULITE system (Diagnostic Products Corporation, DPC, Los Angeles, CA). Serum cytokines were measured by Biochip array technology (Randox, CRP). ELISA was used in the culture supernatant was measured by commercially available ELISA kits (R&D, UK).

- **Statistical analysis and data presentation:** Results are expressed by MEAN ± SEM of the % decrease in inflammation (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without glucocorticoid). SEM was used to calculate the difference between two means. For more than two means, ANOVA was used.

RESULTS
Depressed patients an activation of the inflammatory system in the presence of hyporesponsiveness

- **Figure 1:** Cortisol and Cytokines serum levels in treatment resistant depressed (TRD) and healthy subjects (n=21). Means ± S.E.M. are indicated. Statistical significance was considered when p<0.05.

**Figure 2:** Monocyte chemotactic protein in the serum levels in depressed patients with a moderately treatment-resistance. The white bar represent patients who did not respond and the hashed bar represent patients who responded to antidepressant treatment. Means ± S.E.M are indicated. Statistical significance was considered when p<0.05.

**Figure 3:** Lypo-polysaccharide (LPS)-induced IL-6 levels (ppm/L) in depressed patients and healthy controls before and after 50μg clomipramine (10 μM) incubation in responders and nonresponders to clinical antidepressant treatment. Data are shown as mean ± SEM. *p<0.05

CONCLUSIONS
In conclusion, therapeutic response to antidepressants is predicted by the effect of antidepressants on mineralocorticoid receptor function in vitro and the inhibition of inflammation.

In vitro studies are of particular relevance to understand the molecular mechanisms underlying GR abnormalities in patients with major depression and its regulation by antidepressant treatment.

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