Persistent effects of mifepristone (RU-486) on cortisol levels in bipolar disorder and schizophrenia

Peter Gallagher, BSc (Hons), MPhil
Research Associate

Stuart Watson, MB BS, MD, MRCPsych
Senior Lecturer and Consultant Psychiatrist

Cordelia Elizabeth Dye, BSc (Hons)
Research Assistant

Allan H. Young, MB ChB, MPhil, PhD, FRCPsych, FRCPC
Professor of Psychiatry

I. Nicol Ferrier, FRCP, MD (Hons), FRCPsych
Professor of Psychiatry

School of Neurology, Neurobiology and Psychiatry; Newcastle University, UK

Address for correspondence:
Peter Gallagher,
School of Neurology, Neurobiology and Psychiatry,
Newcastle University,
Leazes Wing (Psychiatry),
Royal Victoria Infirmary,
Newcastle upon Tyne, NE1 4LP, UK.
Telephone: +44 (0)191 282 4065    Fax: +44 (0)191 222 6162
E-mail: peter.gallagher@newcastle.ac.uk

WORD COUNT: abstract (191) + main text (1,687)
**ABSTRACT**

Recent pre-clinical and clinical studies have examined the potential use of anti-glucocorticoid drug augmentation – including glucocorticoid receptor (GR) antagonists – as a method of improving treatment response in severe psychiatric illness. However, the direct and persistent effects such drugs exert on the hypothalamic-pituitary-adrenal (HPA) axis are unclear. We examined afternoon cortisol levels in 39 patients (19 with bipolar disorder, 20 with schizophrenia) at baseline, following treatment with mifepristone (600mg/day for 7 days) or placebo and at +21 days. Following treatment with mifepristone (day +7) there was a significant increase in cortisol levels from baseline (mean change=60,434 nmol/L×min, 95%CI=44,755 to 76,112; t=7.803, df=38, p<0.0001) which significantly decreased from this point by day +21 (mean change=-64,487 nmol/L×min, 95%CI=-49,974 to -79,001; t=8.995, df=38, p<0.0001). Cortisol levels at day +21 were significantly lower than they were at baseline (mean change=-4,054 nmol/L×min, 95%CI=-456 to -7,652; t=2.281, df=38, p=0.028).

No significant changes occurred following placebo. These results provide preliminary evidence that subtle but significant reductions in HPA axis activity (measured by peripheral cortisol levels) are evident 14 days after cessation of treatment with the GR-antagonist mifepristone. This may in part underlie the putative therapeutic effects of such drugs.
1. **INTRODUCTION**

Hypothalamic–pituitary–adrenal (HPA) axis dysfunction has been hypothesised to play a central role in the pathogenesis and aetiology of many psychiatric illnesses (Holsboer, 2000; Raison & Miller, 2003). This dysfunction manifests peripherally as hypercortisolaemia, a phenomenon first described in depressed patients over 50 years ago (Board et al., 1956) which has subsequently been replicated in many studies, across a range of disorders (e.g.; Gallagher et al., 2007; Gibbons, 1964; Halbreich et al., 1985). The origins of this abnormality are evident at multiple structural and functional levels of HPA axis regulation and manifest as decreased glucocorticoid receptor (GR) mRNA post-mortem (Perlman et al., 2004; Webster et al., 2002); increased ACTH and cortisol responses to the dexamethasone/corticotrophin releasing hormone (dex/CRH) challenge test (Lammers et al., 1995; Watson et al., 2004) and cortisol non-suppression following dexamethasone challenge (DST; Dewan et al., 1982; Greden et al., 1982; Nelson & Davis, 1997).

It has been argued that a possible consequence of HPA axis abnormalities in psychiatric illness is that they may cause or exacerbate neurocognitive impairment and depressive or psychotic symptoms (Gispen-de Wied, 2000; McAllister-Williams et al., 1998; Walder et al., 2000). Indirect evidence for this link has been found in a number of studies reporting impaired neurocognitive function or the emergence of depressive or psychotic symptoms following exogenous or endogenous (marked or chronic) increases in glucocorticoid levels (e.g.; de Quervain et al., 2000; Forget et al., 2000; Newcomer et al., 1999; Starkman et al., 2001). Studies have shown that in major depression lower baseline HPA axis activity (assessed using the dex/CRH test) or a normalisation of response following the commencement of antidepressants is associated with better treatment response, measured in terms of depressive symptoms (Brouwer...
et al., 2006; Ising et al., 2007) and working memory performance (Zobel et al., 2004). Specifically targeting such abnormalities may therefore improve outcome.

A number of recent pre-clinical and clinical studies have examined the potential use of anti-glucocorticoid drug augmentation – including glucocorticoid receptor (GR) antagonists – as a method of improving treatment response in severe psychiatric illness (DeBattista & Belanoff, 2006; Gallagher & Young, 2006; Johnson et al., 2007). However, what is unclear from these studies is the immediate and persistent effect that GR antagonists exert on the hypothalamic-pituitary-adrenal (HPA) axis. It has been suggested that a ‘resetting’ of the HPA axis may occur (Belanoff et al., 2001; Belanoff et al., 2002); there is evidence that single doses of mifepristone can suppress circulating glucocorticoid bioactivity for up to two weeks (Heikinheimo et al., 2003) and also preliminary evidence that specific GR-antagonists may be particularly effective in subjects with more pronounced HPA axis abnormalities (Høyberg et al., 2002). We therefore sought to examine the effects of a GR-antagonist on peripheral HPA axis activity in patients with severe psychiatric illness. We hypothesise that treatment with the GR-antagonist mifepristone will cause an initial elevation of cortisol followed by suppression of HPA axis activity.

2. METHODS

2.1 Subjects

Patients aged 18 to 65 years with a SCID confirmed (Structured Clinical Interview for DSM-IV; First et al., 1995) diagnosis of bipolar disorder or schizophrenia were recruited. The study formed part of a research program examining the efficacy of glucocorticoid antagonists in schizophrenia and bipolar disorder, the results of which are reported elsewhere (Gallagher et al., 2005; Young et al., 2004). A
specific attempt was made to recruit those who were in a stable clinical condition, but who remained symptomatic. Illness characteristics, clinical ratings and medication history were determined by trained psychiatrists using full history, case-note and medication review and standardized rating scales. All patients were on medication at the time of testing which had remained stable for a minimum of 6 weeks (for details see Gallagher et al., 2007). After a complete description of the study, written informed consent was obtained from all participants; the study received full approval from the local ethics committee.

2.2 Design and procedure
The same design was utilised in both studies. Following an initial baseline assessment, patients were randomly assigned to receive (double-blind) 600mg/day mifepristone or placebo for 7 days. Neuroendocrine profiling was performed at baseline, immediately after the week’s treatment period (day +7) and then two weeks later (day +21). At this point, the groups crossed over and the alternative treatment (placebo or mifepristone) was administered, again with neuroendocrine profiling after the week’s treatment period and two weeks later. To perform the neuroendocrine assessment of plasma cortisol secretion, blood samples were collected at 30 minute intervals from 1 pm to 4 pm. Cortisol levels were determined using Corti-cote radioimmunoassay kits (ICN Pharmaceuticals, Costa Mesa, CA).

2.3 Statistical analysis
Prior to analysis, the area-under-the-curve (AUC) was calculated for 1 pm to 4 pm samples using trapezoid integration. As a result of missing data points, AUC values could not be calculated for at least one visit for 2 patients with schizophrenia and 4 patients with bipolar disorder – these values were replaced
using the AUC\textsubscript{mean} of the group. Data were analysed by repeated measures
analysis of variance (ANOVA) or analysis of covariance (ANCOVA) where
appropriate. The relationship between cortisol levels and symptoms was
examined using Spearman’s correlation coefficient ($r_s$). All analyses were
performed using SPSS for Windows version 14 (SPSS, 2006).

3. RESULTS

In total, data from 39 patients (schizophrenic patients: 18 male, 2 female; bipolar
patients: 18 male, 1 female) were available for analysis. There was a significant
difference in the mean age of the groups (schizophrenic patients: mean=42.1
years, SD=10.3; bipolar patients: mean=49.0 years, SD=10.9; $t=2.044$, df=37,
$p=0.048$) although the groups did not differ in severity of symptoms on the BPRS
(schizophrenia patients: mean=29.7, SD=9.8; bipolar patients: mean=29.8,
SD=7.1; $t=0.050$, df=37, $p=0.960$).

3.1 Cortisol data

See table 1.

Following treatment with mifepristone a repeated-measures ANOVA revealed a
significant main effect of visit ($F=68.338$, df=2,76, $p<0.0001$). Pair wise
comparisons indicated that following treatment with mifepristone (day +7) there
was a highly significant increase in cortisol AUC from baseline (mean
change=60,434 nmol/L×min, 95%CI=44,755 to 76,112; $t=7.803$, df=38,
$p<0.0001$) which had decreased significantly from this point by day +21 (mean
change=-64,487 nmol/L×min, 95%CI=-49,974 to -79,001; $t=8.995$, df=38,
$p<0.0001$). Cortisol AUC at day+21 was significantly lower than at baseline
(mean change=-4,054 nmol/L×min, 95%CI=-456 to -7,652; $t=2.281$, df=38,
$p=0.028$).
Repeated measures ANOVA revealed no significant main effect of visit following treatment with placebo (F=1.040, df=2,76, p=0.359) and consequently no pairwise differences at any time point (p>0.15 for all).

To confirm the effect at day +21, a repeated measures ANCOVA was performed directly comparing AUCs at this point (mifepristone vs. placebo) with the inclusion of age and baseline cortisol AUC as covariates. Again the main effect of treatment was significant, with AUCs being significantly lower following mifepristone compared with placebo at day +21 (F=5.832, df=1,36, p=0.021).

3.2 Exploratory analyses
The effect of treatment order was examined by rerunning the day +21 ANCOVA, directly comparing AUCs at this point (mifepristone vs. placebo) with the inclusion of order (mifepristone/placebo vs. placebo/mifepristone) as a between subjects factor, and age and baseline cortisol AUC as covariates. The main effect of treatment remained significant with AUCs being significantly lower following mifepristone compared with placebo at day +21 (F=5.810, df=1,35, p=0.021). However there was no significant main effect of order (F=0.126, df=1,35, p=0.725) or no treatment by order interaction (Following mifepristone treatment: active first mean=40,046, SD=10,606; placebo first mean=42,048, SD=12,561; Following placebo treatment: active first mean=45,169, SD=8,957; placebo first mean=41,290, SD=10,996; F=3.368, df=1,35, p=0.075).

Similarly, the effect of diagnosis was examined by repeating the day +21 ANCOVA, directly comparing AUCs at this point (mifepristone vs. placebo) with the inclusion of diagnosis (bipolar disorder vs. schizophrenia) as a between subjects factor, and age and baseline cortisol AUC as covariates. Again, the main
effect of treatment was significant \( F=6.349, \, df=1,35, \, p=0.016 \) but there was no significant effect of diagnosis \( F=0.444, \, df=1,35, \, p=0.509 \) and no diagnosis by treatment interaction \( F=1.077, \, df=1,35, \, p=0.306 \).

Finally, the effect of diagnosis on the cortisol AUC at the point of cessation of treatment (day +7) was examined. A significant main effect of diagnosis was observed, with the cortisol AUC being significantly greater in patients with schizophrenia compared to patients with bipolar disorder \( F=5.684, \, df=1,35, \, p=0.023 \). A diagnosis by treatment interaction was also observed \( F=7.349, \, df=1,35, \, p=0.010 \). Univariate comparisons confirmed that the significant difference between the groups was only observed following active treatment \( F=6.797, \, df=1,35, \, p=0.013 \) and not following placebo \( F=0.050, \, df=1,35, \, p=0.824 \).

3.3 Relationship between cortisol levels and symptoms

There were no significant correlations between the percentage change in BPRS scores and percentage change in AUC at any time point \( r_s<0.26, \, p>0.1 \) for all.

4. DISCUSSION

This study sought to examine changes in HPA axis function (as measured using afternoon plasma cortisol levels) following treatment with the GR-antagonist mifepristone in patients with schizophrenia or bipolar disorder. In line with our original hypothesis, cortisol levels were significantly elevated following administration of mifepristone for 7 days, while 2 weeks later (day +21) a significant reduction in cortisol AUC was observed compared to baseline levels. No significant change was found at any time point following placebo.
It could be argued that the reduction in HPA axis activity following mifepristone was secondary to an effect on symptoms. Following treatment with mifepristone, significant changes from baseline in BPRS scores were observed in bipolar patients at day +14 (Young et al., 2004) and in schizophrenic patients at day +21 (Gallagher et al., 2005); no significant changes occurred following placebo. However, there were no significant correlations between changes in BPRS scores and changes in cortisol AUC at any time point. Nevertheless, we cannot fully exclude this possibility and future studies should consider exploring the relationship between the time course and magnitude of HPA axis changes to GR-antagonists and symptomatic response. It should also be noted that the primary outcome measure in these trials was neurocognitive functioning, which improved in bipolar patients but not in patients with schizophrenia. It is therefore of interest that no significant effect of diagnostic group was observed in cortisol AUC at day +21, although the patients with schizophrenia did show a more robust HPA axis response to active treatment i.e. at day +7 (see section 3.2). We have also previously demonstrated that both groups exhibited baseline hypercortisolaemia compared to a healthy control group, although the patients with schizophrenia also exhibited elevated dehydroepiandrosterone levels (Gallagher et al., 2007). Neurocognitive impairment in schizophrenia may be more ‘hardwired’ and resistant to functional alteration of the HPA axis. Future studies should consider potential clinical and diagnostic predictors of response to GR antagonists which could be explored more precisely in larger cohorts. This would also permit examination of the effects of concomitant medications which may differentially affect HPA axis and symptomatic or neurocognitive responses to such drugs, especially across heterogeneous clinical populations.

Effects of mifepristone on afternoon cortisol levels have been observed in patients with psychotic depression. In an early case-series, Belanoff and colleagues (2001) reported a normalisation of afternoon cortisol rhythm in one patient whilst in a
second patient who was tested at follow-up 8 weeks later, afternoon cortisol levels were found to be notably lower than at baseline. In a subsequent open-label trial, Belanoff and colleagues (2002) reported that doses of 600mg/day or 1,200mg/day mifepristone for 7 days resulted in statistically significant increases in serum cortisol compared with the 50mg dose. However no data was reported following cessation of treatment. It has also been demonstrated that changes in mean (6pm to 1am) ACTH following mifepristone are correlated with improvement in positive symptoms (trend level) and significantly so with improvement in BPRS total score, such that greater improvement was seen in patients with greater decreases in mean (6pm to 1am) ACTH levels (Flores et al., 2006). Again, no data was reported following cessation of treatment. Measuring longer-terms effects on both cortisol and ACTH levels is warranted.

Chronic hypercortisolaemia and reduced GR signalling, for example via reduced GR sensitivity (Raison et al., 2003; Watson & Mackin, 2006), have been postulated to contribute to morbidity in mood disorder patients. The efficacy of mifepristone which, during treatment, blocks GR and causes hypercortisolaemia may therefore appear paradoxical. However, acute hypercortisolaemia may be a possible compensatory adaptive response to a central hypocortisolaemic state (Pariante, 2003). Recent work has shown that many antidepressant drugs have actions on blood-brain barrier steroid transporters (such as multidrug resistance p-glycoprotein) which increase the access of cortisol to the brain (Pariante et al., 2004). This may restore glucocorticoid-mediated negative feedback of the HPA axis. Furthermore, one could speculate that the impact of unrestrained cortisol may cause an increase in mineralocorticoid receptor (MR) number or function, as is supported by an in-vitro study (Bachmann et al., 2003). This in turn, if persistent, may be expected to lower cortisol levels and improve neuropsychological performance and/or symptoms.
These results provide preliminary evidence that subtle but significant reductions in HPA axis activity (measured by peripheral cortisol levels) are evident 14 days after cessation of treatment with the GR-antagonist mifepristone. This effect may in part underlie the putative therapeutic actions of mifepristone.
5. REFERENCES


Lammers, C. H., Garcia-Borreguero, D., Schmider, J., Gotthardt, U., Dettling, M., Holsboer, F., & Heuser, I. J. Combined dexamethasone/corticotropin-


Perlman, W. R., Webster, M. J., Kleinman, J. E., & Weickert, C. S. Reduced glucocorticoid and estrogen receptor alpha messenger ribonucleic acid levels in the amygdala of patients with major mental illness. Biological Psychiatry 2004;56:844-852.


Elevated cortisol levels in Cushing’s disease are associated with cognitive

Walder, D. J., Walker, E. F., & Lewine, R. J. Cognitive functioning, cortisol
release, and symptom severity in patients with schizophrenia. Biological

Watson, S., Gallagher, P., Ritchie, J. C., Ferrier, I. N., & Young, A. H.
Hypothalamic-pituitary-adrenal axis function in patients with bipolar

Watson, S., & Mackin, P. HPA axis function in mood disorders. Psychiatry
2006;5:166-170.

Webster, M. J., Knable, M. B., O'Grady, J., Orthmann, J., & Weickert, C. S.
Regional specificity of brain glucocorticoid receptor mRNA alterations in
subjects with schizophrenia and mood disorders. Molecular Psychiatry
2002;7:985-94.

N. Improvements in neurocognitive function and mood following
adjunctive treatment with mifepristone (RU-486) in bipolar disorder.

Zobel, A. W., Schulze-Rauschenbach, S., von Widdern, O. C., Metten, M.,
Freymann, N., Grasmader, K., Pfeiffer, U., Schnell, S., Wagner, M., &
Maier, W. Improvement of working but not declarative memory is
correlated with HPA normalization during antidepressant treatment.
Table 1. Cortisol AUC (1pm to 4pm)

<table>
<thead>
<tr>
<th></th>
<th>Mifepristone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean a (SD)</td>
<td>Mean a (SD)</td>
</tr>
<tr>
<td>Baseline</td>
<td>45,075 (11,724)</td>
<td>45,075 (11,724)</td>
</tr>
<tr>
<td>Day +7</td>
<td>105,509 (49,489)</td>
<td>42,271 (10,774)</td>
</tr>
<tr>
<td>Day +21</td>
<td>41,021 (11,490)</td>
<td>43,279 (10,062)</td>
</tr>
</tbody>
</table>

*a nmol/L×min