Rifampicin: a glucocorticoid receptor ligand?

To the editor—Calleja *et al.* reported that the antibiotic rifampicin was capable of binding to the glucocorticoid receptor (K_d 9.9 nM) and transactivating it[1], and it has been suggested that rifampicin's immunosuppressive properties may be mediated by the glucocorticoid receptor[1,2].

Glucocorticoid action on pituitary corticotroph cells is well-defined, and suppression of secreted adrenocorticotrophin (ACTH) is a marker of glucocorticoid action on an endogenous gene. This effect is mediated by the binding of activated glucocorticoid receptors to two 5' promoter-negative glucocorticoid response elements on the ACTH precursor gene pro-opiomelanocortin. If rifampicin has glucocorticoid-like activity it would be expected to suppress pituitary ACTH secretion and endogenous glucocorticoid production, resulting in a compensated state. However patients on stable glucocorticoid replacement therapy treated with rifampicin do not have this confounding effect but usually require higher doses of hydrocortisone, as rifampicin is a potent inducer of hepatic glucocorticoid metabolizing enzymes.

The apparent ability of rifampicin, a molecule with no steroid homology, to activate the glucocorticoid receptor has important implications for understanding receptor physiology. Therefore, we examined the action of rifampicin (Sigma R3501) on a pituitary corticotroph cell line, AtT20. The synthetic glucocorticoid dexamethasone at a concentration of 100 nM suppressed ACTH secretion from 2651 (375 ng/l to 1024 ± 401 ng/l, whereas 100 nM rifampicin was without effect (2336 ± 142 ng/l) (Fig. 1*a*). Because rifampicin did not trans-repress ACTH expression, we examined whether it was able to have any effect on a well-characterized positive glucocorticoid reporter gene (MMTV-luc) in AtT20 cells. After 16 hours of incubation, 100 nM dexamethasone caused a 6.4-fold induction in reporter activity, but rifampicin (1000 nM) had no effect. The lack of effect on AtT20 cells could not be explained by metabolism of rifampicin. Although its final concentration in conditioned medium dropped from 100 nM (calculated) to 18 ± 4 nM (measured by HPLC and ultraviolet absorbance at 254 nM) after 48 hours of incubation, this is well above the previously reported K_d for receptor binding (9.9 nM).

The possibility remained that rifampicin action was dependent on cell-type specific factors in the COS 7 cells used by Calleja *et al.* Accordingly, the action of rifampicin was measured in COS 7 cells co-transfected with a human glucocorticoid receptor expression vector and MMTV-luc reporter (Fig. 1*b*). Dexamethasone at a concentration of 100 nM caused a 47-fold induction in reporter activity (from 629 ± 65 to 29742 ± 5479 light units) but rifampicin (100 nM) had no effect (624 ± 108 light units).

These data indicate that rifampicin is not a biologically significant ligand for the glucocorticoid receptor in the pituitary corticotroph cell. The earlier report by Calleja *et al.* may reflect the action of cell-type or promoter-type specific rifampicin metabolites or, possibly, contamination of rifampicin by other compounds.

D.W. Ray[1], A.M. Lovering[3], J.R.E. Davis[1] & A. White[2]

[1]Department of Medicine, [2]School of Biological Sciences, University of Manchester
Manchester M13 9PT
[3]Regional Antimicrobial Reference Laboratory
Southmead Hospital, Bristol BS10 5NB

Calleja et al. reply—Genes encoding interleukin-2 and pro-opiomelanocortin (POMC) offer interesting models of negative regulation of gene transcription by glucocorticoids (GCs), but may differ considerably from other, more direct GC regulatory systems. In the POMC gene, binding of activated glucocorticoid receptors (GRs) to negative glucocorticoid response elements (nGREs) could lead to steric occlusion of positive transcription factors or activity[3]. In contrast, activated GR can directly interact with and inhibit transcription factors such as c-jun in activated Jurkat T-cells, leading to inhibition of interleukin-2 expression.

Ray *et al.* measured ACTH secretion, normally suppressed by GCs through the activated GR, and found that rifampicin had no effect. We agree that cell type is likely an important determinant of rifampicin activity. Indeed previous work has demonstrated a species specificity of rifampicin effects. Mouse and rat hepatocytes are refractory to rifampicin but not DEX-mediated induction of cytochrome P450 3A (CYP3A) genes[4] or a GRE-driven reporter gene (unpublished data), in contrast to human hepatocytes[5].

Furthermore, studies in knockout mice and data obtained in our laboratory indicate that cellular efflux of rifampicin by the P-glycoprotein transporter (a product of the murine multi-drug resistance gene *mdr1a*) is involved in this unresponsive rifampicin phenotype: rifampicin induces CYP3A gene expression in mdr1a-null mice but not in normal mice[6]. Moreover, verapamil, a well documented inhibitor of p-glycoprotein, restores rifampicin-mediated CYP3A induction in rat hepatocytes. Because AtT20 cells are of murine origin, the 18-nM rifampicin concentration measured in the extracellular medium may not reflect the intracellular concentration.

As the molecular mechanisms driving GC repression of POMC expression involve several pathways, it is also possible that rifampicin- and GC-activated GR differ in their abilities to form protein–protein interactions with co-receptors, co-activators and other transcription factors.

C. Calleja[1], J.M. Pascussi[1], J.C. Mani[2], P. Maurel[1] & M.J. Vilarem[1]

[1]INSERM U128
CNRS BP5051
1919Route de Mende
34033 Montpellier, France
[2]UMR 9921 Faculté de Phamacie
15 Avenue Flahault
34060 Montpellier, France

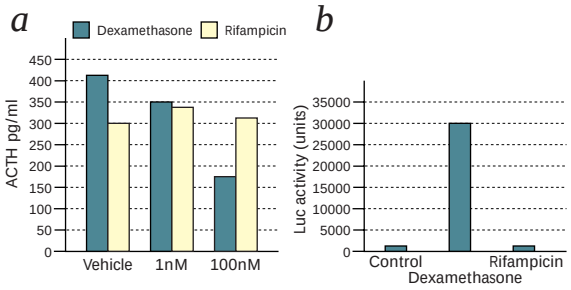

Fig. 1 ***a***, AtT20 cells were incubated with vehicle alone, dexamethasone or rifampicin. ACTH concentration in conditioned medium was measured by two-site IRMA after 24 hours. ***b***, COS 7 cells were transfected with both MMTV-luc reporter and hGR expression vectors before treatment with dexamethasone (100nM) or rifampicin (100nM) for 24 hours.