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CORTIVAZOL MEDIATED INDUCTION OF GLUCOCORTICOID RECEPTOR MESSENGER RIBONUCLEIC ACID IN WILD-TYPE AND DEXAMETHASONE-RESISTANT HUMAN LEUKEMIC (CEM) CELLS*

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Summary—Cortivazol is a phenylpyrazolo glucocorticoid of high potency and unusual structure. In both wild-type and highly dexamethasone(dex)-resistant clones of the human leukemic cell line CEM, exposure to cortivazol leads to cell death. It has been shown recently that in wild-type CEM cells but not in a dex-resistant, glucocorticoid receptor(GR)-defective clone ICR-27 TK-3, dex induces GR mRNA. To test the hypothesis that cortivazol acts in dex-resistant cells by making use of the residual GR found there, wild-type and dex-resistant clones were treated with various concentrations of cortivazol and induction of GR mRNA was studied. Cortivazol significantly induced GR mRNA in the normal CEM-C7 as well as in two classes of dex-resistant clones, although the dex-resistant clones needed at least 10 times more cortivazol than the normal cells for significant GR mRNA induction. Increased levels of GR mRNA were noticed as early as 3 h after treatment. A general correlation between induction of GR mRNA and lysis of the normal and dex-resistant cells was found. Positive induction of GR mRNA might be one of the earliest crucial steps in the lysis of normal and dex-resistant CEM cells, or might serve as a marker for the process. However, the lysis pathway in the dex-resistant cells is defective in that dex-resistant clones needed significantly more cortivazol than the normal cells for lysis of the cells.

INTRODUCTION

In a ligand-dependent manner, glucocorticoid receptor (GR) modulates transcription of various genes, either positively or negatively [1–6]. In some cases, a specific gene can be either up-regulated or down-regulated by glucocorticoids in a tissue-dependent manner. For example, the corticotropin-releasing hormone gene is up-regulated in human placenta whereas it is down-regulated in hypothalamus [7], and the rRNA gene is up-regulated in rat hepatoma cells [8] but down-regulated by glucocorticoid in lymphosarcoma cells [9]. Interestingly, transcription of the GR gene itself is modulated by its own ligand. GR gene transcription is down-regulated in a variety of tissues [10–13], but it is up-regulated in CEM-C7 [14, 15] and OPM-2 myeloma cells [16].

Of considerable scientific and practical interest is the ability of glucocorticoids to cause lysis of certain T-cell originated leukemic and lymphoma cells [17–19]. The T lymphoblast human leukemic CEM cell line has provided an array of useful information about GR mediated cellular responses, including lymphocytolysis. Upon treatment with receptor-saturating concentrations, glucocorticoids arrest the growth and eventually lyse the wild-type CEM-C7 cells through a pathway which requires functional GR [20–22]. Several dexamethasone(dex)-resistant clones have been isolated from the CEM-C7 cell line. Analysis of these dex-resistant clones has revealed that their GR is functionally and quantitatively impaired [23–27]. In understanding the GR mediated functions, including lymphocytolysis, these dex-resistant clones have proved to be very valuable.

Although dex cannot lyse the aforesaid dex-resistant CEM clones, the novel pyrazolosteroid, cortivazol can. Cortivazol is considerably more potent than dex in lysing normal and dex-resistant CEM cells [28–31]. Cortivazol binds specifically to GR [28] in CEM-C7 cells on two distinct binding sites with K_d values

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of about 11 and 0.7 nM, respectively [30, 32]. Both the binding sites of cortivazol are on the GR [32]. Cells of the "receptor-deficient" ICR-27 clone contain some residual GR which show little dex binding but can bind cortivazol [31]. In this same clone, dex has a single binding site with a K_d of about 10 nM [28]. Clones 4R4 and 3R43 contain "activation-labile" GR that can bind cortivazol. All three mutant clones grow well in up to 10^{-5} M dex. Since cortivazol is able to lyse all these dex-resistant clones, it could be predicted that it should carry out other GR mediated responses. If induction of GR mRNA is an important correlate of lysis and if cortivazol mediated lysis is truly by way of the mutant GRs, cortivazol should induce GR mRNA in these clones. This study explores the prediction that corticazol should induce GR message in the dex-resistant clones ICR-27, 4R4 and 3R43.

MATERIALS AND METHODS

Cortivazol (11α,13β,21-trihydroxy-6-16α-dimethyl-2′-phenyl-2′H-pregna-2,4,6-trieno [3.2-C]-pyrazal-20-onc-21-acetate) was a gift from Roussel-UCLAF (Paris, France) courtesy of Dr J. Raynaud. All the reagents used in the isolation of RNA and electrophoresis were either of analytical or molecular biology grade. Cesium chloride, guanidine hydrochloride, vanadyl ribonucleotide complex (VRC), the gel electrophorisis unit and the nick-translation kits were obtained from BRL (Gaithersberg, Md, U.S.A.). Electrophoresis grade agarose was obtained from IBI (New Haven, Conn., U.S.A.), MOPS [3-(4-morpholino 2) sulfonic acid], formaldehyde and formamide were purchased from Fisher Scientific (Houston, Tex., U.S.A.); *N*-lauroyl sarcosine, polyvinyl pyrrolidone, bovine serum albumin (BSA) (fraction 5) and cell culture supplements (insulin, transferrin and sodium selenite) were obtained from Sigma (St Louis, Mo., U.S.A.); [^{32}P]dCTP was obtained from ICN (Costa Mesa, Calif., U.S.A.). Nitrocellulose filters were purchased from Schleicher & Schuell (Keene, N.H., U.S.A.); the dot blotting unit was from Bio-Rad (Richmond, Calif., U.S.A.); RPMI 1640 was obtained from Cellgrow, Fisher Scientific (Houston, Tex., U.S.A.); Ficoll was from Pharmacia (Pleasant Hill, Calif., U.S.A.); and cell culture flasks were purchased from Corning & Falcon (Houston Tex., U.S.A.).

Cells. The wild-type CEM-C7 cell line was originated from a female patient with acute lymphoblastic leukemia [33]. The "receptor-deficient" ICR-27 cell clone was derived from the wild-type cells by chemical mutagenesis and the "activation-labile" clones 4R4 and 3R43 were selected for spontaneous dex-resistance [23–25]. All three clones were selected for high dex-resistance by a single step process.

Cell culture

CEM cells were grown in RPMI 1640 medium supplemented with 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml sodium selenite [34] in plastic culture flasks at 37°C in a humidified incubator aerated with 95% air and 5% CO_2.

Treatment with steroids

The cells were grown in the above-mentioned medium for about 1 week prior to treatment. At the time of treatment the cells were diluted in the medium at a cell density of (2–2.5) × 10^5 cells/ml and treated with various concentrations of steroids from ethanolic stock solutions for 18–20 h.

RNA analysis

Total RNA was isolated by the procedure of Glisin *et al.* [35]. Samples of total RNA were either subjected to electrophoresis on 1% agarose gel containing 3% formaldehyde using the MOPS system [36] and subsequent northern transfer; or were dot blotted in serial dilutions on nitrocellulose filters. For electrophoresis, samples of 20 μg/lane of total RNA were run on 1% agarose gel containing 3% formaldehyde, MOPS and ethidium bromide. The running buffer contained MOPS [36]. After electrophoresis, the RNA was transferred to a nitrocellulose filter following the procedure of Maniatis *et al.* [37] with some modifications. The gel was not treated with 0.1 M ammonium acetate; instead it was rinsed for few seconds with water and soaked with 50 mM NaOH and 10 mM NaCl for 30 min. The gel was then neutralized with 0.1 M Tris–Cl (pH 7.5) for 30 min, rinsing briefly with water before and after the neutralization step. Next, the gel was soaked with 10× SSC (0.15 M NaCl and 0.15 M trisodium citrate, pH 7.0) for 10 min before transfer of RNA onto nitrocellulose filters using the wick method. The blotting buffer was 10× SSC. For dot blots, the aqueous stock solutions of RNA were diluted with 3 vol of a solution containing 18.5% formaldehyde and 10× SSC, heated

to 65°C for 5 min, chilled on ice and applied on nitrocellulose filters under vacuum using a Bio-Rad dot blotting unit. Northern blots and dot blots were air dried and baked under vacuum at 80°C for 2 h.

Hybridization with the human GR probe

The prehybridization solution contained 6× SSC, 5× Denhardt's mixture (1% Ficoll, 1% BSA, 1% polyvinyl pyrrolidone), 200 μg/ml denatured Salmon sperm DNA and 0.2% SDS. The blots were prehybridized for 4–6 h at 65°C. The hybridization solution contained fresh prehybridization solution and (1–2) × 10^6 cpm/ml of denatured ^{32}P-labeled nick-translated human GR cDNA OB7 [38] (sp. act. $\geqslant 10^8$ cpm/μg DNA). After incubation overnight at 65°C to allow hybridization, the blots were washed at 65°C twice for 30 min with 2× SSC, 0.1% SDS; then once for 30 min with 0.1× SSC, 0.1× SDS. After air drying, the blots were exposed to Kodak X-ray films (X Omat AR) at −70°C for about 1 day.

Normalization of the GR mRNA levels

The blots were first probed with nick-translated and ^{32}P-labeled hGR probe (OB7), then reprobed with ^{32}P-labeled α-tubulin probe after removing the GR probe from the blots by keeping the blots in boiling water for 15 min. The autoradiographic signals thus obtained were densitometrically scanned using a gel scanning program of the Beckman DU-8 spectrophotometer.

Lysis of CEM cells by cortivazol

Treated or untreated cells were grown in 2 ml medium in the wells of tissue culture cluster disks (Gibco; Long Island, N.Y., U.S.A.) for 4 days. At the time of treatment and 4 days later 0.1 ml of the cultures were withdrawn. Tryptan blue was added to the samples, and viable (dye-excluding) cells were counted by hemocytometer. The average of two independent counts was used for the analysis.

RESULTS

Induction of GR message by cortivazol in the wild-type and dex-resistant clones

Dex had been shown to induce GR mRNA in CEM-C7 cells, but cortivazol had not been tested [14]. We therefore first wished to establish whether cortivazol was an inducer of GR mRNA in these cells. As an example, induction of hGR message by cortivazol in CEM-C7 is demonstrated in the Northern blot shown in Fig. 1 (data obtained from this Northern blot were not used in calculating the fold induction of GR mRNA). Human GR probe OB7 specifically hybridizes with the 7 kb hGR message, as is shown in upper panel of Fig. 1. Northern blots showing clear induction of GR mRNA in dex-resistant mutants were also obtained (not shown). The lower panel of Fig. 1 shows the levels of α-tubulin message in this Northern blot. This data suggests that cortivazol does not affect *a*-tubulin mRNA levels in these cells. To further confirm this point, we compared the

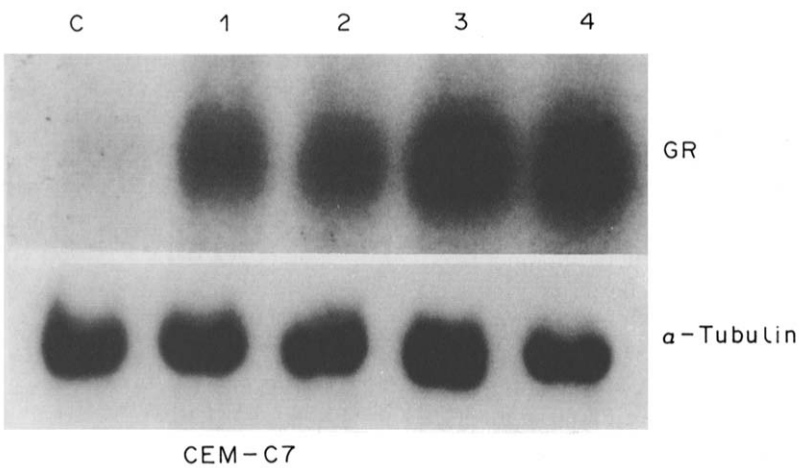

Fig. 1. Autoradiograph of a Northern blot showing induction of GR mRNA in normal CEM-C7 cells: lane C, untreated control cells; lanes 1–4, cells treated with 10^{-11}, 10^{-10}, 5×10^{-10} and 10^{-9} M cortivazol. 20 μg/lane of total RNA was loaded on the gel. The filter was probed with ^{32}P-labeled nick-translated human GR probe (upper panel) and it was re-probed with ^{32}P-labeled nick-translated α-tubulin probe for normalization (lower panel).

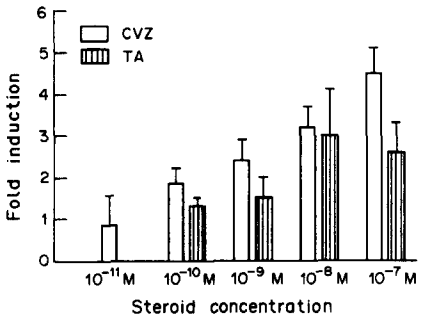

Fig. 2. Induction of GR mRNA by cortivazol and TA in CEM-C7 cells: Total RNA samples (1, 2 and 4 μg) were dot blotted in 3 experiments (each experiment had 3 replicates). The blots were first probed with nick-translated ^{32}P-labeled human GR probe (OB7), then re-probed with α-tubulin probe after removing the OB7 probe by keeping the blots in boiling water for 15 min. The signals on autoradiographs were densitometrically scanned and fold induction of GR mRNA (mean of n = 3 $\pm$ SD) was calculated. The data were plotted on Sigmaplot (Scientific Graph Sigmaplot System, version 3.1 Jandel Scientific, Corte Madera, Calif., U.S.A.). The level of GR mRNA from untreated cells was set at 1 for comparisons. Testing for significance by Student's t-test showed induction by cortivazol at all concentrations $\geq 10^{-10}$ M to be significant with >99% confidence except 10^{-9} M, significant at >95% confidence limit. Concentrations of TA of 10^{-8} and 10^{-7} M produced fold induction statistically significant at greater than the 95% confidence limit.

level of rRNA—shown by ethidium bromide stain—on the same filter with the α-tubulin message. Again, it was clear that the α-tubulin message was not regulated by cortivazol (not shown). For further quantification of effects, we decided to use only dot blots.

Results from 3 representative experiments to determine to cortivazol induction of GR mRNA levels in the wild-type CEM-C7 and dex-resistant clones ICR-27 TK-3, 4R4 and 3R43 are presented here.

Figure 2 shows induction of GR mRNA by cortivazol and triamcinolone acetonide (TA) in wild-type CEM-C7 cells. The data shown in Fig. 2 are the mean $\pm$ SD from the dot blots of the 3 experiments, each of which included replicates. It is evident from the results that cortivazol significantly induced GR mRNA levels in CEM-C7 cells in a dose-dependent manner from 10^{-10} to 10^{-7} M. There was no induction when 10^{-11} M cortivazol was used. Concentrations of cortivazol 10^{-11}M failed to induce GR mRNA levels in CEM-C7 cells (not shown). Figure 2 also includes the induction patterns of GR mRNA when CEM-C7 cells were treated with another glucocorticoid of high potency (TA). As expected from an earlier study in which CEM-C7 cells were treated with dex [14], TA in concentrations from 10^{-10} to 10^{-7} M also induced GR mRNA in CEM-C7 cells. However, TA was less potent than cortivazol in its ability to induce GR mRNA levels

Having established that cortivazol reliably induced GR mRNA levels in CEM-C7 cells, we next wished to examine the possibility whether cortivazol could induce GR mRNA levels in the dex-resistant clones and whether cortivazol was unique in doing so. Three dex-resistant clones, ICR-27 TK3 (GR deficient), 4R4 and 3R43 (activation labile GR) were treated with 10^{-9}–10^{-7} M cortivazol, and total RNA samples were analyzed for induction of GR mRNA levels. Data shown in Fig. 3 represent mean $\pm$ SD from 3 experiments in which GR mRNA levels were found to be induced by 10^{-9}–10^{-7} M cortivazol in all 3 dex-resistant clones tested. Corticazol concentrations $< 10^{-9}$ M failed to induce GR mRNA in the dex-resistant mutants (not shown). In these experiments, two other commonly used steroids, namely dex and TA, were also tested for their ability to induce GR mRNA in dex-resistant clones. Even high concentrations of these steroids at doses adjusted to give roughly equivalent potency (dex, 10^{-6} M; TA, 10^{-7} M) failed to induce GR mRNA levels in the dex-resistant clones. Statistical analysis of the data of the 3 experiments showed that there was no statistically significant induction of GR mRNA in the activation labile clones (not shown).

Time course of GR mRNA induction

The wild-type CEM-C7 and the dex-resistant cells were treated with 10^{-9} and

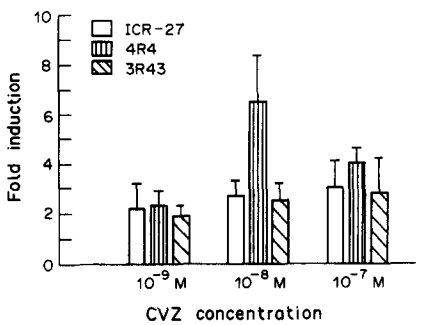

Fig. 3. Induction of GR mRNA by cortivazol in dex-resistant mutants: conditions were same as for Fig. 2. Total RNA (1, 2 and 4 μg) from 3 dex-resistant clones ICR-27, 4R4 and 3R43 were dot blotted in 3 experiments (each experiment has 3 replicates). Fold induction of GR mRNA (mean of $n = 3 \pm$ SD) was calculated, relative to control, as in Fig. 2. t-Test for significance showed that all inductions but 10^{-9} M cortivazol in ICR-27 cells and 10^{-7} M in 3R43 cells were significant at least at the 95% confidence limit. The wider standard deviation around those two points caused them to drop just below the 95% confidence limit.

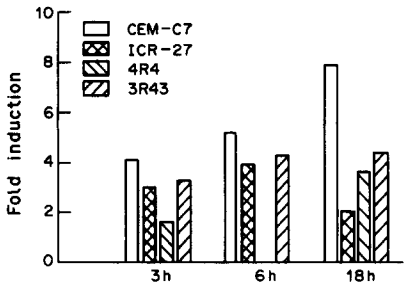

Fig. 4. Time course of GR mRNA induction by cortivazol in CEM cells: CEM-C7 cells were treated with 10^{-9} M cortivazol (cell density 2.5×10^5 cells/ml). Dex-resistant clones ICR-27, 4R4 and 3R43 were treated with 10^{-8} M cortivazol. 1, 2 and 4 μg of total RNA samples extracted from cells after 3, 6 and 18 h of treatment were dot blotted and GR mRNA levels were determined by probing the blot with ^{32}P-labeled nick-translated human GR probe. The blot was reprobed with α-tubulin probe for quantitation. The normalized GR mRNA levels were compared for each time point vs the initial values, and the results plotted as fold induction. Data from 1 experiment, representative of the 2 experiments carried out.

10^{-8} M cortivazol, respectively. At 3, 6 and 18 h after the treatment to cells were harvested, total RNAs isolated and GR mRNA levels analyzed by dot blotting. Figure 4 shows the quantitated levels of GR mRNA normalized to α-tubulin on RNA levels in 1 of 2 time course experiments which gave similar results. It is evident from the data presented in Fig. 4 that GR mRNA induction was well underway in all the cells by 3 h of treatment with cortivazol.

Lysis of CEM cells by cortivazol

The wild-type and dex-resistant clones were grown in serum free medium as described in Materials and Methods. The cells were treated with various concentrations of cortivazol for 4 days in the wells of tissue culture dishes. The cells were counted by hemocytometer at the time of treatment and 4 days later, using the trypan blue exclusion method. The results of 2 such experiments are summarized in Table 1. It is evident from the results that the wild-type and dex-resistant clones were lysed by cortivazol to varying degrees and with differing sensitivity to steroid concentration. Among the dex-resistant clones, 3R43 was the most susceptible to lysis. Essentially 100% of these cells lysed at 10^{-7} M of cortivazol, whereas about 21% of ICR-27 and 16.5% of 4R4 cells survived at this time point even at 10^{-7} M cortivazol. The cells were also treated with TA. The results of 2 such experiments are summarized in Table 2. Only wild-type CEM-C7 cells were completely lysed by 10^{-7} M TA after 4 days of treatment,

Table 1. Viability of CEM cells upon treatment with cortivazol

Cell type	Percentage of viable cells	
	Expt 1	Expt 2
CEM-C7 (control)	92	94
CEM-C7 (10^{-11} M cortivazol)	87	90
CEM-C7 (10^{-10} M cortivazol)	71	60
CEM-C7 (10^{-9} M cortivazol)	27	33
CEM-C7 (10^{-8} M cortivazol)	7	2
CEM-C7 (10^{-7} M cortivazol)	0	0
ICR-27 (control)	90	95
ICR-27 (10^{-10} M cortivazol)	79	92
ICR-27 (10^{-9} M cortivazol)	60	66
ICR-27 (10^{-8} M cortivazol)	35	44
ICR-27 (10^{-7} M cortivazol)	17	25
4R4 (control)	92	93
4R4 (10^{-10} M cortivazol)	84	81
4R4 (10^{-9} M cortivazol)	64	51
4R4 (10^{-8} M cortivazol)	25	33
4R4 (10^{-7} M cortivazol)	15	18
3R43 (control)	93	89
3R43 (10^{-10} M cortivazol)	68	59
3R43 (10^{-9} M cortivazol)	27	36
3R43 (10^{-8} M cortivazol)	10	15
3R43 (10^{-7} M cortivazol)	0	2

Cells were treated with cortivazol at a cell density of $(2.0–2.5) \times 10^5$ cells/ml. The data shown here represent the cell count after 4 days of treatment. The cells were counted by hemocytometer using the trypon blue exclusion method. The percentage of viable cells was calculated by an average cell count obtained from two values.

whereas all the 3 dex-resistant mutants were largely resistant to lysis by TA.

DISCUSSION

Glucocorticoid receptor is subject to down-regulation by its ligand in various tissues [10–13] but to positive regulation in CEM-C7 and myeloma cells [14–16]. The presence of one or more

Table 2. Viability of CEM cells upon treatment with TA

Cell type	Percentage of viable cells	
	Expt 1	Expt 2
CEM-C7 (control)	92	93
CEM-C7 (10^{-8} M TA)	56	48
CEM-C7 (10^{-7} M TA)	0	0
ICR-27 (control)	95	88
ICR-C7 (10^{-8} M TA)	90	86
ICR-27 (10^{-7} M TA)	88	86
4R4 (control)	86	90
4R4 (10^{-8} M TA)	88	89
4R4 (10^{-7} M TA)	83	86
3R43 (control)	85	87
3R43 (10^{-8} M TA)	85	86
3R43 (10^{-7} M TA)	77	75

Cells were treated with TA at a cell density of $(2.0–2.5) \times 10^5$ cells/ml. The data shown here represent the cell count after 4 days of treatment. The cells were counted by hemocytometer using the trypan blue exclusion method. The percentage of viable cells was calculated by an average cell count obtained from two values.

glucocorticoid response elements (GREs) in the promoter regions of responsive genes is generally a prerequisite for GR mediated positive transcriptional regulation of such genes [1–6]. Glucocorticoid-dependent down-regulation of GR in various tissues was explained by GR interaction with a specific sequence in the 3′ untranslated region of GR gene or by post-transcriptional and post-translational mechanisms [10, 12, 13]. It is not yet clear why the GR message is up-regulated in CEM-C7 and OPM-2 myeloma cells, whereas it is down-regulated in various other tissues. In this laboratory about 3 kb of GR promoter region from a human lymphocyte genomic library has been sequenced, but no GRE has been found [39]. We have proposed that therefore the GR message is regulated by some indirect mechanism involving direct GR: regulatory factor interactions or the induction/repression by GR of factors that do act on the GR promoter region.

The data reported here demonstrate that cortivazol significantly induces GR message both in the wild-type CEM-C7 and dex-resistant clones ICR-27, 4R4 and 3R43. The dex-resistant clones needed at least 10 times more cortivazol than the wild-type cells for noticeable GR mRNA induction. This observation is consistent with the defective nature of the GR in these dex-resistant clones. The "activation labile" GRs in clones 4R4 and 3R43 form unstable complexes with ligand during the activation step [23–25]. It is likely that the residual GR in ICR-27 cells also forms unstable complexes (J. M. Harmon, personal communication), so that the steroid is not retained as well during activation in all 3 dex-resistant clones as it is in normal cells. Thus we propose that they need both considerably more cortivazol than wild-type cells as well as the special binding properties for induction of the GR message. We have proposed that the GR gene is heterozygous in our clones of CEM cells [27, 40]. Cortivazol appears to be unique in its ability to utilize the residual GR in the dex-resistant clones to cause a GR mediated response.

There is a direct correlation between endogenous GR levels and glucocorticoid sensitivity in mouse T-cells [41] and mouse WEHI-7 cells [42]. This observation was confirmed by more direct studies involving transfection of exogenous GR into responsive cells to show that in a given cell type, the degree of GR mediated cellular responses is proportional to the number of GR molecules expressed in the cells [43, 44]. A correlation between cellular GR content and clinical response to glucocorticoid therapy has been recorded in the treatment of some types of lymphoma and leukemia patients (for a review, see Ref. [45]). The requirement for a functional GR for responses like growth inhibition, cytolysis, induction of glutamine synthetase, of GR itself, and for down-regulation of *c-myc* gene has been extensively documented in various studies on CEM cells [14, 15, 19, 46, 47]. It was also reported recently that the human metallothionein II gene is induced by dex in CEM-C7 cells but not in ICR-27 cells [48]. The present study shows a direct correlation between the extent of GR mRNA induction and cortivazol mediated lysis of the wild-type CEM-C7 cells. The dex-resistant clones also followed, generally, a similar correlation between the extent of GR mRNA induction and their lysis.

The mechanism of lysis of CEM cells by dex or cortivazol is not fully understood. It is known that cell growth of CEM cells is inhibited after 24 h of treatment with steroids and the lysis follows thereafter [19, 29, 31]. Continuous presence of steroids is needed for the lysis process to be completed. From the data presented here and in earlier studies [19, 23, 24, 28–34, 49], it appears that two steps might play a crucial role in GR mediated cytolysis process: (a) proper binding of steroid to GR to convert GR to its lysis-triggering conformation; (b) ability of the steroid to be retained long enough on the GR during or after the activation step. The unique structure of each steroid within a class might determine the fate of these steps. The role of the steroid itself in maintaining the action of activated GR is not clear. Transfected GR genes lacking the DNA sequence that codes for the glucocorticoid binding region are constitutively (though weakly) active in inducing expression from cotransfected genes [43, 44]. It has not been shown, however, that they induce endogenous genes of the transfected cells. The action of two antiglucocorticoids shows that simply activating GR to its DNA binding, GRE-specific form is not a guarantee of agonist activity. Antiglucocorticoid RU35586 was shown to block glucocorticoid action by preventing dissociation of the heat shock protein (hsp90) from the GR [50]. Dex-21 mesylate leaves dex covalently bound to a cysteine in the steroid binding region of the GR [51, 52]. The resultant receptor is activated and specificially binds to the GRE [53, 54]; yet dex-mesylate is only weak agonist, with mostly antagonist

activity [55]. Perhaps the steroid *must* be able to dissociate from the GR in order for it to interact properly with GRE sites. Cortivazol, because of its phenylpyrazolo moiety, binds to GR with two-site kinetics, and the higher affinity site is tighter-binding than that for all other known glucocorticoids [30]. Furthermore, cortivazol retains a high affinity site in the 3 GR mutant clones studied here, even in ICR 27 cells, whose residual GR under normal circumstances can hardly be shown to bind dex at all [24]. Thus, because of its unique phenylpyrazolo moiety, cortivazol is able to initiate lysis-triggering steps in the wild-type and dex-resistant cells with great potency. However, the requirement of at least about 10-fold more cortivazol for the lysis of the dex-resistant clones suggests that the GR in these cells is defective in other than its ability to bind steroid. It is possible that cortivazol is not properly retained during activation steps due to the altered structure of GRs from dex-resistant cells, or that some other factor besides GR is needed in the lysis process and the GR cannot correctly interact with this factor. The lysis pathway itself, at least in ICR 27 cells seems intact, since the normal response to dex is restored if the cells are supplied with a normal GR gene by transfection [56].

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