0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(5):2994–3000

doi: 10.1210/jc.2004-2298

Differential Regulation of Synthetic Glucocorticoids on Gene Expression Levels of Glucocorticoid-Induced Leucine Zipper and Interleukin-2

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Individual glucocorticoid (GC) sensitivity was determined by measuring the effects of several clinically used GCs on transactivation of the GC-induced leucine zipper (GILZ) gene and on transrepression of the IL-2 gene using quantitative real-time PCR. A clear difference in relative potencies for transactivation and transrepression of the various GCs was observed, suggesting differential effects. To determine whether the *in vitro* outcomes could predict *in vivo* effects of GCs, 15 individuals underwent a 0.25-mg dexamethasone (DEX) suppression test (DST) while determining GILZ and IL-2 mRNA levels in their peripheral blood mononuclear cells incubated with hydrocortisone, DEX, budesonide, and prednisolone. No correlations were found between the DST and the two expression assays. However, significant correlations existed between hydrocortisone and DEX ($r = 0.52$; $P = 0.046$), hydrocortisone and budesonide ($r = 0.48$; $P = 0.069$), and hydrocortisone and prednisolone ($r = 0.86$; $P = 0.007$) regarding GILZ mRNA levels, and between hydrocortisone and DEX ($r = 0.62$; $P = 0.014$), hydrocortisone and budesonide ($r = 0.71$; $P = 0.003$), and hydrocortisone and prednisolone ($r = 0.71$; $P = 0.047$) regarding IL-2 mRNA levels. In conclusion, intra- and interindividual variations in GC sensitivity were observed using two expression assays representing GC-mediated transactivation and transrepression. The two expression assays did not correlate with each other or with the results of the DST. This suggests that regulation of the hypothalamic-pituitary-adrenal axis is more complex. However, within an individual person, these two tests combined might predict what type and dosage of GC will be preferable in individual patients for its inhibitory clinical effects, together with relatively fewer transactivating effects related to adverse effects. (*J Clin Endocrinol Metab* 90: 2994–3000, 2005)

GLUCOCORTICOIDS (GCs) PLAY a crucial role in the regulation of transcription of many genes and are important regulators of diverse physiological systems, including the immune and cardiovascular systems (1–3). The effects of GCs are exerted through the GC receptor (GR), a member of the nuclear receptor superfamily (4). Upon ligand-induced dimerization of the GR, the complex translocates to the nucleus and binds to conserved DNA motifs known as GC response elements (GREs) and negative GREs (nGREs), to stimulate (GREs) and suppress (nGREs) gene expression. GC-mediated regulation also occurs independently of interactions of the GR with DNA, mediated by protein-protein interactions of the GR with other transcription factors such as activating protein-1 and nuclear factor-κB (4, 5). The antiinflammatory effects of GCs are routinely used in the pharmacological GC treatment of patients with chronic inflammatory or autoimmune diseases. However, severe side effects (including diabetes and osteoporosis) are associated with GC treatment, limiting its therapeutic usefulness (4, 6). Although some patients develop side effects on relatively low doses of topically administered GCs, others appear to be less sensitive to GCs, because they do not show an adequate improvement in response to treatment even on high doses (7). Some patients are even resistant to the antiinflammatory effects of GCs while at the same time showing side effects known to reflect normal sensitivity to GCs, including suppression of the hypothalamic-pituitary-adrenal axis (7, 8). Cellular GC sensitivity can be measured using several different assays based upon 1) receptor protein characteristics of mononuclear leukocytes (9), 2) inhibition of phytohemagglutinin-induced T lymphocyte proliferation (10), or 3) changes in gene expression levels (11).

It is generally thought that transactivation is the predominant mechanism by which GCs exert many of their metabolic and cardiovascular side effects (12–14). Conventional GCs do not dissociate transactivation from transrepression. Strategies to develop new GCs aim to maintain transrepression of immune genes in the absence of significant transactivation of GRE-dependent promoters (15).

In this study, we aimed to assess individual GC sensitivity by measuring the effects of several clinically used GCs directly on gene expression in human peripheral blood mononuclear cells (PBMCs). For this purpose, the effects of GCs on transactivation of the GC-induced leucine zipper (GILZ) gene and on transrepression of the IL-2 gene were determined by means of quantitative real-time PCR. Because we were also interested whether these *in vitro* outcomes could

First Published Online February 22, 2005

Abbreviations: AC, Acetonide; DEX, dexamethasone; DP, dipropionate; DST, DEX suppression test; E2, 17β-estradiol; GC, glucocorticoid; GILZ, GC-induced leucine zipper; GR, GC receptor; GRE, GC response element; nGRE, negative GRE; HPRT, hypoxanthine phosphoribosyltransferase; MPA, 6α-methyl-17α-hydroxyprogesterone acetate; PBMC, peripheral blood mononuclear cell; PR, progesterone receptor.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

predict *in vivo* potencies of GCs, a group of 15 healthy volunteers underwent a 0.25-mg dexamethasone suppression test (DST) while determining GILZ and IL-2 expression levels in their PBMCs incubated with hydrocortisone and the synthetic GCs dexamethasone (DEX), budesonide, and prednisolone.

Subjects and Methods

Materials and subjects

The steroids hydrocortisone, DEX, prednisolone, triamcinolone acetonide (AC), budesonide, methylprednisolone, beclomethasone dipropionate (DP), deoxycorticosterone, D-aldosterone, megestrol acetate, progesterone, 6α-methyl-17α-hydroxyprogesterone acetate (MPA), and 17β-estradiol (E2) were all purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The GR antagonists RU 38486 (mifepristone) and ZK 98299 (onapristone) were from Sigma-Aldrich, whereas Org 31806 was obtained from Organon, Oss, The Netherlands.

For the determination of transactivation and transrepression activities of GCs, peripheral blood from healthy volunteers (all Caucasian) was used. For the first series of experiments in which we tested the whole range of GCs, we obtained cells from a healthy 52-yr-old male, whereas a study group consisting of 10 males and five females (aged 23–37 yr; mean age, 27.7 ± 1.0 yr; not using GCs or oral contraceptives) was used for testing of inter-individual variation. Informed consent from all subjects and approval from the institutional human research committee was obtained.

DST

The 0.25-mg DST was performed as previously described (16). Briefly, venous blood for serum cortisol measurements was obtained between 0800 and 0900 h after an overnight fast. Subjects were asked to ingest a tablet of 0.25 mg DEX at 2300 h. The next morning, fasting blood was drawn by venapuncture at the same time as the previous day.

Blood cell preparations

Peripheral blood was collected by venapuncture in heparinized tubes, and PBMCs were obtained after density centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) as previously described (17). For the hormone measurements, blood was drawn by venapuncture and allowed to coagulate for at least 30 min. Subsequently, serum was separated by centrifugation and quickly frozen at −20 C.

Cortisol measurements

Serum cortisol concentrations were determined using the Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA). Between-assay variability was less than 10.4%.

Cells and culture conditions

The acute lymphoblastic T cell leukemia cell line CCRF-CEM (no. CCL-119; American Type Culture Collection, Manassas, VA) was cultured in RPMI 1640 medium containing L-glutamine (Life Technologies, Inc. Europe, Breda, The Netherlands) supplemented with 4.5 g/liter glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (Life Technologies, Inc. Europe). Cortisol levels in the culture medium were below detection limits (data not shown). Cells (4×10^6 per incubation) were incubated at a density of 10×10^6 cells/ml for 4 h at 37 C with 10^{-7} M steroids (including GR antagonists), after which they were collected.

PBMCs were suspended in RPMI 1640 medium containing L-glutamine (Life Technologies, Inc. Europe) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (Life Technologies, Inc. Europe). Cells were incubated for 30 min at 37 C in a shaking water bath to remove endogenous cortisol. Afterward, medium was replaced and 4×10^6 cells per well were precultured overnight in 48-well plates at a density of 10×10^6 cells/ml. The next day, PBMCs were incubated for 4 h with 0, 10^{-9}, 10^{-8}, and/or 10^{-7} M GCs together with 10 μg/ml phytohemagglutinin (Sigma-Aldrich). Afterward, cells were collected.

RNA isolation

Total RNA was isolated from CCRF-CEM cells and PBMCs using an RNA isolation kit (High Pure RNA Isolation Kit; Roche, Mannheim, Germany) and directly frozen at −80 C.

RT-PCR

An RT-PCR was performed using 200 ng of total RNA per reaction (400 ng for CCRF-CEM cells). For this, we used a 50-μl reaction volume, containing the desired amount of RNA, 5.5 mM $MgCl_2$, 5 μl reverse transcriptase buffer, 2 mM dNTP mixture (0.5 mM each), 5 μM random hexamers, 0.2 μM oligo $d(T)_{16}$, 20 U RNase inhibitor, and 62.5 U reverse transcriptase (TaqMan Reverse Transcriptase Reagents; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands).

Quantitative real-time PCR

For quantitative real-time PCR analysis, the TaqMan technology (7700 Sequence Detector; Applied Biosystems) was applied according to the manufacturer's instructions. We determined the gene expression levels of GILZ and IL-2 while correcting for the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT). Primers and probes (Biosource International, Camarillo, CA) were chosen using the Primer Express software (Applied Biosystems) and are listed in Table 1. A reaction volume of 25 μl was used, containing 2.5 μl cDNA (obtained from RT-PCR), 12.5 μl Universal Master Mix (Roche, Branchburg, NJ), 0.3 pmol/μl forward and reverse primer (0.5 pmol/μl for HPRT), and 0.1 pmol/μl probe (0.2 pmol/μl for HPRT). Standard PCR conditions, as supplied by the manufacturer, were used for analysis on the 7700 Sequence Detector.

The samples from the CCRF-CEM cell line and the 52-yr-old male were analyzed in at least two independent assays with duplicate samples, whereas the samples from the 15 persons were analyzed in one assay with duplicate samples. For calculation of the relative amounts of GILZ, IL-2, and HPRT mRNA, the comparative CT method was used, as described in the ABI PRISM 7700 Sequence Detection System User Bulletin 2 (http://docs.appliedbiosystems.com).

Statistical analysis

Data were analyzed using SPSS for Windows, release 10.1 (SPSS, Chicago, IL). Spearman rank correlation was used for analyzing rela-

TABLE 1. Primer and probe sequences for GILZ, IL-2, and HPRT used in quantitative real-time PCR

	Sequence
GILZ, forward primer	5′-GCA CAA TTT CTC CAT CTC CTT CTT-3′
GILZ, reverse primer	5′-TCA GAT GAT TCT TCA CCA GAT CCA-3′
GILZ, probe	5′-6FAM-TCG ATC TTG TTG TCT ATG GCC ACC ACG-TAMRA-3′
IL-2, forward primer	5′-TTT GAA TGG AAT TAA TAA TTA CAA GAA TCC-3′
IL-2, reverse primer	5′-TTC TAG ACA CTG AAG CTG TTT CAG TTC-3′
IL-2, probe	5′-6FAM-CAG GAT GCT CAC ATT TAA GTT TTA CAT GCC C-BHQ-3′
HPRT, forward primer	5′-CAC TGG CAA AAC AAT GCA GAC T-3′
HPRT, reverse primer	5′-GTC TGG CTT ATA TCC AAC ACT TCG T-3′
HPRT, probe	5′-FAM-CAA GCT TGC GAC CTT GAC CAT CTT TGG A-TAMRA-3′

tionships between data, and data points were fitted with regression lines using the least-squares method. Statistical significance was set at $P < 0.05$. EC_{50} values and maximal values were calculated using Instat software version 2.01 (GraphPad Software, Inc., San Diego, CA).

Results

Optimization of GILZ and IL-2 expression assays

We optimized the GILZ and IL-2 expression assays using PBMCs from a healthy volunteer. The relative increase and decrease of GILZ and IL-2 mRNA levels under the influence of DEX are shown in Figs. 1 and 2. Because intra- and inter-individual differences were more pronounced at the concentration at which suboptimal effects were achieved (data not shown), we used a DEX concentration of 10^{-7} M in additional experiments. From Fig. 1, we concluded that an incubation time of 4 h is the most suitable.

Specificity of the regulation by GCs and the GR in CCRF-CEM cells

To investigate whether up-regulation of GILZ gene expression is specifically regulated by GCs, CCRF-CEM cells were incubated for 4 h at 37 C with 10^{-7} M of different steroids after which GILZ mRNA levels were determined. The housekeeping gene HPRT was not influenced by GC treatment (data not shown). The GCs DEX and hydrocortisone were both able to increase GILZ mRNA levels to levels that were, respectively, 5028 and 1869% higher than in non-stimulated cells (Fig. 3). When cells were incubated with DEX together with RU 38486, the increase in GILZ mRNA was highly suppressed. The GR antagonists (RU 38486, Org 31806, and ZK 98299), the progesterone receptor (PR) agonist progesterone, the mineralocorticoid receptor agonists (deoxycorticosterone and aldosterone), and the estrogen receptor agonist E2 were all unable to increase GILZ mRNA levels. However, incubation of the cells with the PR agonists megestrol acetate and MPA led to a slight induction of GILZ gene expression levels compared with nonstimulated cells.

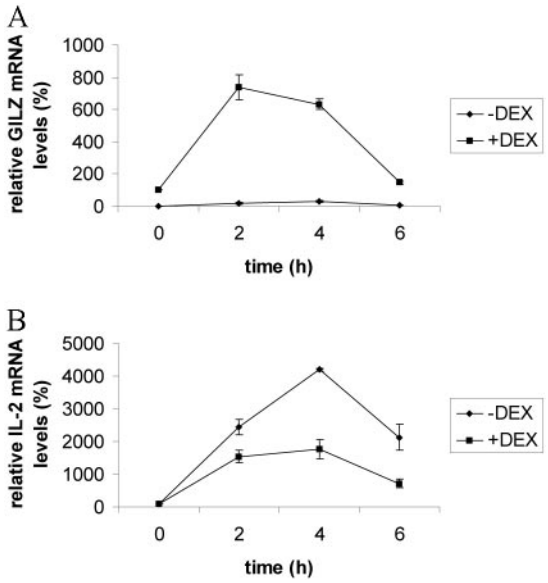

FIG. 1. Relative GILZ (A) and IL-2 (B) mRNA levels after a 0- to 6-h incubation with 10^{-7} M DEX in PBMCs of a healthy volunteer. *Lines* represent means ± SEM.

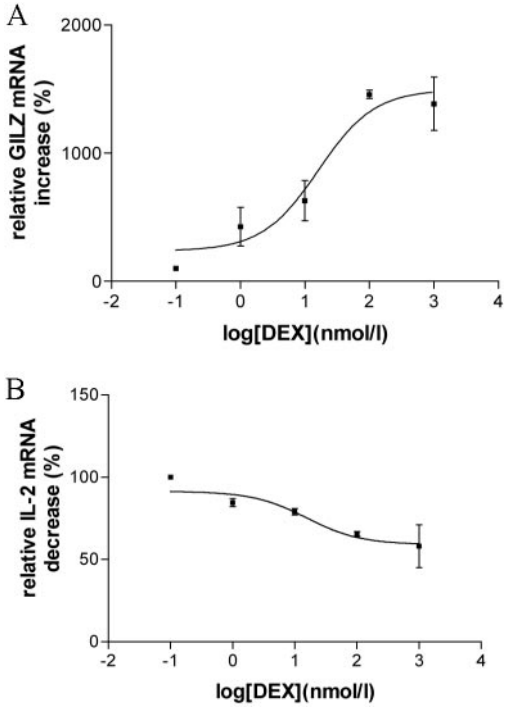

FIG. 2. Relative increase in GILZ (A) and relative decrease in IL-2 (B) mRNA levels after a 4 h incubation at the indicated concentrations of DEX in PBMCs of a healthy volunteer. *Bars* represent means ± SEM.

Transactivation and transrepression capacities of GCs in a healthy volunteer

When incubating PBMCs from a healthy volunteer for 4 h at 37 C with 10^{-9}, 10^{-8}, and 10^{-7} M of seven clinically used GCs (hydrocortisone, DEX, prednisolone, triamcinolone AC, budesonide, methylprednisolone, and beclomethasone DP), all GCs were able to induce GILZ gene expression levels and to repress IL-2 gene expression levels. Maximal effect and the concentration at which half of the maximal effect is achieved (EC_{50}) were calculated from the mean value of at least two separate assays. The results show that there existed considerable differences between the GCs used (Table 2), both with respect to the EC_{50} and to the maximal effect. Interestingly, high transactivating activity (low EC_{50}/high maximal effect in the GILZ expression assay) did not necessarily correspond to high transrepression activity (low EC_{50}/high maximal effect in the IL-2 expression assay).

Effects of GCs on GILZ and IL-2 gene expression levels in the study group

When incubating PBMCs from 15 healthy volunteers for 4 h at 37 C with 10^{-7} M hydrocortisone, DEX, budesonide, and prednisolone (n = 8), large intra- and inter-individual differences in transactivation and transrepression capacities were found for the different GCs (Table 3). However, studying the whole group, we found correlations in transactivation levels (GILZ) between hydrocortisone and DEX ($r = 0.52$; $P = 0.046$), hydrocortisone and budesonide ($r = 0.48$; $P = 0.069$),

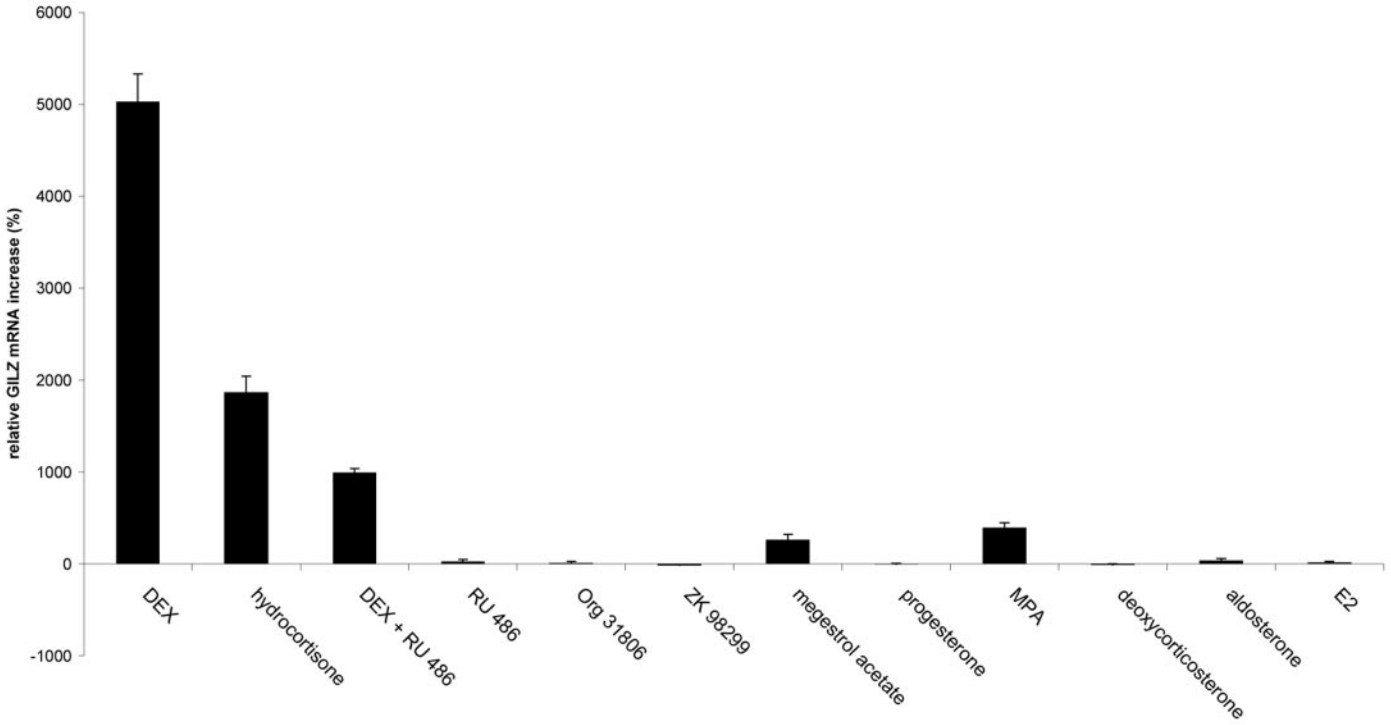

FIG. 3. Relative increase in GILZ mRNA levels in CCRF-CEM cells incubated for 4 h with 10^{-7} M steroids. *Bars* represent means ± SEM.

and hydrocortisone and prednisolone (r = 0.86; $P = 0.007$) (Fig. 4) and in transrepression levels (IL-2) between hydrocortisone and DEX (r = 0.62; $P = 0.014$), hydrocortisone and budesonide (r = 0.71; $P = 0.003$), and hydrocortisone and prednisolone (r = 0.71; $P = 0.047$) (Fig. 5). However, no correlations were found between the two expression assays.

Furthermore, we observed a difference in mean ranking of the relative potencies and in the absolute effects (Table 4) of the GCs tested between the GILZ and IL-2 expression assays. In the GILZ expression assay, the order in potencies for the different GCs was, from greatest to least, DEX, budesonide, prednisolone, and hydrocortisone, whereas this was budesonide, DEX, prednisolone, and hydrocortisone in the IL-2 expression assay.

Effects of 0.25 mg DEX on serum cortisol concentrations in the study group

All 15 subjects underwent a 0.25-mg overnight DST, and serum cortisol levels before and after the administration of DEX were measured (Table 5). No correlations were found between the results of the DST and GILZ and IL-2 expression assays.

Discussion

In this study, we investigated individual sensitivity toward several clinically used GCs by determining the effects on gene expression levels in PBMCs. For this purpose, we developed two new assays for the determination of the potencies of these GCs in transactivation and transrepression using quantitative real-time PCR analysis. Tonko *et al.* (18) identified several GC-regulated genes in the CCRF-CEM cell line using DNA chip technology. We chose the GILZ protein, first identified by D'Adamio *et al.* (19), for our experiments on transactivation, because this protein showed the highest levels of up-regulation induced by DEX in these cells. We selected IL-2 for studying transrepression activity, because it is the prototype of a key immune gene repressed by GCs (20–22). From basal experiments, we chose a GC concentration of 10^{-7} M and an incubation time of 4 h for our subsequent experiments.

TABLE 2. Maximal relative effect and EC_{50} values calculated from the relative increase in GILZ and relative decrease in IL-2 mRNA levels in PBMCs from a healthy control donor incubated for 4 h with the indicated concentrations of GCs

Glucocorticoid	GILZ EC_{50} (nM)	Maximal activation (%)	IL-2 EC_{50} (nM)	Maximal suppression (%)
Hydrocortisone	56.7 ± 1.3	698 ± 79	1.1 ± 2.4	33.9 ± 4.4
DEX	4.1 ± 1.9	1341 ± 160	14.3 ± 1.5	76.3 ± 8.3
Prednisolone	88.5 ± 4.0	1680 ± 1075	6.1 ± 2.0	38.0 ± 5.2
Triamcinolone AC	2.8 ± 2.3	1074 ± 145	1.5 ± 3.2	61.6 ± 10.2
Budesonide	0.3 ± 1.9	1216 ± 85	0.3 ± 2.3	73.8 ± 6.7
Methylprednisolone	13.8 ± 1.7	998 ± 139	0.2 ± 6.5	64.0 ± 7.6
Beclomethasone DP	2.8 ± 2.2	806 ± 114	2.2 ± 1.7	63.6 ± 5.2

Data represent means ± SEM.

TABLE 3. Relative increase of GILZ and relative decrease of IL-2 mRNA levels compared in PBMCs from 15 healthy subjects stimulated for 4 h with 10^{-7} M hydrocortisone, DEX, budesonide, and prednisolone

Subjects	Relative increase in GILZ mRNA levels (%)				Relative decrease in IL-2 mRNA levels (%)			
	Hydrocortisone	DEX	Budesonide	Prednisolone	Hydrocortisone	DEX	Budesonide	Prednisolone
1	1057 ± 412	2248 ± 159	1914 ± 167	1907 ± 892	−18 ± 25	32 ± 5	47 ± 11	9 ± 24
2	265 ± 81	1619 ± 330	870 ± 72	474 ± 174	61 ± 12	61 ± 5	84 ± 4	41 ± 20
3	662 ± 19	1250 ± 117	2223 ± 257	1228 ± 129	−2 ± 3	46 ± 13	52 ± 1	22 ± 4
4	273 ± 55	776 ± 71	612 ± 72	312 ± 82	24 ± 7	73 ± 2	74 ± 3	62 ± 3
5	268 ± 22	1399 ± 525	1038 ± 547	ND	63 ± 20	79 ± 3	90 ± 3	ND
6	816 ± 120	1022 ± 135	1554 ± 174	ND	62 ± 11	66 ± 2	72 ± 2	ND
7	1099 ± 190	3503 ± 392	1992 ± 562	ND	70 ± 12	76 ± 5	80 ± 1	ND
8	209 ± 20	1362 ± 28	2312 ± 238	ND	43 ± 6	68 ± 7	83 ± 2	ND
9	1058 ± 146	2765 ± 448	2726 ± 88	ND	63 ± 5	73 ± 4	86 ± 5	ND
10	532 ± 78	2050 ± 75	1539 ± 77	1052 ± 157	73 ± 2	95 ± 1	83 ± 1	78 ± 3
11	307 ± 52	2355 ± 39	708 ± 77	515 ± 183	47 ± 14	30 ± 0	79 ± 7	72 ± 2
12	520 ± 94	2121 ± 370	1386 ± 83	ND	−17 ± 25	57 ± 9	22 ± 7	ND
13	803 ± 149	2226 ± 659	2215 ± 319	ND	43 ± 5	64 ± 8	72 ± 8	ND
14	648 ± 2	2571 ± 144	944 ± 96	548 ± 23	51 ± 1	33 ± 6	67 ± 2	58 ± 7
15	537 ± 28	1874 ± 319	2466 ± 120	1357 ± 208	70 ± 2	60 ± 5	81 ± 1	68 ± 8

Data represent means ± SEM. ND, Not determined.

To determine whether GC effects on GILZ gene expression are really mediated by the GR, we incubated CCRF-CEM cells with GCs (DEX and hydrocortisone), GR antagonists (RU 38486, Org 31806, and ZK 98299), PR agonists (megestrol acetate, progesterone, and MPA), mineralocorticoid receptor agonists (deoxycorticosterone and aldosterone) and the estrogen receptor agonist E2. The results demonstrate that GILZ gene expression is indeed regulated via the GR, because only the GCs and the steroids with known GC activity (megestrol acetate and MPA) were able to induce GILZ mRNA levels. Furthermore, coincubation of DEX-stimulated cells with the GR antagonist RU 38486 led to a considerable suppression of the transactivation of GILZ by DEX.

When comparing the regulation of GILZ and IL-2 gene expression levels by several GCs in one person, large differences in both EC_{50} and maximal values were shown. Such a variation was also found in other studies using different tests of *in vitro* sensitivity (23, 24). Because it is thought that most known side effects of GCs are driven by transactivation rather than transrepression (12–14), the ideal GC would be a relatively weak inducer of GILZ gene expression while at the same time being a strong suppressor of IL-2 gene ex-

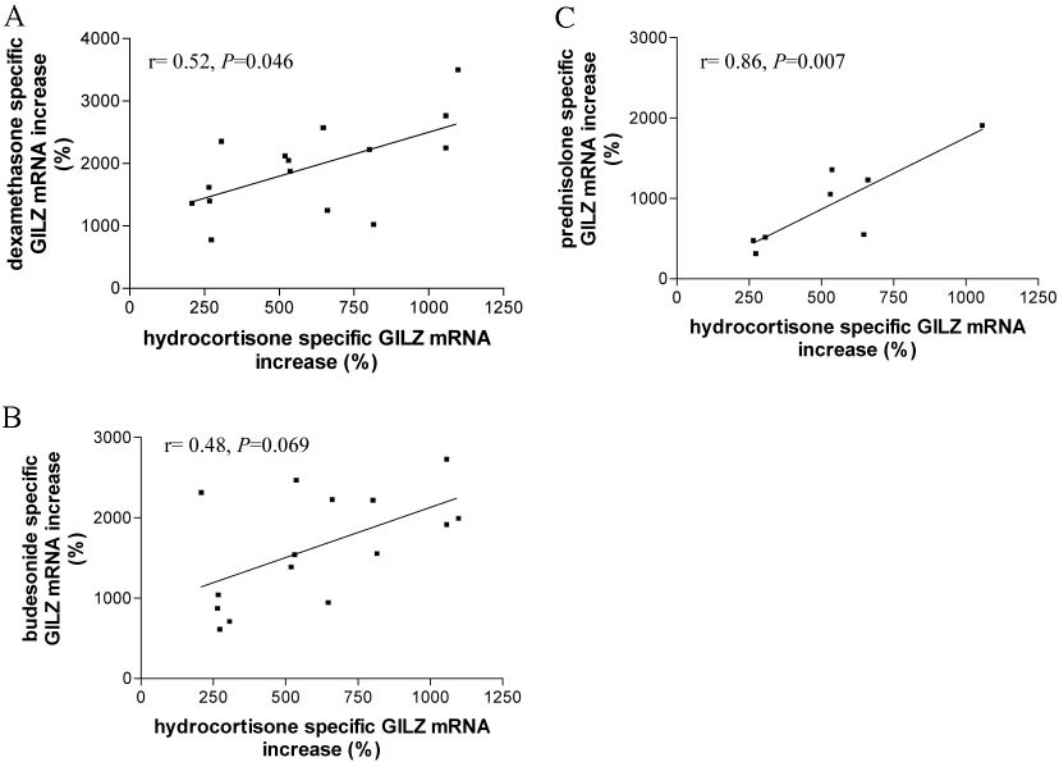

FIG. 4. Correlation analysis (Spearman's correlation) between induction of GILZ mRNA levels by 10^{-7} M hydrocortisone and DEX (A), budesonide (B), and prednisolone (C) in PBMCs of 15 healthy volunteers.

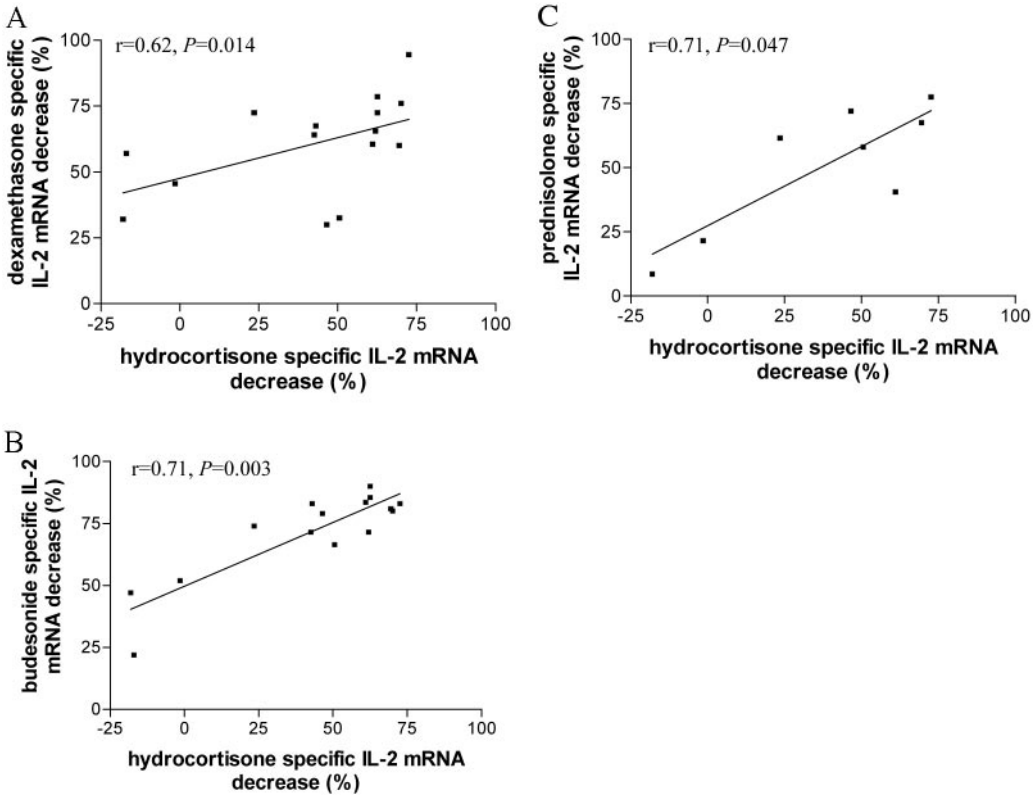

FIG. 5. Correlation analysis (Spearman's correlation) between repression of IL-2 mRNA levels by 10^{-7} M hydrocortisone and DEX (A), budesonide (B), and prednisolone (C) in PBMCs of 15 healthy volunteers.

pression. The GCs showing a high EC_{50} and a low maximal value in the GILZ expression assay in combination with a low EC_{50} and a high maximal value in the IL-2 expression assay might therefore be an indication for a beneficial outcome with minor adverse effects. For the person described in Table 2, methylprednisolone for instance might be a suitable GC. The EC_{50} in the GILZ expression assay is higher than the EC_{50} in the IL-2 expression assay, indicating a relatively weak transactivation activity and a relatively strong transrepression activity.

We were also interested whether there is a relationship between the *in vitro* outcomes from our expression assays and the *in vivo* effects of GCs. For this purpose, we selected a group of 15 healthy volunteers (not using GCs or oral contraceptives) and subjected them to a 0.25-mg DST. In parallel, we measured *in vitro* the induction of the expression of GILZ and the suppression of the expression of IL-2 by hydrocortisone, DEX, budesonide, and prednisolone in their PBMCs. No significant correlations were found between the cortisol response to DEX in the 0.25-mg DST and the outcomes from the GILZ and IL-2 expression assays.

Although we found large intra- and inter-individual variation in the GILZ and IL-2 assays, in the whole study group, correlations were found for GILZ and IL-2 mRNA levels for hydrocortisone with DEX, budesonide, and prednisolone. So, for an individual person, the potency of DEX, budesonide, and prednisolone can be predicted from knowing only

TABLE 4. Mean rank and mean effect of 10^{-7} M hydrocortisone, DEX, budesonide, and prednisolone in the GILZ expression assay and the IL-2 expression assay

Glucocorticoid	GILZ expression assay		IL-2 expression assay	
	Mean rank	Mean activation (%)	Mean rank	Mean repression (%)
Hydrocortisone	3.7 ± 0.1	603 ± 80	3.4 ± 0.2	42 ± 8
DEX	1.3 ± 0.1	1943 ± 187	2.3 ± 0.3	61 ± 5
Budesonide	1.7 ± 0.1	1633 ± 178	1.1 ± 0.1	71 ± 5
Prednisolone	3.1 ± 0.1	924 ± 196	2.9 ± 0.2	51 ± 9

Data were calculated from Table 3 and represent means ± SEM.

TABLE 5. Serum cortisol levels of 15 healthy volunteers before and after a 0.25-mg overnight DST

Subjects	Cortisol concentrations (nmol/liter)		
	Before DEX	After DEX	Before − after
1	465	170	295
2	472	228	244
3	370	298	72
4	692	71	621
5	394	231	163
6	373	274	99
7	727	416	311
8	284	255	29
9	540	400	140
10	425	570	−145
11	570	358	212
12	469	448	21
13	351	225	126
14	457	197	260
15	362	142	220

the potency of hydrocortisone. However, it is uncertain whether this also accounts for other GCs.

Furthermore, differences in mean ranking of the relative potencies of the GCs were observed between the two expression assays. With the GILZ expression assay, the order was, from greatest to least, DEX, budesonide, prednisolone, hydrocortisone, whereas it was budesonide, DEX, prednisolone, hydrocortisone in the IL-2 expression assay. Whelan *et al.* (25) compared in their study the potency of budesonide, DEX, and hydrocortisone (and beclomethasone DP) in inhibition of IL-5 and IFN-γ and found a ranking of, from greatest to least, budesonide, DEX, and hydrocortisone, which is similar to our results in the IL-2 expression assay.

In conclusion, the present report describes the use of two new expression assays using quantitative real-time PCR in which the potencies of different clinically used GCs were determined. Our data show that there is a large intra-individual variation in potency in transactivation and transrepression of GC-induced genes when using PBMCs from one healthy control subject. However, we show that in a larger group (n = 15), correlations exist between the potency of hydrocortisone and DEX, budesonide, and prednisolone in the GILZ and IL-2 expression assays. As we expected, differences were found between the GCs with respect to their relative potencies for transactivation and transrepression. But surprisingly, the order of potencies was not only different for transactivation and transrepression but also between individuals. Also, neither the results of the GILZ nor the IL-2 expression assay correlated with the results of the DST. This lack of correlation between the DST and the GILZ and IL-2 expression assays may reflect the mechanistic differences between stimulation and repression of gene expression *in vitro* and the process of hypothalamic-pituitary-adrenal axis regulation *in vivo*. However, these assays may be useful in determining the optimal type and dosage of GC in individual patients.

Acknowledgments

Received November 23, 2004. Accepted February 16, 2005.

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This work was supported by Grant 903-43-093 from The Netherlands Organization for Scientific Research (NWO).

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