Early Adrenocortical Recovery after Glucocorticoid Therapy in Children with Leukemia

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The duration of glucocorticoid-induced inhibition that occurs in the hypothalamic-pituitary-adrenal (HPA) axis after discontinuation of treatment is controversial. The main objective of this prospective study was to evaluate the inhibition of the HPA axis by dexamethasone in children and adolescents with acute lymphoid leukemia. Thirty-five patients (median age of 6.9 yr) were evaluated. A stimulus test with ovine CRH (1 μg/kg) was performed before the introduction of dexamethasone (6 mg/m²·d for 28 d), in the 8th and 28th days of glucocorticoid therapy, and 48 h and 1 month after discontinuation of glucocorticoid therapy. Suppression of the basal secretion as well as the maximum concentration of ACTH occurred during glucocorticoid therapy (P < 0.01). The pituitary function before the introduction of dexamethasone was similar to the one seen 48 h and 1 month after withdrawing it. Suppression of the adrenal function was detected during glucocorticoid therapy, which persisted for 48 h after the steroid was removed from treatment (P < 0.01). One month after ceasing the administration of the glucocorticoid, the adrenal function was similar to that before glucocorticoid therapy. According to these results, a clinical and laboratory follow-up of the HPA axis in the month after the cessation of dexamethasone therapy is suggested to determine glucocorticoid replacement.

Subjects and Methods

Subjects and study protocol

Thirty-five children were enrolled, 30 from the same institution, and were treated according to the Brazilian Group for the Treatment of ALL, 1993 protocol (GBT11-93) (5). After this protocol, 11 patients were included in a high relapse risk group, 12 in a basic risk group, and five in a true basic risk group. This classification takes into account age, initial leukometry, and the patient’s response to treatment (5).

Dexamethasone (6 mg/m²·d, twice daily) was given for 28 d; dose reduction was done over 10 d (50% each 3 d, with complete withdrawal on the 10th day).

The study obtained approval from the local ethics committee. The families involved agreed to participate, giving written consent after receiving detailed information about the study.

Patients who had been given glucocorticoids during the year before ALL diagnosis or who had a personal or family history of hypothalamic, pituitary, and adrenal gland-related diseases, or who presented hemodynamic instability after the initial assessment were not included in the study. After inclusion, loss of patients in this study occurred due to inadequate glucocorticoid intake, death (two cases), and family abandonment of further participation in the program.

The patients’ median age was 6.9 yr (minimum, 1.2 yr; maximum, 14.4 yr). Twenty-nine patients were prepubertal (Tanner I) (6, 7), and 17 were male patients. The children underwent the ovine CRH (oCRH) stimulus test (1 μg/kg, iv, at 0800 h) five times: before introduction of dexamethasone, on the 8th and 28th days of dexamethasone use, and 48 h and 1 month after cessation of dexamethasone. Patient assessment before the treatment was the parameter used to compare data obtained during and after glucocorticoid use. Patients remained at rest during the procedure, accompanied by their parents. Vital signs were measured. Patients were hydrated, hemodynamically stable, and afebrile during the stimulus test.

Freeze-dried oCRH (500 μg/ampoule) was obtained from Peninsula Laboratories, Inc. (Belmont, CA), and diluted at the Federal University of Minas Gerais Hospital sector responsible for sterile preparations. Solutions with a final 10-ml volume containing 12.5 or 25.0 μg of oCRH were stored at −80°C for up to 3 months.

Blood samples for ACTH and cortisol measurements were obtained immediately before and 30, 60, and 90 min after oCRH use. Blood samples (4 ml) were collected using a peripheral venous blood access route kept patent using saline (0.9%). Freeze-dried EDTA (Merck, West Point, PA)-cooled plastic syringes and tubes were used to collect blood for ACTH measurements. Plastic syringes and tubes with no anticoagulant were used to collect blood for cortisol measurements. Samples thus obtained were kept at 4°C during the test and transportation of material, and refrigerated-centrifuge processing was done immediately after the
end of the test. Serum and plasma were stored in plastic tubes at \(-80\) C for up to 6 months. All tests for each single patient were done simultaneously using the same kit.

An ELISA-ACTH solid-phase radioimmunometric assay (CIS; Atomic Energy Laboratory of Biochemical Products, Gif-sur-Yvette, France) was used to measure ACTH. The intra-assay coefficient of variation was 6.1%. The interassay coefficient of variation was 5.3%. The baseline concentration reference level adopted by the laboratory was 9–32 pg/ml.

A Coat-A-Count solid phase RIA (Diagnostic Products Corporation, Los Angeles, CA) was used to measure cortisol. The intra-assay coefficient of variation was 6.4%. The baseline concentration reference level adopted by the laboratory was 5–25 µg/dl.

**Statistical analysis**

Baseline and 30-, 60-, and 90-min post-CRH plasma ACTH and serum cortisol concentrations were determined for each child. CRH stimulus tests were done before initiating glucocorticoid therapy (n = 35), on the 8th day of treatment (n = 28), on the 28th day of treatment (n = 28), 48 h after cessation of glucocorticoid therapy (n = 27), and 1 month after interruption of dexamethasone (n = 20). Baseline and maximum median plasma ACTH and serum cortisol concentrations were calculated (maximum levels were obtained 30, 60, or 90 min after oCRH stimulus).

The ACTH and cortisol levels observed during and after corticotherapy were compared with the ones observed before treatment. Consequently, the evaluation of the patients before therapy was the parameter for comparison of values obtained during and after corticotherapy. Because the distribution was not normal (Kolmogorov-Smirnov test), the Wilcoxon test, using Bonferroni’s correction (\(P < 0.01\)), was used to compare medians (8).

**Results**

**Clinical assessment**

Seven patients presented infection (six with febrile neutropenia of indeterminate origin and one with acute otitis media) at the exam done before glucocorticoid therapy; 11 presented infection on the 8th day of treatment (10 patients with febrile neutropenia and one with urinary tract infection). On the 28th day of glucocorticoid therapy, two patients presented febrile neutropenia. All of these patients were hemodynamically stable and had no fever during the CRH stimulus tests.

During the test done on the 8th day of treatment, two patients presented slight and transient blushing around 30 min after being given oCRH. No changes in vital signs were observed.

Nine patients (32% of 28) and 20 patients (71% of 28) presented the cushingoid moon face on the 8th day of treatment and on the 28th day of glucocorticoid therapy, respectively. Nineteen (70% of 27) patients presented similar features 48 h after cessation of glucocorticoid use. Four (20% of 20) still had this sign 1 month after cessation the period of this study (68 d).

**Hormonal evaluation**

The median baseline and maximum levels obtained before, during, and after glucocorticoid therapy are shown in Tables 1 and 2.

A statistically significant baseline ACTH reduction was seen from the pretreatment levels compared with the 8th and 28th days of glucocorticoid therapy (\(P < 0.001\) for both days). Baseline ACTH levels obtained 48 h and 1 month after cessation of dexamethasone did not show statistically significant differences compared with ACTH levels measured before glucocorticoid therapy (\(P = 0.53\) and 0.58, respectively).

Inhibition of maximum ACTH concentration during glucocorticoid therapy compared with the concentration observed before treatment was observed for tests done on the 8th and 28th days (\(P = 0.001\) and \(P < 0.0001\), respectively). Maximum concentrations measured 48 h and 1 month after the end of treatment were higher than those observed before glucocorticoid therapy (\(P = 0.16\) and 0.05, respectively) (Fig. 1).

Thus, suppression of baseline ACTH and pituitary response after oCRH stimulus was observed during glucocorticoid therapy, with recovery after dexamethasone withdrawal.

Inhibition of baseline cortisol during glucocorticoid therapy (8th and 28th days) and 48 h after cessation of dexamethasone occurred when compared with pretoglucocorticoid therapy levels (\(P = 0.01\), respectively, for the three tests). The baseline cortisol level, although slightly lower, did not show a statistically significant difference compared with pregluocorticoid therapy levels 1 month after interruption of treatment with glucocorticoids (\(P = 0.1\)).

A statistically significant reduction in the maximum cortisol concentration during dexamethasone treatment and 48 h after cessation of treatment was observed, compared with the pretreatment level (\(P < 0.0001\) for the three tests). The maximum concentration 1 month after the end of glucocorticoid therapy was similar to the pretreatment level, although slightly lower (\(P = 0.162\)) (Fig. 2).

Before corticotherapy, nine patients (26% of 35) already showed basal cortisol levels higher than 25 µg/dl (superior limit of reference), and five (14% of 35) had basal ACTH levels above 52 pg/ml (superior limit of reference).

**TABLE 1.** Median ACTH concentrations (picograms per milliliter and picomoles per liter) at baseline and after CRH stimulus (maximum levels), prior to, during, and after glucocorticoid therapy (GT) in children with ALL

<table>
<thead>
<tr>
<th>CRH stimulus</th>
<th>Baseline</th>
<th></th>
<th>Maximum</th>
<th></th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before GT</td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
<td>n</td>
</tr>
<tr>
<td>8th day</td>
<td>23.0 (0.5)</td>
<td>4.0–337.2 (0.08–7.42)</td>
<td>44.0 (0.96)</td>
<td>11.8–339.5 (0.25–7.47)</td>
<td>35</td>
</tr>
<tr>
<td>28th day</td>
<td>8.0* (0.17)</td>
<td>2.0–225.0 (0.04–5.17)</td>
<td>12.5* (0.27)</td>
<td>2.9–90.0 (0.06–1.98)</td>
<td>28</td>
</tr>
<tr>
<td>48 h after GT</td>
<td>18.0 (0.39)</td>
<td>3.0–152.0 (0.06–3.34)</td>
<td>66.0 (1.45)</td>
<td>9.0–717.0 (0.19–15.78)</td>
<td>27</td>
</tr>
<tr>
<td>1 month after GT</td>
<td>29.0 (0.63)</td>
<td>11.4–54.8 (0.25–1.2)</td>
<td>69.0 (1.51)</td>
<td>12.8–231.0 (0.28–5.08)</td>
<td>20</td>
</tr>
</tbody>
</table>

Conversion factor: pmol/liter = pg/ml \(\times 0.2202\).

* \(P < 0.01\) (Wilcoxon’s test), compared with pregluocorticoid therapy levels.
Discussion

A review of literature shows divergences related to glucocorticoid withdrawal and replacement protocols after cessation of glucocorticoid therapy. There are classical strategies, such as replacement during the year after the end of therapy under stressful conditions. They were based on studies that assessed the time needed for recovery of the HPA axis after prolonged and supraphysiological dose glucocorticoid therapy (3, 9–12). In one of these studies by Graber et al. (9), it was observed that plasma ACTH levels were normal or elevated 2–5 months after interruption of glucocorticoid use, but that adrenal function remained inhibited; 6–9 months after glucocorticoid therapy, adrenal function had become normal (9).

In our study, we observed the same sequence described by Graber et al., although during a much shorter period. Probably this discrepancy is due to the lower glucocorticoid exposure time (3, 12). Whereas Graber assessed patients who had received glucocorticoids during 1–10 yr, our patients received dexamethasone during 38 d, including the dose reduction period. Therefore, if the glucocorticoid posttreatment 1-yr replacement strategy was used in the population we studied, this would imply an unnecessary glucocorticoid use.

Comparing the pituitary-adrenal response to oCRH obtained during and after glucocorticoid therapy with levels determined before dexamethasone use, we observed recovery of pituitary function and persistence of adrenal suppression 2 d after cessation of glucocorticoid use.

After withdrawal of glucocorticoid therapy, a low cortisol

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### TABLE 2. Median cortisol concentrations (µg/dl and nmol/liter) at baseline and after CRH stimulus (maximum levels), prior to, during, and after glucocorticoid therapy (GT) in children with ALL

<table>
<thead>
<tr>
<th>CRH stimulus</th>
<th>Baseline</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before GT</td>
<td>17.5 (482.82)</td>
<td>7.6–40.9 (209.68–1128.43)</td>
</tr>
<tr>
<td>8th day</td>
<td>1.2* (33.10)</td>
<td>0.9–132.7 (24.83–3661.93)</td>
</tr>
<tr>
<td>28th day</td>
<td>0.9* (24.83)</td>
<td>0.9–12.6 (24.83–182.09)</td>
</tr>
<tr>
<td>48 h after GT</td>
<td>2.4* (66.21)</td>
<td>0.9–11.2 (24.83–500.0)</td>
</tr>
<tr>
<td>1 month after GT</td>
<td>12.4 (342.11)</td>
<td>1.8–29.0 (49.66–800.11)</td>
</tr>
</tbody>
</table>

Conversion factor: nmol/liter = µg/dl × 27.59.

* P < 0.01 (Wilcoxon’s test), compared with preglucocorticoid therapy levels.
concentration is expected to stimulate ACTH release—resulting in adrenal recovery—as observed in this study. Forty-eight hours after glucocorticoid withdrawal, adrenal inhibition and a marked pituitary response were observed. One month after ceasing dexamethasone, adrenal function was similar to pretreatment levels.

Few papers have assessed the function of the HPA system in leukemic patients using glucocorticoid therapy (Table 3).

Spiegel et al. (13) studied 14 leukemia or lymphoma patients receiving prednisone (40–100 mg/m²d) during 1–4 wk. Adrenal function suppression was observed 24 h after ceasing glucocorticoid use, persisting in five patients on the 7th post end-of-treatment day, assessed using the synthetic ACTH test (13).

Lightner et al. (14) studied 13 ALL patients using prednisone (2 mg/kg-d) for 1 month. Baseline cortisol concentration inhibition was seen 36 h after abrupt glucocorticoid withdrawal, with eventual recovery 9 d later.

Felner et al. (15) studied 10 ALL children who had used dexamethasone (6 mg/m²d, for 28 d) 24 h and 4 wk after abrupt drug withdrawal. All patients presented adrenal suppression 1 d after abrupt glucocorticoid removal, as assessed using the synthetic ACTH test. Recovery of adrenal function was demonstrated in seven of these after 1 month, and in a further three after 60 d post-glucocorticoid withdrawal (15).

Kuperman et al. (16) studied 15 ALL children receiving dexamethasone (6 mg/m²d) for 42 d followed by abrupt glucocorticoid withdrawal. Baseline and post-CRH stimulus ACTH secretion recovery was demonstrated on the 7th day after cessation of dexamethasone. Adrenal function remained suppressed up to 14 d after glucocorticoid withdrawal at the time of the last assessment (16).

Petersen et al. (17) studied 17 ALL children after receiving prednisolone (60 mg/m²d for 5 wk) during remission induction, and/or dexamethasone (10 mg/m²d for 3 wk) during reinduction therapy. The adrenal function was assessed by an ACTH stimulus test within 2 wk after discontinuing glucocorticoid therapy, and repeated at 3- to 5-wk intervals until recovery or the end of follow-up. Recovery of adrenal function up to 7 wk was demonstrated in six of 10 children who had used prednisolone, and four of seven who had used dexamethasone plus prednisolone (17). Adrenal function remained suppressed in three patients up to 4–8 months after glucocorticoid withdrawal at the time of the last assessment.

We assessed the hypothalamus-pituitary-adrenal axis in a greater number of patients reducing the individual variability. We found adrenal recovery after 30 d post-glucocorticoid withdrawal.

According to the data presented, we observed that recovery of adrenal function occurs between the 14th and the 30th day after dexamethasone withdrawal in most patients. The longest recovery time for the adrenal function would be 2 months, seen in a few patients (15).

Considering the two studies that used dexamethasone and noted adrenal recovery, we see that recovery took place 1 month after glucocorticoid withdrawal, whether removed abruptly, as in the Felner et al. (15) study, or gradually, as in this study.

We also observed that recovery of adrenal function took place earlier in studies using prednisone (13, 14). Literature describes dexamethasone as having an 80–150 higher inhibitory power over the hypothalamus, pituitary, and adrenal gland system compared with hydrocortisone (18, 19). The results showed by Petersen et al. (17) help confirm this statement. These results are also in agreement with other studies in which the authors found longer periods of adrenal function inhibition after prolonged and supraphysiological dose glucocorticoid therapy (3, 9–12).

From a clinical standpoint, we observed that no child had signs and symptoms suggesting adrenal failure as in other published studies, regardless of reported episodes of stress (13–16).

In this study, 32% of the patients had signs of hypercortisolism (cushingoid moon face) on the 8th day of therapy. Seventy percent of the patients had the aforementioned sign, immediately after the end of glucocorticoid therapy, and 48 h after withdrawal, coinciding with moments of adrenal function suppression. Although the laboratory values already showed pituitary-adrenal function recovery 1 month after glucocorticoid withdrawal, 20% of the children still had a cushingoid moon face at this point.

According to Orth and Kovacs (20), patients with clinical signs of hypercortisolism during glucocorticoid therapy usually present HPA axis suppression. There is a correlation between clinical signs of hypercortisolism and tests showing HPA inhibition, although this is not a rule. Thus, laboratory work-up for the hypothalamic, pituitary, and adrenal glands should be emphasized when dealing with glucocorticoid-related Cushing’s syndrome.

Among the current tests to evaluate HPA axis integrity, insulin tolerance test (ITT) is considered the most reliable. On the other hand, it is known to possibly cause potentially

### TABLE 3. Glucocorticoid therapy in ALL

<table>
<thead>
<tr>
<th>Studies</th>
<th>n</th>
<th>Type</th>
<th>Dose (mg/m²d)</th>
<th>Duration (d)</th>
<th>Cessation</th>
<th>HPA recovery (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiegel et al. (13)</td>
<td>14</td>
<td>Pred.</td>
<td>40–100</td>
<td>7–28</td>
<td>Abrupt</td>
<td>7</td>
</tr>
<tr>
<td>Lightner et al. (14)</td>
<td>13</td>
<td>Pred.</td>
<td>2 mg/kg-d</td>
<td>30</td>
<td>Abrupt</td>
<td>9</td>
</tr>
<tr>
<td>Felner et al. (15)</td>
<td>10</td>
<td>Dex.</td>
<td>6</td>
<td>28</td>
<td>Abrupt</td>
<td>30</td>
</tr>
<tr>
<td>Kuperman et al. (16)</td>
<td>15</td>
<td>Dex.</td>
<td>6</td>
<td>42</td>
<td>Abrupt</td>
<td>7b</td>
</tr>
<tr>
<td>Petersen et al. (17)</td>
<td>15</td>
<td>Prednisolone</td>
<td>60</td>
<td>35</td>
<td>9 d</td>
<td>49</td>
</tr>
<tr>
<td>Cunha et al. (this study)</td>
<td>35</td>
<td>Dex.</td>
<td>6</td>
<td>28</td>
<td>10 d</td>
<td>30</td>
</tr>
</tbody>
</table>

Dex., Dexamethasone; Pred., prednisone.

a Leukemia or lymphoma patients.

b ACTH and cortisol suppressed until d 7 and 14, respectively.
severe adverse effects and is not indicated in infancy. The synthetic ACTH test was not selected, because the test results may not correlate to the results seen under stressful conditions. The CRH stimulus test shows a good correlation with the ITT, which in its turn is a stressful condition (21). Thus, the oCRH stimulus test was selected, because in our experience and in accordance with the literature, it is commonly free of adverse effects, and in order to avoid complications in leukemic children who had just been diagnosed and had started treatment. Safety of the CRH stimulus test is an advantage for the diagnosis in children (22–25). In this study, blushing—the most frequent side effect reported (25)—was the only sign noted.

Published data show a 2- to 4-fold increase in ACTH concentration levels in 95% of normal individuals, reaching 20–100 pg/ml 10–30 min after the CRH stimulus. Cortisol usually reaches levels between 20 and 25 µg/dl 30–60 min after CRH use (20). Pituitary-adrenal response to the CRH stimulus may be compromised during glucocorticoid-induced suppression (26). In this study, maximum ACTH and cortisol levels were similar or even higher than those numbers, except during the inhibition period. Meanwhile, we observed some children who did not show an expected response, even before corticotherapy. Maximum stimulation of HPA axis, probably related to stressful conditions before CRH stimulus, may have inhibited ACTH and cortisol response to CRH (27). In fact, before corticotherapy, nine patients showed basal cortisol levels higher than 25 µg/dl, and five had basal ACTH levels above 52 pg/ml. It seems that the CRH test shows good sensibility but not specificity. We thus underline the remarks of Clark and Lipworth (26) that CRH is better used in comparative rather than individual patient screening.

Taking these results together with previous reports, we suggest that clinical and laboratory follow-up of the HPA axis is necessary during the month after dexamethasone withdrawal. Although the CRH stimulus test shows some benefit for the use in children, there is large interindividual variation on the response of ACTH and cortisol that limits its use in clinical practice (26, 28). Basal serum cortisol is considered a marker of adrenal function (26) and shows good correlation with adrenal response to CRH stimulus test and ITT (21). Therefore, we suggest that a serial morning cortisol evaluation should be done in the month after corticotherapy discontinuation, until recovery of the HPA axis function, and at any moment a stressful condition is suspected. If a stressful condition is present, replacement with glucocorticoids should be started. A morning cortisol evaluation should be done before the beginning of corticotherapy. If one decides for the evaluation with the CRH stimulus test, we believe that is necessary to establish the child’s pattern of response before the therapy starts to allow latter comparisons. Replacement with glucocorticoids during stressful conditions should be started in those cases presenting evidence of inadequate adrenal function on laboratory tests. These cases may also require further follow-up.

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