The Effects of Glucocorticoid Therapy on Glucocorticoid Receptors in Leukemia and Lymphoma

By G. F. Shipman, C. D. Bloomfield, K. A. Smith, B. A. Peterson, and A. Munck

Measurement of glucocorticoid receptors appears to be useful for selecting which patients with leukemia and lymphoma should receive glucocorticoid therapy. To determine the effect of recent or concurrent glucocorticoid therapy on the number of measurable tumor glucocorticoid receptor sites, 18 patients with leukemia and lymphoma were studied. Baseline determinations of numbers of glucocorticoid receptors were performed on the malignant cells circulating in the patients' peripheral blood. Glucocorticoid therapy was then instituted consisting of dexamethasone 4 mg p.o. every 6 hr. Repeat determinations of the number of glucocorticoid receptor sites were performed within 24 hr and at various subsequent times from the start of therapy. When compared to baseline receptor numbers, 16 of the 18 patients demonstrated a decrease in receptor number (median decrease 1651 sites/cell) after the start of glucocorticoid therapy. The magnitude of the change in receptor number was independent of the initial number of receptors. Our results suggest that in order to accurately interpret glucocorticoid receptor numbers in patients with leukemia and lymphoma, glucocorticoid should not be administered for 3 wk prior to determinations of receptor levels.

Many chemotherapy regimens presently used for the treatment of patients with lymphoma and leukemia contain a glucocorticoid. Although glucocorticoids are effective antitumor agents in many of these patients, adverse effects associated with their use can complicate patient management. It would therefore be of clinical utility to have a tumor marker that would predict sensitivity to glucocorticoids, so that only patients who might benefit from their inclusion would receive these drugs. We and others have suggested that measurement of the number of tumor glucocorticoid receptor sites in non-Hodgkin's lymphoma and acute lymphoblastic leukemia may be useful in selecting those patients likely to benefit from the inclusion of a glucocorticoid in the chemotherapy of their disease. However, frequently patients have received glucocorticoid just prior to referral for the glucocorticoid receptor assay, and what effect this might have on the measured number of glucocorticoid receptors of the malignant cells is unknown. Consequently, this study was undertaken to determine the effect of single agent glucocorticoid therapy on the number of glucocorticoid receptor sites measurable in malignant cells of patients with leukemia and lymphoma. Our results indicate that glucocorticoid administration causes the measurable number of glucocorticoid receptors to fall.

Materials and Methods

Patient Characteristics

The patient population was comprised of 18 adults with leukemia or non-Hodgkin's lymphoma who demonstrated circulating malignant cells. Nine patients were male and 9 female, ranging in age from 21 to 87 yr (median 63). Eleven patients had previously never received a glucocorticoid. The remaining seven patients had not received a glucocorticoid for at least 3 wk prior to this study.

Patients were diagnosed as having lymphoma on the basis of lymph node histology or as having leukemia based on bone marrow aspirate using standard criteria. Four patients had acute myeloid leukemia; three, acute lymphoblastic leukemia (ALL); six, chronic lymphocytic leukemia (CLL); and five, lymphocytic lymphoma (ML). Immunologic classification of the patients with lymphoproliferative disorders was based on surface immunoglobulin elaboration and sheep erythrocyte, complement, and Fe receptor analyses using techniques and criteria previously described. The three patients with ALL were of non-T, non-B type (i.e., did not demonstrate any of the above lymphocyte surface markers). The 11 patients with lymphoma or CLL were of B-cell type (i.e., demonstrated monotypic surface immunoglobulin).

Laboratory Studies

Baseline pretreatment laboratory studies done on the peripheral blood included leukocyte count and differential, lymphocyte surface markers, and glucocorticoid receptor analysis of the malignant cells. Malignant cells were identified on the basis of morphology in the myeloid malignancies and on the basis of morphology and lymphocyte surface markers in the lymphoid malignancies. All but two patients demonstrated greater than 60% tumor cells in their peripheral blood. For the glucocorticoid receptor assay, 60 ml of heparinized blood were diluted one part in four in RPMI medium and cultured for 24 hr at ambient temperatures. The malignant cell population of the suspension was then enriched by Ficoll-Hypaque
THERAPY AND GLUCOCORTICOID RECEPTORS

Gradient centrifugation, such that all samples studied had greater than 80% malignant cells. To promote dissociation of endogenously bound glucocorticoid, the cells were washed 3 times in serum-free RPMI 1640 medium with a 30-min equilibration at 37°C between each centrifugation. Since we had previously found that the T1/2 is approximately 12 min for dissociation of glucocorticoid from rat thymocyte receptors at 37°C, it was felt that this washing procedure would minimize "masking" of receptors by endogenously bound glucocorticoid. Additionally, in other studies (Longo PA, Munck A, Smith KA: unpublished), we have found that such a washing procedure promotes maximal dissociation of glucocorticoid from the human promyelocytic cell line, HL-60.

The methods used for determining receptor sites per malignant cell have been previously described in detail. Briefly, the cells were incubated with a near saturating concentration (40 nM) of tritiated dexamethasone (specific activity 35 Ci/mole, New England Nuclear, Boston, Mass.) with and without an excess of unlabeled dexamethasone (2μM) for 30 minutes at 37°C. The cell suspension was then cooled to 3°C. Cytoplasmic receptors were determined by lysing the cells with a rapid dilution into hypotonic MgCl2 (1.5 mM) containing dextran-coated charcoal to absorb free glucocorticoid. After centrifugation, an aliquot of the released cytosol was removed and counted by liquid scintillation. Nuclear receptor sites were determined similarly by lysing the cells in hypotonic MgCl2. The released nuclei were then pelleted, the cytosol removed, and the nuclear pellet counted. Data are expressed as total glucocorticoid receptor sites per cell (RТ), which represents the sum of the measured cytoplasmic and nuclear receptor sites per cell.

In order to evaluate the amount of change in glucocorticoid receptor numbers that occurred after the institution of glucocorticoid therapy, the percent of change in total glucocorticoid receptors (%RT) was calculated according to the following equation:

\[
%RT = \frac{R_T \text{ (on therapy)} - R_T \text{ (pretherapy)}}{R_T \text{ (pretherapy)}} \times 100
\]

A negative value represents a decrease in RТ while on glucocorticoid therapy.

Administration of Single Agent Glucocorticoid Therapy

Informed consent was obtained in writing from all patients. After the baseline laboratory studies were obtained, each patient was begun on single-agent glucocorticoid therapy. This consisted of dexamethasone 4 mg p.o. every 6 hr for 11 patients and either prednisone 40 mg p.o. every 6 hr or prednisolone 30 mg i.v. every 6 hr in 7 patients followed by dexamethasone 4 mg p.o. every 6 hr. The maximum duration of the prednisone/prednisolone therapy prior to switching to dexamethasone was 48 hr. The duration of single-agent glucocorticoid therapy varied from 24 to 336 hr (median 120 hr).

Repeat determinations of the initial laboratory studies were obtained from each patient within 24 hr of starting glucocorticoid therapy. The shortest time from initiating glucocorticoid therapy to repeat laboratory sampling was 10 hr. Thirteen patients had at least two repeat determinations of initial laboratory studies while on glucocorticoid therapy.

RESULTS

The effect of glucocorticoid therapy on the number of total glucocorticoid receptor sites measurable within 24 hr of the start of therapy is shown in Fig. 1 for each patient. In 12 of the 18 patients the receptor level fell (median decrease 1651 receptor sites/cell, range 679–13,283). The decrease in receptor number was found to occur as early as 10 hr from the start of therapy (i.e., after only 2 doses of glucocorticoid). There was no significant change in receptor number in 5 of the patients (indicated by the dotted lines), and there was an increase in receptors in the cells from one patient (Fig. 1, lymphoma group).

To evaluate the degree of change in receptor level irrespective of the wide range of initial pretreatment values, the percent change in receptor level was determined (%RT). For those patients where a fall in receptor number occurred, the median %RT was −32, with a range of −63 to −24. The magnitude of the fall in receptor number was independent of the initial receptor level, gender, age, diagnosis, immunologic classification, prior exposure to glucocorticoid, or type of glucocorticoid used in this study.

The effect of continued glucocorticoid therapy on glucocorticoid receptor levels was determined in 13 patients (Fig. 2). Among the 12 patients where a fall in receptor number was observed within the first 24 hr of therapy, an additional determination was made in 8

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patients 32–336 hr from the institution of therapy (Fig. 2A). The receptor level continued to be depressed as compared to the initial value in all of these patients, and in 7 patients, there was a further decline in detectable receptor sites per cell (range of additional \%R_T\ _14 to \_40).

Among the five patients whose \%R_T\ values demonstrated no significant change at 24 hr from the start of glucocorticoid therapy (i.e., were within 15% of baseline levels), four had repeat \%R_T\ determinations more than 24 hr after the start of therapy (range 35–336 hr) (Fig. 2B). Three of the four patients subsequently were found to demonstrate a fall in \%R_T\ (additional \%R_T\ _31). One patient had an increase in receptor number after being on glucocorticoid therapy for 38 hr (\%R_T\ _40).

The one patient whose malignant cells initially demonstrated an increase in \%R_T\ after 11 hr of glucocorticoid therapy showed a decrease by 36 hr (\%R_T\ _36) (Fig. 2C) and remained at this level after 107 hr of therapy.

Overall, the malignant cells from 16 of 18 patients (89%) demonstrated a decrease from baseline numbers of glucocorticoid receptors within 72 hr of starting single-agent glucocorticoid therapy. The maximum median \%R_T\ in the 18 patients was –45 (range –60 to 40).

To determine if glucocorticoid therapy altered the composition of the peripheral blood in some consistent way that might explain this fall in glucocorticoid receptor levels, we compared the leukocyte counts and percent malignant cells in the sequential samples for each patient. We also compared the percent malignant cells in the aliquot to which the radiolabeled dexamethasone was added following the 24-hr culture, the Ficoll-Hypaque separation, and the washing procedures. As illustrated in Table 1 for 7 representative patients, the percentage of malignant cells in the specimen sampled (peripheral blood) and assayed were remarkably consistent within each patient over time. Although in all patients in the Table the receptor level decreased markedly, the leukocyte counts varied widely, remaining essentially the same in some patients (pt 1), decreasing in some (pts 2–3), and increasing in others (pts 4–7). Thus, we could not find any consistent alterations in the peripheral blood that would explain the observed fall in receptor levels in these patients.

**DISCUSSION**

This study indicates that the administration of glucocorticoid results in a fall in measurable glucocorticoid receptors within the malignant cells of patients with leukemia and lymphoma. The decline has been demonstrated to have occurred as early as 10 hr after the beginning of glucocorticoid therapy and to vary considerably in magnitude during time on therapy and between patients. Studies of HeLa cells in culture and

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**Fig. 2.** Effects of glucocorticoid therapy on \%R_T\. The change in \%R_T\ over time in the 13 patients studied more than once posttreatment is indicated. For each patient, the initial point is the \%R_T\ within 24 hr of therapy; the subsequent 1–3 points are the \%R_T\ 32–336 hours following the start of therapy.
THERAPY AND GLUCOCORTICOID RECEPTORS

have similarly demonstrated a decrease in
sive washing procedures, or it may represent the selec-
exogenous glucocorticoid.

explanation to account for the fall in receptor number
could not demonstrate this change. A final possible
cation; again, our cytologic and immunologic studies
with a different, generally lower, receptor concentra-
tion; this may be an intrinsic regulation by the cell in response
to exogenous steroid. Whatever the explanation, this
observed decline in receptor number may have import-
ance in the management of patients with leukemia
and lymphoma.

The role of glucocorticoid receptors in identifying
those patients who will respond to glucocorticoid ther-
apy has not been totally defined. However, among
patients will ALL and lymphoma, two of the three
groups correlating tumor receptor levels with response
to single-agent glucocorticoid therapy have found
receptor levels to have predictive values. Among patients
with B-cell lymphomas, for example, we have
found that we could accurately predict response to
glucocorticoid therapy in 30 (86%) of 35 patients
using a value of 3000 receptor sites per malignant
cell. Twenty-one of 24 patients with more than 3000
receptors per cell responded to glucocorticoid therapy
alone, while 9 of 11 patients with fewer than 3000
receptors per cell failed to respond. Similarly Mastrangelo
and his colleagues could accurately
predict response to a short course of glucocorticoid
therapy in 15 (79%) of 19 children with ALL using a
value of 4000 receptor sites per malignant cell. Eight
of 12 patients with more than 4000 receptor sites per
cell responded and none of seven patients with fewer
than 4000 sites per cell. Homo et al. reported a lack of
correlation of response to glucocorticoid therapy with
receptor level in 11 children with ALL, but data for
individual patients were not presented.

The significance of the present study, if glucocorti-
coid receptor levels are confirmed to be important in
selecting which patients should receive glucocorticoid
therapy, is obvious. The data in this study indicate that
an aberrantly low receptor value may be obtained if
the patients have received a glucocorticoid within 24
hr of the collection of the sample for the receptor
assay. Thus, such patients might falsely be assigned to
a nonresponder category. The length of time required
for the receptor levels to return to baseline values
following glucocorticoid therapy is unknown, since the
patients who comprised this report could not be stud-
ied sequentially after the discontinuation of glucocor-
ticoid therapy. However, we found no difference
between receptor levels in cells from patients who had
received no glucocorticoid for at least 21 days
compared to those patients who had never received
glucocorticoid therapy. Thus, it appears that an inter-
val of 21 days from the last dose of glucocorticoid may
allow accurate assessment of glucocorticoid receptor
levels in cells from such patients. The significance of
the decreased receptor number that occurs upon the
institution of glucocorticoid on the clinical (antitu-
more) response of these patients is unknown, but is the
subject of continuing investigation.

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<th>Table 1. Sequential Studies of Receptor Level ($R_\alpha$), Leukocyte Count, and Percent Malignant Cells in the Peripheral Blood and Aliquot Assayed for $R_\alpha$</th>
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*Total glucocorticoid receptor sites per cell.
ND, not done.

thymocytes and liver cells of adrenalectomized mice in vivo have similarly demonstrated a decrease in measured glucocorticoid receptor levels in response to exogenous glucocorticoid.

The etiology of the change in receptor numbers is unknown. It may represent masking of receptor sites by the exogenous glucocorticoid despite laboratory measures taken to remove the steroid, including extensive washing procedures, or it may represent the selective destruction of malignant cells with high receptor concentrations, although we could not document this with sequential cytologic or immunologic studies (see Table 1). Alternatively, glucocorticoid might alter the composition of the peripheral blood in some other way, causing a relative increase in a subpopulation of cells with a different, generally lower, receptor concentration; again, our cytologic and immunologic studies could not demonstrate this change. A final possible explanation to account for the fall in receptor number may be an intrinsic regulation by the cell in response to exogenous steroid.
REFERENCES


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