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Glucocorticoid Receptors and Sensitivity in Leukemias*

A. D. Ho[1], W. Hunstein[1], and W. Schmid[2]

[1] Medizinische Universitäts-Poliklinik, Hospitalstraße 3, D-6900 Heidelberg, Federal Republic of Germany
[2] Deutsches Krebsforschungszentrum, Im Neuenheimer Feld, D-6900 Heidelberg, Federal Republic of Germany

Glucocorticoid-Rezeptoren und -Sensitivität bei Leukämien

Zusammenfassung. Zur Prüfung der Frage, ob eine positive Korrelation zwischen dem Steroid-Rezeptorgehalt von Leukämiezellen und dem Erfolg einer Steroid-Therapie besteht, haben wir Glucocorticoid-Rezeptoren bei Leukämien und Lymphomen untersucht. Die Bestimmung erfolgte nach Baxter und Tomkins. Untersucht wurden 46 Patienten mit Leukämien und 18 Kontrollpersonen. Normale Lymphozyten haben durchschnittlich 3875 spezifische Bindungsstellen pro Zelle. Die Anzahl der Glucocorticoid-Rezeptoren in den Blasten von 17 Patienten mit akuter myeloischer Leukämie zeigten starke Schwankungen (Bereich 0 bis 15 295 Rezeptoren pro Zelle). Sechs von 15 Patienten mit chronischer lymphatischer Leukämie wurden seit Jahren mit Glucocorticoiden behandelt und sprachen zur Zeit der Steroid-Rezeptor-Bestimmung auf diese Therapie nicht mehr an. Die Anzahl der Steroid-Bindungsstellen der Lymphozyten dieser Patienten erwies sich im Mittel als niedriger (2000 je Zelle) als bei den unbehandelten Patienten (4500 je Zelle). Bei 24 Patienten haben wir auch die In-Vitro-Sensitivität der Leukämiezellen gegenüber Dexamethason untersucht. Es konnte keine eindeutige Korrelation zwischen Rezeptorengehalt und In-Vitro-Sensitivität festgestellt werden. Weitere Analysen mit klinischen Daten weisen darauf hin, daß die Bestimmung von Glucocorticoid-Rezeptoren für die Planung und Durchführung einer Therapie bei den lymphatischen Leukämien Bedeutung erlangen kann, jedoch wahrscheinlich nicht bei den myeloischen Leukämien.

Schlüsselwörter: Glucocorticoid-Rezeptoren – Sensitivität – Leukämie

Summary. In an attempt to investigate the utility of glucocorticoid receptor determination to predict clinical responsiveness in human leukemias we have

* Supported by the Tumorzentrum Heidelberg/Mannheim and Karl- und Maria-Biesinger-Stiftung, D-6932 Hirschhorn, Federal Republic of Germany

Offprint requests to: A. D. Ho, MD (address see above)

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studied glucocorticoid receptors in the leukemic cells from 46 patients and in the lymphocytes from 18 normal donors. In the normal lymphocytes there were 3,875 (Median) specific binding sites per cell. The blasts from 17 patients with ANLL had on average higher levels of binding sites per cell (Median = 7,250, range: 0 to 15,295) than the other leukemias. Of the 15 patients with CLL, six had received glucocorticoid treatment for 3 to 5 years. Their lymphocytes had lower number of receptors (Median = 2,000) than the other cases which were newly diagnosed (Median = 4,500). Four patients had ALL/AUL, three patients had blast crisis as terminal phase of CML, and seven had leukemic Non-Hodgkin lymphomas (Median = 3,500 sites/cell). In 24 patients we have also studied the in vitro sensitivity of the leukemic cells to dexamethasone. There was no marked correlation between glucocorticoid receptor levels and in vitro sensitivity. An attempt to correlate receptor levels with clinical responsiveness demonstrated that glucocorticoid receptor determination might be of value in patients with lymphoid malignancies but probably not in patients with other leukemias.

Key words: Glucocorticoid receptors – Sensitivity – Leukemias

Introduction

Glucocorticoids have been shown to be of value in the treatment of a variety of leukemias and lymphomas. While some patients with these diseases respond well to hormonal treatment, others are primary resistant or develop resistance with time. It has been estimated that about 90% of acute lymphoblastic leukemias [28], and only some 5–15% of acute myelogenous leukemias [22] respond primarily to glucocorticoid therapy, and that chronic lymphocytic leukemia responds moderately. As treatment with steroid hormones is associated with serious complications it is desirable to develop a method to distinguish those patients who could benefit from endocrine therapy from those who would not.

In recent years, knowledge of steroid hormone action has advanced. It has been demonstrated that specific cytoplasmic receptor proteins for steroids are present in the target organs and that binding of glucocorticoids to these receptors is the first step required for the glucocorticoid effect [2, 11, 25].

Experimental data have shown that most thymocytes and certain human and rodent lymphoma cell lines [1, 24] are lysed by glucocorticoids. Specific receptors for glucocorticoids could be demonstrated in these cell clones whereas steroid resistant variants from mouse lymphoma cells have lower or qualitatively altered glucocorticoid receptors [17]. Thus a plausible explanation for the glucocorticoid effect in leukemias and lymphomas is that the hormones act directly to lyse the leukemic cells, and this effect depends on an initial interaction between steroid and receptor.

In human breast cancer determination of oestrogen receptors has greatly improved our ability to predict hormonal responsiveness. Furthermore Cooke et al. [6] could demonstrate that the level of oestrogen receptor is useful in predicting the prognosis of this disease regardless of treatment. Based on these considerations various attempts have been made to examine human leukemic cells for glucocorticoid receptors in order to establish an eventual correlation between the receptor content

and response to steroid therapy. In childhood acute lymphoblastic leukemia earlier results from Lippman et al. showed that clinical responsiveness seems to correlate with receptor level [19]. Recent results from other authors [3,4,7,8,12,13,15,16,21, 23] are still controversial. In an attempt to investigate the applicability of steroid receptor determination to predict clinical responsiveness in adult leukemias we have studied glucocorticoid receptors in a variety of haematological neoplasias.

Patients and Methods

Glucocorticoid receptors were determined in the leukemic cells of 46 adult (> 18 years) patients with different forms of leukemias: 17 with acute non-lymphoblastic leukemia (ANLL), four with acute lymphoblastic leukemia or acute undifferentiated leukemia (ALL/AUL), three with blast crisis, 15 with chronic lymphocytic leukemia (CLL) and seven with leukemic malignant lymphomas (NHL). Diagnosis was established by the usual criteria: in case of the acute leukemias according to morphologic and cytochemical characteristics [10] and in the Non-Hodgkin lymphomas according to histologic and immunological criteria [18]. There were 29 males and 17 females; their age range was from 22 to 72 yr.

Except in six patients with CLL, blood samples were always drawn at diagnosis and before any specific treatment. Leukemic blast cells and lymphocytes were prepared from heparinized peripheral blood by density sedimentation on Ficoll-Isopaque (Lymphoprep, Nyegaard & Co. A/S, Oslo, Norway). At other times, in patients with less than 50% blasts, leukemic blasts were prepared by means of discontinuous gradients. Lymphoprep was mixed with different amount of ficoll to give a specific density of 1,040 to 1,070. Myeloblasts settled down at the layer between 1,040 and 1,050. Cells so prepared were washed twice in Hank's solution and then suspended at a density between 2.5 to 5×10^6 cells/ml in RPMI 1640 supplemented with 25 mM Hepes buffer (Grand Island Biological Co., Glasgow, Scotland). Viability was always greater than 95%, as determined by trypan blue exclusion. A smear of the cell preparation was always made by means of a cytocentrifuge (Cytospin, Shandon Southern Ltd., England). Preparations with less than 75% leukemic blasts were discarded.

Determination of glucocorticoid receptors in cell suspensions was performed by applying a modification of the method described by Baxter and Tomkins [1]. Aliquots of 1.0 ml were transfered into 12×75 mm glass tubes, and then incubated with ^{3}H-dexamethasone (specific activity: 26.0 to 32.0 Ci/mmol. Radiochemical Centre, Amersham, England) at multiple concentrations from 0.1 to 8.0×10^{-8} mol, each case in the presence or absence of $100 \times$ concentration of non-radioactive dexamethasone. In some experiments, because of limited cell numbers, triplicate determinations of binding at a single concentration (8×10^{-8} M), estimated to be at least 4 times the dissociation constant were used. Incubations were performed at 37°C for 15 min. At the end of this period 2 ml of ice-cold phosphate-buffered saline (PBS, pH 7.4) were rapidly added to each tube. Cell pellets were collected by rapid centrifugation, resuspended and washed twice in PBS. After washing, 1 ml of ethanol was added to assist in extraction of protein bound steroid. This suspension was transferred to liquid scintillation vials and counted in 10 ml of Insta-Gel (Packard Instruments, Co. Inc., Illinois, USA). Binding sites per cell were usually estimated by Scatchard analysis. Since in some experiments only the value from the incubation at 8×10^{-8} M ^{3}H-dexamethasone was available, this value was then chosen for comparisons throughout the study.

Simultaneously, in vitro sensitivity of the cells to dexamethasone was investigated. Briefly, cells were suspended in RPMI 1640 supplemented with fetal calf serum (5%), penicillin and streptomycin. In some experiments the cells were then plated in triplicate into tissue culture plates with flat bottom wells (Microtest II, Falcon, USA) at a density of 2.5×10^6 cells/ml. In other experiments cells were incubated in triplicate in 1 ml cultures at 2.5×10^6 cells/ml. Dexamethasone dissolved in dimethylformamide (DMF) at final concentrations of 1×10^{-6} M to 2.5×10^{-8} M was added. Equal concentrations of DMF were added to controls (final concentration of DMF always less than 0.1%). After 20 h incubation at 37°C in a humidified 5% CO_2-incubator, ^{3}H-thymidine or ^{3}H-uridine (The Radiochemical Centre, Amersham, England) was

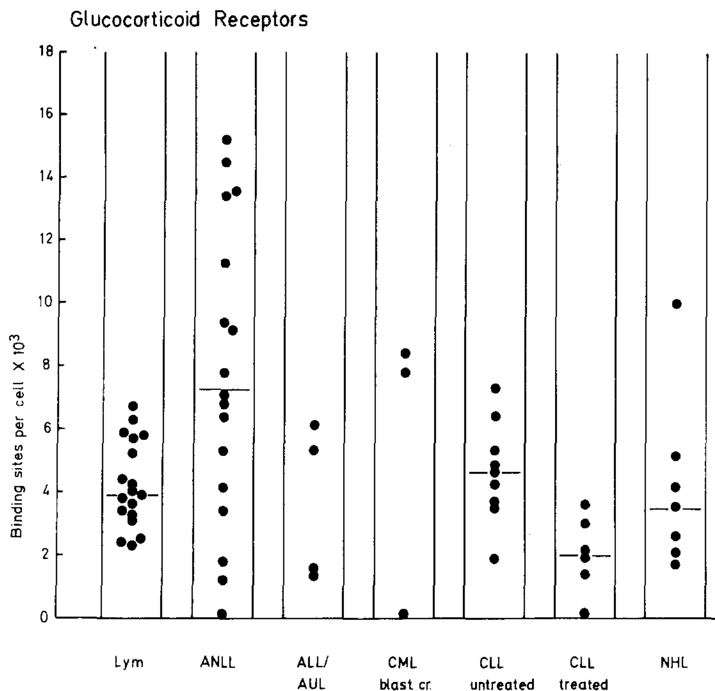

Fig. 1. Levels of glucocorticoid receptors in lymphocytes from 18 normal donors and in the leukemic cells from 46 patients. Lym = normal lymphocytes, ANLL = acute non-lymphoblastic leukemia, ALL/AUL = acute lymphoblastic/undifferentiated leukemia, blast cr CML = chronic myeloid leukemia in blast crisis, CLL untreated = chronic lymphatic leukemia newly diagnosed, CLL treated = chronic lymphatic leukemia treated for a long time with prednisone, NHL = leukemic Non-Hodgkin lymphomas. Each dot represents one patient or normal person and the horizontal bars represent the medians in each group

added and 4 h later the cells were harvested and were collected on glass fiber filters. Five percent trichloracetic acid was used to precipitate the newly synthesized nucleic acids. After air drying the filters were placed in scintillation vials, solubilized and counted in a liquid scintillation counter. Inhibition, or in some cases stimulation, is given in percent of acid insoluble radioactivity incorporated under the influence of dexamethasone as compared to controls. Since the experiments were always performed in triplicate,

$$\text{Inhibition} = \left(1 - \frac{\text{mean of cpm incorporated under dexamethasone}}{\text{mean of cpm of controls}}\right)\%$$

Deviations in such experiments were less than $\pm$ 11%.

Wherever possible, clinical response to therapy regimens which contain glucocorticoids was evaluated after 2 treatment courses according to the usual criteria [26,28]. Differences among groups are evaluated for significance at the $p < 0.05$ level using the median test.

Results

In Fig. 1 the results of the glucocorticoid receptor determination are summarized. As explained under methods the binding values at 8×10^{-8} mol ^{3}H-dexamethasone are given. In the lymphocytes from 18 normal donors there were 3,875 binding sites/cell

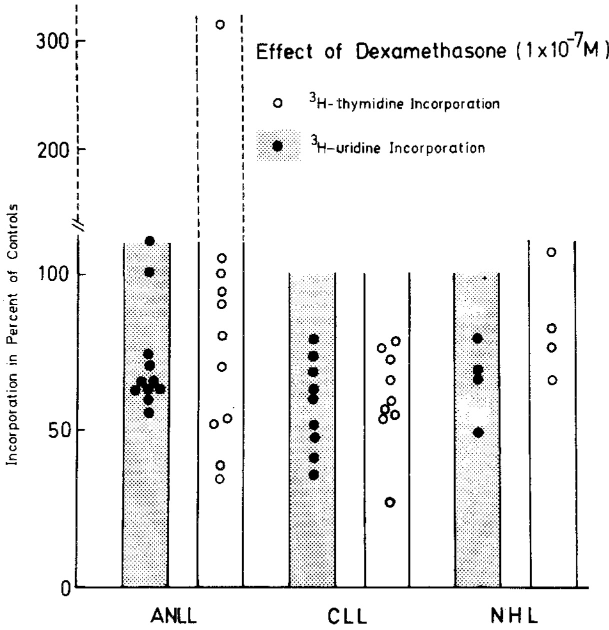

Fig. 2. The effects of dexamethasone at a single concentration (1×10^{-7} mol) on the incorporation of ^{3}H-uridine and ^{3}H-thymidine of leukemic cells from 24 patients. Values are given in percent of incorporated precursors as compared to control wells

(range 2,312–6,856). The blasts from the 17 patients with ANLL had widely varying levels of specific binding sites, ranging from 0 to 15,295 per cell. Only three of the 17 patients were treated with a combination therapy containing glucocorticoids. The blasts of these three patients had >6,000 receptor sites/cell and in all three patients a partial remission was attained. Due to participation in multicentre therapy study the other patients were treated with a regimen which did not contain glucocorticoids so that no attempt could be made to correlate the binding data with clinical responsiveness.

In the four patients with ALL/AUL the two patients with >5,000 receptor sites both showed a response after 2 treatment courses with combination therapy containing glucocorticoid, whereas the other two with receptor levels of <2,000 died shortly after diagnosis.

Among the three patients with blast crisis, all received primarily vincristine and prednisone and the patient with no detectable amount of receptors was resistant to this treatment. Of the two patients with >6,000 receptor sites/cell, one responded readily whereas the other showed progression of disease. Of the 15 patients with CLL, six had been treated for 3 to 5 years with a combination of glucocorticoid and chlorambucil and were resistant to this treatment. Their lymphocytes had lower number of specific binding sites ($p > 0.05$) than the other CLL cases which were newly diagnosed. Determination in those patients receiving glucocorticoid therapy was always performed at least 3 days after interruption of therapy. In three of these

patients the receptor level was determined on 2 or 3 occasions within 8 weeks and only a slight variation of at most 17% was observed.

Seven patients had leukemic malignant lymphomas, which included 5 immunocytomas, 1 centrocytic, centroblastic lymphoma and 1 hairy cell leukemia. (Classified according to Kiel-classification, histology performed by Prof. K. Lennert). Only three of these patients received glucocorticoid therapy and two of them attained a partial remission.

In 24 of the 46 patients the in vitro sensitivity of the leukemic cells to dexamethasone was also determined. Preliminary experiments with cells from patients with chronic lymphatic leukemia showed that an inhibition of ^{3}H-thymidine and ^{3}H-uridine incorporation was observed which was dose-dependent and was maximal at 10^{-7} mol. Figure 2 summarizes our results to date with a single concentration, i.e. 10^{-7} mol, of dexamethasone on RNA and DNA synthesis in the leukemic cells of patients with various forms of leukemia. Each dot or ring represents the mean of three determinations. We consider that there is a response to dexamethasone if the incorporation is decreased or increased by $>20\%$. Except in four patients with ANLL and one patient with NHL an inhibition of both ^{3}H-uridine- or ^{3}H-thymidine-incorporation was observed. Of the three patients without detectable receptors the in vitro effect could be investigated in two (one ANLL-patient and one CML-patient) and both were non-responsive in vitro. Only the patient with CML in blast crisis was treated with glucocorticoid and was clinically resistant.

Discussion

In almost all cases of leukemia in adults we were able to find specific glucocorticoid receptors. Of 46 cases only three appeared to have no detectable receptors. Among the ANLL patients the wide range of 0 to 15,295 receptor sites/cell was at first thought to be due to heterogeneity of this group. An attempt to correlate receptor content to subclassifications of ANLL was not successful. In contrast to earlier results from a smaller number of patients with ANLL [13] we could now establish no marked correlation between glucocorticoid receptor levels and in vitro sensitivity in this group. Since most of the ANLL cases investigated had higher receptor content (Median = 7,250) than the other groups and since ANLL were known to respond to glucocorticoid treatment in at most 15% it is very unlikely that a correlation might exist between receptor levels and clinical responsiveness in this group.

In the CLL cases the fact that patients who were resistant to glucocorticoid treatment at the time of steroid receptor determination had significantly lower levels of binding sites in their lymphocytes suggests that receptor determination might be of value in predicting clinical response in this group, and that there is a replacement of sensitive cells by resistant ones. However some authors have established fluctuations of receptor amount with cell cycle and have demonstrated an increase of receptors during the proliferative phase [5, 27]. Since our patients with pretreated CLL were in relapse at the time of receptor determination and since most of them had a relative high percentage of lymphoblasts this suggestion does not seem to be a plausible explanation for the difference. It is quite possible that long term glucocorticoid treatment results in a selection of resistant clones with less glucocorticoid receptors. How-

ever, pretreatment levels were not known and the possibility of lowered receptor levels due to chlorambucil could not be excluded in this study.

The utility of glucocorticoid receptor determination in human leukemias is thus still controversial. While Bloomfield et al. could relate receptor levels positively to clinical responsiveness in malignant lymphomas [4] and Lippman et al. to remission duration in childhood ALL [19,20], other authors could not establish any such correlations in their investigations in acute leukemia [3,14,15], childhood ALL [16,21], thymomas [23] and blast crisis in CML [3]. Our present data suggest that it might be of value to predict clinical responsiveness in those patients with lymphoid malignancies and probably not in patients with ANLL.

Analysis of the binding data with clinical responsiveness is the most difficult problem since almost all therapy regimens contain a combination of two to three cytostatic drugs other than glucocorticoids. Some authors put their patients on therapy trials with glucocorticoid monotherapy for a limited duration, e.g. 3 days before applying the usual combination therapy [8], which is too short to allow any conclusive statement. Bloomfield et al. applied a glucocorticoid monotherapy to 28 patients with malignant lymphomas up to 14 days but this can only be done on patients with low grade malignancies [4].

Another problem is that the usual assay method can only give an average of the number of binding sites/cell. Homo et al. could by means of discontinuous gradients establish evidence that there is a pronounced heterogeneity of cells as regards to response to glucocorticoid in patients with acute leukemia [14]. It is probable that some clones of leukemia cells have high content of receptors and are mixed with resistant clones with very low content of receptors. Recently antibodies to specific dexamethasone binding proteins from rat liver cytosol have been elicited in rabbits. These antibodies have been used successfully to demonstrate the intracellular glucocorticoid receptors in hepatoma cells by immunofluorescence microscopy [9]. Attempts are now being made to develope antibodies to binding proteins from human lymphoid tissue. Perhaps with this immunological method we can better demonstrate the presence of receptors in the individual leukemic cells.

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