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Reduction in glucocorticoid receptors in renal biopsies of patients with lupus nephritis

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Abstract

Objectives: The first-line treatment for lupus nephritis is the administration of glucocorticoids (GC) that mediate their effects via the glucocorticoid receptor (GR). The aim of this study was to investigate the expression of GR protein in the cortical area of renal parenchyma of normal and diseased renal biopsies from treated and untreated patients.

Design and methods: The immunohistochemical EnVision/HRP technique was performed on renal tissue to detect GR protein. Statistical analysis was performed by SAS (2001).

Results: The antigen was mainly detected in glomerular podocytes and in tubules. The number of GR-positive podocytes of the controls was significantly higher than in the untreated patients, which was accordingly higher than in patients who were under medication.

Conclusions: The lower number of GR-positive cells in the diseased kidney compared to controls is possibly linked to tissue-specific GC resistance, whereas the decreased GR expression in podocytes of treated compared to untreated patients may be due to a down-regulation effect after GCs' administration.

Keywords: Glucocorticoid receptor; Glucocorticoids; Lupus nephritis; Renal biopsy; SLE; Immunocytochemical method

Introduction

Glucocorticoids (GCs) are widely used as potent anti-inflammatory and immunosuppressive drugs administered to many patients suffering from immunologically mediated diseases including systemic lupus erythematosus (SLE) nephritis. Early studies have shown an increased survival rate for patients with lupus nephritis treated with GCs [1–3]. Both renal and patient survival have been dramatically improved over the last decades due to modern therapeutic regimens that include daily or alternate-day high-dose oral steroids, antimetabolites such as azathioprine, alkylating agents such as cyclophosphamide, cyclosporine, and supportive care [4,5]. Most regimens use a combination of steroids and cyclophosphamide in order to halt and possibly reverse existing features of activity and prevent recruitment of additional glomeruli into the active nephritis.

GCs mediate their effects via their intracellular receptor, GRα and GRβ isoforms. GRα is the classic GR located primarily in the cytoplasm bound to heat shock proteins (HSPs) 90, 70, 50 and 20 and possibly to other proteins, as well. Upon binding to GCs, GR undergoes conformational changes, dissociates from HSPs, partly homodimerizes and translocates into nucleus where it interacts directly with its specific DNA sequences in the promoter regions of target genes, the glucocorticoid response elements (GREs) known as transactivation function [6]. GC-activated GRα monomers heterodimerize with other transcription factors (such as NF-κB, AP-1, p53, CREB, STATS and others) via protein–protein interactions,

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thereby influencing indirectly the activity of the latter on their own target genes, known as transrepression effect [7–9]. GCs inhibit inflammation via the inhibitory GR-mediated signal transduction cascade onto transcription factors NF-κB and AP-1. GRβ isoform does not bind GCs, heterodimerizes with GRα and regulates negatively the GRα–GRE-induced transcriptional activity [9]. Kino et al. [10] showed that the β-component of the G-protein complex binds to a specific site on the amino-terminal domain of GRα, influencing the GRα-mediated transcription, thereby implicating that extracellular signals which stimulate G-protein-coupled receptors may also influence GR-mediated activity.

It is apparent that excess activation of the above intracellular GR-interacting factors will reduce the number of effective GRs, thereby decreasing GC-sensitivity. An effective density of functional GRs is usually required to mediate the GC–GR-induced transrepression and transactivation functions. GCs are used as a standard anti-inflammatory therapy in SLE, whereas an adequate GR signalling cascade is necessary to inhibit inflammation. Given that the number of available GRs is among the important aspects of GC–GR-mediated activity, because an increased number of GRs is required to transrepress the NF-κB-mediated inflammatory activity and to increase tissue sensitivity to GCs, we decided to determine the GR protein content in various anatomic kidney compartments in patients with SLE nephritis.

Decisions on proper GCs dosage are of particular importance to further improve the prognosis of patients with lupus nephritis through minimizing not only treatment failure but also undesirable side effects. Despite the extensive use and the crucial role of GCs in the management of lupus nephritis, there is scant information concerning the immunodetection of GR in target organs. Existing studies have focused on the relationship between GR density measurements on peripheral blood mononuclear cells (PBMCs) and glucocorticoid effect in lupus nephritis patients [11–13] demonstrating the significance of GR levels in PBMCs for prediction of therapeutic responsiveness. Since patients would benefit from the development of a test which would help the clinician decide on proper dose and duration of treatment, our aim was to examine GR expression in the various anatomic compartments of the kidney in patients with SLE nephritis who receive glucocorticoids as their therapeutic modality, those who receive no medication and controls.

Methods

To elucidate this issue, we examined renal biopsy specimens from 29 SLE patients (24 females and 5 males), ranging in age from 12 to 62 years (30.9±11.6, mean±SD). They all satisfied at least four of the American Rheumatism Association (ARA) revised criteria for SLE [14]. All clients attended the Nephrology Department of the Evangelismos Hospital. During observation, all patients experienced disease exacerbation and biopsies had been obtained by percutaneous needle technique from patients who had any evidence for renal involvement such as active urinary sediment, proteinuria, or increase in serum creatinine by more than 30%. At this time, 14 patients were under medication with less than 30 mg/day prednisolone to treat extra renal clinical symptoms (Group A) and 15 patients had never received any treatment for SLE (Group B). No patient had received high-dose glucocorticoid therapy (1 mg of prednisolone/kg/day or more) to treat lupus nephritis. Ten tissue specimens obtained from non-affected portions of kidney surgically removed for renal cell carcinoma from an equal number of subjects who had no personal history of SLE or any other autoimmune disorder were used as controls. Ethical consent was obtained from the Renal Pathology and Nephrology Departments of the Evangelismos Hospital and the Medical School of the University of Athens ethical committees conforming to standards currently applied in Greece.

The specimens were fixed in bouin's fluid and embedded in paraffin. Serial sections were cut at 2 μm and stained with haematoxylin–eosin (H–E) and periodic acid–Schiff (PAS). In all cases, diagnosis of lupus nephritis was based on the characteristic findings by light microscopy, immunofluorescence and in some cases electron microscopy. The histological classification of the biopsies according to the criteria of the expanded and refined World Health Organization (WHO) [15] and the scores of the modified activity and chronicity indices [16,17] were assessed. The relevant clinical data are summarized in Tables 1 and 2.

The immunohistochemical EnVision/HRP technique was applied to 2-μm sections of renal tissue to label different cell types with the antibody GR. Deparaffinized through xylene and hydrated through graded alcohol series sections were incubated with freshly made 3% hydrogen peroxide/methanol in order to block endogenous peroxidase activity. After microwave treatment in 2.1% citrate buffer (pH 6.1) for antigen retrieval,

Table 1
Clinical characteristics in 29 patients with lupus nephritis

WHO classification	I	II	III	IV	V	VI	Total
No. of patients	2	3	5	9	8	2	29
Under steroids	0	1	3	5	4	2	14/29
F/M ratio	2/0	2/1	3/2	7/2	8/0	2/0	24/5
Age at submission (year)	16 (12–20)[a]	23 (17–20)[a]	31 (15–46)[a]	35 (19–62)[a]	25 (15–38)[a]	40 (38–41)[a]	30.9 (12–62)[a]
Activity index	0	2.3 (2–3)[a]	2.8 (2–3)[a]	6.9 (4–14)[a]	6.5 (3–11)[a]	–	5 (0–14)[a]
Chronicity index	0	1.3 (1–2)[a]	2.8 (1–8)[a]	2.3 (1–6)[a]	1.25 (0–4)[a]	10.5 (10–11)[a]	2.4 (0–11)[a]
Proteinuria	0	1	4	9	7	2	23/29
Hematuria	0	0	4	4	3	1	12/29

[a] Mean (range).