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ORIGINAL ARTICLE

The Expression of Heat Shock Proteins 27 and 70 in Lupus Nephritis

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ABSTRACT

BACKGROUND: Heat shock protein (HSP) up-regulation is a cytoprotective response following stress insults (toxic, ischemic, inflammatory and oxidative).

OBJECTIVE: To study the localization of HSP27 and HSP70 in the renal tissue of patients with lupus nephritis (LN) and correlate our findings with the severity of histological involvement (activity and chronicity indices) and the degree of renal function impairment.

PATIENTS AND METHODS: Seventy patients with LN (diffuse proliferative n=31, focal proliferative n=20, and membranous n=19) were included in the study. The distribution of HSP27/HSP70 was studied by immuno-histochemistry in renal biopsy sections. A double staining method for vimentin, a-smooth muscle actin, CD34 and CD68 (+) cells were performed to identify the type of glomerular cells expressing HSPs. The severity of immunostaining for HSP27/70 was evaluated semiquantitively.

RESULTS: HSP27 and HSP70 were identified within the cytoplasm of tubular epithelial cells of all patients. Increased HSP27 expression was noted within intrinsic glomerular cells in diffuse lupus nephritis whereas no glomerular expression was observed in focal proliferative and membranous LN. A significant positive correlation was found between HSP27 expression in diffuse proliferative nephritis and the activity and total (activity + chronicity) indices. The severity of histological involvement was also related to the degree of renal function impairment. Conclusions: Up-regulation of heat shock protein expression was identified in patients with various types of LN, especially those with diffuse proliferative nephritis. The severity of HSP 27 expression was related to the activity and total indices. These results suggest a possible defensive role for HSP27 in severe lupus nephritis.

KEY WORDS:
Lupus Nephritis, Actine Heat Shock Proteins

INTRODUCTION

Heat shock proteins (HSPs) represent adaptive or defensive mechanisms of cells that play an important role in their survival in a hostile and continuously changing environment. They were initially described as a group of proteins whose synthesis is induced following cellular exposure to increase temperature [1]. However, recent data showed that HSP synthesis is up-regulated as a defense mechanism following a wide variety of "stress" insults (toxins, oxidative stress, trauma, inflammation) [2]. In the molecular level they act as chaperones, participating in protein assembly and folding, stabilizing the newly formed proteins and helping them to acquire their func-

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tional three-dimensional configuration [3]. In the cellular level HSPs are involved in the control of protein remodeling via facilitating their refolding. Proteins that fail this "remodeling procedure" are destined to proteolysis from the ubiquitin-proteasome system [4].

HSP27 belongs to the low molecular weight HSPs and shows considerable homology (concerning its aminoacid sequence) with proteins having the a-crystallin structure that are abundant in the lens [5]. HSP70 is one of the most abundant HSPs with diverse functions. In the human kidney, both of them are constitutively expressed in the proximal, distal and collecting ducts while HSP70 shows minimal expression in podocytes [6,7].

Lupus nephritis is characterized by a variety of histological lesions ranging from minimal changes to diffuse proliferative nephritis. The type of histological lesion as well as the activity and chronicity indices represent the main factors that determine the clinical outcome of patients with lupus nephritis. The purpose of this study is to localize HSPs27 and 70 in the renal tissue of patients with various types of lupus nephritis and to identify any potential correlation between HSP27/70 expression and the severity of histological involvement (activity and chronicity indices) as well as renal function impairment.

PATIENTS AND METHODS

PATIENTS

Seventy patients with biopsy-proven lupus nephritis, refered to our unit between 1980 and 1999 (10 males, 60 females aged 18-61), were evaluated in this study. According to the renal histology, 31 patients had diffuse proliferative (class IV), 20 focal proliferative (class III) nephritis and 19 membranous (class V). The clinical and histological features of all patients are shown in table 1.

CONVENTIONAL HISTOPATHOLOGY AND GRADING OF HISTOPATHOLOGICAL INVOLVEMENT

The diagnosis was made by adequate renal biopsy on light-microscopy (LM) and immunoflurescence (IF). The lupus nephritis class was based on the WHO classification. Renal tissues were taken with a Vim-Silverman or Tru-cut 14G biopsy needle, were fixed in 10% neutral formalin, embedded in paraffin and were examined on LM in multiple sections (4μm each). The histological study included hematoxylin and eosin (H & E) stain, periodic acid schiff (PAS), Masson trichrome and reticulin silver methenamine stains. The activity and chronicity indices were identified in each renal biopsy according to the Morel-Maroger [8] (modified by Austin and associates) [9] classification (referred to as the NIH index). There are six features of activity, including degree of intraglomerular proliferation, wire loop subendothelial deposits, glomerular neutrophilic infiltration, glomerular necrosis (karyorrhexis, pyknosis, or fibrinoid necrosis), cellular crescents, and interstitial inflammation. Each feature is graded on a scale of 0 (absent), 1+ (mild), 2+ (moderate), or 3+ (severe). Necrosis and cellular crescents are weighted in double (multiplied by 2) because of their greater significance. The individual scores for each of the six histologic features are summed to give the final activity index (scale 0 to 24). In a similar way, the chronicity index (scale 0 to 12) is derived by adding individual semiquantitative scores (0 to 3+) for each of the four histologic features of glomerulosclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed on 3-5 μm thick paraffin sections of all biopsy specimens and in 4 control biopsies (normal part of kidney removed because of hypernephroma). After de-parafinization, they were pretreated with hydrogen peroxide to abolish endogenous peroxidase activity and then covered with the primary antibody (dilution 1:20), a mouse monoclonal antibody against mammalian HSP27 (HSP27-Novocastra) or against HSP 70 (HSP 70- Novocastra) and incubated for 1 hour at room temperature. The biotinylated secondary antibody was followed by the streptavidin complex and the color was developed using the diaminobenzidine as the chromogen. In order to precisely identify the type of cell in which HSP27 expression is upregulated, we used a double staining method (alkaline phosphatase as chromogen) for vimentin (epithelial cell), CD34 (endothelial cell), CD 68 (macrophage) and a-SM actin (mesangial and smooth muscle cell). Sections from all the cases including normal controls were incubated with normal mouse IgG instead of the primary antibodies and were designated as negative controls. Tissue localization and intensity of staining to HSP27/70 were evaluated semiquantitively as 1+ to 4+. Total HSP 27/70 resulted from the sum of glomerular + tubular expression of HSP 27/70.

STATISTICAL ANALYSIS

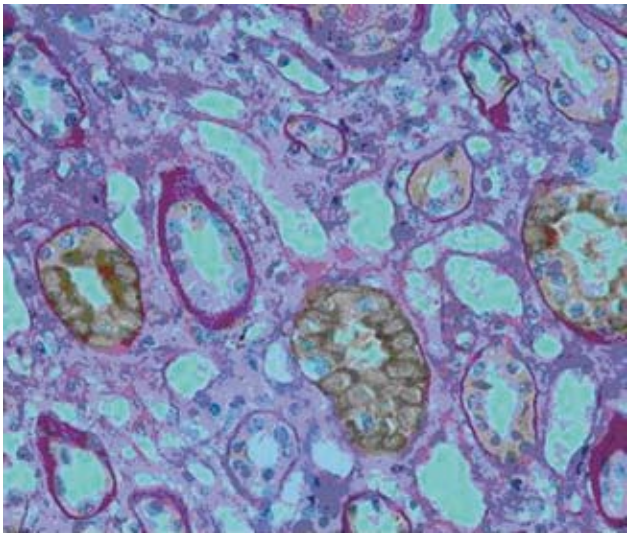
Since all data were not distributed normally, non-parametric tests (Spearman's rho) were used for all correlations. A p-value of less than or equal to 0.05 was considered as significant.

RESULTS

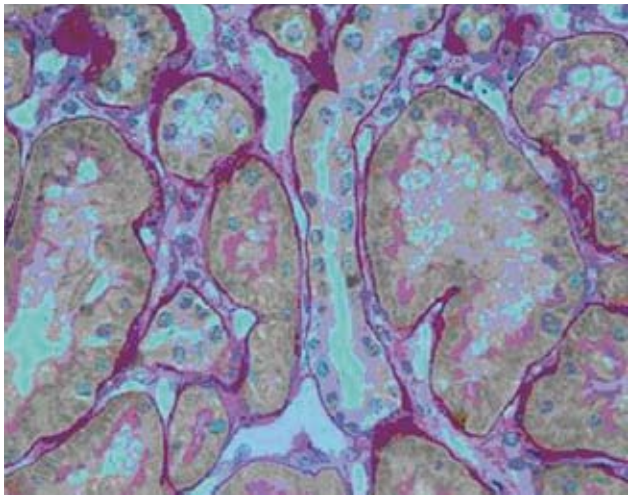
PATTERN OF IMMUNOSTAINING FOR HSP27 AND HSP70

The pattern of immunostaining for HSP27 and 70 in both patients with various types of lupus nephritis and controls is shown in table 2. HSP27 and HSP70 were identified within the cytoplasm of tubular epithelial cells in proximal and distal tubules and in the collecting duct of both patients and con-

trols. However, the severity of HSP27/70 expression within the tubular epithelial cells of patients with lupus nephritis was higher compared to that in the controls (Picture 1A, B, C). Increased HSP 27 expression was detected in the glomeruli of patients with diffuse lupus nephritis (Picture 1D) but no glomerular expression of HSP 27 and 70 was observed in patients with focal proliferative or membranous lupus nephritis. The double immunostaining for HSP 27, vimentin, a-SM actin, CD34 and CD 68 positive cells showed that the positive HSP 27 cells within the glomeruli of patients with diffuse lupus nephritis were intrinsic glomerular cells (mesangial, epithelial and endothelial cells) (Picture 1E). No expression of HSP 27 was identified within inflammatory (CD 68 +) cells in the glomeruli.



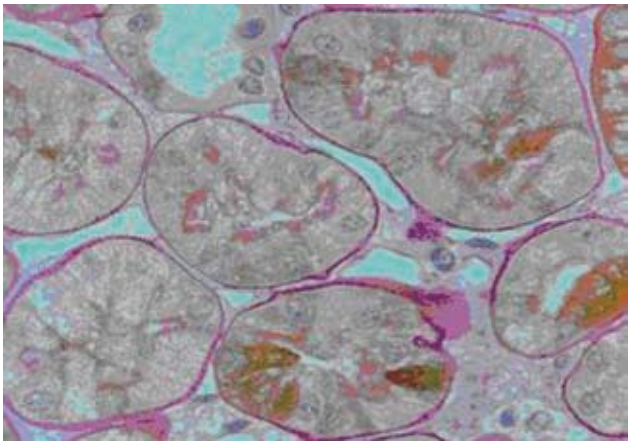
PICTURE 1A. Renal medulla. Focal, granular cytoplasmic staining of low tensity mainly in collecting ducts and distal tubules in normal kidneys. (PAS stain).



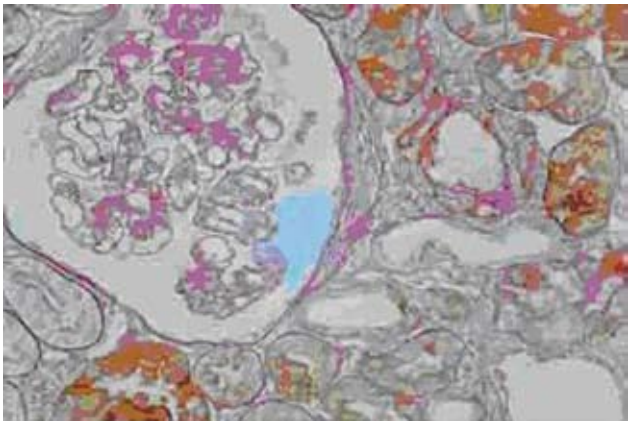
PICTURE 1B. Renal cortex. Diffuse fine cytoplasmic staining for HSP 27 in proximal epithelial cells and focal in distal tubules in normal kidneys (PAS stain).

CORRELATION BETWEEN VARIABLES

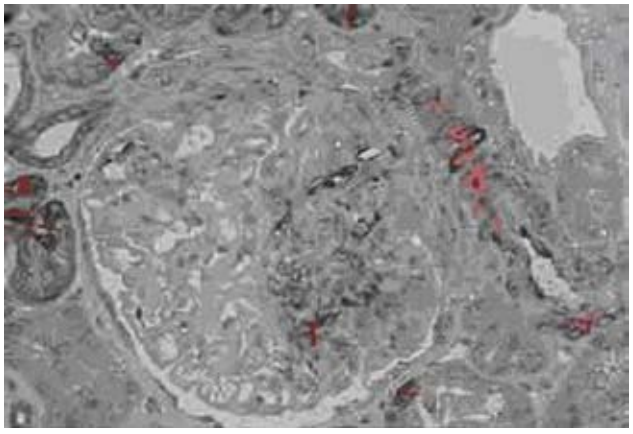
A positive correlation of the severity of HSP 27 expression (glomerular and tubular) in the kidney of patients with diffuse proliferative nephritis (class IV) and both the activity ($r=0.539$, $p<0.003$) and total (activity+chronicity) index score ($r=0.563$, $p<0.001$) was observed (fig.2A, B). A positive correlation



PICTURE 1C. Renal tubules. Focal cytoplasmic staining in a patient with class III lupus nephritis (DAB-brown color).



PICTURE 1D. Class IV lupus nephritis. Increased HSP 27 expression in endothelial and mesangial cells (DAB-brown color).



PICTURE 1E. Class IV lupus nephritis. Double stain for HSP27 (DAB-brown color) and for a-SM actin (alkaline phosphatase-red color). Positive reaction for HSP 27 in endothelial and mesangial cells. HSP is upregulated in endothelial cells of the afferent arteriole, in isolated smooth muscle cells and in cells of the crescent.

of the activity index (AIS) and total (AIS+CIS) scores with serum creatinine ($r=0.311$, $p<0.003$) and an inverse relation to creatinine clearance ($r=-0.290$, $p<0.048$ and $r=-0.349$, $p<0.016$ respectively) was evident in patients with class IV lupus nephritis.

No correlation was found between renal expression of HSP 27, serum creatinine, ClCr and proteinuria in patients with lupus nephritis.

DISCUSSION

In this study, the localization of HSPs 27 and 70 in the renal tissue of patients

with lupus nephritis and its possible correlation with the well-known histologic indices (the AIS and CIS), used to assess the severity and potential reversibility of renal injury, is investigated. According to our results, HSP27 is constitutively expressed in the proximal, distal, collecting tubules and vessels of all patients and also in the glomeruli of patients with severe glomerular inflammation (class IV lupus nephritis). In these cases, intrinsic glomerular cells (epithelial, endothelial and mesangial) showed an increased expression of this protein while transient inflammatory cells did not seem to interfere with this process. In cases with either minimal (class III) or no glomerular inflammation (class V lupus nephritis), no HSP27 glomerular expression was noted. In addition, a significant correlation of HSP27 expression with the histologic indices of activity chronicity of the disease were found in patients with class IV nephritis. The abovementioned findings suggest that HSP27 is up-regulated in cases of lupus nephritis with severe glomerular inflammation and proliferation. Whether this represents a defensive mechanism of the glomerulus to the inflammatory insult is not known. However, it is of note that up-regulation of HSP27 synthesis occurs only in intrinsic glomerular cells. HSP27 has been found to protect cytoskeleton of energy depleted renal epithelial cells against disruption. The beneficial effect of HSP27 in preservation of cell integrity against various stimuli is related to actin [10] Contractile proteins (actin, myosin, caldesmon, tropomyosin) immuno-precipitate with HSP27 leading to sustained smooth muscle contraction [12]. A direct actin-sHSP interaction probably occurs to inhibit actin polymerization and to participate in the in-vivo regulation of actin filament dynamics [11].

This finding suggests that HSP27 might be the link between signal transduction cascade and the contractile proteins.

Thus, increased HSP27 expression in intrinsic glomerular cells is observed in response to a marked cellular infiltrate of the glomerulus; this probably represents an effort of these cells to stabilize cytoskeletal components and preserve their integrity. The absence of glomerular HSP27 expression in classes III and V of lupus nephritis implies that a severe insult is required (diffuse glomerular infiltrate) to induce this cytoprotective response. The degree of HSP27 expression was related to the AIS and the total (AIS+CIS) scores of patients with proliferative (class IV) lupus nephritis. This finding of increased HSP27 expression in cases with severe histological involvement is also suggestive of an important role of HSP27 in cellular defense in this setting.

Regression analysis showed a linear relationship between the AIS and total HSP27 expression (tubular + glomerular), suggesting that the intensity of HSP27 can predict the AIS in class IV lupus nephritis.

HSP70 was constitutively expressed in renal tubules but was characteristically absent in the glomeruli in all renal specimens. This suggests that this protein does not participate, at least not as an important cell defense mechanism, in lupus nephritis. HSP70 expression is induced (mainly in tubules) in a variety of renal diseases such as acute vascular rejection, acute tubular necrosis, CMV infection and minimal change disease [13]. In anti-GBM nephritis [15] and acute renal injury from folic acid [5], its synthesis is down-regulated due to increased apoptosis. Our findings suggest that HSP70

expression was neither enhanced nor suppressed in lupus nephritis even when a severe glomerular infiltrate is present. These results are consistent with previous reports [13] that showed that HSP72 (the induced form) was absent in cases where the mechanism of injury was primary immunological (acute cellular rejection, lupus nephritis).

In conclusion, up-regulation of heat shock protein expression in the kidney was identified in patients with various types of lupus nephritis and, in particular, in those with diffuse proliferative nephritis. The severity of HSP27 expression was related to the activity and total (activity+chronicity) indices of the disease. These findings are suggestive of a defensive role

for HSP27 in severe lupus nephritis. However, further research is required in order to elucidate the role of heat shock protein expression in human diseased kidney.

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