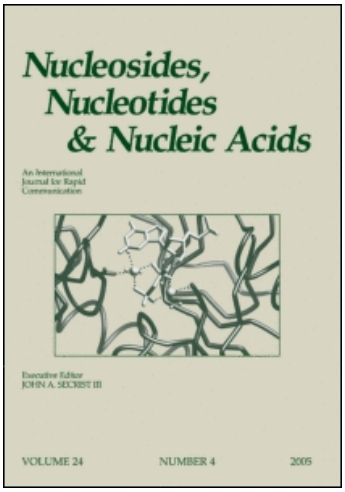

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B. Cerboni[a]; A. Di Stefano[a]; V. Micheli[a]; G. Morozzi[b]; G. Pompucci[a]; S. Sestini[a]
[a] Department of Molecular Biology, Section of Biological Chemistry, University of Siena, Siena, Italy [b] Clinical Medicine and Immunological Sciences Department-Division of Rheumatology, University of Siena, Italy

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PARP ACTIVITY AND NAD CONCENTRATION IN PMC FROM PATIENTS AFFECTED BY SYSTEMIC SCLEROSIS AND LUPUS ERYTHEMATOSUS

B. Cerboni,[1] A. Di Stefano,[1] V. Micheli,[1] G. Morozzi,[2] G. Pompucci,[1] and S. Sestini[1]

[1]*Department of Molecular Biology, Section of Biological Chemistry, University of Siena, Siena, Italy*
[2]*Clinical Medicine and Immunological Sciences Department-Division of Rheumatology, University of Siena, Italy*

□ *The enzyme poly(ADP-ribose) polymerase (PARP-1, EC 2.4.2.30) is activated by DNA strand breaks caused by several agents and utilizes NAD to form polyADPR, bound to acceptor proteins. The involvement of PARP-1 in autoimmune diseases has been suggested: antiPARP autoantibodies are described in systemic lupus erythematosus (SLE), DNA strand breaks have been evidenced in systemic sclerosis (SSc). We tested poly(ADP-ribosyl)ation activity and NAD concentration in PMC from patients affected by SLE or SSc and from controls. Lower PARP-1 activity and higher NAD concentration were observed in pathological conditions than controls, supporting the role of PARP-1 activation in modulating NAD concentration.*

Keywords NAD; PARP-1; SLE; systemic sclerosis

INTRODUCTION

Besides other important functions, NAD is the substrate of poly(ADP-ribose) polymerase-1 (PARP-1, E.C. 2.4.2.30), the major responsible enzyme of polyADP-ribosylation reactions. The enzyme is activated after DNA damage and utilizes NAD to form long and branched ADP-ribose polymers, covalently bound to a large array of acceptor proteins (topoisomerase I and II, histones, p53 etc.) thus playing a relevant role in many cellular processes.[1] It has also been reported to be involved in the pathophysiology of some diseases, such as autoimmune diseases.[2,3] Aberrant apoptosis has been found to be involved in the aetiopathogenesis of systemic lupus erythematosus (SLE), an autoimmune disease in which anti-nuclear, anti-PARP

Address correspondence to B. Cerboni, Department of Molecular Biology, Section of Biological Chemistry, University of Siena, via Fiorentina, 1, 53100, Siena, Italy. E-mail: cerboni@unisi.it

and anti-poly(ADP-ribose) auto-antibodies,[4,5] and lower poly(ADP-ribose) synthesis[6] have been demonstrated.

Systemic sclerosis (SSc) is a connective tissue disorder in which chromosomal fragility has been demonstrated;[7] two-subsets are identified according to LeRoy et al.:[8] limited systemic sclerosis (lSSc), which is characterized by limited cutaneous symptoms and occurs more frequently, and diffused systemic sclerosis (dSSc), presenting with more rapidly advancing and diffuse cutaneous symptoms. Anti-topoisomerase I antibodies have been demonstrated to be prevalent in dSSc patients.[8] In this article, we investigated PARP activation and NAD content of peripheral blood mononuclear cells (PMC) from controls, SLE and SSc patients, in the aim to investigate the relationship between the ability to respond to DNA damage by PARP activation and NAD content.

MATERIALS AND METHODS

Five female patients with each of the above disorders were tested; in particular, SLE patients (mean age 40 years ± 16 SD) were all receiving steroid treatment plus hydroxychloroquine or cyclophosphamide; lSSc patients (mean age 56.1 ± 22 SD) and dSSc (mean age 58.1 ± 18 SD) were not subjected to UVA therapy. Ten sex- and age-matched volunteers were tested as controls. All subjects gave their informed consent to the study. Blood samples from patients and controls were obtained as part of the treatment program or provided by voluntary donors and thus ethical committee approval was not required.

PARP activation was assayed according to Berger's modified method[9] on PMC isolated by Lymphoprep. The enzyme activation was calculated as the percentage of activity in irradiated cells with respect to that of nonirradiated ones. NAD concentration was detected in nonirradiated PMC according to our HPLC-linked method,[10] and was expressed as picomoles per million cells (pmoles 10^{-6} cells); data represent the mean values ± SE. The flow cytometric method described by Nicoletti et al.[11] was used to quantify the percentage of apoptotic cells in PMC from one dSSc patient and one control 16 hours after UV irradiation and in nonirradiated cells. All the experiments were conducted in triplicate and statistical analysis was performed using ANOVA (* $P \leq 0.050$, ** $P \leq 0.010$).

RESULTS

As shown in Figure 1 PARP activation in control PMC was 95% and NAD concentration was 40.4 ± 5.9 pmoles 10^{-6} cells.[12] In PMC from SLE patients PARP activation was 42%; NAD concentration was 60.7 ± 17.8 pmoles 10^{-6} cells. In lSSc patients, the percentage of PARP activation was 34% and NAD

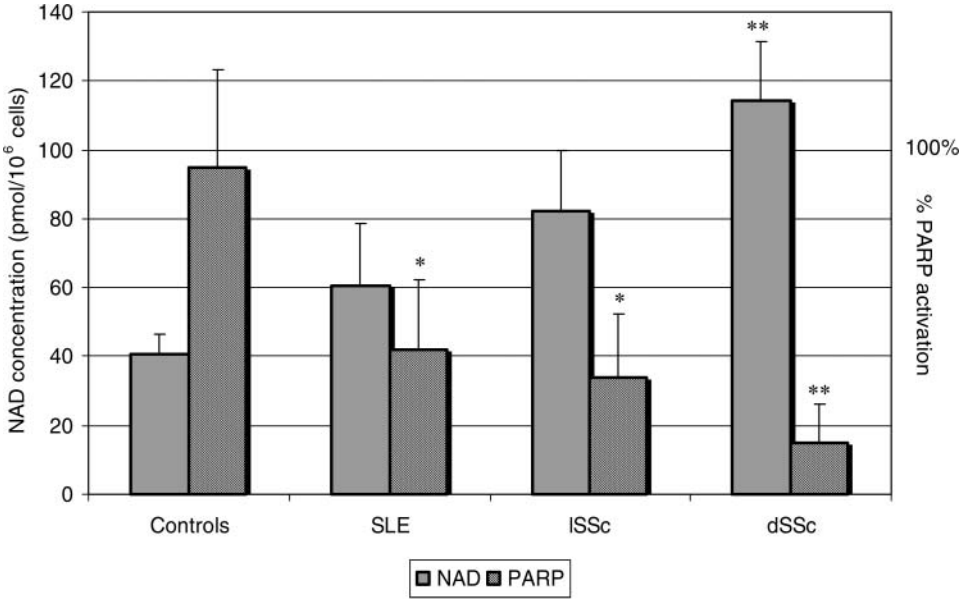

FIGURE 1 Intracellular basal NAD concentration in nonirradiated PMC and PARP percent activation following irradiation. The statistically significant difference patients-versus-controls is shown (*P ≤ 0.050, ** P ≤ 0.010).

content was 82.1 ± 17.7 pmoles 10^{-6} cells, while in dSSc patients PARP activation was 15% and NAD concentration 114.3 ± 17.2 pmoles 10^{-6} cells. The flow cytometry showed the onset of apoptosis 16 hours after irradiation in a dSSC sample, unlike control cells (Figure 2).

DISCUSSION

The present investigation has been conducted to check whether the DNA fragility reported in SSc and some features occurring in SLE (the sensitivity to UV radiations, the presence of antiPARP and anti poly(ADP-ribose) autoantibodies etc.) might be related to PARP-1 activity. As shown by our results, PARP activation produced by UV irradiation was remarkably lower in SLE and SSc patients than in controls, thus suggesting the impairment of PARP in these pathologies, especially in dSSc, in which apoptosis measured by flow cytofluorimetric analysis in one patient was twice his control. Moreover, NAD content was higher in all the patients' PMC compared to controls, reaching a three-fold increase in dSSc. Low basal NAD concentration and high PARP activation were detected in controls, while high NAD content and low PARP activation were shown in all groups of patients, thus confirming that NAD accumulation in patients' PMC may derive from its underutilisation by PARP.

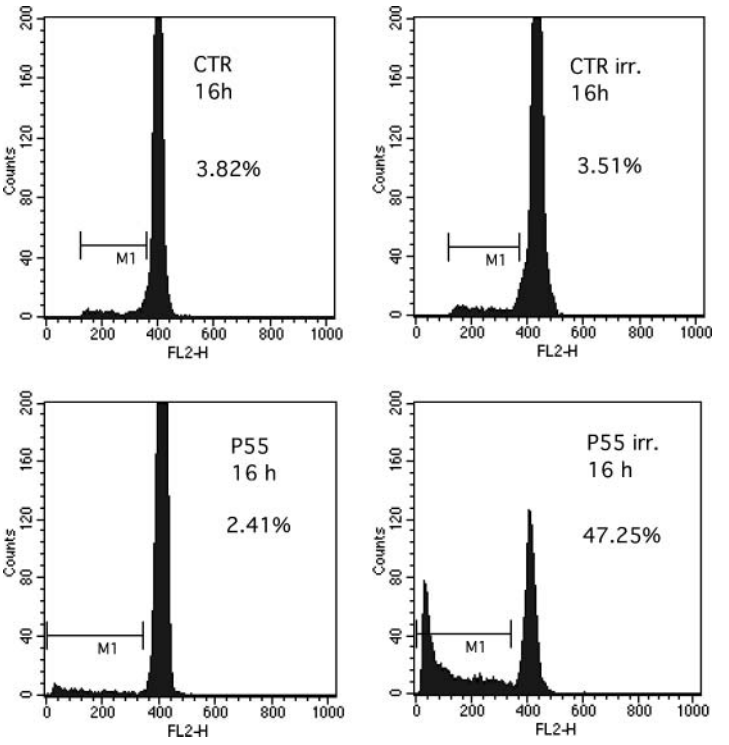

FIGURE 2 Frequency distribution histograms of DNA content of PI-stained nonirradiated (left) and irradiated (right) PMC cells from a dSSc patient (bottom) and a control (top).

This investigation in patients with known defects of DNA repair provides further evidence of the importance of NAD catabolic pathways, such as utilization by PARP, in regulating its concentration.

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