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Poly(ADP-ribose) polymerase activity in systemic lupus erythematosus and systemic sclerosis

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ABSTRACT

The aim of this study is to investigate the role of poly(ADP-ribose) polymerase (PARP), involved in DNA repair and in autoimmune pathologic conditions such as systemic lupus erythematosus (SLE) and both limited systemic sclerosis (lSSc) and diffuse systemic sclerosis (dSSc), to assess its possible implication in the pathogenetic processes. The relationship between PARP activity and the intracellular concentration of its substrate nicotinamide adenine dinucleotide (NAD) is also investigated. Peripheral mononuclear cells (PMC) from controls and patients with SLE, lSSc, and dSSc were irradiated with ultraviolet light (UV) and PARP activity was assayed by a radiochemical method. Pyridine nucleotide concentrations were assayed by a high-performance liquid chromatography–linked method. PARP activity was detectable in nonirradiated cells and showed similar values in all groups. The activity significantly increased after UV irradiation in control, SLE, and lSSc cells, but not in dSSc cells. Irradiated PMC from both SLE and dSSc showed lower enzyme activity with respect to irradiated controls. Higher intracellular NAD content was found in all of the pathologic conditions in comparison to values in the control; this difference was statistically significant in dSSc. Our data demonstrate a lower PARP activity in response to UV damage in PMC from patients affected by the above pathologic conditions compared with controls. An inverse relationship between PARP activity and NAD content was also observed.

1. Introduction

Systemic lupus erythematosus (SLE) [1] is an autoimmune disease characterized by the production of various autoantibodies, particularly antinuclear antibodies, and by widespread inflammation affecting virtually every organ in the body. The exact etiology of SLE is unknown; although no genetic predisposing factors have been identified in most cases, a strong familial trend is observed, indicating that multiple environmental factors, such as exposure to sunlight, may be responsible for the occurrence of the disease. Recently, aberrant apoptosis and disturbed clearance of apoptotic material have been implicated in the etiopathogenesis of SLE [2]. The variability of symptoms required a classification of SLE on the basis of defined criteria according to the disease activity index [3–5].

Scleroderma, or systemic sclerosis (SSc), is a connective tissue disorder producing skin sclerosis and systemic symptoms such as fibrosis, vascular pathology, and immunologic abnormalities in its classical form. The extreme variability of SSc prompted a number of clinical investigators to divide patients into subsets. A two-subset scheme was proposed by LeRoy *et al.* [6] and is the most commonly used: limited systemic sclerosis (lSSc), characterized by stable, limited cutaneous symptoms, represents the disease in the majority of patients; and diffuse systemic sclerosis (dSSc), presenting with more rapidly advancing and diffuse cutaneous symptoms, represents the disease in a minority of patients.

The nuclear enzyme poly(ADP-ribose) polymerase–1 (PARP-1, E.C. 2.4.2.30) [7] catalyzes the polyADP-ribosylation of nuclear proteins as an immediate cellular response to DNA damage caused by either chemical or physical agents [8]. In approximately 15% of cases, such post-translational modification may be catalyzed by other less abundant members of the PARP family, the physiologic functions of which are still under study [9]. PARP-1 is activated by binding to DNA strands and uses nicotinamide adenine dinucleotide (NAD) as a substrate to form long and branched ADP-ribose polymers, covalently binding it to a large array of acceptor proteins, thus playing a relevant role in DNA repair [10], replication, transcription [11], differentiation, apoptosis [12], and activation of cellular defense against DNA damage. The removal of the polymer from the acceptor protein is catalyzed by poly(ADP-ribose) glycohydrolase (PARG) [13]. PARP-1 has also been reported to be involved in the pathophysiology of different diseases, such as acute

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and chronic inflammatory disorders [8], stroke, diabetes, obstructive pulmonary disease [14], and autoimmune diseases [15].

Abnormalities in poly(ADP-ribose) metabolism have been evidenced to play some role in the development or manifestation of SLE. Exposure to sunlight is an environmental factor known to induce and exacerbate SLE; UV rays are known to cause DNA damage (DNA strand breaks and apoptosis), which in turn are related to PARP. Lower poly(ADP-ribose) synthesis has been reported in lymphocytes isolated from patients affected by SLE compared with controls [16], possibly because of defective gene transcription or mRNA turnover [17]. Elevated levels of anti-PARP and anti-poly(ADP-ribose) autoantibodies [1,14,18,19] have been detected in this disorder. Moreover, autoantibodies against the catalytic domain of recombinant PARPs have been demonstrated in the sera of patients with SLE, suggesting their possible use as serologic diagnostic markers of the disease [20]. A specific PARP allele (85bp) has been reported to be associated with SLE [21], thus suggesting that PARP gene is either involved *per se* in SLE susceptibility or is located in close proximity to such a gene, although this association has not been confirmed [22].

The relationship between PARP activity and SSc, unlike SLE, has been insufficiently investigated. Lim *et al.* [19] and Jeoung *et al.* [20] failed to demonstrate autoantibodies against PARP-1 or PARP catalytic domain in the sera of SSc patients. Anti-topoisomerase I antibodies (anti-Scl70) have been demonstrated to be prevalent in dSSc patients [6], in which DNA fragility has also been described. In the presence of NAD, topoisomerase I is known to be inactivated by PARP-1 through ADP-ribosylation, whereas in the absence of NAD it is activated by protein–protein interaction with PARP-1 [23–25].

We have previously demonstrated chromosomal fragility both in SSc and in ACA-positive Raynaud's syndrome by cytogenetic studies [26]; these findings were confirmed by micronucleous assay and fluorescent *in situ* hybridization (FISH) analysis [27]. The association between DNA fragility, PARP-1, and topoisomerase I stimulated further investigation into PARP in SSc patients.

In this study we investigated the overall PARP activity in control, SLE, and SSc peripheral blood mononuclear cells (PMC) before and after UV irradiation. The aim was to test the ability of individuals with the above conditions to respond to DNA damage by activating the enzyme, and to ascertain whether NAD cellular content may be influenced by PARP activation.

2. Subjects and methods

All reagents were of the highest quality available; when not indicated, reagents of analytical grade were purchased from Sigma-Aldrich (St Louis, MO).

2.1. Patients

Nineteen consecutive SLE patients and 28 consecutive patients affected by SSc were investigated. SLE patients (18 female and one male, mean [SD] age 34 ± 16 years, mean disease duration 8.5 years) fulfilled American College of Rheumatology criteria (revised in 1981 and updated in 1997) [3,4]. The patients' clinical disease activity, as assessed using the SLE Disease Activity Scale–2000 (SLEDAI-2000), ranged from 2 to 28 [5]. All SLE patients were receiving steroid treatment (mean, 10 mg/day) plus hydroxychloroquine (five patients, 400 mg/day) or cyclophosphamide (three patients, 1 g endovenously/month). SSc patients were defined according to the criteria of LeRoy *et al.* [6]; 11 (all female, mean age 56.1 years ± 22 SD, mean disease duration 8.4 years) were affected by lSSc and 17 (14 females and 3 males, mean age 58.1 years ± 18 SD, mean disease duration 7.0 years) were affected by dSSc. Clinical data were obtained from medical records; blood was drawn at enrolment of patients. None of the above patients has been subjected to photopheresis or UV-A therapy. Ten healthy volunteers (9 females and 1 male, mean age 44 years ± 11 SD) were used as controls. Patients and controls gave informed consent to the study.

2.2. PMC isolation

Blood was drawn by venipuncture using heparin to prevent coagulation. PMC were isolated by Ficoll-Hypaque sedimentation using Lymphoprep (Nyegaard, Oslo, Norway). The isolated cells were harvested and washed three times with Dulbecco's Modified Eagle's Medium (DMEM, Cambrex Bio Science, Verviers, Belgium) supplemented with 10% fetal calf serum (FCS, Gibco BRL Invitrogen, Carlsbad, CA) and antibiotics. Contaminating erythrocytes were removed by hypotonic lysis followed by washing with DMEM. Cells were counted and resuspended in the same medium (1.5×10^6 cells/ml). A 1-ml quantity of PMC suspension was then inoculated in each well of a 48-well plate and kept overnight in a 5% CO_2 atmosphere at 37°C.

2.3. Enzyme activity assay

PARP activity was assayed according to the method of Berger *et al.* [28] with minor modifications. This method, using whole cells instead of nuclei [29] or cell lysate [30], was chosen to better mimic *in vivo* cell behavior. UV light was chosen as the preferred damaging agent because of its well known ability to provoke DNA breakdown, particularly in SLE patients. The wavelength used in this study (UV-C, 250 nm), is far from the UV-A emission (340–400 nm) recently used to perform phototherapy in patients with localized scleroderma, with good results [31].

Cell irradiation duration (referred to here as "irradiation") sufficient to produce breaks in DNA strands and incubation time for the enzyme assay (referred to here as "incubation") were chosen on the basis of five preliminary experiments performed in control cells. PARP activity was detectable even in basal conditions, before UV irradiation, and increased after irradiation. Maximal effect was reached after 20 minutes of irradiation, as shown in Fig. 1A; this

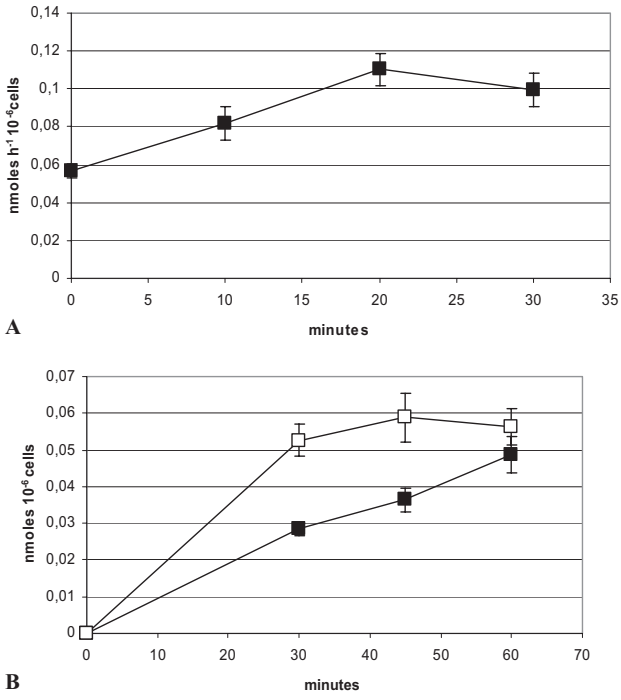

Fig. 1. PARP activity in control PMC before (0 minute) and after different irradiation times (10, 20, and 30 minutes) (A). Amount of incorporated [^{3}H]NAD in control PMC after different incubation times of the assay mixture at 37°C (■ before and □ after 20 minutes of irradiation) (B).