Archives of Biochemistry and Biophysics 635 (2017) 8–16

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Genotoxic effect and antigen binding characteristics of SLE auto-antibodies to peroxynitrite-modified human DNA

Md Asad Khan[a,*], Khursheed Alam[c], Syed Hassan Mehdi[b], M. Moshahid A. Rizvi[b]

[a] Department of Biochemistry, Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India
[b] Department of Biosciences, Jamia Millia Islamia, New Delhi, India
[c] Department of Biochemistry, Jawaharlal Nehru Medical College, A.M.U., Aligarh, India

ARTICLE INFO

Keywords:
Genotoxicity
Human placental DNA
Peroxynitrite
Autoimmunity
Comet assay

ABSTRACT

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease characterized by auto-antibodies against native deoxyribonucleic acid after modification and is one of the reasons for the development of SLE. Here, we have evaluated the structural perturbations in human placental DNA by peroxynitrite using spectroscopy, thermal denaturation and high-performance liquid chromatography (HPLC). Peroxynitrite is a powerful potent bi-functional oxidative/nitrative agent that is produced both endogenously and exogenously. In experimental animals, the peroxynitrite-modified DNA was found to be highly immunogenic. The induced antibodies showed cross-reactions with different types of DNA and nitrogen bases that were modified with peroxynitrite by inhibition ELISA. The antibody activity was inhibited by approximately 89% with its immunogen as the inhibitor. The antigen-antibodies interaction between induced antibodies with peroxynitrite-modified DNA showed retarded mobility as compared to the native form. Furthermore, significantly increased binding was also observed in SLE autoantibodies with peroxynitrite-modified DNA than native form. Moreover, DNA isolated from lymphocyte of SLE patients revealed significant recognition of anti-peroxynitrite-modified DNA immunoglobulin G (IgG). Our data indicates that DNA modified with peroxynitrite presents unique antigenic determinants that may induce autoantibody response in SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem, inflammatory autoimmune disorder of connective tissues with an unknown etiology [1]. It involves both humoral and cell mediated immune response of the innate and acquired immune system and is demonstrated by the presence of auto-antibodies [2] directed against components of cell nucleus, cytoplasm, cell membranes and others [3] in the sera of patients. Autoantibodies against double-stranded DNA (dsDNA) [4], modified self-antigens [5,6], self-proteins that cross-react with native DNA [7,8] and anti-nucleosome antibodies are among the pathogenic antibodies found in SLE patients. It is known that native dsDNA is non-immunogenic [8,9] and several studies have shown that reactive oxygen species (ROS) and reactive nitrogen species (RNS)-modified DNA and polynucleotides are immunogenic in experimental animals and reveal SLE like autoantibody characteristics [8,10].

Generation of nitric oxide *in vivo via* nitric oxide synthase mediated arginine oxidation is one of the common cellular pathways [11,12] and nitric oxide (NO) serves as secondary messenger and mediator for inflammatory response [13]. Frequently, NO combine with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$) leading to DNA damage [14]. Several studies have shown that peroxynitrite reacts with DNA causing nitration, mutation, strand break and structural changes leading to the induction and progression of autoimmune diseases [15,16]. Peroxynitrite induces nitrosative stress, modifies DNA and induces mutations in human lymphoblastoid cell lines [17] suggesting that cytotoxicity and mutagenicity associated with inflammation process may lead to autoimmune diseases [17–20].

In the present study, human placental DNA was damaged by peroxynitrite formed by the experimentally combination of nitric oxide and superoxide anions. Peroxynitrite mediated alterations in DNA were investigated by physicochemical techniques, spectroscopy, melting profile and HPLC. The antibodies induced against peroxynitrite-modified DNA were used as a probe for the diagnosis of neo-epitopes on damaged DNA and circulating antibodies in SLE patients. The induced antibodies were also evaluated for antigenic epitope between and DNA isolated from SLE patients and induced antibodies against peroxynitrite-modified DNA in experimental animals.

* Corresponding author.
E-mail address: asad1amu@gmail.com (M.A. Khan).

http://dx.doi.org/10.1016/j.abb.2017.10.008
Received 3 November 2016; Received in revised form 12 October 2017; Accepted 13 October 2017
Available online 16 October 2017
0003-9861/ © 2017 Elsevier Inc. All rights reserved.

2. Material and methods

2.1. Materials

Human placental DNA, ethidium bromide (EtBr), low melting point agarose (LMPA), methylated bovine serum albumin (mBSA), alkaline phosphatase conjugate, substrate (*p*-nitrophenyl phosphate), complete and incomplete adjuvants, Tween-20, *N*-Methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), and trypan blue dye were purchased from Sigma–Aldrich, USA. Chloroform, NaOH, isoamyl alcohol and EDTA were from purchased from Qualigens, India. ELISA plates were purchased from Nunc, Denmark. All other reagents/chemicals were of the high grade and highest purity available.

2.2. Collection of sera

A sum of 60 patients were characterized and marked as SLE at the rheumatology clinic at the J. N. Medical College and Hospital, A.M.U, Aligarh, India, and were selected for the present study follow the rules of American College of Rheumatology [21]. Informed consent was taken from individual patients/guardian and blood sample was withdrawn in plain vials and then sera were separated [22]. The serum samples from 35 normal and healthy people free from all infectious diseases served as control [23]. The proposed research work was approved by the institutional ethics committee and awarded ethical clearance by J. N. Medical College, Faculty of Medicine, Aligarh Muslim University. Aligarh, India.

2.3. Modification of human placental DNA by peroxynitrite

Peroxynitrite was prepared by rapid quenched flow method from sodium nitrite and acidified hydrogen peroxide and stored in 1.2 M NaOH at −20 °C [24]. Concentration of stored peroxynitrite was determined by recording absorbance at 302 nm using molar extinction coefficient of 1670 M^{-1} cm^{-1}. Human placental DNA was purified as described earlier [25] and the purity of DNA was confirmed by A260/A280 ratio. Human placental DNA (25 μg/ml) was modified with increasing concentration of peroxynitrite (100 μM, 200 μM and 400 μM) at 37 °C for 30 min in 0.01 M sodium phosphate buffer, pH 7.4 containing 150 mM sodium chloride and 100 μM of DTPA (metal ion chelator). The samples were dialyzed against PBS, pH 7.4.

2.3.1. Spectroscopic and fluorescence spectroscopy analysis

The spectral profile of native human placental human DNA and different concentration of peroxynitrite-modified DNA were estimated by a spectrophotometer (Shimadzu UV-1700) and spectrofluorophotometer (Shimadzu RF-5301-PC). The excitation wavelength of sample at 525 nm, using external chromophore ethidium bromide (2.5 μg/ml). Percent decrease in fluorescence intensity was calculated using the following formula [26];

$$\%\ \text{loss in fluorescence intensity} = \frac{\text{Emission of native DNA} - \text{Emission of peroxynitrite modified DNA}}{\text{Emission of native DNA}} \text{x}100$$

2.3.2. Melting temperature profile

The profile of melting temperature of unmodified and peroxynitrite-modified DNA was described previously [27]. % denaturation was computed by following equation:

$$\%\ \text{denaturation} = \frac{A_T - A_{30}}{A_{max} - A_{30}} \times 100$$

2.3.3. Measurement of 8-nitroguanine in peroxynitrite-modified DNA by HPLC

Quantitation of 8-nitroguanine was determined by HPLC (BioRad, USA) as described earlier [28] with slight modifications. Briefly, 25 μg/ml of native and 400 μM peroxynitrite-modified DNA were heated in 0.1 N HCl at 100 °C for 35 min and dried under vacuum. The release of free bases were re-suspended in 150 μl of 100 mM Tris–HCl buffer, pH 8.5 and filtered by 0.42 μM membrane. The flow rate of eluent buffer was maintained at 1 ml/min. The peak of 8-nitroguanine was identical to the peak at a retention time of true 8-nitroguanine. The value of 8-nitroguanine in the modified DNA was calculated from the standard curve of known concentrations of standard 8-nitroguanine [28].

2.4. Isolation of DNA from human lymphocytes of SLE patients

Blood samples were collected from both SLE patients and normal subject [29]. The DNA was purified from these samples by using the Qiagen guidelines (Qiagen, USA) [30]. Heparinized blood samples (5 ml) from healthy subjects were taken by venipuncture and suitably diluted in phosphate buffered saline (PBS) free from metal ions (pH 7.4).

2.4.1. Comet assay of peroxynitrite modified lymphocytes DNA

Lymphocytes were isolated from blood using histopaque 1077 and the cells were suspended in media RPMI 1640 [31]. The viability of cells was checked by trypan blue exclusion assay [32,33] and was found to be more than 90%. Lymphocytes (1×10^5 cells) were exposed with increasing concentration of peroxynitrite (200 μM and 400 μM) respectively and untreated cells serve as control. The sample was centrifuged at 5000 rpm and then pelleted of lymphocytes were re-suspended in 150 ml of PBS and continued for comet assay as described previously [34].

2.5. Immunization of peroxynitrite modified and unmodified DNA

The modified and unmodified DNA was immunized in female rabbits as described earlier [35]. Briefly, four experimental rabbits (two each for modified and unmodified DNA) were immunized intramuscularly mixed with 1:1 ratio (w/w) of 50 μg antigen and ethylated BSA emulsified with Freund's complete adjuvant (an equal volume) at multiple sites. The Freund's incomplete adjuvant was injected as a booster at seven days intervals up to two months of antigens with same amount. The Institutional ethics committee considered the proposal and did not find any objectionable/un-ethical *vis-a-vis* animal subjects in this research work. So the ethical clearance was awarded by Central animal house, J.N Medical College, Faculty of Medicine, A.M.U., Aligarh, India.

2.6. Purification of IgG

Immunoglobulin G (IgG) was purified from sera of rabbits and SLE patients by affinity chromatography as described previously [36].

2.7. Enzymes linked immunosorbent assay

2.7.1. Inhibition ELISA

ELISA was carried for the diagnosis of autoantibodies against unmodified and modified DNA as described previously [37]. Briefly, the wells were coated with 100 μl (2.5 μg/ml) of the peroxynitrite modified human placental DNA for 2 h at room temperature and overnight at 4 °C followed by extensive washing with TBS-T. Unoccupied sites were blocked with 150 μl of 1.5% BSA in TBS for 4 h at room temperature. After incubation the plates were washed four times with TBS-T. The test serum, serially diluted in TBS, was coated onto the plate (100μl/well) and adsorbed for 2 h at 37 °C and over night at 4 °C. Bound antibodies were assayed with anti-human IgG alkaline phosphatase conjugate,

Download English Version:

https://daneshyari.com/en/article/8288902

Download Persian Version:

https://daneshyari.com/article/8288902

Daneshyari.com