ELSEVIER

Cardiovascular Pathology 20 (2011) 195–203

CARDIOVASCULAR PATHOLOGY

Original Article

iNOS induction and PARP-1 activation in human atherosclerotic lesions: an immunohistochemical and ultrastructural approach

Ida Perrotta[a,*], Elvira Brunelli[a], Alfonso Sciangula[b], Francesco Conforti[c], Enrico Perrotta[a], Sandro Tripepi[a], Giuseppe Donato[c], Mauro Cassese[b]

[a]*Department of Ecology, University of Calabria, Rende, Cosenza, Italy*
[b]*Department of Cardiothoracic Surgery and Cardiology, Sant'Anna Hospital, Catanzaro, Italy*
[c]*Department of Pathology, School of Medicine, University Magna Graecia, Catanzaro, Italy*

Received 7 August 2009; received in revised form 2 October 2009; accepted 2 June 2010

Abstract

Background: Several lines of clinical and experimental evidence have demonstrated that reactive oxygen species and nitrogen species are generated in unregulated amounts during diverse cardiovascular disorders. It has been previously reported by our group and others that augmented expression of nitric oxide synthase isoforms is associated with human atherogenesis and that the activity of the enzymes in an atherosclerotic environment may promote the formation of peroxynitrite. Among the downstream mechanisms triggered by oxidants, poly (ADP-ribose) polymerase-1 activation has recently been implicated in the pathogenesis of acute and chronic myocardial dysfunction, diabetes, hypertension, aging, and various forms of shock. **Methods:** Based on these observations, we performed immunohistochemical and immunogold labeling analyses to evaluate the expression profile and the subcellular localization of inducible nitric oxide synthase and poly (ADP-ribose) polymerase-1 in healthy and atherosclerotic human aortae. **Results:** We have demonstrated that inducible nitric oxide synthase colocalizes with poly(ADP-ribose) polymerase-1 within vascular cells of atherosclerotic human aortae. We have reported for the first time, to our knowledge, the ultrastructural localization of poly(ADP-ribose) polymerase-1 within the nuclei of lesional smooth muscle cells. Finally, we have evidenced that poly(ADP-ribose) polymerase-1 induction within cells of the diseased aorta strongly correlates with alterations in mitochondrial morphology. **Conclusions:** Our data imply the possibility of a significant role for cross-talk between inducible nitric oxide synthase and poly(ADP-ribose) polymerase-1 in human atherosclerotic lesions. We conclude that the prooxidant milieu of the plaque might exert damaging effects on mitochondria via a poly(ADP-ribose) polymerase-1-mediated mechanism since the absence of the enzyme results in a corresponding lack of changes in mitochondrial morphology. The present report may open avenues for further researches that could have important therapeutic consequences for the treatment of atherosclerosis and its clinical sequelae. © 2011 Elsevier Inc. All rights reserved.

Keywords: Atherosclerosis; iNOS; PARP-1; Oxidative stress; Mitochondrial damage

1. Introduction

Nitric oxide (NO) is a biological messenger molecule synthesized by the catalytic action of a small family of enzymes called nitric oxide synthases (NOS): neuronal NOS (nNOS or bNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). NO is an important regulator of cardiovascular homeostasis, but it may also play an important role in the pathogenesis of several disease states [1]. It is well established that dysregulation of the NO system occurs in atherosclerosis and hypercholesterolemia [2,3]. The putative mechanisms underlying this abnormality have been the subject of numerous investigations. Recent studies have pointed out the possibility that altered NOS expression or function may drastically decrease NO bioavailability, increase oxidative stress, and impair vascular function. Up-regulation of iNOS expression has been described in

Financial support: The authors declare that no funding was available for this work.

* Corresponding author. Department of Ecology, University of Calabria, Arcavacata di Rende (Cosenza) 87036, Italy. Tel.: +39 0984 492976, +39 3471734173; fax: +39 0984 492986.

E-mail address: idaperrotta@yahoo.it (I. Perrotta).

1054-8807/10/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.carpath.2010.06.002

hypercholesterolemic animal models [4], as well as in human atherosclerotic lesions [5]. Although the specific effects of iNOS on the vascular wall still remain uncertain, one pathway of iNOS-mediated cell damage might be via activation of the enzyme poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 is a highly conserved DNA binding protein that plays a pivotal role in regulating genome integrity, chromatin remodeling, and cell survival [6,7]. In response to oxidative DNA damage, PARP-1 becomes activated and facilitates repair processes through modulation of protein–protein and protein–DNA interactions.

It has been shown that iNOS expression is required for oxidative DNA damage and PARP-1 activation during allergen-induced eosinophilia [8]. The functional synergy between iNOS and PARP-1 has also been demonstrated in an experimental model of focal cerebral ischemia [9]. There is substantial evidence that elevated levels of PARP-1 are present in human atherosclerotic plaques and rat carotid arteries after balloon injury [10,11]. Moreover, it has been proved that pharmacological inhibition of PARP-1 may interfere with atherosclerotic events by promoting factors of plaque stability [12].

Here we have used immunohistochemistry and immunoelectron microscopy to evaluate the precise ultrastructural distribution of iNOS and PARP-1 in atherosclerotic human aortae and to determine whether any relationship may exist between these enzymes and cellular damage. Our experimental data show that iNOS and PARP-1 are strongly activated within human atheroma. We also demonstrate that PARP-1 expression clearly correlates with the appearance of mitochondrial alterations.

These observations led us to propose that a potential link between iNOS and PARP-1 may exist in human atherosclerotic plaques and that this reciprocal interaction may participate in cell injury and ultimately in tissue damage via a complex interplay between the nucleus and mitochondria.

2. Materials and methods

2.1. Study materials

Surgical specimens of human ascending aortae were obtained from 12 male and 8 female (n=20) nondiabetic patients (mean age, 58.1±13.7 years) who had been operated on for aortic atherosclerotic aneurysm at the Sant'Anna Hospital (Catanzaro, Calabria, Italy) between the years 2007 and 2009. None of these patients presented other cardiovascular complications. Control specimens (n=9; mean age, 66.6±6.7 years) were obtained from ethnicity- and age-matched healthy subjects (seven male and two female subjects) at autopsy. All autopsies were performed no later than 24 h after death. Control subjects died of diseases not related to atherosclerosis and had no history or pathomorphological evidence of hypertension, diabetes, or hypercholesterolemia. Four samples for each specimen were prepared and individually submitted for routine histological processing, immunohistochemical evaluation, and ultrastructural evaluation.

2.2. Light microscopy

For light microscopy, specimens of healthy and atherosclerotic aortae were fixed by immersion in Bouin solution and embedded in paraffin. Four-micrometer transverse sections were stained with hematoxylin–eosin, observed, and photographed with a light microscope (LEITZ Dialux EB20).

2.3. Immunofluorescence double-labeling assay

Serial sections adjacent to those used for light microscopy were analyzed by immunohistochemical staining.

Bouin-fixed samples were dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax. Four-micrometer transverse sections were cut and mounted on positively charged slides.

Immunofluorescence staining was carried out using an indirect immunofluorescence technique [13]. All tissue-fixed sections were deparaffinized, cleared, and hydrated to phosphate-buffered saline (PBS; pH 7.4) using a descending series of ethanol. After the sections had been washed with PBS, they were incubated with normal goat serum (Sigma-Aldrich, Milan, Italy) diluted 1:50 in PBS in a moist chamber at 4°C for 10 min. Unwashed sections were then incubated overnight at 4°C with a mixture of two primary antibodies: mouse monoclonal antibody to iNOS (Sigma-Aldrich) and rabbit monoclonal antibody to PARP-1 (Santa Cruz Biotechnology) in PBS in appropriate dilution (1:100). This was followed by washes with PBS. Subsequently, the sections were incubated with a mixture of two fluorescein-conjugated secondary antibodies—fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin (1:50; Sigma-Aldrich) and tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse immunoglobulin (1:50; Sigma-Aldrich)—for 30 min at room temperature in a humidifying chamber.

To check the specificity of immunolabeling (negative control), we substituted the primary antiserum with nonimmune serum at a 1:100 dilution in PBS in control sections. Specimens were examined with a Leica TCS SP2 Confocal Laser 22 Scanning Microscope.

2.4. Electron microscopy

For routine transmission electron microscopy, samples were fixed in 3% glutaraldehyde solution in 0.1 M phosphate buffer (pH. 7.4) for 2 h at 4°C, postfixed in osmium tetroxide (3%) for 2 h, dehydrated in graded acetone, and embedded in Araldite (Fluka, Buchs, Switzerland). Ultrathin sections were contrasted using both

Download English Version:

https://daneshyari.com/en/article/2898807

Download Persian Version:

https://daneshyari.com/article/2898807

Daneshyari.com