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Original Contribution

Role of poly(ADP-ribose) glycohydrolase in the development of inflammatory bowel disease in mice

Salvatore Cuzzocrea [a,b,*], Emanuela Mazzon [a,b], Tiziana Genovese [a], Concetta Crisafulli [a], Woo-Kee Min [c], Rosanna Di Paola [a], Carmelo Muià [a], Jia-He Li [d], Giuseppe Malleo [a], Weizhen Xu [a], Edmond Massuda [e], Emanuela Esposito [f], Jie Zhang [d], Zhao-Qi Wang [c]

[a] *Department of Clinical and Experimental Medicine and Pharmacology, Torre Biologica, Policlinico Universitario, 98123 Messina, Italy*
[b] *IRCCS Centro Neurolesi "Bonino-Pulejo," Messina, Italy*
[c] *International Agency for Research on Cancer, 69008 Lyon, France*
[d] *Guilford Pharmaceuticals Inc., Baltimore, MD 21224, USA*
[e] *Lilium Pharmaceuticals, Cockeysville, MD 21030, USA*
[f] *Department of Experimental Pharmacology, University of Naples "Federico II," Via D. Montesano 49, 80131 Napoli, Italy*

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Abstract

Poly(ADP-ribose) is synthesized from nicotinamide adenine dinucleotide (NAD) by poly(ADP-ribose) polymerase 1 (PARP-1) and degraded by poly(ADP-ribose) glycohydrolase (PARG). The aim of the present study was to examine the role of PARG in the development of experimental colitis. To address this question, we used an experimental model of colitis, induced by dinitrobenzene sulfonic acid (DNBS). Mice lacking the functional 110-kDa isoform of PARG ($PARG_{110}$KO mice) were resistant to colon injury induced by DNBS. The mucosa of colon tissues showed reduction of myeloperoxidase activity and attenuated staining for intercellular adhesion molecule 1 and vascular cell adhesion molecule 1. Moreover, overproduction of proinflammatory factors TNF-α and IL-1β and activation of cell death signaling pathway, i.e., the FAS ligand, were inhibited in these mutant mice. Finally pharmacological treatment of WT mice with GPI 16552 and 18214, two novel PARG inhibitors, showed a significant protective effect in DNBS-induced colitis. These genetic and pharmacological studies demonstrate that PARG modulates the inflammatory response and tissue injury events associated with colitis and PARG may be considered as a novel target for pharmacological intervention for the pathogenesis.

Keywords: Colitis; PARG inhibitor; PARG110 deletion; Inflammation; TNF-α; IL-1β; Apoptosis

Introduction

The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, have become important health problems in recent years. With an actual prevalence of 200–500 per 100,000 people in Western countries and an incidence of about 20 per 100,000 people, the occurrence of these pathologies in high incidence areas almost doubles every 10 years. Indeed, these are diseases of a lifetime, affecting people at a young age. In the last decade a shift toward Crohn's disease in areas with high incidence of IBD has been observed, and IBD has tended to occur at all ages. While this disease has a worldwide distribution, its pathogenesis is not clearly understood [1].

Abbreviations: DNBS, dinitrobenzene sulfonic acid; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule; IL-6, interleukin 6; MPO, myeloperoxidase; PAR, poly (ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; PARP-1, poly(ADP-ribose) polymerase 1; PBS, phosphate-buffered saline; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α.

* Corresponding author. Institute of Pharmacology, School of Medicine, University of Messina, Torre Biologica, Policlinico Universitario Via C. Valeria, Gazzi, 98100 Messina, Italy. Fax: +39 090 694951.
E-mail address: salvator@unime.it (S. Cuzzocrea).

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Several experimental animal models of inflammatory bowel diseases have been developed to define the different components of the pathophysiological processes that characterize these disorders [2–5]. Among these models, the intrarectal administration of 2,4-dinitrobenzene sulfonic acid (DNBS) has been extensively used to study the mechanisms of colonic inflammation and to test anti-inflammatory drugs [6,7]. Infections, trauma, or chemical insults are believed to induce several cellular reactions, which eventually lead to an inflammatory status of the colon. At the same time, however, protective mechanisms aimed at preventing the pathological outcome of proinflammatory insults are also induced. Hence, the overall balance between pro- and anti-inflammatory mechanisms is likely to determine the progression and severity of colitis.

The biological action of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) is the main factor in the pathogenesis of Crohn's disease, and the regulation of this process is very important in controlling the disease [8]. In addition, recruitment of inflammatory cells from the circulation is an important process to augment inflammatory response [9]. TNF-α and IL-6 induce the expression of adhesion molecules in the vascular endothelium, and invasion of inflammatory cells into mucosal layer subsequently occurs [10]. Moreover, a growing body of data indicates that oxygen-derived free radicals such as superoxide ($O_2^{-\bullet}$), nitric oxide ($NO^{\bullet}$), and hydroxyl radicals ($OH^{\bullet}$) have a role in mediating intestinal damage in inflammatory bowel disease [11,12]. The proinflammatory roles of reactive oxygen species (ROS) are well known ranging from recruitment of neutrophils at sites of inflammation, formation of chemotactic factors, depolymerization of hyaluronic acid and collagen, and lipid peroxidation to the release of cytokines such as TNF-α and interleukin-1β (IL-1β) [13]. Moreover, ROS can cause DNA damage resulting in the activation of the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) and poly (ADP-ribose) synthase-driven cell death.

PARP-1 is a member of the PARP enzyme family consisting of PARP-1 and several recently identified novel poly(ADP-ribosylating) enzymes. PARP-1 is an abundant nuclear protein functioning as a DNA nick-sensor enzyme. On binding of DNA, activated PARP cleaves NAD^+ into nicotinamide and ADP-ribose and polymerizes the latter onto nuclear acceptor proteins including histones, transcription factors, and PARP itself. Despite its function in DNA repair, overactivation of PARP has long been recognized to induce cell death under some conditions [14]. PARP inhibitors and PARP-1 gene disruption can reduce cell death resulting from oxidative stress [15], radiation [16], nitric oxide, peroxynitrite [17], and other agents that damage DNA [17,18]. Poly(ADP-ribose) glycohydrolase (PARG, EC 3.2.1.143) is responsible for the degradation of poly (ADP-ribose) (PAR) polymer and causes depletion of NAD when the pathway is overactivated. Within minutes after its synthesis by PARP-1, the PAR is hydrolyzed by PARG to ADP-ribose [19]. The biochemical action of PARG has been proposed to include three steps: (i) endoglycosidic cleavage; (ii) endo plus exoglycosidic cleavage, progressive degradation; (iii) exoglycosidic, distributive degradation. PARG cleaves the ribose–ribose bonds of linear and branched portion of polymer, specifically the glycosidic linkages of PAR. The final products of the reaction are mono-ADP-ribosyl protein and ADP-ribose. ADP-ribose is known to be a weak PARG inhibitor with an IC50 of 0.1 mM [20].

Because PARP-1 is inhibited by extensive poly(ADP-ribosyl)ation, PARG inhibitors could thereby indirectly inhibit PARP-1 activity. Prior work has shown that the PARG inhibitor gallotannin can markedly reduce death of astrocytes after oxidative stress [21]. We have developed several families of small-molecule PARG inhibitors with improved potency [22]. One such novel PARG inhibitor, *N*-bis-(3-phenyl-propyl)9-oxo-fluorene-2,7-diamide (GPI 16552), a nontannin small molecule that is related to the tilorone family of PARG inhibitors, reduces infarct volume in an in vivo model of brain ischemia/reperfusion injury [23]. More recently we have demonstrated that GPI 18214, another novel PARG inhibitor, exerts a protective effect against organ injury associated with nonseptic shock [24]. The IC50 for GPI 16552 and 18214 are 1.7 and 4.2 μM, respectively [25]. These pharmacological studies demonstrate that PAR polymer degradation may be an important step, at which cell death may be regulated through PARG. However, genetic studies have not validated this hypothesis. Recently, Cortes and colleagues [26] have generated mutant mice, by a deletion of the 110-kDa isoform of the PARG protein ($PARG_{110}KO$ mice), which are viable and fertile. It has been clearly demonstrated that inflammatory processes associated with ischemia and reperfusion (kidney and intestine) are significantly reduced in these $PARG_{110}KO$ [27,28]. Thus, $PARG_{110}KO$ mice represent a useful system for studying the function of PARG in a rodent model of diseases and validate the pathways that may be targets for pharmaceutical application/intervention.

This study investigates the role of PARG activation in the pathogenesis of experimental colitis. In particular, we have determined the following endpoints of the inflammatory response: (i) the degree of colonic injury, (ii) the rise in myeloperoxidase (MPO) activity (mucosa), (iii) the production of TNF-α and IL-1β (colon levels), (iv) the increase of staining (immunohistochemistry) for FAS ligand, as well as (v) the increased expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) caused by DNBS in the colon.

Methods

Animals

Wild-type mice and mice with a targeted disruption of the PARG [26] (8–10 weeks old, 20–22 g) in 129/Sv/Ola background were kept in the pathogen-free facility of the International Agency for Research on Cancer, Lyon. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) and with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).