ELSEVIER

European Journal of Pharmacology 415 (2001) 85–94

ejp

www.elsevier.nl/locate/ejphar

GPI 6150, a poly (ADP-ribose) polymerase inhibitor, exhibits an anti-inflammatory effect in rat models of inflammation

Emanuela Mazzon [a], Ivana Serraino [b], Jia-He Li [c], Laura Dugo [b], Achille P. Caputi [b], Jie Zhang [c,1], Salvatore Cuzzocrea [b,*]

[a] *Department of Biomorphology, School of Medicine, University of Messina, Messina Italy*
[b] *Institute of Pharmacology, School of Medicine, University of Messina, Torre Biologica-Policlinico Universitario, Via C. Valeria-Gazzi, 98100 Messina Italy*
[c] *Guilford Pharmaceuticals Inc., Baltimore, MD 21224 USA*

Received 30 November 2000; received in revised form 24 January 2001; accepted 30 January 2001

Abstract

Poly (ADP-ribose) polymerase, a nuclear enzyme activated by DNA strand breaks, has been show to play an important role in the pathogenesis of inflammation. Here, we investigate the effects of GPI 6150 (1,11*b*-dihydro-[2*H*]benzopyrano [4,3,2-*de*]isoquinolin-3-one), a new poly (ADP-ribose) polymerase inhibitor, in animal models of acute and chronic inflammation (carrageenan-induced paw edema, adjuvant-induced arthritis and zymosan-induced multiple organ failure) where oxygen radicals, nitric oxide and peroxynitrite are known to play a crucial role in the inflammatory process. The results show that the poly (ADP-ribose) polymerase inhibitor GPI 6150 inhibits the inflammatory response (paw swelling, and organ injury). The present results demonstrate that inhibition of poly (ADP-ribose) polymerase by GPI 6150 exerts potent anti-inflammatory effects. Part of these anti-inflammatory effects may be related to a reduction of neutrophil recruitment into the inflammatory site. © 2001 Published by Elsevier Science B.V.

Keywords: ADP-ribose; Polymerase; Anti-inflammatory

1. Introduction

In vitro studies have demonstrated that DNA strand breakage, which results from various forms of oxidant injury, triggers the activation of the nuclear enzyme poly (ADP-ribose) polymerase, which in turn, can result in energy depletion and cellular injury (Cochrane, 1991; Szabó et al., 1996a,b). Main triggers of DNA single strand breakage and poly (ADP-ribose) polymerase activation include hydrogen peroxide and hydroxyl radical (Cochrane, 1991) and NO or peroxynitrite (Zhang et al., 1994; Szabó et al., 1996a).

The role of oxyradical formation in various forms of inflammation is well-established. Recent data demonstrate that the expression of the inducible isoform of nitric oxide (NO) synthase also plays important pathologic roles in various forms of inflammation (Clancy and Abranson, 1995; Szabó, 1995; Pfeilschifter et al., 1996). NO and superoxide react to form peroxynitrite, a cytotoxic oxidant species (Beckman et al., 1990), which mediates an important part of the cytotoxic effect of peroxynitrite during shock and inflammation (Crow and Beckman, 1995; Beckman and Koppenol, 1996; Szabó, 1996a).

Weak poly (ADP-ribose) polymerase inhibitors, such as nicotinamide and 3-aminobenzomide has been found to abate inflammation in perochromate or carrageenan-induced arthritis in rodents (Szabó et al., 1998; Bowes et al., 1999). We have synthesized a novel poly (ADP-ribose) polymerase inhibitor, GPI 6150, 1,11*b*-dihydro-[2*H*]benzopyrano[4,3,2-*de*]isoquinolin-3-one, which has been shown to be protective in rat models of cerebral focal ischemia, rat lateral fluid percussion model of traumatic brain injury, rat model of regional heart ischemia, and

Abbreviations: ATP, adenosine triphosphate; NAD, nicotinamide adenine dinucleotide; NO, nitric oxide; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species

* Corresponding author. Tel.: +39-90-221-3644; fax: +39-90-694-951.
E-mail addresses: Zhang_J@guilfordpharm.com (J. Zhang), salvator@www.unime it (S. Cuzzocrea).
[1] Also a corresponding author.

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00809-3

mouse models of septic shock, streptozotocin-induced diabetes and MPTP-neurotoxicity (Li et al., 1999; Lu et al., 2000; Zhang et al., 1998, 2000; LaPlaca et al., 1999; Walles et al., 1998). The present work was designed to elucidate whether a novel poly (ADP-ribose) polymerase inhibitor, GPI 6150, could affect the course of the inflammatory response in carrageenan-induced paw edema, adjuvant-induced arthritis, and zymosan-induced multiple organ failure. In these experimental models, previous work has demonstrated the anti-inflammatory potential of various therapeutic approaches aimed at the scavenging of oxyradicals and at inhibition of NO synthesis and peroxynitrite formation (Tracey et al., 1995; Salvemini et al., 1996a,b; Cuzzocrea et al., 1997a). The results confirm that the poly (ADP-ribose) polymerase inhibitor displays potent anti-inflammatory effect, which was initially observed in these models with weak and less selective poly (ADP-ribose) polymerase inhibitors (Szabó et al., 1998; Cuzzocrea et al., 1998).

2. Materials and methods

2.1. Rat model of adjuvant arthritis

A group of five Long Evans-derived, male or female rats, weighing 150 ± 20 g were used. A well-ground suspension of killed *Mycobacterium tuberculosis* (0.3 mg in 0.1 ml of light mineral oil; Complete Freund's Adjuvant, CFA) was administered into the subplantar region of the right hind paw on day 1. Hind paw volumes were measured by water displacement on days 0, 1, 5, 14, and 18; rats were weighed on days 0 and 18. GPI 6150 was dissolved in 100% dimethyl sulfoxide (DMSO) and administered i.p. (50 mg/kg) on 5 consecutive days, beginning 1 h before injection of complete Freund's adjuvant. For complete Freund's adjuvant-injected vehicle control rats, the increase in paw volume on day 5 relative to day 1 (acute phase of inflammation) was generally between 0.7 and 0.9 ml; those on day 18, relative to day 14 (delayed phase of inflammation) was generally between 0.2 and 0.4 ml. Thus, anti-inflammatory activity of this model may be denoted by values calculated during the acute phase as well as the delayed phase. Animals were also weighed on days 0 and 18; complete Freund's adjuvant-injected vehicle control animals generally gain between 40 and 60 g body weight over this time period.

2.2. Rat model of carrageenan-induced inflammation

A group of five male Long Evans-derived, overnight-fasted rats, weighing 150 ± 20 g were employed. Three doses (5, 20, 60 mg/kg) of GPI 6150 were administered i.p., the control group of three animals received 100% DMSO alone. The animals were injected with carrageenan (0.1 ml of 1% suspension intraplantar) to the right hind paw 1 h after test compound administration. Hind paw edema, as a measure of inflammation, was recorded 3 h after carrageenan administration using a plethysmometer with water cell (25-mm diameter).

2.3. Zymosan-induced MOF

Animals were randomly divided into four groups ($n = 10$ for each group). The first group was treated with saline solution (0.9% NaCl) intraperitoneally (i.p.) and served as a sham group. The second group was treated with zymosan (500 mg/kg, suspended in saline solution, i.p.). In the

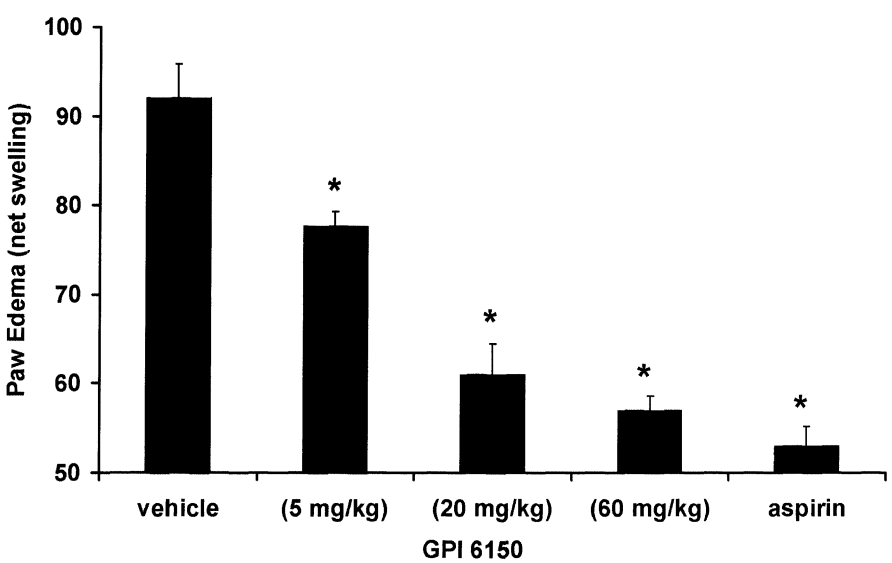

Fig. 1. GPI 6150 dose-responsively reduces paw edema elicited by carrageenan. The net swelling of the paw edema is expressed by the difference between the left (control) and right (carrageenan-injected) as measured by a plethysmometer. Data are means ± SEM of 10 rats for each group. $^{*}P < 0.01$ vs. vehicle.

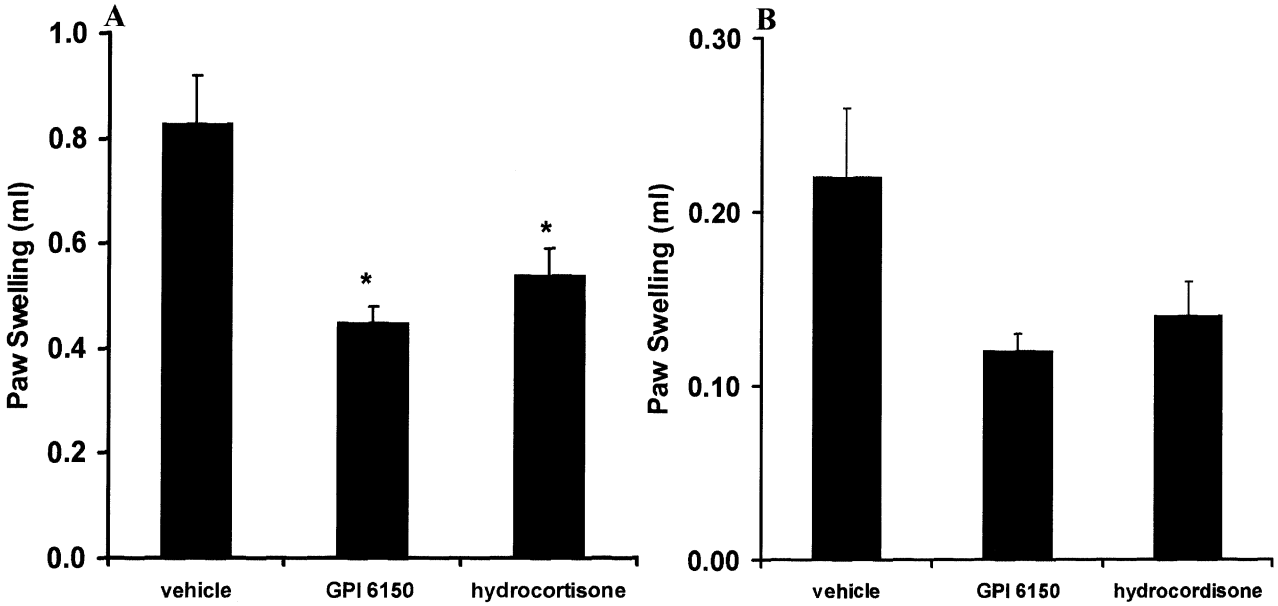

Fig. 2. GPI 6150 reduces acute (A) and delayed (B) phases of adjuvant-induced paw edema in rats. Data are means ± SEM of 10 rats for each group. * $P < 0.01$ vs. vehicle.

third and fourth groups, rats received a new poly (ADP-ribose) synthase inhibitor (40 mg/kg, intraperitoneally), 1 and 6 h after zymosan or saline administration, respectively. After zymosan or saline injection, animals were monitored for evaluation of systemic toxicity (conjunctivitis, ruffled fur, diarrhea and lethargy), loss of body weight and mortality for 72 h. These evaluations were performed in a blinded fashion.

2.4. Acute peritonitis assessment

Eighteen hours after zymosan injection, animals were sacrificed under ether anesthesia in order to evaluate the development of acute inflammation in the peritoneum. Through an incision in the linea alba, 3 ml of phosphate buffer saline (composition, in mM: NaCl 137, KCl 2.7, NaH_2PO_4 1.4, Na_2HPO_4 4.3; at pH 7.4) were injected into the abdominal cavity. Washing buffer was removed by a plastic pipette and was transferred into 10-ml centrifuge tubes. The total volume of peritoneal exudate were evaluated, removing 3 ml of the buffer. Peritoneal exudate were centrifuged at $7000 \times g$ for 10 min at room temperature and the supernatant was utilized for $NO_2^- + NO_3^-$ (NO_x) assay. Cells were suspended in phosphate buffer saline and counted with optical microscope by Burker's chamber after vital Trypan Blue stain.

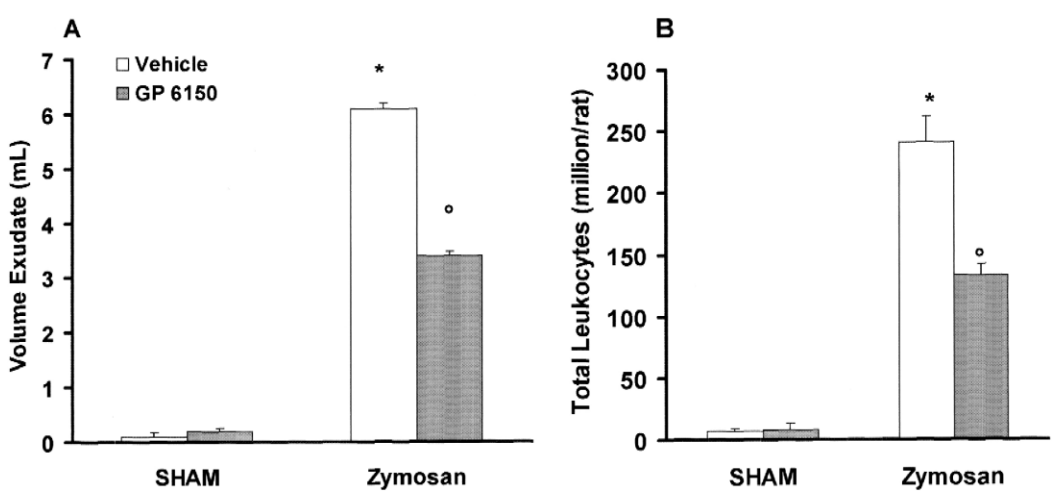

Fig. 3. Volume exudate (A) and leukocyte accumulation (B) in peritoneal cavity at 18 h after zymosan injection. GPI 6150 treatment induced a significant decrease of peritoneal exudate and leukocyte migration. Data are means ± SEM of 10 rats for each group. * $P < 0.01$ vs. sham. ° $P < 0.01$ vs. zymosan.

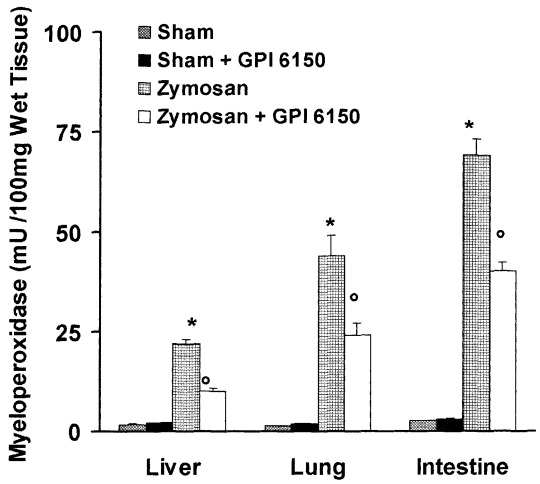

Fig. 4. MPO activity was significantly increased in zymosan-treated rats in comparison to sham rats ($P < 0.01$). GPI 6150 treatment prevented myeloperoxidase activity increase. Data are means ± SEM of 10 rats for each group. $^{*}P < 0.01$ vs. sham; $^{\circ}P < 0.01$ vs. zymosan.

2.5. Metabolic changes

Plasma samples were taken 18 h after zymosan injection and levels of bilirubine, lactate dehydrogenase, (LDH), phosphatase alcaline and alanine aminotransferase were determined by a clinical laboratory.

2.6. Myeloperoxidase activity

The usefulness of measuring myeloperoxidase (MPO) activity to assess neutrophil infiltration has been previously reported (Mullane et al., 1988). Briefly, after weighing, segments of various organs were suspended in 0.5% hexadecyltrimethylammonium bromide (pH 6.5, 50 mg of tissue per ml) and then homogenized. After freezing and thawing the homogenate three times, the tissue levels of myeloperoxidase were determined by utilizing 0.0005% hydrogen peroxide as a substrate for the enzyme. One unit of myeloperoxidase activity is defined as that degrading 1 μmol of peroxide per minute at 25°C and is expressed in units per gram weight (U/g) of wet tissue.

2.7. Light microscopy

Lung and small intestine samples were taken 18 h after zymosan injection. The tissue slices were fixed in Dietric solution (14.25% ethanol, 1.85% formaldehyde, and 1% acetic acid) for 1 week at room temperature, dehydrated by

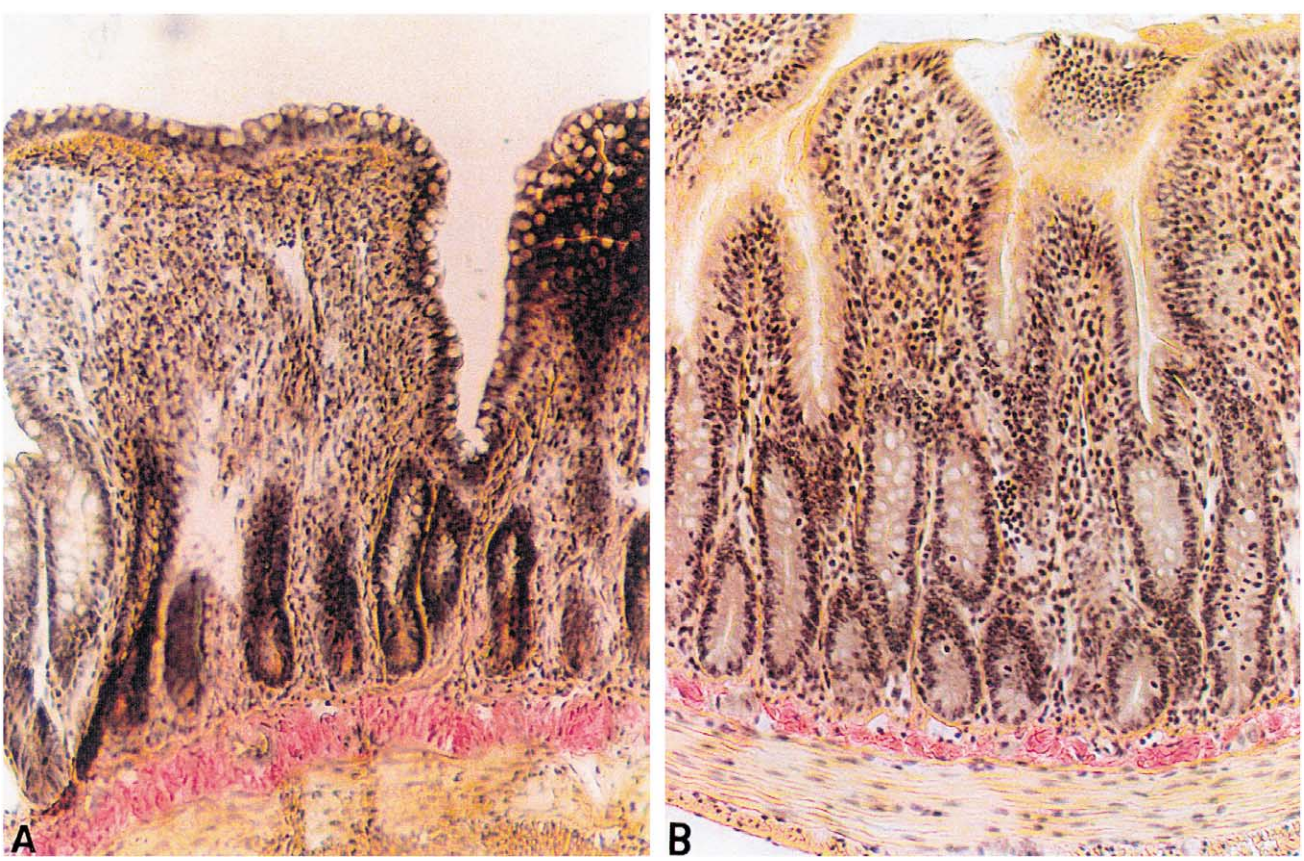

Fig. 5. Representative lung sections from zymosan-treated rat (A) demonstrating necrosis, interstitial haemorrhage, neutrophil and macrophage accumulation. Lung section from a zymosan-treated rat that received GPI 6150 (B) demonstrating disappearance of necrosis and a lesser cellular infiltration. Original magnification: ×62.5.

graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, NJ). Sections (thickness, 7 μm) were deparaffinized with xylene, stained with trichromic Van Gieson and observed in a Dialux 22 Leitz microscope.

2.8. Data analysis

All values in the figures and text are expressed as means ± standard error of the mean of n observations, where n represents the number of animals studied. Data sets were examined by one- and two-way analysis of variance and individual group means will then be compared with Student's unpaired t-test. Statistical analysis for survival data (non-parametric data) will be calculated by Fisher's exact test. A P-value < 0.05 is considered significant.

2.9. Materials

Biotin blocking kit, biotin-conjugated goat anti-rabbit IgG, primary anti-nitrotyrosine antibody and avidin–biotin peroxidase complex were obtained from DBA, Milan,

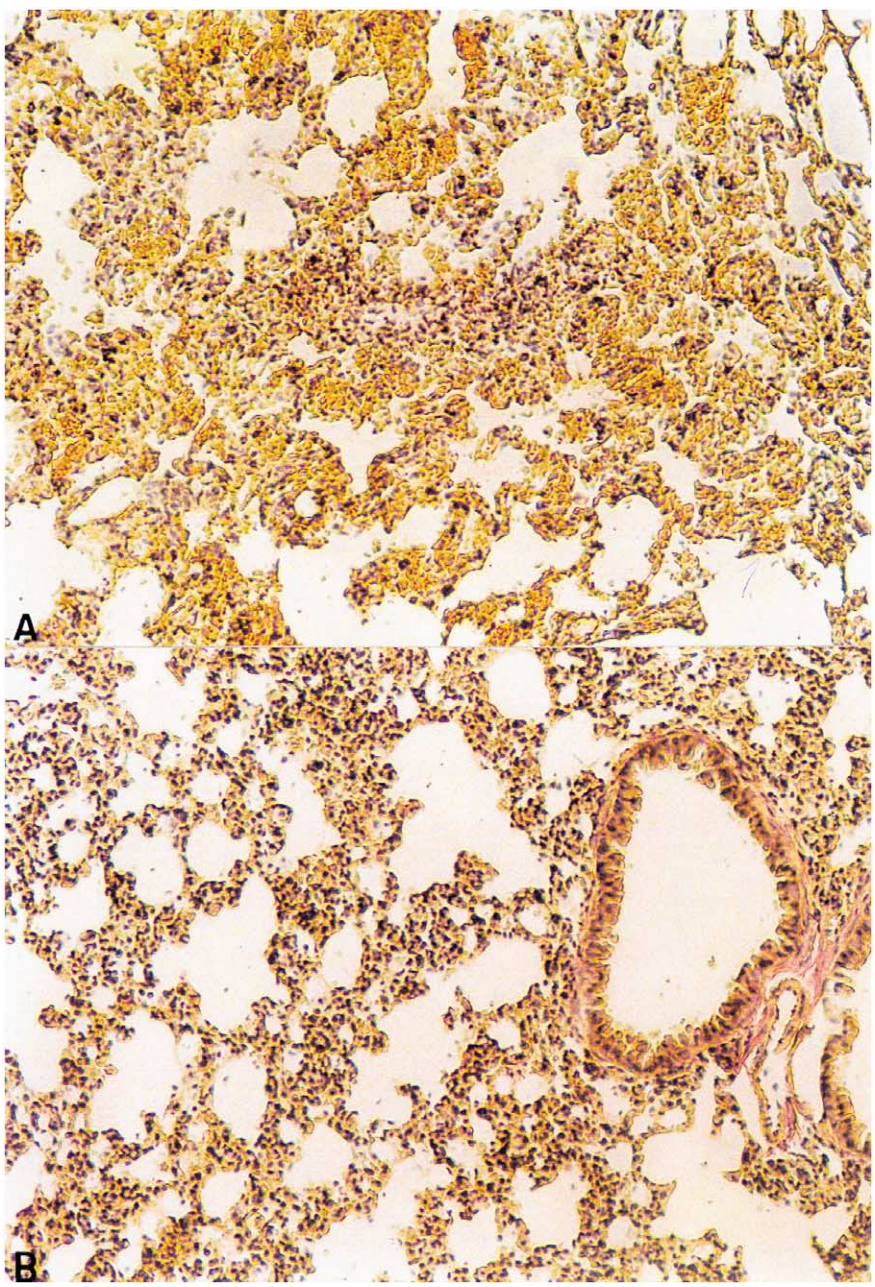

Fig. 6. Distal ileum from a zymosan-treated rat (A) demonstrating edema of the distal portion of the villi and necrosis of the epithelium at the villus tips. Distal ileum from a zymosan-treated rat that received GPI 6150 (B) treatment prevented partially zymosan-induced mucosal injury. Original magnification: ×62.5.

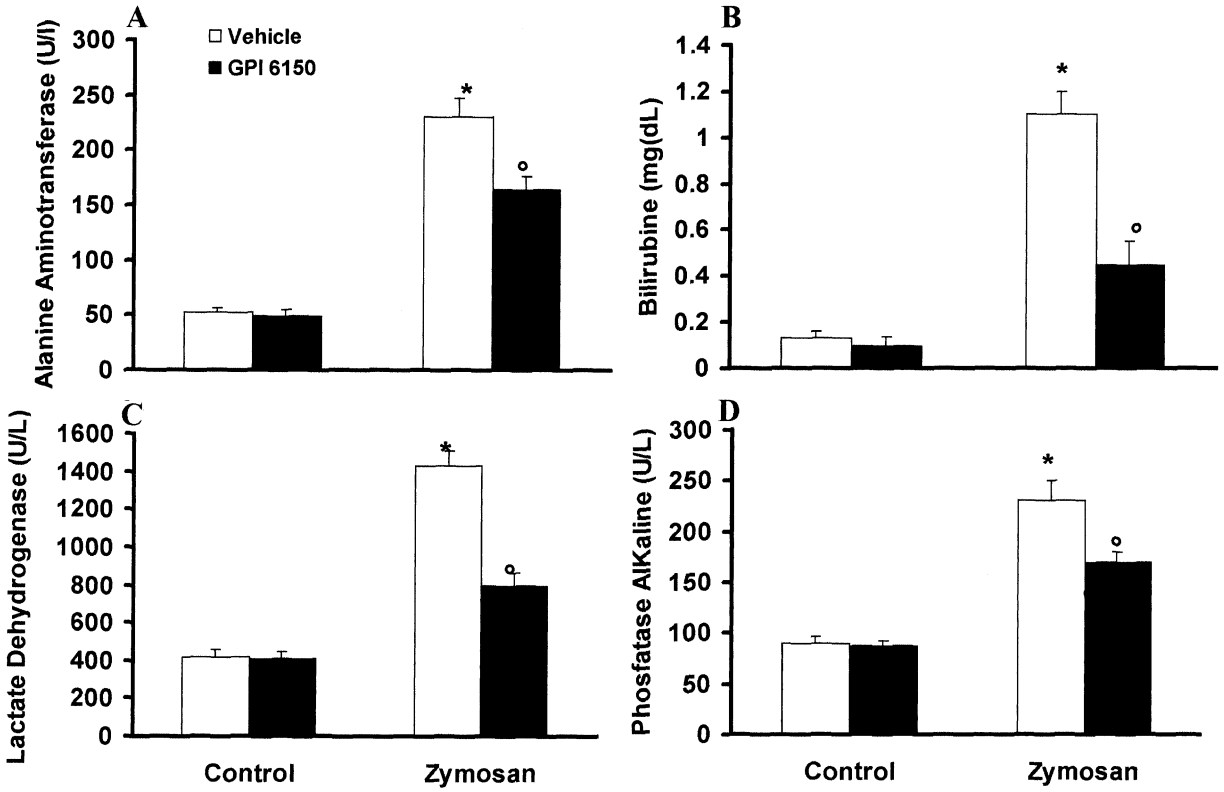

Fig. 7. Plasma level of alanine aminotransferase (A), bilirubine (B), LDH (C) and phosphatase alkaline (D). Zymosan-treated rats exhibit a dysmetabolic change. Administration of GPI 6150 reduced metabolic modification. Data are means ± SEM of 10 rats for each group. * $P < 0.01$ vs. sham; ° $P < 0.01$ vs. zymosan.

Italy. All other reagents and compounds used were obtained from Sigma (Sigma, Milan, Italy).

3. Results

3.1. Effects of GPI 6150 on carrageenan-induced paw edema

Intraplantar injection of carrageenan in rats led to an increase in paw volume (Fig. 1). GPI 6150 inhibited carrageenan-induced local inflammatory response in a dose responsive manner (Fig. 1). Given i.p. 1 h prior to carrageenan, 5 mg/kg GPI 6150 reduced paw swelling by 13%. A 20 mg/kg treatment achieved a significant reduction of paw edema by 32% (61 ± 3.5, $n = 5$, vs. 90 ± 3.8, $n = 3$, $P < 0.01$). Maximal reduction of paw edema was observed at 60 mg/kg GPI 6150 treatment (57 ± 1.6, $n = 5$, $P < 0.01$). In the same model, a single p.o. dose of 150 mg/kg aspirin resulted in a 41% reduction of paw edema (53 ± 2.2, $n = 3$, $P = 0.01$).

Table 1
Systemic toxicity at various hours after treatment

Group	Symptomatology	Time after injection (h)					
		3	6	18	24	48[a]	72[a]
Zymosan	ruffled fur	0.83 ± 0.14	1.5 ± 0.2	2.4 ± 0.3	2.7 ± 0.2	2.6 ± 0.4	1.3 ± 0.1
	lethargy	0.24 ± 0.12	0.78 ± 0.1	1.6 ± 0.10	2.5 ± 0.18	2.3 ± 0.2	1.5 ± 0.25
	conjunctivitis	0.33 ± 0.07	0.42 ± 0.09	0.91 ± 0.06	1.82 ± 0.1	1.6 ± 0.2	0.7 ± 0.1
	diarrhea	0.38 ± 0.06	0.26 ± 0.09	0.82 ± 0.2	1.94 ± 0.1	0.8 ± 0.1	0.55 ± 0.09
Zymosan + GPI 6150	ruffled fur	0.64 ± 0.09	1.1 ± 0.16	1.5 ± 0.13 *	1.7 ± 0.14 *	1.8 ± 0.1 *	0.8 ± 0.07 *
	lethargy	0.12 ± 0.05	0.60 ± 0.07	1.15 ± 0.09 *	1.82 ± 0.1 *	1.2 ± 0.1 *	0.8 ± 0.03 *
	conjunctivitis	0.28 ± 0.08	0.35 ± 0.08	0.77 ± 0.08 *	1.32 ± 0.03 *	0.8 ± 0.07 *	0.5 ± 0.06 *
	diarrhea	0.27 ± 0.06	0.26 ± 0.09	0.72 ± 0.07 *	1.2 ± 0.08 *	0.6 ± 0.09 *	0.3 ± 0.03 *

[a] Data obtained from survivor rats. Scores are: 0 = absence of symptomatology, 1 = mild, 2 = moderate, 3 = serious. Data are means ± SEM.
* $p < 0.05$ vs. zymosan.

Table 2
Decrease of body weight (g) at various hours after treatment

Group	Time after injection (h)			
	0	24	48	72
Sham	315 ± 5 ($n = 10$)	321 ± 4 ($n = 10$)	329 ± 3 ($n = 10$)	350 ± 4 ($n = 10$)
Zymosan	327 ± 3 ($n = 10$)	$276 \pm 4^{*}$ ($n = 8$)	285 ± 5 ($n = 4$)	290 ± 4 ($n = 4$)
Zymosan + GPI 6150	321 ± 4 ($n = 10$)	$295 \pm 2^{*}$ ($n = 8$)	$312 \pm 4^{**}$ ($n = 7$)	$320 \pm 3^{**}$ ($n = 7$)

Zymosan-treated rats exhibit a significant loss of body weight. Treatment with GPI 6150 induced a recovery of the body weight. Data are means ± SEM.
$^{*}p < 0.01$ vs. time 0.
$^{**}p < 0.05$ vs. time 24 h.

3.2. Effects of GPI 6150 on adjuvant-induced arthritis

In the rat adjuvant arthritis model, injection of complete Freund's adjuvant with killed *M. tuberculosis* into the subplantar region of the right hind paw resulted in acute and delayed phases of inflammation, which can be measured by the paw edema. In the acute phase, there was a net swelling of 0.83 ± 0.09 ml (means ± SEM, $n = 5$) in adjuvant-induced hind paw between days 5 and 1 (Fig. 2(A)). In the delayed phase, there was a net swelling of 0.22 ± 0.04 ml between days 18 and 14 (Fig. 2(B)). GPI 6150 was administered i.p. (50 mg/kg) on 5 consecutive days, beginning 1 h before adjuvant injection. GPI 6150 treatment significantly reduced paw edema for both acute phase (0.45 ± 0.03, $n = 5$, $P < 0.01$) and delayed phase (0.12 ± 0.01, $P = 0.05$) of inflammation. In the same model, hydrocortisone treatment (30 mg/kg × 5, p.o.) caused a reduction of paw edema to 0.54 ± 0.05 ml ($n = 5$) for the acute phase and 0.14 ± 0.02 ml for the delayed phase. The 45% and 46% reduction of the acute and delayed inflammation by GPI 6150 were at the same level of 33% and 36% reduction achieved by hydrocortisone.

3.3. Effects of GPI 6150 on zymosan-induced multiple organ failure

All zymosan-injected rats developed an acute peritonitis, as indicated by the production of turbid exudate (Fig. 3(A)). Trypan blue stain revealed a significant increase in the polymorphonuclear leukocytes in comparison to sham rats (Fig. 3(B)). Sham animals demonstrated no abnormalities in the pleural cavity or fluid. The degree of peritoneal exudation and polymorphonuclear migration were significantly reduced in rats treated with GPI 6150 (Fig. 3). GPI 6150 treatment did not cause significant changes in these parameters in sham rats (Fig. 3).

At 18 h after zymosan administration, lung, small intestine and liver were investigated for tissue damage by histological examination and myeloperoxidase activity. As shown in Fig. 4, myeloperoxidase activity was significantly increased in all organs ($P < 0.01$) at 18 h after zymosan injection. In histological examinations, lung and small intestine reveal pathologic changes (see representative sections in Figs. 5 and 6). Lung biopsy examination revealed extravasation of red cells, and neutrophil and macrophage accumulation (Fig. 5(A)). Sections from the distal ileum revealed significant edema in the space bounded by the villus and epithelial separation from the basement membrane (Fig. 6(A)). Hepatic damage was characterised by necrosis of hepatocytes (data not shown). Hepatic cellular injury was correlated with a significant ($P < 0.01$) increase of alanine aminotransferase and LDH blood levels (Fig. 7(A–C)). Treatment with GPI 6150 significantly reduced, in all organs, the zymosan-induced increase in myeloperoxidase activity ($P < 0.01$) and organ injury, indicating a greater inhibiting activity on neutrophil infiltration and an anti-inflammatory effect (Figs. 4, 5(B) and 6(B)). GPI 6150 treatment also reduced the increase of alanine aminotransferase and LDH (Fig. 7(A–C)).

Zymosan-treated rats also show abnormal metabolic changes. In this group of rats, a marked increase of hyperbilirubinemia and alkaline phosphatase was also found 18 h after zymosan injection (Fig. 7(B–D)). Administration of GPI 6150 significantly reduced the increase of bilirubine and alkaline phosphatase levels in blood (Fig. 7).

Administration of zymosan caused a severe illness in the rats, characterised by ruffled fur, lethargy, conjunctivitis, diarrhea (Table 1) and significant loss of body weight (Table 2). The symptoms appear 6 h after zymosan admin-

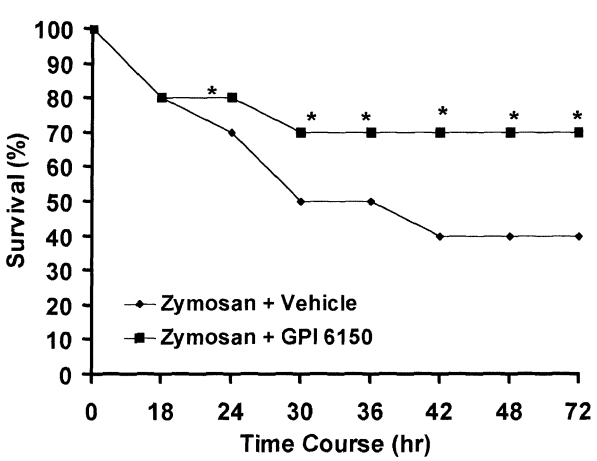

Fig. 8. Effect of GPI 6150 ($n = 10$) treatment on zymosan-induced mortality, $n = 10$ rats for each group. $^{*}P < 0.01$ vs. zymosan.

istration and became more severe within 18–48 h, when animals started to die (Table 1 and Fig. 8). At the end of the observation period (72 h), 60% of zymosan-treated rats were dead (Fig. 8). Sham animals injected only with saline appeared healthy and active through the entire observation period (data not shown). Treatment with received GPI 6150 (40 mg/kg, intraperitoneally) 1 and 6 h after zymosan prevented the development of systemic toxicity (Table 1) and mortality (Fig. 8) and prevented the loss in body weight (Table 2). GPI 6150 treatment did not cause significant changes in these parameters in sham rats (data not shown).

4. Discussion

Inflammatory process is characterised by a production of prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor (PAF) and interleukin 1 (IL-1), by a release of chemicals from tissues and migrating cells (Tomlinson et al., 1994; Vane and Botting, 1987). In particular, the early phase of the inflammatory process is related to the production of histamine, leukotrienes, platelet-activating factor, and possibly cyclooxygenase products, while the delayed phase of the carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radical, as well as to the release of other neutrophil-derived mediators (Oh-Ishi et al., 1989; Dawson et al., 1991; Peskar et al., 1991; Da Motta et al., 1994; Salvemini et al., 1996a,b; Cuzzocrea et al., 1997a). Activation of poly (ADP-ribose) synthase is triggered by single strand breaks in DNA and subsequently catalyses the transfer of ADP-ribose moieties from NAD^+ to various nuclear proteins, including histones and poly (ADP-ribose) synthase (automodification domain) itself (Ueda and Hayaishi, 1985). Continuous or excessive activation of poly (ADP-ribose) synthase produces extended chains of ADP-ribose on nuclear proteins and results in a substantial depletion of intracellular NAD^+, and subsequently ATP, which may ultimately cause cell death (Berger, 1985; Schraufstatter et al., 1986; Hyslop et al., 1988; Thies and Autor, 1991). Radicals including superoxide anions, hydrogen peroxide or hydroxyl radicals cause strand breaks in DNA, activation of poly (ADP-ribose) synthase and depletion of NAD^+ and ATP in cultured cells (Schraufstatter et al., 1986; Hyslop et al., 1988; Thies and Autor, 1991). Production of reactive oxidants such as hydrogen peroxide, superoxide and hydroxyl radical at the site of inflammation contributes to tissue damage (Oh-ishi et al. 1989; Peskar et al., 1991; Cuzzocrea et al., 1999a,b; Da Motta et al., 1994; Salvemini et al., 1996a), strand breaks in DNA (Carson et al., 1986) and ultimately poly (ADP-ribose) synthase activation. Inhibitors of poly (ADP-ribose) synthase activity, including 3-aminobenzamide (3-AB) reduce the development of acute (Cuzzocrea et al., 1998) and chronic inflammation (Szabó et al., 1998).

The main findings of the current study are the following: (1) GPI 6150 reduces the development of carrageenan-induced paw edema; (2) GPI 6150 reduces the development of adjuvant-induced paw edema; (3) GPI 6150 reduces mortality, morphological injury and neutrophil infiltration in zymosan-induced multiple organ failure.

In a variety of in vitro studies in fibroblasts, endothelial cells, epithelial cells, smooth muscle cells and other cell types, it has been established that pharmacological inhibitors of poly (ADP-ribose) synthase, such as 3-aminobenzamide, protect against the cellular oxidant injury in response to oxyradicals, NO generator and peroxynitrite (Cochrane, 1991; Szabó, 1996b).

Based on the present in vivo studies alone, it is not possible to identify the cell type on which GPI 6150 exerts its protection against oxidant injury during inflammation. Nevertheless, it is noteworthy that a marked inhibition by GPI 6150 of polymorphonuclear leukocyte infiltration into the inflammatory site was observed. Therefore, we propose that a reduced neutrophil recruitment represents an important additional mechanism for the anti-inflammatory effects provided by inhibition of poly (ADP-ribose) synthase.

In our study, the increase of the activity of myeloperoxidase, an enzyme specific to granulocyte lysosomes, and therefore directly related to the absolute number of polymorphonucleates (Mullane et al., 1988), was well-correlated with morphological alterations in lung and small intestine at the histological examination. In accord with several previous reports (Faist et al., 1983; Shayevitz et al., 1995; Goris et al., 1991; Demling et al., 1994; Van Bebber et al., 1989) we observe that alteration of organ architecture is also associated with organ malfunctions as demonstrated by modification of biochemical markers of organ function. Our results demonstrate that treatment with GPI 6150 exerts a protective effect on multiple organ failure and support a view that organ damage may be mediated by poly (ADP-ribose) synthase activation. These data are in agreement with previous studies reporting that anti-inflammatory effects of poly (ADP-ribose) synthase inhibitors may be in part related to the inhibition of neutrophil infiltration at the inflamed site (Szabó et al., 1997a). The mechanism of this reduced neutrophil recruitment may be related to a prevention of endothelial oxidant injury (Szabó et al., 1997a,b; Cuzzocrea et al., 1997b; Thiemermann et al., 1997; Zingarelli et al., 1997; Kirkland, 1991). Alternatively, it may be related to inhibition of the expression of adhesion receptors in inflammation (Hiromatsu et al., 1993) and/or to inhibition of the neutrophil–endothelial interaction (Meyer et al., 1991). The mechanism of this action clearly requires further work. Based on the current study and previous observation, (Cuzzocrea et al., 1997a,b; Zingarelli et al., 1997) we propose the following

positive feedback cycle: hydroxyl radical and peroxynitrite production ⇒ poly (ADP-ribose) synthase-related endothelial injury ⇒ neutrophil infiltration ⇒ more hydroxyl radical and peroxynitrite production. Inhibition of poly (ADP-ribose) synthase would interrupt this cycle at the level of endothelial injury.

In conclusion, as with most pharmacological inhibitors, we cannot exclude additional poly (ADP-ribose) synthase-independent effects, which may contribute to the anti-inflammatory effects observed with GPI 6150 in the current study. However, extensive biochemical testing of the compound has established that GPI 6150 is more potent and selective in inhibiting poly (ADP-ribose) synthase than the widely used benzamide and 3-aminobenzamide, which may have side effects independent of poly (ADP-ribose) synthase inhibition at high concentration (Zhang et al., 2000; Milam and Cleaver, 1984).

Taken together, our results strongly support the view that poly (ADP-ribose) synthase plays an important role in the inflammatory process and poly (ADP-ribose) synthase inhibitor may have potential to treat inflammatory injuries.

Acknowledgements

This study was supported by a grant from MURST 40%. The authors would like to thank Giovanni Pergolizzi and Carmelo La Spada for their excellent technical assistance during this study, Mrs. Caterina Cutrona for secretarial assistance and Miss Valentina Malvagni for editorial assistance with the manuscript. Part of the work was reported at the 6th International Meeting of the Biology of Nitric Oxide, Stockholm.

References

Beckman, J.S., Koppenol, W.H., 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am. J. Physiol., 271, C1424–C1427.

Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., Freeman, B.A., 1990. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. U. S. A., 87, 1620–1624.

Berger, N.A., 1985. Poly(ADP-ribose) in the cellular response to DNA damage. Radiat. Res., 101, 4–15.

Bowes, J., Mcdonald, M.C., Piper, J., Thiemermann, C., 1999. Inhibitors of poly (ADP-ribose) synthetase protect rat cardiomyocytes against oxidant stress. Cardiovasc. Res., 41, 126–134.

Carson, D.A., Set, S., Wasson, B., Carrera, C.J., 1986. DNA strand breaks, NAD metabolism and programmed cell death. Exp. Cell Res., 164, 273–281.

Clancy, R.M., Abranson, S.B., 1995. Nitric oxide: a novel mediator of inflammation. Proc. Soc. Exp. Biol. Med., 210, 93–101.

Cochrane, C., 1991. Mechanism of oxidant injury of cells. Mol. Aspects Med., 12, 137–147.

Crow, J.P., Beckman, J.S., 1995. The role of peroxynitrite in nitric oxide-mediated toxicity. Curr. Top. Microbiol. Immunol., 196, 57–73.

Cuzzocrea, S., Filippelli, A., Zingarelli, B., Falciani, M., Caputi, A.P., Rossi, F., 1997a. Role of nitric oxide in a non-septic shock model induced by zymosan in the rat. Shock, 7, 351–357.

Cuzzocrea, S., Zingarelli, B., Costantino, G., Szabo, A., Salzman, A.L., Caputi, A.P., Szabo, C., 1997b. Beneficial effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion. Br. J. Pharmacol., 121, 1065–1074.

Cuzzocrea, S., Zingarelli, B., Gilard, E., Hake, P., Salzman, A.L., Szabó, C., 1998. Protective effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthase in carrageenan-induced models of local inflammation. Eur. J. Pharmacol., 342, 67–76.

Cuzzocrea, S., Costantino, G., Mazzon, E., Caputi, A.P., 1999a. Beneficial effects of raxofelast (IRFI 016), a new hydrophilic vitamin E-like antioxidant, in carrageenan-induced pleurisy. Br. J. Pharmacol., 126, 407–414.

Cuzzocrea, S., Zingarelli, B., Costantino, G., Caputi, A.P., 1999b. Beneficial effects of Mn(III)tetrakis (4-benzoic acid) porphyrin (MnTBAP), a superoxide dismutase mimetic, in carrageenan-pleurisy. Free Radical Biol. Med., 26, 26–33.

Da Motta, J.I., Cunha, F.Q., Vargaftig, B.B., Ferreira, S.H., 1994. Drug modulation of antigen-induced paw oedema in guinea-pigs: effects of lipopolysaccharide, tumour necrosis factor and leucocyte depletion. Br. J. Pharmacol., 112, 111–116.

Dawson, J., Sedgwick, A.D., Edwards, J.C., Lees, P., 1991. A comparative study of the cellular, exudative and histological responses to carrageenan, dextran and zymosan in the mouse. Int. J. Tissue React., 13, 171–185.

Demling, R., Nayak, U., Ikegami, K., LaLonde, C., 1994. Comparison between lung and liver peroxidation and mortality after zymosan peritonitis in the rats. Shock, 2, 222–227.

Faist, E., Baue, A.E., Dittmer, H., Heberer, G., 1983. Multiple organ failure in polytrauma patients. J. Trauma, 23, 775–787.

Goris, R.J.A., Van Bebber, I.P.T., Mollen, R.M.H., Koopman, J.P., 1991. Does selective decontamination of the gastrointestinal tract prevent multiple organ failure? Arch. Surg., 126, 561–565.

Hiromatsu, Y., Sato, M., Tanaka, K., Ishisaka, N., Kamachi, J., Nonaka, K., 1993. Inhibitory effects of nicotinamide on intercellular adhesion molecule-1 expression on cultured human thyroid cells. Immunology, 80, 330–338.

Hyslop, P.A., Hinshaw, D.B., Halsey, W.A., Schraufstatter, I.U., Sauerheber, R.D., Spragg, R.G., Jackson, J.H., Cochrane, C.G., 1988. Mechanisms of oxidant-mediated cell injury: The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. J. Biol. Chem., 263, 1665–1675.

Kirkland, J.B., 1991. Lipid peroxidation, protein thiol oxidation and DNA damage in hydrogen peroxide-induced injury to endothelial cells: role of activation of poly (ADP-ribose) polymerase. Biochim. Biophys. Acta, 1092, 319–324.

LaPlaca, M.C., Raghupathi, R., Verma, A., Pieper, A.A., Saatman, K.E., Snyder, S.H., Mc Intosh, T.K., 1999. Temporal patterns of poly (ADP-ribose) polymerase activation in the cortex following experimental brain injury in the rat. J. Neurochem., 73, 205–213.

Li, J.-H., Zhang, J., Jackson, P.F., Maclin, K.M., 1999. Poly(ADP-ribose) polymerase (PARP) inhibitors, methods and pharmaceutical compositions for treating neural or cardiovascular tissue damage. International Publication Number: WO/99/11645, 1999.

Lu, X.-C.M., Mooney, M., Lin, Q., Steiner, J.P., Ross, D.T., Spicer, D., Guo, H., Howorth, P., Chen, Y., Lautar, S., Li, J.-H., Kalish, V., Zhang, J., 2000. Efficacy of GPI 6150 in mouse models of LPS-septic shock, STZ-diabetes and MPTP-neurotoxicity. Soc. Neurosci. Abstr., 26, 1026.

Meyer, T., Lengyel, H., Fanick, W., Hilz, H., 1991. 3-Aminobenzamide inhibits cytotoxicity and adhesion of phorbol-ester-stimulated granulocytes to fibroblast monolayer cultures. Eur. J. Biochem., 197, 127–134.

Milam, K.M., Cleaver, J.E., 1984. Inhibitors of poly (adenosinediphosphate-ribose) synthesis effect on other metabolic processes. Science, 223, 589–591.

Mullane, K.M., Westlin, W., Kraemer, R., 1988. Activated neutrophils release mediators that may contribute to myocardial injury and dysfunction associated with ischemia and reperfusion. Ann. N. Y. Acad. Sci., 524, 103–121.

Oh-ishi, S., Hayashi, I., Hayashi, M., Yamaki, K., Utsunomiya, I., 1989. Pharmacological demonstration of inflammatory mediators using experimental inflammatory models: rat pleurisy induced by carrageenin and phorbol myristate acetate. Dermatologica, 179, 68–71.

Peskar, B.M., Trautmann, M., Nowak, P., Peskar, B.A., 1991. Release of 15-hydroxy-5,8,11,13-eicosatetraenoic acid and cysteinyl-leukotrienes in carrageenin-induced inflammation: effect of non-steroidal anti-inflammatory drugs. Agents Actions, 33, 240–246.

Pfeilschifter, J., Eberhardt, W., Hummel, R., Kunz, D., Muhl, H., Nitsch, D., Pluss, C., Walker, G., 1996. Therapeutic strategies for the inhibition of inducible nitric oxide synthase-potential for a novel class of anti-inflammatory agents. Cell Biol. Int., 20, 51–58.

Salvemini, D., Wang, Z.Q., Bourdon, D.M., Stern, M.K., Currie, M.G., Manning, P.T., 1996a. Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. Eur. J. Pharmacol., 303, 217–220.

Salvemini, D., Wang, Z.Q., Wyatt, P., Bourdon, D.M., Marino, M.H., Manning, P.T., Currie, M.G., 1996b. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br. J. Pharmacol., 118, 829–838.

Schraufstatter, I.U., Hinshaw, D.B., Hyslop, P.A., Spragg, R.G., Cochrane, C.G., 1986. DNA strand breaks activate poly adenosine diphosphate–ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. J. Clin. Invest., 77, 1312–1320.

Shayevitz, J.R., Miller, C., Johnson, K.J., Rodriguez, J.L., 1995. Multiple organ dysfunction syndrome: end organ and systemic inflammatory response in a mouse model of nonseptic origin. Shock, 4, 389–396.

Szabó, C., 1995. Alterations in the production of nitric oxide in various forms of circulatory shock. New Horiz., 3, 2–32.

Szabó, C., 1996a. The role of peroxynitrite in the pathophysiology of shock, inflammation and ischemia-reperfusion injury. Shock, 6, 79–88.

Szabó, C., 1996b. DNA strand breakage and activation of poly-ADP ribosyltransferase: a cytotoxic pathway triggered by peroxynitrite. Free Radical Biol. Med., 21, 855–869.

Szabó, C., Zingarelli, B., O'Connor, M., Salzman, A.L., 1996a. DNA strand breakage, activation of poly-ADP ribosyl synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite. Proc. Natl. Acad. Sci. U. S. A., 93, 1753–1758.

Szabó, C., Zingarelli, B., Salzman, A.L., 1996b. Role of poly-ADP ribosyltransferase activation in the nitric oxide- and peroxynitrite-induced vascular failure. Circ. Res., 78, 1051–1063.

Szabó, C., Cuzzocrea, S., Zingarelli, B., O'Connor, M., Salzman, A.L., 1997a. Endothelial dysfunction in endotoxic shock: importance of the activation of poly (ADP ribose) synthetase (PARS) by peroxynitrite. J. Clin. Invest., 100, 723–735.

Szabó, C., Lim, L.H.K., Cuzzocrea, S., Getting, S.J., Zingarelli, B., Flower, R.J., Salzman, A.L., Peretti, M., 1997b. Inhibition of poly (ADP-ribose) synthetase exerts anti-inflammatory effects and inhibits neutrophil recruitment. J. Exp. Med., 186, 1041–1049.

Szabó, C., Viràg, L., Cuzzocrea, S., Scott, G.S., Hake, P., O'Connor, M., Zingarelli, B., Ma, Y., Hirsch, R., Boiovin, G.P., Salzman, A.L., Kun, E., 1998. Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly (ADP-Ribose) synthetase. Proc. Natl. Acad. Sci. U. S. A., 95, 3867–3872.

Thiemermann, C., Bowes, J., Mynny, F.P., Vane, J.R., 1997. Inhibition of the activity of poly (ADP-ribose) synthase reduces ischaemia-reperfusion injury in the heart and skeletal muscle. Proc. Natl. Acad. Sci. U. S. A., 94, 679–683.

Thies, R.L., Autor, A.P., 1991. Reactive oxygen injury to cultured pulmonary artery endothelial cells: mediation by poly (ADP-ribose) polymerase activation causing NAD depletion and altered energy balance. Arch. Biochem. Biophys., 286, 353–363.

Tomlinson, A., Appleton, I., Moore, A.R., Gilroy, D.W., Willis, D., Mitchell, J.A., Willoughby, D.A., 1994. Cyclo-oxygenase and nitric oxide isoforms in rat carrageenin-induced pleurisy. Br. J. Pharmacol., 113, 693–698.

Tracey, W.R., Nakane, M., Kuk, J., Budzik, G., Klinghofer, V., Harris, R., Carter, G., 1995. The nitric oxide synthase inhibitor, L-N^G-monomethylarginine, reduces carrageenan-induced pleurisy in the rat. J. Pharmacol. Exp. Ther., 273, 1295–1299.

Ueda, K., Hayaishi, O., 1985. ADP-ribosylation. Ann. Rev. Biochem., 54, 73–100.

Walles, T., Wang, P., Pieper, A., Li, J.-H., Zhang, J., Snyder, S.H., Zweier, J., 1998. Demonstration that poly(ADP-ribose) accumulation occurs in the post-ischemic heart and is associated with myocardial necrosis. Circulation, Suppl., 98, I-260.

Van Bebber, I.P.T., Boekholz, K.F., Goris, R.J., Schillings, P.H., Dinges, H.P., Bahrami, S., Redl, H., Schlag, G., 1989. Neutrophil function and lipid peroxidation in rat model of multiple organ failure. J. Surg. Res., 47, 471–475.

Vane, J., Botting, R., 1987. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J., 1, 89–96.

Zhang, J., Dawson, V., Dawson, T., Snyder, S., 1994. Nitric oxide activation of poly (ADP-ribose) synthetase in neurotoxicity. Science, 263, 687–689.

Zhang, J., Li, J., Lautar, S., 1998. Post-ischemia protection by a potent PARP inhibitor in transient cerebral focal ischemia. Soc. Neurosci. Abstr., 24, 1226.

Zhang, J., Lautar, S., Huang, S., Ramsey, C., Cheung, A., Li, J., 2000. GPI 6150 prevents H_2O_2-cytotoxicity by inhibiting poly (ADP-ribose) polymerase. Biochem. Biophys. Res. Commun., 278, 590–598.

Zingarelli, B., Cuzzocrea, S., Zsengeller, Z., Salzman, A.L., Szabo, C., 1997. Protection against myocardial ischemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly (ADP-Ribose) synthetase. Cardiovasc. Res., 36, 205–215.