J Clin Periodontol 2007; 34: 95–102 doi: 10.1111/j.1600-051X.2006.01016.x

Journal of Clinical Periodontology

5-aminoisoquinolin-1(2*H*)-one, a water-soluble poly (ADP-ribose) polymerase (PARP) inhibitor reduces the evolution of experimental periodontitis in rats

Rosanna Di Paola[1]*, Emanuela Mazzon[1,2]*, Carmelo Muià[1], Dino Terrana[1], Salvatore Greco[1], Domenico Britti[3], Domenico Santori[4], Giacomo Oteri[5], Giancarlo Cordasco[5] and Salvatore Cuzzocrea[1,2]

[1]Department of Clinical and Experimental Medicine and Pharmacology, Torre Biologica, Policlinico Universitario, Messina, Italy; [2]IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy; [3]Università della Magna Græcia di Catanzaro (UMG), Italy; [4]Dipartimento di Scienze Cliniche Veterinarie, Università degli Studi di Teramo, viale Crispi Teramo; and [5]Istituto Policattedra di Odontostomatologia Universita degli Studi di Messina, Italia.

Di Paola R, Mazzon E, Muià C, Terrana D, Greco S, Britti D, Santori D, Oteri G, Cordasco G, Cuzzocrea S. 5-aminoisoquinolin-1(2H)-one, a water soluble poly (ADP-ribose) polymerase (PARP) inhibitor reduces the evolution of experimental periodontitis in rats. J Clin Periodontol 2007; 34: 95–102. doi: 10.1111/j.1600-051X.2006.01016.x.

Abstract

Background: Poly (ADP-ribose) polymerase (PARP), a nuclear enzyme activated by strand breaks in DNA, plays an important role in the tissue injury associated with ischaemia-reperfusion and inflammation. Recent studies have demonstrated that PARP activation plays a crucial role in the pathogenesis of acute periodontal injury.

Aim: We have investigated the effect of 5-aminoisoquinolin-1(2*H*)-one (5-AIQ), a water-soluble PARP inhibitor, in a rat model of periodontitis.

Materials and Methods: Periodontitis was induced in rats by placing a 2/0 braided silk ligature around the lower left first molar. At day eight, the gingivomucosal tissue encircling the mandibular first molar was removed for biochemical and histological analysis.

Results and Conclusions: Ligation significantly induced an increased neutrophil infiltration and a positive staining for PARP activation. Ligation significantly increased Evans blue extravasation in gingivomucosal tissue and alveolar bone destruction. Intraperitonial injection of 5-aminoisoquinolin-1(2*H*)-one (5-AIQ) (5 mg/kg daily for eight days) significantly decreased all of the parameters of inflammation as described above. This suggests that inhibition of PARP may represent a novel approach for the treatment of periodontal disease.

Key words: alveolar bone loss; neutrophil infiltration; PARP inhibitor; periodontal diseases; poly (ADP-ribose) polymerase (PARP)

Accepted for publication 29 September 2006

Periodontitis, an inflammatory disorder of the periodontium, damages the bone and soft connective tissue that supports the teeth. The severity of periodontitis is characterized by the degree of marginal bone loss, the depth of periodontal pockets, the degree of attachment loss and the number of teeth with furcation development (Ridgeway 2000). In recent years, more attention has been focused on the role of reactive oxygen species, lipid peroxidation products and antioxidant systems in the pathology of periodontitis. Recent medical and dental research in this area has been geared towards the prevention of free radical-mediated diseases by using specific nutrient antioxidants (Battino et al. 1999). Elevated lipid peroxidation and disturbed antioxidant status have been reported in experimental periodontitis (Di Paola et al. 2004a, b).

Various evidences from in vitro and in vivo studies have demonstrated that reactive oxygen species (ROS) produce strand breaks in DNA that trigger the activation of the nuclear enzyme poly (adenosine 5′-diphosphate ribose) polymerase (PARP) (Ikai & Ueda 1983).

PARP is a ubiquitous, chromatin-bound enzyme, which is abundantly

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest.

The study was supported by a grant from PRA 2003 from the University of Messina.

*These authors contributed equally to this work.

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

present in the nuclei of numerous cell types (Ikai & Ueda 1983). Continuous or excessive activation of PARP produces extended chains of ADP-ribose on nuclear proteins and results in a substantial depletion of intracellular NAD^+ and subsequently, adenosine triphosphate (ATP), leading to cellular dysfunction and ultimately, cell death (Berger 1985, Schraufstatter et al. 1986a, b, Hyslop et al. 1988, Thies & Autor 1991). More recently, other important mechanisms of PARP activation e.g. release of apoptosis-inducing factor from mitochondria and changes in cell membrane transport have been described (Gagne et al. 2006).

Chemically distinct inhibitors of PARP activity such as benzamides [e.g., 3-aminobenzamide (3-AB), nicotinamide] and isoquinolinones [e.g., 1,5-dihydroxyisoquinoline (5-hydroxyisoquinolin-1(2*H*)-one) (1,5-DHIQ), 3,4-dihydro-5-(4-(1-piperidinyl)butoxyl)-1(2*H*)-isoquinolinone), (DPQ)] can reduce the degree of injury associated with inflammation, and these investigations have provided the basis for potential clinical applications of PARP inhibitors (Virag & Szabò 2002).

Furthermore, the degree of gingivomucosal tissue injury caused by experimental periodontitis is attenuated in mice in which the gene for PARP has been disrupted by gene targeting (Lohinai et al. 2003).

Therefore, PJ34 (*N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide), a novel PARP inhibitor, has been previously examined as a potential novel therapeutic intervention against gingivomucosal tissue injury associated with experimental periodontitis (Lohinai et al. 2003). Specifically, this latter study has demonstrated that PJ34 can attenuate PARP activation and provide beneficial actions against gingivomucosal tissue injury and dysfunction in vivo (Lohinai et al. 2003). However, in contrast, isoquinolinone derivatives such as 1,5-DHIQ and DPQ, 3-AB and nicotinamide are weak inhibitors of PARP activity that do not readily cross cell membranes (Banasik et al. 1992, Szabo & Dawson 1998). Furthermore, although the potency of recently developed PARP inhibitors has improved greatly, most lack good solubility in water, making it difficult to find a biocompatible vehicle for utilization in vivo. Thus, there is still a great need for the development of potent, water-soluble inhibitors of PARP activity. Much effort has been made to develop new PARP inhibitors with better potency, selectivity and water solubility, and there are now 13 chemical classes of PARP inhibitors (Cosi 2002). Twelve years ago, Suto et al. (1991) used a cell-free preparation of PARP (purified 900-fold from calf thymus) to demonstrate that 5-aminoisoquinolinone [5-aminoisoquinolin-1(2*H*)-one] (5-AIQ) is a water-soluble inhibitor of PARP activity. As previously published reports of the synthesis of 5-AIQ reported problems of low yield and unreliability (Wenkert et al. 1964, Suto et al. 1991), Mc Donald et al. (2000) have recently developed a novel and more efficient method for the synthesis of 5-AIQ . We have previously demonstrated that 5-AIQ can reduce ischaemia/reperfusion injury of the heart, intestine and liver (Wayman et al. 2001, Mota-Filipe et al. 2002), and 5-AIQ has been shown to provide beneficial effects in rodent models of heart transplantation (Szabó et al. 2002) and lung injury (Cuzzocrea et al. 2002).

The present study was designed to evaluate the effects of 5-AIQ treatment in a rodent model of ligation-induced periodontitis. In order to gain a better insight into the mechanism(s) of action of the observed anti-inflammatory effects of 5-AIQ, we have also investigated the effects of 5-AIQ on (i) the degree of gingivomucosal tissue injury, (ii) the increase in myeloperoxidase (MPO) activity (mucosa), (iii) the increase in immunohistochemical staining for poly (ADP-ribose) (PAR), as well as (iv) the vascular permeability associated with experimental periodontitis.

Materials and Methods

Surgical procedure

Male Sprague–Dawley rats (280–400 g) were lightly anaesthetized with surgical doses of sodium pentobarbitone (35 mg/kg). Sterile, 2–0 black-braided silk thread was placed around the cervix of the lower left first molar and knotted medially as previously described (Di Paola et al., 2004a, b). After the rats had recovered from the anaesthetic, they were allowed to eat commercial laboratory food and drink tap water ad libitum. Animals and the study protocol were approved by the Institutional Animal Care and User Committee of the University of Messina.

Measurement of arterial blood pressure indirectly in conscious rat

The mean arterial blood pressure in conscious rats was measured by a blood pressure recorder (UGO BASILE, Biological Research Apparatus, 21,025 Comerio, Italy) as previously described (Di Paola et al. 2004a, b). After a week , rats were treated as described below and blood pressure was measured 30 min. before and after each i.p. injection, on each of the 8 days of treatment.

Experimental groups

Rats were randomly allocated into the following groups: *ligature+vehicle group:* rats were subjected to ligature-induced periodontitis and animals received vehicle intraperitoneally (saline solution i.p.; daily treatment for 8 days). *Ligature+5-AIQ group:* rats were subjected to ligature induced periodontitis and animals received 5-AIQ (5 mg/kg i.p., daily for 8 days). At 8 days after the ligature-induction of periodontitis, the rats ($N = 10$ from each group for each parameter) were sacrificed under isoflurane anaesthesia in order to evaluate the various parameters described below.

Measurement of vascular permeability by Evans blue extravasations

Vascular permeability was determinated as previously described (Gyorfi et al. 1994). Briefly, animals received Evans blue (2.5% dissolved in physiological saline, at a dose of 50 mg/kg) via a femoral venous catheter. Extravasated Evans blue in the excised gingivomucosal tissue samples was extracted with 1 ml formamide for 48 h at room temperature for spectrophotometric determination at 620 nm and expressed as microgram per gram gingivomucosal tissue (Gyorfi et al. 1994).

Measurement of alveolar bone loss

In the same set of experiments, the distance from the cementoenamel junction of the first lower molars to the alveolar crest was measured with a modification of the method by Crawford et al.(1978). Recordings were made along the median axis of the lingual surface of the mesial and mediolingual roots of the lower first left and right molars as previously described (Di Paola et al. 2004a, b). These measure-

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

ments were performed by an independent investigator who was unaware of the treatment regimens. The alveolar bone loss induced by the ligature was expressed as the difference between the left and the right side.

Histological examination

For histopathological examination, biopsies of gingiva and mucosa tissue from the buccal and lingual aspect of the teeth were taken 8 days after the ligature induction of periodontitis. The tissue specimen slices were fixed in 10% neutral-buffered formaldehyde for 5 days, embedded in paraffin, and sectioned. The sections (5 μm thickness), oriented longitudinally from the teeth crowns, were stained with trichrome stain. The total number of infiltrating leucocytes (e.g., neutrophils and mononuclear cells) in cortical interstitial spaces from gingiva and mucosa tissues was assessed quantitatively by counting the number of polymorphonuclear cells in 20 high-power fields.

Radiography

Mandibles were placed on a radiographic box at a distance of 90 cm from the X-ray source. Radiographic analysis of normal and ligated mandibles was performed by an X-ray machine (Philips X12, Munich, Germany) with a 40 kW exposure for 0.01 s. A radiographic examination 8 days after ligature placement revealed bone resorption in the lower left first molar region after ligation as previously described (Di Paola et al. 2004a, b).

Myeloperoxidase activity

Myeloperoxidase activity, an indicator of polymorphonuclear leucocyte (PMN) accumulation, was determined in gingivomucosal tissue as previously described (Mullane et al. 1985). The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol/min. of peroxide at 37°C and was expressed in milliunits/gram of wet tissue.

Immunohistochemical localization of PAR

After deparaffinization, endogenous peroxidase was quenched with 0.3% (v/v) H_2O_2 in 60% (v/v) methanol for 30 min. The sections were then incubated overnight with primary monoclonal anti-poly(ADP-ribose) antibody (1 : 500 dilution, Alexis, Milan, Italy), with control solutions including buffer alone or non-specific purified rabbit IgG. Specific labelling was detected with a biotin-conjugated goat anti-rabbit IgG and avidin-biotin peroxidase complex (DBA, Milan, Italy). Immunocytochemistry photographs ($n = 5$ photos from each sample collected from all rats in each experimental group) were assessed by densitometry analysis by using Optilab Graftek software on a Macintosh personal computer.

Materials

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

Data analysis

All values in the figures and text are expressed as mean ± 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 were then compared with Bonferroni's or Student's unpaired t-test. A p-value of < 0.05 was considered significant. In the experiments involving histology or immunohistochemistry, the figures shown are representative at least three experiments (histological or immunohistological staining) performed on different experimental days on the tissues section collected from all the animals in each group.

Results

Effects of 5-AIQ on PARP activation in periodontitis

Sections of gingivomucosal tissues from the contralateral side from vehicle (Fig. 1a) and 5-AIQ (Fig. 1b) treated rats did not reveal any immunoreactivity for PAR, an indicator of PARP activation, within the normal architecture. At 8 days following ligation, a positive staining for PAR (Fig. 1c) was found in the gingivomucosal tissues from ligature-operated rats mainly localized in the cell nuclei (see arrows Fig. 1c). No positive staining for PAR was found in the gingivomucosal tissues from rats that have received 5-AIQ treatment (Fig. 1d).

Effects of 5-AIQ on plasma extravasation and neutrophil infiltration in periodontitis

Before the measurement of Evans blue extravasation, the mean arterial pressure of vehicle-treated and 5-AIQ-treated animals was recorded. In agreement with previous studies (Di Paola et al. 2004a), 5-AIQ treatment did not affect the mean arterial blood pressure (vehicle treated: 118 ± 2 mm Hg; $n = 10$ and 5-AIQ treated: 112 ± 2 mm Hg; $n = 10$). Ligation significantly increased Evans blue extravasation in gingivomucosal tissue compared with the contralateral side (Fig. 2b). 5-AIQ treatment prevented this increase in Evans blue extravasation, but did not change the Evans blue content of the contralateral side (Fig. 2b). Myeloperoxidase activity was significantly elevated ($p < 0.001$) at 8 days after the ligature (Fig. 2a) and 5-AIQ treatment significantly reduced these levels (Fig. 2a). No significant changes of myeloperoxidase activity were observed in the gingivomucosal tissues from the controlateral side (Fig. 2a).

Effect of 5-AIQ on tissue damage and bone destruction

When compared with gingivomucosal tissue sections taken from the contralateral side from vehicle (Fig. 3a)- and 5-AIQ (Fig. 3b)-treated rats, histological examination of gingivomucosal tissues sections of ligature-operated rats showed oedema, tissue injury as well as infiltration of the tissue with inflammatory cells (Fig. 3c). 5-AIQ treatment reduced the degree of gingivomucosal tissues injury (Fig. 3d). Quantification of infiltrating polymorphonuclear cell into gingivomucosal tissue showed that there was only a minimal number of polymorphonuclear cells in tissue from the controlateral side (Fig. 3d). However, a large number of infiltrating polymorphonuclear cells was observed in the gingivomucosal tissue of ligated rats (Fig. 3e). 5-AIQ administration significantly reduced the numbers of polymorphonuclear cells infiltrating into gingivomucosal tissue (Fig. 3e). A radiographic examination of the mandibles, at day 8 after ligature placement, revealed bone matrix resorption in the lower left first molar region after ligation (Fig. 4a). There was no evidence of

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

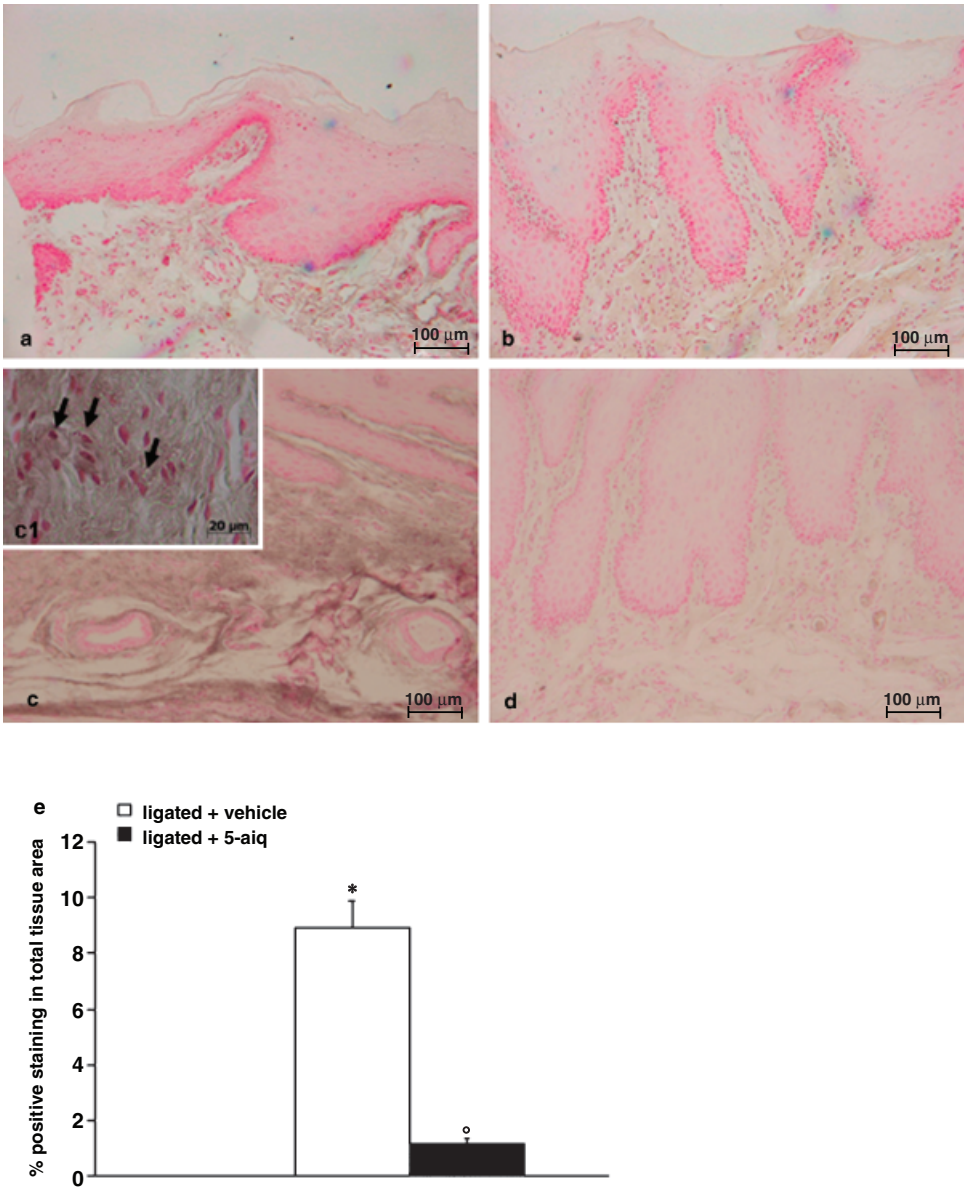

Fig. 1. Immunohistochemical staining for poly (ADP-Ribose) (PAR) formation and densitometry analysis. Sections of gingivomucosal tissues from the contralateral side from vehicle (a) 5-aminoisoquinolin-1(2*H*)-one (5-AIQ) (b)-treated rats did not reveal any immunoreactivity for (PAR), an indicator of PARP activation. Positive staining for (PAR) was observed in gingivomucosal tissue 8 days after ligature (c). In gingivomucosal tissue of 5-AIQ-treated rats, no positive staining for (PAR) (d) was observed. Densitometry analysis of Immunohistochemical photographs ($n = 5$ photos from each sample collected from all rats in each experimental group) for PAR from gingivomucosal tissue was carried out (e). Figure is representative of at least three experiments performed on different experimental days. $^{*}p < 0.01$ *versus* non-ligated; $^{\circ}p < 0.01$ *versus* ligated.

pathology in theright first molar (data not shown). 5-AIQ markedly reduced the degree of bone resorption in the lower left first molar region after ligation (Fig. 4b).

In addition, a significant increase in the distance between the cemento-enamel junction and alveolar crest at the mediolingual root of the first molar was observed in ligature-treated rats. 5-AIQ treatment significantly reduced the increase in the distance between the cemento-enamel junction and alveolar crest (Fig. 4c).

Discussion

We demonstrate here that the treatment with 5-AIQ significantly reduced (i) the degree of gingivomucosal tissue injury, (ii) the degree of vascular permeability, (iii) the infiltration of the gingivomucosal tissues with PMNs, (iv) the positive staining (immunohistochemistry) for PAR, as well as (v) the alveolar bone loss associated with experimental periodontitis. All these findings support the view that PARP activation plays an important role in the development of experimental periodontitis and that potent PARP inhibitors like 5-AIQ may be useful in the therapy of periodontitis. What, then,

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

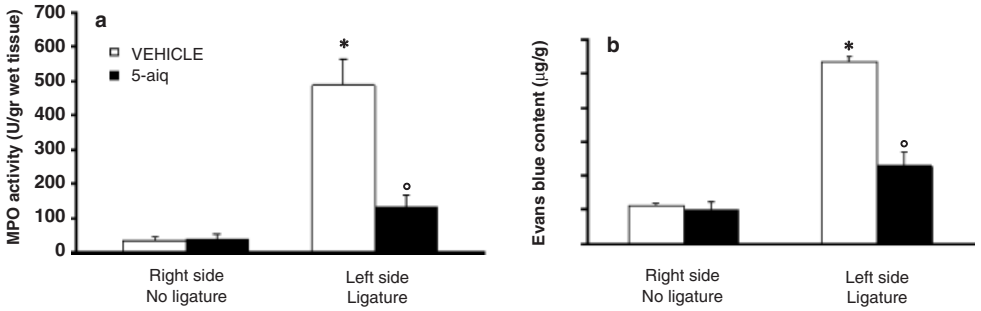

Fig. 2. Myeloperoxidase activity and Evans blue content in gingivomucosal tissue. Myeloperoxidase activity (a) and Evans blue content (b) in gingivomucosal tissue were significantly increased by ligature compared with the contralateral side. 5-aminoisoquinolin-1(2*H*)-one 5-AIQ significantly reduced Evans blue content and myeloperoxidase activity levels. Densitometry data are expressed as percent of total tissue area. Data are mean ± SEM. from $n = 10$ rats for each group. $^{*}p < 0.01$ *versus* non-ligated; $^{\circ}p < 0.01$ *versus* ligated.

is the mechanism by which 5-AIQ inhibits gingivomucosal tissues inflammation caused by ligation-induced periodontitis?

Using a new and potent PARS inhibitor (5-AIQ), we confirm here as previously described the an activation of PARP plays a role in the development of acute exudative vasculitis of the inflamed gingivomucosal tissue. Our study also confirmed earlier findings that one of the characteristic signs of inflammation, Evans blue extravasation, was higher on the ligated side on the eighth day, than on the opposite side (Gyorfi et al. 1994). In 5-AIQ-treated rats subjected to ligation-induced periodontitis, the Evans blue extravasation was largely attenuated. The mechanism behind a PARP-induced increase in epithelial permeability has been suggested to be a reduction in cellular ATP levels and a resultant breakdown of tight junctional integrity (Szabo & Dawson 1998). Moreover, Lohinai et al.(2003) have also pointed out the role of PARP in epithelial permeability, which is supported by the observation that endothelial cells were highly reactive for PARP, and that pharmacological and genetic PARP inhibition significantly reduced the ligature-induced extravasation. This observation is in agreement with a previous study, which has clearly demonstrated that the PARP activation was associated with endothelial dysfunction that was prevented or eversed by PARP inhibition (Szabo et al. 1997, Soriano et al. 2001, Jagtap et al. 2002).

In addition, in the present study, using 5-AIQ, we confirmed as previously described (Cuzzocrea et al. 2002) that inhibition of PARP activity leads to a reduction of inflammatory cells, infiltration as assessed by the specific granulocyte enzyme MPO and to the moderation of the tissue damage as evaluated by histological examination. This observation supports the role of PARP activity in the interaction of PMNs and endothelial cells. It is noteworthy, however, that tissue MPO activity was not completely abolished. This result is consistent with previous studies demonstrating that constitutive levels of ICAM-1 appear to be sufficient to support a lower degree of CD11/CD18-dependent trans-endothelial migration of activated PMNs (Furie et al. 1991, Kukielka et al. 1994).

Reduction of PMN infiltration was also paralleled with the inhibition of PAR immunoreactivity. Our finding (i.e. that PAR staining is reduced in 5-AIQ animals), coupled with the protective effects of PARP inhibition, proves the existence of a self-amplifying suicide cycle in which early oxidant production by endothelium activates PARP; the consequent endothelium injury with activation of PMN-attractive factors (e.g. ICAM-1) and PMN infiltration leads to further production of oxidants, which ultimately are responsible for the gingivomucosal tissue. Inhibition of PARP would intercept this cycle at the level of endothelial injury.

Several in vitro and in vivo studies have demonstrated that the catalytic activity of the nuclear enzyme PARP, induced by single DNA strand breakage, is a direct result of oxidant injury in conjunction with a variety of immunological stimuli, including bacterial endotoxin and cytokines. More specifically, it has been previously reported that oxidant injury by ROS induces metabolic changes and cytotoxicity in association with the intracellular elevation of PARP activity in macrophages and in pulmonary epithelial, smooth muscle and endothelial cells (Szabo & Dawson 1998). Recently, in vitro experiments with HUVEC cells have demonstrated that oxidant injury by peroxynitrite or TNF-α stimulation induces an up-regulation of P-selectin and ICAM-1 surface expression, a process that is prevented by the PARP inhibitor 5-AIQ (Cuzzocrea et al., 2002). Thus, due to its water solubility (as well as its potency), 5-AIQ currently represents a good pharmacological tool to elucidate the role of PARP in the pathophysiology of inflammation and other disease states (Thiemermann 2002).

In contrast to ''prototypical or classical'' PARP inhibitors such as benzamide analogues, which have been available for over 20 years, there is currently a dearth of information available on the selectivity and potential side-effects of more recently developed and potent PARP inhibitors such as the isoquinolinones (Szabo & Dawson 1998). In addition, the chronic use of 5-AIQ has now demonstrated side effects (Cuzzocrea et al. 2004). Although it has been established that 5-AIQ displays 1000-fold selectivity for PARP over mono(ADP-ribosyl) transferase (Mc Donald et al. 2000), the effects of 5-AIQ on other enzymes have not been established. As with most pharmacological inhibitors, we cannot exclude that additional, PARP synthetase independent effects may contribute to the anti-inflammatory effects observed with 5-AIQ in the current study. Furthermore, only limited pharmacodynamic and pharmacokinetic data exist for these novel PARP inhibitors and, therefore, appropriate caution should be exercised in experimental design and in the interpretation of data

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

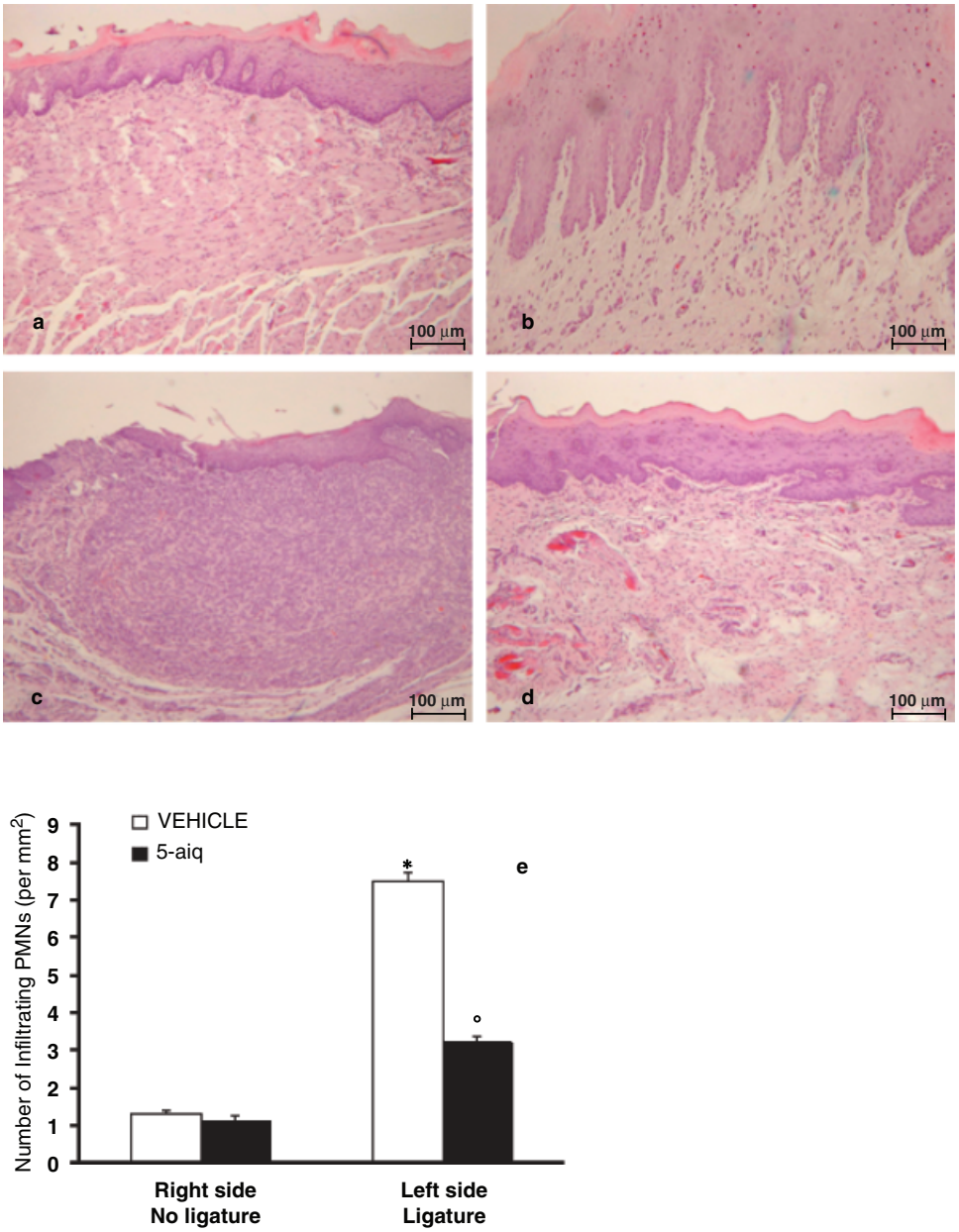

Fig. 3. Gingivomucosal section from the contralateral side from vehicle (a)- and 5-aminoisoquinolin-1(2*H*)-one (5-AIQ) (b)- treated rats demonstrating no tissue damage. Inflammatory cell infiltration and oedema were observed in the gingivomucosal section from ligature-treated rats (c). Significantly less oedema and inflammatory cells infiltration was observed in gingivomucosal section from ligature-treated rats that had been treated with 5-AIQ (d). The total number of infiltrating leucocytes (e.g., neutrophils and mononuclear cells) in gingivomucosal tissue was assessed quantitatively by counting the number of polymorphonuclear cells in 20 high-power fields (e). Figure is representative of at least three experiments performed on different experimental days. The tissue sections, oriented longitudinally from the teeth crown, were stained with trichrome stain. Data represent the mean ± SEM for 20 counts obtained from the gingivomucosal tissue of each treatment group. $^{*}p<0.01$ *versus* non-ligated; $^{\circ}p<0.01$ *versus* ligated.

obtained using compounds such as 5-AIQ.

Based on a previous study (Lohinai et al. 2003) as well as from the present study, we support a role for PARP in the process of gingivomucosal tissue injury and put forward the hypothesis that inhibition of PARP represents a novel anti-inflammatory strategy. The potential risk of this strategy is reduced by the demonstration that cells from the PARS knockout mice do not have a compromised DNA repair, although poly (ADP-ribose) synthetase is traditionally viewed as an enzyme that is important for the DNA repair processes (Wang et al., 1995). Further studies are needed to compare the efficacy of poly (ADP-ribose) synthetase inhibition with that of other, established or experimental anti-inflammatory approaches.

Acknowledgements

The authors would like to thank Lelio Di Paolo, Giovanni Pergolizzi and Carmelo La Spada for their excellent technical assistance during this study, Mrs. Caterina Cutrona for secretarial assistance and

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

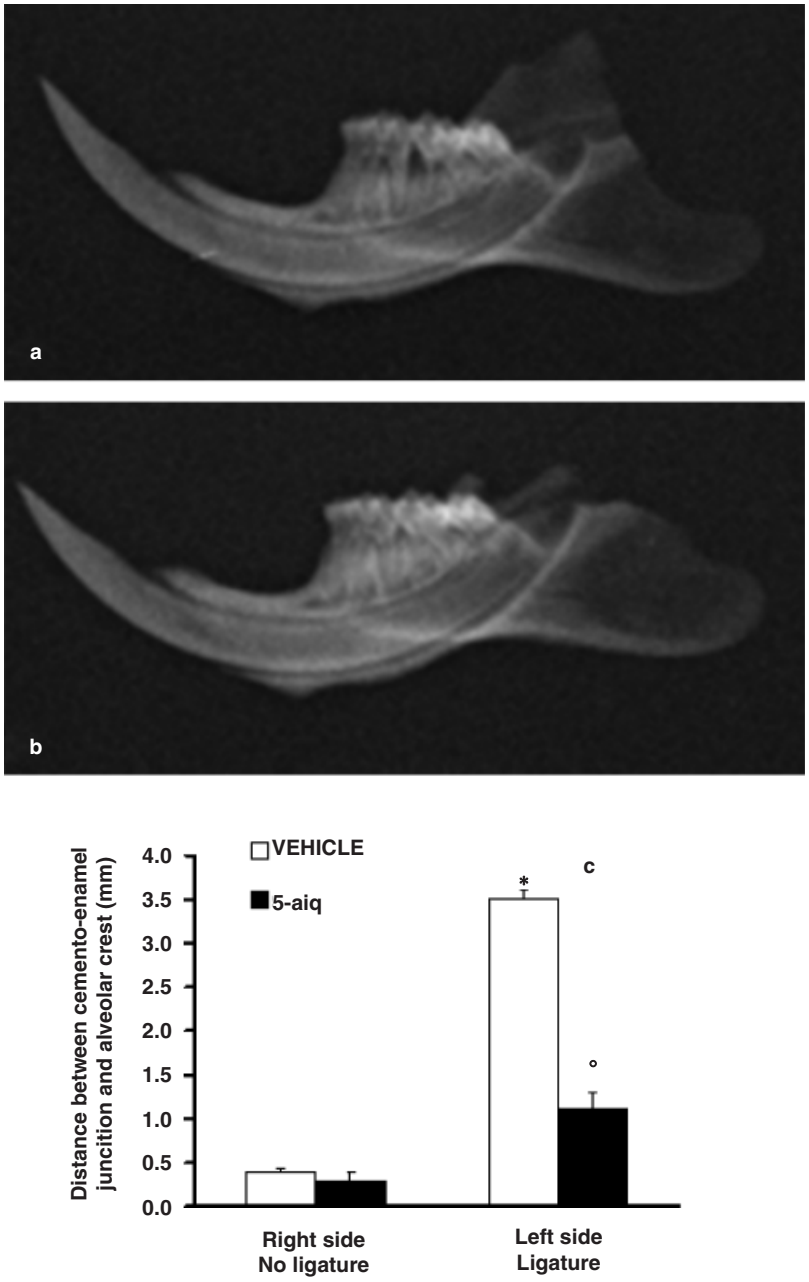

Fig. 4. The alveolar bone from ligated (8 days) rats demonstrated alveolar bone resorption (a). 5-aminoisoquinolin-1(2*H*)-one (5-AIQ) treatment suppressed alveolar pathology in the rat alveolar bone (b). A significant increase in the distance between the cemento-enamel junction and alveolar crest at mediolingual root of the first molar was observed in ligature-treated rats. 5-AIQ treatment significantly reduced the increase in the distance between the cemento-enamel junction and alveolar crest (c). Radiographic figure is representative of at least three experiments performed on different experimental days. Data represent the data from 20 counts obtained from the gingivomucosal tissue of each treatment group. $^{*}p<0.01$ *versus* non-ligated; $^{\circ}p<0.01$ *versus* ligated.

Miss Valentina Malvagni for editorial assistance with the manuscript.

References

Banasik, M., Komura, H. & Shimoyama, M. (1992) Specific inhibitors of poly (ADP-ribose) synthetase and mono (ADP-ribose) transferase. *Journal of Biological Chemistry* **267**, 1569–1157.

Battino, M., Bullon, P., Wilson, M. & Newman, H. (1999) Oxidative injury and inflammatory periodontal diseases; the challenge of anti-oxidants to free radicals and reactive oxygen species. *Critical Reviews in Oral Biology and Medicine* **10**, 458–476.

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

Cosi, C. (2002) New inhibitors of poly (ADP-ribose) polymerase and their potential therapeutic targets. *Expert Opinion on Therapeutic Patents* **12**, 1047–1071.

Crawford, J. M., Taubman, M. A. & Smith, D. J. (1978) The natural history of periodontal bone loss in germfree and gnotobiotic rats infected with periodontopathic microorganisms. *Journal Periodontal Research* **13**, 316–325.

Cuzzocrea, S., Mazzon, E., Di Paola, R., Genovese, T., Patel, N. S., Muia, C., Threadgill, M. D., De Sarro, A. & Thiemermann, C. (2004) 5-Aminoisoquinolinone reduces colon injury by experimental colitis. *Naunyn-Schmiedebergs Archives of Pharmacology* **370**, 464–473.

Cuzzocrea, S., Mc Donald, M. C., Mazzon, E., Dugo, L., Serraino, I., Threadgill, M., Caputi, A. P. & Thiemermann, C. (2002) Effect of 5-aminoisoquinolinone, a water-soluble, potent inhibitor of the activity of poly (ADP-ribose) polymerase, in a rodent model of lung injury. *Biochemical Pharmacology* **63**, 293–304.

Di Paola, R., Genovese, T., Caputi, A. P., Threadgill, M., Thiemermann, C. & Cuzzocrea, S. (2004a) Beneficial effects of 5-aminoisoquinolinone, a novel, potent, water-soluble, inhibitor of poly (ADP-ribose) polymerase, in a rat model of splanchnic artery occlusion and reperfusion. *European Journal of Pharmacology* **492**, 203–210.

Di Paola, R., Marzocco, S., Mazzon, E., Dattola, F., Rotondo, F., Britti, D., Genovese, T. & Cuzzocrea, S. (2004b) Effect of aminoguanidine in ligature-induced periodontitis in rats. *Journal of Dental Research* **83**, 343–348.

Furie, M. B., Tancinco, M. C. & Smith, C. W. (1991) Monoclonal antibodies to leukocyte integrins CD11a/CD18 and CD11b/CD18 or intercellular adhesion molecule-1 inhibit chemoattractant-stimulated neutrophil transendothelial migration in vitro. *Blood* **78**, 2089–2097.

Gagne, J. P., Hendzel, M. J., Droit, A. & Poirier, G. G. (2006) The expanding role of poly (ADP-ribose) metabolism: current challenges and new perspectives. *Current Opinion Cell Biology* **18**, 145–151.

Gyorfi, A., Fazekas, A., Suba, Z., Ender, F. & Rosivall, L. (1994) Neurogenic component in ligature-induced periodontitis in the rat. *Journal of Clinical Periodontology* **21**, 601–605.

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. *Journal of Biological Chemistry* **263**, 1665–1675.

Ikai, K. & Ueda, K. (1983) Immunohistochemical demonstration of poly (adenosine diphosphate-ribose) synthetase in bovine tissues.

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Journal of Histochemistry and Cytochemistry **31**, 1261–1264.

Jagtap, P., Soriano, F. G., Virag, L., Liaudet, L., Mabley, J. & Szabo, E. (2002) Novel phenanthridinone inhibitors of poly (adenosine 5′ -diphosphate-ribose) synthetase: potent cytoprotective and antishock agents. *Critical Care Medicine* **30**, 1071–1082.

Kukielka, G. L., Youker, K. A. & Hawkins, H. K. (1994) Regulation of ICAM-1 and IL-6 in myocardial ischemia: effect of reperfusion. *Annals of the New York Academy of Sciences* **723**, 258–270.

Lohinai, Z., Mabley, J. G., Feher, E., Marton, A., Komjati, K. & Szabo, C. (2003) Role of the activation of the nuclear enzyme poly (ADP-ribose) polymerase in the pathogenesis of periodontitis. *Journal of Dental Research* **82**, 987–992.

Mc Donald, M. C., Mota-Filipe, H., Wright, J. A., Abdelrahman, M., Threadgill, M. D., Thompson, A. S. & Thiemermann, C. (2000) Effects of 5-aminoisoquinolinone, a water-soluble, potent inhibitor of the activity of poly (ADP-ribose) polymerase on the organ injury and dysfunction caused by haemorrhagic shock. *British Journal of Pharmacology* **130**, 843–850.

Mota-Filipe, H., Sepodes, B. & McDonald, M. C. (2002) The novel PARP inhibitor 5-aminoisoquinolinone reduces the liver injury caused by ischemia and reperfusion in the rat. *Medical Science Monitor* **8**, BR444–BR453.

Mullane, K. M., Kraemer, R. & Smith, B. (1985) Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *Journal of Pharmacological Methods* **141**, 157–167.

Ridgeway, E. E. (2000) Periodontal disease: diagnosis and management. *Journal of the American Academy of Nurse Practitioners* **12**, 79–83.

Schraufstatter, I. U., Hinshaw, D. B., Hyslop, P. A., Spragg, R. G. & Cochrane, C. G. (1986a) DNA strand breaks activate poly adenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. *The Journal of Clinical Investigation* **77**, 1312–1320.

Schraufstatter, I. U., Hyslop, P. A., Hinshaw, D. B., Spragg, R. G., Sklar, L. A. & Cochrane, C. G. (1986b) Hydrogen peroxide induced injury and its prevention by inhibitors of poly (ADP-ribose) polymerase. *Proceedings of the National Academy of Sciences* **83**, 4908–4912.

Soriano, F. G., Pacher, P., Mabley, J., Liaudet, L. & Szabo, C. (2001) Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly (ADP-ribose) polymerase. *Circulation research* **89**, 684–691.

Suto, M. J., Turner, W. R., Arundel-Suto, C. M., Werbel, L. M. & Sebolt-Leopold, J. S. (1991) Dihydroisoquinolinones: the design and synthesis of a new series of potent inhibitors of poly (ADP-ribose) polymerase. *Anti-Cancer Drug Design* **6**, 107–117.

Szabó, G., Bährle, S. & Stumpf, N. (2002) Poly (ADP-ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. *Circulation Research* **90**, 100–106.

Szabo, C., Cuzzocrea, S., Zingarelli, B., O'Connor, M. & Salzman, A. L. (1997) Endothelial dysfunction in a rat model of endotoxic shock: importance of the activation of poly (ADP-ribose) synthetase by peroxynitrite. *Journal of Clinical Investigation* **100**, 723–735.

Szabo, C. & Dawson, V. L. (1998) Role of poly (ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends in Pharmacological Sciences* **19**, 287–298.

Thiemermann, C. (2002) Development of novel, water-soluble inhibitors of poly (adenosine 5′-diphosphate ribose) synthetase activity for use in shock and ischemia-reperfusion injury. *Critical Care Medicine* **30**, 1163–1165.

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. *Archives of Biochemistry and Biophysics* **286**, 353–363.

Virag, L. & Szabò, C. (2002) The therapeutic potential of poly (ADP-ribose) polymerase inhibitors. *Pharmacological Reviews* **54**, 375–429.

Wang, Z. Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M. & Wagner, E. F. (1995) Mice lacking ADPRT and poly (ADP-ribosyl)ation develop normally but are susceptible to skin disease. *Genes and Development* **9**, 509–520.

Wayman, N. S., McDonald, M. C. & Thompson, A. C. (2001) 5-Aminoisoquinolinone a potent inhibitor of poly (adenosine 5'diphosphate ribose) polymerase, reduces myocardial infarct size. *European Journal of Pharmacology* **430**, 93–100.

Wenkert, E., Johnston, D. B. R. & Dave, K. G. (1964) Derivatives of hemimellitic acid. A synthesis of erythrocentaurin. *Journal of Organic Chemistry* **29**, 2534–2542.

Address:
Salvatore Cuzzocrea.
Institute of Pharmacology
School of Medicine
University of Messina
Torre Biologica–Policlinico Universitario
Via C. Valeria–Gazzi 98100 Messina
Italy
E-mail: salvator@unime.it

Clinical Relevance

Scientific rationale for the study: Poly (ADP-ribose) polymerase (PARP) is related to the degree of severity of periodontal disease in rats.

Principal findings: This is likely the result of release of reactive oxygen species by activated phagocytes and fibroblasts in the inflamed periodontal tissues.

Practical implications: The results of our study suggest that 5-AIQ, a water-soluble PARP inhibitor, may be a useful adjunctive treatment for periodontitis.

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.