PHYTOTHERAPY RESEARCH
Phytother. Res. **22**, 1075–1082 (2008)
Published online 20 June 2008 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2458

Phytomodulatory Potentials of *Aloe vera* against *Salmonella* OmpR-mediated Inflammation

Praveen Rishi[1]*, Anjali Rampuria[1], Rupinder Tewari[2] and Ashwani Koul[3]

[1]Department of Microbiology, Panjab University, Chandigarh 160014, India
[2]Department of Biotechnology, Panjab University, Chandigarh 160014, India
[3]Department of Biophysics, Panjab University, Chandigarh 160014, India

Mediators released during inflammatory response play an essential role in eliminating microbes or microbial products. However, the uncontrolled release of cytotoxic substances characterized by extensive inflammation may adversely affect normal tissues. Under such conditions it is important to manage the hyperinflammation in order to change the clinical manifestations of the disease. Accordingly, the present study was designed to evaluate the modulation of *Salmonella* OmpR mediated inflammation by *Aloe vera*, a plant known to contain antiinflammatory ingredients. It was observed that outer-membrane proteins (OMPs) extracted from the wild type strain of *S. typhimurium* caused inflammation of greater magnitude compared with the OMPs extracted from its mutant construct as evident from the oedema test as well as the hyperalgesic (flicking) response of the animals under experimental conditions. However, *Aloe vera* applied topically, administered intraperitoneally or in combination modulated the inflammatory response. The maximum effect was observed with the combined formulation indicating modulation at local as well as systemic levels. The results reveal that this modulation could be due to the potential of *Aloe vera* to decrease peroxidative damage via a decrease in the levels of monokines (TNF-α, IL-1 and IL-6) and an increase in the level of superoxide dismutase (SOD). Moreover, the presence of SOD in *Aloe vera* itself might be responsible for enhancing its levels in the macrophages. On the other hand, no significant change in the catalase activity was observed by *Aloe vera* treatment. The use of *Aloe vera*, therefore, seems to have a promising role in the modulation of *Salmonella* OmpR mediated inflammation.
Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: Aloe vera; S*almonella* OMPs; oxidative stress; inflammation; monokines.

INTRODUCTION

Inflammation is an important biological implication, which occurs in response to noxious stimuli and to local injury. *Salmonella* is responsible for a variety of infections in humans and domestic animals. This may range from gastroenteritis (an acute localized inflammation of the intestine) caused by *Salmonella typhimurium* to severe systemic disease caused by *Salmonella typhi* (Monack *et al.*, 2004). In Salmonellosis, the ensuing inflammatory response of the intestinal mucosa has long been associated with *Salmonella* virulence (Giannella, 1979) due to several determinants expressed by this pathogen. There is a vast amount of information regarding the expression of outer-membrane proteins (OMPs) regulated by *OmpR* and their role in virulence, immunogenicity as well as antimicrobial resistance (Chanana *et al.*, 2005; Choudhary *et al.*, 2005; Sood *et al.*, 2005; Ausiello *et al.*, 2006). The *ompR* and *envZ* genes form a two-gene operon. OmpR has been identified as a positive activator of gene expression (Kawaji *et al.*, 1979; Noroika *et al.*, 1986), while EnvZ acts as an environmental sensor and transmits signal to OmpR, which then modulates transcription of various genes (Hall and Silhavy, 1979). However, their role in the induction of inflammation is not well understood.

The uncontrolled release of cytotoxic substances (Miesel and Zuber, 1993) and proinflammatory mediators including cytokines (tumor necrosis factor and interleukins) (Stenson, 1990; Klimpel *et al.*, 1995) by the migrating cells resulting into oxidative stress, may damage the host tissues as well. Therefore, under such conditions, it is necessary to manage the hyperinflammation to a useful level, to change the clinical manifestation of the disease. One potential approach is the use of anti-inflammatory drugs. Apprehending the undesirable effects of drugs such as dexamethasone and indomethacin, the scientific interest has now been diverted towards the natural compounds which are biocompatible, safe and are also cost effective. Thus, efforts are continuously being made to identify such agents and to validate their scientific authenticity. This could lead to their rational use as chemopreventive/therapeutic agents.

Aloe vera is one such plant with enormous medicinal values. It is a perennial, drought-resisting, succulent plant belonging to the Liliaceae family. Clinical evaluations have revealed that the pharmacologically active ingredients of this plant are concentrated in both the

* Correspondence to: Associate Professor Dr Praveen Rishi, Basic Medical Sciences Building, Department of Microbiology, Panjab University, Chandigarh 160014, India.
E-mail: rishipraveen@yahoo.com

Received 18 April 2007
Revised 11 October 2007
Accepted 7 November 2007

Copyright © 2008 John Wiley & Sons, Ltd.

gel and the rind of *Aloe vera* leaves (Green, 1996; Heggers, 1996). These active ingredients are thought to have acute and chronic antiinflammatory activities (Langmead *et al.*, 2004; Sarkar *et al.*, 2005). On the basis of this information, the present study was designed to explore the potential of *Aloe vera* to modulate *Salmonella typhimurium*-OmpR mediated inflammation, if any, and the underlying mechanism involved in it.

MATERIAL AND METHODS

***Aloe vera* preparation.** Commercially available 90% w/w green leaf preparation of *Aloe vera* (Brihans) was evaluated for its antiinflammatory potential.

Bacterial strain and growth conditions. In the present study, the wild type strain of *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) and its mutant construct (*ompR*$^-$) were used. The strains were checked for purity and were characterized biochemically as well as serologically. These strains were maintained in 10% glycerol broth and also stored as lyophilized ampules at −80 °C (U41085, Ultra Low Freezer, New Brunswick Co. USA). Bacteria were grown on nutrient agar plates for 24 h at 37 °C and a single colony was used to prepare seed culture. A 0.1% inoculum from seed culture was used for growing the bacteria.

Analysis of outer membrane proteins (OMPs). After growing the bacteria, their outer membrane proteins were extracted and the protein profile was analysed using SDS-PAGE as described earlier (Chanana *et al.*, 2006).

Animals. Balb/c mice (25–30 g) were procured from the Central Animal House, Panjab University, Chandigarh. Animals were kept in polypropylene cages bedded with clear rice husk under well-aerated conditions at room temperature (25 ± 2 °C). The mice in all the groups had free access to standard animal pellet diet (Ashirwad Industries Ltd, Ropar, Punjab) and clean drinking water throughout the experiment. All experimental protocols were approved by the Institutional Animals Ethics Committee, Panjab University, Chandigarh (India).

Experiment design. Animals were divided into the following groups each comprising six mice. Each mouse was injected intradermally with 0.1 mL of the following preparations in the dorsal foot pad of the left paw: (i) 1% w/v of carrageenan (Hainan Kaiyang Trade Co. Ltd, China) (positive control); (ii) OMPs extracted from the wild type strain of *S. typhimurium*; (iii) OMPs extracted from the mutant construct of *S. typhimurium*; (iv) *Aloe vera* administration i.p. 3 h prior to OMPs extracted from wild type strain; (v) *Aloe vera* administration i.p., 3 h prior to OMPs extracted from mutant strain;. (vi) *Aloe vera* applied (topically) immediately before injection of OMPs from wild type strain; (vii) *Aloe vera* applied (topically) immediately before injection of OMPs from mutant strain; (viii) Combined treatment (i.p. + topical application) with *Aloe vera* before infection of OMPs from wild type strain; (ix) Combined treatment (i.p. + topical application) with *Aloe vera* before infection of OMPs from mutant strain; (x) normal saline (negative control).

Assessment of antiinflammatory potential of *Aloe vera*

Oedema test. The effect of *Aloe vera* on OMPs induced inflammation was assessed by the oedema test qualitatively as well as quantitatively. All the mice were checked at regular intervals up to 3 h for inflammation in the dorsal foot pad of left paw. Quantitative assessment of oedema was done using the plethysmometer, which is a simple apparatus containing mercury. The groups of mice listed above were marked on both the hind paws (right and left), just beyond the tibiotarsal junction so that every time the paw was dipped to the same level in the mercury column of plethysmometer. The mercury displacement due to dipping of the paw can be directly read from the scale attached to the mercury column.

Hyperalgesia test. The effect of *Aloe vera* on thermal hyperalgesia during inflammation was assessed by the paw immersion (warm water) test. The animals were marked as described above, so that every time the mouse paw was dipped to the same level in the water bath. The mouse paws were immersed in the warm water bath (47 ± 0.5 °C) until paw withdrawal (flicking response) or signs of struggle were observed (cut off 10 s). The paw flicking response in terms of the time in each of the above groups of mice was recorded.

Assessment of inflammatory mediators released by macrophages

Isolation of peritoneal macrophages and their interaction with OMPs. Every time fresh murine peritoneal macrophages were isolated according to the method of Chander *et al.* (2005). The cell viability was checked with 0.2% trypan blue staining. For the interaction, 10^6 macrophages/mL was treated with 10 μg of protein and incubated in a humidified atmosphere containing 5% CO_2 at 37 °C for 16 h in the absence/presence of *Aloe vera*. Each reaction mixture contained 10 μL of commercially available *Aloe vera* aqueous extract (90% w/w). The dose of the protein and time of interaction was optimized after thorough standardization using 2, 5, 7, 10 and 15 μg of OMPs for 2 h, 5 h, 10 h and then for 15 h, 16 h, 17 h, 18 h, 19 h and 20 h. The culture supernatants of macrophages were used for the following experiments.

Estimation of malondialdehyde (MDA) as an index of lipid peroxidation. The quantitative measurement of lipid peroxidation, in the culture supernatants of macrophages, was performed according to the method of Wills (1966). The amount of MDA formed (a measure of lipid peroxidation) was assayed by the reaction with thiobarbituric acid (TBA). In brief, to 0.5 mL of culture supernatant, 0.5 mL of Tris-HCl buffer (0.1 M, pH 7.4) was added and the mixture was incubated at 37 °C for 2 h. Following incubation, 1.0 mL of 10% (w/v) ice-cold trichloroacetic acid (TCA) was added and the mixture was centrifuged at 3000 × **g** for 10 min. To 1.0 mL of supernatant (obtained after centrifugation), 1.0 mL of 0.67% (w/v) TBA was added and the mixture was kept in a boiling water bath for 10 min. After cooling the tubes with tap water, 1.0 mL of distilled water was added and the absorbance was measured at

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

532 nm. The results were expressed as nanomoles of MDA per milligram of protein.

Estimation of superoxide dismutase (SOD) activity. SOD activity was assayed according to the method of Kono (1978). The reaction was initiated by the addition of 0.5 mL of hydroxylamine hydrochloride to the reaction mixture containing 2.0 mL of nitro blue tetrazolium (NBT) and 0.1 mL of culture supernatant. The change in absorbance was measured spectrophotometrically at 560 nm. The SOD activity was expressed as units of SOD per milligram of protein where one unit of activity is defined as the amount of SOD required to inhibit the rate of reduction of NBT by 50%. Finally the result was calculated by taking the SOD inhibition of the control as 100%.

Estimation of catalase activity. The catalase activity was assayed by the method of Claiborne (1985). The assay mixture consisted of 1.95 mL phosphate buffer (0.05 M, pH 7.0), 1.0 mL of hydrogen peroxide and 0.05 mL of culture supernatant in a final volume of 3.0 mL. The change in absorbance was recorded spectrophotometrically at 240 nm. The results were expressed in terms of K min^{-1}.

Monokine assays. The cell free culture supernatants were collected at the indicated time. The cytokine (IL-1α, IL-6 and TNF-α) activity in the culture supernatants was assessed by enzyme-linked immunosorbent assay (ELISA) using mouse cytokine ELISA kits (Chemicon, USA) according to the manufacturer's instructions. Briefly, 96-well microtitre plates precoated with monoclonal antibodies generated against mouse IL-1α, IL-6 and TNF-α were used to capture the respective monokines produced in the culture supernatants of the macrophages. The assays were visualized using streptavidin alkaline phosphatase conjugate for IL-1α and TNF-α. For IL-6 the assay was visualized using a goat anti-rabbit- alkaline phosphatase conjugate and an ensuing chromagenic substrate reaction. The ELISA was sensitive to 0.2 pg/mL of the cytokines released.

Statistical analysis. The results were expressed as mean ± SD. The inter group variation was measured by one-way analysis of variance (ANOVA) followed by Fischer's LSD test. The statistical analysis was done using Jandel Sigma Stat Statistical Software version 2.0. The statistical significance of the results were calculated as at least $p < 0.05$.

RESULTS

Analysis of outer membrane proteins (OMPs)

A number of proteins were found to be expressed by *S. typhimurium* with varying intensities as assessed by SDS-PAGE analysis (Fig. 1). In the wild type strain, two proteins shown with arrows in the range of 28–55 kDa were found to be expressed with enhanced intensity compared with their respective mutant construct (*ompR*$^-$) in lanes 3 and 4. Another band marked by an arrow head between 35–55 kDa was observed in the wild type strain (lanes 1, and 2) but could not be observed in its respective mutant construct (*ompR*$^-$) (lanes 3 and 4).

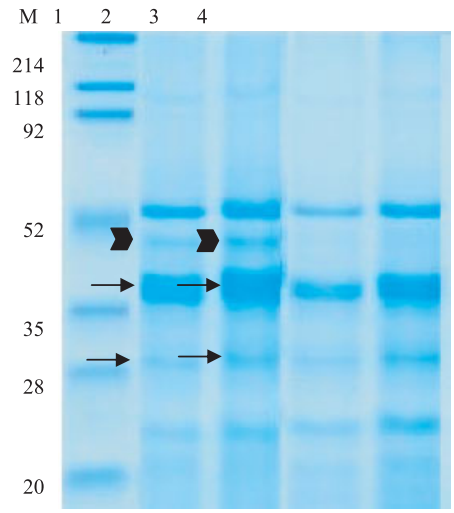

Figure 1. Analysis of OMPs from wild type and mutant construct of *S. typhimurium* by SDS-PAGE. Lane M: standard molecular weight; Lane 1 and 2: OMPs from wild strain of *S. typhimurium* under normal condition; Lane 3 and 4: OMPs from mutant strain of *S. typhimurium* under normal conditions.

Qualitative assessment of inflammation

Inflammation in all the groups was found to be maximum at 3 h post injection. No inflammation was observed in the right paw of mice injected with normal saline which served as negative control. Inflammation was found to be maximum in the mice injected with carrageenan Fig. 2A(a) compared with all the groups. Figures 2A(b) and (c) show inflammation of the mice hind paw due to OMPs extracted from wild type and mutant strain.

In the groups treated with *Aloe vera* prior to OMPs administration, a maximal reduction in inflammation, compared with their respective untreated groups was observed when the mice were administered *Aloe vera* by the intraperitoneal route as well as topical application (Figs 2A(d) and (e).

Quantitative assessment of oedema

Inflammation induced by wild type strain under normal conditions was significantly ($p < 0.001$) higher than that of its mutant construct. Oedema was significantly ($p < 0.01$) reduced upon treatment with *Aloe vera* (Fig. 2B). In the treated groups, oedema observed in the case of the mutant strain showed significant ($p < 0.01$) reduction compared with its corresponding wild type. Intraperitoneal administration as well as topical application of *Aloe vera* significantly ($p < 0.01$) reduced oedema compared with that observed when treatment was given separately.

Hyperalgesic response

Figure 3 depicts the thermal hyperalgesia response of mice with inflamed paws. The time required for the withdrawal of the paw, injected with OMPs extracted from wild type and its mutant construct, was significantly shorter than the control paw, indicating hyperalgesia. Thermal hyperalgesia during inflammation induced by wild type strain was significantly ($p < 0.001$)

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

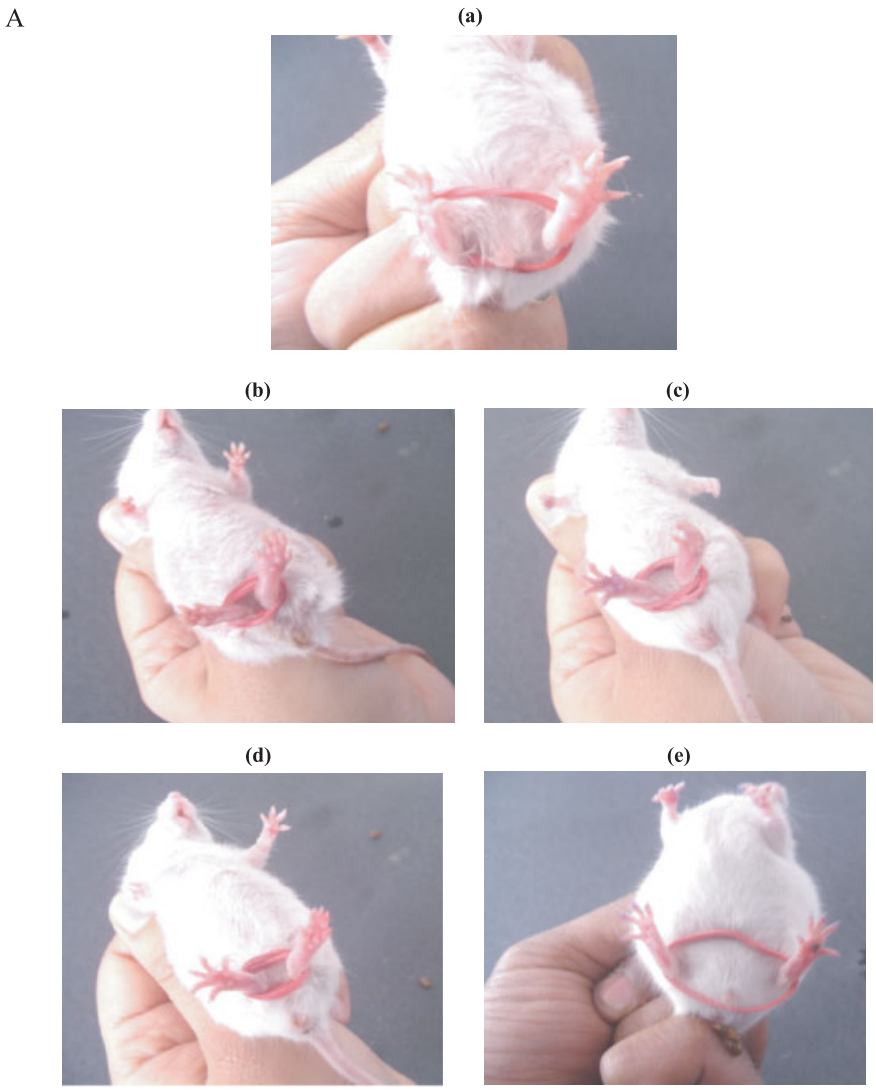

Figure 2A. (a) Mouse injected with carrageenan (positive control) on the left foot paw showing oedema. (b) Mouse showing inflammation in the left paw injected with OMPs extracted from the wild type strain; (c) Mouse showing inflammation in the left paw injected with OMPs extracted from the mutant construct strain; (d) Mouse showing inflammation in the left paw injected with wild type OMPs after treatment with *Aloe vera* (i.p. + topical), indicating marked reduction in inflammation; (e) Mouse showing inflammation in the left paw injected with mutant construct OMPs after treatment with *Aloe vera* (intraperitoneal route and topical application), indicating marked reduction in inflammation.

higher than that induced by its mutant construct. The hyperalgesic response was significantly ($p < 0.01$) reduced upon treatment with *Aloe vera* (Fig. 3). In the treated groups, the hyperalgesia response observed in the case of the mutant strain showed a significant ($p < 0.01$) reduction compared with its corresponding wild type. Combined treatment with *Aloe vera* significantly ($p < 0.01$) reduced the hyperalgesia response when compared with the individual topical application or intraperitoneal administration.

Malondialdehyde (MDA) levels as an index of ROS generation

A significant increase in the MDA level was observed in the macrophages treated with OMPs compared with the control macrophages with the levels being highest in the macrophages treated with OMPs from the wild type strains. MDA levels observed in the macrophages interacted with *Aloe vera* showed a significant ($p < 0.001$) decrease compared with the macrophages which were interacted with OMPs in the absence of *Aloe vera*. In fact, the MDA levels of macrophages interacted with OMPs in the presence of *Aloe vera* were comparable to the control group ($p < 0.001$).

Superoxide dismutase and catalase activity

The activity of the antioxidant defense enzyme SOD was significantly lower in the macrophages treated with OMPs compared with the control, with the activity being the lowest in the wild type OMPs treated macrophages. When compared with SOD levels of macrophages in the absence of *Aloe vera*, there was a significant increase ($p < 0.001$) in the SOD levels of macrophages in the presence of *Aloe vera*. However, the highest SOD level was observed in macrophages interacted with the mutant construct OMPs ($p < 0.001$) in the presence of *Aloe vera* (Table 1). However, no significant change in the catalase activity was observed in these groups.

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

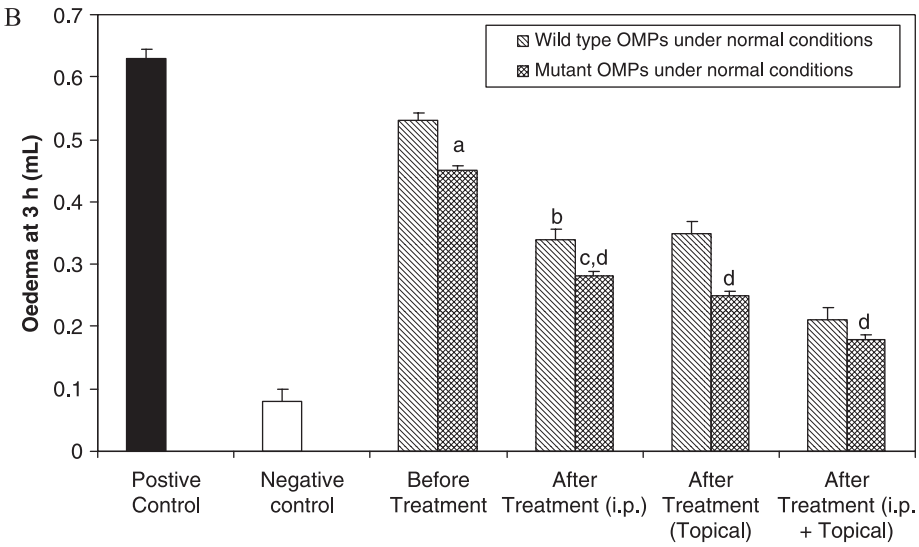

Figure 2B. Oedema measured by plethysmometer scale, in the left paw injected with OMPs from wild type and its mutant construct in the presence/absence of *Aloe vera*.
'a' depicts statistical significance ($p < 0.005$) between wild type and mutant OMPs (before treatment); 'b' depicts statistical significance between wild type OMPs before and after the treatment; 'c' depicts statistical significance between mutant construct OMPs before and after the treatment; 'd' depicts statistical significance between wild type and mutant OMPs after treatment with their respective counterparts.

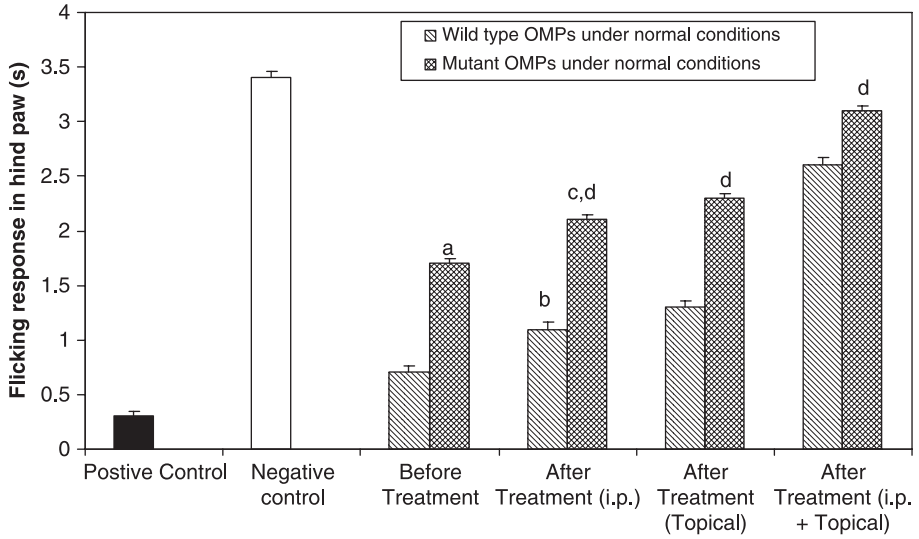

Figure 3. Hyperalgesia response in mice observed after 3 h hind paw injected with wild type and its mutant construct OMPs in the presence and absence *Aloe vera*. 'a' depicts statistical significance ($p < 0.005$) between wild type and mutant OMPs (before treatment); 'b' depicts statistical significance between wild type OMPs before and after the treatment; 'c' depicts statistical significance between mutant construct OMPs before and after the treatment; 'd' depicts statistical significance between wild type and mutant OMPs after treatment with their respective counterparts.

Table 1. Modulatory effect of *Aloe vera* on status of lipid peroxidation and antioxidation defense enzymes

		Before treatment		After treatment	
	Control	Wild type OMPs	Mutant OMPs	Wild type OMPs	Mutant OMPs
MDA	0.08 ± 0.005	0.24 ± 0.007	0.20 ± 0.006[a]	0.12 ± 0.004[b]	0.11 ± 0.007[c]
SOD	0.60 ± 0.015	0.24 ± 0.009	0.42 ± 0.020[a]	0.46 ± 0.013[b]	0.58 ± 0.020[c]
Catalase	0.50 ± 0.010	0.31 ± 0.009	0.29 ± 0.005	0.30 ± 0.012	0.30 ± 0.006

Values are expressed as mean ± SD ($n = 6$).
[a] Statistical significance ($p < 0.005$) between wild type and mutant OMPs (before treatment).
[b] Statistical significance between wild type OMPs before and after treatment.
[c] Statistical significance between mutant construct OMPs before and after treatment.

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

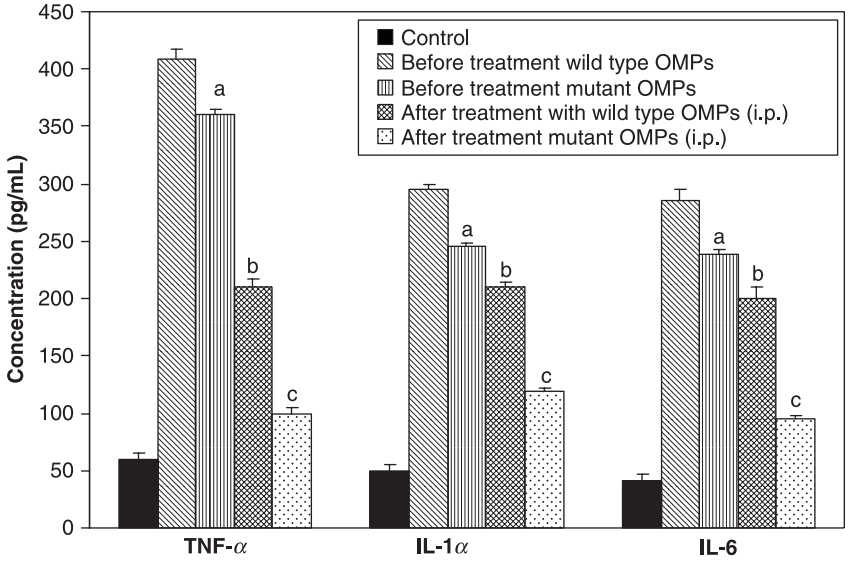

Figure 4. Levels of monokines (TNF-α, IL-1 and IL-6) in the culture supernatants of macrophages interacted with OMPs from *S. typhimurium* wild strain and its mutant construct in the presence and absence of *Aloe vera* after 16 h of incubation. 'a' depicts statistical significance ($p < 0.005$) between wild type and mutant OMPs (before treatment); 'b' depicts statistical significance between wild type OMPs before and after the treatment; 'c' depicts statistical significance between mutant construct OMPs before and after the treatment.

Monokine assays

Enhanced levels of monokines were observed when macrophages were interacted with OMPs compared with the levels in the non-interacted macrophages. However, the level of TNF-α was found to be the maximum compared with the IL-α and IL-6 (Fig. 4). Moreover, the levels were found to be higher in the wild type OMPs treated macrophages compared with the mutant construct OMPs treated macrophages, indicating the *OmpR* mediated inflammation. However, *Aloe vera* treatment could significantly reduce the levels of these monokines, though the levels remained higher in comparison with the levels in the control macrophages (Fig. 4).

DISCUSSION

Of the various virulence determinants, OMPs present on the surface of the *Salmonella* sp., have been found to be immunogenic. They help in the adherence of bacteria to the host cell and play a vital role in the bacterial adaptation to host niches, which are otherwise usually hostile to the invading pathogens (Koebnik *et al.*, 2000; Lin *et al.*, 2002; Choudhary *et al.*, 2005; Uchiyama *et al.*, 2006). It is also known that OMPs have an inflammatory potential, which results in the release of cytokines (Galdiero *et al.*, 1990, 1993; Chanana *et al.*, 2005). In the present study also, OMPs mediated oedema and hyperalgesia might have resulted due to increased diacylglycerol levels thus activating protein kinase C (PKC), which has been implicated in changes in pain perception (Anjaneyulu and Chopra, 2003). This may in turn result in the increased generation of ROS as prostaglandin H synthase has been reported as a potential endogenous substrate for the hydroperoxidase (Eling *et al.*, 1986). Moreover, there is enough evidence in the literature clearly suggesting the excessive production of ROS during infection/inflammation (Rishi *et al.*, 2006; Choudhary *et al.*, 2005).

The controlled production and release of inflammatory mediators play an essential role in the mechanism of phagocytosis and killing of the infectious microorganisms, thereby protecting the host. However, the viability of the normal tissue can be adversely affected during disease processes characterized by extensive inflammation (Arnold *et al.*, 1993; Herbert *et al.*, 1996). It is thus crucial to modulate *S. typhimurium* OMPs induced hyperinflammatory response.

Steroids (e.g. hydrocortisone) suppress inflammation but can cause weight gain, brittle bones and thin, weak skin. Non-steroidal antiinflammatory drugs (e.g. aspirin and ibuprofen) ease swollen joints and improve mobility but may trigger stomach upsets or peptic ulcers. Therefore, attempts are being made to identify biocompatible and cost effective antiinflammatory agents. A number of plant extracts have been shown to modulate antioxidant defense enzymes in various models (Sharma *et al.*, 2000; Koul *et al.*, 2006). Thus, phytochemicals stretch the boundaries of the conventional medicinal paradigm.

Plant steroids in *Aloe vera* can relieve inflammation, help to regenerate new cells and disperse damaged tissue with no known side effects (Davis, 1994a, 1994b). In the present study, it was observed that *Aloe vera* could modulate *Salmonella OmpR* mediated inflammation. It significantly reduced hyperalgesia which may be attributed to inhibition of prostaglandins production (Langmead *et al.*, 2004) and due to the release of an enzyme known as carboxypeptidase which hydrolyses bradykinin (Obata *et al.*, 1993; Shelton, 1991; Bautista-Perez *et al.*, 2004) thus resulting in an analgesic effect. The results coincide well with those reported by Sarkar *et al.* (2005). The maximum effect of *Aloe*

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

vera was observed with the combined formulation of topical application as well as intraperitoneal injection, in the present study, indicating its modulatory effect at the local as well as systemic level.

There is evidence that during *Salmonella* induced infection, there is an excessive production of ROS which leads to lipid peroxidation and finally to tissue damage (Rishi *et al.*, 2006). In the present study also, OMPs enhanced the extent of lipid peroxidation and decreased SOD levels. However, *Aloe vera* treatment reduced the extent of LPO and enhanced the level of antioxidant enzyme SOD. t' Hart *et al.* (1990) have also shown that the aqueous extract of the *Aloe vera* gel inhibits the release of reactive oxygen species. It has also been reported that *Aloe vera* contains antioxidant enzymes such as SOD which are involved in scavenging reduced oxygen species (Sabeh, 1995). In the present condition also, *Aloe vera* might have decreased LPO by up-regulating the levels of antioxidants and therefore, counteracting the oxidative stress resulting in inflammatory response to a useful level.

It is known that stimulation of TNF-α results in a rapid rise in the levels of intracellular ROS in various cells (Larrick and Wright, 1990). Moreover, cellular sensitivity or resistance to TNF-α is correlated with decreased or increased levels of SOD, respectively (Hirose *et al.*, 1993). In the present study TNF-α levels in the presence of *Aloe vera* dropped maximally compared with other monokines i.e. IL-1α and IL-6 levels, indicating the crucial role of TNF-α in the disease process of this pathogen. A similar phenomenon was observed by Sarkar *et al.* (2005) who suggested that *Aloe vera* causes inactivation of macrophages by modulating the production of cytokines, i.e. TNF-α and IL-1.

The results of the present study indicate a central role of TNF-α in conjunction with IL-1 and IL-6 in triggering ROS generation and down-regulating the SOD levels during an *OmpR* mediated inflammatory response. However, the inflammatory response induced by OMPs was observed to be modulated by *Aloe vera*. The use of *Aloe vera*, therefore, seems promising as an antiinflammatory agent against *Salmonella* OMPs.

REFERENCES

Anjaneyulu M, Chopra K. 2003. Quercetin, a bioflavonoid, attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain. *Prog Neuropsychopharmacol Biol Psychiatry* **27**: 1001–1005.

Arnold JW, Niesel DW, Annable CR *et al.* 1993. Tumor necrosis factor-α mediates the early pathology in *Salmonella* infection of the gastrointestinal tract. *Microbiol Pathog* **14**: 217–227.

Ausiello CM, Fedel G, Palazzo R, Spensieri F, Cierve A, Cassone A. 2006. 60-kDa heat shock protein of *Chlamydia pneumoniae* promotes a T helper type 1 immune response through IL-12/IL-23 production in monocyte-derived dendritic cells. *Microbes Infect* **8**: 714 –720.

Bautista-Perez R, Segura-Cobos D, Vasquez-Cruz B. 2004. *In vitro* antibradykinin activity of *Aloe barbadensis* gel. *J Ethnopharmacol* **93**: 89–92.

Chanana V, Majumdar S, Rishi P. 2006. Tumour necrosis factor-α mediated apoptosis in murine macrophages by *Salmonella enterica* serovar Typhi under oxidative stress. *FEMS Immun Med Microbiol* **47**: 278.

Chanana V, Sehgal R, Rishi P. 2005. *Salmonella typhi* iron-regulated outer-membrane proteins cause oedema and hyperalgesia during inflammation induced in a rat model. *J Med Microbiol* **54**: 421–423.

Chander H, Majumdar S, Sapru S, Rishi P. 2005. Macrophage cell death due to *Salmonella enterica* serovar Typhi and its acid stress protein has features of apoptosis. *Microbiol Immunol* **49**: 323–330.

Choudhary A, Tiwari RP, Koul A, Chanana V, Gupta S, Rishi P. 2005. Role of *Salmonella* surface components in immunomodulation of inflammatory mediators. *Mol Cell Biochem* **270**: 167–175.

Claiborne A. 1985. *Handbook of Methods for Oxygen Radical Research.* CRC Press: Boca Raton, 267–278.

Davis RH, DiDonato JJ, Johnson RW, Stewart CB. 1994a. Aloe vera, hydrocortisone, and sterol influence on wound tensile strength anti-inflammation. *J Am Podiatr Med Assoc* **84**: 614–621.

Davis RH, Donato JJ, Hartman GM, Haas RC. 1994b. Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. *J Am Podiatr Med Assoc* **84**: 77–81.

Eling TE, Curtis JF, Harman LS, Mason RP. 1986. Oxidation of glutathione to its thiyl free radical metabolite by prostaglandin H synthase. A potential endogenous substrate for the hydroperoxidase. *J Biol Chem* **261**: 5023–5028.

Galdiero F, Cipollario G, Benedetto N, Galdiero M, Tufano MA. 1993. Release of cytokines induced by *Salmonella typhimurium* porins. *Infect Immun* **61**: 155–161.

Galdiero F, Tufano MA, Galdiero M, Masiello S, Di Rosa M. 1990. Inflammatory effects of *Salmonella typhimurium* porins. *Infect Immun* **58**: 3183–3186.

Giannella RA. 1979. Importance of the intestinal inflammatory reaction in *Salmonella*-mediated intestinal secretions. *Infect Immun* **23**: 140–145.

Green P. 1996. Aloe vera extracts in equine clinical practice. *Vet Times* **26**: 9.

Hall MN, Silhavy TJ. 1979. Transcriptional regulation of *Escherichia coli* K-12 major outer membrane protein 1b. *J Bacteriol* **140**: 342–350.

Heggers JP. 1996. Beneficial effects of *Aloe* on wound healing in an excisional wound healing model. *J Altern Complement Med* **2**: 271–277.

Herbert JM, Corseaux D, Lale A, Bernat A. 1996. Hypoxia primes endotoxin-induced tissue factor expression in human monocytes and endothelial cells by a PAF-dependent mechanism. *J Cell Physiol* **169**: 290–299.

Hirose K, Longo DL, Oppenheim JJ, Matsushima K. 1993. Over expression of mitochondrial manganese superoxide dismutase promotes the survival of tumor cells exposed to interleukin-1, tumor necrosis factor, selected anti cancer drugs, and ionizing radiation. *FASEB J* **7**: 361–368.

Kawaji H, Mizuno T, Mizushima S. 1979. Influence of molecular size and osmolarity of sugars and dextrans on the synthesis of outer membrane proteins O-8 and O-9 of *Escherichia coli* K-12. *J Bacteriol* **140**: 843–847.

Klimpel GR, Asuncion M, Haithcoat J, Niesel DW. 1995. Cholera toxin and *Salmonella typhimurium* induce different cytokine profiles in the gastrointestinal tract. *Infect Immun* **63**: 1134–1139.

Koebnik R, Locher KP, Gelder PU. 2000. Structure and function of bacterial OMP: barrels in a nutshell. *Mol Microbiol* **37**: 239–253.

Kono Y. 1978. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys* **186**: 189–195.

Koul A, Ghara AR, Gangar SC. 2006. Chemomodulatory effect of *Azadirachta indica* on the hepatic status of skin tumor bearing mice. *Phytother Res* **20**: 169–177.

Langmead L, Makins RJ, Rampton DS. 2004. Anti-inflammatory effects of *Aloe vera* gel in human colorectal mucosa *in vitro*. *Aliment Pharmacol Ther* **19**: 521–570.

Larrick JW, Wright SC. 1990. Cytotoxic mechanism of tumor necrosis factor-alpha. *FASEB J* **4**: 3215–3223.

Lin J, Huang S, Zang Q. 2002. Outer membrane proteins: Key players for bacterial adaptation in hot niches. *Microbes Infect* **4**: 325–331.

Miesel R, Zuber M. 1993. Copper-dependent antioxidase

Copyright © 2008 John Wiley & Sons, Ltd.

defenses in inflammatory and autoimmune rheumatic diseases. *Inflammation* **17**: 283–294.
Monack DM, Bouley DM, Falkow S. 2004. *Salmonella typhi-murium* persists within macrophages in the mesenteric lymph nodes of chronically infected Nramp $1^{+/+}$ mice and can be reactivated by IFN-Ó neutralization. *JEM Press* **199**: 231–241.
Norioka S, Ramakishnan G, Ikenaka K, Inouye M. 1986. Interaction of a transcriptional activator, OmpR, with reciprocally osmoregulated genes, *ompF* and *ompC*, of *Escherichia coli. J Biol Chem* **261**: 17113–17119.
Obata M, Ibo S, Beppu H. 1993. Mechanism of anti-inflammatory and anti-thermal burn action of carboxypeptides from *Aloe aborescens miller* var. *natalensis Berger* in rats and mice. *Phytother Res (spec. issue)* **7**: 530–533.
Rishi P, Kaur H, Tirkey N *et al.* 2006. Is the increased liver damage – observed in *Salmonella typhimurium* infected mice pre-exposed to starvation – assessed by increased local TNF and lipid peroxidation? *Microb Infect* **8**: 1695–1701.
Sabeh F. 1995. Studies of the status of antioxidant enzymes and metabolites following burn injury, and the presence of antioxidant enzymes in the *Aloe vera* plant (tumor necrosis factor glutathione). *Dissert Abst Int* **56(12B)**: 6728.
Sarkar D, Dutta A, Das M, Sarkar K, Mandal C, Chatterjee M. 2005. Effects of *Aloe vera* on nitric oxide production by macrophages during inflammation. *Indian J Pharmacol* **37**: 371–375.
Sharma N, Trikha P, Athar M, Raisuddin S. 2000. Inhibitory effect of *Emblica officinalis* on the *in vivo* clastogenicity of benzo-[a]pyrene and cyclophosphamide in mice. *Hum Exp Toxicol* **19**: 377–384.
Shelton MS. 1991. *Aloe vera*, its chemical and therapeutic properties. *Int J Dermatol* **30**: 679–683.
Sood S, Rishi P, Dhawan V, Sharma S, Ganguly NK. 2005. Protection mediated by antibodies to iron-regulated outer-membrane proteins of *S. typhi* in a mouse peritonitis model. *Mol Cell Biochem* **273**: 69–78.
Stenson WF. 1990. Role of eicosanoids as mediators of inflammation in inflammatory bowel disease. *Scand J Gastroenterol Suppl* **72**: 13–18.
t'Hart LA, Nibbering PH, van den Barsellar MT, van DiJk H. van den Berg AJ. 1990. Effects of low molecular constituents from *Aloe vera* gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. *Int J Immunopharmacol* **12**: 427–434.
Uchiyama T, Kawano H, Kusuhara Y. 2006. The major outer membrane protein OmpB of spotted fever group rickettsiae functions in the rickettsial adherence to and invasion of Vero cells. *Microbes Infect* **8**: 801–809.
Wills ED. 1966. Mechanism of lipid peroxidation formation in animal tissues. *Biochem J* **99**: 667–676.

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr