Biochemical and Biophysical Research Communications 381 (2009) 90–95

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Biphasic activity of resveratrol on indomethacin-induced gastric ulcers

Anindya Dey [a], Prasun Guha [a], Subrata Chattopadhyay [b], Sandip K. Bandyopadhyay [a,*]

[a] Department of Biochemistry, Dr. B.C. Roy Post Graduate Institute of Basic medical Sciences & I.P.G.M.E. & R., 244B, Acharya Jagadish Chandra Bose Road, Kolkata, West Bengal 700 020, India
[b] Bio-Organic Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

ARTICLE INFO

Article history:
Received 3 February 2009
Available online 12 February 2009

Keywords:
Resveratrol
Indomethacin
Gastric ulcer
eNOS
iNOS
COX
Angiogenesis

ABSTRACT

Resveratrol showed biphasic activity in indomethacin-induced gastric ulcerated mice. A protective effect at a lower dose (2 mg kg^{-1}) and a contraindicative effect at a higher dose of Resveratrol (10 mg kg^{-1}) were observed. This phenomenon was possibly controlled by a COX-1 and eNOS balance, which ultimately maintained angiogenesis in Resveratrol-treated pre-ulcerated mice. The lower dose of Resveratrol (2 mg kg^{-1}) augmented eNOS expression without altering COX-1 expression, but, at a higher dose (10 mg kg^{-1}), Resveratrol predominantly suppressed COX-1 expression, which significantly reduced both PGE_2 synthesis and angiogenesis. Thus it ultimately resulted in delay healing of indomethacin-induced gastric ulcers. Hence, it could be concluded that COX-1 and eNOS acted as key regulatory factors switching the biphasic effects of Resveratrol in indomethacin-induced ulcerated mice.

Introduction

A vast variety of naturally occurring substances have been shown to protect against experimental carcinogenesis, and an increasing amount of evidence suggests that certain phytochemicals, particularly those included in our daily diet, have marked cancer chemopreventive properties [1,2]. Resveratrol (Resv) is one such dietary chemopreventive phytochemical, and it has recently attracted considerable interest because of its remarkable multifunctional inhibitory effects on multistage carcinogenesis [3]. One of the plausible mechanisms that could account for the chemopreventive activity of Resv occurs through inhibition of cyclooxygenase expression [3]. However, at the same time, cyclooxygenase expression plays a critical role in healing of drug-induced acute gastric ulcers [4]. Brzozowski et al. showed that [4] Resv treatment delayed healing of acetic acid-induced gastric ulcers in rats [5], depending on its cyclooxygenase-1 (COX-1) inhibitory properties. Hence, apart from its chemopreventive activity, Resv also possesses contraindicative ulcerogenic properties.

However, one of the most important features of Resv is its unique biphasic dual effect depending on its variable dosage. Kuwajerwala et al. [6] showed that, at lower doses, Resv caused two- to three-fold increases in DNA synthesis, but at higher doses, inhibited DNA synthesis in prostate cancer cells (LNCaP cells), thus proving its biphasic nature. Here, we made an attempt to determine whether different doses of Resv treatment could exert any kind of biphasic dual effect on indomethacin-induced gastric ulcerated mice in reference to COX and nitric oxide synthase (NOS) expression.

Materials and methods

Chemicals. Indomethacin, 3,3′-diaminobenzidine (DAB), bovine serum albumin (BSA), misoprostol, resveratrol, RNA Later™, hexadecyltrimethylammonium bromide (HTAB) and Gen Elute™ Mammalian Total RNA miniprep kits were purchased from Sigma (Sigma, St. Louis, MO, USA). Other reagents used were hydrogen peroxide (35%, Lancaster, Morecambe, UK), disodium hydrogen phosphate and sodium dihydrogen phosphate from BDH (BDH, Poole Dorset, U.K.), von Willebrand Factor (rabbit anti-human, Chemicon, Temecula, USA), hematoxylin monohydrate and eosin yellow (both from Merck, Mumbai, India), dimethyl formamide and tetramethyl benzidine (TMB) (Acros, Geel, Belgium), a Prostaglandin E_2 EIA kit (Cayman Chemical, Ann Arbor, MI, USA), a Revert Aid™ H minus first strand cDNA synthesis kit, and a Dynamo™ SYBR green® qPCR kit (Fermentas Life Sciences, USA).

Animals. Male Swiss albino mice (6–8 weeks, 25–30 g) that were bred in-house with free access to food and water were used for all experiments. The mice were kept in 12-h light/dark cycles and housed at 25 °C. Animal experiments ($n = 15$) were conducted in accordance with guidelines of the animal ethics committee of the Post Graduate Institute of Basic Medical Sciences, Kolkata (Animal Ethical Committee 507/CPCSEA, Sanction No. IAEC/SB-2/2004/

* Corresponding author. Fax: +91 33 22234844.
E-mail address: sandipkpc@gmail.com (S.K. Bandyopadhyay).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.02.027

UCM-16, dated 06.15.04) and were handled following the International Animal Ethics Committee Guidelines, ensuring minimum animal suffering.

Drug treatment. Drugs were prepared using 1.0% aqueous DMSO solutions of Resv (Sigma), suspended in 2% gum acacia in distilled water as a vehicle. Misoprostol (Sigma) (5 μg kg^{-1}) in the same vehicle was used as a positive control.

Protocol for gastric ulceration and assessment of healing. Acute gastric ulceration in mice was induced by oral administration of indomethacin (18 mg kg^{-1}, single dose) dissolved in distilled water and suspended in 2% gum acacia as a vehicle [7,8]. The animals were deprived of food but had free access to tap water for 24 h before ulcer induction. The normal and untreated control groups received the vehicle only throughout the course of the experiments. The treatment groups received different doses of Resv (10, 5, 3, 2, 1 and 0.5 mg kg^{-1}, p.o.) and misoprostol (5 μg kg^{-1}, p.o.) as a positive control for different periods of time, starting the first dose 6 h after indomethacin administration. On the 1st, 2nd, 3rd, 4th, 7th, 10th and 15th days, mice were sacrificed by cervical dislocation under anesthesia (ketamine, 12 mg kg^{-1}). The stomachs from the normal and treated groups were removed rapidly, opened along the greater curvature, and thoroughly rinsed with normal saline. The ulcerated gastric mucosal areas were visualized using a transparent sheet and a dissecting microscope. The damage score (DS) was assessed [9] by grading the gastric injury on a 0–4 scale, based on the severity of hyperemia and hemorrhagic erosions: 0—almost normal mucosa, 0.5—hyperemia, 1—one or two lesions, 2—severe lesions, 3—very severe lesions, and 4—mucosa full of lesions (lesions—hemorrhagic erosions, hyperemia—vascular congestions). The sum of the total scores divided by the number of animals was expressed as the mean damage score. The experiments were performed by two investigators blinded to the groups and treatment of animals.

Histological analysis. After scoring the damage score (DS), the fundic stomach was sectioned for histological studies. The tissue samples were fixed in 10% formalin and embedded in paraffin. The sections (5 μm) were cut using a microtome, stained with hematoxylin and eosin [9], and assessed under an Olympus microscope (BX41, Hamburg, Germany).

Myeloperoxidase (MPO) assay. The MPO activity was determined following a previously reported method [10] with slight modifications. Gastric ulcer tissues (100–150 mg) were homogenized in a 50 mM phosphate buffer (pH 6.0) containing 0.5% HTAB (hexadecyltrimethylammonium bromide) (Sigma), and was followed by three cycles of freeze and thawing. The homogenate was centrifuged at 12,000*g* for 20 min at 4 °C. The supernatant (50 μl) was collected for the MPO assay and was added to 80 mM phosphate buffer, pH 5.4, 0.03 M TMB (dimethyl formamide and tetramethyl benzidine) (Acros, Geel, Belgium) and 0.3 M H_2O_2 (hydrogen peroxide) (35%, Lancaster, Morecambe, UK), to make a final reaction volume of 500 μl. After incubating the mixture at 25 °C for 25 min, the reaction was terminated by adding 0.5 M H_2SO_4, and the change in the absorbance was measured at 450 nm. Results were expressed as a total number of neutrophils by comparing the OD of tissue supernatants with that of the mice peritoneal neutrophils processed in the same way. A standard curve relating neutrophil numbers and absorbance was obtained by processing purified neutrophils, and assaying the MPO activity with 0.0005% hydrogen peroxide as a substrate. Correlation between the number of neutrophils and units of MPO was determined using a previously reported technique [11]. One unit of MPO activity is defined as an MPO activity converting 1 μmol of hydrogen peroxide to water in one min at 22 °C.

Real-time PCR. Four days after ulcer induction, tissue samples from the normal, untreated, or Resv-treated mice were immediately immersed into an RNA later solution (Sigma). After extracting the total RNA using Gen Elute™ Mammalian Total RNA miniprep kits, and after checking its integrity by gel electrophoresis, the cDNA was synthesized from 5 μg of purified total RNA using a Revert Aid™ H minus first strand cDNA synthesis kit (Fermentas Life Sciences, USA). Expression of COX-1, COX-2, eNOS and iNOS were detected using suitably designed primers (Sigma) (Primer Express program, Applied Biosystems) (Table 1). The expressions of the designated enzymes were normalized using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as an internal reference. The experiments were performed (Real-Time PCR Systems 7500, Applied Biosystems, CA) in triplicate by using a Dynamo™ SYBR green® qPCR kit (Finnzymes, Oy-keilaranta, Finland). The samples were quantified for all genes mentioned above using a comparative Ct (ΔΔCt) method, as described in the Assays-on-Demand Users Manual (Applied Biosystems). The fold values (x) were calculated using the formula $x = 2^{(-\Delta\Delta Ct)}$, where the data for sample and healthy tissues, or untreated cells as a calibrator (a calibrator being, e.g., a healthy tissue or untreated cells), were first normalized against variations of sample quality and quantity.

Table 1
Primers used in Real-time PCR.

Primer pairs	Sequence
cox-1 (Forward)	5′-ccggattggtggaggtaggaactttgac-3′
cox-1 (Reverse)	5′-ggcgcatctctcgggactccttg-3′
cox-2 (Forward)	5′-acccccctgctgcccgacacct-3′
cox-2 (Reverse)	5′-ccagcaacccggccagcaatc-3′
eNOS (Forward)	5′-ccggcgctacgaagaatggaagtg-3′
eNOS (Reverse)	5′-gggcgctgggtgctgaactgac-3′
iNOS (Forward)	5′-gccttggctccagcatgtaccctcag-3′
iNOS (Reverse)	5′-cctgcccactgagttcgtccccttc-3′
GAPDH (Forward)	5′-ctgccacccagaagactgtg-3′
GAPDH (Reverse)	5′-ggtcctcagtgtagcccaag-3′

Table 2
Effects of Resv on ulcer index.

Groups	Ulcer index (damage score) Days of ulceration						
	1	2	3	4	7	10	15
Sham treated	$0.015^{aaa} \pm 0.009$	$0.018^{bbb} \pm 0.010$	$0.020^{ccc} \pm 0.011$	$0.0125^{ddd} \pm 0.009$	$0.013^{eee} \pm 0.010$	0.016 ± 0.008	0.012 ± 0.009
Ulcerated untreated	1.770 ± 0.083	2.013 ± 0.095	2.758 ± 0.123	2.839 ± 0.098	1.209 ± 0.086	0.31 ± 0.043	0.026 ± 0.034
Resv (10 mg Kg^{-1})	1.796 ± 0.140	$2.305^{bbb} \pm 0.098$	$3.456^{c} \pm 0.128$	$3.760^{ddd} \pm 0.125$	$2.650^{eee} \pm 0.068$	$1.356^{fff} \pm 0.113$	$0.46^{ggg} \pm 0.080$
Resv (5 mg Kg^{-1})	1.750 ± 0.170	1.935 ± 0.089	2.780 ± 0.145	2.856 ± 0.096	$1.765^{eee} \pm 0.148$	$0.896^{f} \pm 0.060$	$0.24^{ggg} \pm 0.072$
Resv (3 mg Kg^{-1})	1.815 ± 0.021	1.995 ± 0.125	2.636 ± 0.100	2.866 ± 0.128	$1.586^{eee} \pm 0.069$	0.680 ± 0.095	0.038 ± 0.018
Resv (2 mg Kg^{-1})	1.746 ± 0.051	$1.700^{bbb} \pm 0.130$	$1.550^{ccc} \pm 0.141$	$1.256^{ddd} \pm 0.145$	$0.630^{eee} \pm 0.114$	0.086 ± 0.095	0.016 ± 0.009
Resv (1 mg Kg^{-1})	1.715 ± 0.091	1.856 ± 0.126	$1.986^{cc} \pm 0.114$	$2.130^{ddd} \pm 0.123$	$0.992^{ee} \pm 0.096$	0.129 ± 0.110	0.019 ± 0.006
Resv (0.5 mg Kg^{-1})	1.717 ± 0.071	2.016 ± 0.119	2.798 ± 0.098	2.826 ± 0.112	1.214 ± 0.126	0.36 ± 0.869	0.023 ± 0.010
Misoprostol (5 μg Kg^{-1})	1.615 ± 0.078	$1.547^{bbb} \pm 0.098$	$1.526^{ccc} \pm 0.895$	$0.564^{ddd} \pm 0.115$	$0.310^{eee} \pm 0.009$	0.016 ± 0.008	0.011 ± 0.007

The values are mean ($n = 6$) and (±) indicates standard deviations. $^{a, b, c, d, e, f}$ and ^{g} $P < 0.05$, $^{aa, bb, cc, dd, ee, ff}$ and ^{gg} $P < 0.01$, $^{aaa, bbb, ccc, ddd, eee, fff}$ and ^{ggg} $P < 0.001$ versus ulcerated untreated mice on different days of ulceration.

The $\Delta\Delta C(t)$ were determined using the formula: $\Delta\Delta C(t) = \Delta C(t)\text{sample} - \Delta C(t)\text{calibrator}$, where $\Delta C(t)\text{sample} = C(t)\text{target gene of sample} - C(t)\text{reference}$, and $\Delta C(t)\text{calibrator} = C(t)\text{target gene of calibrator} - C(t)\text{reference}$. The expression of the target genes was normalized to the reference gene and related to the calibrator = $2^{-\Delta\Delta C(t)}$.

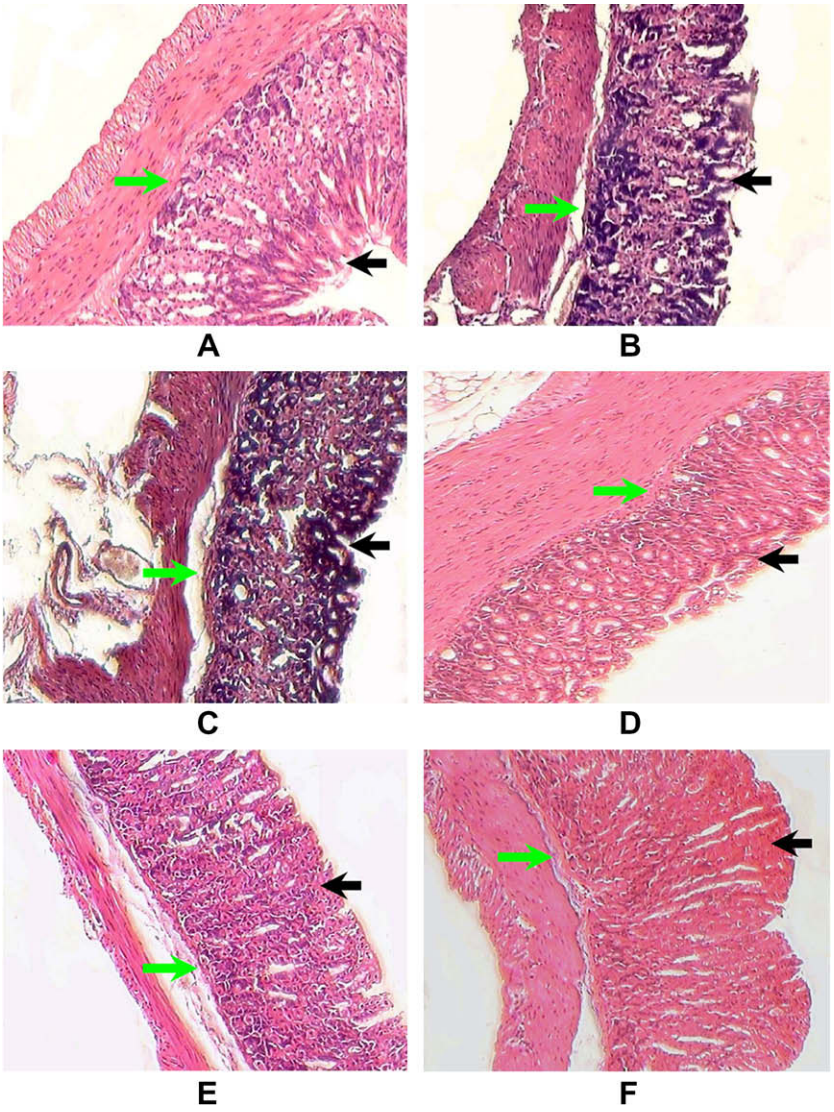

Fig. 1. Histology of mouse gastric tissue after ulcer induction by indomethacin and the effects of Resv. Ulceration in mice was induced by indomethacin (18 mg kg^{-1}, p.o.). Resv at different doses (10 mg kg^{-1}, 2 mg kg^{-1}, 0.5 mg kg^{-1}) and misoprostol (5 μg kg^{-1}) were administered 6 h post ulcer induction as described in "Materials and methods". At the fourth day of ulceration, mice were sacrificed and the stomachs were sectioned for the histological studies. Histological photograph of Sham-treated (A), untreated ulcerated (B), Resv (10 mg kg^{-1})-treated (C), Resv (2 mg kg^{-1})-treated (D), Resv (0.5 mg kg^{-1}) -treated (E), and misoprostol-treated (F) mice. Gastric tissue sections were photographed at a 10× magnification. Mucosal and submucosal layers are shown by black and green arrows, respectively.

Table 3
Effects of Resv on MPO activity.

Groups	MPO activity (U/mg) Days of ulceration						
	1	2	3	4	7	10	15
Sham treated	$0.500^{aaa} \pm 0.105$	$0.572^{bbb} \pm 0.115$	$0.520^{ccc} \pm 0.106$	$0.585^{ddd} \pm 0.110$	$0.498^{eee} \pm 0.189$	$0.506^{fff} \pm 0.125$	0.538 ± 0.160
Ulcerated untreated	1.725 ± 0.141	2.680 ± 0.145	3.574 ± 0.206	3.800 ± 0.146	1.560 ± 0.181	1.065 ± 0.228	0.640 ± 0.110
Resv (10 mg Kg^{-1})	1.678 ± 0.164	2.750 ± 0.148	$3.856^{c} \pm 0.197$	4.250 ± 0.163	$3.026^{eee} \pm 0.165$	$2.124^{fff} \pm 0.116$	$1.096^{ggg} \pm 0.150$
Resv (5 mg Kg^{-1})	1.730 ± 0.150	2.696 ± 0.125	3.680 ± 0.110	4.005 ± 1.856	$2.878^{eee} \pm 0.112$	$2.000^{fff} \pm 0.115$	$1.006^{ggg} \pm 0.114$
Resv (3 mg Kg^{-1})	1.800 ± 0.185	2.569 ± 0.115	3.320 ± 0.111	3.638 ± 0.185	$2.030^{eee} \pm 0.162$	1.126 ± 0.120	0.800 ± 0.125
Resv (2 mg Kg^{-1})	1.758 ± 0.115	$2.120^{bbb} \pm 0.126$	$2.268^{ccc} \pm 0.096$	$1.926^{ddd} \pm 0.114$	$0.920^{eee} \pm 0.165$	$0.682^{fff} \pm 0.112$	0.560 ± 0.096
Resv (1 mg Kg^{-1})	1.685 ± 0.118	$2.325^{bbb} \pm 0.150$	$2.506^{ccc} \pm 0.160$	$2.340^{dd} \pm 0.126$	1.306 ± 0.185	$0.796^{f} \pm 0.112$	0.620 ± 0.089
Resv (0.5 mg Kg^{-1})	1.758 ± 0.122	2.662 ± 0.114	3.640 ± 0.168	3.804 ± 0.156	1.550 ± 0.096	1.105 ± 0.090	0.710 ± 0.099
Misoprostol (5 μg Kg^{-1})	1.650 ± 0.122	1.750 ± 0.145	$1.789^{ccc} \pm 0.136$	$0.980^{ddd} \pm 0.116$	$0.594^{eee} \pm 0.089$	$0.504^{fff} \pm 0.092$	$0.501^{ggg} \pm 0.090$

The values are mean ($n = 6$) and (±) indicates standard deviations. $^{a, b, c, d, e, f}$ and ^{g} $P < 0.05$, $^{aa, bb, cc, dd, ee, ff}$ and ^{gg} $P < 0.01$, $^{aaa, bbb, ccc, ddd, eee, fff}$ and ^{ggg} $P < 0.001$ versus ulcerated untreated mice on different days of ulceration.

Prostaglandin E_2 (PGE_2) assay. Following harvesting of the stomach, the corpus (full thickness) was excised, weighed (100 mg) and suspended in a 10 mM sodium phosphate buffer, pH 7.4 (1 ml). The tissues were finely minced and incubated at 37 °C for 20 min. After centrifugation (9000*g*), the PGE_2 levels in the supernatant were measured by ELISA, following the Prostaglandin E_2 EIA kit (Cayman Chemical) kit instructions.

Quantification of von Willebrand factor (vWF) VIII. The number of microvessels in the ulcer was assessed from vWF VIII, following a previously reported procedure [12] with slight modifications. Briefly, after deparaffinization and rehydration, endogenous peroxidase activity in tissues was quenched with 0.3% hydrogen peroxide/methanol. The sections were incubated with polyclonal rabbit anti-human vWF VIII for 2 h at room temperature, and the bound primary antibody was detected (vWF VIII) by using the cell and tissue staining kit. Any positive-staining endothelial cell or endothelial cell cluster that was clearly separated from adjacent microvessels was considered an angiogenic microvessel. The vascular areas immediately adjacent to the normal tissue of the stomach served as internal quality controls. The microvessels (under 20× magnification) on coded slides in five randomly selected microscopic fields of mucosal erosions were counted in a blind manner, and the data were averaged.

Statistical analyses. The data are expressed as the mean ± SD unless otherwise noted. Comparisons were made between different treatments (ANOVA) using GraphPad InStat software, where an error protecting multiple comparison procedures, namely Tukey–Kramer Multiple Comparison tests, was applied for the analysis of significance of all data.

Results and discussion

The present study demonstrates for the first time the dose-dependent biphasic effects of resveratrol on indomethacin-induced ulcerated mice. Kuwajerwala et al. have already demonstrated [6] the biphasic character of Resv on DNA synthesis of prostate cancer cells. Here we found that at a higher dose (10 mg kg^{-1}), Resv delayed the ulcer healing process, but a lower dose (2 mg kg^{-1}) of Resv reversed the deleterious effects and simultaneously accelerated the ulcer healing process compared to the autohealing.

Our macroscopic examinations revealed that administration of indomethacin caused marked hemorrhagic damage in the stomach within 6 h of ulcer induction. Maximum ulcerative damage was observed on the fourth day after administration of indomethacin (Table 2). Mice receiving only the vehicle showed no lesions in the gastric mucosa. Indomethacin (18 mg kg^{-1}) administration produced typical time-dependent acute mucosal lesions in mice, as assessed by histology (Fig. 1). However, after seven days, the autohealing was evident.

The MPO activity, a marker of neutrophil aggregation at the site of inflammation, is frequently increased under ulcerated conditions and reduced with the healing process [13]. Consistent with this, we also observed ulceration-induced MPO activity up to the fourth day, followed by its gradual reduction on the seventh day (Table 3). At the same time, higher doses of Resv ($\geqslant$5 mg kg^{-1}) aggravated ulceration (Table 2) and delayed the healing process in a dose-dependent manner. The DS results were well-supported by the MPO activity data, where MPO activity was found to be appreciable at higher doses of Resv ($\geqslant$5 mg kg^{-1}) even on the seventh day compared to the ulcerated untreated mice (Tables 2 and 3). This establishes a strong contraindication of Resv use at its higher doses ($\geqslant$5 mg kg^{-1}) regarding gastric ulceration induced by indomethacin in mice. However, Resv at its lower dose (2 mg kg^{-1}) showed a significant healing effect. The healing response was predominantly evident from day 4. On day 7, Resv treatment showed a better rate of healing compared to ulcerated untreated mice. However, Resv at doses 1 and 0.5 mg Kg^{-1} showed no significant effect in comparison with ulcerated untreated mice.

The biphasic effects of Resv could be explained by studying the activity of COX (cyclooxygenase) isozymes as well as of NOS (nitric oxide synthase), which is one of the important angiogenic factors and gastroprotective agents. Resv is a cyclooxygenase inhibitor

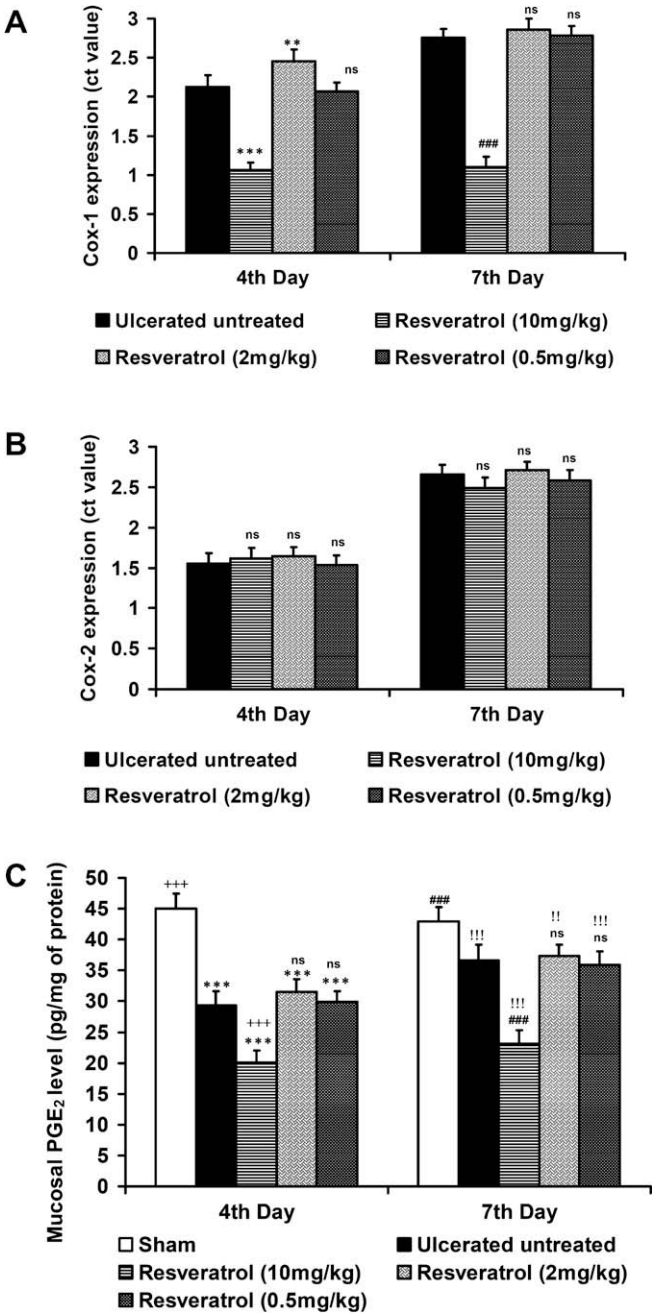

Fig. 2. Effects on COX mRNA expression and PGE_2 synthesis. COX-1 and COX-2 gene expression was studied by real-time PCR on the fourth and seventh day of ulceration. Relative mRNA expression was calculated according to the comparative ΔΔC(t) method, and quantitative mRNA expression of COX-1 (A) and COX-2 (B) were analyzed. The expression values are the means (n = 6), and vertical error bars represent standard deviations. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ versus ulcerated untreated mice on the fourth day, and $^{###}P < 0.001$ on the seventh day of ulcer induction. The PGE_2 levels were measured using an ELISA (C). $^{***}P < 0.001$, versus sham treated mice and +++ P<0.001 versus ulcerated untreated on the fourth day, and $^{###}P < 0.001$ versus sham treated mice and $^{!!!}P < 0.001$, $^{!!}P < 0.01$ versus ulcerated untreated on the seventh day of ulcer induction.

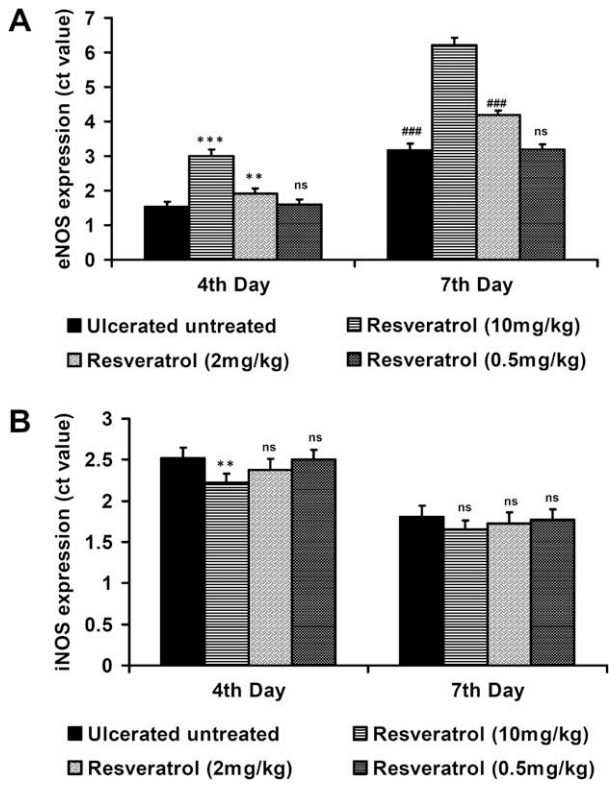

Fig. 3. Effects on NOS mRNA expression. eNOS and iNOS gene expression was studied by real-time PCR on the fourth and seventh days of ulceration. Quantitative mRNA expression of eNOS (A) and iNOS (B) were analyzed, respectively. $^{***}P < 0.001$, $^{**}P < 0.01$ versus untreated ulcerated mice on the fourth day and $^{###}P < 0.001$ on the seventh day of ulcer induction.

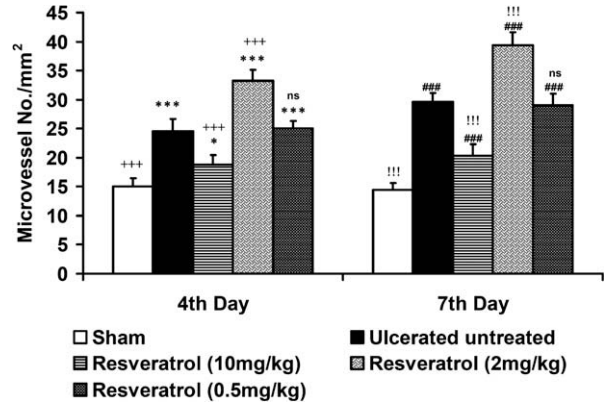

Fig. 4. Effects of Resv treatment on angiogenesis. Effects of Resv on angiogenesis were assessed by immunohistochemistry of the vWF VIII on the fourth and seventh days of ulceration. The numbers of microvessels were determined as stated in "Materials and methods". $^{***}P < 0.001$, versus sham treated mice and $^{+++}P < 0.001$ versus ulcerated untreated on the fourth day, and $^{###}P < 0.001$ versus sham treated mice and $^{!!!}P < 0.001$, $^{!!}P < 0.01$ versus ulcerated untreated on the seventh day of ulcer induction.

(COX) [3] and the expression of the COX isozymes plays a critical role in prostaglandin synthesis, which ultimately renders gastric protection or causes acute gastric ulcers [14]. A 10 mg Kg^{-1} dose of Resv significantly inhibited COX-1 expression (Fig. 2A) and PGE_2 synthesis was significantly suppressed (Fig. 2C) on the 4th and 7th days of ulceration compared to the untreated ulcerated mice. Resv by itself did not suppress COX-2 expression (Fig. 2B). Hence, it could be assumed that Resv at its higher dose acted as a COX-1-specific inhibitor, hindering the autohealing process.

It was interesting that in spite of being a COX-1-specific inhibitor, Resv can still induce eNOS expression [15] even at its higher dose (10 mg kg^{-1}) (Fig. 3A), which might be a cause of ulcer healing, although it is delayed. Yet, our gene expression study also revealed that a 2 mg kg^{-1} dose of Resv did not suppress COX-1 expression nor did it reduce PGE_2 synthesis. However, at a lower dose, it did induce eNOS expression (Fig. 3A) and also reduced iNOS (Fig. 3B) expression, leading to an enhanced eNOS/iNOS ratio and explaining its healing effect. At a dose of 0.5 mg kg^{-1}, Resv did not show any effect on the COX and NOS genes or on PGE_2 synthesis. Hence, the healing effects were not evident at this dose.

Therefore, it could be presumed that at a higher dose, Resv suppressed COX-1 expression significantly in spite of a high eNOS/iNOS ratio, which ultimately abolished its healing effect and delayed the autohealing process.

eNOS expression and PGE_2 synthesis directly modulate angiogenesis [16,17], which plays a major role in the ulcer healing process. Inhibition of angiogenesis is directly related to acute ulceration and delayed healing. We also found that the 10 mg kg^{-1} dose of Resv suppressed angiogenesis on the 4th and 7th days (Fig. 4) after ulceration compared to the untreated ulcerated mice, but at a lower dose, the Resv treatment moderately induced angiogenesis and accelerated the healing process. This effect of Resv on angiogenesis could directly be related to eNOS expression and PGE_2 synthesis. A higher dose of Resv significantly suppressed PGE_2 synthesis, which might be a cause of angiogenesis suppression. At a lower dose, PGE_2 suppression was not evident, but eNOS expression was significant, which probably stimulated angiogenesis, and therefore, the healing response was accelerated.

Conclusion

The results presented show that the biphasic activities of Resv in indomethacin-induced ulcerated mice were switched on and off, depending on COX-1 and eNOS expression. This, in turn, controlled the PGE_2 synthesis and angiogenesis process, ultimately switching between delayed and accelerated healing responses in Resv-treated indomethacin-induced ulcerated mice.

Acknowledgments

The work was financially supported by Life Science Research Board, Defense Research & Development Organization (DRDO) and the Department of Science and Technology (DST), Govt. of India.

References

[1] S.E. Benner, W.K. Hong, Clinical chemoprevention: developing a cancer prevention strategy, J. Natl. Cancer Inst. 85 (1993) 446–1447.
[2] A.R. Waladkhani, M.R. Clemens, Effect of dietary phytochemicals on cancer development, Bioorg. Med. Chem. Lett. 1 (1998) 747–753.
[3] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (1997) 218–220.
[4] T. Brzozowski, P.C. Konturek, S.J. Konturek, Z. Sliwowski, D. Drozdowicz, J. Stachura, R. Pajdo, E.G. Hahn, Cyclooxygenase-1 and cyclooxygenase-2 in healing of ischemia-reperfusion-induced gastric lesions, Eur. J. Pharmacol. 385 (1999) 47–61.
[5] T. Brzozowski, P.C. Konturek, S.J. Konturek, Z. Sliwowski, R. Pajdo, D. Drozdowicz, A. Ptak, E.G. Hahn, Classic NSAID and selective cyclooxygenase (COX)-1 and COX-2 inhibitors in healing of chronic gastric ulcers, Microsc. Res. Tech. 53 (2001) 343–353.
[6] N. Kuwajerwala, E. Cifuentes, S. Gautam, M. Menon, E.R. Barrack, G.P.V. Reddy, Resveratrol induces prostate cancer cell entry into S phase and inhibits DNA synthesis, Cancer Res. 62 (2002) 2488–2492.
[7] D. Banerjee, A.K. Bauri, R.K. Guha, S.K. Bandyopadhyay, S. Chattopadhyay, Healing properties of malabaricone B and malabaricone C, against indomethacin-induced gastric ulceration and mechanism of action, Eur. J. Pharmacol. 578 (2008) 300–312.

[8] P. Guha, A. Dey, B. Sarkar, M.V. Dhyani, S. Chattopadhyay, S.K. Bandyopadhyay, Improved antiulcer and anticancer properties of a trans-resveratrol analog in mice, J. Pharmacol. Exp. Ther. 328 (2009) 1–10.
[9] D. Dokmeci, M. Akpolat, N. Aydogu, L. Doganay, F.N. Turan, L-carnitine inhibits ethanol-induced gastric mucosal injury in rats, Pharmacol. Rep. 57 (2005) 481–488.
[10] K. Suzuki, H. Ota, S. Sasagawa, T. Sakatani, T. Fujikura, Assay method for myeloperoxidase in human polymorphonuclear leukocytes, Anal. Biochem. 132 (1983) 345–352.
[11] P.P. Bradley, R.D. Christensen, G. Rothstein, Cellular and extracellular myeloperoxidase in pyogenic inflammation, Blood 3 (1982) 618–622.
[12] L. Ma, J.L. Wallace, Endothelial nitric oxide synthase modulates gastric ulcer healing in rats, Am. J. Physiol. Gastrointest. Liver Physiol. 279 (2000) 341–346.
[13] M.H.L.P. Souza, H.P. Lemos, R.B. Oliveira, F.Q. Cunha, Gastric damage and granulocyte infiltration induced by indomethacin in tumour necrosis factor receptor 1 (TNF-R1) or inducible nitric oxide synthase (iNOS) deficient mice, Gut 53 (2004) 791–796.
[14] H. Ryo, T. Akiko, T. Mayu, A. Kikuko, K. Shinichi, A. Yasuko, T. Koji, Cyclooxygenase-2/prostaglandin E_2 accelerates the healing of gastric ulcers via EP_4 receptors, Am. J. Physiol. Gastrointest. Liver Physiol. 293 (2007) G788–G797.
[15] C.A. Araj, K.N. Slack, M.B. Harris, Effects of resveratrol on endothelial nitric oxide synthase (eNOS) phosphorylation and interaction with regulatory proteins, FASEB J. 22 (2008) 1154.7.
[16] F. Finetti, R. Solito, L. Morbidelli, A. Giachetti, M. Ziche, S. Donnini, Prostaglandin E2 regulates angiogenesis via activation of fibroblast growth factor receptor, J. Biol. Chem. 283 (2008) 2139–2146.
[17] M. Cudmore, S. Ahmad, B. Al-Ani, P. Hewett, S. Ahmed, A. Ahmed, VEGF-E activates endothelial nitric oxide synthase to induce angiogenesis via cGMP and PKG-independent pathways, Biochem. Biophys. Res. Commun. 345 (2006) 1275–1282.