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N-(2-mercaptopropionyl)-glycine, a diffusible antioxidant, activates HIF-1 by inhibiting HIF prolyl hydroxylase-2: Implication in amelioration of rat colitis by the antioxidant

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ABSTRACT

We investigated anti-colitic effects of N-(2-mercaptopropionyl)-glycine (NMPG), a diffusible antioxidant, in TNBS-induced rat colitis model and a potential molecular mechanism underlying the pharmacologic effect of the antioxidant. NMPG alleviated colonic injury and effectively lowered myeloperoxidase activity. Moreover, NMPG substantially attenuated expression of pro-inflammatory mediators in the inflamed colon. NMPG induced hypoxia-inducible factor-1α (HIF-1α) in human colon carcinoma cells, leading to elevated secretion of vascular endothelial growth factor (VEGF), a target gene product of HIF-1 involved in ulcer healing of gastrointestinal mucosa. NMPG induction of HIF-1α occurred by inhibiting HIF prolyl hydroxylase-2 (HPH-2), an enzyme that plays a major role in negatively regulating HIF-1α protein stability. In *in vitro* Von Hippel-Lindau protein binding assay, the inhibitory effect of NMPG on HPH-2 was attenuated by escalating dose of ascorbate but not 2-ketoglutarate, cofactors of the enzyme. Consistent with this, cell-permeable ascorbate significantly attenuated NMPG induction of HIF-1α in cells. Our data suggest that NMPG is an anti-colitic antioxidant that exerts its pharmacologic effects at least partly through activation of an ulcer healing pathway, HIF-1-VEGF.

1. Introduction

Hypoxia-inducible factor-1 (HIF) is a heterodimeric transcription factor composed of HIF-1α and aryl hydrocarbon receptor nuclear translocator (ARNT, HIF-β) whose activity is mainly regulated by oxygen [1]. In normoxia, HIF-1α is rapidly ubiquitylated and subsequently degraded by the 26S proteasome. The post-translational regulation of the protein is orchestrated by interaction with the E3 ubiquitin ligase, von Hippel Lindau protein (pVHL) [2]. The cellular oxygen-sensing mechanism that determines the HIF-1α/pVHL interaction consists of an oxygen-dependent enzymatic hydroxylation of two highly conserved proline residues [3,4]. To date, four human HIF prolyl-4 hydroxylases (HPHs) have been cloned [5,6]. According to their catalytic mechanism, these HPHs belong to the family of oxygen-, iron-, and 2-oxoglutarate-dependent dioxygenases. HIF-Prolyl-4 hydroxylase-2 (HPH2) is ubiquitously expressed and exhibits the highest specific activity toward HIF-1α [6]. In hypoxia, HPHs are inhibited; HIF-1α is thereby stabilized, accompanied by its nuclear translocation, heterodimerization with HIF-1β, and transcription of genes encoding proteins involved in cellular homeostasis under hypoxia [1].

Acute and chronic gastrointestinal inflammatory disease such as Crohn's disease and ulcerative colitis renders the mucosal surface lined with a monolayer of epithelia and the underlying supportive tissue to undergo structural abnormalities including changes in the microvasculature with tissue hypoxia. Based on this pathological change, it is now appreciated that a common feature of a variety of disease processes is diminished oxygen delivery probably leading to tissue hypoxia. Considering the importance of HIF-1α as a regulator of adaptation to low oxygen levels throughout the body [1] and its protective activity in murine experimental colitis [7], the elevation of HIF-1α levels resulting in the activation of HIF-1 could be a therapeutic approach for the treatment of colonic inflammatory diseases [8,9]. The therapeutic activity of HIF-1 likely involves the regulation of a number of barrier-protective and ulcer healing genes including vascular endothelial growth factor (VEGF) [7,10]. In fact, it is reported that elevated HIF-1α levels in colonic tissue, which were achieved by pharmacologically inhibiting or genetically down-regulating HIF prolyl hydroxylases, ameliorate experimental colitis [8,9,11].

The protective roles of N-(2-mercaptopropionyl)-glycine (NMPG), a diffusible antioxidant and free radical scavenger, have been studied in reactive oxygen species-mediated tissue damage [12,13]. In the investigation of the protective effect of NMPG in experimental colitis in which reactive oxygen and nitrogen species play an important pathological role, we found that the antioxidant alleviated the colitis at a significant level. In addition to its antioxidant activity, NMPG is reported to have the ability to induce HIF-1α [14], which should be beneficial to colitis. Thus, we investigated

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whether the therapeutic effect of the antioxidant on colitis involved activation of HIF-1 and how NMPG activation of HIF-1 occurred at a molecular level.

2. Materials and methods

2.1. Chemicals

N-(2-mercaptopropionyl)-glycine (NMPG) and (+)-5,6-O-isopropylidene-L-ascorbic acid were obtained from Tokyo Chemical Industry (Tokyo, Japan). Echinomycin (NSC-13502), sodium 2-ketoglutarate, sodium ascorbate, and ferrous chloride were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent-grade, commercially available products.

2.2. Cell culture and transient transfection

Human colon carcinoma cells HCT116 cells and human renal cancer UMRC2 and UMRC2/VHL cells which expresses a stably integrated construct encoding VHL (a gift from Dr. J. Issacs, Medical University of South Carolina) were grown in DMEM (Hyclone, South Logan, UT) supplemented with 10% fetal bovine serum (Hyclone) and penicillin/streptomycin (Hyclone). For transfection of a siRNA, chemically synthesized double stranded siRNA specific for HIF-1α (HIF-1α siRNA) were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). The siRNA was transfected (5 nmol/L) using a Fugene transfection reagent (Roche, Indianapolis, IN) according to the manufacturer's instructions. A pre-designed non-targeting siRNA sequence (Integrated DNA Technologies) was used as a nonspecific control.

2.3. Immunoblot analysis

Tissue lysates of the inflamed distal colon were prepared as described previously [15]. Briefly, tissues (1 g) were disrupted and homogenized in 3 mL of ice cold RIPA buffer [50 mM Tris–HCl (pH7.4), 1 mM EDTA, 0.7% Na deoxycholate, 1% NP-40, 150 mM NaCl, 0.3 μM aprotinin, 1 μM pepstatin and 1 mM PMSF] followed by the centrifugation at 10,000*g*, 4 °C for 10 min. COX-2 and iNOS proteins in the tissue homogenates were analyzed by Western blot using a monoclonal anti-COX-2 antibody and anti-iNOS (NOS-2) antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Hypoxia-inducible factor-1α (HIF-1α) was detected in nuclear extracts (30–40 μg) using a monoclonal anti-HIF-1α antibody (BD Biosciences Pharmingen, San Jose, CA). Experiments were performed in duplicate and normalized with antibodies to topoisomerase II (Santa Cruz Biotechnology) for HIF-1α and to α-tubulin (Santa Cruz Biotechnology) for COX-2 and iNOS.

2.4. In vitro VHL capture assay

Biotinylated wild type or proline-hydroxylated peptides (corresponding to HIF residues 556–574, Peptron, Daejeon, South Korea) attached to steptavidin beads (Pierce ImmunoPure, Rockford, IL) were prepared [15]. The beads were suspended in reaction buffer (20 mM Tris pH 7.5, 5 mM KCl, 1.5 mM $MgCl_2$). For each condition, 2 μg peptide/20 μl beads was aliquoted into separate tubes and the reaction buffer was added, along with cofactors (100 μM 2-ketoglutaric acid, 20 μM L-ascorbic acid, 50 μM ferrous chloride. Separate *in vitro* translated (IVT) reactions (Promega) were the source for the HIF prolyl hydroxylase-2 protein (HPH-2 plasmid was kindly provided by S. McKnight (University of Texas Medical Center, Dallas, TX)) [5] and Flag-VHL protein [16]. A 5 μl aliquot of IVT HPH-2 was added to the bead-peptide mixture for 40 min at 30 °C. Prior to this incubation, any inhibitors or competing factors were added to the appropriate tubes. Subsequently, the beads were washed with VHL binding buffer (20 mM Tris pH 8, 100 mM NaCl, 1 mM EDTA, 0.5% NP40) and 10 μl Flag-VHL IVT was added to the beads overnight at 4 °C. The beads were washed, SDS Laemmli buffer was added, the samples were boiled, subjected to SDS–PAGE, and resultant blots were probed for Flag.

2.5. Induction and evaluation of inflammation

Male Sprague-Dawley rats (250–260 g) were lightly anesthetized with ether. TNBS dissolved in 50% (v/v) aqueous ethanol was instilled into the colon via the rubber cannula (20 mg/0.35 mL/rat). For macroscopic evaluation of inflammation, colonic damage score (CDS) and myeloperoxidase (MPO) activity were measured as described previously [17]. The modified scoring system for colonic damage score (CDS) is as follows: Normal Appearance, 0; Localized hyperemia but no ulcer, 1; Linear ulcers without significant inflammation, 2; 2–4 cm site of inflammation and ulceration with scab, 3; Serosal adhesion to other organs, 2–4 cm site of inflammation and ulceration with scab, 4; Stricture, serosal adhesion involving several bowel loops, <4 cm site of inflammation and ulceration with scab, 5. The animal protocol used in this study has been reviewed and approved by the Pusan National University-Institutional Animal Care and Use Committee (PNU-IACUC) based on their ethical procedures and scientific care.

2.6. Elisa

Cells were treated as indicated in the figure legends. Medium was collected following 8 h treatment. An ELISA kit (R&D Systems, Minneapolis, MN) was used to assess secreted VEGF levels from an appropriate volume of medium. Each sample was harvested for quantification of protein, which was used to normalize VEGF levels. To measure cytokine-induced neutrophil chemoattractant-3 (CINC-3) in the inflamed tissues, the inflamed distal colon was homogenized in pH 6 potassium phosphate buffer, which was centrifuged at 10,000*g* and 4 °C for 10 min. An appropriate volume of the supernatants was subjected to CINC-3 ELISA (R&D Systems).

2.7. Data analysis

Results were expressed as mean ± S.D. One-way ANOVA followed by Tukey's (HSD) test was used to test for differences between data. Differences with $P < 0.05$ were considered significant. The XLSTAT® Software (Addinsoft, Inc., Suite 503, NY) was used for the statistical analysis.

3. Results

3.1. N-2-mercaptopropionylglycine (NMPG) ameliorates TNBS-induced colitis of rats

Antioxidants exert a beneficial effect against experimental colitis [18,19]. To test whether NMPG, a diffusible antioxidant, also possesses such a pharmacological property, the antioxidant was administered rectally to colitic rats once a day, beginning 3 days after induction of inflammation. Colonic damage score and myeloperoxidase activity were determined after treatment with 10 mM NMPG for 6 days. The dose of NMPG was decided based on a previous report demonstrating that rectal administration of N-acetylcysteine (20–40 mM), an antioxidant, ameliorates TNBS-induced rat colitis [18]. As shown in Fig. 1A and Supplementary data 1, the normal colon showed no damage, but the control colon (inflamed colon with no medication) was

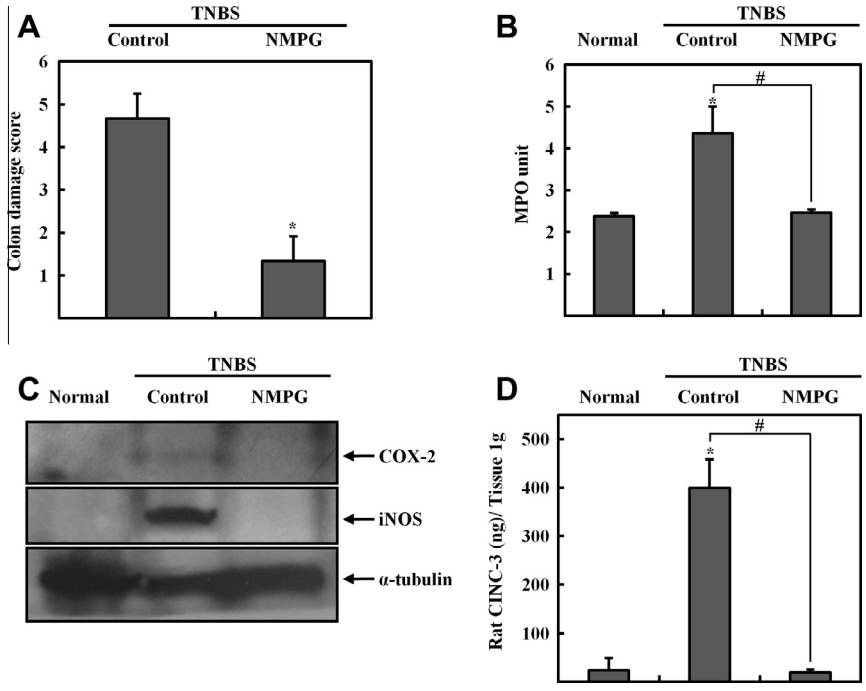

Fig. 1. N-2-mercaptopropionylglycine (NMPG) ameliorates TNBS-induced colitis of rats. NMPG (10 mM, 500 μL) was administered rectally to TNBS-induced colitis rats once a day for 6 days, beginning 72 h after induction of colitis. (A) Colonic damage score (CDS) was assigned according to the modified CDS scoring system. The data are mean ± S.D. (n = 3–5) $^{*}P < 0.005$ vs Control. (B) MPO activity was measured in the distal colon segments (4 cm) of rats. The data are mean ± S.D. (n = 3–5) $^{*}P < 0.01$ vs Control, $^{\#}P < 0.01$. (C) The levels of COX-2 and iNOS protein were determined in the distal colon segments by Western blot. α-Tubulin was used as a loading control. (D) The levels of CINC-3 were measured in the distal colon segments using an ELISA kit. The data are mean ± S.D. (n = 3–5). $^{*}P < 0.005$ vs Control, $^{\#}P < 0.005$.

severely damaged, nearly corresponding to 5 in the scoring system. Rectal administration of NMPG significantly healed the colonic injury. Consistent with this, NMPG lowered the level of MPO activity to the normal level (Fig. 1B). Molecular indices were examined as well. As shown in Fig. 1C and D, the levels of pro-inflammatory mediators, COX-2, iNOS and CINC-3, were elevated in the inflamed colonic tissue, but were significantly attenuated by rectal administration of NMPG.

3.2. NMPG induces HIF-1α protein, leading to increased VEGF secretion

In addition to antioxidant activity of NMPG, we explored a molecular mechanism for the NMPG-mediated therapeutic effects. Since activation of HIF-1α plays a beneficial role in ameliorating experimental colitis [8] and NMPG induces HIF-1α [14], we examined whether NMPG could stimulate the HIF-1 pathway in human colon carcinoma cells HCT116. Cells were treated with NMPG and lysed to obtain nuclear extracts. HIF-1α levels were examined by Western blot. As shown in Fig. 2A, NMPG elevated the level of HIF-1α protein in a dose-dependent manner. VEGF is a HIF-1 target gene that is likely involved in ulcer healing of gastrointestinal mucosa [20]. Thus, we tested whether NMPG was able to increase secretion of VEGF in HCT116 cells. As shown in Fig. 2B, NMPG increased secretion of VEGF. To verify whether NMPG induction of VEGF was dependent on HIF-1, cells transfected with HIF-1α siRNA or pretreated with a specific HIF-1 inhibitor echinomycin [21] were treated with NMPG, and VEGF levels were then measured. As shown in Fig. 2C and D, HIF-1α siRNA but not nontargeting siRNA reduced the level of HIF-1α protein in cells (Fig. 2C). Consistent with this result, HIF-1α siRNA attenuated VEGF secretion. Echinomycin substantially reduced NMPG-induced secretion of VEGF as well (Fig. 2D).

3.3. NMPG inhibits HIF prolyl hydroxylases

Since the α-subunit of HIF is tightly regulated at the posttranslational level by protein degradation [22], we considered whether NMPG modulated HIF-1α stability. HCT116 cells were treated with NMPG followed by addition of the protein synthesis inhibitor cycloheximide, and the disappearance rate of HIF-1α protein was compared. As shown in Fig. 3A, HIF-1α protein was extremely labile, disappearing in 5 min in cells left untreated with NMPG. In marked contrast, a substantial amount of HIF-1α protein remained in NMPG-pretreated cells 40 min after addition of cycloheximide, suggesting that NMPG stabilized HIF-1α protein. The central molecular mechanism for regulating HIF-1α protein stability is VHL-dependent proteasomal HIF-1α degradation following hydroxylation of proline residues in HIF-1α by HIF-prolyl hydroxylases (HPHs) [3,4]. We considered whether NMPG might impact the HIF-regulating pathway. To test this, we first examined the effects of NMPG on HIF-1α expression in either the parental VHL-deficient renal carcinoma cell line UMRC2, or UMRC2/VHL. As shown in Fig. 3B, NMPG increased HIF-1α expression in UMRC2/VHL; however, when this experiment was repeated in the VHL-deficient parental line, NMPG was unable to induce HIF-1α expression. While these data support involvement of VHL in NMPG-mediated HIF induction, it remains unclear how NMPG intervenes in VHL-dependent HIF-1α regulation. HPHs are the key enzymes for VHL-dependent HIF-1α degradation, thus we examined whether NMPG affected HPH-2 activity, which is ubiquitously expressed and exhibits the highest specific activity toward HIF-1α [6]. To do this, we utilized an *in vitro* VHL binding assay with a biotinylated HIF peptide that contains a conserved proline residue subject to HPH-dependent hydroxylation. As shown in Fig. 3C (left panel), the association of VHL with the HIF peptide in the absence of exogenously added cofactors was undetectable

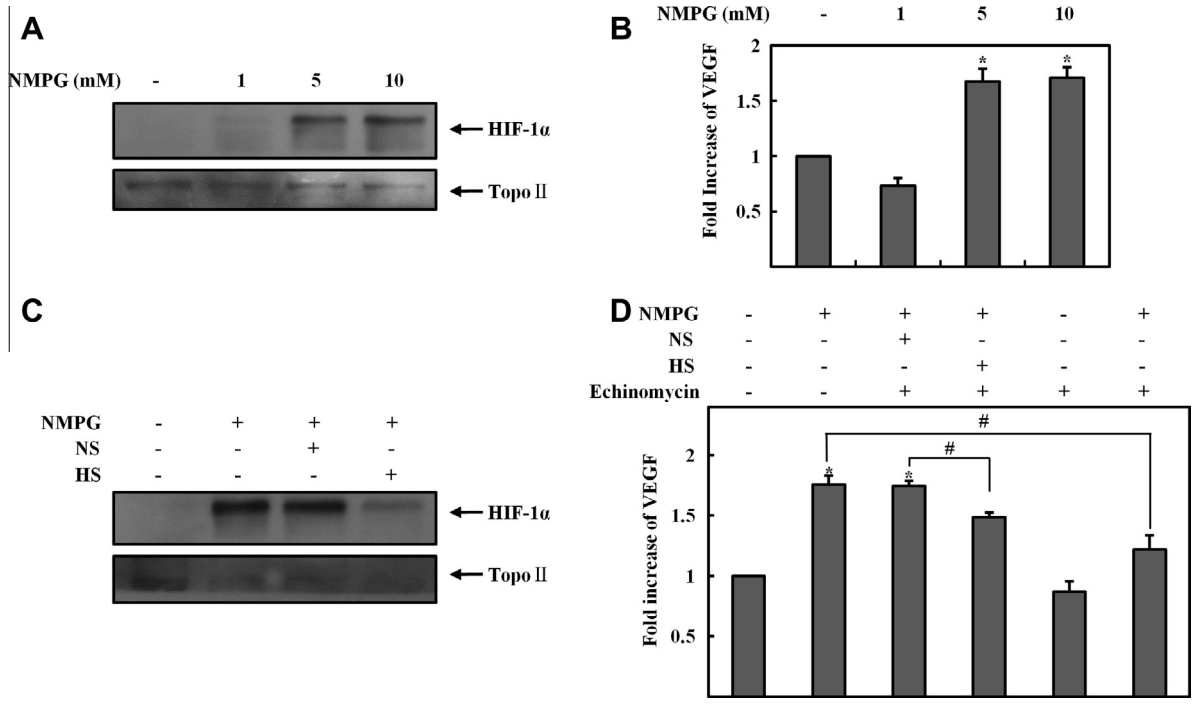

Fig. 2. NMPG induces HIF-1α protein leading to the activation of HIF-1. (A) HCT116 cells were 4 h-treated with the indicated concentrations of NMPG. HIF-1α levels in the nuclear extracts were examined by Western blot. (B) Cells were treated with the indicated concentrations of NMPG for 10 h and VEGF in the cell culture supernatants was analyzed. The data are mean ± S.D. (n = 3–4). *P < 0.01 vs control. (C and D) siRNA specific for HIF-1α (HIF-1α siRNA, HS) was transfected (50 nmol/L) using a Fugene transfection reagent. A nontargeting siRNA (NS) sequence was used as a nonspecific control. Two day post-transfection, cells were treated with NMPG (10 mM) for 4 h and 10 h for Western blot of nuclear HIF-1α (C) and VEGF ELISA (D), respectively. The data are mean ± S.D. (n = 3–4). *P < 0.005 vs Control, # P< 0.01.

(lane "con"). When the required cofactors for HPH were added, the association between the HIF peptide and VHL was markedly enhanced (lane "UT"). 10 mM NMPG substantially attenuated the interaction between these proteins. Finally, we used a chemically synthesized hydroxylated peptide to verify that NMPG directly impacts HPH activity and does not impair VHL protein. As shown in Fig. 3C (right panel), NMPG did not impair the ability of VHL to associate with hydroxylated HIF peptide up to 10 mM. In contrast, HIF peptide in which the two proline residues were mutated to alanine failed to bind VHL under any circumstances (data not shown). Our data strongly support the premise that NMPG is a potent inhibitor of HPH. To test this notion, cells were treated with NMPG and the hydroxylated status of NMPG-induced HIF-1α was monitored. As shown in Fig. 3D, lower levels of hydroxylated HIF-1α were detected in NMPG-induced HIF-1α than in MG132 (proteasome inhibitor)-induced HIF-1α.

3.4. Ascorbate, a cofactor of HPHs, attenuates NMPG inhibition of HPH-2

To investigate a biochemical mechanism underlying NMPG inhibition of HPH-2, we examined whether NMPG interfered with the catalytic activity of HPH-2 by limiting the use of required factors (ascorbate and 2-ketoglutarate) of the enzyme. Some HPH inhibitors are known to inhibit the enzyme by such action modes [15,23]. The inhibitory effect of NMPG on VHL binding to the HIF peptide was monitored with increasing concentrations of the required factors. As shown in Fig. 4A and B, while 2-ketoglutarate did not affect the VHL binding (Fig. 4A), elevated doses of ascorbate substantially attenuated the inhibitory effect of NMPG, thus restoring VHL binding to the HIF peptide to a significant level (Fig. 4B). To confirm this, HCT116 cells were treated with NMPG in the presence of cell-permeable ascorbate and the levels of HIF-1α were monitored. As shown in Fig. 4C, NMPG induction of HIF-1α protein was diminished remarkably (left panel). In contrast, no change in the level of phenanthroline (an iron chelator)-induced HIF-1α was observed (right panel), indicating that the ascorbate effect was specific.

4. Discussion

In this study, we investigated the anti-colitic activity of NMPG in a rat colitis model and a potential molecular mechanism underlying the anti-colitic effects of the antioxidant. Our data showed that NMPG ameliorated TNBS-induced rat colitis and activated an ulcer healing pathway, HIF-1-VEGF, in human colon carcinoma cells. NMPG activation of HIF-1 occurred by inhibiting HPH-2 through interference with the normal function of ascorbate, a required factor for the catalytic activity of the enzyme.

Consistent with a previous paper [14], NMPG induced HIF-1α protein in human colon carcinoma cells. Although NMPG is known to induce HIF-1α by inhibiting proteasome [14], our data suggest that the cellular effect of NMPG is also ascribed to stabilization of HIF-1α protein by inhibition of HPH-2, a hydroxylation enzyme that regulates HIF-1α protein stability [5]. In fact, NMPG delayed HIF-1α protein degradation and inhibited HPH-2 in an *in vitro* VHL binding assay. NMPG inhibition of HPH-2 was confirmed by data showing that NMPG attenuated hydroxylation of cellular HIF-1α.

We suggest that HPH inhibition by NMPG occurs via interference with ascorbate binding to the iron in the active site of HPH-2. For hydroxylation of the substrate HIF-1α by HPH, the iron in the active site needs to be associated transiently with the required factors, 2-ketoglutarate and ascorbate [24,25]. This argument is supported by data showing that (1) escalation of ascorbate dose attenuates the NMPG effect on VHL association in the VHL binding assay, (2) pretreatment with ascorbate prevents HIF-1α protein induction by NMPG and (3) dose change of the other factor,

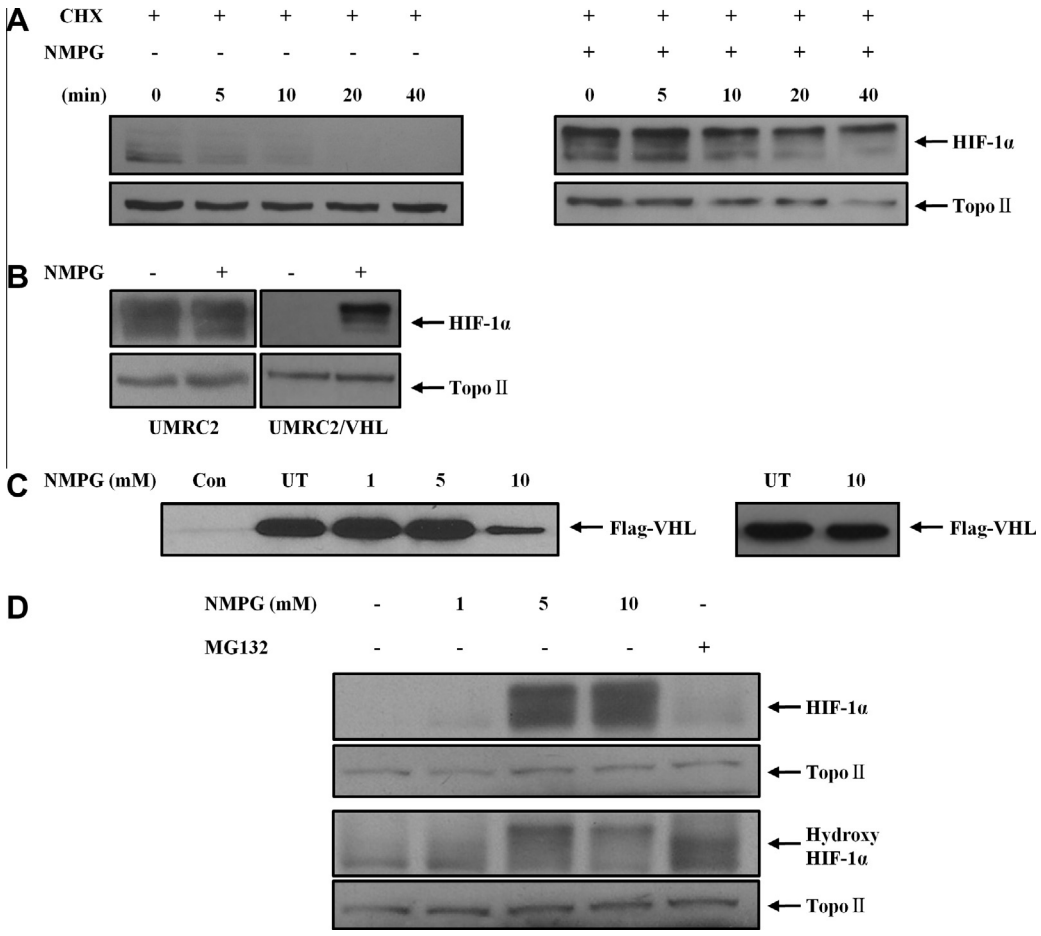

Fig. 3. NMPG inhibits HIF prolyl hydroxylases. (A) HCT116 cells were either left untreated or were pretreated with NMPG (10 mM) for 4 h, followed by addition of cycloheximide (CHX, 200 μM) for the indicated times. Levels of HIF-1α in nuclear extracts were monitored by Western blot. (B) Renal carcinoma cells that are deficient for VHL function (UMRC2), or a clonally selected line with VHL stably expressed (UMRC2/VHL), were treated with NMPG (10 mM) for 4 h, and HIF-1α protein was detected in nuclear extracts. (C) Left panel: The VHL binding assay was performed in the presence of the indicated concentrations of NMPG, and resultant blots were probed for Flag (VHL). The control lane (con) represents the assay in the absence of added cofactors, while the untreated (UT) lane contains all required cofactors. Right panel: The same assay was performed with biotinylated HIF peptide in which the proline residue is already hydroxylated. (D) NMPG at various concentrations was treated in cells for 4 h and HIF-1α and hydroxylated HIF-1α were detected in nuclear extracts. The same experiment was performed with MG132 (10 μM) a proteasome inhibitor.

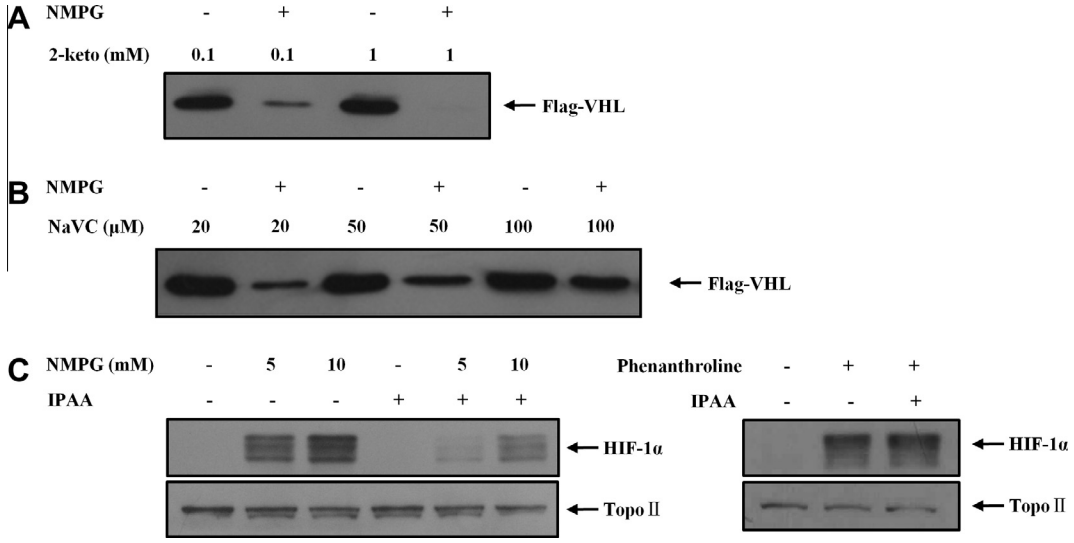

Fig. 4. Ascorbate, a cofactor of HPHs, attenuates NMPG inhibition of HPH-2. (A and B) VHL binding assay was performed in the presence of NMPG (10 mM) following exogenous addition of elevating dose of 2-ketoglutarate (A, 2-keto) or ascorbate (B, NaVC), and resultant blots were probed for Flag (VHL). (C) HCT116 cells were treated with the indicated concentrations of NMPG (left panel) or an iron chelator phenanthroline (50 μM, right panel) in the presence of (+)-5,6-O-isopropylidene-L-ascorbic acid (IPAA, 1 mM) and nuclear HIF-1α was monitored by Western blot.

2-ketoglutarate, does not influence the NMPG effects on VHL association. In addition, exogenous addition of Fe(II) to the reaction mixture attenuated the inhibitory effect of NMPG on VHL binding to the HIF peptide, further supporting that NMPG binds to the iron in the active site of HPH-2 (Supplementary data 2).

In line with numerous papers demonstrating that anti-oxidants have beneficial effects on intestinal inflammation [18,19], rectal administration of NMPG, a diffusible anti-oxidant, effectively alleviated TNBS-induced rat colitis, which should be attributed to anti-oxidative activity of NMPG in the inflamed colon. Considering that HPH inhibition leading to HIF-1 activation is a therapeutic strategy for treatment of colonic inflammation, our cellular data suggest that HPH inhibition is involved in the anti-colitic effects of NMPG as an additional mechanism. Actually, genetic and pharmacological inhibition of HPH mitigates experimental colitis [9,11].

Although NMPG, which was administered rectally at concentration at which it activated the HIF-1-VEGF pathway in cells, exhibited beneficial effects on rat colitis, it is unclear whether the ulcer healing mechanism is involved in the anti-colitic effects of rectal NMPG. Moreover, it should be taken into consideration that oral administration of NMPG, which has the best patient compliance, can achieve millimolar levels of NMPG in the blood. Given that the oral dose of NMPG to achieve such plasma concentrations should be very high, NMPG therapy via oral route may not be practical, which may limit its clinical use. Generally, adoption of colon-specific delivery of a drug drastically increases the therapeutic concentration of the drug at the target site (large intestine), thus simulating the local action of the drug by rectal administration [26]. Therefore, design of a colon-targeted NMPG may be a feasible strategy to develop the antioxidant as an anti-colitic agent with therapeutic mechanisms that include activation of an ulcer healing pathway, HIF-1-VEGF.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.081.

References

[1] G.L. Semenza, Hypoxia-inducible factor 1: master regulator of O_2 homeostasis, Curr. Opin. Genet. Dev. 8 (1998) 588–594.
[2] K. Tanimoto, Y. Makino, T. Pereira, L. Poellinger, Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein, EMBO J. 19 (2000) 4298–4309.
[3] M. Ivan, K. Kondo, H. Yang, W. Kim, J. Valiando, M. Ohh, A. Salic, J.M. Asara, W.S. Lane, W.G. Kaelin Jr., HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing, Science 292 (2001) 464–468.
[4] P. Jaakkola, D.R. Mole, Y.M. Tian, M.I. Wilson, J. Gielbert, S.J. Gaskell, A. Kriegsheim, H.F. Hebestreit, M. Mukherji, C.J. Schofield, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation, Science 292 (2001) 468–472.
[5] R.K. Bruick, S.L. McKnight, A conserved family of prolyl-4-hydroxylases that modify HIF, Science 294 (2001) 1337–1340.
[6] M. Hirsila, P. Koivunen, V. Gunzler, K.I. Kivirikko, J. Myllyharju, Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor, J. Biol. Chem. 278 (2003) 30772–30780.
[7] J. Karhausen, G.T. Furuta, J.E. Tomaszewski, R.S. Johnson, S.P. Colgan, V.H. Haase, Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis, J. Clin. Invest. 114 (2004) 1098–1106.
[8] A. Robinson, S. Keely, J. Karhausen, M.E. Gerich, G.T. Furuta, S.P. Colgan, Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition, Gastroenterology 134 (2008) 145–155.
[9] M.M. Tambuwala, E.P. Cummins, C.R. Lenihan, J. Kiss, M. Stauch, C.C. Scholz, P. Fraisl, F. Lasitschka, M. Mollenhauer, S.P. Saunders, P.H. Maxwell, P. Carmeliet, P.G. Fallon, M. Schneider, C.T. Taylor, Loss of prolyl hydroxylase-1 protects against colitis through reduced epithelial cell apoptosis and increased barrier function, Gastroenterology 139 (2010) 2093–2101.
[10] H. Hashimoto, M. Akimoto, A. Maeda, M. Shigemoto, K. Yamashito, Relation of hypoxia-inducible factor-1alpha to vascular endothelial growth factor and vasoactive factors during healing of gastric ulcers, J. Cardiovasc. Pharmacol. 44 (2004) S407–S409.
[11] E.P. Cummins, F. Seeballuck, S.J. Keely, N.E. Mangan, J.J. Callanan, P.G. Fallon, C.T. Taylor, The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis, Gastroenterology 134 (2008) 156–165.
[12] L.D. Horwitz, P.V. Fennessey, R.H. Shikes, Y. Kong, Marked reduction in myocardial infarct size due to prolonged infusion of an antioxidant during reperfusion, Circulation 89 (1994) 1792–1801.
[13] H.K. Saini-Chohan, N.S. Dhalla, Attenuation of ischemia-reperfusion-induced alterations in intracellular Ca^{2+} in cardiomyocytes from hearts treated with N-acetylcysteine and N-mercaptopropionylglycine, Can. J. Physiol. Pharmacol. 87 (2009) 1110–1119.
[14] V. Srinivas, L.P. Zhang, X.H. Zhu, J. Caro, Characterization of an oxygen/redox-dependent degradation domain of hypoxia-inducible factor alpha (HIF-alpha) proteins, Biochem. Biophys. Res. Commun. 260 (1999) 557–561.
[15] H. Jeon, H. Kim, D. Choi, D. Kim, S.Y. Park, Y.J. Kim, Y.M. Kim, Y. Jung, Quercetin activates an angiogenic pathway, hypoxia inducible factor (HIF)-1-vascular endothelial growth factor, by inhibiting HIF-prolyl hydroxylase: a structural analysis of quercetin for inhibiting HIF-prolyl hydroxylase, Mol. Pharmacol. 71 (2007) 1676–1684.
[16] J.S. Isaacs, Y.J. Jung, E.G. Mimnaugh, A. Martinez, F. Cuttitta, L.M. Neckers, Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway, J. Biol. Chem. 277 (2002) 29936–29944.
[17] S. Hong, S. Yum, H.J. Yoo, S. Kang, J.H. Yoon, D. Min, Y.M. Kim, Y. Jung, Colon-targeted cell-permeable NFkappaB inhibitory peptide is orally active against experimental colitis, Mol. Pharm. 9 (2012) 1310–1319.
[18] A. Siddiqui, H. Ancha, D. Tedesco, S. Lightfoot, C.A. Stewart, R.F. Harty, Antioxidant therapy with *N*-acetylcysteine plus mesalamine accelerates mucosal healing in a rodent model of colitis, Dig. Dis. Sci. 51 (2006) 698–705.
[19] H.R. Ancha, R.R. Kurella, C.C. McKimmey, S. Lightfoot, R.F. Harty, Luminal antioxidants enhance the effects of mesalamine in the treatment of chemically induced colitis in rats, Exp. Biol. Med. (Maywood) 233 (2008) 1301–1308.
[20] A.S. Tarnawski, A. Ahluwalia, Molecular mechanisms of epithelial regeneration and neovascularization during healing of gastric and esophageal ulcers, Curr. Med. Chem. 19 (2012) 16–27.
[21] D. Kong, E.J. Park, A.G. Stephen, M. Calvani, J.H. Cardellina, A. Monks, R.J. Fisher, R.H. Shoemaker, G. Melillo, Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity, Cancer Res. 65 (2005) 9047–9055.
[22] L.E. Huang, J. Gu, M. Schau, H.F. Bunn, Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway, Proc. Natl. Acad. Sci. USA 95 (1998) 7987–7992.
[23] S. Yum, H.J. Doh, S. Hong, S. Jeong, D.D. Kim, M. Park, Y. Jung, Piceatannol, a hydroxystilbene natural product, stabilizes HIF-1alpha protein by inhibiting HIF prolyl hydroxylase, Eur. J. Pharmacol. 699 (2013) 124–131.
[24] K.I. Kivirikko, R. Myllyla, T. Pihlajaniemi, Protein hydroxylation: prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit, FASEB J. 3 (1989) 1609–1617.
[25] K.S. Hewitson, C.J. Schofield, The HIF pathway as a therapeutic target, Drug Discov. Today 9 (2004) 704–711.
[26] Y. Jung, Y.M. Kim, What should be considered on design of a colon-specific prodrug?, Expert Opin Drug Deliv. 7 (2010) 245–258.