Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 317 (2004) 121–127
BBRC
www.elsevier.com/locate/ybbrc

Cysteamine alters redox state, HIF-1α transcriptional interactions and reduces duodenal mucosal oxygenation: novel insight into the mechanisms of duodenal ulceration

Tetyana Khomenko, Xiaoming Deng, Zsuzsanna Sandor, Andrzej S. Tarnawski, and Sandor Szabo*

Pathology and Laboratory Medicine Service, Diagnostic and Molecular Medicine Health Care Group, Department of Medicine, VA Medical Center, CA 90822, USA
Departments of Pathology, Pharmacology, and Medicine, University of California-Irvine, Long Beach, CA 90822, USA

Received 4 March 2004

Abstract

Our recent studies demonstrated a critical role of enhanced transcriptional activity of early growth response factor-1 (Egr-1) in early stages of cysteamine-induced duodenal ulcer in rats. Since cysteamine is also a reducing agent, the aims of this study were to determine the effect of cysteamine on proximal duodenal mucosa: (a) redox status, (b) mucosal oxygenation, (c) expression of hypoxia-inducible factor 1 (HIF-1α) and its binding to DNA, and (d) HIF-1α interaction with Egr-1 and other redox-sensitive transcription factors. Here we demonstrate for the first time that cysteamine treatment reduced the duodenal oxygenation by 19% (vs. baseline) and markedly increased the redox status in duodenal mucosa ($p < 0.05$). Cysteamine increased HIF-1α expression, its binding to DNA, and enhanced the HIF-1α interactions with Egr-1 and other transcription factors (e.g., AP-1, AP-2, L-III BP, NF-E1, NF-E2, STAT4, and MRE), their binding to DNA. Thus, these data demonstrate the involvement of the redox-dependent regulatory mechanisms in the early stages of duodenal ulceration.
Published by Elsevier Inc.

Keywords: Cysteamine; Redox state; Tissue oxygenation; HIF-1α; Egr-1; Transcription factors

Oxidative stress through the changes in the intracellular environment and generation of free radicals such as superoxide, hydrogen peroxide (H_2O_2), hydroxyl free radical or singlet oxygen can lead to lipid peroxidation, protein damage, and mutations in DNA. The redox signaling in the cells constitutes a complex system which serves both defensive mechanisms and the response to oxidative stress.

The duodenal ulcerogen cysteamine ($HS\text{-}CH_2\text{-}CH_2\text{-}NH_2$, β-mercaptoethylamine) is a reducing aminothiol which induces perforating ulcers in the rat proximal duodenum within 24–48 h [1–3]. In vitro studies demonstrated that the cytotoxic effect of cysteamine in variety of cells depends on the generation of H_2O_2 and the oxidation of cysteamine to its corresponding disulfide [4–6]. The thiol-derived H_2O_2 reacts with the reducing transition metals and produces the hydroxyl radical via the Fenton reaction. These radicals are the final mediators of cysteamine toxicity [7,8] as evidenced by the fact that catalase completely inhibits the cysteamine toxicity [4,9]. Thiol–disulfide exchange reactions via redox active disulfides are essential in the control of protein function via the redox state of structural SH groups. This mechanism of thiol-redox control [10] is a major regulatory mechanism in cell and tissue signaling, since reactive oxygen species oxidizing protein thiols have a wide range of functions in cellular physiology and pathological conditions.

In vivo studies in rats demonstrated that cysteamine causes a local release of endothelin-1 (ET-1) [11] (a potent vasoconstrictor that causes tissue ischemia and hypoxia [12]) and decreases duodenal blood flow within 5–15 min after administration [13]. The antioxidant

* Corresponding author. Fax: 1-562-826-5768.
E-mail address: sandor.szabo@med.va.gov (S. Szabo).

0006-291X/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.bbrc.2004.03.017

ebselen or catalase inhibits the ulcerogenic effect of cysteamine [14]. We also demonstrated that cysteamine causes a rapid induction of hypoxia-associated early growth response factor-1 (Egr-1) [15] which was followed by elevation of growth factor production including VEGF [16]. Recently we showed that in the duodenal mucosa Egr-1 function is dependent upon the interaction with other redox-sensitive transcription factors (AP-1, AP-2, NFATc, Egr-2, Sp1, PAX-5, MRE, c-Myb, and CREB), and that cysteamine increases Egr-1 expression and Egr-1/DNA binding through interaction with thioredoxin (Trx) and redox factor (Ref-1)—redox system in early, pre-ulcerogenic stages of duodenal ulceration [14].

Cellular adaptation to hypoxic condition involves also a transcriptional response pathway mediated by hypoxia-inducible factor 1 (HIF-1) [17–20], a heterodimeric complex of HIF-1α and HIF-1β subunits [19,20]. Under normal oxygenation, HIF-1β protein is relatively stable, whereas, HIF-1α is continuously produced but rapidly degraded [21]. Hypoxia stabilizes the HIF-1α protein, induces its translocation to the nucleus, and leads to the formation of the complex with hypoxia-responsive element of target genes involved in angiogenesis (e.g., VEGF), erythropoiesis, and glycolysis [22–24]. The regulation of HIF-1 activity in some cells involves redox-dependent stabilization of HIF-1α protein [25]. Pre-exposure of Hep3B cells to H_2O_2 selectively prevented hypoxia-induced HIF-1 binding by blocking accumulation of HIF-1α protein, whereas treatment of hypoxic cell extracts with H_2O_2 had no effect on HIF-1 binding [25]. These observations suggest that an intact redox-dependent signaling pathway is required for destabilization of the HIF-1α protein. In hypoxic cells, HIF-1 DNA binding was reversibly abolished by SH oxidation [25]. Furthermore, the addition of reduced Trx to cells enhanced HIF-1α DNA binding. Consistent with these results, overexpression of Trx and Ref-1 significantly potentiated hypoxia-induced expression of a reporter construct containing the wild-type HIF-1-binding site.

In this study we tested the hypothesis that cysteamine affects the regional redox status, reduces tissue oxygenation, modifies HIF-1α transcriptional activity, and alters its interaction with Egr-1 and other redox-sensitive transcription factors.

Materials and methods

Animal experiments. This study was approved by the Animal Study Subcommittee of the VA Medical Center in Long Beach. Duodenal ulcers were induced in female Sprague–Dawley rats (180–210 g) by intragastric administration of cysteamine–HCl (Aldrich, Milwaukee, WI). Rats had unlimited access to food and water. Randomized groups of rats ($n = 7$) were given either saline or cysteamine–HCl 25 mg/100 g by gavage once and euthanized 0.5, 1, 2 or 6 h later. In other groups cysteamine was given three times in 4 h intervals and rats were euthanized 12 h after the first dose of cysteamine. The size of duodenal ulcers was measured in mm^2. Mucosal scrapings of proximal duodenum (2.5 cm) were frozen and kept at −80 °C.

Redox state and tissue oxygenation. Animals were treated with saline or cysteamine as described above. Thirty minutes or 2 h later rats were anesthetized using isoflurane (Abbott Laboratories, Chicago, IL) inhalation and after laparotomy the duodenal mucosal oxygenation and redox status were measured using oxygen and redox electrodes (Lazar Research Lab., Los Angeles, CA). The microelectrodes were placed onto gastric or proximal duodenal mucosa through small incisions in the forestomach.

Western blotting. Nuclear proteins were extracted from duodenal mucosa using the Nuclear Extraction Kit (Panomics, Redwood City, CA). Protein concentration in all samples was measured by the Bradford assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of proteins (100 μg) were subjected to SDS–PAGE analysis. Proteins were transferred onto Hybond-ECL nitrocellulose membranes (Amersham, Buckinghamshire, England) and then incubated for 3 h at 20 °C with mouse monoclonal anti-HIF-1α antibody (Novus Biologicals, Littleton, CO). Blots were treated with secondary antibody and visualized using the ECL detection system (Amersham, Buckinghamshire, England). The density of protein bands in Western blots was assessed by a digital imaging system, Eagle Eye II (Stratagene, Austin, TX). Each experiment was repeated four times.

HIF-1α transcriptional activity. HIF-1α binding to the DNA consensus binding sequences of HIF-1α was detected using BD Mercury Transfactor Kit (BD Biosciences Clontech, Palo Alto, CA) according to the manufacture's manual. This assay is an enzyme-linked immunosorbent assay (ELISA)-based 96-well format containing the consensus binding sequences for HIF-1 coated on the wells. When nuclear tissue extracts containing the transcription factor are incubated in the wells, the transcription factor binds to its consensus sequence. Bound transcription factor is then detected by a specific mouse monoclonal anti-HIF-1α antibody (Novus Biologicals, Littleton, CO). A horseradish peroxidase-conjugated secondary antibody is then used to detect bound primary antibody. The enzymatic product was measured with microtiter plate reader.

Transcription factor interaction assay. To determine the changes in Egr-1 transcriptional activity in rat duodenal mucosa induced by cysteamine we used the TranSignal TF–TF Interaction Array Kit (Panomics, Redwood City, CA). Nuclear extracts from duodenal tissues were incubated with the kit's biotin-labeled oligonucleotides, which represent a library of *cis*-elements, and then the HIF-1α proteins were immunoprecipitated with mouse monoclonal anti-HIF-1α antibody (Novus Biologicals, Littleton, CO). After washing of the free *cis*-elements and non-specific-binding proteins, eluted labeled cDNA probes were hybridized to the TranSignal Protein/DNA Array membranes. The spot signals of DNA probes were visualized according to manufacturer's protocol. The density of each hybridized dot was measured by the Eagle Eye II.

Statistical analysis. Statistical significance of changes was determined by the non-parametric Mann–Whitney *U* test. Differences resulting in p value <0.05 were considered to be statistically significant.

Results

The effect of cysteamine on redox potential and tissue oxygenation in rat proximal duodenum

The redox potential was decreased from baseline value of 1342.5 ± 22.98 to 1089.3 ± 45.61 and to 1082 ± 11 mV in duodenal mucosa at 0.5 and 2 h after cysteamine administration, respectively ($p < 0.05$) (Fig. 1A). The

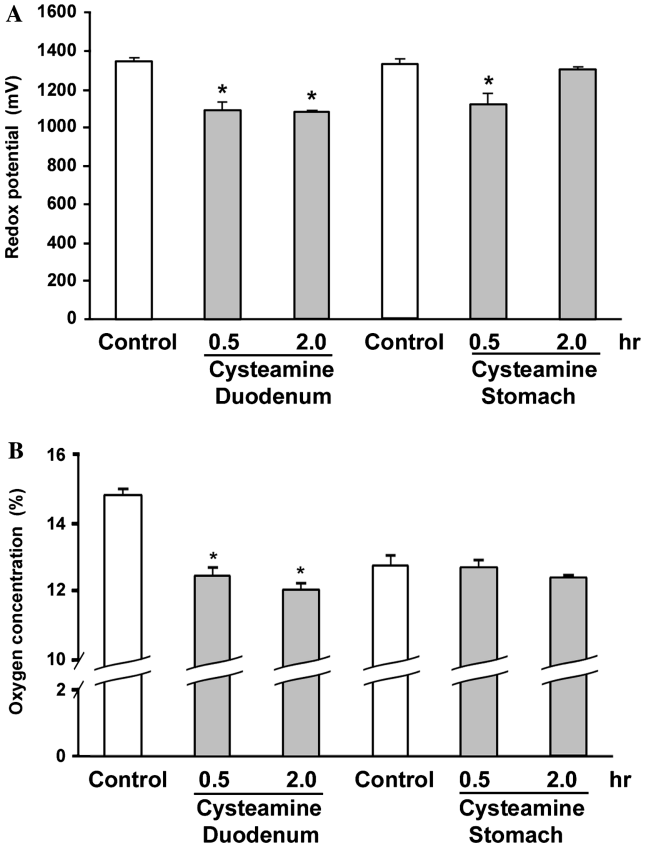

Fig. 1. Effect of the duodenal ulcerogen cysteamine on redox potential (A) and oxygen concentration (B) in duodenal and gastric mucosa. The measurements were performed using the microelectrodes, 0.5 and 2 h after treatment of rats with saline or cysteamine. * $p < 0.05$ vs. saline-treated animals (control).

duodenal tissue oxygenation was stable at the baseline and was reduced by 17% and 19% ($p < 0.05$) at 0.5 and 2 h after cysteamine administration, respectively. In gastric mucosa cysteamine did not induce any changes in tissue oxygenation (Fig. 1B). Cysteamine decreased the redox potential in gastric mucosa from 1338.6 ± 24.12 to 1130 ± 53.15 mV ($p < 0.05$) 0.5 h after administration and the values returned to the control level 2 h after cysteamine treatment (Fig. 1A). The decrease in redox potential reflects the elevation of redox state in rat duodenal mucosa after cysteamine administration.

The distribution of expression HIF-1α protein in nuclear and cytoplasmic fractions of duodenal mucosa

Rats treated with cysteamine exhibited an increase in HIF-1α protein expression in the proximal duodenal mucosa. Western blot analysis of nuclear and cytoplasmic extracts showed a cysteamine-induced redistribution of HIF-1α protein from the cytoplasm into the nucleus within 12 h of cysteamine treatment. Two hours after cysteamine, the expression of HIF-1α in nuclear fraction was increased by 42% and at 12 h by 77% compared to controls. Cytoplasmic distribution of HIF-1α was decreased in a time-dependent manner except a slight elevation at 6 h after cysteamine (Fig. 2).

HIF-1α transcriptional activity

The DNA-binding activity of HIF-1α nuclear protein does not always correlate with HIF-1α expression level. To determine whether an increase in HIF-1α transcriptional activity accompanies the increase in its expression, we performed the HIF-1α transcription activity assay which is more sensitive than electrophoretic mobility shift assay (EMSA). The results showed that 0.5 h after cysteamine administration, the level of HIF-1α protein binding to DNA increased vs. control (Fig. 3), independently from nuclear HIF-1α protein expression (Fig. 2), which was not affected by cysteamine. The elevation of the HIF-1α/DNA binding reached more than 33% at 2 h after cysteamine treatment and declined within 12 h of cysteamine exposure.

HIF-1α transcriptional interactions with other transcription factors

To determine the changes of HIF-1α binding to its DNA consensus sequence in duodenal mucosa after cysteamine administration and to detect the possible HIF-1α transcription interactions with other nuclear proteins we used the TranSignal TF–TF Interaction Array Kits from Panomics. These arrays contain the HIF-1 specific DNA *cis*-elements, which are able to activate ET-1 promoter activity (HIF-1: AGCTTGC CCTACGTGCTGTCTCAGA) [26], and two other HIF-1-binding sites (HBS: CAGTGCATACGTGGGC TCCA) with its downstream HIF-1 ancillary sequence (HAS: GCTCCAACAGGTCCTCTTCCT) identified previously as HIF-1 induced VEGF promoter activity by hypoxia [27]. The membrane was spotted with 96 different consensus sequences. The results demonstrated that in control duodenal mucosa HIF-1α binds to HIF-1 and HBS/HAS sequences and cysteamine increased this binding at 0.5 and 2 h after treatment (Fig. 4). However, HIF-1α did not bind to HBS or HAS sequences located separately on the membrane. The prominent HIF-1α binding with upstream stimulating factor (USF), which binds to the L-III BP oligonucleotide (binding element III for pyruvate kinase L gene), occurred. Cysteamine treatment increased HIF-1α binding to transcription factors that recognize the *cis*-elements such as XRE, ADR1, AhR/Arnt, $RREB_{(1,2)}$, Pax-4, GATA, MT-Box, and c-Rel (Fig. 4).

To determine the possible transcriptional interaction between HIF-1α and Egr-1 we used another array, containing oligonucleotides for the detection of redox-sensitive transcription factors. The results demonstrate

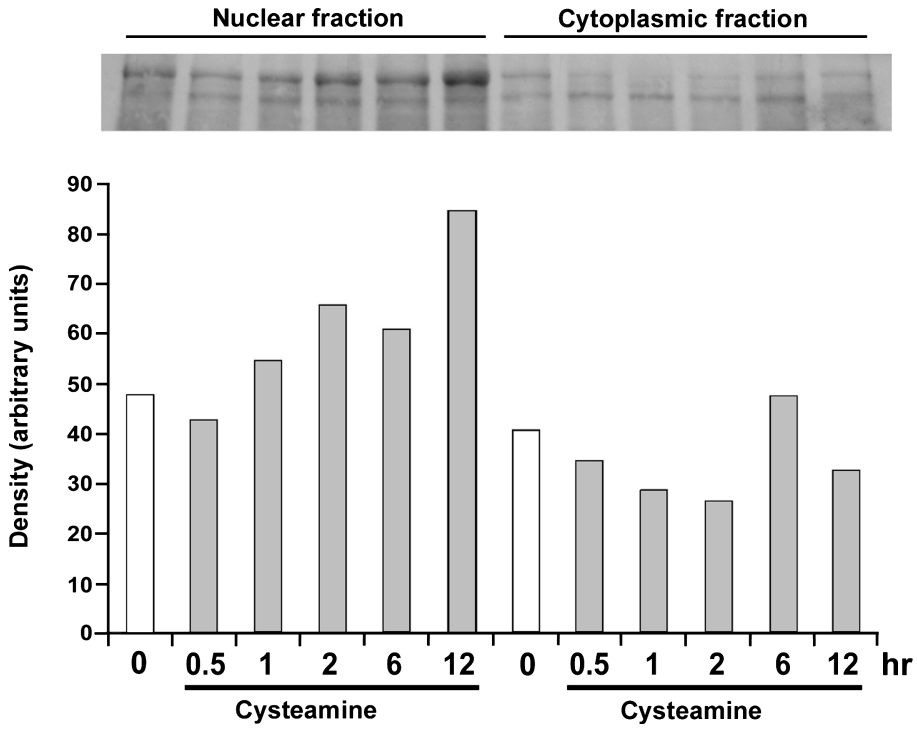

Fig. 2. Western blots for detection of HIF-1α in nuclear and cytoplasmic fractions of duodenal mucosa after administration of saline (control) or cysteamine at different times (0.5–12 h) in rats. These time points precede development of duodenal ulceration, which occurs at 24–48 h after cysteamine treatment.

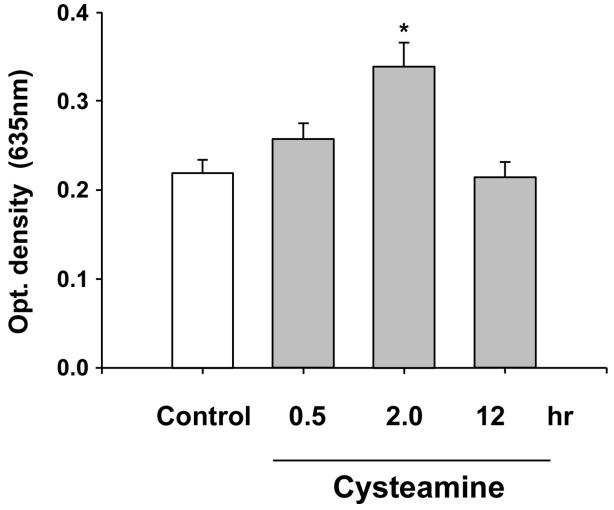

Fig. 3. Effect of cysteamine on HIF-1 binding to DNA in the proximal duodenal mucosa in rats. This assay is an ELISA-based 96-well format containing the consensus binding sequences for HIF-1 coated on the wells. Values are means ± SEM of duplicate determinations from four separate experiments. $*p < 0.05$ vs. saline treated animals.

that HIF-1α transcription factor binds strongly to Egr-1 in normal duodenal mucosa, and the binding was increased by cysteamine at 0.5 and 12 h after its administration. HIF-1α was also able to form the complexes with redox-sensitive transcription factors such as AP-1, AP-2, STAT4, MRE, c-Myb, GATA, and Pax-5 (Fig. 5) and cysteamine treatment co-activated their binding.

Discussion

This study demonstrates for the first time that treatment with cysteamine increases the redox status and reduces the tissue oxygenation of duodenal mucosa at early stage, before ulcer development. Cysteamine treatment increased HIF-1α expression, its binding to DNA, and enhanced the HIF-1α interactions with transcription factors (e.g., AP-1, AP-2, L-III BP, NF-E1, NF-E2, STAT4, and MRE) as well as their binding to DNA. Another important finding of this study is that cysteamine enhances transcriptional interaction in duodenal mucosa between HIF-1α and Egr-1, which is also redox-sensitive and hypoxia-inducible transcription factor.

Diverse mechanisms are involved in the activation of transcription factors in response to specific environmental signals. There is growing evidence that hypoxia and redox-signaling regulate transcription of a broad spectrum of genes through a common mechanism of O_2 sensing [28]. Moreover, redox chemistry appears to play a critical role in the trans-activation of genes involved in a protective or reparative cellular response to the damaging effects of oxidative stress and in promoting angiogenesis and cell survival [29].

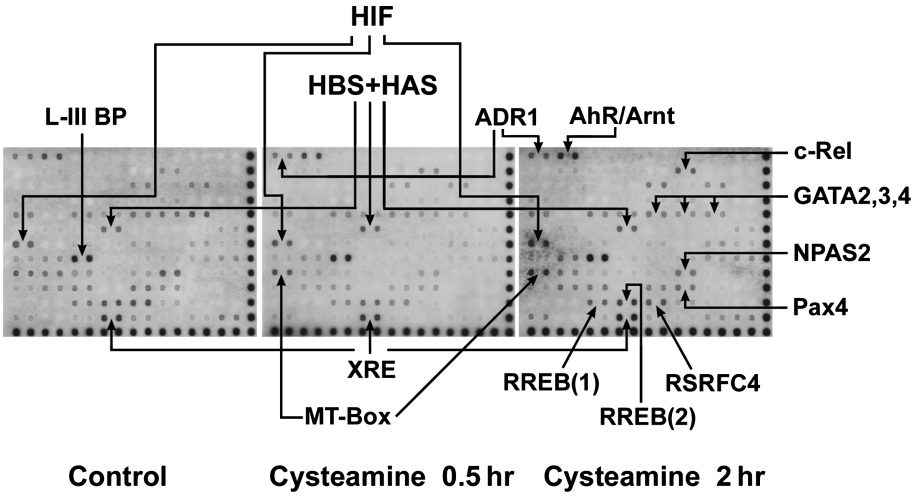

Fig. 4. TranSignal TF–TF Interaction Assay II: detection of changes in HIF-1/DNA binding and HIF-1α/transcription factor interactions in nuclear fraction of rat duodenal mucosa after administration of saline or cysteamine (0.5 and 2 h). The genes on the array are spotted in duplicate: the first row is DNA spotted normally, the second row is DNA diluted 1:10. Darker spots indicate more intense binding. The time-dependent cysteamine-induced increase in HIF-1α bindings was seen with transcription factors which bind to transcription factor *cis*-elements such as L-III BP (pyruvate kinase L gene-binding element III), HIF-1 (hypoxia-inducible factor 1), HAS (hypoxia-inducible factor ancillary sequence, *cis*-element for VEGF), HBS (HIF-1-binding site, *cis*-element regulating VEGF), XRE (xenobiotic response element), ADR1 (alcohol dehydrogenase regulatory gene 1-binding element), AhR/Arnt (aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator-binding element), $RREB_{(1,2)}$ (Ras-responsive transcription elements 1 and 2), GATA2,3,4 (GATA-binding protein 2,3,4), c-Rel (NFkB p75kDa protein), NPAS2 (neuronal PAS domain protein 2), and MT-Box (tentative new binding domain).

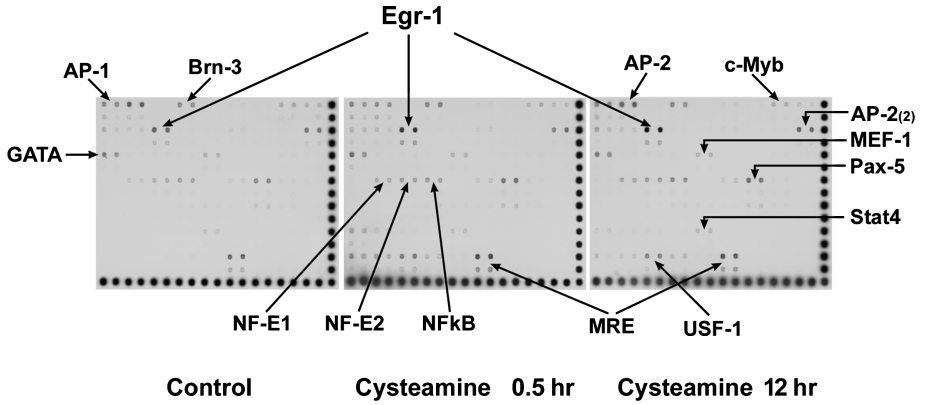

Fig. 5. TranSignal TF–TF Interaction Assay I: detection of changes in HIF-1α/redox transcription factor interactions in nuclear fraction of rat duodenal mucosa after administration of saline or cysteamine (0.5 and 12 h). The genes on the array are spotted in duplicate: the first row is DNA spotted normally, the second row is DNA diluted 1:10. HIF-1α forms the complex with redox-sensitive transcription factors such as Egr-1, AP-1, AP-2, STAT4, NFkB, MRE (mineralocorticoid response element), c-Myb, GATA, Pax-5, USF-1, MEF-1, Brn, and cysteamine co-activated their binding.

Cysteamine alters the reduction/oxidation to reduction state in the proximal duodenal mucosa in the early, pre-ulcerogenic stage. Such alterations in the redox balance might lead to the reduction of protein disulfide groups and increased formation of proteins that contain the free sulfhydryls. At the same time, cysteamine significantly decreases the oxygen content in duodenal mucosa leading to generation of the reactive oxygen radicals. We performed the measurement of tissue oxygenation and redox state 0.5 and 2 h after a single dose of cysteamine. However, in our model we need to give cysteamine three times in 4 h intervals to induce the perforating duodenal ulcer. Thus, our dose was only 33% of ulcer inducing dose. Cysteamine does not induce gastric ulcers, and in our present study cysteamine did not change the level of oxygenation in the gastric mucosa and only slightly decreased the redox state in the rat stomach.

Our work further demonstrates the role of hypoxia in the pathogenesis of duodenal ulceration and explains its association with significant redox potential changes in the proximal duodenal mucosa. The best characterized mechanism triggering adaptation to hypoxia involves the transcriptional regulator HIF-1α. Activation of

HIF-1α by low oxygen tension results in expression of genes which activate metabolic and other cellular processes that enhance cell survival, angiogenesis, and healing [22,24,30]. The activation of HIF-1α and resulting induction of VEGF expression was demonstrated during gastric and esophageal ulceration [31,32]. Cysteamine induced VEGF expression, increased ET-1 release and synthesis in our previous studies [11,16], which might be related to the cysteamine-enhanced HIF-1α transcriptional activity in duodenal mucosa.

Although HIF-1α is critical for restoring cellular homeostasis under hypoxia, the molecular events triggered by hypoxia extend beyond HIF-1α. Our study demonstrated that cysteamine-induced hypoxia and increased redox state in proximal duodenal mucosa lead to activation of complex interactions between hypoxia-related transcription factors such as Egr-1, AP-1, AP-2, L-III BP, NF-E1, NF-E2, STAT4, MRE, and others. Another report characterized HIF-1-binding site in the human ET-1 promoter which activates of ET-1 expression in endothelial cells [26]. However, the HIF-1-binding site alone is not sufficient for the response to hypoxia and requires an additional 50 bp of flanking sequence that includes binding sites for the factors such as activator protein-1 (AP-1), GATA-2, and CAAT-binding factor (NF-1). These results suggest an essential role for AP-1, GATA-2, and NF-1 in stabilizing the binding of HIF-1 and promoting recruitment of p300/CBP to the ET-1 hypoxia response complex. Site-directed mutational analysis of VEGF gene promoter revealed that an HIF-1-binding site (HBS) and its downstream HIF-1 ancillary sequence (HAS) within hypoxia response element are required as *cis*-element for transcriptional activation of VEGF by hypoxia [27], and that the AP-1 site is required for its optimal response [33]. The response of c-jun/AP-1 to chronic hypoxia is also HIF-1α dependent [34].

Recently we demonstrated that the cysteamine-induced increase in Ref-1 and Trx expression might activate the transcriptional activity of Egr-1 in the duodenal mucosa [16]. Both of these proteins, Ref-1 and Trx, were identified as reductive activators of redox-sensitive transcription factors. Other investigators showed that Trx-1 increases hypoxia-induced HIF-1α transcriptional activity [25]. Trx-1 transfection also caused a significant increase in the protein products of hypoxia-responsive genes, including VEGF [35]. Transfection of a redox-inactive Trx-1 markedly decreased levels of HIF-1α protein, HIF-1 transactivating activity, and VEGF protein in MCF-7 cells compared with empty vector controls [35].

Thus, this is the first demonstration that the duodenal ulcerogen cysteamine reduces duodenal mucosal oxygenation, causes profound changes in the redox balance and activates HIF-1α in the proximal duodenum. These changes trigger a cellular defensive response leading to the activation of redox-sensitive transcription factors and are essential for growth, differentiation, angiogenesis, and healing. These defensive responses include not only the transcriptional activation of hypoxia-inducible transcription factors HIF-1α and Egr-1, but also transcriptional interaction of these proteins with other redox-sensitive transcription factors in the duodenal mucosa prior to ulcer development.

Acknowledgment

The present study was supported by a Department of Veterans Affairs, Medical Research Service Merit Review.

References

[1] H. Selye, S. Szabo, Experimental model for production of perforating duodenal ulcers by cysteamine in the rat, Nature 244 (1973) 458–459.
[2] S. Szabo, Animal model of human disease. Duodenal ulcer disease. Animal model: cysteamine-induced acute and chronic ulcer in the rat, Am. J. Pathol. 93 (1978) 273–276.
[3] S. Szabo, G. Pihan, Development and significance of cysteamine and propionitrile models of duodenal ulcer, Chronobiol. Int. 4 (1987) 31–42.
[4] J.E. Biaglow, R.W. Issels, L.E. Gerweck, M.E. Varnes, B. Jacobson, J.B. Mitchell, A. Russo, Factors influencing the oxidation of cysteamine and other thiols: implications for hyperthermic sensitization and radiation protection, Radiat. Res. 100 (1984) 298–312.
[5] G. Capozzi, G. Modena, Oxidation of thiols, in: S. Patai (Ed.), The Chemistry of the Thiol Group, John Wiley, London, 1974, pp. 785–840.
[6] H.I. Tahsildar, J.E. Biaglow, M.M. Kligerman, M.E. Varnes, Factors influencing the oxidation of the radioprotector WR-1065, Radiat. Res. 113 (1998) 243–251.
[7] B. Halliwell, J.M.C. Gutteridge, Free Radical in Biology and Medicine, Clarendon Press, Oxford, 1989.
[8] T.M. Jeitner, D.A. Lawrence, Mechanisms for the cytotoxicity of cysteamine, Toxicol. Sci. 63 (2001) 57–64.
[9] K.D. Held, J.E. Biaglow, Mechanisms for the oxygen radical-mediated toxicity of various thiol-containing compounds in cultured mammalian cells, Radiat. Res. 139 (1994) 15–23.
[10] A. Holmgren, Thioredoxin, Annu. Rev. Biochem. 54 (1985) 237–271.
[11] S. Szabo, A. Vincze, Z. Sandor, M. Jadus, Z. Gombos, A. Pedram, E. Levin, J. Hagar, G. Iaquinto, Vascular approach to gastroduodenal ulceration: new studies with endothelins and VEGF, Dig. Dis. Sci. 43 (1998) 40S–45S.
[12] M. Yanagisawa, H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, K. Goto, T. Masaki, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 322 (1988) 411–415.
[13] H. Tanaka, K. Takeuchi, S. Okabe, M. Murakami, Pathogenesis of the earliest epithelial cell damage induced by mepirizole and cysteamine in the rat duodenum, Jpn. J. Pharmacol. 51 (1989) 509–519.
[14] T. Khomenko, X.M. Deng, M.R. Jadus, S. Szabo, Effect of cysteamine on redox-sensitive thiol-containing proteins in the duodenal mucosa, Biochem. Biophys. Res. Commun. 309 (2003) 910–916.

[15] T. Khomenko, S. Szabo, X.M. Deng, H. Matsumoto, H. Ishikawa, Aggravation of experimental duodenal ulcers by the suppression of transcription factor Egr-1 synthesis with egr-1 antisense, Gastroenterology 120 (2001) A144.
[16] X.M. Deng, S. Szabo, T. Khomenko, M. Yoshida, W.H. Florsheim, Changes in endogenous vascular endothelial growth factor (VEGF) in duodenal ulceration, Gastroenterology 116 (1999) A601.
[17] G.L. Semenza, G.L. Wang, A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation, Mol. Cell. Biol. 12 (1992) 5447–5454.
[18] G.L. Wang, G.L. Semenza, Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia, J. Biol. Chem. 268 (1993) 21513–21518.
[19] G.L. Wang, B.H. Jiang, E.A. Rue, G.L. Semenza, Hypoxia-inducible factor 1 is a basic-helix–loop–helix–PAS heterodimer regulated by cellular O_2 tension, Proc. Natl. Acad. Sci. USA 92 (1995) 5510–5514.
[20] G.L. Wang, G.L. Semenza, Purification and characterization of hypoxia-inducible factor 1, J. Biol. Chem. 270 (1995) 1230–1237.
[21] S. Salceda, J. Caro, Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin–proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes, J. Biol. Chem. 272 (1997) 22642–22647.
[22] J.A. Forsythe, B.H. Jiang, N.V. Iyer, F. Agani, S.W. Leung, R.D. Koos, G.L. Semenza, Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1, Mol. Cell. Biol. 16 (1996) 4604–4613.
[23] H.F. Bunn, J. Gu, L.E. Huang, J.W. Park, H. Zhu, Erythropoietin: a model system for studying oxygen-dependent gene regulation, J. Exp. Biol. 201 (1998) 1197–1201.
[24] J.E. Nor, J. Christensen, D.J. Mooney, P.J. Polverini, Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression, Am. J. Pathol. 154 (1999) 375–384.
[25] L.E. Huang, Z. Arany, D.M. Livingston, H.F. Bunn, Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit, J. Biol. Chem. 271 (1996) 32253–32259.
[26] K. Yamashita, D.J. Discher, J. Hu, N.H. Bishopric, K.A. Webster, Molecular regulation of the endothelin-1 gene by hypoxia. Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, and p300/CBP, J. Biol. Chem. 276 (2001) 12645–12653.
[27] H. Kimura, A. Weisz, T. Ogura, Y. Hitomi, Y. Kurashima, K. Hashimoto, F. D'Acquisto, M. Makuuchi, H. Esumi, Identification of hypoxia-inducible factor 1 ancillary sequence and its function in vascular endothelial growth factor gene induction by hypoxia and nitric oxide, J. Biol. Chem. 276 (2001) 2292–2298.
[28] H.F. Bunn, R.O. Poyton, Oxygen sensing and molecular adaptation to hypoxia, Physiol. Rev. 76 (1996) 839–885.
[29] G.L. Semenza, Surviving ischemia: adaptive responses mediated by hypoxia-inducible factor 1, J. Clin. Invest. 106 (2000) 809–812.
[30] D.A. Elson, G. Thurston, L.E. Huang, D.G. Ginzinger, D.M. McDonald, R.S. Johnson, J.M. Arbeit, Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1alpha, Genes Dev. 15 (2001) 2520–2532.
[31] I.L. Szabo, H. Kawanaka, M.K. Jones, R. Pai, B.A. Soreghan, D. Baatar, S.S. Husain, A.S. Tarnawski, Activation of hypoxia inducible factor-1alpha in gastric mucosa in response to ethanol injury: a trigger for angiogenesis?, Life Sci. 69 (2001) 3035–3044.
[32] D. Baatar, M.K. Jones, K. Tsugawa, R. Pai, W.S. Moon, G.Y. Koh, I. Kim, S. Kitano, A.S. Tarnawski, Esophageal ulceration triggers expression of hypoxia-inducible factor-1 alpha and activates vascular endothelial growth factor gene: implications for angiogenesis and ulcer healing, Am. J. Pathol. 161 (2002) 1449–1457.
[33] H. Kimura, A. Weisz, Y. Kurashima, K. Hashimoto, T. Ogura, F. D'Acquisto, R. Addeo, M. Makuuchi, H. Esumi, Hypoxia response element of the human vascular endothelial growth factor gene mediates transcriptional regulation by nitric oxide: control of hypoxia-inducible factor-1 activity by nitric oxide, Blood 95 (2000) 189–197.
[34] K.R. Laderoute, J.M. Calaoagan, C. Gustafson-Brown, A.M. Knapp, G.C. Li, H.L. Mendonca, H.E. Ryan, Z. Wang, R.S. Johnson, The response of c-jun/AP-1 to chronic hypoxia is hypoxia-inducible factor 1 alpha dependent, Mol. Cell. Biol. 22 (2002) 2515–2523.
[35] S.J. Welsh, W.T. Bellamy, M.M. Briehl, G. Powis, The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1 alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis, Cancer Res. 62 (2002) 5089–5095.