Inhibition of TNF-α induced ICAM-1, VCAM-1 and E-selectin expression by selenium

By: Fan Zhang, Wei Yu, James L. Hargrove, Phillip Greenspan, Roger G. Dean, Ethan W. Taylor, and Diane K. Hartle

Fan Zhang, Wei Yu, James L. Hargrove, Phillip Greenspan, Roger G. Dean, Ethan W. Taylor, and Diane K. Hartle. Inhibition of TNF-α induced ICAM-1, VCAM-1 and E-selectin expression by selenium. *Atherosclerosis.* Volume 161, Issue 2, April 2002, Pages 381-386. https://doi.org/10.1016/S0021-9150(01)00672-4

Abstract:

The initiation of an atherosclerotic lesion involves an endothelial cell pro-inflammatory state that recruits leukocytes and promotes their movement across the endothelium. These processes require endothelial expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial-leukocyte adhesion molecule-1 (E-selectin). Tumor necrosis factor-α (TNF-α) is a powerful inducer of these adhesion molecules. Selenium status is known to affect the rate of atherosclerosis. These experiments tested whether selenium alters cytokine-induced expression of these adhesion molecules. Human umbilical vein endothelial cells (HUVECs) were pretreated for 24 h with sodium selenite (0–2 μM) and then treated with 0 or 50 U/ml TNF-α in the presence of 0–2 μM selenite. ICAM-1, VCAM-1 and E-selectin were detected by ELISA and their mRNAs were evaluated by Northern blots. Selenite significantly inhibited TNF-α-induced expression of each adhesion molecule in a dose-dependent manner and reduced the level of the respective mRNAs. Nuclear factor-κB (NF-κB) is required for transcription of these adhesion molecule genes. Western blot analysis revealed that selenite did not inhibit the translocation of the p65 subunit of NF-κB to the nucleus. In conclusion, these data indicate selenium can modulate cytokine-induced expression of ICAM-1, VCAM-1 and E-selectin in HUVECs without interfering with translocation of NF-κB.

Keywords: Selenium | ICAM-1 | VCAM-1 | E-selectin | NF-κB | HUVEC | Endothelium

Article:

1. Introduction

One function of the vascular endothelial cell is to recruit leukocytes into underlying tissue during inflammation. The transmigration of leukocytes across the endothelial lining of the blood vessel is regulated in part by specific endothelial–leukocyte adhesion molecules [1]. The leukocyte transmigration process is basic to both acute and chronic inflammatory states [1], [2], [3]. A major event in the development of the pro-inflammatory state is the expression of endothelial

cell adhesion molecules for the recruitment of leukocytes to the site of injury. The regulation of NF-κB activation is one key step in controlling inflammatory processes. Suppression of the pro-inflammatory endothelial cell state will limit the atherosclerotic process.

The induced expression of ICAM-1, VCAM-1 and E-selectin requires the activation of NF-κB. NF-κB is an inducible transcriptional factor, which exists in the cytoplasm in its inactive form as the protein heterodimer, p50/p65, associated with an inhibitor protein, IκB. Extracellular inducers attach to specific cell surface receptors that recruit binding proteins. These proteins initiate a cascade of kinases that result in the activation of IκB kinases. Phosphorylation of IκB releases it from the p50/p65 heterodimer and marks it for proteosomal degradation. Activated NF-κB translocates to the nucleus and binds DNA at specific κB sites in promotor regions [4], [5].

Selenium is a micronutrient whose antioxidant functions are mediated through selenoproteins such as the glutathione peroxidases and thioredoxin reductases [6]. Selenium deficiency decreases protection from oxidative stress, decreases glutathione peroxidase activity, increases lipid peroxidation (including LDL oxidation) and increases the risk of atherosclerosis. Conversely, supplementation with selenium activates the glutathione system, protects against peroxidation of LDL and other lipids and slows the atherosclerotic process [7], [8], [9]. Selenium deficiency also enhances neutrophil adhesion to endothelial cells [10].

This study tested whether physiological levels of selenium would alter the expression of TNF-α-induced expression of ICAM-1, VCAM-1 and E-selectin in HUVECs. Follow-up studies determined the effects of selenium on the activation of NF-κB caused by TNF-α treatment.

2. Methods

2.1. Cell culture

HUVECs and an EGM-Bulletkit were purchased from Clonetics (San Diego, CA). HUVECs were cultured at 37 °C in endothelial cell basal medium supplemented with 10 ng/ml human recombinant epidermal growth factor, 1 μg/ml hydrocortisone, 50 μg/ml gentamicin, 50 ng/ml amphotericin-B, 12 μg/ml bovine brain extract and 2% fetal bovine serum (FBS). The tissue culture plates and flasks were coated with 0.1% gelatin and fourth-passage cells were used for these experiments.

2.2. Enzyme linked immunosorbent assays (ELISA)

ICAM-1, VCAM-1 and E-selectin on cell surfaces were quantified by ELISA with slight modifications to method described by Chen et al. [11]. HUVECs were cultured to confluence and pretreated with sodium selenite (0, 0.5, 1.5, and 2 μM) for 24 h at 37 °C. These pretreated cells were then incubated with fresh growth medium, which contained TNF-α (0 or 50 U/ml) and the same concentration of sodium selenite as in the pretreatment medium. Cells were incubated with TNF-α/EGCG for 4, 6, and 8 h for measurement of E-selectin, VCAM-1 and ICAM-1, respectively. After this incubation, the cells were washed with PBS and fixed with 1% paraformaldehyde for 15 min, and then treated with 2% BSA overnight at 4 °C. After another

PBS wash, the cells were incubated with monoclonal antibodies (Chemicon, Temecula, CA) against either E-selectin, VCAM-1, or ICAM-1 for 2 h at 37 °C. The concentration of each antibody was 1 μg/ml. Then the cells were washed three times in PBS with 0.05% Tween 20 (PBS-T) and incubated for 2 h at 37 °C with 1 μg/ml goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma, St Louis, MO). The cells were washed three times with PBS-T and then incubated with 1.0 mg/ml substrate *p*-nitrophenyl phosphate for 30 min. Samples were read spectrophotometrically at 405 nm. Test and control samples were assessed in triplicate in each experiment, a total of three experiments were performed. The degree of specific antibody binding was calculated by subtracting the mean negative control value from each test value, and expressing the results as a percentage of the TNF-α-treated control value.

2.3. Northern blot analyses

Cultured HUVECs were pretreated with sodium selenite (0 and 2 μM) for 24 h. The pretreated cells were then incubated with fresh medium, which contained TNF-α (0 or 50 U/ml) and the same concentration sodium selenite as in the previous incubation. Total RNA was isolated using a SV Total RNA Isolation System (Promega, Madison, WI). Electrophoresis of 20 μg RNA per slot was performed in a 1.2% agarose and 2% formaldehyde gel. The RNA was transferred to a HybondTM-N+ membrane (Amersham, Piscataway, NJ) and immobilized by ultraviolet illumination. cDNA probes were labeled by fluorescein random primer labeling (Amersham, Piscataway, NJ). The membranes were hybridized at 60–65 °C overnight with probes specific for human ICAM-1, VCAM-1 and E-selectin. After hybridization, the membranes were washed with 0.5×SSC (0.3 M NaCl and 0.03 M sodium citrate, pH 7) and 0.1% SDS for 15 min at 60–65 °C, followed by a stringent wash with 0.1× SSC and 0.1% SDS at 60–65 °C. Next, the membranes were blocked and then incubated with an anti-fluorescein alkaline phosphatase conjugate (Amersham, Piscataway, NJ). After washing three times with 0.3% (v/v) Tween 20 in buffer A (100mM Tris–HCl, 300mM NaCl pH 9.5), the detection reagent was added, and films were exposed and developed.

2.4. Western blot analyses

HUVECs were pretreated with sodium selenite (0 and 2 μM) for 24 h, then incubated for 1 h with fresh medium containing TNF-α (0 or 50 U/ml) and the same concentration of sodium selenite as in the previous incubation. Nuclear extracts were prepared as described by Schreiber [12]. Briefly, 10^6 cells were washed in 10 ml PBS, scraped, and pelleted by centrifugation at 1500×*g* for 5 min. The cell pellet was then resuspended in 400 μl of cold buffer A (10 mM HEPES, pH 7.9; 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA, 1 mM DTT; 0.5 mM PMSF; 2 μg/ml leupeptin, and 2 μg/ml aprotinin). After incubating for 15 min at 4 °C, 25 μl of 10% NP-40 was added and the suspension was vortexed at for 10 s. After recentrifugation for 30 s, the nuclear pellet was resuspended in 50 μl of ice-cold buffer B (20 mM HEPES, pH 7.9; 0.4 M NaCl; 1 mM EDTA; 1 mM EGTA, 1 mM DTT; 1 mM PMSF; 25% glycerol; 2 μg/ml leupeptin and 2 μg/ml aprotinin). Thereafter it was homogenized, and shaken vigorously for 15 min at 4 °C. The nuclear extract was then centrifuged for 5 min at 4 °C. Protein concentrations were determined by the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). Equal amount of extracts (40 μg) were subjected to electrophoresis on 12% SDS-polyacrylamide gels and the fractionated proteins were electrophoretically transferred to Hybond ECL nitrocellulose

membranes (Amersham, Piscataway, NJ) in a tank transfer system. The membranes were blocked and then incubated with 1 μg/ml rabbit anti-NF-κB p65 subunit polyclonal antibody (Chemicon, Temecula, CA) for 1 h, washed three times with PBS-T (PBS with 0.05% Tween 20), then incubated for 1 h with a polyclonal donkey antirabbit IgG Ab conjugated with horseradish peroxidase. After washing five times with PBS-T, the ECL detection reagents were added, and the membranes were exposed to HyperfilmTM (Amersham, Piscataway, NJ).

2.5. Statistical analyses

The ELISA data are expressed as the mean±S.E.M. Comparisons among groups were made using one-way analysis of variance (ANOVA), followed by Fisher's Least Significant Difference posthoc comparison test. A value of $P \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. ELISA results

Sodium selenite did not alter the expression of the three adhesion molecules in non-TNF-α treated control cultures but significantly inhibited TNF-α induced expression in a dose-dependent manner (Fig. 1). Percent inhibition by 2 μM selenium was 53, 81, and 71% for ICAM-1, VCAM-1, and E-selectin, respectively. Significant inhibition of the expression of the three adhesion molecules was observed at 1 μM sodium selenite treatment. The highest concentration of sodium selenite (2 μM) used in our experiments did not alter cellular viability as assessed by cell number, cellular morphology, and trypan blue exclusion (data not shown).

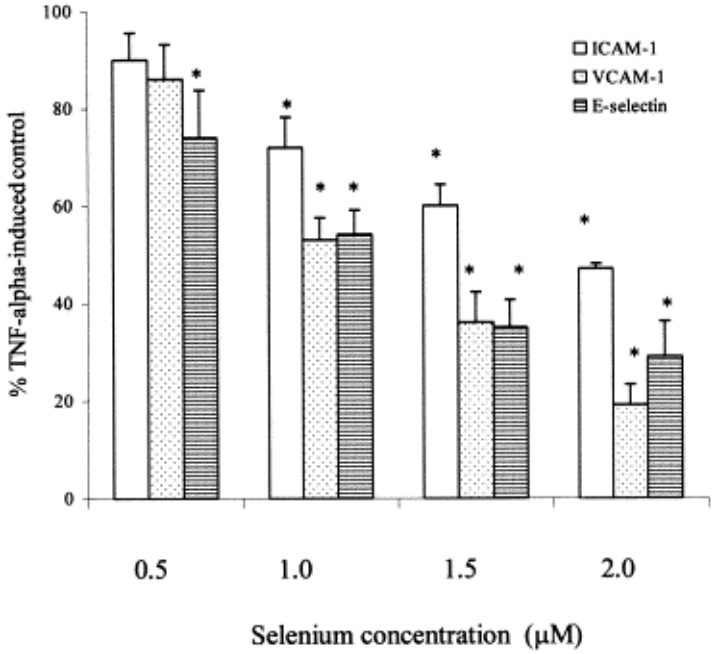

Fig. 1. ELISA for detection of cell surface expression of ICAM-1, VCAM-1 and E-selectin. HUVECs were pretreated with sodium selenite and induced by TNF-α in the presence sodium selenite. The data are given as the mean ± S.E.M. A value of $P \leq 0.05$ was considered to be statistically significant.

3.2. Northern blot results

Selenium inhibited the cell surface expression of ICAM-1, VCAM-1 and E-selectin stimulated by TNF-α. Furthermore, mRNA expression was evaluated by Northern blotting (Fig. 2). Ethidium bromide staining for 28 S ribosomal RNA indicated all lanes contained identical amounts of RNA. TNF-α increased the expression of mRNAs for each of these cell adhesion molecules compared with non-TNF-α treated cells. The accumulation of the specific mRNAs after induction with TNF-α was reduced with treatment with 2 μM sodium selenite. These data correlated with the decreased expression of the respective proteins, suggesting that the regulatory action of selenium on the expression of these adhesion molecules may be at the transcriptional level.

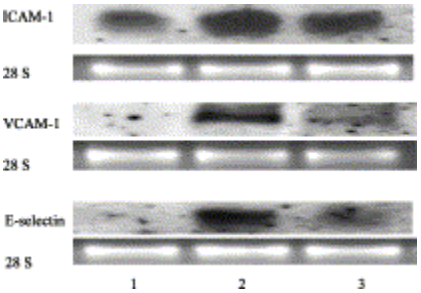

Fig. 2. Northern blot for evaluation of mRNA of ICAM-1, VCAM-1 and E-selectin. The bottom panels contain 28 S ribosomal RNA stained by ethidium bromide to show equal loading. Lane 1 was a negative control without sodium selenite and TNF-α treatments. Lane 2 was a positive control in which HUVECs were induced by TNF-α. Lane 3 was a test group in which cells were pretreated with sodium selenite and induced by TNF-α in the presence of sodium selenite.

3.3. Western blot results

Nuclear p65 was detected by Western blotting to assess the translocation of NF-κB (Fig. 3). The p65 subunit increased in the nuclear fraction of HUVECs induced by TNF-α compared with non-TNF-α treated control cultures. Sodium selenite treatment did not reduce p65 translocation to the nucleus after TNF-α treatment. The selenium concentration used in this test was the same amount as that which reduced cell adhesion molecule mRNAs and protein expression after TNF-α induction.

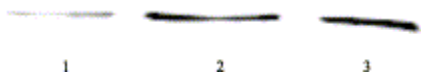

Fig. 3. Nuclear p65 was detected by Western blotting to analyze the translocation of NF-κB. Lane 1 was a negative control without sodium selenite and TNF-α treatments. Lane 2 was a positive control in which HUVECs were induced by TNF-α. Lane 3 was a test group in which cells were pretreated with sodium selenite and induced by TNF-α in the presence of sodium selenite.

4. Discussion

Human epidemiological and animal studies have associated low physiological levels of selenium with decreased antioxidant status and increased risk of atherogenesis [7], [8], [9]. Since atherosclerosis is a chronic inflammatory disease associated with increased oxidative stress on the vascular endothelial cell, it is reasonable to conclude that the anti-atherosclerotic effects of selenium are due to its ability to support anti-oxidative defense mechanisms.

In these studies, we demonstrated that selenium suppressed expression of three key endothelial adhesion molecules after HUVECs were stimulated with the inflammatory cytokine, TNF-α. Expression of these adhesion molecules is a hallmark event in the development of the pro-inflammatory state of the endothelial cell. This state is an early step in atherosclerotic plaque formation that promotes the attraction, adherence and subendothelial migration of monocytes in the area of dysfunctional endothelium. The interactions between endothelial cells and leukocytes occur via two-way chemical signaling that controls gene expression in each cell type.

There are at least two mechanisms by which free radicals are involved in regulation of the expression of endothelial–leukocyte adhesion molecules. The first is by producing oxidative damage to macromolecules of the endothelium. The second involves the intracellular formation of oxidative signals in response to extracellular stimuli or membrane signal transduction events [2], [13]. Antioxidants can intervene in either or both of these oxidative steps. Selenium is known to affect the activity of both extracellular and intracellular antioxidant enzymes. It, therefore, could alter both external and internal oxidative activators of cell adhesion molecule expression.

ICAM-1, VCAM-1 and E-selectin are three adhesion molecules that are expressed in the pro-inflammatory state and mediate the migration of leukocytes through the endothelial barrier. The expression of these endothelial adhesion molecules is regulated by activation of NF-κB [2], [3]. NF-κB can be activated by a large number of extracellular stimuli, including the cytokine, TNF-α, which was used in this study [4], [5]. There is considerable evidence that reactive oxidative intermediates, namely, lipid hydroperoxides, may be a common intracellular signal for at least some activators of NF-κB [14]. We reasoned that if TNF-α signaled NF-κB activation through a pathway that involved reactive oxidative intermediates, selenium pretreatment might bolster antioxidant defenses and thereby suppress activation of NF-κB. We found that with combined selenium and TNF-α treatment, NF-κB still translocated to the nucleus, but mRNAs of ICAM-1, VCAM-1 and E-selectin were decreased when compared with the TNF-α induced control. We also observed dose-dependent decreases in ICAM-1, VCAM-1 and E-selectin protein expression as determined by ELISA. We concluded that selenium blocked the cytokine-induced expression of cell adhesion molecules via a mechanism independent of the translocation of NF-κB. It can be inferred that if the signaling pathway did involve free radical signals, selenium-dependent oxidative defenses were not able to quench the specific signal.

Recent research shows that selenium inhibits TNF-α induced NF-κB activation by two pathways. First, the activity, as well as the expression, of glutathione peroxidases are regulated by selenium status [15]. These enzymes catalyze the reduction of peroxides that can damage cells [6]. Glutathione peroxidase mimics have been shown to strongly inhibit TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin as well as to inhibit leukocyte recruitment by HUVECs [16], [17]. In comparing these experiments with our present data, it is important to note

that the concentration of these seleno-organic compounds used in prior experiments was quite high, 10 μM. Although these experiments demonstrated the ability of selenium-based glutathione peroxidase mimics to suppress cytokine-induced cell adhesion molecule expression, it was unclear whether the suppression was causally related to glutathione peroxidase activity or to some other action of the seleno-compounds. Overexpression of phospholipid hydroperoxide glutathione peroxidase in a human endothelial cell line inhibited the NF-κB activation induced by interleukin-1, (IL-1) [18]. That work suggested that a phospholipid hydroperoxide intermediate mediated IL-1 activation of NF-κB. As far as we know, this same experiment has not been conducted using TNF-α. It is known that TNF-α and IL-1 have different intracellular signaling cascades.

Interactions of selenium with TNF-α induced expression of adhesion molecules have been described in other experimental systems [10]. Omission of selenium in cultured bovine mammary endothelial cells caused glutathione peroxidase activity to decrease to 10% of normal. While the actual selenium concentration was not reported, this degree of deficiency significantly enhanced the ability of TNF-α to increase neutrophil adherence and mRNA expression for E-selectin and ICAM-1 [10]. In another study, selenium supplementation was demonstrated to inhibit the expression of ICAM-1, VCAM-1 and E-selectin induced by interferon-γ in a dose-dependent manner. The half-maximal inhibitory concentrations of selenium were 0.5, 4, and 3.8 μg/ml [19]. These results were akin to the present results except that the inducing agent used was interferon-γ and the concentrations of selenium were above the physiological range (0.02–10 μg/ml or 0.25–126.6 μM). Plasma and serum selenium normally ranges from 0.049 to 0.168 μg/ml (0.6–2.1 μM) for healthy individuals [20]. The concentration of selenium in our experiments in the physiological range (0.5, 1.0, 1.5. 2 μM).

Makropoulous et al. demonstrated that in the selenium-deprived Jurkat and Esb-LT lymphocytes, 100 nM selenite repletion for 3 days produced maximum increases in glutathione peroxidase activity and inhibition of NF-κB binding to κB site. The dose-response of NF-κB inhibition roughly corresponded to the ability of selenium to increase glutathione peroxidase activity. It was inferred that selenium altered cellular redox systems and indirectly inhibited IκB dissociation [21]. This led us to postulate that selenium might inhibit the translocation of NF-κB in HUVECs during a TNF-α challenge. The Western blot analysis in the present experiments showed that selenium did not inhibit the translocation of NF-κB even at the highest physiological dose employed, 2 μM. We concluded that its actions in blocking cell adhesion molecule expression must be at a later step of NF-κB activation or independent of the NF-κB activation pathway.

Another possible point of intervention for selenium would be for it to interfere with the ability of NF-κB to bind to its κB sites in the promoter region. After activation of NF-κB, the essential cysteine residues required for NF-κB binding to DNA are maintained in the reduced state through the action of thioredoxin and thioredoxin reductase. It has been shown that selenite can decrease NF-κB binding to DNA by about 40% at 1 μM and can completely abolish binding at 7 μM. In addition, NF-κB binding could be completely restored by 2 mM dithiothreitol, even in the presence of 10 μM selenite with Jurkat T cell nuclear extracts precubated with 0.1 nM human TNF-α for 20 min [22]. The binding of NF-κB to its κB binding sites depends on critical cysteine residues remaining in a reduced state in Jurkat T cells [23]. Selenite inhibition of NF-κB binding

activity was, therefore, interpreted to be due to RS-Se-SR adduct formation of selenium with the essential thiols of NF-κB [22]. We did not study NF-κB binding to DNA in these experiments. However, the same concentration of selenite that reduced NF-κB binding by 40% [22], significantly inhibited TNF-α induced expression of ICAM-1 (−28%), VCAM-1 (−47%) and E-selectin (−46%) in cultured HUVECs.

In conclusion, selenium inhibits TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin in HUVECs. The mechanism of inhibition may occur after the translocation of NF-κB into the nucleus. Our data are consistent with at least two mechanisms that could explain this action. Selenium treatment may inhibit NF-κB binding by directly forming adducts with the essential thiols of NF-κB [22]. Secondly, the selenoprotein, thioredoxin/thioredoxin reductase, regulates the activity of NF-κB through redox control of the cysteine residues necessary for its DNA-binding activity [23], [24]. Interestingly, although selenium is required for the activity of thioredoxin/thioredoxin reductase, it can also inactivate this enzyme by forming a stable diselenide derivative [25]. The reduction of the activity of thioredoxin/thioreductase, coupled with the direct ability of selenium to form adducts with NF-κB would significantly alter the availability of the critical cysteine residues for NF-κB binding to DNA. The present data are consistent with an ability of selenium to interfere with the cell adhesion molecule expression, possibly by blocking NF-κB binding to DNA.

Selenium has multiple actions that it can be considered to be anti-inflammatory and anti-atherosclerotic. The above work describes yet another important function of this micronutrient. The significance of the ability of selenium to inhibit activation of NF-κB in response to TNF-α may also help explain anti-tumor growth and anti-metastatic effects of selenium that have been observed both in vivo and in vitro. Dietary supplementation with selenium significantly reduced the incidence of metastasis, the number of lung tumors, the tumor cross-sectional area and the tumor volume in a mouse metastatic melanoma model [26]. In an in vitro cell culture model, others have demonstrated that pre-exposure of HeLa cells to micromolar concentrations of selenite reduced their ability to attach to a solid matrix [27]. Metastatic tumor cells engage in upregulation of cell adhesion molecules to promote their ability to invade and metastasize. Regulation of endothelial cell adhesion molecules by tumor cells promotes angiogenesis and neovascularization in tumors [28]. In addition, tumor cells that have been shed into the vasculature are able to extravasate at distal sites by using tumor cell/endothelial cell adhesion molecule interactions. There is increasing evidence that circulating levels of cellular adhesion molecules in human serum samples are significantly higher in various types of metastatic cancer e.g. [29], [30]. It is probable that selenium may exert some of its anti-cancer actions via dampening mechanisms involved in up-regulating cell adhesion molecule expression and thereby decreasing tumor cell attachment, extravasation, and neovascularization.

Acknowledgements

This work was funded by the Georgia Gerontology Center Seed Grant Program to Dr Diane K. Hartle and the SE Affiliate of the American Heart Association to James L. Hargrove.

References

[1] Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994;76:301–14.

[2] Collins T. Biology of disease, endothelial nuclear factor-_B and the initiation the atherosclerotic lesion. Lab Invest 1993;68:499–508.

[3] Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. FASEB J 1995;9:899–909.

[4] Ghosh S, May MJ, Kopp EB. NF-κB and rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998;16:225–60.

[5] Foo SY, Nolan GP. NF-κB to the rescue. RELs, apoptosis and cellular transformation. Trends Genet 1999;15:229–35.

[6] Holben DH, Smith AM. The diverse role of selenium within selenoproteins: a review. J Am Diet Assoc 1999;99:836–43.

[7] Bonithon-Kop C, Coudray C, Berr C, Touboul PJ, Feve JM, Favier A, Ducimetiere P. Combined effects of lipid peroxidation and antioxidant status on carotid atherosclerosis in a population aged 59–71 year: the EVA study. Am J Clin Nutr 1997;65:121–7.

[8] Hussein O, Rosenblat M, Refael G, Aviram M. Dietary selenium increases cellular glutathione peroxidase activity and reduces the enhanced susceptibility to lipid peroxidation of plasma and low-density lipoprotein in kidney transplant recipients. Transplantation 1997;63:679–85.

[9] Schwenke DC, Behr SR. Vitamin E combined with selenium inhibits atherosclerosis in hypercholesterolemic rabbits independent of effects on plasma cholesterol concentrations. Circ Res 1998;83:366–77.

[10] Maddox JF, Aherne KM, Reddy CC, Sordillo LM. Increased neutrophil adherence and adhesion molecule mRNA expression in endothelial cells during selenium deficiency. J Leukoc Biol 1999;65:658–64.

[11] Chen CC, Rosenbloom CL, Anderson DC, Manning AM. Selective inhibition of E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 expression by inhibitors of IκB-a phosphorylation. J Immunol 1995;155:3538–45.

[12] Schreiber E, Matthias P, Muller MM, Schaffner W. Rapid detection of octamer binding proteins with mini-extracts, prepared from a small number of cells. Nucl Acids Res 1989;17:6419–9.

[13] Kunsch C, Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. Circ Res 1999;85:753–66.

[14] Bowie A, O'Neill AJ. Oxidative stress and nuclear factor-κB activation. Biochem Pharmacol 2000;59:13–23.

[15] Wingler K, Bocher M, Flohe L, Kollmus H, Brigelius-Flohe R. mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy if selenoproteins. Eur J Biochem 1999;259:149.

[16] Moutet M, D'Alessio P, Malette P, Devaux V, Chaudiere J. Glutathione peroxidase mimics prevent TNFα- and neutrophilinduced endothelial alterations. Free Radic Biol Med 1998;25:270–81.

[17] D'Alessio P, Moutet M, Coudrier E, Darquenne S, Chaudiere J. ICAM-1 and VCAM-1 expression induced by TNF-α are inhibited by a glutathione peroxidase mimic. Free Radic Biol Med 1998;24:979–87.

[18] Brigelius-Flohe R, Friedrichs B, Maurer S, Schultz M, Streicher R. Interleukin-1-induced nuclear factor κB activation is inhibited by overexpression of phospholipid hydroperoxide glutathione peroxidase in a human endothelial cell line. Biochem J 1997;328:199–203.

[19] Horvathova M, Jahnova E, Gazdik F. Effect of selenium supplementation in asthmatic subjects on the expression molecules in culture. Biol Trace Elem Res 1999;69:15–26.

[20] Versieck J, Cornelis R. Trace Elements in Human Plasma or Serum. Boca Raton, FL: CRC Press, 1989:77.

[21] Makropoulos V, Bruning T, Schulze-Osthoff K. Selenium-mediated inhibition of transcription factor NF-κB and HIV-1 LTR promoter activity. Arch Toxicol 1996;70:277–83.

[22] Kim LY, Stadtman TC. Inhibition of NF-κB DNA binding and nitric oxide induction in human T cells and lung adenocarcinoma cells by selenite treatment. Proc Natl Acad Sci USA 1997;94:12904–7.

[23] Toledano MB, Leonard WJ. Modulation of transcriptional factor NF-κB binding activity by oxidation–reduction in vitro. Proc Natl Acad Sci USA 1991;88:4328–32.

[24] Mitomo K, Nakayama K, Fujimoto K, Sun X, Seki S, Yamamoto K. Two different cellular redox systems regulate the DNA-binding activity of p50 subunit of NF-kappa B in vitro. Gene 1994;145:197–203.

[25] Ganther HE. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis 1999;20:1657–66.

[26] Yan L, Yee JA, McGuire MH, Graef GL. Effect of dietary supplementation of selenite on pulmonary metastasis of melanoma cells in mice. Nutr Cancer 1997;28(2):165–9.

[27] Yan L, Frenkel GD. Inhibition of cell attachment by selenite. Cancer Res 1992;52(20):5803–7.

[28] Regidor PA, Callies R, Regidor M, Shindler AE. Expression of the cell adhesion molecules ICAM-1 and VCAM-1 in the cytosol of breast cancer tissue, benign breast tissue and corresponding sera. Eur J Gynaecol Oncol 1998;19(4):377–83.

[29] Banks RE, Geraring AJ, Hemingway IK, Norfolk DR, Perren TJ, Selby PJ. Circulating intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular adhesion molecule-1 (VCAM-1) in human malignancies. Br J Cancer 1993;68(1):122–4.

[30] Sun JJ, Zhou XC, Liu YK, Tang ZY, Feng JX, Zhou G, Xue Q, Chen J. Invasion and metastasis of liver cancer: expression of intercellular adhesion molecule 1. Cancer Res Clin Oncol 1999;125(1):28–34.