Journal of Trace Elements in Medicine and Biology 51 (2019) 106–114

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Physiology

Selol (Se IV) modulates adhesive molecules in control and TNF-α-stimulated HMEC-1 cells

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ARTICLE INFO

Keywords:
Selol
Reactive oxygen species
Adhesion molecules
TNF-α
NF-B

ABSTRACT

Selol, an organic selenitetrigliceride formulation containing selenium at +4 oxidation level, has been suggested as anticancer drug. One of the causes of several diseases including cancer may be inflammation. This study aimed at determining the activity of Selol via measuring its effect on reactive oxygen species (ROS) generation, nuclear factor kappa B (NF-κB) activation, intercellular cell adhesion molecules-1 (ICAM-1), vascular cell adhesive molecule-1 (VCAM-1), and plateled-endothelial cell adhesive molecule-1 (PECAM-1) levels on control and on tumor necrosis factor-α (TNF-α)-stimulated human microvascular endothelial cells (HMEC-1). Cells were treated either with Selol 5% (4 or 8 μgSe/mL) or TNF-α (10 ng/mL) alone or with Selol concomitant with TNF-α.

Selol treatment resulted in ROS generation, activation of NF-κB, downregulation of PECAM-1, VCAM-1 and slight upregulation ICAM-1 expression on the cell surface. TNF-α treatment reflected in sharp NF-κB activation, upregulation of both ICAM-1 and VCAM-1 in parallel with the downregulation of PECAM-1 expression on cell surface. Exposure to both compounds upregulated ICAM-1 and VCAM-1, downregulated PECAM-1 level on cell surface in parallel with no changes in level of NF-κB activation as compared with effects mediated by TNF-α alone.

These results points to new look at Selol action since it shows a pro-inflammatory activity in parallel with effects on CAMs expression on the cell surface of human microvascular endothelial cells. However, since Selol enhances CAMs expression level when is present concomitantly with TNF-α this fact might suggest that selenium present in the condition of inflammation will make it worse.

1. Introduction

Inflammation is a fundamental homeostatic response triggered by both endogenous and exogenous noxious stimuli. However, inflammation may be the cause of several diseases including neurodegenerative disorders, atherosclerosis, and cancer, among others [1].

Microvascular endothelium provides an important site of regulation and amplification of the inflammatory responses [2]. Many inflammatory mediators e.g. tumor necrosis factor (TNF)-α and interleukin-1 (IL-1) contribute to the development of an activated endothelium [3]. TNF-α can induce chemotactic factors, other cytokines, and cell adhesion molecules via activation of transcription nuclear factor (NF)-κB [4]. Inactive NF-κB occurs in the cytosol of unstimulated cells, associated with an inhibitor protein I-κB.

After inactivation of I-κB through proteolysis, NF-κB is translocated from cytoplasm to the nucleus where it induces expression of many pro-inflammatory genes. In endothelial cells activated NF-κB upregulates expression of endothelial cellular adhesion molecules (CAMs), including vascular cell adhesive molecule-1 VCAM-1 (CD106), intercellular cell adhesion molecules-1 ICAM-1 (CD54), and plateled-endothelial cell adhesive molecule-1 PECAM-1 (CD31) [5–9]. Cellular adhesion molecules arelocated on the endothelial surface of the vessel wall and facilitate the adhesion and transendothelial migration of leukocytes from circulation to extravascular tissues during inflammation

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https://doi.org/10.1016/j.jtemb.2018.10.005
Received 28 June 2018; Received in revised form 13 September 2018; Accepted 4 October 2018

[10].

VCAM-1 on the endothelium functions as both a scaffold for leukocyte migration and a trigger of endothelial signaling through NADPH oxidase-generated ROS. VCAM-1 signaling is a target for intervention by pharmacological agents and by antioxidants during inflammatory diseases [11]. ICAM-1 is expressed on endothelial and epithelial cells where it is essential for proliferation, differentiation and immunological functions. The increased expression of ICAM-1 may be associated with a better prognosis, which is a base for suspecting an improvement of the function of the body's immune system [12,13]. In physiological conditions, PECAM-1 controls junctional and adhesive properties in endothelial cells. During inflammation, loss of PECAM function leads to increased adhesion of neutrophils and other leukocytes to endothelium, decreased vascular integrity and greater leukocyte transmigration to the intima media [14,15].

Selol is a mixture of selenitetriglicerides containing selenium (Se) at +4 oxidation state. The role of Se in cancer prevention is very well documented in the literature [16–20]. As an important antioxidant, Se also displays anti-inflammatory properties [18]. The highest biological activity as an antioxidant and anticancer agent is assigned to selenium compounds containing tetravalent Se (IV) [21]. The data on toxicity and pharmacokinetics of Selol have been published by Jastrzębski et al. [22,23]. Previous in vitro studies revealed that Selol 5% is a promising anticancer drug [24–27]. Studies of Falqueiro et al. [28] have demonstrated activity of Selol to overcome the cell resistance to doxorubicin. Flis et al. [29] showed that the anticancer activity of Selol involves perturbation of the redox regulation in the androgen dependent human prostate cancer cells (LNCaP). Additionally, Selol 5% supplementation in vivo affected the selenoenzymes activities and antioxidant status in the blood of mice [30]. Recently, Dominiak et al. has shown that Selol dose-dependently improved the survival and decreased the percentage of apoptosis in rat pheochromocytoma (PC12) dopaminergic cells exposed to sodium nitroprusside (SNP)-evoked oxidative/nitrosative stress [31] and prevented lipopolysaccharide induced oxidative stress and inflammatory reaction in the rat brain [32].

Despite the increasing interest in the pharmacological and molecular effects of Selol as chemopreventive and anticancer drug, the effects of Selol on the endothelium have not been described. In the present study, we investigated the effects of Selol 5% in untreated microvascular endothelial cells and in inflammation activated by TNF-α. Our results point to new look at Selol 5% as therapeutic drug, since it shows a pro-inflammatory activity in parallel with effects on CAMs expression on the cell surface of human microvascular endothelial cells. However, since Selol enhances CAMs expression when is present concomitantly with TNF-α this fact might suggest that Se present in the condition of inflammation will make it worse.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grade and purchased from commercial sources as follows: Selol is a mixture of selenitetriglycerides obtained from sunflower oil in the Department of Drug Analysis at Medical University of Warsaw [33]. The structure of Selol based on ^{1}H and ^{13}C NMR study [34] is presented (Fig. 1). In the present study Selol 5% was used, which designates the declared content of Selenium (IV) as 5%. Trypan blue (TB), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), tumour necrosis factor α (TNF-α), non-enzymatic cell dissociation solution and all other general laboratory chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trypsin − EDTA solution and phosphate buffered saline (PBS), attachment factor were supplied by Gibco BRL. Microvascular growth supplement (MVGS) was purchased from Cascade Biologics (Portland, OR, USA). Cell Wash Buffer and all cell culture plastics were purchased from Becton Dickinson (San Diego, CA, USA). Dihydrorhodamine 123 (DHR 123), 2',7'- dichlorofluorescein diacetate (chloromethyl-DCF-DA), and hydroethidine (HE) were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). The anti-ICAM-1 (CD54) (Cat. No:555511), anti-VCAM-1 (CD106) (Cat. No:551148), anti-PECAM-1 (CD31) (Cat. No:555445) antibody labeled with PE, PerCP, and FITC, respectively, and primary mouse antibody anti-human NF-κB subunit p65 (Cat. No:sc-8008) labeled with FITC were purchased from Becton Dickinson (San Diego, CA, USA). Secondary goat anti-mouse FITC conjugated antibody (Cat. No:F5387-5 mL) and mouse monoclonal antibody anti-β-actin conjugated with horseradish peroxidase (Cat. No:A3854) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Rabbit polyclonal antibody anti-p65 NF-κB (Cat No:sc-372) and anti-histone H1 (Cat No:sc-10806) were obtained from Santa Cruz Biotechnology. The secondary goat anti-rabbit IgG peroxidase conjugate antibody (Cat. No:DC03L) was supplied by Calbiochem (USA). Fluorescent mounting medium was purchased from Dako (North America, Inc., Carpinteria, CA, USA) and PVDF (polyvinylidene difluoride) membrane from Millipore (Bedford, MA, USA). ECL plus Western Blotting Detection System was purchased from GE Healthcare (Uppsala, Sweden).

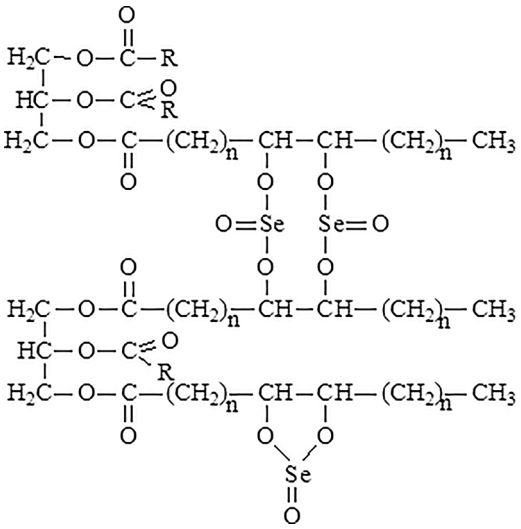

Fig. 1. Structure of Selol containing 5% Se (IV) built into fatty acid chains of sunflower oil.

2.2. Cell culture and Selol treatment

Cell culture of human microvascular endothelial cells HMEC-1 (ATCC, No CRL-10636) was obtained from American Type Culture Collection (Teddington, UK) and cultured according to its instructions (MCDB 131 medium in a 95% air, 5% CO_2 humidified incubator at 37 °C). All media (Gibco BRL) were supplemented with 10% fetal bovine serum (FBS), L-glutamine (200 μM), sodium bicarbonate (1.18 g/L), 100 U/mL penicillin, 100 μg/mL streptomycin, and 5% microvascular growth supplement (MVGS).

After HMEC-1 cells grew into the logarithmic phase (80% confluence), the culture medium in each flask or plate was replaced completely with 10% FBS-MCDB 131 medium supplemented with either 10 ng/mL TNF-α or Selol (0–10 μg Se/mL) alone or with the combination of both TNF-α (10 ng/mL) and Selol (0–10 μg Se/mL), and the cells were cultured at the same conditions for subsequent experiments.

2.3. Cell viability assays

Cell viability was measured in HMEC-1 cells (8×10^5/6-well plate) treated for 18 h with Selol (0–10 μg Se/mL) or TNF-α (10 ng/mL). Cells were harvested and cell viability was evaluated by the trypan blue (TB)

exclusion assay. Untreated cells were used as the 100% viability value. Cells were counted using Bürker chamber under light microscope. All experiments were performed in triplicate.

The growth inhibition was evaluated by MTT dye reduction assay, which measures mitochondrial respiratory function. In brief, HMEC-1 cells (2×10^4/well) were seeded into 96-well plates and incubated 48 h to attach to the bottom of the well in medium (200 µl) and treated with Selol (0–10 µg Se/mL) or TNF-α (10 ng/mL) for 18 h. Then, medium was removed and MTT (0.5 mg/mL) in fresh growth medium without serum was added to each well and plates were incubated in dark for 4 h. The liquid in each well was removed and replenished with 200 µl DMSO: isopropanol (1:1) to dissolve the generated formazon crystals. The optical density was measured with an UVM 340 (ASYS Hitech GmbH, Austria) microplate reader at 570 nm. Replicates of three wells for each dosage including vehicle control were analyzed for each experiment. The experiments were conducted in triplicate. Results are expressed as mean percentage of viable cells after treatment. Untreated cells were used as the 100% viability value.

2.4. ROS detection: DHR 123, DCFH-DA, and HE assay

ROS generation was evaluated by spectrofluorometric method using the dihydrorhodamine 123 (DHR 123), 2′, 7′- dichloro-dihydro-fluorescein diacetate (DCFH-DA) or hydroethidine (HE), a sodium borohydride-reduced derivative of ethidium bromide. Method is based on the ROS-dependent oxidation of the compounds to fluorescent rhodamine 123, dichlorofluorescein (DCF) [35], and ethidium (Et), respectively. HMEC-1 were seeded onto 96-well plates (5×10^4/well) and allowed to adhere for 24 h. Then cells were rinsed with PBS and incubated with DHR123 (1 µM), DCFH-DA (5 µM) or HE (5µM) on 96-well plates for 30 min at 37 °C in the dark. Thereafter, cells were rinsed with PBS, treated for 1 or 3 h at 37 °C with red phenol free culture medium containing Selol (4 µg Se/mL or 8 µg /mL) to observe short-living ROS. A sample with H_2O_2 (1.5 mM) was a positive control and a sample without any reagent was a negative control. Maximum excitation and emission spectra for rhodamine 123 was 500 nm and 536 nm, for DCF: 492 nm and 527 nm, and for Et: 510 nm and 590 nm, respectively. The generation of H_2O_2 or $O_2^{\cdot -}$ was measured by Microplate Spectrofluorometer BioTek Synergy™ (BioTek Instruments, USA) and expressed as fluorescence intensity (FI). Values from three experiments performed in triplicate were analyzed.

2.5. Endothelial cell NF-κB response to Selol and TNF-α. Indirect analysis of NF-κB induction with laser scanning confocal microscope (LSCM)

Activation of *NF-κB* in HMEC-1 cells treated with Selol was observed in laser scanning confocal microscope (LSCM) as previously described Kurpios-Piec et al. [36]. HMEC-1 cells (6×10^4/well) were grown on glass cover slips in 6-well plates. After cells reached 60–70% confluence, they were incubated in complete medium with Selol (4 or 8 µg Se/mL) and/or with 10 ng/mL TNF-α for 1 h. Thereafter, HMEC cells were washed two times with cold PBS, fixed with 4% paraformaldehyde in PBS for 20 min at room temperature and permeabilized with 0.2% Triton X-100 in PBS for 20 min. Then, cells were washed three times with cold PBS, treated with 3% bovine serum albumin (BSA) in PBS containing 0.05% Tween overnight at 4 °C and then incubated with primary mouse antibody anti-human NF-κB subunit p65 (1:300) overnight at 4 °C. After three washes with PBS/3% BSA, secondary goat anti-mouse FITC conjugated antibody (1:150) was applied to the cells for 60 min at room temperature. After washing with PBS, coverslips were mounted with fluorescent mounting medium and analyzed with a Radiance 2000 confocal microscope equipped with a 40-x glycerol immersive objective. The sections were assessed using laser line of 488 nm for FITC and filter 500–560 nm. On average, five to six slides from four independent experiments were examined by LSCM with similar results, and representative experiments are shown in the corresponding photographs.

2.6. Western blot analysis of NF-κB activation

Nuclear and cytoplasm extracts of HMEC-1 cells stimulated with TNF-α and/or Selol (4 or 8 µg Se/mL) were prepared according to procedure described in NE-PER® Nuclear and Cytoplasm Extraction Reagents (Pierce, Thermo Scientific; Rockford, IL, USA). Western blot analysis of NF-κB/p65 subunit protein was performed as previously described [36]. Proteins (10 µg per sample) were separated by 10% polyacrylamide gel electrophoresis and transferred onto PVDF membrane. The membrane was blocked in 10% skim milk for 1 h and washed with 3% skim milk in TBS-T (TBS containing 0.05% Tween 20). Then, it was incubated with primary antibodies against NF-kB/p65 and H1 (1:500 dilution), and against β-actin (1:4000 dilution) overnight at 4 °C. After extensive washing with 3% skim milk in TBS-T the secondary goat anti-rabbit IgG was added to p65 and H1 (1:2000 dilution) for 60 min at room temperature. Proteins were detected by chemiluminescent immunodetection system (ECL plus Western Blotting Detection System). Band density was determined by Image Analysis System UVI-KS4000 Syngen Biotech, Scion Co. (Frederick, MD, USA). Assays were repeated three times with similar results.

2.7. Flow cytometry analysis

Cell surface presentation of adhesion molecules on HMEC-1 cells was analyzed by flow cytometry as previously described [36]. Cells were grown in 6-well plates (8×10^4/well) and incubated for 18 h in complete medium with Selol (4 or 8 µg Se/mL) or TNF-α (10 ng/mL) alone or with TNF-α (10 ng/mL) with Selol (4 or 8 µg Se/mL). Cell viability was unaffected during the exposition time. It was assessed by the trypan blue (TB) staining time. After the treatment, cells were washed with Wash Buffer, carefully removed from culture plates using non-enzymatic cell dissociation solution. Cells were stained with monoclonal antibodies against ICAM-1/PE, VCAM-1/PerCP, and PECAM-1/FITC at 4 °C for 30 min in dark according to the standard procedure provided by the company. Three-color analysis was performed on FACS Calibur (Becton-Dickinson, San Jose, CA, USA). Results of four independent experiments are presented as the percentage of positively stained cells. The expression of adhesion molecules present on the cell surface was determined by the mean fluorescence intensity (MFI). Analysis was performed using the CellQuest program. Cells stimulated by TNF-α were used as a positive control.

2.8. Statistical analysis

All results are represented as the mean ± standard deviation (± SD) of at least three independent experiments. The Student's *t*-test was used to determine the significances between two mean values. Changes in the expression of cell adhesion molecules were evaluated by the Kolmogorov-Smirnov statistic. Representative histograms are presented. Statistically significant differences between the experimental and control groups are denoted in the figures. Differences were considered significant if the probability (*P*)-value was $P < 0.05$ and $P < 0.001$.

3. Results

3.1. Cell viability

Two tests were used to assess the potential cytotoxicity of Selol: trypan blue exclusion test and MTT reduction assay (Fig. 2A,B). Selol-treated cells exhibited no changes in cell viability measured by TB assay up to 10 µg Se/mL. In MTT assay we observed proliferating effect of Selol at the lowest concentration (2 µg Se/mL) and no effect up to 10 µg Se/mL. TNF-α in tested concentration did not attribute to any cytotoxic

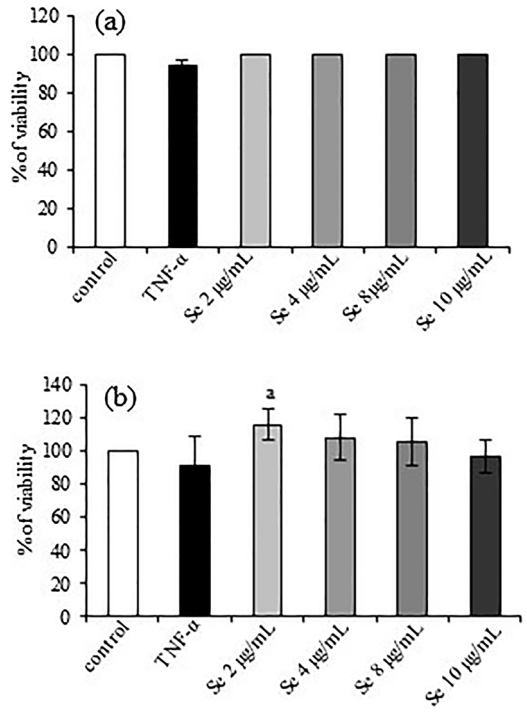

Fig. 2. Cell viability of Selol treated HMEC-1 cells using TB exclusion assay (a) and MTT test (b). Values are expressed as percentages of viable cells with respect to untreated cells (control). All data represent the means ± SD of three experiments, each of them performed in triplicate. $^{a}P < 0.05$ versus control cells.

effect in HMEC-1 cells. Additionally, the viability HMEC-1 cells incubated with TNF-α in combination with Selol was not altered as assessed by both TB and MTT assay (data not shown). According to these results, 4 and 8 μg Se/mL concentrations were selected for experiments. Both were not toxic and no changes in cell morphology were observed.

3.2. ROS production

In HMEC-1 cells incubated with either DHR123 or DCF-DA the statistically significant increase of fluorescence intensity was observed after 3 h incubation with 4 and 8 μg Se/mL (Fig. 3A) and only after 1 h incubation with 4 μg Se/mL (Fig. 3B) as compared to unexposed cells, respectively. Observed changes in fluorescence result of DHR123 and DCF-DA oxidation by H_2O_2 generated by Selol. There was no observed increase intensity of fluorescence in cells loaded with HE after 1 or 3 h incubation with both concentration of Selol.

3.3. Cellular localization of NF-κB p65 subunit in HMEC-1 cells

The cellular localization of NF-κB was detected by indirect immunofluorescence and confocal microscopy (40 x magnification) with FITC-labeled NF-κB/p65 (fluorescence in Fig. 4). No signal was observed in untreated HMEC-1 cells labeled in the absence of primary antibody to NF-κB/p65 (data not shown). Control HMEC-1 cells labeled with FITC-NF-κB p65 revealed diffuse distribution of NF-κB p65 with cytoplasm (Fig. 4A). After incubation with Selol (4 or 8 μg Se/mL) alone for 1 h immunoreactive NF-κB/p65 moved to nucleus (Fig. 4B,C). Treatment with TNF-α (10 ng/mL) alone resulted in dense accumulation of NF-κB/p65 within the nucleus (Fig. 4D). Concomitant treatment of cells with TNF-α and Selol at both concentrations (4 or 8 μg Se/mL) resulted in keeping dense accumulation of immunofluorescence within the nucleus (Fig. 4E,F).

3.4. Western blot analysis of NF-κB p65 subunit in HMEC-1 cells

The protein level of NF-κB/p65 subunit in untreated, Selol and/or TNF-α stimulated cells was analyzed by Western blot analysis (Fig. 5a,b). In untreated HMEC-1 cells the amount of NF-κB/p65 subunit was higher in cytoplasm (ratio 1.12) than in nucleus (ratio 0.7). After stimulation with TNF-α or Selol alone, the translocation of p65 from the cytoplasm to nucleus occurred within 60 min. Thus, the amount of NF-κB/p65 subunit was higher in nucleus of all treated cells: with TNF-α, Selol 4 or 8 Se μg/mL (ratio 1.16, 1.1 and 1.22, respectively) than in cytoplasm (ratio 0.71, 0.865 and 0.85, respectively). Concomitant stimulation by both TNF-α and Selol 4 or 8 Se μg/mL resulted in slighty increase of NF-κB amount in nucleus (ratio 1.2 and 1.27) as compared to cells stimulated with TNF-α alone (ratio 1.16). All results were statistically significant $P < 0.05$.

3.5. Cell surface presentation of adhesion molecules in HMEC-1 cells

It is well known that activation of NF-κB increases the expression of cell adhesion molecules and their amount on cell surface increases [37,38]. Therefore, in our study, we investigated the effect of Selol on the expression of PECAM-1, ICAM-1, and VCAM-1 by flow cytometry analysis (Fig. 6a,b,c). Treatment of HMEC-1 with Selol at both concentrations of Se resulted in statistically significant changes of the surface presentation of all tested cell adhesion molecules. Expression of PECAM-1 was decreased by 9% and 7% for 4 and 8 μg Se/mL, respectively ($P < 0.001$), ICAM-1 expression increased by 9% and 15%, respectively ($P < 0.001$) as well as VCAM-1 expression decreased by 40% and 41%, respectively ($P < 0.001$), as compared to the untreated cells. Treatment with TNF-α alone caused a statistically significant increase in the surface expression of both, ICAM-1 (by 1300%, $P < 0.001$) and VCAM-1 (by 95%, $P < 0.001$). However, TNF-α significantly inhibited the PECAM-1 expression (by 33%, $P < 0.001$), as compared to the untreated cells. The stimulating TNF-α effect on ICAM-1 and VCAM-1 expression was potentiated by concomitant treatment of TNF-α with Selol (4 and 8 μg Se/mL). Expression of ICAM-1 increased by 21% and 15%, ($P < 0.001$), respectively, and VCAM-1 increased by 32% and 14% ($P < 0.001$), respectively, as compared with level of expression in TNF-α-treated cells. Opposite effect was observed in level of PECAM-1. After concomitant treatment of cells with TNF-α and Selol expression of PECAM-1 was decreased by 14% ($P < 0.001$) only at 4 μg Se/mL as compared with TNF-α treated cells. Expression level of PECAM-1 at 8 μg Se/mL concomitant with TNF-α was not changed as compared with effect of TNF-α alone.

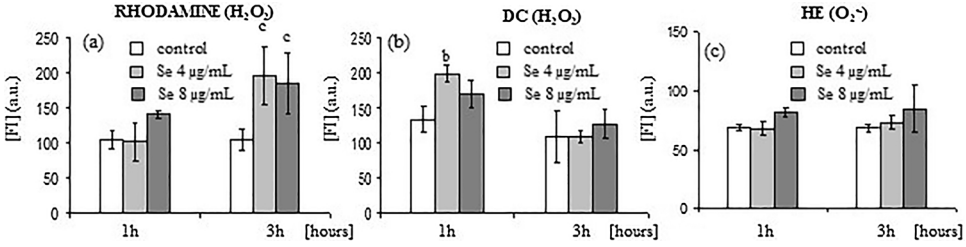

Fig. 3. Level of reactive oxygen species in HMEC-1 cells stimulated with Selol.
(a) Fluorescence intensity (FI) of the probe Rhodamine (5 μM), (b) fluorescence intensity of the probe DCF (5 μM), (c) fluorescence intensity of the probe Ethidium (5 μM) in the presence of Selol (4 or 8 μg Se/mL) for 1 or 3 h. All data represent the means ± SD of three experiments, each of them performed in triplicate. $^{a}P < 0.02$ versus control cells, $^{b}P < 0.05$ versus control cells.

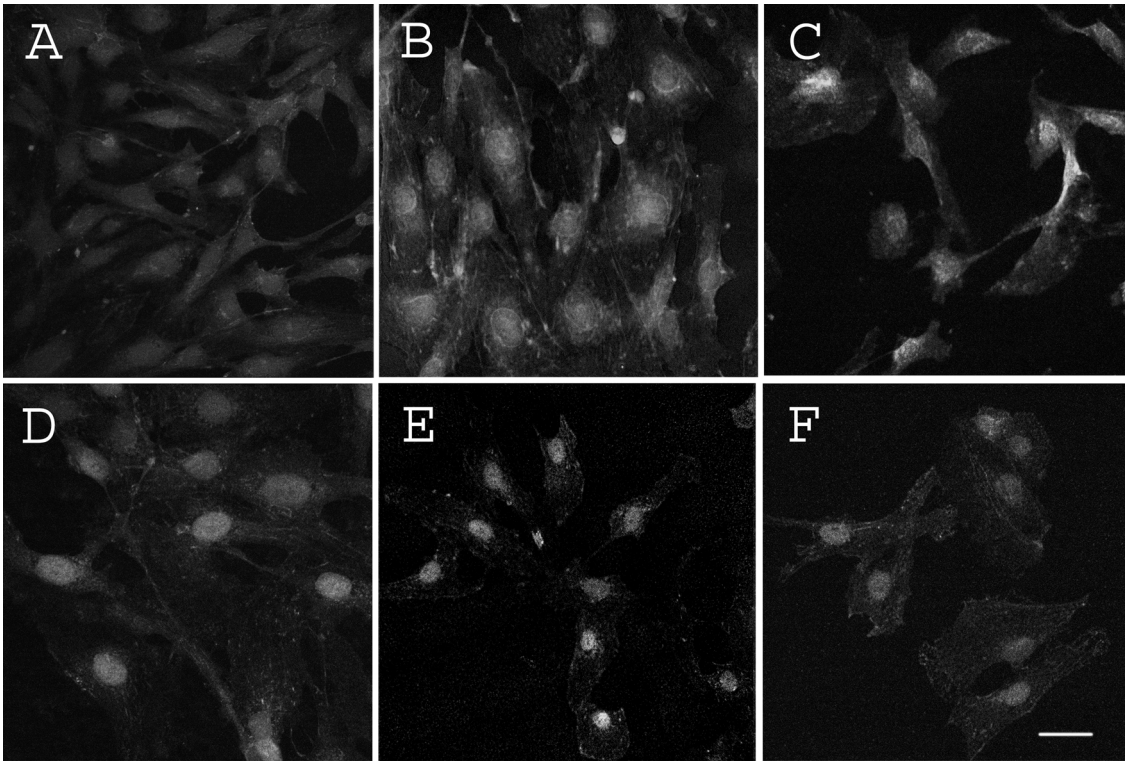

Fig. 4. Indirect immunofluorescence and confocal microscopic analysis demonstrating the subcellular localization of NF-κB/p65 in HMEC-1 cells treated with Selol or with TNF-α.
(A) The non-treated HMEC-1 cells (control), (B) cells treated with Selol (4 μg Se/mL), (C) cells treated with Selol (8 μg Se/mL), (D) cells treated with TNF-α (10 ng/mL), (E) cells treated with Selol 4 μg Se/mL together with TNF-α (10 ng/mL), (F) cells treated with Selol 8 μg Se/mL together with TNF-α (10 ng/mL). Results are representatives of four independent experiments. Bar 40 nm.

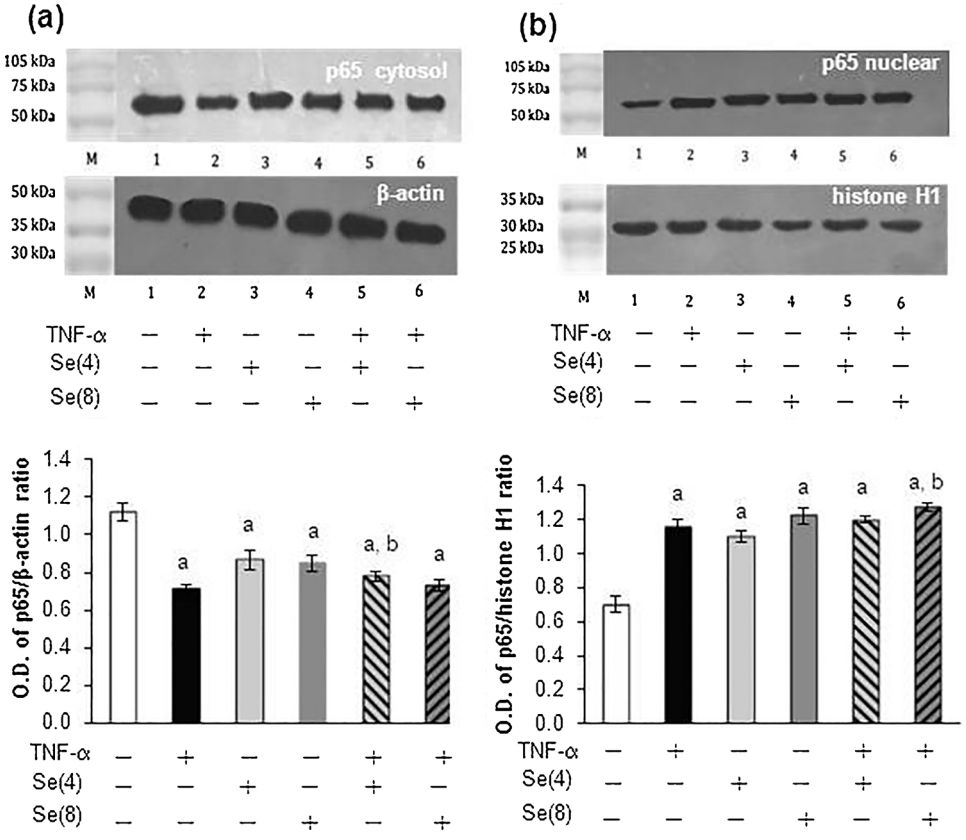

Fig. 5. Effect of Selol on NF-κB/p65 subunit expression in non-treated and Selol or/and TNF-α stimulated HMEC-1 cells.
The NF-κB/p65 protein levels in cytosol (A) and nucleus (B) were determined by Western blot analysis. Cells were treated with Selol (4 or 8 Se μg/mL) alone or concomitant with TNF-α (10 ng/mL) for 18 h. The β-actin protein level was considered as an internal control in cytosol and histone H1 protein level was considered as an internal nuclear control. Experiments were performed in triplicate and representative gels are presented. The intensity of the bands was quantitated by densitometry. The levels of NF-κB/p65 are in arbitrary units, and data were normalized to respective amounts of β–actin and histone H1. $^{a}P < 0.05$ versus untreated (control) cells, $^{b}P < 0.05$ versus cells treated with TNF-α.

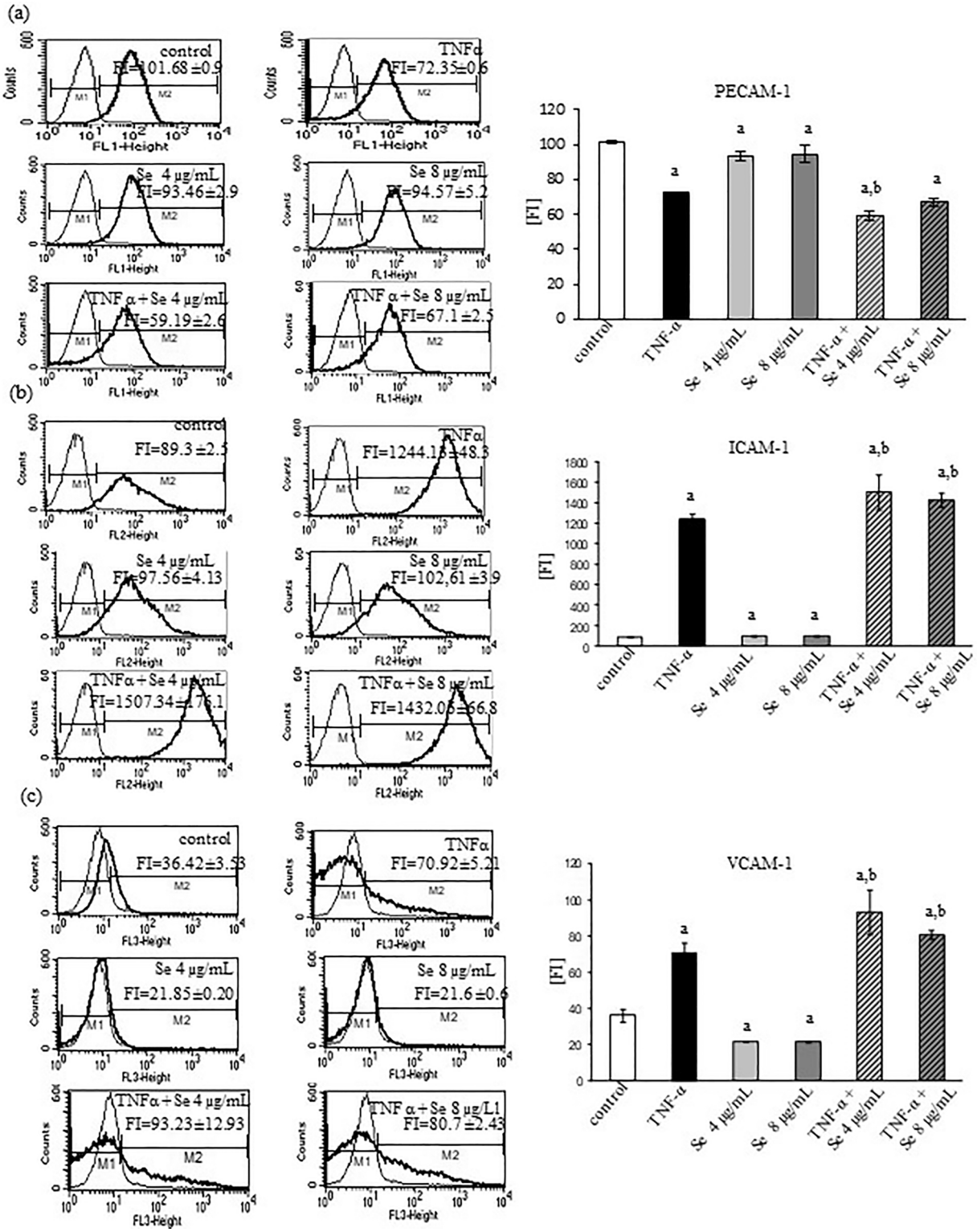

Fig. 6. Expression of adhesion molecules on the surface of HMEC-1 cells treated with Selol or with TNF-α.
Expression of PECAM-1 (a), ICAM-1 (b), and VCAM-1 (c) was determined by flow cytometry. HMEC-1 cells were treated either with Selol (4 or 8 µg Se/mL), TNF-α (10 ng/mL) alone or with Selol (4 or 8 µg Se/mL) concomitant with TNF-α (10 ng/mL) for 18 h. Experiments were performed in triplicate and representative histograms are presented. Fluorescent intensity (FI). $^{a}P < 0.001$ versus untreated (control) cells, $^{b}P < 0.001$ versus cells treated with TNF-α.

4. Discussion

Adhesive interactions between cells or cells and the extracellular matrix have been found to be crucial to tissue functions including the inflammatory response. A major event in the development of the pro-inflammatory state is the expression of the endothelial cell adhesion molecules (CAMs) for the recruitment of leukocytes to the site of injury [39]. The inflammatory cytokine TNF-α mediates changes in the barrier properties of endothelium, among them changes in the profile of cell adhesive molecules. The complexity of this process has been reviewed

by Marcos-Ramiro et al. [40]. The decrease of endothelial barrier function is central to the long-term inflammatory response. In humans, aberrant expression of endothelial CAMs has been reported in various pathological conditions [41–45]. Thus, the data of their profile at development the cellular level is of great significance as an aid for therapeutic drug.

Supportive function of Se in health and disease (cancer, cardiovascular and autoimmune diseases) is documented in large number of clinical examinations. However, exact mechanism of its action as preventive and therapeutic element is still studied and analyzed [17,18,31,32,46,47]. A low physiological level of Se can be associated with decreased antioxidant status due to e.g. reduced expression of glutathione peroxidase (GPX) observed in patients with severe burn injuries or traumas [48]. Additionally, Se may have an impact on the course and outcome of a number of etiologically inflammatory diseases. Many studies indicate that there is a strong indication that viral, bacterial, or stress induced inflammation may be variably influenced by Se availability [48].

Antioxidant properties of Se are mainly associated with its incorporation into selenoproteins as well as the fact that it can occur in the form of Se +4 oxidation state. Selol, an organic compound which contains Se (IV) reveals lover potential for toxicity than sodium selenite, an inorganic Se (IV) compound. It is believed that Se provided in the form of Selol is metabolized to selenocysteine incorporated into selenoproteins [49–51]. Sochacka et al. [30] revealed that Selol 5% supplementation increased activity of selenoenzymes such as selenium-dependent glutathione peroxidase (SeGPX) and thioredoxin reductase (THNRD) in healthy animal's model. Flis et al. [29] also observed increased concentration of ROS and significant changes in GPX and THNRD activity in healthy animals and in cancer cells.

It is well documented that ROS, cytokine TNF-α, and disturbance of oxidative balance are signals which activate NF-κB [52,53]. Its regulation is one key step in controlling inflammatory process. The activation of NF-κB is required, among others, to regulate CAMs expression. Changes in their expression are a hallmark event in the development of the pro-inflammatory state of the endothelial cells [54]. Many researchers have studied effects of selenium on adhesive molecules profile and mechanisms of their regulation in pathological conditions [20,55–60]. The principal finding of our study is that Se in form of Selol slightly induced ROS, activated NF-κB as well as it slightly increased ICAM-1, but decreased VCAM-1, and PECAM-1 cell surface presentation compared to untreated HMEC-1 cells. We also observed that co-administration of Selol with TNF-α to HMEC-1 cells potentiated effects of TNF-α on CAMs, i.e. enhanced ICAM-1 and VCAM-1 in parallel with decreasing PECAM-1 expression, proven by immunofluorescence detection. Selol presented two opposite effects on CAMs expression depending on whether TNF-α is present or not.

The effect of selenium on ICAM-1 expression observed in our work can be the result of increased ROS amount. Flis et al. [29,61] observed that Selol has an initial strong pro-oxidative and antineoplastic effect, while in the second phase it exhibits antioxidant and repair properties. Many studies have provided evidences that expression of ICAM-1 is ROS dependent and it can be inhibited by antioxidants [62,63]. Moreover, the increased ICAM-1 expression in TNF-α and Selol treated cells in comparison to cells treated with TNF-α alone can be explained by greater ROS accumulation in HMEC-1 cells incubated with both compounds. It is well known, that TNF-α can induced intracellular ROS production, redox-sensitive transcription pathway activation and adhesion molecule expression. In endothelial cells NF-κB activation and ICAM-1 expression by TNF-α is dependent on oxidants that are generated by the polymorphonuclear neutrophils (PMN) NADPH oxidase complex [64].

Surprisingly, we observed decreased VCAM-1 expression in Selol treated cells and opposite effect when Selol and TNF-α were added together. Although ICAM-1 and VCAM-1 play important functions during inflammation and are expressed under the influence of many of the same pro-inflammatory stimuli there is evidence that some factors (cytokines, the cell redox state) can trigger specific mechanisms that allow the expression of one particle independent of the expression of the second [65–68]. The specific regulation of VCAM-1 expression is possible, among others due to the presence in the promoter region of this adhesive particle of motifs allowing the attachment of GATA binding transcription factors (GATA binding proteins) and IRF1 (IF-regulatory factor). The presence of these motifs distinguishes VCAM-1 from other adhesion particles [69]. In addition, many studies have shown that the expression of VCAM-1 depends on the cell type and stimulant [70–73].

The presented results provide new information on effect of Selol action in human endothelial cells. We showed that Selol can induce ROS production, activate NF-kB and modulate expression of ICAM-1, VCAM-1 and PECAM-1 in HMEC-1 cells. Our study demonstrated that Selol shows a pro-inflammatory activity in parallel with effects on CAMs expression on the cell surface of human microvascular endothelial cells. Moreover, increased ICAM-1 and VCAM-1 expression in HMEC-1 cells treated with both Selol and TNF-α might suggest that Selol present in the condition of inflammation will make it worse. Further studies will be required to understand the exact mechanism by which Selol modulates the inflammatory response in endothelial cells.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was supported by Ministry of Science and Higher Education, Warsaw, Poland (grant NN402210235) and Warsaw Medical University, Warsaw, Poland (grant 1WK/WP4/10).

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