



Research Article

P38 MAPK-dependent Targets of Gaseous Transmitters Proapoptotic Action in Jurkat Cells

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Abstract

Background: p38 MAPK participates in gases-induced apoptosis signal transmission though it hasn't molecular structure necessary for interaction with gaseotransmitters.

Methodology: annexin positive cells and cells with decreased mitochondrial transmembrane potential were detected by flow cytometry. Caspase 3 and 9 activity was investigated via spectrophotometry. Protein contents of Bcl-2 family members were measured with western blot analysis.

Results: Caspase-3 and -9 activity and the number of cells with decreased mitochondrial membrane potential showed the apoptotic reaction vector when p38 MAPK-dependent pathways of NO and CO action inhibited. Inhibition of p38 kinase in H₂S treated cells led to caspase-3 activation accompanied with the decrease of the number of apoptotic cells. Proteins Bcl-2 and Bad were the p38 MAPK-dependent targets of all three gases action. P38 MAPK influenced on protein Aven when intracellular H₂S concentration increased and on Bcl-x1 protein in CO-treated cells. In both cases p38 kinase was the negative regulator of these proteins and promoted the proapoptotic action of gases abolishing the gases-mediated increase of the Aven and Bcl-x1 content.

Conclusion: p38 MAPK acted proapoptotically in the cases of intracellular increase of NO and H₂S and antiapoptotically in CO-treated cells.

Keywords: p38 MAPK; apoptosis; nitric oxide; hydrogen sulfide; carbon monoxide

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1. Introduction

The group of endogenous gaseous transmitters currently includes three molecules: nitric oxide, carbon monoxide and hydrogen sulfide. Gaseous messengers chemically modify intracellular proteins thus cellular metabolism is changed in the more rapid manner. Vascular relaxation (the main physiological effect of gases) is achieved by nitric oxide and carbon monoxide interaction with guanilate cyclase. Hydrogen sulfide relaxes vascular wall via the change of potassium ATP-sensitive channels activity. NO influences the cell metabolism through the binding of heme-containing transcriptional factors. The same mechanism has been demonstrated for CO but its ability of interaction with heme is significantly lower than that for NO. NO also nitrosylates sulfhydryl groups of cysteine. Metabolic enzymes, ion channels, neurotransmitter receptors, a sodium-pumping enzyme, and structural proteins are nitrosylated at the basal level. The sulfhydration reaction mediates molecular mechanisms of H₂S action (the analogy of nitrosylation for nitric oxide) [1].

The important role of gases was shown in vascular tone regulation, neurotransmission, inflammation [2]. It is shown that NO is the regulator of apoptosis also. Nitric oxide inhibits programmed cell death within inflamed tissues. On the other hand it promotes apoptosis of cancer cells [3, 4]. The role of the other two members of gaseotransmitters family in apoptosis regulation is also controversial. CO and H₂S increase apoptosis of smooth muscle cells, Jurkat cells and decrease of endothelial cells, β -pancreatic cells, neutrophils [5-10].

It was shown that p38 MAP kinase is involved in signal transduction by gaseotransmitters [11, 12]. P38 MAPK is a member of the family of stress-activated kinases and has proapoptotic function in the most cell systems in vivo and in vitro. But in some type of cancer cells (Jurkat, B-lymphoma cells) it protects cells from apoptosis [13, 14].

In our study we tried to establish the role of p38 MAPK in gases-induced apoptosis activation in Jurkat cells. The dependence of the changes in mitochondrial membrane permeabilisation, caspase 3 and 9 activity, protein levels of bcl-2 family members was tested during variation of intracellular concentration of gases.

The present study results improve understanding of molecular mechanisms governing apoptotic cell death

via gaseotransmitters. Identification of p38 MAPK-dependent and independent targets in gases signal transduction mechanism is important in understanding of gases action and allow new directions for medications that affect apoptosis.

2. Materials and methods

2.1 Reagents and cells

Human Jurkat T-lymphocytes were obtained from Russian cell culture collection (Saint-Petersburg, Russia) and grown in RPMI-1640 (Vector-Best, Russia) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (Invitrogen, USA), 0,3 mg/ml L-glutamine, 100 μ g/ml gentamicin sulfate. Cells were cultured at 37 °C with 5% CO₂ and passaged twice weekly. The donors of intracellular gaseous transmitters

3-[2-hydroxy-1-(1-methylethyl)-2-nitrosohydrazino]-1-propanamine (NOC-5) (100 μ M), sodium hydrosulfide (NaHS) (10 mM) and tricarbonyldichlororuthenium(II) dimer (CORM-2) (50 μ M) were purchased from Sigma Chemical Co. (Louis, MO). The CORM-2 was dissolved in dimethyl sulfoxide (DMSO), NOC-5 and NaHS were dissolved in PBS. Donors were immediately added to the cell cultures at proapoptotic concentrations. Cells were incubated with NO and CO donors for 24 h and for 15' in the case of H₂S donor application. Control cultures were treated as above in the absence of donors.

For p38 MAPK inhibition Jurkat cells were treated with 0,2 μ M selective inhibitor of p38 MAP-kinase SB203580 (Sigma, USA) for 20' prior gases donors application.

2.2 Detection of apoptotic cells

Apoptosis was evaluated by using the Annexin V FITC Kit (Abcam, USA). Cells were washed with cold PBS twice and then resuspended in 1x binding buffer. Five microliters of Annexin V were added and vortexed. After incubated at room temperature in the dark for 15 min, 400 μ l of cold PBS was added, and samples were evaluated by flow cytometry (FACS CantoII, BD Biosciences).

2.3 Detection of cells with decreased mitochondrial transmembrane potential

The number of cells with decreased mitochondrial membrane potential was evaluated using «MitoScreen» kit («BD Pharmigen», USA). Cells were washed twice in cold PBS. 500µl of freshly prepared JC-1 (according to manufacturer instruction) was added to the cells. Cells were reslurred, incubated for 10-15 min at 37°C and washed twice. Colored with JC-1 Jurkat cells were analyzed on flow cytometer FACS CantoII (BD Biosciences).

2.4 Caspase 3 and 9 activity assay

Activity of caspase 3 and 9 was measured spectrophotometrically with «Abcam», USA kits. Cells were washed twice in cold PBS, reslurred in 50 µl of lysis buffer and incubated for 10 min on ice. After centrifugation supernatants (cytosolic extracts) were used for protein content and caspases' activity measurement. 50-200 mg of protein were reslurry in 50 µl of lysis buffer, 50 µl of reaction buffer and 5 µl of caspases' substrates (DEVD-pNA for caspase 3 and LEHD-pNA for caspase 9) were added and incubated for 24 h at 37°C. Then dilution buffer was added and caspases' activities were measured at 400-405 wave opposite cytosolic extracts and buffers for each sample.

2.5 Western blot analysis

Western blot was performed to assess the protein level of Bcl-2, Bad, Aven and Bcl-xL. Untreated cells served as the control, and GAPDG was used as the control for equal loading as the internal loading test. Briefly, cells were harvested in the presence of protease inhibitor cocktail (Sigma-Aldrich, St Louis, USA) in lysis buffer. Soluble proteins were subjected to 10% SDS-PAGE and transferred electrophoretically to nitrocellulose membranes. Nonspecific binding was blocked with 1% gelatin in TBST [50 mM Tris (pH 7.5), 0.9% NaCl, and 0.1% Tween-20] at room temperature (RT) for 20 min. Membranes were then incubated with anti-Bcl-XL (1:10,000; Sigma, USA),

anti-Bcl-2 (1:10,000; Sigma, USA), anti Bad (1:10,000; Sigma, USA) and anti-AVEN (1:2,000; Sigma, USA) at RT for 30 min. After 5 min washed with TBST, the membranes were incubated with Anti-rabbit or anti-mouse immunoglobulin G (IgG)-horse radish peroxidase (HRP) (Biosource, USA) for 1 h. The membranes were washed four times for 5 min each with TBST. Bound antibody chemiluminescence was detected using NOVEX ECL Chemiluminescent Substrate Reagent kit (Invitrogen, USA). The optical density was determined using a scanning densitometer and analyzed using Quantity One software (Bio-Rad).

2.6 Statistical analysis

The data obtained were tested for normality for the distribution of variables using the Shapiro-Wilk test [15]. The reliability of differences was evaluated with the use of the Kruskal-Wallis test [16, 17]. Differences were considered significant at a significance level of $p < 0.05$.

3. Results

3.1 Time and dose dependent activation of Jurkat cells apoptosis after treatment with donors of NO, CO and H₂S

Number of annexin-V-FITC positive cells was evaluated after 15 min incubation of cells with 10 mM NaHS compared with untreated control. Activation of apoptosis of Jurkat cells was achieved after 24 h treatment of cells with 100 µM NOC-5 and 50 µM CORM-2 (Figure 1).

3.2 Alteration of gases-induced apoptosis after p38 MAPK inhibition

Pre-incubation of cells with 0.2 µM SB203580 didn't cause changes of apoptosis of untreated cells but reduce the percentage of annexin-V-FITC positive cells after NOC-5 and NaHS treatment compared with the results after isolated gases treatment. Application of p38 MAPK inhibitor prior CORM-2 treatment led to increase of the number of apoptotic cells (Figure 1).

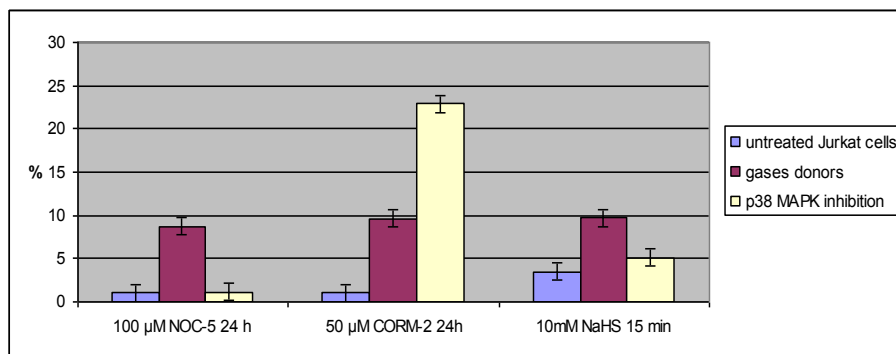


Figure 1 Number of apoptotic cells after gases donors application

3.3 Mitochondrial membrane permeabilisation status after treatment of Jurkat cells with donors of NO, CO and H₂S and inhibition of p38 MAPK

The number of cells with decreased $\Delta\psi$ enlarged 70 times after NO donor application, 7 times after H₂S donor application and 15 times after CO donor

application. P38 MAPK inhibitor application led to increase in number of cells with decreased $\Delta\psi$ in case of treatment with H₂S and CO but didn't cause changes in NO-treated cells culture compared with the results after isolated gases treatment (Table 1).

Table 1 Number of cells with decreased mitochondrion membrane potential and caspase 3 and 9 activities in Jurkat cells after NO, CO and H₂S donors application

	Number of cells with decreased mitochondrion membrane potential, %	Caspase 3 activity, arbitrary units	Caspase 9 activity, arbitrary units
Untreated cells	1,1(0,7-1,2)	1(0,99-1,01)	1(0,99-1,01)
Application of H ₂ S	8,4 (7,8-12,6), (p ₁ <0,05)	1,64 (1,59-1,68) (p ₁ <0,05)	2,71 (2,69-2,74) (p ₁ <0,05)
Application of p38 MAPK inhibitor and H ₂ S	16,2 (15,0-21,5), (p ₁ <0,05, p ₂ <0,05)	2,08 (1,99-2,13) (p ₁ <0,05, p ₂ <0,05)	2,19 (2,13-2,24) (p ₁ <0,05, p ₂ <0,05)
Application of NOC-5	80,7 (76,2-84,3) (p ₁ <0,05)	1,62 (1,57-1,66) (p ₁ <0,05)	0,96 (0,91-1,01) (p ₁ >0,05)
Application of p38 MAPK inhibitor and NOC-5	89,9 (88,0-92,3) (p ₁ <0,05, p ₂ <0,05)	0,80 (0,76-0,84) (p ₁ >0,05, p ₂ <0,05)	0,38 (0,36-0,40) (p ₁ <0,05, p ₂ <0,05)
Application of CORM-2	16,4 (15,9-18,5) (p ₁ <0,05)	1,46 (1,20-1,31) (p ₁ <0,05)	1,67 (1,61-1,70) (p ₁ <0,05)
Application of p38 MAPK inhibitor and CORM-2	30,3 (29,1-30,4) (p ₁ <0,05, p ₂ <0,05)	3,65 (3,59-3,70) (p ₁ <0,05, p ₂ <0,05)	3,25 (3,23-3,29) (p ₁ <0,05, p ₂ <0,05)

P₁ < 0,05 versus intact Jurkat cells; P₂ < 0,05 versus gase's donor action

3.4 Activity of caspases-3 and -9 after treatment of Jurkat cells with donors of NO, CO and H₂S and inhibition of p38 MAPK

Activity of caspases-3 and -9 was evaluated after CORM-2 and NaHS application. NOC-5 caused the activation of caspase-3 but didn't affect the activity of caspase-9. Pre-incubation of cells with SB203580 led to inhibition of caspase-3 and -9 after NOC treatment. Application of SB203580 prior NaHS treatment caused activation of caspase-3 and didn't cause changes in caspase-9 activity compared with the isolated gas treatment. Inhibition of p38 MAPK dependent pathways in CORM-2 induced apoptosis

lead to induction of caspases-3 and -9 compared with isolated CO action (Table 1).

3.5 Protein level of Bcl-2, Bcl-xl, Bad and Aven after treatment of Jurkat cells with donors of NO, CO and H₂S and inhibition of p38 MAPK

Incubation of Jurkat cells with NOC-5 for 24 h led to increase of Bcl-2, Bad and Aven level compared with untreated cells. P38 inhibition caused Bcl-2 content augmentation and Aven level decrease compared with NOC-5-treated cell (Figure 2).

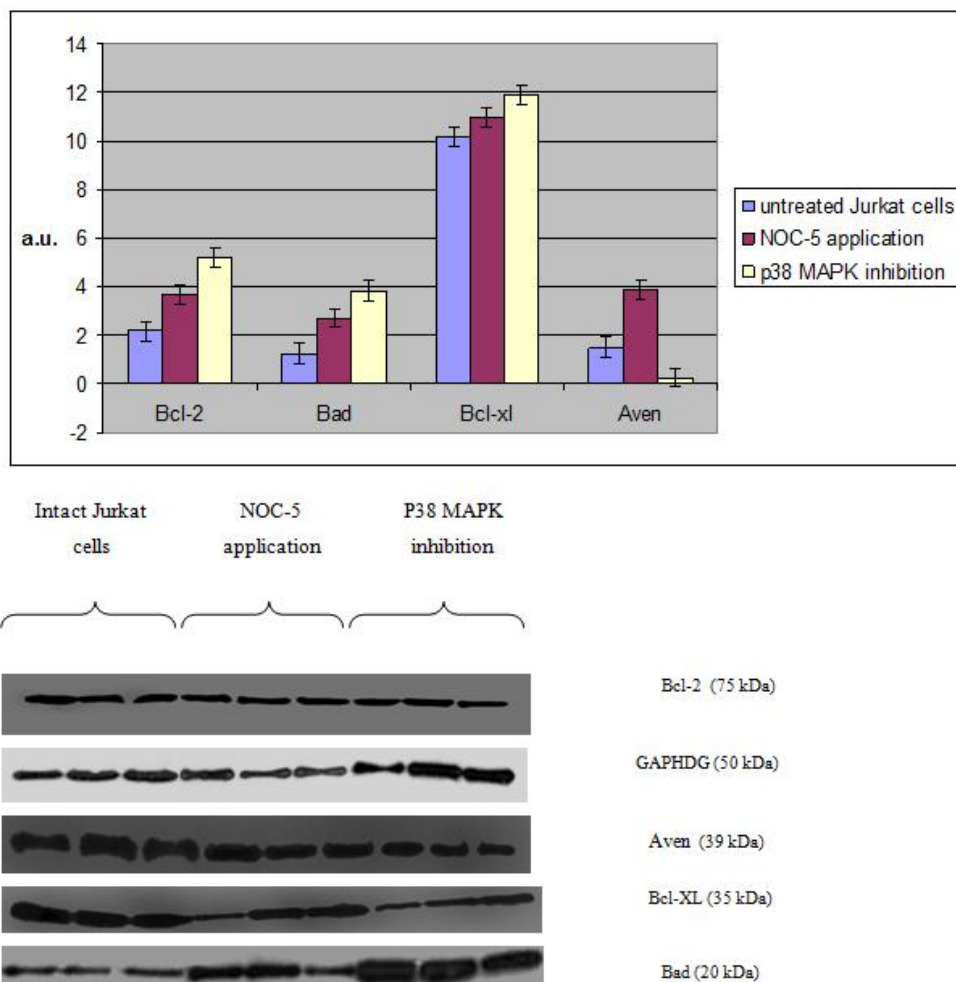


Figure 2 Proteins content after NOC-5 treatment and p38 MAPK pathways inhibition

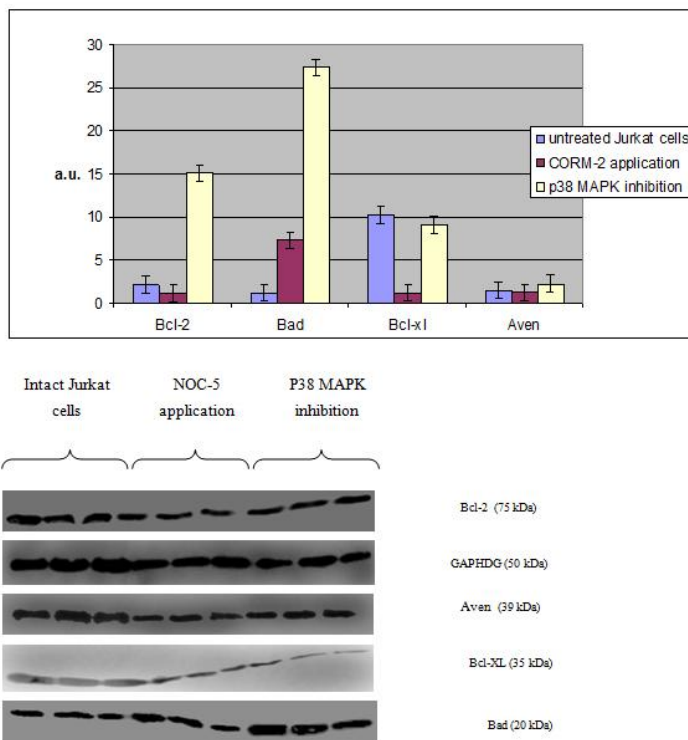


Figure 3 Proteins content after CORM-2 treatment and p38 MAPK pathways inhibition

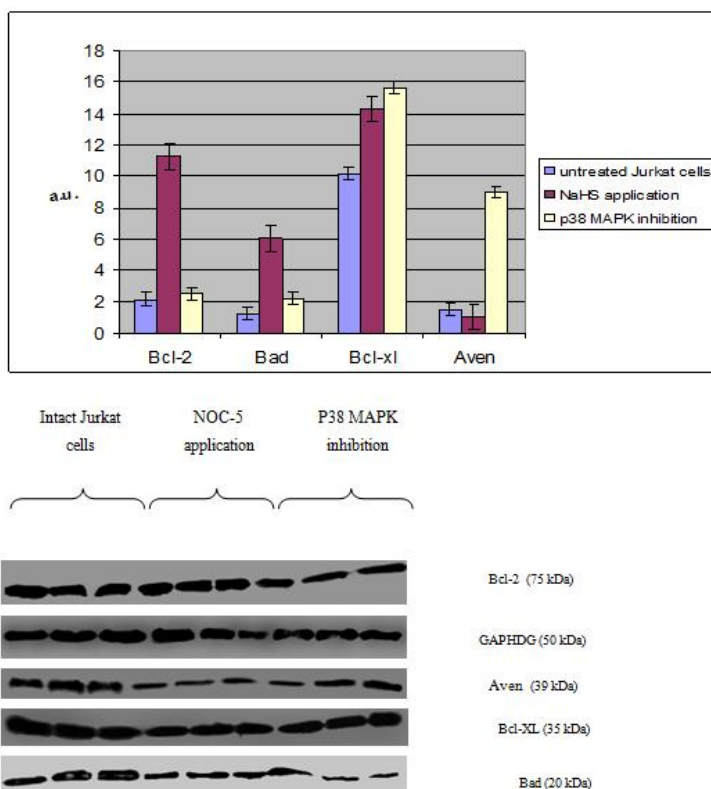


Figure 4 Proteins content after NaHS treatment and p38 MAPK pathways inhibition

Donor of hydrogen sulfide increased level of Bcl-2 and Bad proteins compared with untreated Jurkat cells. P38 MAPK pre-incubation abolished H₂S-induced Bad and Bcl-2 augmentation. Bcl-2 content didn't vary from that in untreated cells but Bad level was still higher than the same parameter in intact cells. Application of SB203580 led to increase level of Aven in H₂S treated cells (Figure 3).

Incubation of Jurkat cells with carbon monoxide donor led to decrease of antiapoptotic protein Bcl-xl and to increase of proapoptotic Bad compared with untreated control. Pre-incubation of cells with p38 MAPK inhibitor elevated level of Bcl-2, Bcl-xl and Bad compared with CORM-2-treated cells (Figure 4).

4. Discussion

Investigation of apoptotic response of Jurkat cells show that intensification of apoptosis is revealed after application of nitric oxide donor NOC-5 100 μM (in 24 h), hydrogen sulfide donor 10 mM NaHS (in 15 min) and carbon monoxide 50 μM CORM-2 (in 24h) (Figure 1). We tested the response of mononuclear leucocytes, derived from the blood of healthy donors, and THP1 cell line for gases' donors application. The above mentioned cells weren't sensible for gases-induced apoptosis or died necrotically [18].

It is known that mitochondria are the main sensors of gases concentration in the cells. In this work we have measured the change of index reflected the mitochondrial membrane integrity – mitochondrial transmembrane potential. The number of cells with decreased mitochondrial membrane potential enlarged 70 times after NO donor application. NO (or ONOO) reacts with heme-containing, Fe- and S-containing proteins a great number of which are in mitochondria [19]. Carbon monoxide also binds with the heme-containing proteins but its ability to do that is some times lower than that of NO [20]. Lower CO affinity to heme-containing mitochondrion proteins could be the reason of more stability of mitochondrial membrane in CO-treated Jurkat cells. H₂S disturbs the mitochondrial membrane integrity comprehensively less than NO and CO. It is known that H₂S is able to open K_{ATP} mitochondrial membrane canals, this ability

could lead to the promoting the safety of mitochondrial membrane integrity [21].

Proteins of Bcl-2 family are the connecting part between the mitochondrial function and apoptosis development. Antiapoptotic members of this family (Bcl-2, Bcl-XL, Bcl-w, Mcl-1, A1) locate in outer mitochondrion membrane, prevent the pore formation via binding and neutralization of proapoptotic proteins (Bax, Bak), the latter induce mitochondrial membrane permeabilization. The proapoptotic members subclass of this family (Bim, Puma, Bid, Bad, Bik, Bmf, Hrk, Noxa) cannot influence the mitochondrial membrane permeability by themselves but promote cell's death via binding antiapoptotic or promoting the effect of proapoptotic proteins [22].

The increase of Bcl-2 and Bad proteins was observed in NO and H₂S-treated cells. Apoptosis intensification in these gases-treated cells showed the abolishment of antiapoptotic Bcl-2 function which may be due to binding with proapoptotic protein Bad. CO decreased the Bcl-xl level in cells and increased the level of Bad, as a result of that the number of cells with decreased mitochondrial transmembrane potential grew and the cells died.

Disturbances of mitochondrial membrane integrity lead to proapoptotic factors (cytochrom *c*, Smac/DIABLO, AIF, endonuclease G) withdrawal to cytosol. These factors activate the basic apoptosis effectors – caspases family. Thus, combination of cytosolic procaspase-9, ATP, Apaf-1 and cytochrom *c* leads to the formation of apoptosome complex [23]. We have demonstrated that incubations of Jurkat cells with 10 mM NaHS and 50 μM CORM-2 led to caspase-9 activation in comparison with the same value in untreated cells. Cells incubation with NOC-5 didn't cause activation of caspase-9. It is known that NO nitrosylates and thus inhibits several members of caspases family [24]. Also abolition of apoptosis on caspases level can occur as a result of caspases connection with caspases inhibitors (proteins of IAP family, some members of Bcl-2 family). It has been shown that NO in physiological concentration changes the expression of genes including apoptosis regulators [25]. Absence of effect from mitochondrial proapoptotic factors withdrawal on the caspase-9 activation can be the result of micromolar NO-induced

proteins-caspase inhibitors synthesis.

Caspase-9 has initiative function among the members of this enzyme group and caspase-effectors -3, -6, -7 execute apoptotic program [26]. We have found donor application of all three gases resulted in caspase-3 activation compared with intact cells. Caspase-3 activation can be caused by the effect of caspases-8, -9, -10, CPP32 activating protease, grandzyme B on procaspase-3 [27]. The registered enzyme activation increasing after NaHS, CORM-2 cells treatment is the result of caspase 9 catalytic effect. Incubation of cells with 100 μ M NOC-5 led to activation of caspase-3 without caspase-9 activation as a result of other enzymes functions involved in caspase-3 induction.

Phosphorylation plays the key role among the signal transduction mechanisms based on posttranslation modification of target proteins. Eucariotic cell has wide range of kinases (518 in human cells) a great number of which are not well investigated [28]. It has been shown MAPK family takes part in the most ways of signal transmission. MAPKs can be activated by a

wide variety of different stimuli, but in general, p38 kinase is more responsive to stress stimuli ranging from osmotic shock and ionizing radiation to cytokine stimulation [29].

P38 MAPK had proapoptotic function when NO influenced Jurkat cells. It is known that the Bcl-2 proteins imbalance contributes to mitochondrial membrane permeability. P38 kinase stabilized mitochondrial membranes of 100 μ M NOC-5-treated cells. P38 MAPK inhibition led to increase of the levels of antiapoptotic protein Bcl-2 and proapoptotic Bad, which could abolish the antiapoptotic function of the first one. Final increase of Bcl-2 and Bad content after p38 MAPK inhibitor and NOC-5 application was accompanied with augmentation of cells with decreased mitochondrial transmembrane potential. The caspase-3 and -9 activity decrease after p38 MAPK inhibition could be the result of negative p38 MAPK effect absence on the caspase inhibitors-proteins level. But protein Aven having the property of caspase-3 inhibition decreased after p38 MAPK inhibition (Figure 5).

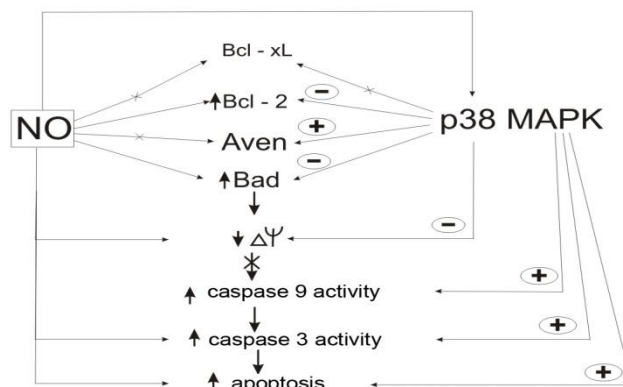


Figure 5 p38 MAPK-dependent targets of proapoptotic action of NO

Incubation of Jurkat cells with NOC-5 for 24 h led to the increase of Bcl-2, Bad and Aven level compared with untreated cells. P38 MAPK was the negative regulator of Bcl-2 and Bad content and positive regulator of Aven level. NO through Bcl-2 family members imbalance and directly increased the number of cells with decreased mitochondrial transmembrane potential, activated caspase-3 and apoptosis. P38 MAPK had the ability to activate caspase-3 and -9 thus leading to apoptosis induction.

P38 kinase stabilized mitochondrial membranes of 100 μ M NOC-5-treated cells.

CORM-2 and p38 MAPK inhibitor led to intensification of Jurkat cells apoptosis so p38 MAPK had antiapoptotic function when carbon monoxide concentration increased. P38 MAPK was the negative regulator of caspase 3 and 9 activation and stabilized mitochondrial membranes. P38 kinase inhibition caused the Bcl-xL level rising but Bcl-xL level decreased when the CO acted alone. Bcl-2 protein intracellular content didn't change after CO donor

application but it increased while p38 MAPK-dependent pathways of CO action were inhibited. So, in Bcl-2 and Bcl-xL cases p38 MAPK revealed proapoptotic properties as the negative regulator of these antiapoptotic proteins content. Partly p38 MAPK antiapoptotic function could be due to the negative regulation of Bad content under CO effect. This protein has the ability to connect and inhibit the antiapoptotic Bcl-2 family proteins - this way it leads to apoptosis promotion. Increasing of Bad could abolish the Bcl-2 and Bcl-xL rising that led to apoptosis intensification under intercellular CO action and p38 MAPK inhibition. Thus, proteins Bcl-xL and Bad are p38 MAPK-dependent targets of CO action for apoptosis signal transduction (Figure 6).

The evidence of p38 MAPK participation in H₂S – induced apoptosis is the decreasing number of apoptotic cells after p38 kinase–dependent signal cascades inhibition. Thus p38 MAPK is the proapoptotic factor by the hydrogen sulfide action. We have got controversial results when p38 MAPK inhibition leads to the increase of the number of cells with decrease $\Delta\psi$ and caspase-3 activation and simultaneously to decrease in the number of apoptotic cells. P38 MAPK decreased proapoptotic H₂S action via reduction of the number of cells with decreased

mitochondrial transmembrane potential and caspase-3 activity (both parameters increased after p38 kinase inhibition). In our investigation the signal to the maintenance of some Bcl-2 family members changes was transmitted through p38 MAPK after Jurkat cells were treated with H₂S. Thus, the greater Bad content under the effect of hydrogen sulfide depended from p38 MAPK. P38 MAPK is not the only one transmitter of signal from H₂S to Bad level change as the Bad content didn't reach the intact cells level. The signal for the increasing of antiapoptotic Bcl-2 protein level via H₂S was transmitted exclusively with p38 MAPK. Abolition of Bcl-2 increasing during p38 MAPK inhibition could result in augmentation of number of cells with decreased mitochondrial transmembrane potential. Within the presence of H₂S p38 MAPK was the transmitter of both the proapoptotic and antiapoptotic changes which resulted in apoptosis decreasing when inhibited. The Aven level increased by the p38 MAPK inhibition, consequently p38 MAPK was the negative regulator of this protein content under H₂S effect. The Aven content increasing could be the reason of caspase-9 activation abolition by p38 MAPK inhibition in spite of mitochondrial membrane permeability rising (Figure 7).

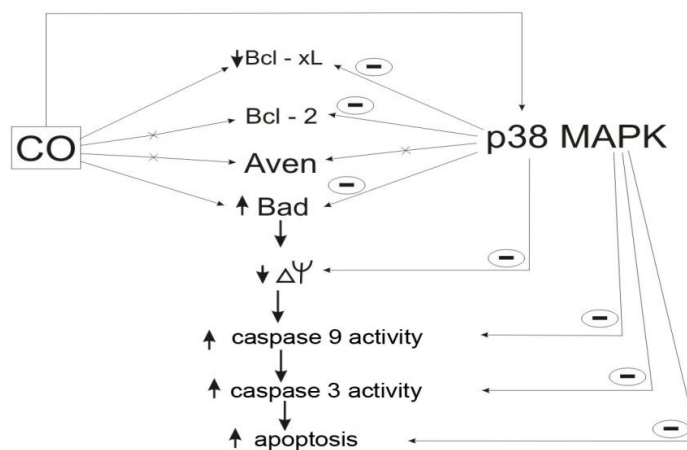


Figure 6 p38 MAPK-dependent targets of proapoptotic action of CO

Increase of the number of cells with decreased $\Delta\psi$ in CO-treated cells was the result of Bcl-2 family members imbalance. Activation of caspase -3 and -9 and apoptosis induction followed after mitochondrial membrane permeabilization. P38 MAPK was the negative regulator of Bcl-XL, Bcl-2 and Bad in CO-treated cells. Preventing of

Bcl-XL and Bcl-2 rising by p38 MAPK favoured CO-mediated apoptosis induction. But on the other side p38 MAPK inhibited mitochondrial membrane permeability, caspase -3 and -9 activity thus this molecule acted antiapoptotically in the case of CO application.

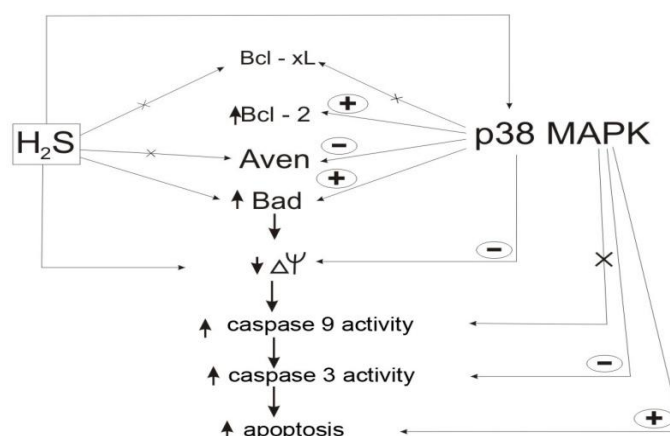


Figure 7 p38 MAPK-dependent targets of proapoptotic action of H₂S

Mitochondrial permeabilisation and caspase-3 activity decreased after p38 MAPK inhibition in cells treated with H₂S. But hydrogen sulfide increased the apoptotic cell death via p38 MAPK induction. The signal for the increasing of antiapoptotic Bcl-2 protein level via H₂S was transmitted exclusively with p38 MAPK. P38 MAPK inhibition leads to the Aven rising and decreasing of Bad content.

Therefore, protein Bcl-2 was the p38 MAPK-dependent target of hydrogen sulfide action and the changes of Bcl-2 content resulted in apoptosis modulation of Jurkat cells. The changes of other indexes under our test during p38 inhibition (decrease of Bad, Aven rising) didn't contribute to the proapoptotic H₂S action.

p38 MAPK acted proapoptotically in the cases of intracellular increase of NO and H₂S and antiapoptotically in CO-treated cells. It was shown that hemoxigenase-1 enzyme responsible for CO synthesis is overexpressed in many cancer types [30]. Also it is known that in cancer cells p38 MAPK is involved in survival potential and contributes to metastases [31]. The received antiapoptotic uncanonical p38 kinase action could be resulted by the CO concentration increase in cancer cells. Further detail molecular mechanisms investigation of this phenomenon is needed. In whole the caspase 3 and 9 activity and the number of cells with decreased mitochondrial transmembrane potential showed the apoptotic reaction vector when p38 MAPK-dependent pathways of NO and CO action inhibited. Inhibition of p38 kinase in H₂S treated cells led to caspase 3 activation

accompanied with the decrease of the number of apoptotic cells. This result needs further investigation. Proteins Bcl-2 and Bad were the p38 MAPK-dependent targets of all three gases action. But both proteins content augmentation after p38 MAPK-dependent pathways of NO and CO action inhibition resulted in apoptosis intensification when carbon monoxide intracellular concentration increased and in decrease of apoptosis in NO-treated cells. H₂S action when p38 MAPK inhibited led to decrease of Bcl-2 and Bad level. Thus context factors modified by gases could differently change the activity and content of molecules participating in apoptosis regulation. P38 MAPK influenced on protein Aven when intracellular NO and H₂S concentration increased and on Bcl-xl protein in CO-treated cells. In both cases p38 kinase was the negative regulator of these proteins and promoted the proapoptotic action of gases abolishing the gases-mediated increase of the Aven and Bcl-xl content.

Acknowledgments

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