Summary

Ovarian cancer is one of the most common cause of death from the gynecologic malignancies among women worldwide. Despite a significant improvements in the conventional cancer therapies, late diagnosis, as well as tumor cell resistance to various cytostatic drugs, remains a relevant problem. Therefore, the new cancer treatment strategies, based on the use of novel compounds/therapeuthics with anti-tumoral activity to aid the standard therapies, are being developed. Among many agents that have been studied for their potential anti-cancer activity, the most promising are the nitric oxide (NO) donors - farmacologicaly active syntethic compounds that release NO *in vivo* and/or *in vitro*. In the last years there have been on-going research on the posibility of their application in the treatment of various types of cancers, including ovarian cancer.

The aim of this study was the assessment of the direct effect of NO donors on some of the cancer cells' specific features. The main emphasis has been put on: the uncontrolled proliferation, over-activation of particular signaling pathways, high resistance to therapeutics and elevated expression and secretion of invasiveness/metastatic factors. The above-mentioned attributes are crucial for intensive growth and progress of cancer tumors.

In this research, SK-OV-3 and OVCAR-3 ovarian cancer cell lines have been used. Both of the cell lines originate from the ascites of patients in the advanced stage of the cancer disease but differ in the degree of aggressiveness. Additionally, HEK 293 (human embryonic kidney 293) cell line has been used as the reference control. The activity of two members of NONOate family, with different half-life times: spermine nitric oxide complex hydrate (SPER/NO $t_{1/2}$ = 30 minutes) and diethylenetriamine nitric oxide adduct (DETA/NO, $t_{1/2}$ = 20 hours), has been evaluated. The cell lines cultures with the addition of NO donors, in three concentrations, has been incubated in the predetermined time frames: 24-48 hours (SPER/NO) and 48-96 hours (DETA/NO). The cell cultures without addition of NO donors were used as a control samples.

The first stage of the research focused on the impact of NO donors on the ovarian cancer cells viability. Both, SPER/NO and DETA/NO, presented a significant growth inhibiting effect the SK-OV-3 and OVCAR-3 cancer cell lines, at the concentration of 100μM and 1000μM. Moreover, this effect was different for each type of cancer cell line, being also time and dose-dependent. It is worth to point out that the effect of DETA/NO was stronger than SPER/NO on both cancer cell lines and SK-OV-3 cells were more resistant to both NO donors than OVCAR-3 cells. What is crucial, the non-cancer

HEK 293 cells proved to be less susceptible to both NO donors than both ovarian cancer cell lines. In the following research, the ability of SPER/NO and DETA/NO to enhance the cytotoxic activity of cisplatin on SK-OV-3 and OVCAR-3 cells has been evaluated. The data indicate that pre-incubation of ovarian cancer cells with NO donors made OVCAR-3 but not SK-OV-3 more susceptible to this cytostatic drug. What is more, simultaneous addition of NO donors and cispaltin to the ovarian cancer cell lines cultures enhanced the cytotoxic activity of cisplatin on both SK-OV-3 and OVCAR-3 cell lines.

The next stage of study aimed to determine, whether the NO donors' cytotoxic activity mechanism is based on the induction of apoptosis or rather on the induction of necrosis. In order to answer this question, the evaluation of particular symptoms, specific for apoptosis, was perfomred. These symptoms included: the loss of mitochondrial membrane potential, increased activity of caspase-3 and the presence of phosphatidylserine on the cells' surface. The received data indicate that NO donors induced above-mentioned symptoms in both ovarian cancer cell lines, what points at pro-apoptotic mechanism of their action. However, it should also be noted that the number of late apoptotic/necrotic SK-OV-3 and OVCAR-3 cells rose significantly in a dose-dependent manner, up the highest value at the concentration of 1000μM for SPER/NO and DETA/NO. This fact suggests that although the mechanism of NO donors' action is mainly based on the induction of cells apoptosis, at higher concentrations and after longer exposition time, it can also induce cells necrosis.

Subsequent research evaluated the impact of NO donors on the level and activity of crucial, for cancer cells' survival, signaling proteins, such as signal transducer and activator of transcription 3 (STAT3) and serine-threonine protein kinase (AKT). Results show that both NO donors, at the concentration of 1000μM, significantly decreased total level of STAT3 and AKT, as well as their phosphorylation in the SK-OV-3 and OVCAR-3 cell lines. It is worth to point out that DETA/NO proved to be stronger inhibitor of both signaling proteins activity than SPER/NO and SK-OV-3 cells were more resistant to both NO donors action than OVCAR-3 cells. What is more, application of specific STAT3 and AKT inhibitors revealed that inhibition of these proteins' phosphorylation reduced the viability of both ovarian cancer cell lines. Therefore, it may be suggested that mechanism of NO donors' cytotoxic activity is also related to the inhibition of constitutively activated signaling pathways, responsible for uncontrolled growth and proliferation of cancer cells.

Equally important part of research was the evaluation of NO donors' impact on the secretion of factors that enhance cancer cells invasiveness, such as VEGF-A,

metaloproteinases (MMP-2, -9), and TGF-β1. Additionally, the level of mRNA for each of the above-mentioned factors was determined in the cancer cells after their exposure to NO donors. Data indicate that SK-OV-3 cells released considerably higher amounts of VEGF-A, MMP-2 and TGF-β1 than OVCAR-3 cells, what highlights their more invasive nature. It was noted that SPER/NO and DETA/NO, at the concentration of 100μM and 1000μM, significantly inhibited the secretion of VEGF-A by the OVCAR-3 cell line only. Yet, the inhibition of MMP-2 release was observed in both cell lines. What is more, it was demonstrated that SPER/NO and DETA/NO, at the concentration of 1000μM, decreased the activity of MMP-2 in the SK-OV-3 cells' supernatants. It is worth to point out that only DETA/NO, at the concentration of 1000μM, decreased the secretion of TGF-β1 by the SK-OV-3 cells in a significant manner. The assessment of VEGF-A and MMP-2 mRNA revealed the increased expression of VEGF-A mRNA after the treatment with DETA/NO and decreased expression of MMP-2 mRNA after the treatment with SPER/NO in the SK-OV-3 cells. Neither of NO donors influenced the expression of VEGF-A and MMP-2 mRNA in the OVCAR-3 cells. Similarly, SPER/NO and DETA/NO did not affect the expression of MMP-9 and TGF-β1 in both ovarian cancer cell lines. Obtained data implicate that NO donors, at the highest concentration, inhibit the secretion of pro-metastatic factors (VEGF-A, MMP-2, MMP-9, TGF-β1) by ovarian cancer cell lines. However, both SPER/NO and DETA/NO have very little impact on the mRNA expression of aforementioned factors in the SK-OV-3 and OVCAR-3 cells. This allows the suggestion that low secretion of pro-metastatic factors may be caused by increased apoptosis/necrosis of the ovarian cancer cells in the presence of NO donors.

The final stage of research emphasized on the impact of NO donors on the secretion of immunoregulatory cytokines, such as IL-6, IL-10 and TNF-α, by both ovarian cancer cell lines. Results point out that the level of IL-6 secretion was rising in a dose-dependent manner, with the highest values at the concentration of 1000μM of both NO donors. Since IL-6 is a known inducer of STAT3 signaling pathway, which is responsible for cancer cells survival, it may be suggested that increased secretion of this cytokine is a type of defense mechanism against NO donors action. The level of IL-10 and TNF-α secretion by the SK-OV-3 and OVCAR-3 cells could not be measured because it was below the sensitivity threshold of ELISA assays.

To sum up, both NO donors demonstrated a wide range of action on both ovarian cancer cell lines. These compounds inhibited ovarian cancer cells growth and proliferation, making them also more susceptible to the cisplatin cytotoxic activity. What is more, both

NO donors decreased activity of constitutively activated, crucial for cancer cells survival, signaling proteins. Similarly, SPER/NO and DETA/NO decreased the secretion of pro-metastatic factors, responsible for cancer cells invasiveness. It is worth to point out that NO not only exerts anti-cancer activity but also mitigate many side effects of chemotherapeutics (high blood pressure, thrombosis), due to the overall systemic actions. Hence, NO donors have a high potential of being a supporting compounds in the cancer therapies.