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„THE EFFECTS OF SILDENAFIL ON PERIPHERAL BLOOD MONONUCLEAR CELLS AND GRANULOCYTES OF HEALTHY MEN”

SUMMARY

Time needed to produce and to investigate a new therapeutic product is estimated at more than ten years until the launch of the drug to the pharmaceutical market. This contributes to search for novel applications for authorized drugs whose bioavailability, pharmacokinetics and toxicity are known (so called ‘drug repurposing’). Of particular interest are drugs which may find use in the treatment of cancer, autoimmune deseases and inflammatory diseases.

Nowadays, sildenafil has Federal Drug Administration (FDA) approval for the treatment of erectile dysfunction (ED) and pulmonary artery hypertension (PAH). As a phosphodiesterase type 5 (PDE5) inhibitor, the drug increases intracellular level of cyclic guanosine monophoshate (cGMP), which plays an important role in the regulation of activity of a number of cell populations, including immune cells. Intracellular level of cGMP is regulated by the enzyme - phosphodiesterase (PDE), which catalyzes the hydrolysis of cGMP to inactive 5'-monophosphates. In addition, cGMP production is regulated by the level of nitric oxide (NO), which activates guanosine cyclase, (enzyme which converts guanosine-5'-triphosphate [GTP] to cGMP). In addition, sildenafil increases cGMP level by stimulation of NO production (by increasing the expression of inducible nitric oxide synthase (iNOS).

Data in the literature indicate that NO exerts multiple immunomodulatory effects. Moreover, iNOS expression is observed in various cel types, including macrophages, dendritic cells and neutrophils. In addition, it was demonstrated that PDE5 is expressed in T lymphocytes, macrophages and dendritic cells but not in B cells. However PDE5 inhibitors can modulate the activity of B cells by modulation of T helper lymphocytes (Th1 and Th2).

Some analyzes performed in animals and very few observational studies in humans suggested that sildenafil can modulate immune system function. Therefore, the aim of the

study was to determine the immunomodulatory effects of sildenafil on selected immune cells in healthy men.

The present study was performed *in vitro* on peripheral blood mononuclear cells (PBMCs) and peripheral blood granulocytes, isolated by density gradient centrifugation from healthy blood donors (16 men). Cells were cultured in medium supplemented with sildenafil (concentrations: 400 ng/ml, 4 μg/ml and 10 μg/ml). Controls cultures contained equivalent amounts of medium. Next, the effects of sildenafil on the proliferation of mitogen-stimulated T and B cells, as well as alloantigen-stimulated PBMCs in a mixed lymphocyte reaction (MLR) were analized. The proliferation was examined on the DNA synthesis level by measurements of ^{3}H-thymidine incorporation. The next stage of the work was to examine the impact of sildenafil on production of proinflammatory cytokines: TNF-α, IL-1β, IL-6 and osteopontin (OPN) by PBMCs (by enzyme-linked immunosorbent assay - ELISA), as well as the effect of sildenafil on osteopontin gene expression (by real time PCR). The study also assessed the effect of sildenafil on pheripheral blood NK cells activity (by flow cytometry), as well as on the ability of granulocytes to produce superoxide anion radicals (by cytochrome c reduction test).

The present study demonstrated that sildenafil (concentrations of 4 μg/ml and 10 μg/ml) significantly decreased the activity of NK cells ($p < 0,05$) but did not significantly affect T and B lymphocyte proliferation as well as production of superoxide anions by granulocytes. Moreover, it was showed that sildenafil (concentrations of 4 μg/ml, 10μg/ml) significantly decreased osteopontin (OPN) gene expression ($p < 0,05$) as well as protein production ($p < 0,05$) in PBMCs. In addition it was demonstrated that sildenafil (400 ng/ml) significantly increased production of TNF-α ($p < 0,05$) PBMCs, but had no influence on IL- 1β and IL-6 production.

The results of this study strongly suggest that sildenafil affects functions of certain immune cells in healthy men, however, due to the fact that these analyzes were conducted for the first time, the results should be treated as preliminary. Therefore, further *in vitro* and *in vivo* studies in larger populations are required. Moreover, it is necessary to evaluate the molecular mechanisms which may be associated with the immunomodulatory effects of phosphodiesterase type 5 inhibitor, sildenafil.