9. Summary

The evaluation of the impact of type A botulinum toxin (BTX A) on the skin fibroblasts is the current and promising direction for anti-aging medical research. Finding a method of increasing the efficiency of fibroblasts is a way to regenerate and rejuvenate the skin. Determining whether type A botulinum toxin can be used as fibroblasts stimulating agent and not only as a wrinkle reduction agent may intensify repairing processes and delay the skin ageing.

The aim of this study was to evaluate the influence of Botox (Bx, onabotulinum toxin A) and Bocouture (Bc, incobotulinum toxin A) preparations, used in doses administered to humans, on the functions of human skin fibroblast cell line (CRL-1474) in terms of: viability, DNA synthesis, hyaluronic acid (HA) concentration, glutathione peroxidase (GPx) activity, uric acid (UA) concentration and the evaluation of the total antioxidant status. The research enabled to indicate whether fibroblasts respond with changes in their functions after administration of BTX A preparations and whether this effect depends only on the presence of the pure toxin (Bocouture) or also on the presence of complexing proteins (Botox). Moreover, it was assessed how the above parameters change with time and whether the results depend on the applied doses. Experiments were performed on fibroblasts and a culture medium using the MTT test, thymidine incorporation assay, and colorimetric and immunoenzymatic analyses. It has been demonstrated that Bocouture (2IU) decreases skin fibroblast viability but stimulates DNA synthesis in the fibroblasts within 6 hours after administration, while Botox (2 IU) stimulates DNA synthesis within 48 hours after application. Fibroblasts viability and DNA synthesis after 48 hours of administration of Bc and Bx were similar to those in control. Botox (2 IU) and Bocouture (4 IU) reduced the total protein content in fibroblasts after 48 hours, but Bocouture (4 IU) increased the total protein content 6 and 24 hours after administration of this drug. Bocouture (2 IU and 4 IU) causes an increase in HA concentration within 24 and 48 hours of exposure, while Botox (2 IU) decreases HA concentration (versus relevant groups) in the same times of exposition. However, with increasing doses of Bx and times of exposition, HA concentration is growing in comparison with a smaller dose and the initial time of application. Bocouture (2 IU) causes less antioxidant mobilization (TAS, GPx and UA measurement) than Botox just after 6 hours, but Bocouture and Botox (2 IU and 4 IU)

increase TAS within 48 hours after administration. Botox also causes an increase in UA concentration within 24 and 48 hours after administration.

Based on the research results, the following conclusions have been formulated:

1. Bocouture and Botox differ in their impact on fibroblast viability and DNA synthesis, mainly due to the time of exposure.
2. Bocouture and Botox have a similar influence on cell viability and DNA synthesis, and comparable for both used doses.
3. Bocouture increases hyaluronic acid concentration in fibroblasts. Both agents cause an increase in the concentration of HA with increasing doses and times of exposition.
4. Antioxidant mobilization of fibroblasts was noted after Bocouture but weakened after Botox application, which suggests a protective role of complexing proteins in this extent.
5. Bocouture and Botox differ in their effectiveness in terms of skin fibroblasts viability, DNA synthesis, HA concentration, total protein content, and antioxidant protection.