DESCRIPTION FOR THE GENERAL PUBLIC

Our research project is aimed at investigations on the effects of exendin-4, a drug used to treat diabetes mellitus, on functions of fibroblasts (connective tissue cells involved in wound healing) isolated from skin of diabetic animals. Type 2 diabetes is a disease of civilization whose prevalence is growing rapidly both in Poland and in the world. One of the most severe diabetic disturbances is so-called diabetic foot. Diabetic foot is caused by disturbed healing of foot injury sometimes leading to the development of a non-healing wound and foot amputation. Fibroblasts, the major connective tissue cells, play a crucial role in the scar formation. They synthesize components of extracellular matrix that fills in spaces between cells in tissues, such as collagen and glycosaminoglycans (GAGs). After injury, fibroblasts move (migrate) into the wound, divide (proliferate) and begin to synthesize collagen and GAGs. The tissue repair process is controlled by enzymes referred to as metalloproteinases and by their tissue inhibitors as well as C-reactive protein (CRP). Metalloproteinases break up extracellular matrix proteins which, in turn, are replaced by new proteins synthesized by fibroblasts. CRP inhibits wound healing. Growth factors, such as vascular endothelial growth factor (VEGF) also play an important regulatory role in wound healing. It stimulates fibroblast activity. Similarly, microRNA, particles are regulators of wound healing. Importantly, diabetes impairs markedly all the aforementioned fibroblast functions and alters the concentration of metalloproteinases, their inhibitors, CRP as well as VEGF production in a way which is unbeneficial for wound healing; this is likely the major cause of the abnormal healing. To date, no satisfactory method improving fibroblast function has been discovered. A gut hormone glucagon-like peptide-1 (GLP-1), detected also in the skin, might improve diabetic fibroblast activity. Compounds similar to this hormone, such as exendin-4, are used to treat diabetes. Medications increasing the GLP-1 content in wounds accelerate healing of ulcers in patients with diabetes. Thus, GLP-1 might control wound healing through the effect on fibroblasts. So far, however, no studies on the GLP-1- or exendin-4-induced effects on diabetic dermal fibroblasts have been performed. Therefore, the goal of the this research project is to check how exendin-4, injected to rats with the inherited type 2 diabetes, affects functions of fibroblasts crucial for wound healing. Skin fibroblasts will be obtained from the skin of diabetic rats and then grown outside the body (an ex vivo method). To this end, rats will be implanted with polypropylene mesh pieces similar to those used to treat abdominal hernia in humans. Then rats will be injected daily with exendin-4 or, in the case of control animals, with 0.9% NaCl. for 4 weeks. During this period, the amount of food consumed, body weight and blood glucose levels will be measured in each animal. Then the rats will be anesthetized and mesh pieces coated with the granulation tissue will be removed. Isolated fibroblasts will be grown in an incubation fluid. The proliferating cells will be then used for further experiments. In an additional series of experiments, we will examine mechanisms by which exendin-4 affects fibroblast activity, i.e., we will identify receptor through which this drug affects fibroblasts as well as intracellular compounds and microRNAs mediating exendin-4 action on the particular fibroblast functions. Understanding of these mechanisms is crucial for the possible future application of exendin-4 not only as a drug counteracting hyperglycemia but also improving complicated wound healing in people with diabetes. In both series of experiments, we will compare the fibroblast ability to produce collagen and GAGs. Using a special chamber consisting of two compartments separated by a porous membrane, we will examine whether this drug alters fibroblast migration ability. Additionally, fibroblast cultures will be treated with antibodies binding with the cell-derived substances that control cell death (apoptosis) and DNA repair, and flow cytometry will be used to determine whether exendin-4 affects proliferation, apoptosis and repair of DNA damage in fibroblasts. Based on methods of molecular biology we will investigate if exendin affects expression (activity) of genes responsible for manufacturing of metalloproteinases, their inhibitor, and VEGF in fibroblast colonies. Also, we will determine the levels of metalloproteinase, its inhibitor and CRP in the blood plasma of rats to check if exendin-4 is able to restore the diabetes-disturbed level of these compounds towards more beneficial values. In the second series of experiments in vitro (i.e., performed outside organism on isolated cells), we will use wound fibroblasts derived from the skin of diabetic humans. The aim of this series is to explain mechanisms by which exendin-4, applied directly to fibroblast colonies, affects their function. In this regard, we will examine the exendin-4 effects on collagen and GAGs production, cell viability, migration, proliferation and expression of the aforementioned microRNA molecules. Owing to these experiments, we will check whether these agents mediate exendin-4 action and gain more information about the way this drug affects cell functions. The results of our experiments will indicate whether exendin-4 will improve diabetic fibroblast activity and might be applied to accelerate wound healing in diabetic individuals.