An innovative method of treating acute liver failure using amniotic stem cells isolated from the human placenta.

Studies on the mechanisms of hepatotoxicity and new therapies of the liver damaged by drugs, toxins, viruses, or as a result of autoimmune diseases are important from an application point of view due to the limited effectiveness of the currently used therapeutic methods, the large number of people awaiting organ transplantation, high treatment costs, and the need to take immunosuppressive drugs for life. One such disease is acute liver failure (ALF), defined as extensive hepatocyte necrosis, which is characterized by an average mortality rate of 50%. At the stage of preclinical studies, an appropriate experimental model of ALF induction is intoxication of experimental animals with the hepatotoxic agent D-galactosamine (D-GaIN). Its action also mimics damage caused by human hepatitis viruses, which cannot be used directly in an animal model.

The currently used ALF therapy is largely supportive. Its main purpose is to predict, prevent, and treat complications as well as facilitate the regeneration of the patient's liver. A fairly effective form of ALF treatment is transplantation of the liver or hepatocytes alone, but in both cases one of the key problems is their availability. Studies on the influence of mesenchymal stem cells in diseases such as ALF indicate that liver parameters may improve, but the isolation of cells from the bone marrow is insufficient, invasive and painful for the patient. Currently, only one clinical trial using commercial stem cells is registered with ClinicalTrials.com for the treatment of ALF. The few publications describing the role of cell therapy in treating this disease focus on mesenchymal cells, but the limitations in the use of these cells motivate the search for further alternatives. An alternative form of treatment may be therapy using human amniotic epithelial cells (hAECs), which have a high differentiation potential, are characterised by a low immunogenic profile, secrete inflammation inhibiting agents, and, unlike many other stem cells, do not form cancerous tumours after transplantation. Their acquisition from the placenta, compared to cells isolated from other human tissues, is more efficient, safe, and is not ethically controversial. Previous studies have indicated significant therapeutic potential of amniotic cells but its association with therapy of acute liver failure has not been defined.

Due to the above, it was hypothesized that cells isolated from the human amnion and administered intrasplenically as a part of cellular therapy to recipient mice intoxicated with D-galactosamine can effectively colonize liver and participate in preventing liver damage. The multistage assessment of liver damage (RT-PCR, immunocytochemistry, histopathological assessment) will help to determine the relationship between the effectiveness of the injection of the injected cells and the therapeutic effect they caused. Applying the two methods for identification hAEC (DNA and markers of human cells) will increase the probability of detecting the transplanted hAEC in the organs and blood of the recipient-mouse, as well as the precision of quantitative evaluation of transplanted cells, even if their number is very small

The experiments will be carried out on adult female BALB/c mice. Healthy mice will be divided into two groups receiving single injections of, respectively, a placebo and hAECs administered intrasplenically. The mice subjected to intraperitoneal D-GaIN intoxication will be subdivided into two groups, one of which will receive a single dose of hAECs. Next, 3, 21, and 69 hours after administration of hAECs and 6, 24, and 72 hours after administration of D-GaIN, organs (brain, lungs, spleen, liver, heart, and kidneys) and blood will be collected from the recipient mice. The degree of liver injury will be assessment by detection of number of proliferation (Ki-67^{+}) and apoptosis (Caspase-3+) cells, blood morphology, histopathological changes (haematoxylin-eosin staining), and determination of the expression of oxidative stress genes. Additionally, the detect of injected hAECs will be carried out by an immunohistochemically method (NuMa, CK14, B7H3) and polymerase chain reaction (AlyY8b,cytochrome B).

The novelty of the presented experiment is the use of hAEC for the treatment of acute liver injury, which has not been studied so far. This experimental model has not been used so far to assess a potential of hAEC as preclinically therapy. The relationship between the number of cells administered, the number of implanted cells and their therapeutic effects has also not been studied. Obtaining such data would facilitate the planning of effective clinical treatment of ALF based on hAEC application to the patient.