ABSTRACT

The innovative part of the study required establishment of the most accurate *in vitro* O_2 level corresponding to the hypoxia level prevailing in myocardium after MI. So far, no study has previously reported such an experiment. Thus, human wild type myoblastst were cultured *in vitro* in different concentrations of oxygen (1%, 2%, 3%, 5%, 7%, 10%, and 15%) and expression of *HIF-1α* was verified at time intervals of 24 h and 7 days of *in vitro* culture. Thus, we determined the *HIF-1α* expression curve depending on the O_2 level in cultured *in vitro* wild type myoblasts. Myoblast cells exhibited the highest expression of *HIF-1α* when cultured *in vitro* for 24 h in 3% oxygen. To compare *in vitro* hypoxia to conditions prevailing in post-infarcted hearts, *HIF-1α* expression was examined in human myoblasts transplanted into murine hearts immediately after myocardial infarction. Highly elevated expression of *HIF-1α* in human was detected in samples of myoblasts at 24 h after cell transplantation and at 7 days after transplantation *in situ*. After comparison of the *in vitro* and *in vivo* experiments, it turned out that only cells cultured for 24 h in 3% oxygen exhibited similar *HIF-1α* levels with no statistically significant differences, similarly to *HIF-1α* expression in myoblasts transplanted into murine post-infarcted hearts. Therefore, 3% oxygen for *in vitro* hypoxic myoblast cell culture was considered equivalent to post-infarcted heart environment.

After establishment of in *in vitro* O_2 level corresponding to the hypoxia level prevailing in myocardium after MI, human myoblasts cells were genetically modified using vectors containing following proangiogenic genes: *FGF-4/VEGF* and *PlGF*. Subsequently, the influence of oxygen concentrations on the biological functions (proliferation, differentiation, apoptosis) was evaluated. Additionally, to define how O_2 conditions may impact selected gene expression (of transgenes) and protein secretion, the expression of introduced transgenes was evaluated and the functional test for *in vitro* primitive vessel-like structures was performed.

Studies using SCID mice contributed to establishement of gene expression profile of proangiogenic genes in different time points after induction of heart infarction. The interest was focused on proangiogenic genes (*Vegf isoforms a, b, c, d; Plgf*) and genes coding for respective receptors (*Flt-1; Kdr*). To evaluate the influence of hypoxia in the hearts of mice, the expression of *Hif-1α* was also examined. Mice were sacrificed at 24 h, 7 days and 28 days after coronary artery ligation and the espression of listed above genes was evaluated. Understanding the dynamics of proangiogenic genes expression in mouse pos-infarction model allowed for

selection of an appropriate regenerative approach by applying cell therapy with genetically modified cells using the *PlGF* gene in further preclinical studies.

The final phase of the study involved the transplantation of genetically modified myoblasts using *PlGF* into post-infarcted mouse hearts. The therapy was successful not only at the functional level but also at the molecular level, in which the introduced factors could enhance the expression of pro-angiogenic genes. It is worth noting, that the cellular therapy provided a prolonged benefit since the results at endpoint were assessed three months after MI induction, which is significant period of time in the life of a mouse. Echocardiography of the left ventricle showed improvement in the haemodynamic properties of the heart after therapy with both genetically modified myoblasts and WT myoblasts in contrast to treatment with 0.9% NaCl solution. Additionally after cell therapy, the most prominent pro-angiogenic genes (*Vegf-a, Vegf-b,* and *Vegf-c*) and their receptors (*Flt-1* and *Kdr*) were upregulated in the myocardium three months after MI induction.