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Editorial

Stem Cell Therapy in Iran

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During the last years, stem cell research have found great public interest in Iran because of the establishment of research institutions and their activities in Iran's public media. In contrast to this public propaganda, only the results of bone marrow transplantation in the hematopoietic disorders, especially in those with thalassemia major[1, 2] and in few patients with multiple sclerosis[3] have been published in scientific literature.

In this issue of *Archives of Iranian Medicine*, two articles with the results of transplantation of autologous stem cells from bone marrow in two different diseases, i.e., advanced liver cirrhosis and myocardial infarction, are presented.[4, 5] It is the results of activities and collaboration of one center where the stem cells are prepared and two clinical centers in Iran where the patients were selected, treated, and followed.

In one article, stem cells from bone marrow of each of four patients with nonvirus-induced advanced liver cirrhosis (three without ascites or encephalopathy) were aspirated, cultured in media for 64 – 85 days, phenotyped, and found viable. A total number of 30 – 45×10^6 autologous cells suspended in 20 mL normal saline containing 1% human albumin were injected intravenously in each of the same patients. They were then followed for 12 months. The routine liver function tests, model for end-stage liver disease score calculation, measurement of liver volume by computed tomography, and assessment of quality of life were done before and after the stem cell transplantation. The patients tolerated the therapy well. The ethics committee approved the study.

The improvement of liver function tests was observed first in two patients, but remained stable in only one during the follow-up. Increase of liver volume, observed in three patients, was considered optimistically as the result of hepatic cell regeneration. No liver biopsy specimen was taken for proving the results or further studies. At the end of therapy, in three patients, neither improvements nor deterioration of liver function tests were observed; three patients had a Child-Pugh score of <8. The one-year survival of such patients is usually good.[6]

In the other article, autologous stem cells were injected intracoronary in three patients and intramusculary in scar areas in five patients at the time of coronary artery bypass graft surgery. Eight patients with the same surgical procedure were selected without stem cells injection and served as the control group. The cell numbers injected varied from 2.3 to 9.1×10^6 cells in those patients who received stem cells intramuscularly. But it was constant with 6 – 6.5 $\times10^6$ cells in those five patients who received cells intracoronary. All patients had heart failure due to myocardial infarction. Both groups were followed for six months.

The heart function was evaluated by New York Heart Association (NYHA) staging system and left ventricular ejection fraction derived through echocardiography and thallium scan before and six months after the injection. Selection criteria of the control patients are not well-described. The mean age of the control group was 53 years, four years more than the treatment group. It is also not explained whether one surgeon performed all surgical procedures or not. The risk factors of patients, i.e., body mass index, hypertension, diabetes, hyperlipidemia, the angiographic findings, and the kind and intensity of therapy during the follow-up period in patients of both groups were also not presented. It is not clear whether the patients were blinded to the examiner by evaluation of myocardial function and whether all examinations were performed and evaluated by one person. The method of echocardiography and the way the calculated values of this examination were the results of repeated single examination in one session, are not mentioned too. Remarkable inter-observer variation may be found by

echocardiographic examination depending on the method used.[7, 8]

For lack of necessary data, comparison of the characteristics of the few patients in two groups is questionable.

In both studies no tracking of injected stem cell were performed.

Therapy of genetically-induced metabolic liver disease with hepatic cells derived from cadaver liver injected into the portal vein has been of partial success as an alternative to whole liver transplantation.[9, 10] The necessity of repeated injections of high number of well-preserved hepatic cells and the immunosuppressive therapy has remained a therapeutic challenge over the last years.

Although the transdifferentiation of stem cells into other cells in the culture under optimal conditions is evident and proven.[11, 12] There is still some doubts that the stem cells injected to the patients can be differentiated into functionally intact hepatocytes[13, 14] or into cardiomyocytes. In rat xenomodel, the human mesenchymal cells could be identified after injection into myocardium and its differentiation could be followed. In this experiment, the differentiated injected cells were identified as being fibroblasts and not cardiomyocytes.[15]

In patients with liver cirrhosis, after injection of autologous mesenchymal cells, its engraftment and its migration into the liver remain the main problem. In one experimental study, the tracking of bone marrow-derived mesenchymal cells in mice showed that only 1.6% of cells could be detected in the liver over a six-month period.[16] Therefore, the number of stem cells engrafted in the liver and its differentiation to functionally intact hepatocytes are also unclear.

The human liver contains approximately 250×10^9 cells, $175\times10^9$of which are hepatocytes. For the improvement of the liver cell function injection, of much higher numbers of intact liver cells into the portal vein is necessary. This higher numbers of liver cells increases the pressure of portal vein and provides conditions for the development of thrombosis. The number of injected stem cells in this study was <1:1,000 of cells in the human liver. Only a very small number of these injected cells by transvenous route can be engrafted, finds it homing in the liver, and when transdifferentiated to hepatocytes, can exert its function.

In inherited metabolic liver diseases for improvement of liver function, more than 10^9 hepatocytes from cadaver liver were injected through portal vein,[10] which is more than 50 – 100 times the number of stem cells injected intravenously in this study.

When we consider that only very few stem cells could find their route in the liver, it is not surprising that this procedure could contribute little to the liver parenchymal regeneration.[14]

However, from animal experiment a reduction of hepatic fibrosis in a CCl_4 acutely-injured liver was observed after bone marrow mesenchymal cell injection.[17] Outside of the differentiation of mesenchymal cells to hepatocytes, the infusion of macrophages and precursor cells may express cytokines, growth factors, and metalloprotease matrix proteins, which are able to degrade collagen and fibrosis resolution in cirrhotic liver.[18] This can improve the liver function.

In order to prevent thrombosis and to improve the liver function, injection of higher number of stem cells can be performed repeatedly over short intervals, when the patients tolerate the repeated injections. Such treatment is a great challenge for the patients and is not free of complication.

The authors of the study in liver cirrhosis in this issue have recently published the results of a study, in which four patients with liver cirrhosis received almost the same amount of autologous stem cells by hepatic artery route. One of the patients with very advanced liver disease at baseline developed hepatorenal syndrome few days after injection and died. In other three patients, no improvement of liver function occurred during six months of observation. The authors concluded that the hepatic artery injection is not a suitable route for stem cell transplantation.[19]

There are few human studies with stem cell transplantation in liver diseases. In one study, the patients had hepatectomy due to hepatocellular carcinoma in the right lobe.[20] In this study, 2.4 – 8.8×10^6 bone marrow-derived cells were injected into the portal vein, which was tolerated. The three injected patients developed hypertrophy of the left lobe as compared to three controls without injection. In a further small group of nine patients with compensated cirrhosis, a very large number of cells ($5.2\times l0^9$) were injected by intravenous route, also hundred times more than in the study published in this journal. Significant improvement of the serum albumin level and decrease of the

serum bilirubin level occurred after six months.[21]

From theoretical standpoint, the number of mesenchymal cells injected had not been enough at the level that could be useful for the improvement of liver cell function, but its effect for regeneration of liver cells and the potential of regression of liver fibrosis remain to be elucidated in a large controlled trial. It should be considered that cancer can derive from stem cells, as has been seen in experimental studies for gastric carcinogenesis.[22] This should be a great concern for any stem cell transplantation in humans.

In the treated patients with myocardial infarction study authored by Mohyeddin-Bonab et al,[5] any improvement of cardiac function may be the consequence of coronary artery bypass graft surgery and not due to stem cell therapy. The patients in the control group cannot be compared, as mentioned earlier, to that of the treatment group. In the few randomized controlled trials, in which stem cells were injected intracoronary in patients with myocardial infarction, some improvement of cardiac function could be verified.[23 – 25] In a randomized controlled trial on 204 patients whose autologous bone marrow-derived stem cells (about 10,000 times more cells than what used in the study published in this issue of the journal), were injected intracoronary to themselves (without any surgical procedures), the absolute change of ejection fraction, five days after injection, observed in those with greater left ventricular dysfunction (ejection fraction of <49 % at baseline), was 7.5% in the treatment group compared to 2.5% in the control group. After three months of injection, five patients in the control group, but none in the treatment arm suffered from infarction (P=0.06). Death due to heart failure occurred in nine of 103 placebo-treated patients as compared to two of 101 in the treatment arm (P= 0.033). [25]

The study of Mohyeddin-Bonab et al[5] in which the patients has undergone bypass graft surgery at the time of bone marrow cell injection cannot prove the efficacy of cell therapy on the course of myocardial infarction.

The two articles published in this issue with small numbers of injected stem cells in a small group of patients may prove the safety of the autologous stem cell injection and can only be considered as phase I study, as emphasized in one study, and do not justify the rectification of stem cell therapy outside the clinical trials.

As intact and viable autologous stem cells are available in Iran, large well-prepared randomized controlled trials should be done in order to understand the importance and role of cell therapy in treatment of chronic diseases.

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