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RESEARCH ARTICLE

A COMPAR AISON BETWEEN INTRAVENOUS AND INTRAPERITONEAL INJECTION OF HUMAN STEM CELL (CD34+) WITH/ OR WITHOUT MESENCHYMAL STEM CELL IN ALBINO RATS

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ABSTRACT

This study aimed to assess the *in vivo* engraftment and proliferation of purified human haemopoietic stem cell infusion or combined infusion containing haemopoietic stem cells plus mesenchymal stem cells in albino rats through intravenous and intraperitoneal infusion. Twenty female albino rats were used for human cells engraftment, whereas 10 served as a control group. 20 to 40 ml of cord blood samples and length of 12 cm of umbilical cords for Wharton jelly samples were obtained from 10 newborns delivered after full term following ethical consent. Ficoll Paque was used to obtain the mononuclear cells from cord blood samples while trypsin enzymatic treatment was used for Wharton jelly samples. Purification of CD34+ cells was done using RosetteSep and EasySep techniques. The expression and count of CD44+, CD34+and CD45+ cells was analyzed using flowcytometer. The study confirmed the success of *in vivo* engraftment, self renewal and proliferation of immune isolated human umbilical cord HSC/CD34+ by albino rats and differentiation to CD45+ cells, beside the highly increase of the harvested mononuclear cells from the spleen and bone marrow without any case of GvHD within 3 weeks period. Moreover, best engraftment obtained from the infusion that contains CD34+ cells and MSCs.

INTRODUCTION

Umbilical cord blood contains circulating stem cells, and the cellular contents of umbilical cord blood appear to be quite distinct from those of bone marrow and adult peripheral blood. Cord blood shows decreased graft versus host reaction compared with bone marrow, possibly due to high interleukin-10 levels produced by the cells and/or decreased expression of the beta-2-microglobulin. Cord blood stem cells have been shown to be multipotent by being able to differentiate into neurons and liver cells (Rogers and Casper, 2004). While most of the attention has been on cord blood stem cells and more specifically their storage for later use, there have also been reports that matrix cells from the umbilical cord contain potentially useful stem cells (Mitchell *et al.,* 2003). In a recent review of umbilical cord blood transplantation, Laughlin *et al* cites evidence that, cord blood causes less graft-versus-host disease (Laughlin *et al*., 2001). The characteristics of hematopoietic stem cells (HSCs) in umbilical cord blood have recently been clarified.

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These cells could be expanded in vitro without losing their "stemness" or self-renewal capacity. In conjunction with HSCs, allogeneic MSCs have been transplanted without graft rejection or major toxicities. Laughlin et al reported that, it is yet to be determined whether umbilical cord blood HSCs are, in fact, longer lived in a transplant recipient or not (Caplan, 2000). In laboratory and mouse-model experiments comparing CD34+ cells from human cord blood with CD34+ cells derived from adult bone marrow, researchers found that, cord blood had greater proliferation capacity (Kaufman and Thomson, 2002). In the present study we aimed to assess the *in vivo* engraftment and proliferation of purified human haemopoietic stem cell infusion or combined infusion contain haemopoietic stem cells plus mesenchymal stem cells in albino rats through intravenous and intraperitoneal infusion.

MATERIALS AND METHODS

This study is a pilot comparative study conducted at the faculty of medicine, Cairo University, Egypt and the faculty of medical laboratory sciences, Al Neelain University, Sudan.

Samples collection

About 20 to 40 milliliter (ml) of umbilical cord blood, and 12 cm of umbilical cord (used for extraction of Wharton jelly samples) were collected, following 10 deliveries.

Experimental albino rats

Thirty healthy female albino rats, aged 5-6 weeks and weighted 40-60 grams were enrolled in this study. Twenty of them were used for transplantation, whereas the remaining 10 were used as a control group.

Purification of (CD34+) stem cells from umbilical cord blood

Each 2 ml of the cord blood samples were used for CD34+ cells isolation either by RosetteSep Technique (negative selection) or EasySep technique (positive selection). both of the protocols have been referred by "STEM CELL TECHNOLOGIES" {Version 1.0.1, Catalog 15026 and Version 1.0.0, Catalog No 18096, 2007}.

Preparation of MSCs (CD44+) from Wharton jelly sample

About 12 cm umbilical cord sample was used for extraction of MSCs from Wharton jelly. The preparation of MSCs from Wharton jelly was done according to the method previously reported by Mather (2008), while the culturing and identification of MSCs in Dulbecco modified Eagle Medium (DMEM) was prepared as described by Wang *et al.* (2004).

Manipulation and injection of albino rats

The Wistar albino rats were exposed to 3.5 Gy dose of radiation for 7 minutes prior to transplantation. After 72 hours from radiation 3 rats were died from the radiation toxicity; the remaining alive rats were divided according to the type of injected cells and the site of injection into five groups; group one (G1=5 rats) were injected by 300 µL of CD34+ cells intravenously, group two (G2=4 rats) were injected by 200µL of CD34+ cells plus 100µL MSCs intravenously, group three (G3=5 rats) were injected by 300µL of CD34+ cells through intraperitoneal injection, group four (G4=4 rats) were injected by 200µL of CD34+ cells plus 100µL MSCs through intraperitoneal injection, and group five(G5=9 rats) were left without transplantation (control group).

Harvesting the mononuclear cells

After 3 weeks from the manipulation and injection the rats were dissected to harvest the femurs, tibia and spleen for mononuclear cells isolation. After that the cells suspension was prepared and filtered by 45µm filter to get rid of clumps and fibers; the suspension was then used for the mononuclear cells enumeration and identification.

Enumeration of mononuclear cells, CD44+, CD34+cells, and CD45+ cells

Enumeration of MSCs (CD44+), HCS (CD34+) and (CD45+) cells was done by dual platform flowcytometer (Becton Dickinson), while the mononuclear cells enumeration was done by automated hematology analyzer.

Ethical considerations

This study was approved by the ethical committee of faculty of medicine, Cairo University. Furthermore, written informed consent was obtained from all the mothers before sample collection.

RESULTS

Total white blood cells of rats in blood circulation

The means count of the total white blood cells of negative controls rats after 3 weeks of radiations was 1.042.857/ml, while after 3 weeks from Human CD43 engraftment, the count was 6.257.142/ml. By T.test those means count showed a significant statistical difference (*P.value*:0.01).

The Bone marrow - mononuclear cells count of rats

The mean count of mononuclear cells in bone marrow and spleen suspensions, after 3 weeks fromradiation, in control rats (G5) was 385.714/ml, while the mean count of mononuclear cells for the intravenously injected rats (G1 and G2) were 1.400.000/ml and 1.750.000/ml respectively. On the other hand, the mean counts of mononuclear cells in the intraperitoneal injected rats (G3 and G4) were 1.940.000/ml and 5.075.000/ml respectively. The mean percent count of injected CD34+ cells in rats was 2.8% while after 3 weeks from engraftment the count was increased to 15.5%. The difference was statistically significant (*P.value*:.01). Moreover the harvested MNC expressed CD45+ (34.2%). That revealed a successful self renewal and differentiation of human stem cell.

DISCUSSION

The study confirmed the success of *in vivo* engraftment, self renewal and proliferation of human CD34+ cells isolated either by RosetteSep (negative selection) or EasySep (positive selection) in albino rats, and differentiation to CD45+ cells, beside the highly increased harvested mononuclear cells from the spleen and bone marrow without fatal Gv HD in a period of three weeks. Furthermore, the study showed that, the best site for CD34+ cells transplantation and engraftment in albino rats is through intravenous injection. In agreement with Rasmusson *et al.* (2008), the study showed an elevation in the mononuclear cell count harvested from the rats that injected by both CD34+ cells and MSCs either by intraperitoneal or intravenous injection. We believe that, the allogeneic haemopoietic stem cells transplantation in hematological malignancies may be improved by the administration of MSCs and this may minimize and control the severity of GVHDs. It is questionable whether the results observed in rats can be extrapolated to human samples as allogeneic transplantation or not, because of the differences in the genetic materials in human and albino rats. MSCs have the potential to be used as immunosuppressants because of their powerful suppressive effects but the off-the-shelf use of allogeneic MSCs for tissue repair and regeneration should be questioned.

Conclusion

The study confirmed the success of *in vivo* engraftment, self-renewal and proliferation of immuno isolated human umbilical

cord CD34+ cells by albino rats , which differentiates into CD45+ cells, beside the highly increase of the harvested mononuclear cells from the spleen and the bone marrow without any case of GvHD until 3 weeks period. Moreover the best engraftment obtained from the infusion that contains HSC CD34+and MSCs CD44+.

Also, we concluded, that the best site for CD34 transplantation and engraftment in albino rats through intravenous injection. Umbilical cord can be viewed as the most promising source of stem cells for research and clinical applications. Wharton jelly MSCs look to have the same properties of bone marrow stroma, when it is combined with the haemopoietic stem cell (CD34) in case of in vivo engraftment. It is abundant supply, immunological immaturity and high plasticity made it superior to other sources of stem cells. Pre-clinical trials on animal models showed promising results toward moving into clinical trials and treatment of hematological diseases.

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