Journal of Infertility and Reproductive Biology

Journal of Infertility and Reproductive Biology, 2013, Volume 1, Issue 1, Pages: 7-11

A Study of the Glycoconjugates Distribution of Umbilical Cord; a Lectin Histochemical Study

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Receive: 04/06/2013 **Accept:** 01/08/2013

Abstract

Umbilical cord can be considered as a source of stem cells with a wide differentiates capacity. It also supplies the embryos with blood contains oxygen and vital nutritional elements and may change by some abnormalities during embryo development. The study of different aspects of umbilical cord biology is critical for understanding of pathology and the way of its application in a stem cell therapy protocol. The objective of this study was to find the glycoconjugates distribution pattern in various regions of the umbilical cord. To do this, umbilical cords from normal pregnancy were fixed, sectioned and stained with WGA, PNA, Concanavalin A, PHy, DBA, SBA and UEA lectins. The lectins were FITC-conjugated. The slides were counterstained with DAPI and observed by fluorescence microscopy. The data showed amnioblasts were reacted with BSA, ConA, UEA, DBA and WGA and endothelial cells with BSA and UEA. Smooth muscle also took up BSA, UEA and WGA. Mesenchymal cells at the interarterial zone were stained with BSA, ConA and UEA. In conclusion, the staining pattern with lectins was different in various zones of umbilical cord. Any modification of this glycoconjugates distribution may be exerted effects on the proper umbilical cord function or structure.

Keywords: Umbilical cord, Lectin, Glycoconjugates

1- Introduction

Umbilical cord as a biological waste product is a good source of mesenchymal stem cells (MSC) and can be considered as a cell source for regenerative medicine. Umbilical cord MSCs (UCMSC) have greater expansion and differentiation capacity (1) and also express pluripotency and mesenchymal CD markers (2, 3). These make it as a potential resource for cell therapy. The characterization of the cell population in umbilical cord is a pre-requisition for using it in a routine cell therapy protocol.

Umbilical cord has three regions; the perivascular zone, the intervascular zone and the sub-amniotic part. These three regions contain different cell number and nature (4). For instance, the cell isolated from sub-amniotic region has more proliferation rate while, the cells isolated form intervascular zone near the blood vessels have wider differentiation potential (5). The most common region to isolate MSC is intervascular and sub-amniotic part. However; the perivascular zone is also used as a source of MSCs (6). Two common methods that are used to isolate MSC from umbilical cord is explants-culture and enzymatic digestion. In both methods, the isolated cells encounter risk to contaminate with other cell types such as blood and endothelial cells (4). Therefore; it is necessary to find a method to reduce the cell contamination.

Cell surface glycoconjugates play roles in cell physiology. They involve in cell-cell and cell matrix interaction (7) and also cell growth and differentiation (8). Lectins are a valuable reagent for studying the cell surface glycoconjugates. Cell surface glycoconjugates affinity can be used to separate and purified a specific cell type among different cell in a cell suspension (9).Lectin reactivity of sugar residues in human umbilical cord was compared in normal pregnancy with pregnancy complicated by intra-uterine growth retardation (10) and also gestational diabetes mellitus (11). The correlation was also demonstrated in lectin reactivity of the human umbilical cord with various coiling indices and it was shown that the alternation in lectin reactivity in the connective tissue may be related to morphological and functional changes and the coiling of the umbilical cord (12).

With regards to this consideration, the aim of this study was to investigate the regional difference in lectin reactivity. Especially, the main question that this study tried to answered it was if the cell surface glycoconjugates were various in different cell population in umbilical cord.

2- Material and methods:

2-1-Sample collection

This research is a descriptive study. Four umbilical cords from full term and normal pregnancywere collected from Hafez hospital, Shiraz. All umbilical cords were belonged to the healthy normal offsprings. The parents of all donors were volunteers and signed the informed consent according to the headlines of University ethic committee.

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2-2-Lectinhistochemistry

The umbilical cords were washed in phosphate buffer saline (PBS) and flashed to remove the blood in the umbilical veins. Then the umbilical cords were fixed in buffer formalin and prepared histologically. The paraffin blocks were prepared and sectioned 5µm thickness. The sections were then deparaffinized with xylon, dehydrated in graduated alcohol and washed with PBS.

The samples were incubated with FITC-conjugated lectins (Sigma) for 2 h at room temperature. The lectins was peanut agglutinin (PNA), Concanavalin A (Con A), Wheat germ agglutinin (WGA), Ulexeuropaeus (UEA) ,soybean agglutinin (SBA), Dolichosbiflorus (DBA), Phaseolus vulgaris-leucoagglutinin (PHA). The lectins react to Galactose/N-acethylgalactose amine, manose, N-acetyl glucoseamine (and also sialic acid), fucose, N-acetyl galactoseamine (and also galactose), n-acetyl galactoseamine and glucose/manose/N-acetylglucoseamine, respectively. The samples were counterstained with 6-diamidino-2-phenylindole (DAPI) for 1 min and washes with PBS6-diamidino-2-phenylindole (DAPI). The samples were studied by fluorescent microscopy. The intensity of the reaction was evaluated by a arbitrary scaling system as "very weak", "weak", "moderate" and "strong".

3- Results

The data showed amnioblasts were reacted with BSA, ConA, UEA, DBA and WGA; however, DBA and WGA stained just the apical surface and basal lamina of the amnioblast. The mesenchymal cells located in the sub-amniotic zone could take up the BSA, ConA and UEA. The extracellular matrix was also reacted with BSA and ConA “weakly”, with UEA and DBA “moderately” and with PNA and WGA “strongly”. The pattern of the lectin reaction to PNA showed different distribution compared to the others, so that the island in the extracellular matrix was reacted to the lectin more intense and between the islands the intensity of the reaction to the PNA was “moderate” (Fig 1). The reaction of the amnioblast, mesenchymal cells at the sub-amniotic zone and extracellular matrix were negative for PYT. Intervascular zone Mesenchymal cells at the intervascular zone were stained “very weakly” with BSA, ConA and UEA. The extracelluar matrix also reacted with PNA, DBAand PHY“weakly” and with WGA “moderately” (Fig 2).

Sub-endothelial zone and Endothelium Endothelium of the vessels were reacted with BSA “moderately” and with UEA “strongly”. WGA also reacted with apical surface of the endothelial cell. Sub-endothelial cells consist of smooth muscle also took up BSA, UEA” moderately” and WGA “strongly”; however, WGA just reacted with the cell surface. Smooth muscle cells located in the media of arteries and vein did not show any reaction with PNA, ConA and PHY. DBA just reacted with cell surface of the smooth muscle; however, the cytoplasm of the cells did not show any reaction with this lectin. Extracellular matrix in the sub-endothelial connective tissue reacted “strongly” with PNA and WGA and “weakly with PHY. Basement membrane of the endothelial cells covering the vein and internal elastic membrane of the arteries stained with ConA and DBA. Elastic fibers stained with PHY. The smooth muscles located in the media of the vein and artery showed autoflorescence (Fig 3).

4- Discussion

The glycoconjugates play various structural and functional roles in different tissues. For instance, DBA has been demonstrated to accelerate the ossification capacity of the mesenchymal cells extracted from mouse limb bud (13). Lectins such as UEA and BSA have been known to be as endothelial cell and endothelial precursor markers (14). Our data also showed the endothelia covering the umbilical cord vessels were reacted with UEA and BSA. Peanut agglutinin has been considered as a mitogen agent, in vitro and in vivo.

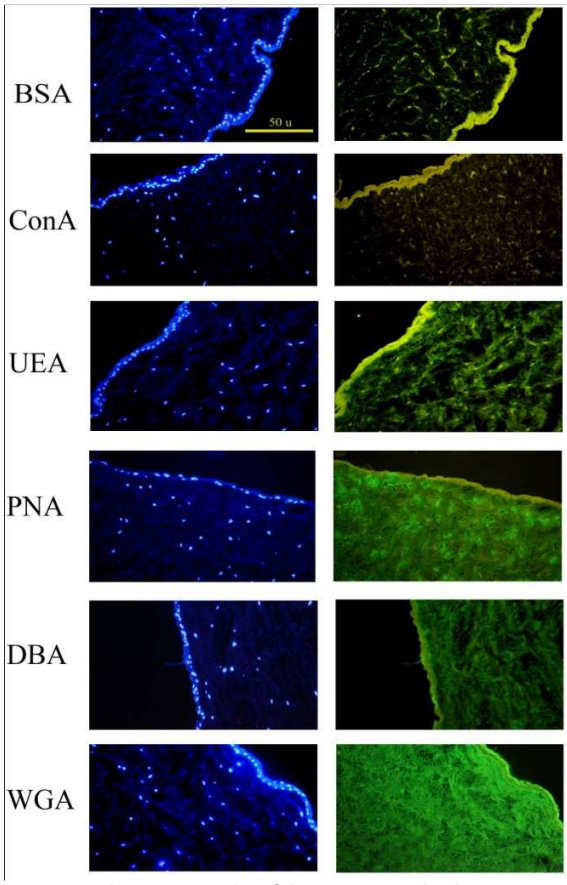

Figure 1: The micrographs of the amnion and sub-amniotic regions of the umbilical cord stained with different lectins. DAPI (Left) and FITC-conjugated lectins (right).

It can be act through the receptors for growth factors (15). Our date showed more intense reaction with PNA in the sub-amniotic region than intervascular zone. Mesenchymal cells are located in the intervascular zone has been shown to have more proliferation rate (5). More PNA-reacted substance at this location may be related to the higher proliferation rate.

Umbilical cord matrix contains sialic acid, d-Galactose (beta1-->3)-N-acetyl-d-galactosamine and N-acetyl-d-galactosamine as indicated by reacting with WGA, PNA and DBA. These data confirmed the previous study (10). Glycoconjugates distribution has been demonstrated a correlation with structural characterization (16).

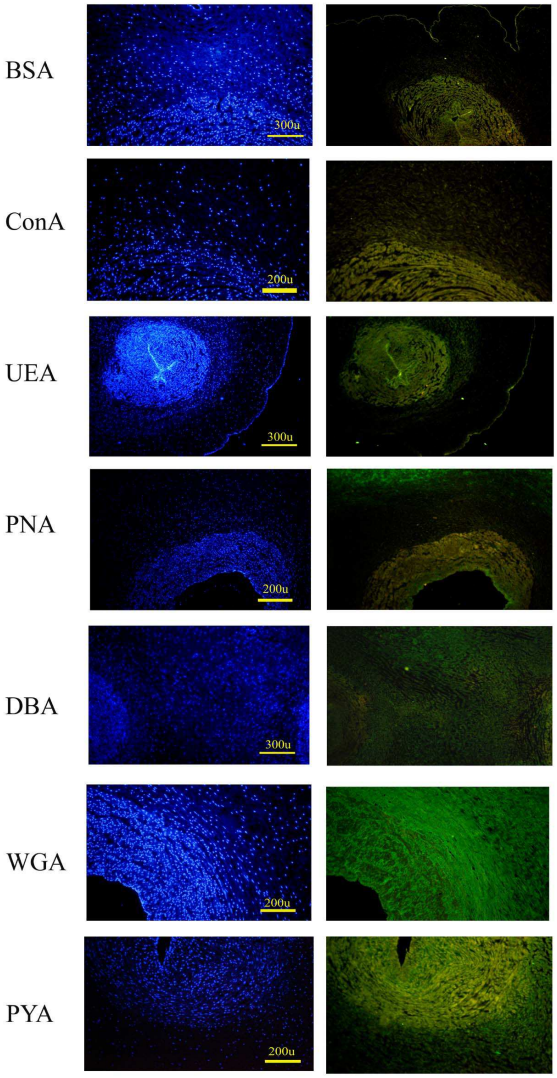

Figure2: The micrographs of the intervascular zone of the umbilical cord stained with different lectins. In all sections a part of arteries were also demonstrated. DAPI (Left) and FITC-conjugated lectins (right).

Glycoconjugates with negative charge is highly hydrated (17). Hydration resulted from glycoconjugates, provides a mechanical stress in the extracellular metrix (18). This force can be responsible for tortuous state of umbilical cord (10).

Although, the cells in various zones of umbilical cord showed a similarity in lectin reactivity, some variation was also shown. This may indicated the different nature of the cells in these zones. Our data showed WGA-positive reactivity for smooth muscle cell surface. This can be a base for isolation and purification of mesenchymal cells originated from intervascular and sub-amniotic zones from smooth muscle contamination.

In conclusion, the glycoconjugates distribution was shown a regional difference in various parts of umbilical cord.

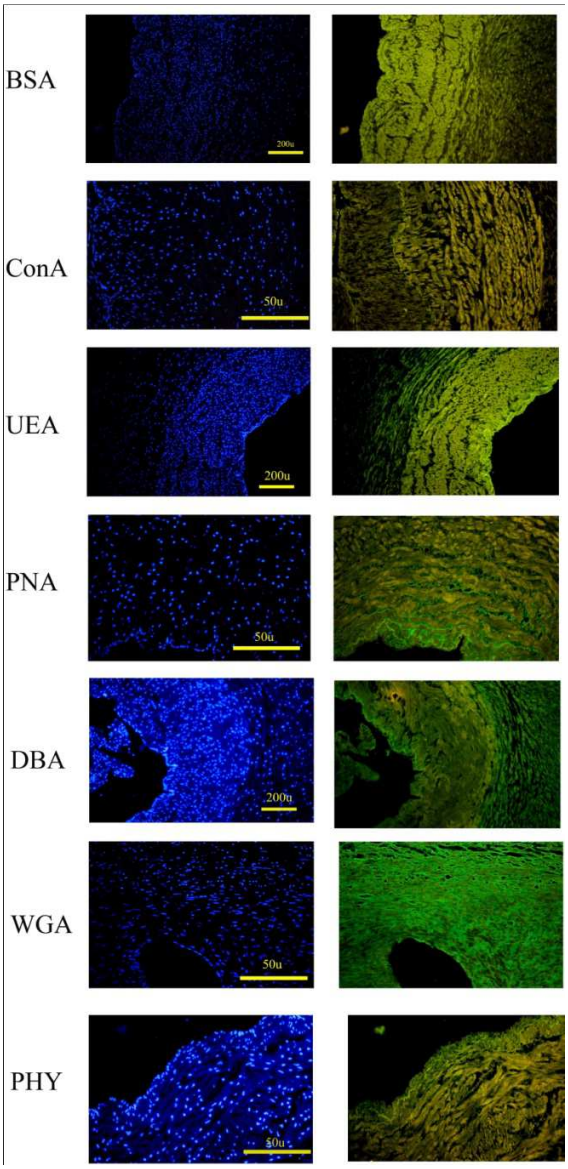

Figure 3: The micrographs of the vascular zone of the umbilical cord stained with different lectins. DAPI (Left) and FITC-conjugated lectins (right).

Acknowledgment

The authors wish to thank Vic chancellor of research deputy of Shiraz University of Medical Sciences for

financial support and offering the grant no 4533 and also Mr Ezad Nori for technical support.

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