Short Report

# Unique Carbohydrate Appearance of the Floor Plate During Early Neurolation

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**ABSTRACT**

**During early neurogenesis, the floor plate plays an essential role(s) in the differentiation of the ventral portion of neural tube. In this study, we detected the specific distribution of unique glycoconjugate during the floor plate differentiation. Formalin fixed paraffin sections of BALB/c mice (10-14 embryonic days) were processed for histochemical studies using horseradish peroxidase-labelled N-acetylgalactosamine (GalNAc) sensitive lectins. Histochemical analysis has revealed the presence of unique Wisteria floribunda (WFA)-sensitive glycoconjugate reaction in the floor plate area and surrounding extracellular matrix. Weak reaction also was observed in the outer surface of the basal zone of the neural tube. Extensive differences among the GalNAc lectins were demonstrated during the sensitive time of motoneuron differentiation. There was no reaction with other tested GalNAc lectins. The duration and distribution of WFA lectin reactions suggest that these molecules may play a key role(s) in tissue interactions and subsequent formation of the adjacent tissues including floor plate and basal plate differentiation during critical morphogenic period. Furthermore, our findings indicate that among considered early neuronal morphogenic (embryonic) days, WFA reactions were increased and expanded near the end of gestation period.** *Iran. Biomed. J. 7 (3): 133-137, 2003*

*Keywords:*Floor plate, Cell interactions, Lectin histochemistry

## INTRODUCTION

Carbohydrate-containing macromolecules of the cell surface and in the extracellular matrix are thought to play a significant role in many developmental phenomena, including cell-cell interaction and differentiation [1-4]. These molecules have also been shown to undergo structural modification and differentiation during development [3, 5]. In early neurogenesis, floor plate of the neural tube plays a significant role in differentiation of the ventral portion of the neural tube into the basal plate in which motoneurons develops. A number of molecules are involved in this complicated cellular differentiation and development [6- 9]. One well-studied factor is a morphogenic sonic hedgehog (Shh), a glycoprotein acting as a local inducer of floor plate differentiation [10-13].

It has been proposed that sugar that occupy a terminal position on glycoprotein side chains may function as tissue-specific stimulants that can initiate morphogenetic events leading to normal development of many biological systems [1, 3, 14, 15]. Precise histological distribution and the origin of specific terminal sugar in the ventral portion of

the neural tube are not known [16]. The purpose of this study was to localize and characterize terminal sugars of glycoconjugates *in situ* during early neurogenesis with particular interest in ventral portion of the neural tube.

## MATERIALS AND METHODS

BALB/c mice embryos from day 10 to 14 of gestation (embryonic period) were used. Female mice were mated with males of the same strain overnight and day 0 of the gestation was assigned at the appearance of the vaginal plug. At gestational stages 10 through 14, pregnant mice were sacrificed by cervical dislocation and the embryos were dissected from the uterus and extraembryonic membranes. Whole embryos were fixed in 1% sodium acetate, 6% mercuric chloride and 0.1% glutaraldehyde solution overnight [2, 15]. After dehydration, the embryos were embedded in paraffin blocks in two different orientations that permitted frontal and transverse sectioning.

**Table 1.** Lectins used in this study.

| Lectin tested | Abbreviation | Carbohydrate binding specificity |
|---|---|---|
| *Dolichos biflorus* | DBA | α-D-Gal Nac |
| *Glycine max* | SBA | α, β, D-Gal Nac>D-Gal |
| *Wisteria floribunda* | WFA | D-Gal Nac |
| *Vicia vilosa* | VVA | D-Gal Nac |

***Lectin histochemistry.*** All lectins used in this histochemical study (Table 1) were purchased from Sigma Chemical Co. (USA). The lectins were conjugated to horseradish peroxidase (HRP) references pertaining to the carbohydrate binding specificity of the lectins used [7, 18, 19]. Sections (5 µm) were treated with Lugol solution to remove mercuric salts prior to staining. The selected sections of the early neural tube (from the rostral to the caudal of the tube) flooded with a solution containing 10-20 µg/ml of lectin-HRP conjugate in 0.1 M PBS, pH 7.2 at 4ºC for two hours. After prolonged rinsing with PBS, the sections were incubated in a diaminobenzidine (DAB)-hydrogen peroxidase substrate medium (pH 7.0) at room temperature for 15 minutes. All sections were counterstained with 1% solution of Alcian blue at pH 2.5 for 5 minutes.

Only lectin from Wisteria floribunda (WFA) bound preferentially to the floor plate zone, and thus the standardize concentration was used for a direct comparison of the staining results at different levels of the neural tube as well as at different developmental periods.

## RESULTS

Among the four GalNAc binding lectins tested, only WFA reacted to the neural tube during development. The other GalNAc specific lectins: *dolichs biflorus* (DBA), *glycine max* (SBA), and *vicia vilosa* (VVA), failed to bind to any part of the neural tube during early embryogenesis (Table 1).

***From day 10 to early day 12 of gestation.*** Cell surface of the luminal face of the neuroepithelial cells reacted with WFA. Weak reaction was also observed in the outer part of the basal zone of the neural tube. Notochord and floor plate reacted only with the Alcian blue (Fig. 1A).

***From day 12 to 14 of gestation.*** The molecular changes occurred in the area of the floor plate of the neural tube and around the notochord. The cells of these areas showed increasingly WFA reaction in the cephalocaudal direction. Notochord was no longer in contact with the neural tube (Figs. 1B, 1C and 1D).

***From early day 14 to late day 15 of gestation.*** Large WFA reacted cells appeared in the area of future anterior horn close to the floor plate (Fig. 1D). Anterior funiculus also appeared during day 14 of gestation and showed weak reaction with WFA.

## DISCUSSION

A floor plate is defined as a region of specialized group of midline neuroepithelial cells that appears to regulate cell differentiation and axonal growth in the developing nervous system [6, 10, 20]. Motor neurons develop from the embryonic basal plate, an anterolateral area of the early neural tube. Differentiation of the basal plate is believed to result from complex interactions of the notochord with the floor plate and then developing floor plate

interacts with the basal plate [10, 21]. A number of molecules are involved in these complicated pathways. One well-studied factor is Shh, a morphogenic molecule, which plays a major role during early neural tube formation including ventral patterning of the neural tube [9,11,13,20,22,23]. Also, it has critical roles in other biological systems

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during early morphogenesis [10, 18]. Other factors such as carbohydrate-containing macromolecules of the cell surface and within the extracellular matrix are also thought to play a significant role in cell-cell interactions during the development of the nervous system [3, 4]. These glycoconjugates particularly

**Fig. 1. (A),** Cross section of the neural tube at thoracic level of early day 11 mouse embryo incubating with WFA and counterstained with Alcian blue. Notochord (arrow) is in touch with the floor plate (parenthesis) of the neural tube. Only internal limited line of the neural tube and part of external line of the basal palate (B) had weak reaction with WFA. N (neural crest forming, dorsal root ganglion); V (vessels); **(B),** Cross section of the 12-day-old mouse embryo neural tube at the level of developing medulla incubating with WFA shows reaction in the floor plate (the area between two hollow arrows). Notochord (arrow) is separated from the tube and mesenchymal cells have reaction with this lectin. B (Basal, plate); V (vessels); **(C),** Cross section of the floor plate (star) at the thorasic level of 13-day-old mouse embryo incubating with WFA. Floor plate shows extensive reaction. There is no reaction in the area of forming ventral horn (vh); **(D),** Cross section of the ventral part of the thorasic part of the neural tube in the 14-day-old mouse embryo shows intense reaction with WFA in the floor plate (star) and also in the large cells (arrows) in the area of forming ventral horn (vh) and forming anterior funiculus (F). Ependymal layer facing the luminal (L) also has reaction with this lectin.

their terminal sugar, interact with components of surrounding cells and the extracellular matrix after morphological changes of the developing cells [1, 5, 16, 17].

In this investigation, we presented evidences that in the early stages of neural tube development, WFA-sensitive glycoprotein uniquely appears in the floor plate of the neural tube prior to theformation of the basal plate. It has been shown that WFA is a lectin that labels selectively N-acetyl-galactosamines (GalNAc β→3 Gal) residues of glycoproteins within the extracellular matrix of the neurons and different cortical areas of the rodent brain [17, 19].

Based on our results, the temporal and the spatial pattern of the WFA reacted glycoconjugates in floor plate and its surrounding areas is similar to that of Shh, an active signaling molecule of the floor plate. It is well documented that Shh is involved in early development of the basal plate precursor of the motoneurons of the central nervous system [8-10]. Attempts are underway to obtain monoclonal antibodies that react with Shh molecules to compare their exact distribution with that of WFA-positive areas.

Other N-acetylgalactosamine sensitive lectins were also tested. None of these lectins had reaction with floor plate or neural tube tissue. Lectins with a similar specificity toward monosacchraides may differ in their affinity for particular glycoprotein [2, 3, 15, 18]. In this study, WFA was the only GalNAc lectin that reacted with floor plate and formed the anterior funiculus of the neural tube during limited time of development. WFA reaction was increased and expanded near the end of embryonic period about day 14 of gestation.

Further study need to understand how this unique and specific glycoconjugate molecule and its GalNAc terminal sugar cause morphological changes during short period. Also, it is also necessary to determine the relationship between this glycoconjugate and Shh molecules in differentiation of the basal plate that leads to formation of the motor neurons.

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