Comparative Lectins Histochemical Study of Vertebrates' Retina.

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دراسة كيميانسجية مقارنة بواسطة اللكتينات لشبكية الفقريات

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الخلاصة:

أستخدمت عينة من ٣٠ حيوان؛ ١٠ اسماك كارب، ١٠ حمامات، و١٠ جرذان بيض و أخذت العينان من كل حيوان. حضرت ٥ شرائح بارافين زجاجية من كل عين بسمك ٦ مايكرون للدراسة الكيميانسجية لمعاملتها بخمس لكتينات هي :UEAI, PNA, SBA, Con-A, and LFA

الهدف من الدراسة كان لمقارنة أنواع الكاربوهيدرات الموجودة في شبكية العين بواسطة اللكتينات في ثلاث حيوانات فقرية تعيش في ظروف بيئية مختلفة.

أظهرت الدراسة اختلافا واضحا في نوعية الكاربوهيدرات الموجودة في الشبكية في الفقريات المدروسة في كل طبقة من طبقات الشبكية العشرة.

Abstract:

30 experimental animals of three species of vertebrates was used; 10 adult Carp fishes, 10 adult doves, and 10 adult Albino rats. Both eyes were taken. 5 paraffin sections were prepared from each eye with 6 µm thickness for histochemical study with 5 lectins which are: UEAI, PNA, SBA, Con-A, and LFA.

The aims of the study were comparing the types of carbohydrates by lectin histochemistry that present in the retina of three vertebrates species habituating in different environmental conditions.

This study found a clear variation in types of carbohydrates in each one of the ten layers of vertebrates' retina.

Key words:

Retina, Lectins, Carbohydrates Histochemistry.

Introduction:

The vertebrate retina has ten distinct layers [(11)]. From innermost to outermost, they include:

1. Inner limiting membrane - Müller cell footplates.
2. Nerve fiber layer.
3. Ganglion cell layer - Layer that contains nuclei of ganglion cells and gives rise to optic nerve fibers.
4. Inner plexiform layer.
5. Inner nuclear layer contains bipolar cells.
6. Outer plexiform layer - In the macular region, this is known as the Fiber layer of Henle.
7. Outer nuclear layer.

8. External limiting membrane - Layer that separates the inner segment portions of the photoreceptors from their cell nuclei.
9. Photoreceptor layer - Rods / Cones.
10. Retinal pigment epithelium.

Retinal carbohydrates were studied thoroughly in the last few years by different methods. The distribution of N-acetyl-lactosamine (NALA), a cell-surface carbohydrate epitope of the lactoseries, has been studied in the retina of representative species of all vertebrate classes by light microscope immunohistochemistry [(2)].

Monosaccharides on the apical processes of the retinal pigment epithelium were examined using lectin-affinity cytochemical methods [(15)].

Lectins are carbohydrate binding proteins of non-immune origin that agglutinate cells and/or precipitate glycoconjugates. The lectins have no enzymatic activity, may be soluble or membrane bound, and are of bacterial, animal or plant origin [(10)]. Lectin histochemistry may highlight subtle differences and changes that are not detectable with monoclonal antibodies [(8)].

Aims of the study:

This study was intended for comparing the carbohydrates histochemistry by lectins in the retinal layers in three vertebrates; namely the Carp fish, the Dove, and the Albino rat. These vertebrates were selected in consideration of the different living environmental circumstance.

Materials and Methods:

The laboratory animals were selected as healthy young adults as follows:-

I. 10 Carp fishes (*Cyprinus carpio*) (Common carp); from Al-Swaira breeding lakes, as a model of fish. Their ages were between (2-2.5 years), their length was (30-35 cm) [(17)].

II. 10 Pigeons *(Streptopelia capicola)* (Ring-necked Doves); from the market, as a model of birds. Their ages were (5-6 months) [(9)].

III. 10 Albino rats (*Rattus rattus norvegicus albinus*); from the animal house in the Institute of embryo researches and infertility treatment/ Al-Nahrain University, as a model of mammals. Their ages were (6-7 weeks) [(7)].

The animals had been sacrificed and dissected under dissecting microscope to take out both eyes and fixed immediately (within less than 30 minutes)in the fixative solution (Bouin's solution) to avoid ischemic damage of the retinal tissue [(13)].

The samples then dehydrated in ascending concentrations of ethanol alcohol (70% for 24 hours- 90% for 6 hours- and 100% for 2 hours), clearing by xylene for 30 minutes, and embedded in molten paraffin wax for 2- 2.5 hours [(3)]. The paraffin blocks then sectioned from the equator of the eyeball into 6 µm thickness on glass slides.

Procedure for fluorescin isothiocyanate labeled lectins:-

1. Hydrated paraffin sections were washed in phosphate buffered saline (PBS) for (10 minutes).
2. To keep the slides swamped by the lectin-PBS solution; the slides were unsoiled at the margins of each section by a cotton wool in order to isolate the sections. To provide an edge that prevents spillage of the lectin-PBS solution, each slide was fitted in a pre-prepared pit on a sheet of softened dental wax.
3. Sections were swamped by lectin-PBS solution and kept for 1.5 hours in a humid closed chamber to prevent from drying and to protect from light.

4. Sections were washed in PBS and mounted in non-fluorescent Fractoil (BDH) mount solution. Then examined under fluorescence microscope.

The lectins used in this study were fluorescin isothiocyanate (FITC) labeled. Table (1) shows the lectins used in this study and their specifications.

Table (1): Lectins used in this study and their specifications. (Gal = Galactose, GalNAc =N-Acetylgalactosamine, Neu5Ac =Acetylneuraminic (sialic) acid)

Lectin (Common name) Abbreviation	***Saccharide specificity***
Ulex europaeus (gorse seed) UEA-I	α- L-Fucose
Arachis hypogaea (peanut agglutinin) PNA	Galβ1,3GalNAc> α- & β-Gal
Glycine maximus (soya bean) SBA	α- & β GalNAc> α- & β Gal
Canavalia ensiformis (jack bean) Con-A	α- Mannose
Limax flavius LFA	Neu5Ac

Results:

The binding patterns of the retinal layers in the three species studied with the 7 lectins showed either positive or negative reaction and listed in tables (2) – (8). Figures (1) – (7) showing fluorescence of retinal layers with the 7 lectins. The retinal layers were numbered from inside outward, i.e. the internal limiting membrane is number 1 and the pigment epithelium is number 10.

1. Binding with UEAI lectin: Table (2)

	Carp Fish	Dove	Albino rat
positive	All but 1, 2, 10	All but 1,2	9 O.S.
negative	1, 2, 10	1,2	All but 9 O.S.

2. Binding with PNA lectin: Table (3)

	Carp Fish	Dove	Albino rat
positive	All but 1, 10	All but 1, 10	All but 1, 10
negative	1, 10	1, 10	1, 10

3. Binding with SBA lectin: Table (4)

	Carp Fish	Dove	Albino rat
positive	All but 1,10	All but 1	2, 3, 4, 6, 9
negative	1, 10	1	1, 5, 7, 10

4. Binding with LFA lectin: Table (5)

	Carp Fish	Dove	Albino rat
positive	5, 7, 9	3, 5, 6, 7, 9	3, 7, 9 O.S.
negative	others	others	others

5. Binding with Con-A lectin: Table (6)

	Carp Fish	Dove	Albino rat
positive	All but 1, 10	All but 1, 2	All but 1, 2, 10
negative	1, 10	1, 2	1, 2, 10

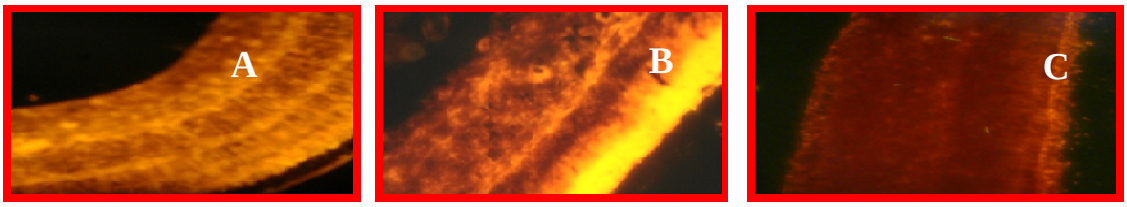

Figure (1): UEAI binding with retina in: A (Carp fish), B (Dove), C (Rat).

Figure (2): PNA binding with retina in: A (Carp fish), B (Dove), C (Rat).

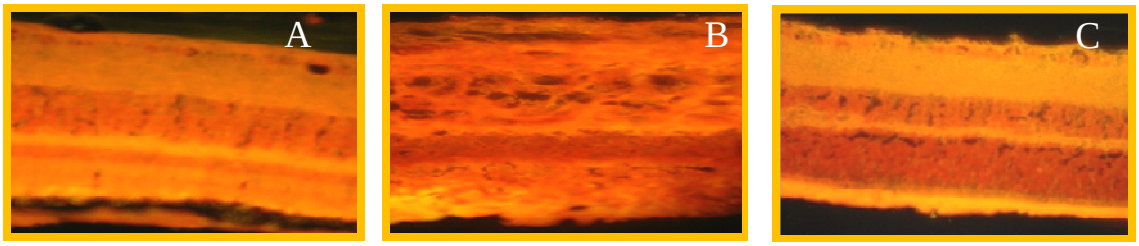

Figure (3): SBA binding with retina in: A (Carp fish), B (Dove), C (Rat).

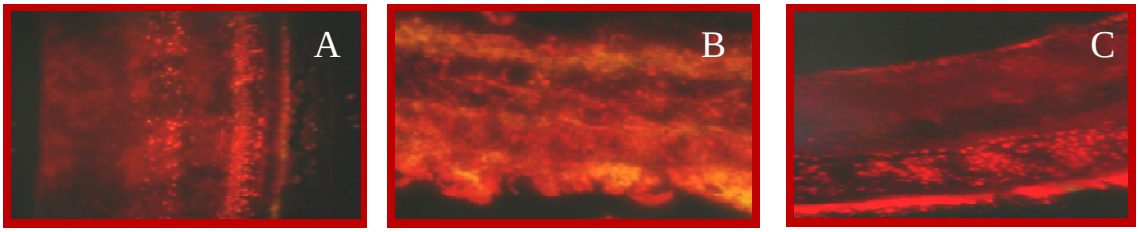

Figure (4): LFA binding with retina in: A (Carp fish), B (Dove), C (Rat).

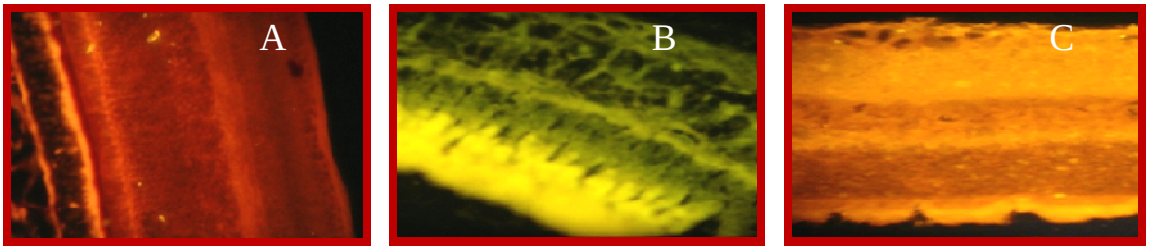

Figure (5): Con-A binding with retina in: A (Carp fish), B (Dove), C (Rat).

Discussion:

1- Lectins binding pattern in the nerve fiber layer: The PNA and SBA lectins were markers for the retinal nerve fiber layer in the three animals used. Con-A was a marker of this layer in the Carp fish retina. These results were similar to a previous study that made a comparable study of lectin binding in fish and rat retina [(12)]. This indicates presence of α and β galactose and N-Acetylgalactosamine in this layer of the three species. The Carp fish nerve fiber layer also contained α-mannose.

2- Lectins binding pattern in the ganglion cell layer: This layer was marked in all species by positive binding with PNA, SBA, and Con-A. The UEAI and LFA was marker of this layer in the Dove retina. UEAI binding was shown in Carp fish retina, and LFA binding was shown in the Albino rat retina. These results were in agreement with previous studies that study lectin binding in vertebrates' retina [(6), (16)]. These results indicated the presence of fucose, galactose, N-acetylgalactosamine, sialic acid, and mannose in the ganglion cells of the three species studied except fucose that was not present in the albino rat and sialic acid that was not present in the Carp fish ganglion cells.

3- Lectins binding pattern in the inner plexiform layer: The inner plexiform layer in the all species was marked by PNA, SBA, and Con-A. In the Carp fish and Dove retina, this layer was marked by UEAI. These results were similar to those of previous study on canine retina [18]. The results of this study indicated that the inner plexiform layer of the retina of the three species of vertebrates contain galactose, N-acetylgalactosamine, and mannose. Fucose was present in this layer of the Carp fish and Dove retina.

4- Lectins binding pattern in the inner nuclear layer: This layer was marked in the all species by PNA, and Con-A. The lectins UEAI, SBA, and LFA marked this layer in the Carp fish and the Dove retina. These results were similar to previous studies on avian and rat retinas [4, 5]. The results of this study indicate that the inner nuclear layer in the three species studies contain galactose, N-acetylgalactosamine, and mannose. Fucose and sialic acid were found in this layer in the Carp fish and Dove retinas but not found in the Albino rat retina.

5- Lectins binding pattern in the outer plexiform layer: This layer was marked in the all species by PNA, SBA, and Con-A. In Dove retina this layer was labeled by UEAI and LFA. This layer was marked by UAEI in Carp fish retina. These results were similar to previous studies [6]. These results indicated that outer plexiform layer in the three species studied contain galactose, N-acetylgalactosamine, and mannose. Fucose was present only in Carp fish and Dove. Sialic acid found only in the Dove.

6- Lectins binding pattern in the outer nuclear layer: The outer nuclear layer was marked in all species by PNA, Con-A, and LFA. In the Carp fish and Dove retina this layer was marked by UEAI and SBA. These results were similar to previous studies [14]. These results indicated that the outer nuclear layer contain galactose, N-acetylgalactosamine, mannose, and sialic acid in the three species studied. Fucose, α and β N-acetylgalactosamine were found in the Carp fish and Dove retina.

7- Lectins binding pattern in the photoreceptor layer: The inner segment of photoreceptor layer was marked in all species by PNA, SBA, Con-A. In the Carp fish and Dove retina this layer was labeled also with UEAI and LFA. The outer segment of photoreceptor layer was labeled in all species by all the lectins; PNA, SBA, UEAI, Con-A, and LFA. These results were similar to previous studies in human [1, 14]. The results of this study indicated that the inner segment of photoreceptor layer in the three species studied contain α and β galactose, N-acetylgalactosamine, and mannose. The Carp fish and Dove retina contain fucose and sialic acid in this layer. The outer segment of photoreceptor layer in all animals studied contain fucose, galactose β1,3, α and β galactose, N-acetylgalactosamine, mannose, and sialic acid.

8- Lectins binding pattern in the pigment epithelium: The retinal pigment epithelium layer was labeled in the Dove only by UEAI, SBA, and Con-A. This layer was not labeled with any lectin in the Carp fish and rat retina. These results were in agreement with previous studies[6]. These results suggested that the Dove retinal pigment epithelium only contain fucose, α and β galactose, galactose β1,3, N-acetylgalactosamine, and mannose.

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