Turkish Journal of Diabetes and Obesity / *Türkiye Diyabet ve Obezite Dergisi*
Original Article / *Özgün Araştırma*

Lectin Staining of Extensor Digitorum Longus Muscle Cell Membranes in Alloxan Diabetic Rats

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ABSTRACT

Aim: Effect of alloxan-diabet on Extensor digitorum longus (EDL) skeletal muscle of rats were observed by lectin staining techniques in light microscope.

Material and Methods: After 30 days alloxan (dose of alloxan: 55 mg/kg) injected by intravenously, samples of muscles were obtained from control and diabetic rats. The muscle samples sections were cutted by cryostat microtome and stained by four biotinylated lectins [Wheat Germ Agglutinin (WGA), Pea Nut Agglutinin (PNA), Concavalin A (ConA), *Griffonia simplicifolia* I (GS)]. The lectins were fixed by avidin-peroxidase complex. All lectins were bounded to EDL muscle cell membranes of control and diabetic rats.

Results: GS and WGA lectins were strongly (+++) stained extensor digitorum muscle cell membranes of alloxan-diabetic rats. Not only cell membranes but also cytoplasmic miyofibrills of diabetic muscle cells were stained by GS lectin. PNA was moderate (++) stained diabetic muscle cell membranes. Con A was weakly (+) stained diabetic muscle cell membranes with respect to control cell membranes. According to our findings, alloxan-diabetes altered the molecular structure of glycoproteins in cell membranes of EDL skeletal muscles of rats.

Conclusion: We suggested that this study will contribute to diabetes research to show the damaging effects of diabetes on cell membranes.

Key Words: *Extensor digitorum longus muscle cell membrane, Lectin stainning, Diabetes mellitus, Alloxan, Wistar rat*

Alloxan Diyabetli Sıçanlarda Extensor Digitorum Longus Kas Hücresi Membranlarının Lektin Boyanması

ÖZET

Amaç: Alloksan diyabetin sıçanların Extensor Digitorum Longus (EDL) iskelet kasına etkisi lektin boyama tekniği ile ışık mikroskobunda incelendi.

Gereç ve Yöntemler: İntravenöz yoldan alloksan enjekte edildikten 30 gün sonra, kontrol ve diyabetik grubu sıçanlardan kas örnekleri alındı. Kas örneklerinden kryostatlı mikrotom ile kesitleri alındı ve dört biyotinli lektin [Wheat Germ Agglutinin (WGA), Pea Nut Agglutinin (PNA), Concavalin A (ConA), *Griffonia simplicifolia* (GS)] ile boyandı. Lektinler, avidin-peroksidaz kompleksi ile tespit edildi. Lektinlerin hepsi kontrol ve diyabetik sıçan grubunun EDL iskelet kasına bağlandı.

Bulgular: GS ve WGA lektinleri alloksan diyabetik sıçanların EDL iskelet kaslarını kuvvetli (+++) boyadı. Özellikle GS lektini sadece hücre zarına değil aynı zamanda kas fibrillerini kuvvetli boyadı. PNA lektini orta derecede (++) EDL kaslarını boyadı. Con A ise kontrol grubuna göre diyabetik kasları daha zayıf (+) boyadı. Elde ettiğimiz bulgulara göre alloksan-diyabet sıçanların EDL iskelet kaslarının hücre zarlarındaki glikoproteinlerin moleküler yapısını değiştirmiştir.

Sonuç: Bu çalışma diyabetin hücre zarlarına hasar verici etkisini göstermek açısından diyabetle yapılan araştırmalara katkıda bulunacaktır.

Anahtar Sözcükler: *Extensor digitorum longus iskelet kası hücre zarı, Lektin boyama, Diabetes mellitus, Alloksan, Wistar sıçan*

DOI: 10.25048/tjdo.2017.17

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Received / *Geliş tarihi* : 16.08.2017
Revision / *Revizyon tarihi* : 22.11.2017
Accepted / *Kabul tarihi* : 26.12.2017

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INTRODUCTION

As a known, EDL muscle is skeletal muscle in the vertebrate organism. EDL muscles are consist of type I (slow oxidative), type IIA (fast oxidative) and type IIB (fast glycolytic) fibers (1). Type IIB or fast-twitch glcolytic fibers undergo the severe atrophy due to the oxidative stress mediated by hyperglycaemia (2). Several authors pointed out that diabetes causes to myopathological effects on muscles of organisms (3-8). It was designed to explore dosage schedules which might improve rabbit responsiveness to and survival after alloxan treatment (9). Diabetic myopathy is occure by induction of Streptozotocin (STZ) in rats (10). According to this research, STZ causes to generate nitric oxide intracellularly, which causes alkylation and fragmentation of deoxyribonucleic acid (DNA). At the end of serial reactions, depletion of ATP inhibits insulin synthesis and secretion by β cells of pancreas (11). In vitro studies of contraction and electrical properties of the EDL muscle were analyzed at various periods following cessation of insulin treatment (12). The effects of streptozotocin (STZ) diabetes and the antihyperglycaemic agent metformin on the contractile characteristics of the limb skeletal muscles were examined in rats and concluded that diabetes-induced depolarisation in the EDL and soleus muscles (13). Histochemical staining for the oxidative marker succinic dehydrogenase (SDH) revealed marked disruption of reaction product distribution in soleus (14).

Lectins are present from prokaryotic cells to eukaryotic cells and their protein or glycoprotein structure which are binds to specific carbohydrate moieties such as monosaccharides, disaccharides, acetylated sugars (15-18). Lectins have been reported to function in the cell-cell expression and cell adhesion (15,19). They have obtained from plants especially plant seeds and conjugated to various markers (enzymes, florescent dyes, biotin) which are used for the diagnosis of diseases and animal species (20-27). The authors diagnosed disorders of human muscles by lectin histochemistry (21,22). Anadolu et al., (24) used PNA in case of squamous cell carcinoma and observed that the cancer cell membrane receptor of PNA was concentrated to one side with respect to the control cell membranes. Also, we studied on abnormalities of EDL muscle cell membrane of alloxan-diabetic rats with lectin histochemistry.

Our purpose is to determine alterations of carbohydrate residues on the cell membrane and cytoplasm of alloxan diabetic rats EDL muscle by avidin-biotin technique.

MATERIALS and METHODS

Forty Wistar albino rats were obtained from the Animal Laboratories of Ankara University. Alloxan with 55 mg/kg dissolved in sterile physiological saline was intravenously injected into 22 rats ranging in weight from 160-230 g (28). Eighteen rats ranging in weight from 160-230 g were used as a normal group. After 24 h administration of alloxan, glucose levels in urine were determined by Diastix test strips (Bayer, Germany). The alloxan-injected animals were kept under controlled conditions for 30 days. After 30 days, blood glucose concentrations were measured as >400 mg dl^{-1}, determined with Glucostix strips (Bayer, Germany) from the cut tip of the tail of the alloxan-injected rats.

After experimental days, the animals were then decapitated under ether anesthesia. Extensor muscles were dissected free, cleaned of excess fascia, blotted dry. All muscles were transected at the mid-belly region. Their proximal and distal portions were then mounted in gum tragacanth on cork, quick-frozen by immersion in isopentane cooled to about -160°C, sealed in plastic bags, and stored at -20°C. Transverse serial sections (10-12 mm thickness) were obtained with a freezing cryostat.

The lectin-peroxidase conjugates (Sigma Chemical Co, UK. STA), their hapten sugars and the appropriate concentrations, which allowed optimum staining with minimum background staining, are listed in Table1. Appropriate lectin concentrations were used in the present study (22). The lectins were applied to unfixed, frozen sections for 60 min at room temperature and the sections then washed twice with phosphate-buffered saline (PBS; pH 7.4) Binding was visualized by incubating for 45 min with avidin-biotin-peroxidase complex, washing twice with PBS, and incubating with diaminobenzidine (0.6 mg/ml with 3 ml of hydrogen peroxide) for 5 min. The sections were lightly counterstained with haematoxylin before dehydrating, clearing, and mounting under coverslips. Specificity of staining was checked by omitting the lectins from the staining schedule and by preincubation of the lectins with the appropriate inhibitory carbohydrates in 0.1 M solution (Table 1) (22).

RESULTS

In this study, we observed the effects of alloxan-diabetes on extensor muscle cells of rats by biotinylated lectins. The EDL muscle membrane receptors of control and diabetic rats were different bound used biotinlayed lectins (Table 2).

PNA weakly (+) stained or unstained for EDL muscle cell membranes of control rats whereas EDL muscle cell membranes of diabetic rats were moderate (++) stained by PNA (Figure 1, 2).

Con A was also exhibited moderate (++) staining for control muscle cell membranes of rats (Figure 3), whereas no or weak (+) stainability was detected in that of diabetic rats (Figure 4). Alloxan diabetic and control EDL muscle cell membranes were differently stained by biotinylated PNA and Con A lectins, but their cytoplasms were not stained in cryostat sections.

The control EDL muscle cell membranes were moderately (++) stained for WGA (Figure 5). Alloxan diabetic EDL muscle cell membranes were more intensely (+++) stained for WGA (Figure 6). But WGA was not stained EDL muscle cell cytoplasm of control and diabetic rats.

The best staining was detected for biotinylated-GS lectin. The control muscle cell membranes were strongly (+++) stained for GS (Figure7). Incubation with GS, which is known to bind preferentially to the α-D-Galactose residue, resulted in an intense reaction with membranes of EDL

Table 1: Used Lectins and their carbohydrate specificities.

Source of lectin	Lectins Specificity and inhibitory for sugars	Used Concentration (mg/ml) of lectins
Griffonia simplicifolia (GS)	α-D-Galactose	50
Canavalia ensiformis (Con-A)	α-D-Mannose > α-D-Glucose	2.5
Triticum vulgare (WGA)	N-Acetylglucoseamine>sialic acid	1.0
Arachis hypogaea (PNA)	b-D-Galactose-(1→3)-D-N-Acetyl-Galactoseamine	5.0

Table 2: Used Lectins and their stainning EDL muscle cells.

Lectins	Control EDL muscle cell membrane	Diabetic EDL muscle cell membrane
Griffonia simplicifolia (GS)	+++	+++
Canavalia ensiformis (Con-A)	++	+
Triticum vulgare (WGA)	++	+++
Arachis hypogaea (PNA)	+	+ +

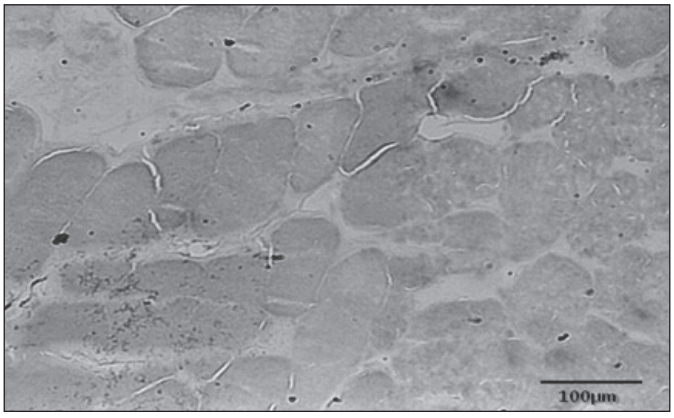

Figure 1: PNA no /weakly (+) stained control muscle cells membranes.

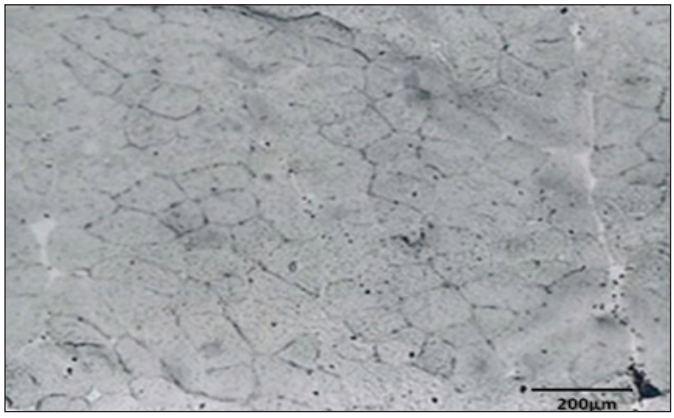

Figure 2: PNA moderate (++) stained alloxan diabetic muscle cells membranes.

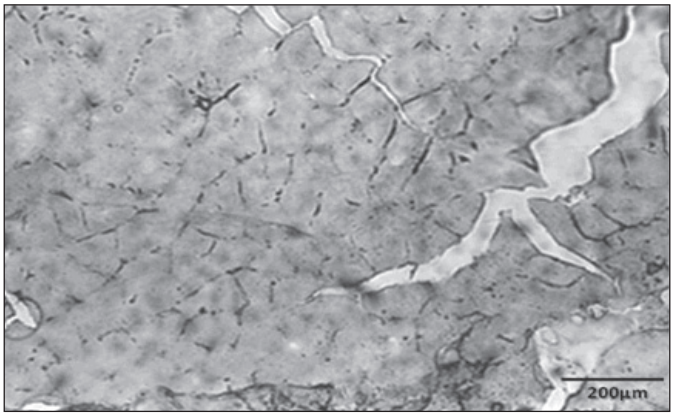

Figure 3: Con A exhibited moderate (++) staining for control muscle cells membranes.

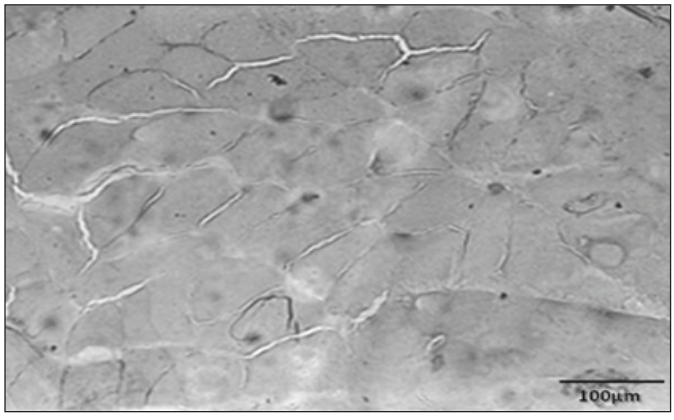

Figure 4: Con A weakly (+) stained alloxan diabetic muscle cells membranes.

muscle cells of diabetic rats; intensely (+++) staining was also observed in the sarcoplasmic myofibrills (Figure 8). This staining pattern was different from all of the used lectins in this study.

DISCUSSION

Our study is demonstrated that there is difference lectin binding to lectin receptors in Extensor digitorum longus muscles cell membranes of control and diabetic rats. There are several manuscripts with lectin binding cell membranes and cell components for example myofibrills (24, 29-31). The binding characteristic of lectins with varying sugar specificities were investigated by Dunn et al., (24). The authors showed that Con A and WGA and the β-D- galactose specific lectins were always binding to the perimeter of each muscle fiber in biopsies from dystrophic patients whereas the lectins were not bound to control muscle. In our study, Con A showed moderate staining for control muscle cell membranes but no or weak staining for diabetic muscle cell membranes. On the other hand, WGA showed moderate staining for control and alloxan-diabetic EDL muscle cell membranes. It was reported that GS lectin was bound to cytoplasm myofibrills in the fiber of human which had undergone neurogenic atrophy (22). According to our findings, GS lectin was intensely staining EDL muscle cell membranes of diabetic rats.

Fifteen lectins-horseradish peroxidase conjugates have been used in a comprehensive histochemical study of human skeletal muscle (31). According to study, Con A and WGA were similarly stained for membranes of vastuslateralis muscle cells of the healthy human. Capaldi et al., (31) reported that staining of Con A and WGA were moderately stained for control EDL muscle sarcolemma like as staining of human skeletal muscle cells. Where as in our study, Con A was moderate stained control EDL muscle cell membranes and WGA weakly stained that of control rats. Yamagami et al., (32) were studied on histochemical characteristics of individual muscle fibers of rats by lectin staining. According to their study results, Con A was strongly stained for skeletal muscle of rats. WGA was weakly stained for skeletal muscle of rats. PNA was unstained for skeletal muscle. Our findings demonstrated that Con A moderate stained, PNA and WGA lectins weakly or unstained for control EDL muscle

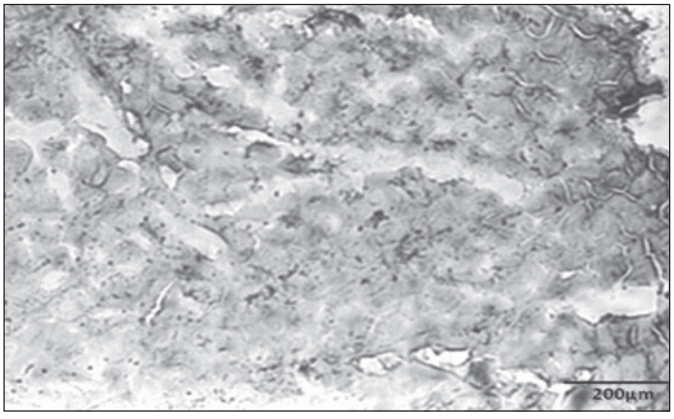

Figure 5: Control muscle cell membranes were weakly (+) stained with WGA.

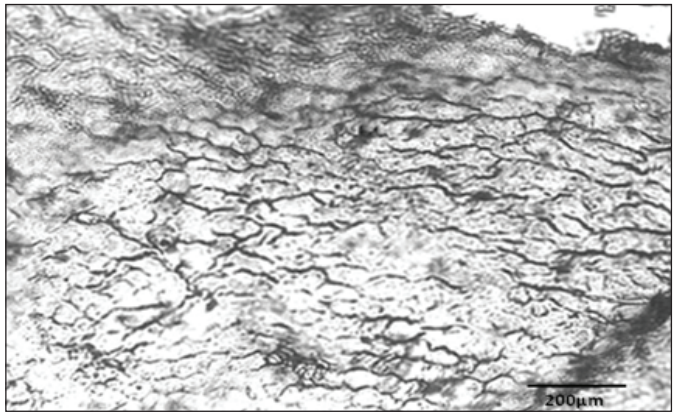

Figure 6: WGA showed intensely (+++) staining for alloxan diabetic muscle cell membranes.

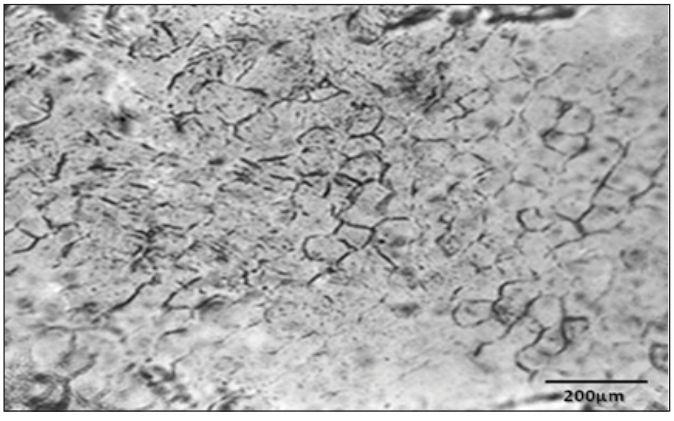

Figure 7: Control muscle cell membranes were strongly (+++) stained with GS.

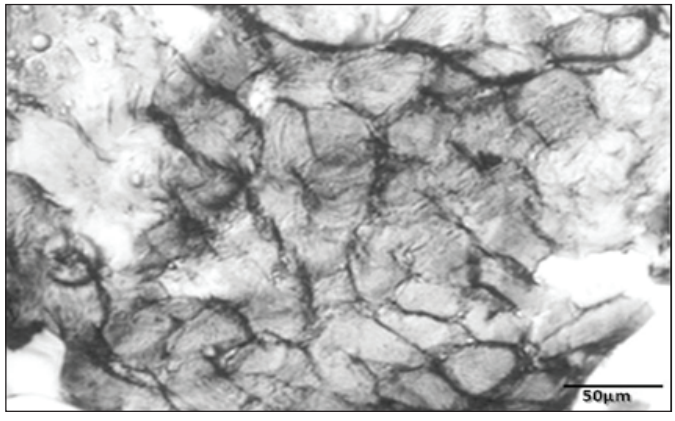

Figure 8: Not only cell membranes but also miyofibrils of diabetic muscle cells showed intensely (+++) staining for GS.

cell membranes. According to findings with PNA and WGA lectins staining are similar to our finding with PNA and WGA lectins. But Con A binding was not similarly stained according to Yamagami's et al.,(32).

We hope that presented this study will be reference concern with diabetes and lectins staining studies.

ACKNOWLEDGMENTS

This study was supported by the Directorate of Scientific Research Projects of Ankara University as a project (Number: 2000.07.05031).

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