

Full length Research Paper

Lectins binding during alloxan-induced diabetes in rat soleus muscle

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Membrane structural changes of soleus muscle of alloxan-diabetic rats were detected with a panel of six biotinylated lectins. Samples of muscles were obtained from normal and diabetic rats. The biotinylated lectins in staining were detected by avidin-peroxidase complex. Lectin staining of soleus muscle cryostat sections from alloxan-diabetic rats were compared with similar cryostat sections from normal rats. All lectins were bounded to soleus muscle cell membranes of normal and diabetic rats except the *Ulex europaeus* agglutinin I (UEA-I). UEA-I was not stained for cell membranes of soleus muscles of normal and diabetic rats. But intercellular areas of normal and diabetic soleus muscle were stained for UEA-I. *Griffonia simplicifolia* I (GS-I) lectin was bounded more strongly to cell membrane of diabetic rats than other lectins. Not only cell membranes but also cytoplasmic myofibrils of diabetic muscle cells were stained by *Griffonia simplicifolia* (GS1) lectin. It was concluded that cell membranes of soleus muscle of diabetic rats were differently stained for lectins than those of normal rats. Our data can supplement on the use of lectins as probes for changes in carbohydrate-containing constituents of the alloxan-diabetic muscle cell membrane information obtained from normal and diabetic

Key words: Diabetes, alloxan, Wistar rat, soleus muscle, lectin staining.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Several pathogenic processes are involved in the development of diabetes. Severe hyperglycemia was detected under conditions of acute infective, traumatic circulatory or other stress may be transitory and should not in itself be regarded as diagnostic of diabetes (WHO, 1999). There are several reports with effects of diabetes mellitus on muscles cells. Effects of experimental diabetes mellitus on skeletal muscle of rats were examined by several authors (Grossie, 1982; Paulus and Grossie, 1983; Cotter et al., 1989). The effect of diabetes mellitus was examined on the proliferative behavior of cultured aortic smooth muscle cell of rabbits (Alpui et al., 1993). The mechanism of diabetic macroangiopathy was studied

from the view point of phenotypic change of aortic smooth muscle cells (Kawano et al., 1993). Some authors indicated that defects in muscle glycogen synthase activity combine to reduce glycogen synthesis in non-insulin dependent diabetes mellitus (NIDDM) (Thorburn et al., 1991). Diabetes decreases $\text{Na}^+\text{-K}^+$ pump concentration in skeletal muscles, heart ventricular muscle, and peripheral nerves of rats (Kjeldsen et al., 1987). Cotter et al. (1989) examined the effect of long-term streptozotocin diabetes on the contractile and histochemical properties of rat muscle. Histochemical properties of skeletal muscle fibers in alloxan-diabetic rats were investigated in our previous study (Cebesoy et al., 2000). Transverse sections of slow-twitch soleus and fast twitch Extensor Digitorum Longus (EDL) muscle from normal and diabetic rats were histochemically assayed for reduced nicotin amid adenine dinucleotide-tetrazolium reductase (NADH-TR-TR).

Lectins are proteins or glycoproteins of nonimmune origin which bind noncovalently to specific carbohydrate groups without modifying them chemically (Sharon, 1977; Lis and Sharon 1986; Sharon and Lis, 1989; Vierbuchen,

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Table 1. Lectins used and their carbohydrate specificities.

Source of lectin	Specificity of lectins and inhibitory sugars	Concentration used ($\mu\text{g/ml}$)
<i>Canavalia ensiformis</i> (Con-A)	α -D- Mannose > α -D-Glucose	2.5
<i>Triticum vulgare</i> (WGA)	N-Acetylglucosamine>sialic acid	1.0
<i>Arachis hypogaea</i> (PNA)	β -D- Galactose-(1 \rightarrow 3)-D-N-Acetyl-Galactoseamine	5.0
<i>Ulex europaeus</i> (UEA-1)	α -L-Fucose	5.0
<i>Dolichos biflorus</i> (DBA)	α -N-Acetyl-D-Galactoseamine	50
<i>Griffonia simplicifolia</i> (GS1)	α -D-Galactose	50

1991). This binding is reversible. Lectins are valuable for identifying and mapping the sugars on the surface of cell. Several reports demonstrate that unique binding ability of lectins can be used to identify specific subpopulations of cells both in normal and diseased tissues, such as human muscle tissues (Pena et al., 1981; Helliwell et al., 1989; Capaldi et al., 1984; Dunn et al., 1982; Kirkeby et al., 1993; Zacccone et al., 1987), retinal and myocardial capillaries following acute ischaemia (Lawrenson et al., 2002).

Individual lectins can be conjugated to various markers; for instance to fluorescent dyes and enzymes, such as peroxidase (Pena et al., 1981; Helliwell et al., 1989; Capaldi et al., 1984; Dunn et al., 1982; Kirkeby et al., 1993; Zacccone et al., 1987; Lawrenson et al., 2002; Schaumburg-Lever et al., 1984). In the bridged avidin-biotin technique, avidin acts as a bridge between biotin-labelled lectin and a biotin-labeled peroxidase enzyme (Pena et al., 1981). The aim of present study is using this technique, to determine of differences between soleus muscle membrane of normal and diabetic rats.

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 (Reuterwing et al., 1987). 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.

The animals were then decapitated under ether anaesthesia. Soleus muscles were dissected free, cleaned of excess fascia, blotted dry. All muscles were transected at the midbelly 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 μm thickness) were obtained with a freezing cryostat.

The lectin-peroxidase conjugates, their hapten sugars and the appropriate concentrations, which allowed optimum staining with minimum background staining, are listed in Table 1.

The biotin-labelled lectins (Table 1) were supplied by Sigma Chemical Co, UK. STA. Appropriate lectin concentrations were used in present study (Helliwell et al., 1989). The lectins were applied to

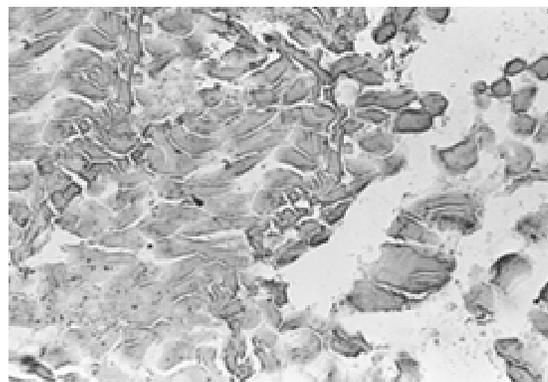


Figure 1. *Arachis hypogaea* (PNA) exhibited moderate staining for normal muscle cells membranes (X 500).

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 μl 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 (Table 1) in 0.1 M solution (Helliwell et al., 1989).

RESULTS

Effects of alloxan-diabetes on soleus muscle cells of rats were examined by biotin-laid lectins (Table 1) in this study. All lectins showed a distinctive binding pattern to the membranes of soleus muscle cells of normal and diabetic rats. *Arachis hypogaea* (PNA) and *Canavalia ensiformis* (Con-A) had similar stainability for soleus muscle cell membranes of normal and diabetic rats. PNA and Con A showed moderate staining for membranes of normal muscle cell (Figures 1 and 2) but no or weak staining for diabetic muscle cell membranes (Figures 3 and 4). In additionally, *Dolichos biflorus* (DBA) was also exhibited moderate staining for normal muscle cell membranes of rats (Figure 5), whereas no or weak stainability was detected in that of diabetic rats (Figure 6). All of the diabetic muscle cell membranes were moderately stained with PNA, Con A and DBA, but their

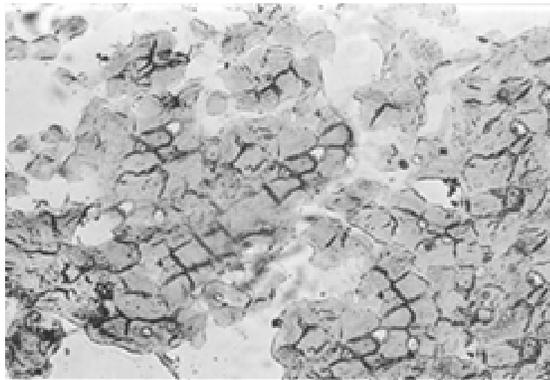


Figure 2. *Canavalia ensiformis* (Con-A) exhibited moderate staining for normal muscle cells membranes (X 435).

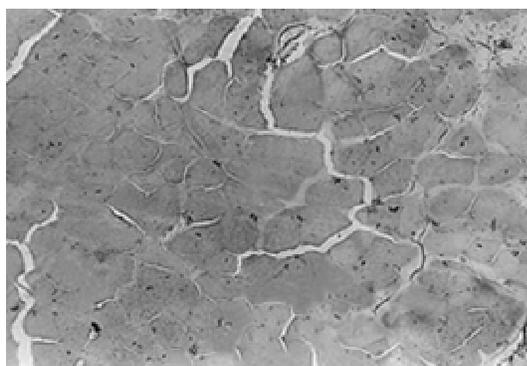


Figure 3. *Arachis hypogaea* (PNA) showed none or weak staining for diabetic muscle cells membranes (X 500).

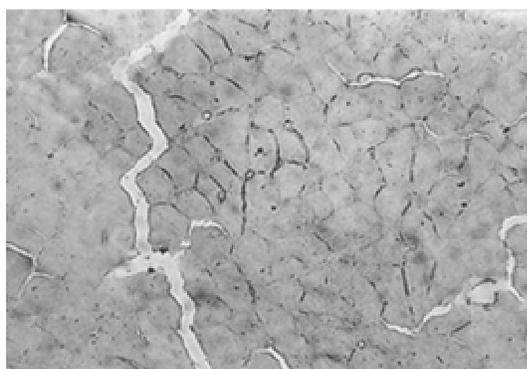


Figure 4. *Canavalia ensiformis* (Con-A) showed none or weak staining for diabetic muscle cells membranes (X 500).

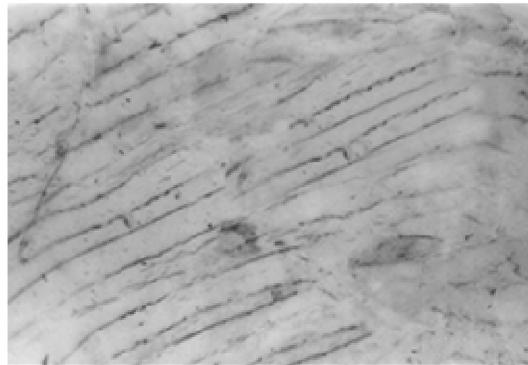


Figure 5. Muscle cell membranes of normal rats showed moderate staining for *Dolichos biflorus* (DBA) (X 500).

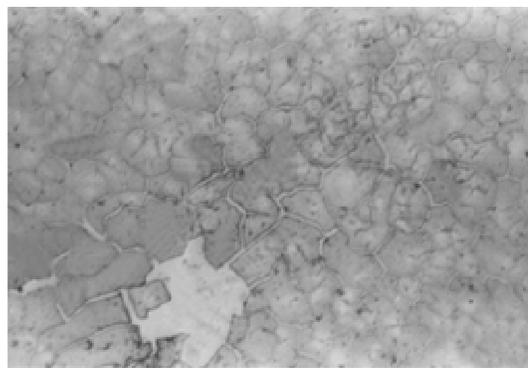


Figure 6. None or weak stainability of *Dolichos biflorus* (DBA) seen in section of the diabetic soleus muscle (X 500).

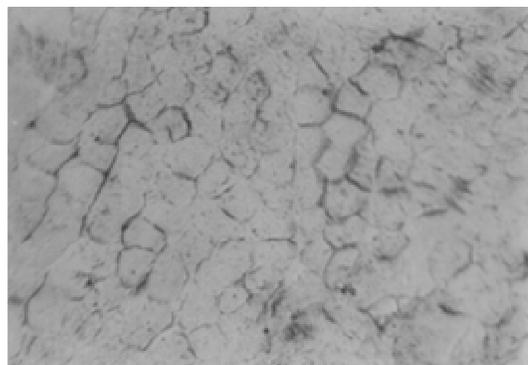


Figure 7. *Triticum vulgare* (WGA) moderate staining in normal muscle sarcolemma (X 335).

cytoplasms were weak stained in cryostat sections. The normal soleus muscle cell membranes were moderately stained for *Triticum vulgare* (WGA) (Figure 7). Alloxan diabetic soleus muscle cell membranes were more mode-

rately stained for WGA than normal muscle cell membranes (Figure 8). Both of muscle cell membranes of normal and diabetic rats were not or weakly stained for *Ulex europaeus* (UEA-1) (Figure 9, 10).

The best staining was detected for GS-I. The normal muscle cell membranes were strongly stained for GS-I

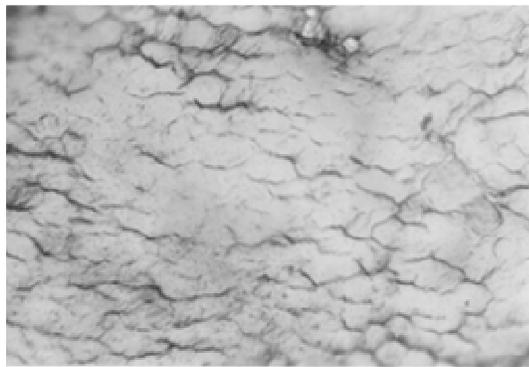


Figure 8. *Triticum vulgare* (WGA) showed moderate staining with alloxan diabetic muscle cell membranes (X 470).

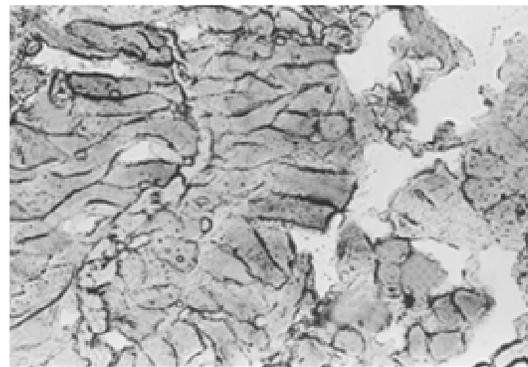


Figure 11. The normal muscle cell membranes were strongly stained with *Griffonia simplicifolia* (GS1) (X 500).

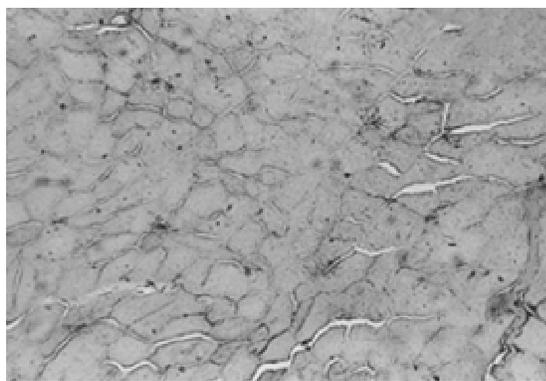


Figure 9. *Ulex europaeus* (UEA-1) was not stained for muscle cell membranes of normal rats (X 500).

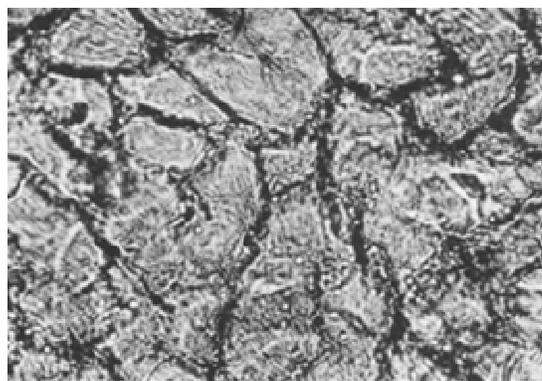


Figure 12. Not only cell membranes but also myofibrils of diabetic muscle cells showed intense staining for *Griffonia simplicifolia*(GS1) (X 920).

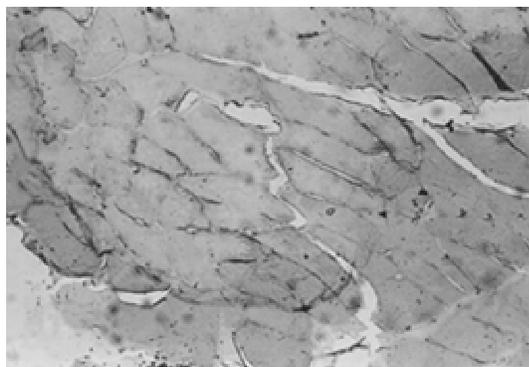


Figure 10. *Ulex europaeus* (UEA-1) was not or weakly stained for diabetic soleus muscle cell membranes (X 500).

(Figure 11). Incubation with GS-I, which is known to bind preferentially to α -D-galactose residue, resulted in an intense reaction with membranes of soleus muscle cells of diabetic rats; strong staining was also observed in the sarcoplasmic myofibrils and interfibre connective tissues

(Figure 12). This staining pattern was different from all of the used lectins in this study.

All of the lectins staining were prevented by the addition of the appropriate inhibitory carbohydrates (Table 1) in 0.1 M solution to the incubation medium.

DISCUSSION

In the present study investigation we have therefore examined lectin histochemistry the distribution of glycoconjugates in soleus muscle cells membranes of normal and diabetic rats. The soleus muscle cells of normal and diabetic rats revealed specific binding patterns, so that they could be characterized according to their particular array of glycoconjugates.

Helliwell et al. (1989) observed that in fiber of human which had undergone neurogenic atrophy, cytoplasmic lectin binding was only with GS-I lectin. GS-I lectin was strongly binding to skeletal muscle cell membrane and myofibrils of cytoplasm in our study.

The binding characteristic of lectins with varying sugar

specificities were investigated in muscle biopsies from normal individuals and from patients with neuromuscular disorders (Dunn et al., 1982). Dunn et al. (1982) showed that Con A and WGA and the β -D- galactose specific lectins were always binding to the perimeter of each muscle fibre in biopsies from dystrophic patients. These lectins were not found in normal muscle. We found different findings on the muscle fiber of diabetic rats. Con A showed moderate staining for normal muscle cell membranes but no or weak staining for diabetic muscle cell membranes. On the other hand, WGA showed moderate staining for normal and alloxan -diabetic soleus muscle cell membranes.

Binding sites for three fucose specific lectins, *Aleuria aurantia* agglutinin (AAA), *Lotus tetragonolobus* agglutinin (LTA) and *Ulex europaeus* I agglutinin (UEA I) were investigated in sections from normal human and rat muscles, in muscle from patients with Duchenne Muscular Dystrophy (DMD) and denervated and devascularized rat muscle (Kirkeby et al., 1993). According to Kirkeby et al. (1993), the normal rat muscle cell membranes were not stained for UEA-I. The same findings were determined in our study.

Capaldi et al. (1985) examined that fifteen lectin-horseradish peroxidase conjugates have been used in a comprehensive histochemical study of human skeletal muscle. Con A and WGA were similar stained for membranes of vastus lateralis muscle cells of healthy human but UEA-I was not stained for that of healthy human. UEA-I only stained blood vessels and capillaries. The findings of Capaldi et al. (1985) were similar to our findings. UEA-I was unstained for soleus muscle cell membranes of normal rats in our study. Also, staining of Con A and WGA were moderately stained for normal soleus muscle sarcolemma like as staining of human skeletal muscle cells (Capaldi et al., 1985).

Yamagami et al. (1985) studies were carried out on histochemical characteristics of individual muscle fibers of rats by lectin staining. According to their results, Con A was strongly stained for skeletal muscle of rats. WGA was weakly stained for skeletal muscle of rats. PNA, DBA and UEA-I were unstained for skeletal muscle. UEA-I was not stained for muscle cells of normal rats in our study. Also, Con A, PNA and DBA were moderately stained for muscle cells sarcolemma of normal rats.

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