ORIGINAL RESEARCH

EFFECTS OF AMINOGUANIDINE ON DERMAL COLLAGEN STRUCTURE AND TGF-BETA EXPRESSION IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of aminoguanidine treatment on dermal extracellular matrix protein expression and collagen structure in a diabetic rat model.

Methods: Twelve Wistar Albino male rats were induced diabetes by streptozosin. Six rats were given aminoguanidine in drinking water; remaining 6 rats were followed as diabetic controls. Additional six rats were followed as healthy controls. After an 8-week follow-up period skin samples obtained and evaluated for light microscopy and immunohistochemistry for collagen IV and TGF- β.

Results: Light microscopy revealed increase in basement membrane thickness, dermal edema, vascular membrane thickness, increase in endothelial proliferation and perivascular degeneration in diabetic rats. Immunohistochemical staining displayed reduction in collagen IV and TGF- β in diabetic group compared to others. All these changes were ameliorated with aminoguanidine treatment.

Conclusion: This study confirms microscopic abnormalities in clinically normal skin of diabetics that can be prevented with aminoguanidine treatment.

Keywords: Diabetes Mellitus, Skin, Aminoguanidine

DİYABETİK SIÇAN MODELİNDE AMİNOGUANİDİN TEDAVİSİNİN EKSTRASELÜLER MATRİKS PROTEİN EKSPRESYONU VE KOLLOJEN YAPISINA ETKİSİ

ÖZET

Amaç: Diyabete bağlı cilt değişiklikleri klinik olarak normal durumdayken başlamaktadır ve metabolik kontrolle doğru orantılıdır. Bu çalışmanın amacı diyabetik sıçan modelinde aminoguanidin tedavisinin ekstraselüler matriks protein ekspresyonu ve kollojen yapısına etkisini incelemektir.

Yöntem: Bu amaçla streptozosin ile diyabet oluşturulmuş 12 sıçandan 6 tanesine içme suyuna aminoguanidin eklenerek, diğerleri ise ilaçsız olarak 8 hafta boyunca izlendi. Altı sıçan da sağlıklı kontrol olarak takip edildi. Takip süresi sonunda cilt dokuları alınarak incelendi.

Bulgular: Işık mikroskopisinde diyabetik sıçanlarda bazal membranda kalınlaşma, dermal ödem, vasküler membran kalınlaşması, endotel proliferasyonu ve perivasküler dejenerasyon gözlendi. İmmünhistokimyasl boyamada kollojen IV ve TGF-β'da azalma görüldü. Tüm bu değişikliklerin aminoguanidin tedavisi ile oluşmadığı izlendi.

Sonuç: Bu çalışma diyabetin klinik olarak normal görülen ciltte mikroskopik bozukluklar oluşturduğunu doğrulamaktadır. Aminoguanidin tedavisi cilt değişikliklerinin gelişimini engellemektedir.

Anahtar Kelimeler: Diabetes Mellitus, Cilt, Aminoguanidin

INTRODUCTION

Studies have demonstrated microscopic abnormalities in the clinically normal skin of diabetic patients [1]. Histological changes in diabetic skin are attributed to metabolic changes in diabetes. Advanced glycosylation of long lived structural proteins of skin found to be associated with structural and functional changes in diabetes [2]. Advanced glycation end products (AGEs) have been reported to accumulate in the dermal skin and modulated cell function of dermal

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fibroblasts [3]. Collagen, which is the most abundant protein of skin, was shown affected from non-enzymatic glycosylation process. Glycosylated skin collagen was found to correlate with duration of diabetes and to various degrees with the severity of retinopathy and nephropathy in patients with type 1 diabetes [2, 4].

Other extracellular matrix components are involved in the pathogenesis of skin changes in diabetes. Decreased glycosaminoglycan content [5,] ratio of fibronectin to collagen produced by skin fibroblasts were reported [6]. Some of the growth factors are known to involve in the wound healing process in diabetes.

Inhibition of advanced glycation end products with aminoguanidine was shown to ameliorate the dermal lesions in diabetic rats [7].

The aim of this study was to evaluate the effects of aminoguanidine treatment on dermal extracellular matrix protein expression and collagen structure in a diabetic rat model.

METHODS

All animal procedures were in accordance with the declaration of Helsinki and the guide for the care and use of laboratory animals. Study protocol was approved by the local ethic committee of Marmara University.

Twelve, 10 weeks old Wistar Albino male rats (180-200 g) were induced diabetes by streptozotocin (STZ, Sigma Mo, USA) injection (65 mg/kg, ip). One week after STZ injection diabetes was confirmed demonstrating that the overnight fasting blood glucose was more than 250 mg/dL. Six rats were given aminoguanidine bicarbonate (AG, Sigma, 1g/L/d), (group DAG) in drinking water add libitum, remaining 6 rats were followed as diabetic controls (group D) and only given tap water. Six rats were included as healthy controls (group H). During the experiments, animals were housed one per cage, maintained under controlled environmental conditions (12-h light/dark cycle, temperature ~21ºC), and provided with standard rat chow and water add libitum. All groups were followed 8 weeks.

At the end of the 8 week period skin samples from middle third of the back were evaluated for following parameters.

Histological evaluation

The samples were fixed in 10% buffered formalin for light microscopic examination. After fixation they embedded in paraffin. Sections 5-mm thick of paraffin embedded tissues were mounted on glass slides and stained with hematoxylin and eosin and alcian blue in critical electrolyte to determine glycosaminoglycan deposition throughout the skin[8].

Using masked slides under the microscope at X20 to X400 magnifications epidermal, basement membrane thickness, dermal edema, small vascular structure, PAS accumulation, endothelial proliferation and perivascular infiltration were evaluated by two blinded investigators and assigned a histological score ranging from 0 to 3.

Immunhistochemistry

Briefly, sections (5mm) of the formalin fixed and paraffin embedded wound samples were placed onto 3-aminopropyltriethoxysilane coated slides (Sigma, St Louis, MO), deparaffinised and rehydrated. Endogenous peroxidase activity was blocked by further pretreatment with 1% H_2O_2/ methanol for 20 minutes at room temperature. The sections were incubated with pepsin (1 mg/mL in 0.01 N HCl) for 60 minutes at 37ºC and subsequently rinsed 2-3 changes of distillated water and tris buffer saline pH 7.0 for 10 minutes. The sections were incubated with polyclonal goat anti-bovine antibody / anti-mouse collagen IV and TGF-b anti-mouse primary antibodies obtained from Novo Castra Laboratories U.K.

Peroxidase conjugates were subsequently localized using diaminobenzidine tetrahydrochloride (DAB) as a chromogen (Zymed Lab Inc. San Francisco, CA).

A semi quantitative scale adapted from O'Brien et al.[9] was used for immunohistochemical evaluation by two pathologists in blind way.

Stained sections was graded as 0 = absent staining, 1+ = weak staining (<20% tissue stained), 2+ = moderate staining (20-50% tissue stained) or 3+ = strong staining (>50 tissue stained).

Assays

Serum glucose was measures with glucose oxidase method.

RESULTS

Serum glucose measurements were 88 ± 9, 291± 91, 281 ± 153 mg /dl for group H, D and DAG, respectively.

Light microscopic evaluation of skin samples are shown in Figure 1.

Pathologic changes were observed in diabetic control group. Increased basement membrane

thickness, dermal edema, vascular membrane thickness, accumulation of PAS positive material, increased endothelial proliferation, perivascular degeneration were prominent changes in diabetic controls compared with other groups (Fig. 1A). AG treated diabetic rats have less edema and perivascular degeneration than diabetic controls (Fig. 1B). No pathologic changes were observed in the skins of healthy rats (Fig. 1C).

Prominent changes were observed in collagen fibers in diabetic rats. Collagen fibers were partially atrophic, swollen and fused to make dense collagen bundles sometimes associated with neutrophilic infiltration. Almost all of these findings are ameliorated in aminoguanidine treated diabetic rats. No significant change was observed in healthy rat skin.

Immunohistichemical staining of skin, collagen IV was reduced in diabetic controls (Fig. 2A) compared with group H (Fig. 2B) and DAG (Fig. 2C).

TGF – β expression was reduced in diabetic rat skins (Fig. 3A). AG treated diabetic rats (Fig. 3C) have a similar pattern with healthy controls (Fig. 3B)

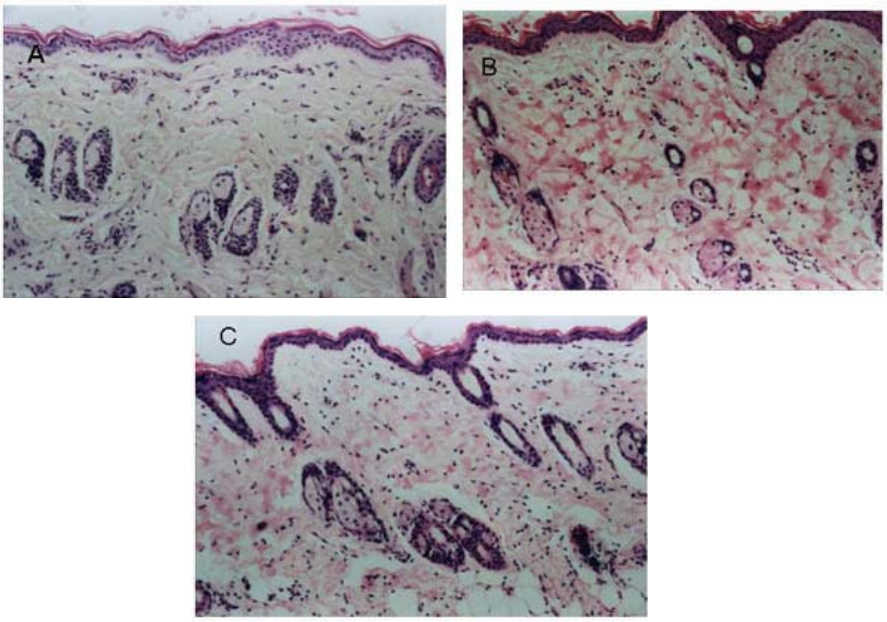

Fig. 1: Light microscopic appearance of intact skin of healthy (A), diabetic control (B) and aminoguanidine treated diabetic rats (C) H-Ex200

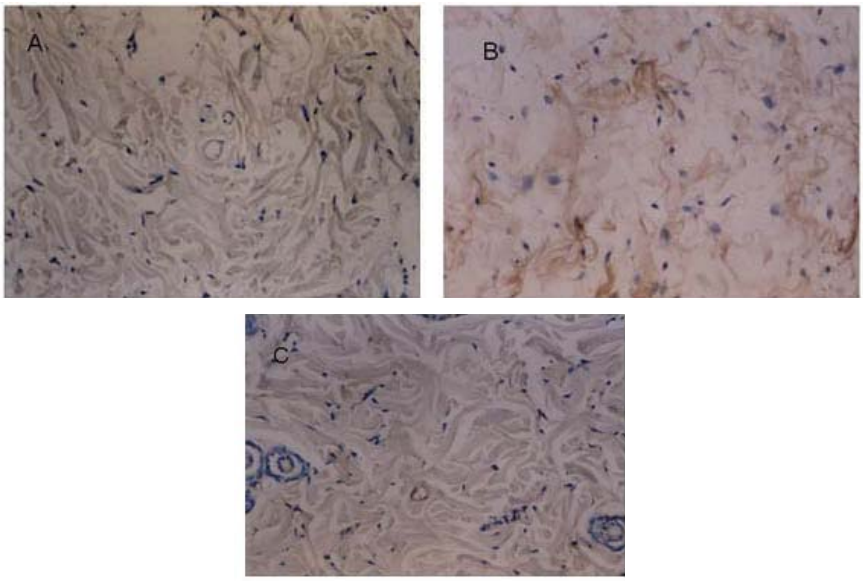

Fig. 2: Collagen IV staining of intact skins of healthy (A), diabetic control (B) and aminoguanidine treated diabetic rats (C) H-Ex 200

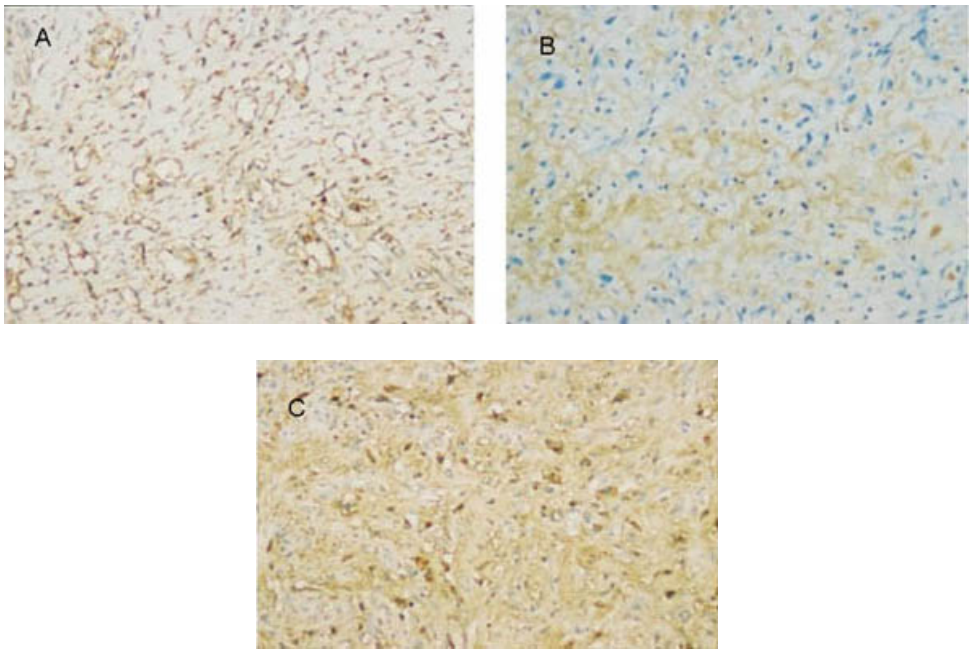

Fig. 3: TGF-beta1 staining of intact skins of healthy (A), diabetic control (B) and aminoguanidine treated diabetic rats (C) H-Ex 200

DISCUSSION

This study confirms the previous studies that observed remarkable histological changes in intact diabetic skin.

In clinical studies scleroderma like changes observed in clinically normal skin of type 1 diabetic patient [10]. Dermal histopathology in diabetic rats resembles with clinical observations. Thickened dermal blood vessel walls, multilaminated basement membrane, degenerative collagen fibers reported in diabetic rat models [1,6].

Collagen, which is the major fibrous element of the skin, was edematous, irregular and fused as bundles in non treated diabetic rats. All those changes ameliorated with aminoguanidine treatment. Prominent finding was the reduced, atrophic, and edematous collagen fibers. In AG treated rats collagen fibers were more regular, less edematous and atrophic and increased compared to diabetic controls. Total mass, synthesis of skin collagen found to diminish in diabetic rats and attributed to the delayed and depressed wound healing [6,11]. These results suggest that the histological changes in diabetic skin were caused by metabolic aberrations such as non-enzymatic glycosylation, peculiar to diabetes.

Collagen IV an extracellular matrix protein, was reduced in non-treated diabetic rats and increased with aminoguanidine treatment.

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