

Protective Effect of Vitamin B5 (Dexpanthenol) on Nephropathy in Streptozotocin Diabetic Rats

Sıçanlarda B5 Vitamininin (Dekspantenol) Nefropatiye Etkisi

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Abstract

Objective: This study aimed to evaluate the effects of vitamin B5 [dexpanthenol, DEX] treatment on the kidney in a streptozotocin (STZ) diabetes animal model.

Materials and Methods: Twenty-four Wistar rats were randomised in one of the four groups: Group 1 served as control, and group 2 was administered high-dose DEX (300 mg/kg/day) as a safety group. Diabetes was induced by intraperitoneal injection of a single dose of STZ (50 mg/kg) in groups 3 and 4. In group 4, DEX was administered as well. All groups were followed up for 6 weeks. Histopathological analyses were performed on renal tissue using periodic acid-Schiff and Hematoxylin-Eosin stains.

Results: Microscopic evaluation of the kidney revealed that the rats demonstrated kidney damage 6 weeks after STZ administration. However, high-dose and long-term DEX administration alone did not damage renal tissue; moreover, kidney damage was prevented if DEX was administered in the early phase of diabetes.

Conclusion: DEX could be a safe and cost-effective vitamin for the prevention of diabetes complications.

Öz

Amaç: Çalışmamızda streptozotosin (STZ) diyabet hayvan modelinde B5 [dexpanthenol (DEX)] vitamini ile tedavinin böbrek üzerindeki etkilerini değerlendirdik.

Gereç ve Yöntemler: Yirmi dört wistar sıçanı dört gruba eşit ve randomize olarak dağıtıldı. Grup 1 kontrol grubu; grup 2 güvenlik grubu olarak yüksek doz DEX (300 mg/kg/gün) verildi. Diyabet, grup 3 ve 4'te tek bir intraperitoneal doz STZ (50 mg/kg) enjeksiyonu ile oluşturuldu. Grup 4 DEX ile tedavi edildi. Tüm gruplar 6 hafta takip edildi. Periyodik asit Schiff ve Hematoksilen-Eosin kullanılarak böbrek dokusu üzerinde histopatolojik incelemeler yapıldı.

Bulgular: Böbreğin mikroskopik değerlendirmesinde, sıçanlarda STZ müdahalesinden 6 hafta sonra böbrek hasarı geliştiği görüldü. Tek başına yüksek ve uzun süreli DEX uygulaması böbrek dokusu için zararlı değildi. Ayrıca diyabetin erken evresinde DEX verilmesi böbrek hasarını önledi.

Sonuç: Diyabet komplikasyonlarını önlemek için DEX güvenli ve uygun maliyetli bir vitamin olabilir.

Introduction

Diabetic nephropathy (DN) is seen in approximately 40% of diabetic patients as being one of the major long-term microvascular complications (1). And characterized with high blood pressure, albuminuria and progressive renal damage that leads to end stage renal failure (2). Metabolic and hemodynamic factors were thought to be the main mechanisms of the diabetes and DN; however, it has been shown that chronic inflammation caused by immunologic and inflammatory mechanisms may have a significant role in the development and progression of the disease (3). Worldwide the number of diabetes patients and patients with DN, are highly increasing and more and more patients will unfortunately be experiencing end-stage renal disease, therefore, it is highly important to investigate and prevent progressive renal disease with the effective treatments (1).

Streptozotocin (STZ) is the main chemical agent used for the induction of diabetic metabolic state in experimental animals (4,5).

Most of the protective effects of pantothenic acid and its reduced derivative, dexpanthenol (DEX) (pantothenol, panthenol) are attributed to its modulator activity on antioxidant capacity (6-8). Decreased apoptosis rate (9), increased coenzyme A (10) and adenosine triphosphate (ATP) synthesis (11) have been reported as well. Bayrak et al. (12) have reported DEX might be a new therapeutic approach by reducing the inflammation and cell infiltration in interstitial cystitis. Meanwhile, increased rate of urinary excretion of pantothenic acid has been reported in both animal model and patients with diabetes in 1967 (4). Since then, the studies conducted regard to the matter of vitamin B5's effect on DN are scarcely close to none. In the present study, we investigated the effect of DEX supplementation on the reversal of STZ-induced DN in rats.

Materials and Methods

Experimental Procedure

Twenty-four male Wistar rats, each weighed 220-240 g, were provided by Aydın Adnan Menderes University, Experimental Animal Center and all experiments were designed and done being in line with the principles and guidelines of Aydın Adnan

Menderes University Animal Ethics Committee's approval (decision no: HEK/2009/64, date: 02.09.2009). The animals were randomly divided in to four groups: The rats in the control group served as healthy animals and the DEX group has taken only intraperitoneal 300 mg/kg DEX once a day for six weeks. The other two groups have injected a single intraperitoneal dose of STZ (50 mg/kg in 1 mL of saline). STZ applied rats with over than 200 mg/dL blood sugar levels (IME-DC® Glucosticks) 72 h after the injection were considered diabetic. STZ+DEX group was treated daily DEX (300 mg/kg) for six weeks as well. DEX doses of animals were adjusted every monday according to their weight changing during 6 weeks. Under the anaesthesia of ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively), kidney tissues were obtained and fixed in 10% neutral buffered formalin solution.

Histological Assessment of Renal Tissue

Kidney tissues embeded into paraffin and afterwards stained with hematoxylin and eosin and periodic acid Schiff (PAS). A bright-field microscope was used to examine these preparations and then photographs were taken (Carl Zeiss Axio Lab A1, Germany). Statistical analysis was not used in the study.

Results

We explained our results by dividing them into two groups histopathologically and histochemically

Histopathological Findings

In groups control and DEX-treated rats (Figure 1A-B), normal renal tubular architecture and normal glomeruli were observed. In the STZ-treated rats (Figure 1C), it was observed that Bowman spaces of a number of glomeruli were narrowed, mesangial matrix was enlarged and tubular dilatations were seen. Additionally; falling of the epithelial cells to the lumens of tubules and Armani-Ebstein lesions were determined. When compared, the renal structural alterations in STZ+DEX-treated (Figure 1D) rats, were less obvious than the untreated diabetic rats.

Histochemical Findings

When compared with control and DEX animals, in the renal histology in untreated diabetic rats, accelerated mesangial expansion, tubular dilatation,

thickening of capsular basement membranes (CBMs), glomerular basement membranes (GBMs), and tubular basement membranes (TBMs) which are characterized by an increase in PAS-positive area, were observed (Figure 2A-B). When compared with untreated diabetic group, it is seen that the treatment of DEX, reduced the glomerular size; thickening of CBMs, GBMs, and

TBMs; increased amounts of mesangial matrix; and tubular dilatation (Figure 2C-2D).

Discussion

In the present study, we considered the possibility that supplementation of DEX, a source of pantothenic acid, might prevent the kidney injury observed in STZ rats. Concerning this hypothesis, we have preferred to use pantothenic acid's high amount of dose to see its safety on the kidney. Histopathologic examination revealed that even if it is taken in long term and high dose, it does not show any adverse effect on the renal tissue. It has been investigated cardiovascular and liver effect of DEX, clinical and biochemical findings have been previously given (13,14). Here, we particularly focused on histopathological evaluation on kidney for avoiding repeating of the data.

STZ is well accepted model of animal diabetes and the toxin can damage the kidney due to inhibition of glucose transporter expression (5). This study has been shown that 6-week was sufficient period to develop a remarkable injury following to the induction of STZ. Introducing treatment of DEX in the early phase of STZ diabetes was highly prevented the progression of DN. These results are in agreement with the previous literatures. One of earlier study about the relationship between diabetic kidney and pantothenic acid demonstrated the increased urinary loss of pantothenic acid and claimed that the body cannot use pantothenic acid for forming ATP; additional to vitamin B5 loss, some expelling mechanism might exist in the diabetic condition such as impaired renal tubular reabsorption of the vitamin or impaired conversion of pantothenic acid into its active form (4). The thickening of the basement membrane, hypertrophy of glomerular structures, and accumulation of extracellular matrix components are the major findings in DN (3). Replacement of depleted vitamin B5 stores lead to an improvement in histological findings such as tubular dilatation, thickening of glomerular and tubular basement membranes probably reducing oxidative stress. DEX protective effect has been reported in different animal model and on variety of tissues including kidney (15), liver (8) and testicular ischemia (7) and these studies explained and supported by improvement of biochemical parameters such as glutathione and malondialdehyde levels. Restoration

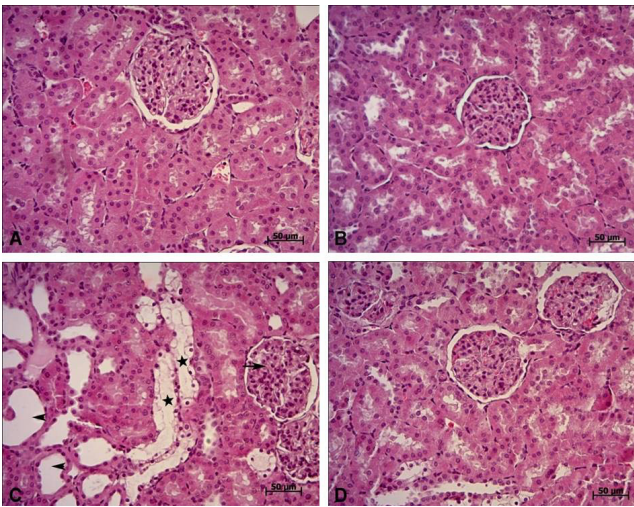


Figure 1. Representative micrographs of rat kidney sections stained with Hematoxylin-eosin. (A-B) Normal kidney morphology of control and DEX treated rats. (C) streptozotocin-treated rats, showing mesangial matrix enlargement (arrow), tubular dilatations (arrowheads) and Armani-Ebstein lesions (asterisks). (D) DEX-treated diabetic rats, close to normal architecture and low tubular damage. Scale bar, 50 µm

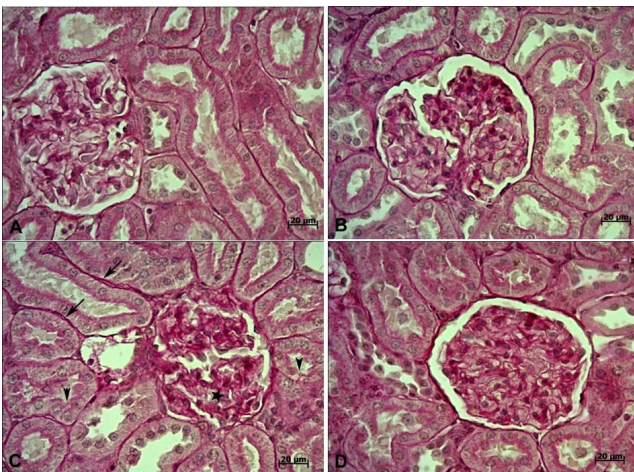


Figure 2. Pediatric acid Schiff (PAS) staining of kidney sections of control (A), dexpanthenol (DEX) (B), streptozotocin (STZ) (C), STZ+DEX (D) rats. In the STZ-treated group (C), increase in mesangial matrix (asterisk), thickening basal membranes (arrows), shorting and ruptures at the brush border (arrowheads) were seen. Scale bar, 20 µm.

of antioxidant defence system may help to improve inflammatory status, hence protects the kidney. Tutun et al. (16) publication also supports our findings that DEX protects the kidney from diabetes by increasing antioxidant activity.

It has been reported that increased infiltration of monocytes and macrophages and activated intrarenal lymphocytes in interstitium are the underlying immunopathological mechanisms of DN (2,3). DEX treatment has been found effective to reduce caspases level to prevent apoptosis and significantly decreased tubular necrosis (15) in renal ischemia-reperfusion injury. Additionally, has demonstrated intravesical DEX application reduce the inflammatory leukocytes and mast cells infiltration in animal model of chemical cystitis (12). Immunohistochemistry staining is required for further research to understand treatment effects of DEX especially on cytokine pathway.

Conclusion

The STZ diabetes model produce kidney damage within 6 weeks and DEX treatment protects the tissue from damage, at some point by regulating the inflammatory responses and oxidant status. DEX is well-tolerated and highly safe adjuvant therapy for preventing diabetes induced complications in kidney.

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Ethics

Ethics Committee Approval: Aydın Adnan Menderes University Animal Ethics Committee's approval for the study (decision no: HEK/2009/64, date: 02.09.2009).

Informed Consent: The study is an animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.G.C., B.D., Design: N.G.Ç., B.D., Supervision: N.G.C., B.D., Fundings: N.G.Ç., K.G., B.D., Materials: M.A., M.Ak., Data Collection or Processing: N.G.C., K.G., M.A., M.Ak., Analysis or Interpretation: N.G.C., M.A., M.Ak., B.D., Literature Search: N.G.C., K.G., M.A., M.Ak., B.D., Critical Review: N.G.Ç., B.D., Writing: N.G.C., K.G., M.A., M.Ak., B.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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