Reduction of podocytes number in late diabetic alloxan nephropathy. 
Prevention by glycemic control

Redução do número de podócitos na fase tardia da nefropatia diabética aloxânica. 
Prevenção pelo controle glicêmico

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ABSTRACT

Purpose: To determine podocyte number and GBM thickness in diabetic rats either under glycemic control or without glycemic control at 6 and 12 months after diabetes induction. Methods: 100 wistar rats weighing 200-300g were divided into 6 groups: Normal group (N6 and N12- 25 rats); Diabetic group (D6 and D12- 25 rats), diabetic treated group ( DT 6 and DT 12- 25 rats) on insulin 1,8- 3,0 IU/Kg associated with acarbose (50mg to 100g of food) daily mixed in chow. Alloxan was injected intravenously in a dose of 42 mg/Kg of weight. Body weight, waterintake, 24-h diuresis,  glycemia and glucosuria were determined before induction, 7 and 14 days after induction and monthly thereafter. Treatment started at day 14. Three groups were sacrificed at 6 months (N6,D6, DT6) and 3 groups at 12 months (N12, D12, DT12) with the renal tissue being prepared for electron microscopy. Results: Glycemia in DT6¨and in DT12 was significantly different from that in D6 and D12 rats and similar to that in N6 and N12 animals. The number of podocytes in DT6 was not different from that in N6 and D6 (median = 11); the number of podocytes in DT12 (median = 11) differed from that in D12 (median = 8), but not from that in N12 (median = 11). GBM thickness in D6 (0.18 micrometers) was lower than in D12 (0.29 micrometers); while in DT6 (0.16 micrometers) it was lower than in D6 (0.18 micrometers). In DT12 (0.26 micrometers), it was lower than in D12 (0.29 micrometers). Conclusion: The control of hyperglycemia prevented GBM thickening in early and late (12 mo) alloxan diabetic nephropathy and podocyte number reduction. 

Key words: Diabetes Mellitus, Experimental. Microscopy, Electron. Podocytes. Rats.

RESUMO

Objetivo: Avaliar o número de podócitos e espessamento da membrana basal glomerular (MBG) em ratos diabéticos com e sem controle glicêmico com 6 e 12 meses da indução. Métodos: 100 ratos Wistar com 200-300g compuseram 6 grupos: Normal (N6, N12 – 25 animais) Diabético (D6,D12 – 25 animais) e diabético tratado com insulina 1,8 a 3,0 U/Kg e acarbose misturada a ração (50g para cada 100g de ração) (DT6 e DT12 – 25 animais). Aloxa na foi ministrada via endovenosa na dose de 42mg/Kg. Peso, ingestão hídrica e diurese de 24 horas e glicemia e glicosúria foram determinados antes da inoculação, 7 e 14 dias após e mensalmente. No 14º dia foi iniciado o tratamento. Três grupos de animais (N6, D6 e DT6) foram sacrificados no 6º mês e três grupos (N12, D12 e DT12), no 12º mês sendo o tecido renal processado para estudo à microscopia eletrônica. Resultados: A glicemia dos animais DT6 e DT12 diferiram significativamente, dos ratos D6 e D12, e não diferiram dos grupos N6 e N12. O número de podócitos do grupo DT6 não diferiu de N6 e D6 (median=11); o número de podócitos de DT12 (median=11) diferiu de D12 (median=8) e não diferiu de N12 (median=11). O espessamento da MBG de D6 (0,18 micrômetros) foi menor que D12 (0,29 micrômetros); de DT6 (0,16 micrômetros) foi menor que D6 (0,18 micrômetros) e de DT12 (0,26 micrômetros) foi menor que D12 (0,29 micrômetros). Conclusão: O controle da hiperglycemia preveniu o espessamento da MBG na nefropatia diabética aloxânica precoce (6 meses) e tardia (12 meses), e a diminuição do número de podócitos. 

Introduction

The importance of podocyte lesions in diabetic nephropathy has been established in patients with Type 2 diabetes. Podocyte loss in early progressive glomerulosclerosis has been described in North-American PIMA Indians, and in patients with Type 1 diabetes. Reduced podocyte number has been seen after 6 months in rats with streptozotocin (STZ)-induced diabetes, compared with normal rats. In alloxan diabetic rats an evident podocyte reduction has been observed concomitantly with an increased excretion of protein in urine 12 months after induction. There is an inverse relationship between glucose homeostasis and renal damage. Maintaining glycemia levels close to normal is seldom achieved by conventional treatment. One of the reasons is that carbohydrate absorption in the intestinal tract does not occur at the same rate and speed as insulin absorption from the injection site. Acarbose inhibits glucosidase, an enzyme that hydrolyzes carbohydrates in the intestine, and decreases postprandial glycemia in diabetic patients treated with insulin. Diabetic animals treated with acarbose combined with insulin have been reported to develop lower glomerular basement membrane (GBM) thickening that was clearly identifiable after six months of diabetes.

The increasing interest in the investigation of podocytes in diabetic nephropathy motivated this experimental study. Our objective was to evaluate whether the early treatment of alloxan diabetes with insulin combined with acarbose for 6 and 12 months results in the preservation of podocyte number and GBM thickening.

Methods

One hundred male and female Wistar rats, approximately three months old and weighing 200-300g, were divided into six experimental groups: Normal Group (N6 and N12), consisting of 25 healthy animals; Diabetic Group (D6 and D12), including 25 rats with alloxa-induced diabetes (42 mg/Kg); and Diabetic Treated Group (DT6 and DT12), comprising 25 rats with alloxa-induced diabetes treated with slow insulin (Monotard R, Novo Nordisk Farmacêutica do Brasil, Ltda, São Paulo, SP) associated with Acarbose (Bayer do Brasil, São Paulo). Insulin was administered at an initial dose of 1.8 to 30 IU/Kg which was adjusted according to daily glycemia and ketonuria. Acarbose (50mg/100 g of food) was mixed in chow and offered to the animals on a daily basis. All measurements were taken until a minimum of 60 slits per animal was obtained at 42,000 X final magnification. Podocyte slits with detectable linear images of the slit diaphragm were photographed and counted. The pores where the sectioning angle made the evaluation of the slit diaphragm impossible were ignored. A total of 115 photomicrographs of the normal group (N6 and N12), 138 of the diabetic group (D6 and D12) and 140 of the diabetic treated group (DT6 and DT12) were taken. Subsequently, the computer image analyzer system “Qwin Lite 2.5-Leica” was used to determine podocyte number, slit diaphragm number and GBM thickness. All measurements were performed by the same technician, who was unaware of the experimental group to which the material belonged. Data were analysed by Mann-Whitney test. The software sigma-stat 2.0 was used in the comparison of data.

Results

Blood glucose levels are shown in Figure 1. No difference between Normal and Diabetic Treated animals was observed (p< 0.001). The number of podocytes (Figure 2) in Normal animals did not differ from that in Diabetic and Diabetic Treated animals at 6 months. At 12 months, however, podocyte number in the Diabetic treated group differed from that in the Diabetic Group, but showed no difference when compared with the Normal group (p< 0.001). Data regarding GBM thickening are presented in Figure 3. In Diabetic animals, GBM thickness showed a statistical difference, i.e., it was lower at 6 months than at 12 months. Nonetheless, in the Diabetic Treated Group, GBM thickness was lower than in the Diabetic Group, both at 6 months and at 12 months (p< 0.001). GBM thickness and podocyte aspect 12 months after diabetes induction in normal, Diabetic and Diabetic Treated rats were compared (Figures 4, 5 and 6). In Diabetic Treated animals, GBM thickness was lower than in diabetic rats and podocyte number was similar to that in normal animals.
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FIGURE 1 - Glycemia (mg/dL) in normal, diabetic and treated animals 6 and 12 months after diabetes induction. Statistics: Letters or numbers over each box represent a statistically significant difference (p < 0.001).

FIGURE 2 - Box plot of podocyte number per 2.5 micrometers of GBM in normal, diabetic and treated animals 6 and 12 months after diabetes induction. Each box represents 25-75% values with median as an internal line, error bar represents 10% and 90%, and circles represent outliers (5% and 95%). Statistics: Letters or numbers over each box represent a statistically significant difference (p < 0.001).

FIGURE 3 - Box plot of GBM thickness (micrometers) in normal, diabetic and treated animals 6 and 12 months after diabetes induction. Each box represents 25-75% values with median as an internal line, error bar represents 10% and 90%, and circles represent outliers (5% and 95%). Statistics: Letters or numbers over each box represent a statistically significant difference (p < 0.001).

FIGURE 4 - GBM of normal thickness and normal podocyte aspect (P) in N12 rats. Magnification - 42,000 X.
Discussion

Podocytes or visceral epithelial cells are highly differentiated glomerular cells which cover GBM. They are unable to replicate, form the filtration slit structure that prevents protein to escape into urine\(^{14,15}\). Considerable evidence in experimental animals indicates that podocyte disruption contributes to the development of glomerular sclerosis\(^{16,17,18}\). An increase in glomerular pressure causes mechanical stress in the glomerular cells podocytes among them. Cyclic mechanical stretch cultures induce hypertrophy in cultured mouse podocytes\(^{19}\). In diabetic nephropathy, due to glomerular hypertrophy and hypertension, podocytes are damaged and lost in urine\(^{20,21}\) producing bare GBM areas with consequent siliccia formation between GBM and Bowman’s capsule and the development of glomerular sclerosis\(^ {22}\). Podocyte number reduction in STZ diabetic rats after 24 weeks as demonstrated by counting the number of slit pores per unit length of the glomerular basement by electron microscopy\(^4\) or by using immunohistochemical techniques\(^5\) has been recently described. In these studies, the experimental groups were treated with agents that decrease glomerular hypertension, and glycemic levels were maintained. Thus podocyte number reduction was attenuated\(^ {4,12}\). The role of glycemic control in renal lesions was investigated herein. In a previous work, we used electron microscopy to study alloxan diabetic rats for 48 weeks, and demonstrated the presence of significant proteinuria, reduction in podocyte number, and increased GMB thickness\(^6\). In this study, glycemic control attenuated glomerular changes; GBM thickness was not so intense 6 and 12 months after diabetes induction and the podocyte number at 12 months was similar to that in normal animals when continuously using insulin combined with an oral antihyperglycemic. These results were not observed in our previous study using insulin alone for 12 months\(^ {23}\). With regard to GBM, it was thicker at 12 months than at 6 months in the three groups. Glycemic control clearly attenuated this type of lesion. These findings are similar to others reported in the literature\(^ {7,13}\). Glycemia differed between treated diabetic rats and diabetic rats. However, the number of podocytes at 6 months did not differ among the three groups. Immuno histochemical techniques would be worthwhile in confirming these results\(^ {5,24,25}\). Glycemic control probably prevented glomerular hypertension and podocyte injury preserving podocyte number. In diabetes, GBM synthesis is rapid and degradation is low. Many mediators have already been identified, among them is growth factor beta that stimulates the glomerular collagen matrix, including GBM, and inhibits enzymes (proteases) that degrade collagen\(^ {28,29,30}\). It is possible that glycemic control avoided these mechanisms. Electron microscopy 6 and 12 months after diabetes induction provides consistent results that reflect the preservation of podocyte number and the attenuation of GBM thickening under relatively tight glycemic control.

Conclusion

That glycemic control prevented podocyte damage and the GBM thickening, two basic elements of experimental diabetic nephropathy. It is important to emphasize that no study describing podocyte changes in rats under glycemic control for this long is currently available in the literature.
References


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