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The effect of *myo*-inositol treatment on basement membrane thickening in the BB/W-rat retina

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Summary

A polyol-pathway related perturbation of *myo*-inositol metabolism has been invoked in the pathogenesis of diabetic complications, including retinal microvasculopathy. Previous studies have demonstrated a beneficiary effect of aldose reductase inhibition on basement membrane thickening of retinal microvessels in diabetic animals. In the present study we demonstrate a significant but partial effect on basement membrane thickening following *myo*-inositol supplementation. Qualitative structural changes, such as nodular swellings, fibrillar changes and basement membrane projections were not effected by *myo*-inositol supplementation, suggesting that although abnormal *myo*-inositol tissue levels may play a role in basement membrane thickening, other factors may be of primary pathogenetic importance.

Key words: Retinal microangiopathy; Basement membrane thickening; *Myo*-inositol; BB/W-rat

Introduction

Metabolic derangements secondary to hyperglycemia, such as activation of the polyol-pathway and the associated decrease in tissue *myo*-inositol (MI) levels have been suggested as major pathogenetic factors in the development of secondary complications in diabetes [1,2]. The key enzyme in the polyol-pathway, aldose reductase, is present in retinal endothelial cells and pericytes [3] and shows a 3-fold increase in gene expression in the retina of diabetic BB/W-rats [4]. The demonstration of elevated sorbitol and decreased *myo*-inositol levels in association with increased vascular permeability in the retina of diabetic rodents, as well as the responsiveness of vascular damage to aldose-reductase inhibition or *myo*-inositol supplementation lend further support to the polyol-pathway/*myo*-inositol theory [5,6]. We have previously demonstrated a complete prevention of basement membrane thickening (BMT) in the deep capillary bed of the diabetic BB/W-rat retina following 6 months of aldose reductase inhibitor (ARI)-treatment. Vigorous insulin-treatment for the same duration prevented

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BMT in both the superficial and deep capillary beds [7] suggesting that proposed mechanisms such as an augmented polyol-pathway [8,9], non-enzymatic glycation [10], or decreased production of specific proteoglycans [11], may be modified by topographic differences [7].

In the present study we examined the effect of *myo*-inositol supplementation on BMT, in order to explore as to whether the effect of ARI-treatment on BMT is mediated by a normalization of the *myo*-inositol metabolism, or if this effect is a consequence of the sorbitol lowering effect.

Materials and Methods

Animals

Pre-diabetic male BB/W-rats and age-matched non-diabetes-prone male BB/W-rats were obtained from the NIH-Colony at the University of Massachusetts, Worcester, MA. To ascertain onset of diabetes, diabetes-prone animals were monitored daily with respect to glucosuria (Test Tape, Eli Lilly Canada, Inc., Toronto, Ont). After detection of glucosuria diabetic rats were started on small daily doses (0.5–3.0 μ/day) of protamine zink insulin (PZI) (Connaught-Novo Inc. Toronto, Ont.). Three weeks after onset of diabetes, the rats were randomly assigned as follows: non-diabetic control rats, $n = 5$; diabetic insulin-deficient rats maintained on small doses of PZI, $n = 5$; and diabetic insulin-deficient rats maintained on PZI and fed a 1% *myo*-inositol supplemented diet, $n = 5$. All animals were maintained in individual air-filtered metabolic cages with free access to rat-chow (non-MI-supplemented chow contained 0.05% MI) (Lab Blox F-6, Wayne Animal Diets, Winnipeg, MB, Canada) and water. Blood glucose levels were examined every second week and glycated hemoglobin every 3 months as previously described [7]. Five animals from each group were sacrificed after 6 months of diabetes.

Tissue collection

Animals were anesthetized with pentobarbital sodium (50 mg/kg body weight) and perfused with 2.5% glutaraldehyde in cacodylate buffer (pH 7.4). Retinal segments within 3 mm of the optic nerve head were taken from the superior temporal quadrant. The tissues were processed and examined electron microscopically as previously described [7].

Morphometric examination

Twenty cross-sectioned capillaries, 10 from the superficial capillary bed and 10 from the deep capillary bed, were examined in each animal. Capillary basement thickness, pericyte and endothelial cell profile areas and lumenal areas (expressed as percentages of total capillary area), and number of pericyte profiles and endothelial cells per capillary were calculated as previously described in detail [12].

Each capillary was examined for the presence of basement membrane abnormalities (nodular swellings, laminated basement membranes, and fibrillary material, [7]), and basement membrane projections. These changes were expressed as a percentage of capillaries showing the abnormalities. The mean value of basement membrane abnormalities was obtained in each capillary bed in individual animals. The investigator (S.C.) was unaware of the identity of the tissue samples.

Statistical analysis

The data are expressed as mean $\pm$ SEM. One way analysis of variance with linear contrast, and linear regression by least squares were used for data analysis.

Results

Clinical data

MI-treated and untreated diabetic rats showed significantly lower body weights ($P < 0.001$)

compared to non-diabetic control rats (392.0 ± 16.6 and 382.4 ± 12.4 vs 497.0 ± 6.5 g respectively). They showed elevated blood glucose levels ($P < 0.002$) (22.0 ± 2.1 and 20.8 ± 2.1 vs 8.1 ± 1.3 mmol/l, respectively) and increased glycated hemoglobin levels ($P < 0.002$) (9.7 ± 0.9 and 10.9 ± 1.6 vs 3.9 ± 0.1%, respectively).

Vascular morphometry

In control as well as diabetic animals the BMT of the superficial capillaries was greater than that of the deep capillaries. MI-treatment showed a significant ($P < 0.001$) but only partial prevention of BMT of the deep capillaries, and had no effect on BMT in superficial capillaries. Structural basement membrane abnormalities were not prevented by MI-treatment nor did this regimen have an effect on basement membrane projections (Table 1). Endothelial cell and pericyte morphometry was not altered by diabetes or MI-treatment (data not shown). Regression analysis of BMT with blood glucose concentrations and glycated hemoglobin levels as independent variables revealed significant positive relationships for both capillary beds (Fig. 1).

Discussion

The mechanisms responsible for basement membrane thickening in diabetes remain obscure. The only hard evidence at hand is that hyperglycemia is somehow required for the accelerated basement membrane thickening as substantiated by the data in the present study. The possibility that this adverse effect of hyperglycemia may at least in part be mediated by an increased activity of the polyol-pathway, is supported by a similar thickening of the retinal capillary basement membranes in galactosemic animals, that is prevented by aldose reductase inhibition [8]. We [7] and others [13] have previously reported that inhibition of the polyol-pathway prevents the BMT in diabetic rats. However, this preventive effect does not appear to be universal, since in our previous study [7] a complete inhibition was achieved only in the deep capillaries of the retina

TABLE 1

Basement membrane thickness (BMT), basement membrane (BM) abnormalities and projections in control, diabetic and MI-supplemented diabetic BB/W-rats

	BMT nm	BM abnormalities	BM projections
Superficial capillary bed			
Control rats ($n = 5$)	129.1 ± 5.5	2.0 ± 2.0	0
	$P < 0.001$	$P < 0.01$	
Diabetic rats ($n = 5$)	162.6 ± 6.7	24.0 ± 5.0	2.0 ± 2.0
Diabetic + MI rats ($n = 5$)	160.8 ± 8.2	22.4 ± 5.7	6.2 ± 3.8
Deep capillary bed			
Control rats ($n = 5$)	86.4 ± 5.7	10.0 ± 6.3	6.0 ± 2.5
	$P < 0.001$	$P < 0.01$	
Diabetic rats ($n = 5$)	120.2 ± 4.87	80.0 ± 6.3	6.0 ± 4.0
	$P < 0.001$		
Diabetic + MI rats ($n = 5$)	105.6 ± 4.9	66.0 ± 6.9	1.8 ± 1.8

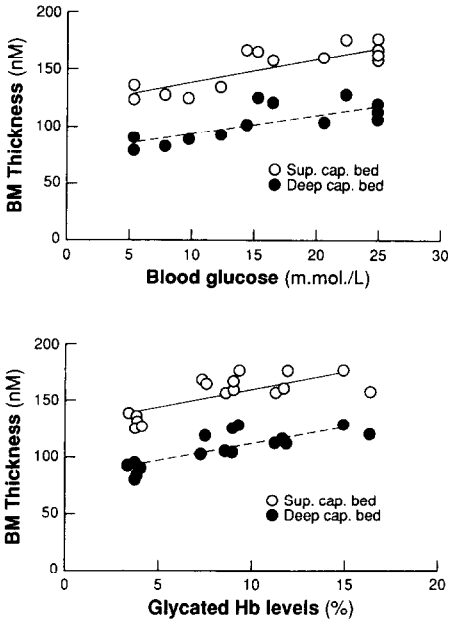

Fig. 1. Regression analysis of retinal capillary BM thickness with (top) blood glucose levels as the independent variable ($r = 0.8$, $P < 0.001$ for superficial and $r = 0.75$, $P < 0.005$ for deep capillary bed) and (bottom) glycated Hb levels as the independent variable ($r = 0.75$, $P < 0.005$ for superficial and $r = 0.78$, $P < 0.001$ for deep capillary bed).

but not in the superficial capillaries, despite similar immunoreactivity in the two capillary beds [3]. These findings suggest, if indeed the polyol-pathway activation and subsequent metabolic abnormalities play a major role in BMT, that the activity of the rate limiting enzyme aldose reductase, or its enzyme kinetics may vary greatly from one capillary bed to another. The failure of an ARI to show an effect on skeletal muscle capillary basement membrane in diabetic subjects would seem to support this notion [14].

In the present study MI-supplementation had a significant but only partial preventive effect on BMT in the deep capillary bed of the diabetic retina in the BB/W-rat. This would indicate that at least part of the complete prevention achieved following ARI-treatment in the same capillary bed is mediated by an effect of the polyol-pathway on MI-metabolism, or alternatively that a 1% MI-supplementation was insufficient to normalize the MI pool. The latter explanation is unlikely, however, since Tilton et al. [15] recently demonstrated a non-significant prevention of BMT in the deep capillary bed of STZ-diabetic rats following 9 months of a 2% MI supplemented diet.

The positive relationship between blood-glucose levels and BMT in both diabetic rodents [7] and man [14], may suggest that non-enzymatic linkage between extracellular matrix proteins and various hexoses could be mainly responsible for BMT, as suggested by Brownlee et al. [10].

This notion is in keeping with our previous suggestion that the demonstrated effect of ARI on BMT [7] may be mediated by a diminished production of fructose by the polyol-pathway, since it has been demonstrated that fructose is a potent glycator of structural proteins [16].

In summary, the present study has demonstrated a significant effect of *myo*-inositol supplementation on BMT in the diabetic BB/W-rat retina. However, the exact mechanism by which this is achieved remains to be investigated.

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