Effects of streptozotocin-induced type 1 diabetes mellitus on protein and ion concentrations in ocular tissues of the rat

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Abstract
This study investigated the effects of streptozotocin (STZ)-induced diabetes mellitus (DM) on protein and cation levels in ocular tissues (lenses, cornea, lacrimal glands and retina with sclera) of rats. Diabetic rats and their lacrimal glands weighed significantly less (p < 0.05) than age-matched controls. Diabetic animals also had significantly (p < 0.05) elevated blood glucose and significantly reduced (p < 0.05) plasma insulin compared to controls. Total protein concentrations in the cornea, lens, lacrimal gland and retina with sclera were markedly reduced compared to controls (circa 50%-90%). Diabetic cornea, lenses, lacrimal glands, and retina with sclera contained more Ca²⁺ (p < 0.05) than age-matched controls (2-3 fold). Levels of Zn²⁺ were also elevated (p < 0.05) in the cornea and retina with sclera of diabetic rats, as compared to control rats (2-3 fold), but were unaffected in lenses and lacrimal glands. In contrast, levels of Cu²⁺, Mg²⁺, Na⁺ and K⁺ were significantly reduced (p < 0.05) in all ocular tissues of diabetic rats when compared to control animals (circa 30%-70%). These results show that STZ-induced DM is generally associated with significant physiochemical changes in ocular tissues of rats with changes observed in body weight, blood glucose, and insulin levels and protein and cation concentrations compared to healthy age-matched controls. Based on these data, it has been speculated that diabetes may induce changes in ocular tissues that include: higher protein turnover through increased protease activity and changes in Na⁺ / K⁺ channel function. It is suggested that these changes may be associated with diabetic retinopathy, diabetic cornea and sight impairment. (Int J Diabetes Metab 2005 13:154-158, 2005)

Keywords: Type 1, diabetes, ocular tissues, protein, cations, rat, retinopathy

Introduction
Diabetes mellitus (DM) is a major global health problem that affects more than 185 million people around the world.¹-³ The disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycaemia.⁴,⁵ This is due to either a lack of insulin or insensitivity of insulin to target cells.⁶ DM can be divided primarily into two types: Type 1 or insulin dependent diabetes mellitus (IDDM) and type 2 or non-insulin dependent diabetes mellitus (NIDDM).⁴ With regards to incidence, around 5% of diabetics suffer from type 1 DM and the remaining 95% suffer from type 2 DM or maturity onset DM.¹

Type 1 DM is due to autoimmune destruction of β-cells, especially in childhood. On the other hand, type 2 DM is mainly due to hereditary factors, affluent lifestyles and obesity.⁴ Both types of DM are associated with a number of common symptoms such as polyuria and polydipsia, and long term complications including retinopathy, cardiomyopathy, nephropathy, neuropathy, foot ulcers and digestive insufficiency, especially if the disease is not diagnosed and treated early.⁴,⁷,⁸

Both type 1 and type 2 DM damage a variety of ocular tissues including the retina, lenses and cornea.⁴,¹¹ Corneal abnormalities are found in more than 70% of diabetic patients ¹² but retinopathy is the most serious eye condition associated with diabetes.¹¹ Retinopathy occurs in two major forms, nonproliferative and proliferative, and these encompass most forms of the diabetes-related disease that affects the retina. Diabetic retinopathy is associated with angiopathy and, in addition to the retina itself, affects other ocular structures such as the cornea, the lens, and surrounding tissues, as well as the lacrimal gland. It has been shown that diabetes causes microvascular abnormalities that lead to retinal vascular pericyte loss and endothelial damage, which in turn cause microvascular occlusion and alterations in retinal vascular permeability often resulting in ischaemia and retinal edema.⁴,⁷ This particular complication of DM is highly prevalent in developing countries and can be particularly severe in persons who have type 1 DM, also occurring frequently with chronic type 2 DM. Approximately one quarter of persons with diabetes have at least some form of diabetic retinopathy, and the incidence increases with the duration of diabetes. At 10 years, the prevalence of retinopathy in patients with diabetes is almost 7% and rises to greater than 90% after 25 years. Diabetic retinopathy can also accelerate during puberty and pregnancy.¹¹

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In many cases, the pathogenic basis of diabetes-induced damage to ocular tissues is unclear at the cellular and molecular levels and recent work has indicated that ion dependent proteins such as calpains may be involved in diabetes-induced ocular damage. The present study investigates the effects of streptozotocin (STZ)-induced type 1 DM on protein concentration and on cation contents in ocular tissues of the rat compared to age-matched healthy controls.

Materials and methods

Materials

Streptozotocin, analar grade chemicals and ion standards were obtained from BDH (Poole, UK).

Experimental procedures

Adult male Wistar rats (150-450 g) were used in this experiment. Ethical clearance was obtained from the Ethics Committee of the University of Central Lancashire. One group of animals (150-200 g and 8 weeks old) were injected intraperitoneally (IP) with 60 mg per kg body weight of STZ dissolved in citrate buffer (pH 4.0) and another group (150-200 g and 8 weeks old) received an equivalent volume of citrate buffer alone. Blood glucose and plasma insulin levels were measured 4 to 5 days following STZ administration, and 6 to 8 weeks later, to confirm DM. Using a glucometer (Johnson and Johnson, UK) and commercial immunoassay kits, respectively. Blood concentrations of glucose in excess of 300 mg dl

The present study also found that, compared to non-diabetic rats, Zn levels [mg. ml⁻¹ 100 g tissue⁻¹] were increased almost threefold in the cornea of diabetic rats with a smaller two-fold increase in the lens and retina (Figure 1) and the results showed the lacrimal glands of diabetic rats exhibited reduced plasma insulin and elevated blood glucose (p < 0.05 compared to non-diabetic animals). In other ocular tissues, the cornea and lens showed no significant differences to those of non-diabetic animals (p > 0.05). In contrast, however, Zn²⁺ levels (mg ml⁻¹ 100 g tissue⁻¹) in the lacrimal glands and lenses of diabetic rats showed no significant differences to those of non-diabetic animals (p > 0.05).

Table 1: Characteristics of animals and weights of rat ocular tissues. Data are mean ± SEM, n=7, * p < 0.05.

<table>
<thead>
<tr>
<th>Parameters measured</th>
<th>Age matched control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of rat (g)</td>
<td>391.83 ± 37.91</td>
<td>190.12 ± 5.41*</td>
</tr>
<tr>
<td>Blood glucose level (mg dl⁻¹)</td>
<td>92.40 ± 2.42</td>
<td>&gt; 500*</td>
</tr>
<tr>
<td>Plasma insulin level (mg dl⁻¹)</td>
<td>20.13 ± 572</td>
<td>4.80 ± 1.38*</td>
</tr>
<tr>
<td>Weight of cornea (mg)</td>
<td>8.03 ± 2.36</td>
<td>10.1 ± 0.89</td>
</tr>
<tr>
<td>Weight of lens (mg)</td>
<td>33.94 ± 5.26</td>
<td>38.44 ± 6.11</td>
</tr>
<tr>
<td>Weight of retina and sclera (mg)</td>
<td>33.11 ± 11.15</td>
<td>25.34 ± 11.74</td>
</tr>
<tr>
<td>Weight of lacrimal (mg)</td>
<td>118.07 ± 4.09</td>
<td>93.77 ± 2.4*</td>
</tr>
</tbody>
</table>

Results and Discussion

In the present study, the effects of STZ-induced diabetes on the levels of protein and cations in ocular tissues of the rat were investigated. Eight weeks after the induction of diabetes, diabetic and control animals were characterised and the results are shown in table 1. When compared to the control group of rats, rats injected with STZ exhibited reduced plasma insulin and elevated blood glucose (p < 0.05 in all cases). These changes confirm the induction of diabetes, resulting from β-cell destruction through the action of STZ. It was also found that diabetic rats weighed significantly less than control animals (p<0.05). In relation to ocular tissues, lacrimal glands from diabetic rats were also found to weigh significantly less (p<0.05) than those from the control group. However, no significant changes in the weights of lens, cornea, and retina with sclera were observed between diabetic and control animals.
Effects of diabetes on rat ocular tissue

Figure 1: Total protein levels in the cornea, lens, lacrimal, and retina with sclera of control (□) and STZ-induced diabetic rats (■). Data are mean ± SEM, n = 7. * p < 0.05 indicating that diabetic tissue contains significantly less protein when compared to the control.

In contrast to the above analyses, it was found that ocular tissues from diabetic rats exhibited levels (mg ml⁻¹ 100 g tissue⁻¹) of Mg²⁺ (Figure 3A), Cu²⁺ (Figure 3B), Na⁺ (Figure 4A) and K⁺ (Figure 4B) that were generally strongly decreased relative to those of non-diabetic animals (p < 0.05) with reductions ranging between 30% and 70%. In the case of these latter ions, Na⁺ and K⁺ channels are well...
known to play important roles in modulating membrane potential and recent studies on STZ-induced diabetic rats have shown that the disorder is able to influence both the membrane potential and $K^+$ channel function of diabetic muscle. Currently, we are investigating the possibility that similar abilities underlie the reduced levels of Na$^+$ and $K^+$ observed in the diabetic ocular tissues of rats in the present study.

In conclusion, the present results show that DM is generally associated with significant physiochemical changes in ocular tissues with regards to body weight, blood glucose, plasma insulin, and protein and cation levels when compared to controls. Based on the data, it has been speculated that diabetes may induce changes in ocular tissues that include higher protein turnover through increased protease activity and changes in Na$^+$/K$^+$ channel function. It is suggested that these changes may contribute to sight impairment and eye disorders such as diabetic retinopathy and diabetic cornea.

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References