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Aqueous flow in galactose-fed dogs

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Abstract

Dogs fed galactose develop diabetes-like ocular complications that include keratopathy, cataracts, and retinopathy. The purpose of this study was to investigate whether galactosemic dogs display reduced aqueous flow similar to that observed in patients with insulin-dependent diabetes mellitus. Twelve male beagles at 9 months of age were divided into three groups of four. The Galactose group was fed diet containing 30% galactose for 97 months and the Reversal group was fed the galactose diet for an initial 38 months then standard dog diet for the remaining period. The Control group was fed standard dog diet for 97 months. Aqueous flow was determined by fluorophotometry in one eye per dog at 96 and 97 months after the initiation of galactose feeding. Intraocular pressure (IOP) was measured once in the morning by pneumatonometry. Anterior chamber depth was measured by A-scan. At the end of the experiment, eyes were enucleated and processed for histological examination. Dogs fed galactose diet for 97 months had significantly ($p < 0.05$) increased body weights but similar IOP and anterior chamber depth compared to the other groups, and significantly ($p = 0.05$) reduced aqueous flow compared to the control group (4.4 ± 2.2 vs. 6.8 ± 2.4 μl/min, mean ± standard deviation, respectively). Additionally, aqueous flow decreased in the Reversal group to 3.1 ± 1.3 μl/min ($p = 0.002$). This decrease correlated with morphological changes of the ciliary processes. Like patients with insulin-dependent diabetes mellitus, galactose-fed dogs demonstrate reduced aqueous flow. This reduction was irreversible and independent of the retinopathy present. This animal model may be useful for the study of aqueous humor dynamics in diabetes.

Keywords: galactose; diabetes; dog; aqueous flow; intraocular pressure; ciliary body

1. Introduction

Diabetes with its prolonged hyperglycemia is linked to a number of ocular complications that affect the cornea, iris, lens and retina (al-Sereiti et al., 1991; Kincaid, 1996; Schertzer et al., 1998). Recent studies suggest that diabetes also may be associated with glaucoma; however, a direct link has not been established. Patients with diabetes have been reported to have higher IOP and increased prevalence of glaucoma than healthy subjects (al-Sereiti et al., 1991; Schertzer et al., 1998). Moreover, diabetes is a risk factor for primary open angle glaucoma (Bonovas et al., 2004). Progressive retinal ganglion cell death that is associated with diabetes also has been reported to contribute to glaucomatous optic neuropathy (Nakamura et al., 2005).

Glaucoma can involve increased intraocular pressure (IOP) that is directly linked to the dynamics of aqueous humor. Several reports suggest that the ciliary processes, which produce aqueous humor, have altered structure and function with diabetes. Increased basement membrane thickening of the ciliary body pigmented epithelium (Engerman and Colquhoun, 1982), altered blood-aqueous barrier permeability (Moriarty et al., 1994), and altered aqueous humor formation (Auricchio and Diotallevi, 1965; Hayashi et al., 1989; Larsson et al., 1995;

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Lane et al., 2001) have been reported. However, the reports on aqueous humor dynamics are conflicting and do not conform to the expected increase in IOP in glaucoma. In one study (Hayashi et al., 1989) aqueous flow was significantly reduced in insulin-dependent diabetes. In another study (Larsson et al., 1995), IOP and aqueous flow decreased relative to the stages of diabetic retinopathy. However, no correlations were observed between aqueous flow and the level of prolonged hyperglycemia as measured by hemoglobin A1c levels. More recently, Lane et al. (2001) reported that, when measured under both euglycemic conditions of relatively high and low insulin concentrations, aqueous flow is decreased in patients with type 1 diabetes without retinopathy or vascular diseases. These results suggest that the decreased aqueous flow is independent of the levels of circulating insulin although higher levels of insulin tended to be associated with lower aqueous flow rates.

To date, no direct correlations between prolonged hyperglycemia or insulin levels and changes in aqueous humor dynamics have been reported. Moreover, results linking decreased aqueous flow with the severity of retinopathy (which directly correlates with hyperglycemia) (The Diabetes Control and Complications Trial Research Group, 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998) have been conflicting. Since the formation and severity of microvascular, neurological and cellular lesions associated with diabetes are linked to the severity of the hyperglycemia (The Diabetes Control and Complications Trial Research Group, 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998), this raises the question of whether the observed changes in ciliary processes structure/function can be defined as a diabetes-linked complication. Noticeably absent are studies in diabetic animals which address potential structural and/or functional changes of the ciliary processes and blood-aqueous barrier. However, there are several reports that indicate both morphological changes of the ciliary body epithelium and increased permeability of the blood aqueous barrier occur in galactose-fed rats (Ikebe et al., 1989; Caspers-Velu et al., 1995). Both of these changes were prevented when the rats were concomitantly treated with aldose reductase inhibitors. Experimentally induced galactosemia, which activates the aldose reductase associated polyol pathway, has been used extensively to explore the pathogenesis of diabetic complications in animals (Robison, 2001). Both glucose and galactose are reduced by aldose reductase to the respective sugar alcohols sorbitol and galactitol through the polyol pathway. This directly reflects the effects of ''hyperhexosemia'' which activates aldose reductase in both diabetes and galactosemia. As a result, similar lesions associated with diabetic complications form in both diabetic and galactosemic animals. Compared to lesions in the diabetic animal, the diabetes-like lesions in galactose fed animals are induced more rapidly and are more severe. The insulin levels, however, remain normal in galactose-fed animals.

Here, we report that in the galactose-fed dog, an animal model that demonstrates clinically and by histology, diabetes-like retinal changes associated with all stages of diabetic retinopathy, aqueous flow is reduced independent of the level of retinopathy present. This reduction mirrors morphological changes of the ciliary body.

2. Materials and methods

2.1. Dogs

All experiments were approved by the Institutional Animal Care and Use Committee of the National Institutes of Health where the study was conducted. The study used 12 9-month-old beagles (Marshall Farms) which were individually housed in 0.9 × 2.7-m runs and fed a daily diet (Bioserve, Frenchtown, NJ) consisting of ca. 450 g of standard dog chow with or without 30% galactose. Four of the eight galactose-fed dogs received the diet for 97 months, while the other four were initially fed galactose diet for 38 months and then control diet for the remaining months of the study. Four age-matched control dogs received standard diet for 97 months.

All dogs were monitored at 3-month intervals by a veterinary ophthalmologist and through clinical blood chemistry profiles which included determinations of complete blood counts with differential, serum T3, thyroxine (T4), glucose, serum urea nitrogen, creatinine, sodium, potassium, chloride, calcium, phosphorus, uric acid, total proteins, albumin, globulin, SGOT, SGPT, GGTP, alkaline phosphatase, LDH, total bilirubin, direct bilirubin, triglycerides, and cholesterol.

2.2. Aqueous flow

In one randomly chosen eye per dog, aqueous flow was measured at 96 months (8 years) of the start of the study. Measurements were repeated one month later. For each measurement day, one drop of 10% fluorescein was applied to the cornea at 5-min intervals for a total of four drops. Five minutes after the fourth drop, the face, lids and lashes were thoroughly cleaned. Four hours after the application of fluorescein the dogs were sedated, if necessary, with a subcutaneous injection of butorphenol or a mixture of butorphenol and valium. The dogs had become accustomed to being handled and examined over the years, which minimized the need for sedation. The fluorescence of the cornea and anterior chamber was measured in duplicate with a scanning ocular fluorophotometer (Fluorotron Master, OcuMetrics). Scans were repeated three times at 60 min intervals for a total of four sets of scans.

Intraocular pressure was measured with a pneumatonometer (Langham tonometer) after topical application of proparacaine HCl 0.5%. Although this tonometer can simultaneously calculate pulsatile ocular blood flow (POBF), the absence of a strong pulse pressure in these dogs made the POBF measurements invalid. Anterior chamber depth, the distance from Descemet's membrane of the central cornea to the anterior lens surface, was measured by ultrasound using an A-scan (Sonomed). Cornea diameter was measured with calipers. Cornea thickness (555 μM) was obtained from the literature (Gilger et al., 1991). Anterior chamber volume was calculated from the values of anterior chamber depth, cornea diameter and cornea