



Validation study:

Effects of STZ induced diabetes as a model of diabetic retinopathy in

Brown Norway Rats

Dean R*, Sukhu R*, Massie B**, Nemeth L*, Meschter C*

*Comparative Biosciences, Inc. Sunnyvale, Ca 94024,

**Phoenix Research Labs, (Pleasanton, CA.

Abstract

STZ-induced hyperglycemia resulted in changes in the rat retinal pigmented layer consisting of increased new vessel formation, reactive endothelium, dilated capillaries distended with either blood or edema fluid, acute inflammation composed of intravascular neutrophils, and neutrophils adhered to vessel walls and extravascularly. Additionally, there were neutrophils free in the rat retina and vitreous and there was some fibrinous debris in the vitreous. Occasional necrolytic debris was also present in the rat retina. These changes were clearly evident histologically. Examination of the rat retina in vivo via fluorescein angiographs obtained with the Micron retinal imaging microscope (Phoenix Research Laboratories, Pleasanton, CA) revealed marked increases in retinal vascularity in STZ treated rats with areas of leakage particularly surrounding the optic nerve. These changes were compatible with and correlated with the histopathologic findings of increased vascularity of the retinal pigmented layer. The Micron III is shown to be a suitable and appropriate device for assessment of retinal changes in STZ hyperglycemic rats.

Introduction

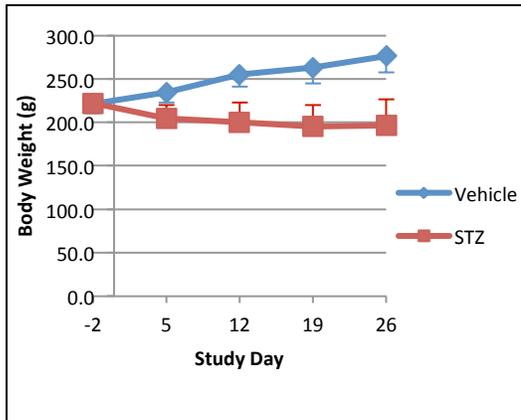
Diabetes is associated with retinal microvascular changes that include leakage, increased neovascularity and increase tortuosity and neutrophil attachment and leukostasis. The Streptozotocin-induced (STZ) model of diabetes induced by STZ administration in rats serves as a validated and reproducible model of retinal hypervascularity in Brown Norway rats (BN). At CBI, this model is induced in male BN rats by STZ administration. Body weights and blood glucose levels were monitored weekly. At 2 and 4 weeks, retinas were examined in vivo using the Micron III retinal

imaging microscope (Phoenix Research Laboratories, Pleasanton, CA). Histologic examination of the eyes was also performed. The in vivo fluorescein angiograms as captured by the Micron retinal imaging microscope as coupled with histopathologic assessment of the vasculature of the retinal pigmented layer allows for a complete assessment of the microvascular retinal effects with this model.



Body weights

STZ-treated rats lost body weight over the 4-week period while untreated animals gained weight.



Blood Glucose Monitoring:

Blood glucose levels were determined pretest and weekly. Hyperglycemia was evident by one week. Control glucose was in the 153 mg/dl±16 range and STZ-treated glucose levels were consistently in the 765±98 mg/dl range from Week 1-4.

Retinal Imaging

Images of the retina were obtained on Days 14 and 28.



Figure 1; Phoenix Research Labs (Pleasanton, CA) Micron retinal imaging microscope for assessment of rodent retinas.

Fluorescein dye was administered intravenously (IV), and optical density staining in the rat retina was visualized using the Micron retinal

imaging microscope. With a

transverse resolution below 4 microns and a depth of focus of 20 microns, the Micron retinal imaging microscope is capable of focusing on different layers in the vascular structure. With some retina motion the best recording mode is full resolution digital video and in this slowly focus through the depths of the retina. To this end the Micron retinal imaging microscope recorded 45 seconds of digital video at 22 frames second and the images at the best focus were selected for extraction and inclusion in this report. In addition videos of the retina obtained immediately after fluorescein dye injection were made using vendor supplied software. For the 4-week videos, 1,600 frame recordings were used for the recording. Selected images from the resulting Week-4 videos were used to quantify the amount of dye leakage from retinal vessels in STZ vs. control animals. In summary the retinal findings are that:

At 2 weeks: Retinal vascularity was similar between control and STZ-treated animals

At 4 weeks: Retinal vascularity was markedly increased in STZ-treated rats with areas of leakage particularly surrounding the optic nerve.

Necropsy

For each animal, both eyes (with optic nerve attached) were collected and fixed overnight in modified Davidson's solution and then transferred to 10% neutral buffered formalin. Following processing, tissues were dehydrated, embedded sagittally in paraffin and serially sectioned (at 3-5 μ) through the center portion of the eye, including the retina-optic nerve region.



Histopathologic findings

Lesions from retinas of eyes for each animal from both groups were evaluated via light microscopy by an ACVP board certified veterinary pathologist. Representative sections of untreated and treated retinas were assessed. Vascular lesions in the retinal pigmented layer were scored 0-4 (0=no lesion present; 1=minimal; 2=mild; 3=moderate; 4=severe) and included vascular dilation, leakage and inflammation.

Untreated eyes were within normal limits. In the STZ-treated rats, there was increased new vessel formation, reactive endothelium, dilated capillaries distended with either blood or edema fluid, acute inflammation composed of intravascular neutrophils, neutrophils adhered to vessel walls and extravascularly. Additionally, there were neutrophils free in the retina, in the vitreous and there was some fibrinous debris in the vitreous. Occasional necrolytic debris was also present in the retina. While lesions were present in all animals, there was considerable variation in severity of the lesion. Vascular lesions corresponded with the retinal scans. In Group 1 capillaries were clearly visible and there was no tortuosity or evidence of leakage, while in the STZ-treated groups, vascular tortuosity and vascular leakage was clearly present.

The histologic scores are presented in detail in Table 2 and are summarized in Table 1.

Conclusion

The in vivo fluorescein angiography as displayed by the Micron retinal imaging microscope (Phoenix Research Laboratories, Pleasanton, CA) coupled with histopathologic assessment of the vasculature of the retinal pigmented layer allows for a complete assessment of the microvascular retinal effects with this model.

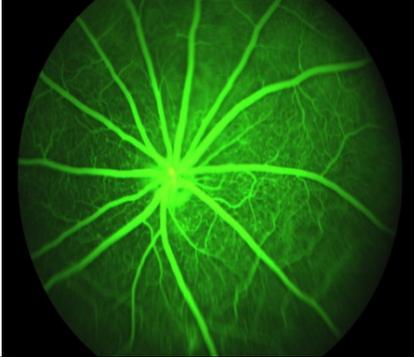
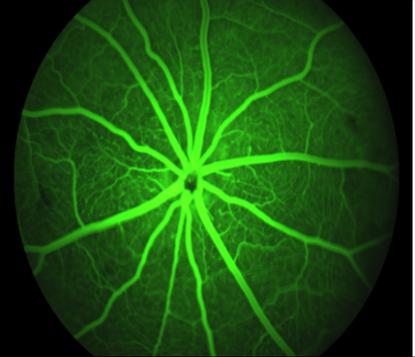
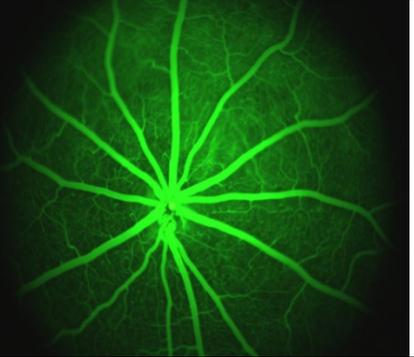
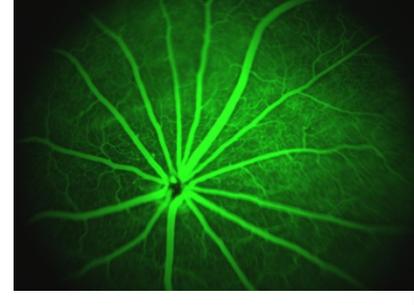
The CBI STZ-induced model of hyperglycemia is a robust and validated model for the assessment of hyperglycemia-induced retinal vascular lesions.



Table 1. Summary of histology measurements*.

Group	Animal No.*	Treatment	New vessel formation score	Edema score	Inflammation score	Total Lesion score
1	101-110	No treatment	0±0	0±0	0±0	0±0
2	201-210	STZ	1.8±1.1	1.8±0.8	2.1±1.0	5.8±2.7

*Group mean and standard deviation. Vascular lesions in the retinal pigmented layer were scored 0-4 (0=no lesion present; 1=minimal; 2=mild; 3=moderate; 4=severe) and included vascular dilation, leakage and inflammation.

		
		
<p>104. Untreated Retinal scan: Two and four week-retinal scans: Normal capillaries are present. HE: Retina is within normal limits. HE 200x</p>	<p>210 STZ-treated Retinal scan: Increased vessel tortuosity and vascular leakage are present. HE: Dilated capillaries filled with edema fluid, interstitial edema and neutrophils. HE 200x</p>	<p>214 STZ-treated Retinal scan: Increased vessel tortuosity and vascular leakage are present particularly around the optic nerve. HE: Markedly dilated capillaries filled with edema fluid, interstitial edema and neutrophils. The retina layer is deviated. HE, 200x</p>