

Effects of Aldose Reductase Inhibitors and Galactose Withdrawal on Fluorescein Angiographic Lesions in Galactose-Fed Dogs

Michael Cusick, BS; Emily Y. Chew, MD; Frederick Ferris III, MD; Terry A. Cox, MD, PhD; Chi-Chao Chan, MD; Peter F. Kador, PhD

Objective: To assess the effect of aldose reductase inhibitor (ARI) M79175 (2-methyl-6-fluoro-spiro-chroman-4-5'-imidazolidine-2',4'-dione) administration and galactose withdrawal on the progression of retinal changes using fluorescein angiography in galactose-fed dogs.

Methods: Thirty male beagles were randomized into 4 groups. Three dogs were fed a normal control diet containing 30% nonnutritive fiber for 74 months (control group), 11 dogs a 30% galactose diet for 74 months (continuous galactose group), 8 dogs a 30% galactose diet for 36 months followed by replacement with a normal diet for 36 months (galactose withdrawal group), and 8 dogs a 30% galactose diet supplemented with M79175 for 34 months followed by replacement with a normal diet and removal of M79175 treatment for 38 months (ARI-treated galactose withdrawal group). Stereoscopic color fundus photography and fluorescein angiography, performed at baseline and follow-up, were assessed for the clinical development of retinopathy, including the first appearance of hyperfluorescence, varying severity of retinal nonperfusion, and retinal neovascularization. Histopathologic features were examined in selected dogs.

Results: All dogs in the 3 groups fed the 30% galactose diet developed areas of hyperfluorescence and nonperfusion. Of these dogs, only those supplemented with the ARI did not develop areas of nonperfusion greater than or equal to half the field and retinal neovascularization. Parametric survival analysis showed significant differences (galactose withdrawal group vs ARI-treated galactose withdrawal group) in the median times to the development of nonperfusion greater than or equal to half the field ($P=.003$) and retinal neovascularization ($P=.03$).

Conclusion: Normalization of glycemic control with galactose withdrawal and ARI treatment may delay the onset and progression of retinal lesions in galactose-fed dogs.

Clinical Relevance: Perfect glycemic control after a period of poor control does not completely prevent the progression of retinal lesions. Therapy with ARIs may potentially be important in the prevention of retinal lesions associated with diabetic eye disease.

Arch Ophthalmol. 2003;121:1745-1751

DIABETIC RETINOPATHY is a microvascular complication of type 1 and type 2 diabetes mellitus and is a leading cause of blindness in adults in the United States.¹⁻³ The clinical progression of this disease has been well described,⁴ and epidemiologic studies⁵⁻⁷ implicate hyperglycemia in the pathogenesis of diabetic complications.

Experimental evidence suggests that excessive sugar alcohol (polyol) formation associated with hyperglycemia may cause retinopathy. Excess polyol formation, catalyzed by the enzyme aldose reductase, has been implicated in the pathogenesis of diabetic eye lesions, including cataract,⁸ microangiopathy,⁹ and retinopathy.¹⁰ Aldose reductase-initiated accumulation of polyols has been demonstrated in isolated retinal microvessels,¹¹ and aldose reductase has

been immunohistochemically demonstrated to be present in pericytes of retinal capillaries isolated by trypsin digestion from human¹² and dog¹³ retinas. Retinal capillary pericytes accumulate sugar alcohols when cultured in vitro in high sugar medium, and this accumulation is inhibited by the presence of aldose reductase inhibitor (ARI).¹⁴ The level of aldose reductase activity is significantly higher in dog retinal capillary pericytes than in endothelial cells,¹⁵ and the rapid polyol accumulation in pericytes when exposed to high galactose levels initiates cell death (apoptosis) that is prevented by the ARI AL1576.¹⁶

The galactose-fed dog is an appropriate experimental animal model for the study of diabetic retinal disease because it develops diabetes-like retinal vessel changes associated with early and advanced stages of retinopathy, including the proliferative

From the National Eye Institute, National Institutes of Health (Mr Cusick and Drs Chew, Ferris, Cox, Chan, and Kador), and Howard Hughes Medical Institute—National Institutes of Health Research Scholars Program (Mr Cusick), Bethesda, Md. The authors have no relevant financial interest in this article.

stage.^{10,17,18} Use of ARIs has been shown to effectively retard the onset and progression of histopathologic changes associated with retinal disease in galactose-fed dogs.¹⁹⁻²³ Intense glycemic control has also been shown to reduce the progression of retinopathy in diabetic²⁴ and galactose-fed²⁵ dogs and in humans with type 1 and type 2 diabetes mellitus.^{26,27} In this study, the common clinical technique of fluorescein angiography is used to evaluate the effects of ARI treatment and galactose withdrawal on retinal lesions, particularly retinal nonperfusion, which precedes the development of retinal neovascularization, in galactose-fed dogs.

METHODS

DOGS

Experiments on all dogs conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Before the study, 30 male beagles underwent phacoemulsification of their lenses bilaterally at age 3 months. The dogs were placed under general anesthesia with ketamine hydrochloride (20-40 mg/kg), xylazine hydrochloride (1 mg/kg), and atropine sulfate (0.04 mg/kg), and the pupil was dilated with 1% cyclopentolate hydrochloride and 10% phenylephrine hydrochloride. After making an incision at the limbus, an anterior capsulotomy was performed using a bent 25-gauge needle on irrigation. A phacoemulsifier-aspirating-infusor probe was then placed in the lens, and the lens nucleus and cortex were removed, leaving the posterior capsule behind. The wound was closed with 9-0 nylon sutures, and 20 mg of gentamicin sulfate was given subconjunctivally. Atropine and dexamethasone-neomycin sulfate-polymyxin B sulfate ointments were then applied daily for 14 days. Subsequently, yttrium aluminum garnet laser photocoagulation was performed on the poster capsules to allow for fundus photography.

At age 9 months, the dogs were randomly divided into 4 groups. Each dog was individually housed in 0.9 × 2.7-m runs and fed a 450-g daily diet (Bioserve, Frenchtown, NJ) consisting of standard dog chow plus the dietary additives indicated per group: (1) control group: 30% nonnutrient filler (n=3); (2) continuous galactose group: 30% galactose for the duration of the study (n=11); (3) galactose withdrawal group: 30% galactose for 36 months followed by replacement of the galactose with the 30% nonnutrient filler diet (n=8); and (4) ARI-treated galactose withdrawal group: 30% galactose plus the ARI M79175 for 34 months followed by replacement of the galactose and the ARI with the 30% nonnutrient filler diet (n=8). M79175 (2-methyl-6-fluoro-spiro-chroman-4-5'-imidazolidine-2',4'-dione) (Eisai Co Ltd, Tokyo, Japan) was supplied in capsule form. The drug was orally administered on weekdays in 3 equal doses (5.0 mg/kg) 1 hour before feeding, 1 hour after feeding, and approximately 8 hours after feeding. On weekends, M79175 was orally administered as 2 equal doses 1 hour before and 8 hours after feeding. Treated dogs were routinely monitored for drug regurgitation approximately 1 hour after inhibitor administration. On completion of the study, all dogs were euthanized with a mixture of pentobarbital sodium and phenytoin sodium (Beuthanasia-D; Schering-Plough Corp, Kenilworth, NJ).

Clinical blood chemistry profiles, conducted at 3- to 4-month intervals, included determinations of complete differential blood cell counts and levels of serum triiodothyronine, thyroxine, glucose, serum urea nitrogen, creatinine, sodium, potassium, chloride, calcium, phosphorus, uric acid, total proteins, albumin, globulin, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, alkaline phos-

phatase, lactate dehydrogenase, total bilirubin, direct bilirubin, triglycerides, cholesterol, and glycosylated hemoglobin. Glycosylated hemoglobin levels were obtained by using high-pressure liquid chromatography at 3-month intervals.

OPHTHALMIC EXAMINATION OF RETINAL LESIONS

Stereoscopic fundus photographs and fluorescein angiograms were obtained during ophthalmoscopic examinations starting at baseline when diets were initiated and then twice yearly after the third annual examination. Pupils were dilated with topical 1% tropicamide hydrochloride, and dogs were anesthetized with an intravenous injection of a mixture of ketamine hydrochloride (10 mg/kg), xylazine hydrochloride (0.5 mg/kg), atropine sulfate (0.05 mg/kg), and diazepam (0.25 mg/kg). Three standard fields of the fundus were photographed in color and with a red-free filter (Zeiss 30° camera; Carl Zeiss Inc, Oberkochen, Germany), including the temporal area centralis, the disc, and the nasal area centralis. Three to 4 mL of 10% fluorescein sodium was then injected into the cephalic vein, and angiograms were taken with the use of a fluorescein fundus camera (model FF3; Carl Zeiss Inc). Multiple frames of each field were taken at the early transit stage up to 35 seconds. Late fluorescein angiograms of each field were taken at approximately 3 to 4 minutes. In dogs with bilateral eyes, 2 fluorescein angiograms with an early transit of each eye were acquired at least 2 days apart.

The photographic data were graded by a masked reader (E.Y.C.) and were assessed for the presence or absence of retinopathy, hyperfluorescence, nonperfusion, and retinal neovascularization at each individual visit. No side-by-side comparisons were performed. The severity of nonperfusion was graded according to the following predetermined levels: 1, no retinopathy; 2, hyperfluorescence; 3, nonperfusion in less than 2 disc areas; 4, nonperfusion in 2 to less than 4 disc areas; 5, nonperfusion in 4 to less than 9 disc areas; 6, nonperfusion in 9 disc areas to less than half a field; 7, nonperfusion in half a field to less than 1 field; and 8, nonperfusion in 1 or more fields. The presence or absence of retinal neovascularization was noted. The outcome of nonperfusion of half a field or more was considered to be a clinically meaningful outcome. Areas of nonperfusion of this extent are indications of severe nonproliferative retinopathy. Such areas are also commonly seen in persons with severe nonproliferative diabetic retinopathy, as defined using the modified Airlie House criteria. Persons with this degree of retinopathy are at high risk of developing proliferative retinopathy and are often considered for scatter photocoagulation.

HISTOPATHOLOGIC ANALYSIS

Retinal neovascularization was confirmed by histopathologic analysis. Selected dog eyes were enucleated after euthanasia for routine histologic examination. The eyes were fixed in 10% buffered formaldehyde. After adequate fixation, the eyes were cut through the pupillary optic nerve head. Gross pictures were taken to document retinal lesions. The eyes were then embedded in paraffin, sectioned, and stained with hematoxylin-eosin and periodic acid-Schiff.

STATISTICAL ANALYSIS

All available eyes were considered for statistical analysis. The times to any nonperfusion (level ≥ 3), nonperfusion greater than or equal to half a field (level ≥ 7), and retinal neovascularization were the primary outcomes. Because the exact time of the events could not be determined by periodic evaluations of the dogs, photographic data were used to determine the interval in which the events occurred. The SAS procedure LIFEREG (SAS Institute Inc, Cary, NC) was used to develop parametric re-

gression models for the interval-censored data. The Weibull accelerated failure time model was applied to estimate survival functions for each group of dogs. Because of correlation of outcomes between eyes of individual dogs, statistical comparisons of median survival times between groups were made using permutation tests ($n \geq 2000$) using the data from both eyes when available for each subject. This approach generated *P* values for differences in median survival times among the groups. All statistical analyses were performed using SAS 8.1 for Windows (SAS Institute Inc).

RESULTS

Dogs were fed the appropriate assigned diet for 74 months. The proportion of chow consumed by each treatment group did not differ: $88\% \pm 8\%$ for the continuous galactose group, $90\% \pm 9\%$ for the galactose withdrawal group, and $93\% \pm 6\%$ for the ARI-treated galactose withdrawal group. The health of each dog was monitored through clinical blood chemistry profiles conducted at 3- to 4-month intervals. No significant abnormalities in blood chemistry profiles were observed. Specifically, the glycosylated hemoglobin level did not differ among treatment groups during the first 36 months of the study. The average glycosylated hemoglobin levels were $2.68\% \pm 0.36\%$ of total hemoglobin for the continuous galactose group, $2.80\% \pm 0.35\%$ of total hemoglobin for the galactose withdrawal group, and $2.41\% \pm 0.25\%$ of total hemoglobin for the ARI-treated galactose withdrawal group. These values did not differ among the treatment groups in the latter part of the study with removal of the galactose diet.

During the study, 1 dog in the continuous galactose group, 1 in the ARI-treated galactose withdrawal group, and 2 in the galactose withdrawal group died of causes unrelated to either the galactosemia or the administration of ARIs.

Grading of the fluorescein angiograms revealed the progression of retinal lesions. The first clinical signs of retinopathy were small areas of hyperfluorescence accompanied by fluorescein leakage (**Figure 1A**). These areas of fluorescein leakage eventually disappeared and progressed into areas of nonperfusion (**Figure 1B**). These areas of nonperfusion increased in size, becoming larger than half of the field size (**Figure 1C**). Increasing ischemia was associated with the development of proliferative disease. Neovascularization was identified by the appearance of vessels that demonstrated massive fluorescein leakage, which was always located around the optic disc (**Figure 2**).

The fluorescein angiographic findings of neovascularization were confirmed using cross-sectional histologic studies. Neovascularization at the optic nerve head was shown by the presence of central retinal vessels extending into the vitreous (**Figure 3A**). Neovascularization elsewhere was suggested by fibrovascular and fibroglial tissue extending from the inner retinal limiting membrane (**Figure 3B**). Other observed pathologic changes include thickening of basement membranes of the retinal endothelial cells, hyalinization of the vascular wall, and the appearance of retinal edema characterized by cystoid spaces within the retina (**Figure 3B**) and the appearance of intravitreal hemorrhage (**Figure 3C**).

Table 1 summarizes the incidence of fluorescein angiographic lesions among groups at each examina-

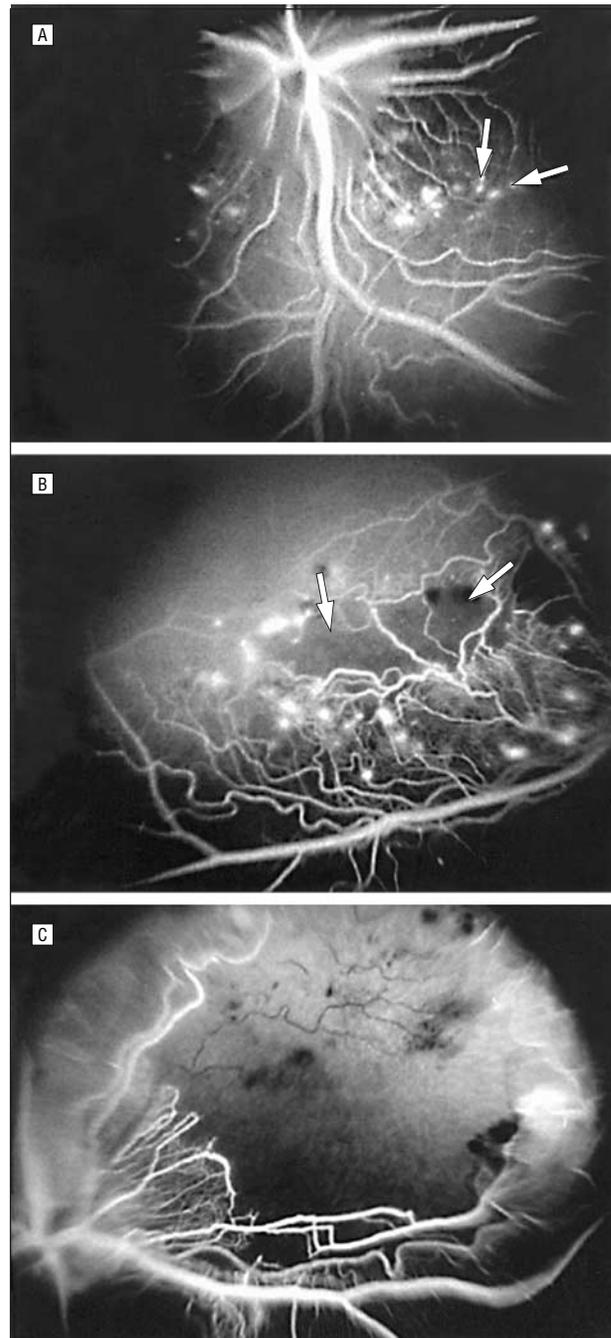


Figure 1. Nonperfusion in fluorescein angiograms of retinas from galactose-fed dogs. A, Focal areas of hyperfluorescence (arrows) were seen at 36 months. B, Areas of nonperfusion (arrows) are indicated by a decreased amount of fluorescein leakage. C, Larger areas of nonperfusion formed in dogs not supplemented with aldose reductase inhibitors.

tion. Fluorescein angiograms from all groups similarly indicated the absence of retinal lesions for the first 27 months of the study, and dogs receiving the normal control diet containing 30% nonnutrient filler continued to show an absence of retinal abnormalities throughout the entire study. After 36 months, dogs in the continuous galactose group showed areas of hyperfluorescence and fluorescein leakage. By 42 months, these dogs showed areas of nonperfusion, which progressed to sizes equal to or larger than half a field by 53 months. Retinal neo-

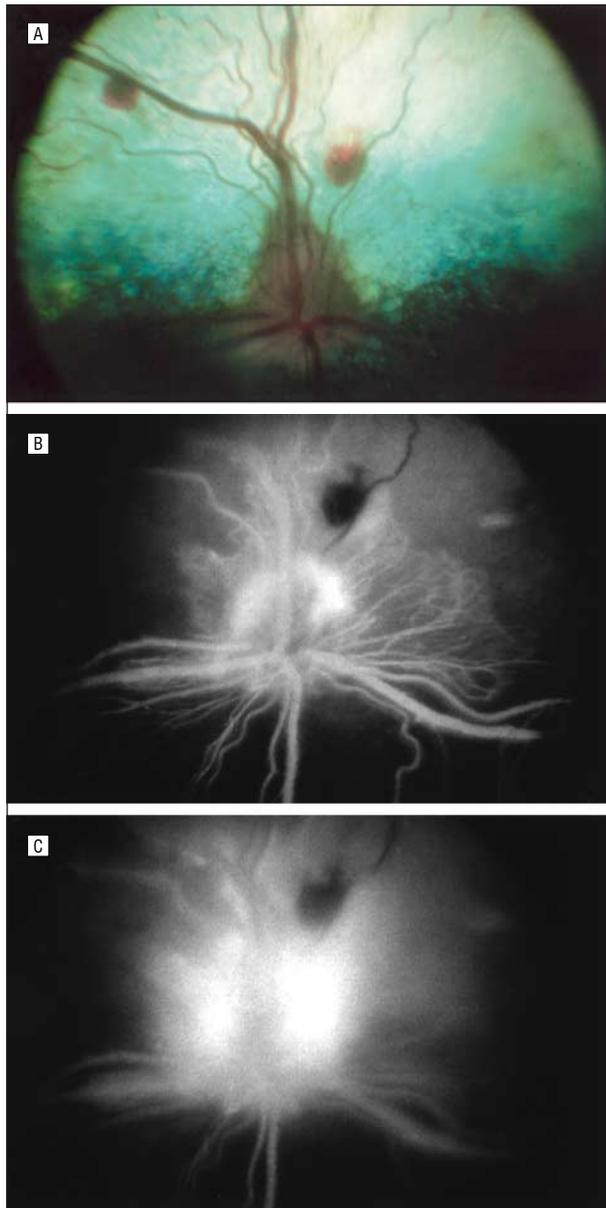


Figure 2. Proliferative changes in a galactose-fed dog's retina. A, Fundus photograph taken near the optic disc. B and C, Early and late angiograms, respectively, after injection of fluorescein show massive fluorescein leakage around the optic disc.

vascularization was first observed in the continuous galactose group after 60 months of galactose feeding. By 74 months, all dogs continuously fed the galactose diet showed areas of nonperfusion greater than or equal to half a field, and 7 of 10 showed proliferative disease.

The appearance of retinal lesions in the galactose withdrawal group, where the galactose diet was removed after 36 months, showed a similar time line for the appearance of retinal lesions as observed in dogs continuously fed the galactose diet. However, the proportion of dogs in the galactose withdrawal group demonstrating retinal lesions was consistently lower than that of dogs continuously fed galactose diet. Of the 6 dogs in the galactose withdrawal group examined after 74 months, 5 showed some evidence of nonperfusion, 3 showed non-

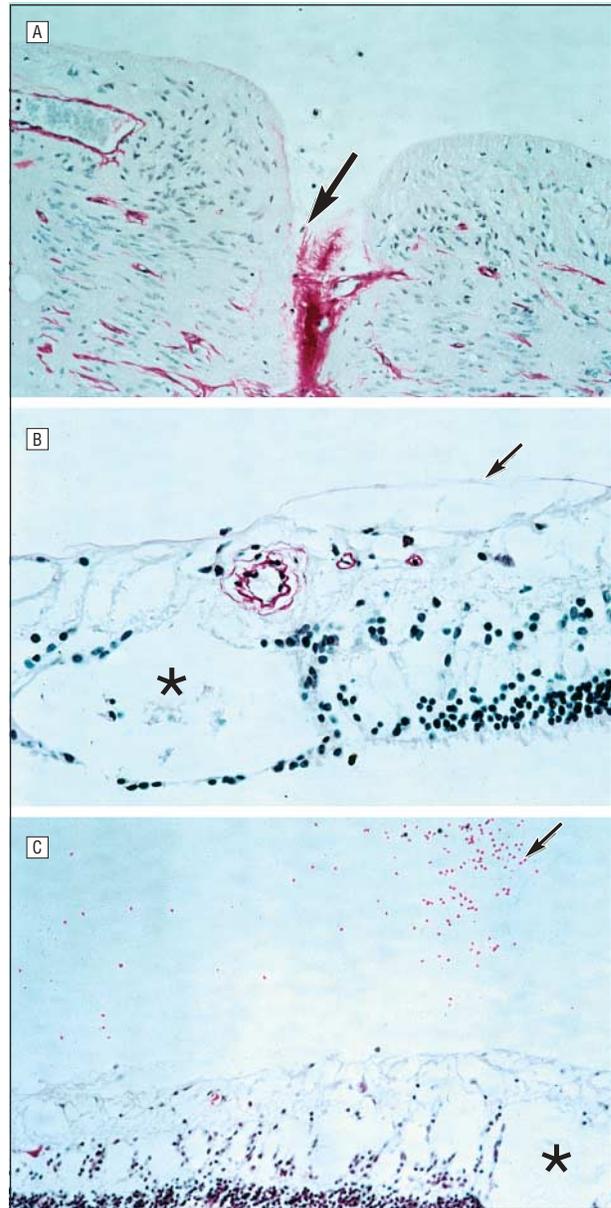


Figure 3. Histopathologic analysis of retinal lesions. A, Cross section at the optic nerve head shows the presence of small vessels extending into the vitreous (arrow), with accompanying leukocytes in the posterior vitreous. B, Glial tissue (arrow) suggests retinal proliferative changes. Cystoid edema (star) in the outer retina and thickening of the basement membrane (in the retinal vascular wall) are seen in diabetic retinopathy. C, Proliferative changes are demonstrated, with intravitreal hemorrhage (arrow) and retinal edema (star) (periodic acid–Schiff staining, original magnification $\times 200$).

perfusion greater than or equal to half a field, and 2 showed signs of neovascularization.

Compared with the continuous galactose group, the ARI-treated galactose withdrawal group showed delayed times to the first appearances of the outcomes. Areas of hyperfluorescence and nonperfusion were not observed in this group until 47 and 53 months, respectively. Unlike the continuous galactose and the galactose withdrawal groups, the ARI-treated galactose withdrawal group did not develop areas of nonperfusion greater than or equal to half a field or retinal neovascularization during the 74-month study. Of the 7 dogs in the ARI-treated galactose

Table 1. Incidence of Fluorescein Angiographic Lesions per Dog Examined*

Group and Outcome	Time After Initiation of Diet, mo											
	0	6	21	27	36	42	47	53	60	67	74	
Control group												
Any nonperfusion	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Nonperfusion \geq 1/2 field	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Neovascularization	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Continuous galactose group												
Any nonperfusion	0/11	0/11	0/11	0/11	0/11	2/10†	7/10	8/10	10/10	10/10	10/10	10/10
Nonperfusion \geq 1/2 field	0/11	0/11	0/11	0/11	0/11	0/10†	0/10	4/10	8/10	9/10	10/10	10/10
Neovascularization	0/11	0/11	0/11	0/11	0/11	0/10†	0/10	0/10	3/10	5/10	7/10	7/10
Galactose withdrawal group												
Any nonperfusion	0/8	0/8	0/7‡	0/7	0/7	1/7	4/7	5/7	6/7	6/7	5/6‡	5/6‡
Nonperfusion \geq 1/2 field	0/8	0/8	0/7‡	0/7	0/7	0/7	0/7	2/7	3/7	3/7	3/6‡	3/6‡
Neovascularization	0/8	0/8	0/7‡	0/7	0/7	0/7	0/7	1/7	1/7	2/7	2/6‡	2/6‡
ARI-treated galactose withdrawal group												
Any nonperfusion	0/8	0/8	0/8	0/8	0/8	0/8	0/7‡	1/8	2/6‡‡	4/7	4/7	4/7
Nonperfusion \geq 1/2 field	0/8	0/8	0/8	0/8	0/8	0/8	0/7‡	0/8	0/6‡‡	0/7	0/7	0/7
Neovascularization	0/8	0/8	0/8	0/8	0/8	0/8	0/7‡	0/8	0/6‡‡	0/7	0/7	0/7

Abbreviation: ARI, aldose reductase inhibitor.

*Data are given as number of dogs with outcome/total number of dogs.

†One dog died before this examination date.

‡Photographic data of 1 dog could not be graded at this examination date.

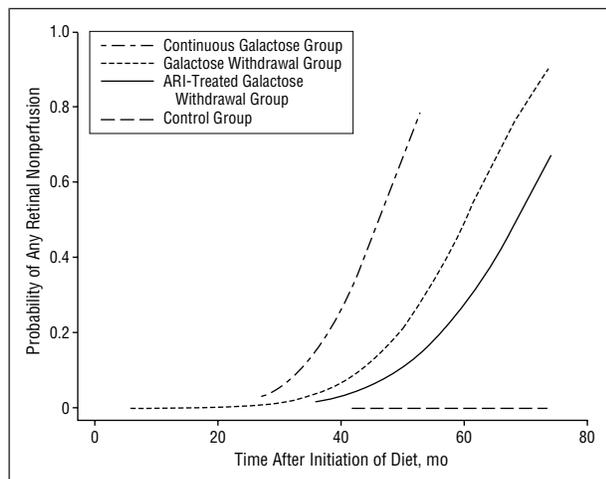


Figure 4. Estimated probability of progression to any nonperfusion in the 4 dog groups. ARI indicates aldose reductase inhibitor.

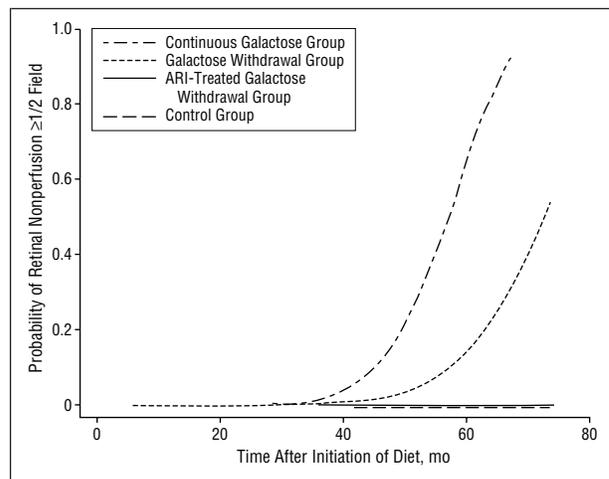


Figure 5. Estimated probability of progression to nonperfusion greater than or equal to half a field in the 4 dog groups. ARI indicates aldose reductase inhibitor.

withdrawal group examined after 74 months, only 4 showed signs of nonperfusion.

Figures 4, 5, and 6 show cumulative distribution functions for progression to any nonperfusion, nonperfusion greater than or equal to half a field, and retinal neovascularization, respectively. The median times to these outcomes, derived from the estimated Weibull survival function, are summarized in **Table 2**. Statistically significant differences in median survival times were seen between the galactose withdrawal and ARI-treated galactose withdrawal groups for the development of nonperfusion greater than or equal to half a field ($P = .003$) and neovascularization ($P = .03$), compared by data permutation. Although not statistically significant, median times to outcomes were longer for dogs in the galactose withdrawal group compared with those in the continuous galactose group.

COMMENT

The present study results illustrate that ARI treatment and galactose withdrawal ameliorate the development and progression of nonperfusion and retinal neovascularization in galactose-fed dogs assessed by fluorescein angiography. Previous studies have assessed the progression of retinal changes in galactose-fed dogs through histologic exami-

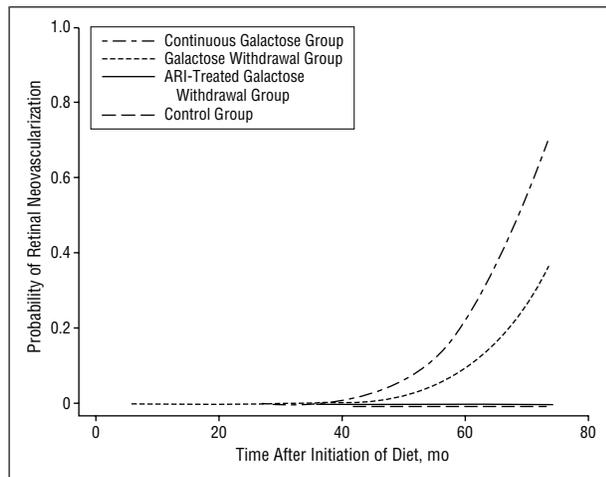


Figure 6. Estimated probability of progression to neovascularization in the 4 dog groups. ARI indicates aldose reductase inhibitor.

Table 2. Median Times to Outcomes by Study Group*

Outcome	Time to Outcome, Median, mo			
	Control Group	Continuous Galactose Group	Galactose Withdrawal Group	ARI-Treated Galactose Withdrawal Group
Any nonperfusion	NA	46.3	60.2	68.5
Nonperfusion \geq 1/2 field	NA	56.9	72.4	NA
Neovascularization	N	68.4	77.5	NA

Abbreviations: ARI, aldose reductase inhibitor; NA, not applicable (no observed outcomes).

*Median times are derived from estimated Weibull survival function.

nation of intact retinal vessels isolated by trypsin digestion. Such histologic analyses include the degeneration of pericytes to form pericyte ghost cells, microaneurysm formation, dot and blot hemorrhages, occluded arterioles, intravitreal hemorrhages, and new vessel growth.^{10,17,18} Use of ARIs has been shown to reduce retinal capillary pericyte degeneration, the formation of microaneurysms and hemorrhages, and areas of capillary acellularity associated with areas of nonperfusion in galactose-fed dogs.¹⁹⁻²³

Fluorescein angiography is used clinically to guide treatment for macular edema and to identify areas of increasing ischemia by identifying sources of fluorescein leakage and capillary loss.²⁸ In the present study, fluorescein angiographic lesions progressed from areas of hyperfluorescence to areas of nonperfusion that became progressively larger with time. The progression of retinal lesions in these galactose-fed dogs parallels the clinical course observed in human studies. The retinal lesions described by fluorescein angiography in this study are also consistent with those described in a previous study of galactose-fed dogs.²⁹

The prespecified main outcome measures in the present study were degrees of nonperfusion and the presence of neovascularization, as determined by fluorescein angiography. To confirm the angiographic findings of neovascularization, histopathologic analysis was performed on selected enucleated eyes. As expected, sections of these eyes

showed the growth of new vessels at the optic nerve head and other areas of the retina, with accompanying hemorrhage into the vitreous. These findings confirm the histopathologic evidence of the development of retinal neovascularization in this animal model.¹⁸

Although the galactosemic dog model is not a true diabetic model, all the angiographic and histopathologic findings that were evaluated during this and other studies exactly mimic the clinical progression of diabetic retinopathy in dogs and people. We believe that this is a useful animal model for evaluating the long-term effects of diabetic retinopathy because of its relative ease of use and the relatively rapid progression of the retinopathy. For this study it was particularly useful because the galactosemia could be easily reversed. The fact that human and dog diabetic studies show that retinopathy progresses after diabetes mellitus is "cured" further validates the appropriateness of the model.

The progression of retinal lesions after strict glyce-mic control was shown to be slowed in patients with type 1 diabetes mellitus in the Diabetes Control and Complications Trial (DCCT).²³ The therapeutic effect of the strict glyce-mic control became apparent after 3 years. In the present study, we instigated perfect glyce-mic control by withdrawing the galactose after a period of galactose administration in the galactose withdrawal group. Despite the removal of galactose, new fluorescein angiographic lesions developed or continued to progress after the withdrawal of galactose. The lesions observed in the galactose withdrawal group, however, neither developed nor progressed as rapidly as those observed in the group continuously fed galactose. Although the benefits of galactose withdrawal were not statistically significant, our results are similar to those of the DCCT in that after intensive therapy, the rates of development of microvascular complications were slowed. Our observation that fluorescein angiographic lesions progressed after galactose withdrawal is consistent with the progression of histopathologic lesions described in a similar group of galactose-fed dogs in which galactose administration was halted.²⁵ In another study³⁰ of galactosemic dogs, retinopathy also continued to progress despite cessation of galactosemia. The outcome measurements in this study were not fluorescein angiographic findings, but results similar to those of the present study were demonstrated.

Observations from the follow-up study of the DCCT, the Epidemiology of Diabetes Interventions and Complications (EDIC) study,³¹ showed the persisting effects of intensive glyce-mic control in patients with type 1 diabetes mellitus. After the close of the DCCT, the levels of glyce-mic control converged among individuals who were initially assigned to either intensive or conventional therapy. Despite this convergence of glyce-mic control, patients previously under intensive therapy continued to show a statistically significant reduction in the progression of retinopathy compared with the conventional group. The findings of the DCCT and the Epidemiology of Diabetes Interventions and Complications study suggest that therapeutic effects on microvascular complications take years to observe and that there seems to be a momentum of retinopathy that takes years to reverse even after controlling hyperglycemia.

It is important to address the limitations of this study. The positive results of this study may have stemmed from the following factors. The minor imbalances in mean glycosylated hemoglobin levels among the treatment groups during the study may contribute to the beneficial effects seen in the ARI-treated group. However, the differences in mean glycosylated hemoglobin levels were not statistically significant, ranging from 2.4% to 2.8%. After galactose withdrawal, glycosylated hemoglobin levels remain unchanged. The control group of dogs given continuous galactose experienced 2 additional months of galactosemia compared with animals in the galactose withdrawal groups with or without ARI administration. It is possible that the additional 2 months may have contributed to the difference in the fluorescein angiographic findings. However, the retinal lesions appeared earlier in the study. Also, it is unlikely that an additional 3% increase in galactose diet during a 6-year study would make a difference in the retinal outcome.

Much debate has surrounded the clinical importance of ARIs. The Sorbinil Retinopathy Trial Research Group³² did not find a clinically important effect on retinopathy from the administration of the ARI sorbinil during a 30-month clinical trial in humans. The relatively short duration of this clinical trial, however, did not allow for possible long-term therapeutic benefits to be observed. In the present study, the combination of ARI therapy with galactose withdrawal showed a statistically significant beneficial effect compared with only galactose withdrawal in the primary outcomes considered in this study. Although we cannot directly compare the ARI-treated galactose withdrawal group with the continuous galactose group owing to the additional benefits associated with the withdrawal of galactose in the ARI-treated group, our results suggest that ARIs, when administered during galactose feeding, may provide protection against the future development of retinal lesions.

The key findings of the present study are that glycemic control is important in slowing the progression of retinopathy and that retinopathy continues to progress for several years even after perfect control of glycemia. The results of this study support the previously reported beneficial effects of ARI therapy in delaying the onset and progression of retinal lesions in galactose-fed dogs. These results suggest a potential role for ARIs in the treatment of human diabetic retinopathy.

Submitted for publication July 2, 2002; final revision received May 28, 2003; accepted July 18, 2003.

Corresponding author and reprints: Emily Y. Chew, MD, Division of Epidemiology and Clinical Research, National Eye Institute/National Institutes of Health, 31 Center Dr, Bldg 31, Room 6A52, Mail Stop Code 2510, Bethesda, MD 20892 (e-mail: echew@nei.nih.gov).

REFERENCES

- Kahn HA, Hiller R. Blindness caused by diabetic retinopathy. *Am J Ophthalmol*. 1974;78:58-67.
- Leibowitz HM, Krueger DE, Maunder LR, et al. The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973-1975. *Surv Ophthalmol*. 1980;24(suppl):335-610.
- Klein R, Klein BEK. Vision disorders in diabetes. In: Hammon R, Harris MWH, eds. *Diabetes in America: Diabetes Data Compiled 1984*. Bethesda, Md: US Public Health Service; 1985. Publication NIH 85-1468.
- Early Treatment Diabetic Retinopathy Study Research Group. Fundus photographic risk factors for progression of diabetic retinopathy: ETDRS report number 12. *Ophthalmology*. 1991;98(suppl):823-833.
- Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. II: prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol*. 1984;102:520-526.
- Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. Glycosylated hemoglobin predicts the incidence and progression of diabetic retinopathy. *JAMA*. 1988;260:2864-2871.
- Chase HP, Jackson WE, Hoops SL, Cockerham RS, Archer PG, O'Brien D. Glucose control and the renal and retinal complications of insulin-dependent diabetes. *JAMA*. 1989;261:1155-1160.
- Kinoshita JH, Kador P, Catiles M. Aldose reductase in diabetic cataracts. *JAMA*. 1981;246:257-261.
- Robison WG Jr, Kador PF, Kinoshita JH. Retinal capillaries: basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. *Science*. 1983;221:1177-1179.
- Engerman RL, Kern TS. Experimental galactosemia produces diabetic-like retinopathy. *Diabetes*. 1984;33:97-100.
- Kern TS, Engerman RL. Hexitol production by canine retinal microvessels. *Invest Ophthalmol Vis Sci*. 1985;26:382-384.
- Akagi Y, Kador PF, Kuwabara T, Kinoshita JH. Aldose reductase localization in human retinal mural cells. *Invest Ophthalmol Vis Sci*. 1983;24:1516-1519.
- Akagi Y, Terubayashi H, Millen J, Kador PF, Kinoshita JH. Aldose reductase localization in dog retinal mural cells. *Curr Eye Res*. 1986;5:883-886.
- Hohman TC, Nishimura C, Robison WG Jr. Aldose reductase and polyol in cultured pericytes of human retinal capillaries. *Exp Eye Res*. 1989;48:55-60.
- Sato S, Secchi EF, Lizak MJ, et al. Polyol formation and NADPH-dependent reductases in dog retinal capillary pericytes and endothelial cells. *Invest Ophthalmol Vis Sci*. 1999;40:697-704.
- Murata M, Ohta N, Fujisawa S, Tsai JY, Kador PF, Sato S. Galactose induces apoptosis of dog retinal capillary pericytes, but not of endothelial cells. *J Diabetes Complications*. 2002;16:363-370.
- Takahashi Y, Wyman M, Ferris F III, Kador PF. Diabetes-like preproliferative retinal changes in galactose-fed dogs. *Arch Ophthalmol*. 1992;110:1295-1302.
- Kador PF, Takahashi Y, Wyman M, Ferris F III. Diabetes-like proliferative retinal changes in galactose-fed dogs. *Arch Ophthalmol*. 1995;113:352-354.
- Kador PF, Akagi Y, Terubayashi H, Wyman M, Kinoshita JH. Prevention of pericyte ghost formation in retinal capillaries of galactose-fed dogs by aldose reductase inhibitors. *Arch Ophthalmol*. 1988;106:1099-1102.
- Kador PF, Akagi Y, Takahashi Y, Ikebe H, Wyman M, Kinoshita JH. Prevention of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by aldose reductase inhibitors. *Arch Ophthalmol*. 1990;108:1301-1309.
- Takahashi Y, Augustin W, Wyman M, Kador PF. Quantitative analysis of retinal vessel changes in galactose-fed dogs. *J Ocul Pharmacol*. 1993;9:257-269.
- Kador PF, Takahashi Y, Sato S, Wyman M. Amelioration of diabetes-like retinal changes in galactose-fed dogs. *Prev Med*. 1994;23:717-721.
- Neuenschwander H, Takahashi Y, Kador PF. Dose-dependent reduction of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by the aldose reductase inhibitor M79175. *J Ocul Pharmacol Ther*. 1997;13:517-528.
- Engerman RL, Kern TS. Progression of incipient diabetic retinopathy during good glycemic control. *Diabetes*. 1987;36:808-812.
- Kador PF, Takahashi Y, Akagi Y, et al. Effect of galactose diet removal on the progression of retinal vessel changes in galactose-fed dogs. *Invest Ophthalmol Vis Sci*. 2002;43:1916-1921.
- Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329:977-986.
- UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998;352:837-853.
- Early Treatment Diabetic Retinopathy Study Research Group. Classification of diabetic retinopathy from fluorescein angiograms: ETDRS report number 11. *Ophthalmology*. 1991;98(suppl):807-822.
- Kobayashi T, Kubo E, Takahashi Y, Kasahara T, Yonezawa H, Akagi Y. Retinal vessel changes in galactose-fed dogs. *Arch Ophthalmol*. 1998;116:785-789.
- Engerman RL, Kern TS. Retinopathy in galactosemic dogs continues to progress after cessation of galactosemia. *Arch Ophthalmol*. 1995;113:355-358.
- Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N Engl J Med*. 2000;342:381-389.
- Sorbinil Retinopathy Trial Research Group. A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. *Arch Ophthalmol*. 1990;108:1234-1244.