Animal Models of Diabetic Complications Consortium
(U01 HL087946)

Annual Report
(2008)

"Dislipidemia, Lipoic Acid and Diabetic Vascular Complications in Humanized Mice"

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Table of Contents

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Part A:

Principal Investigator's Summary

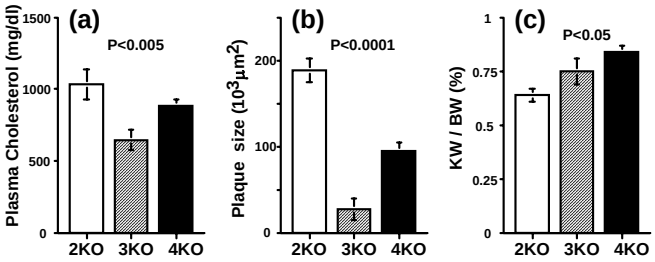
1. Program Accomplishments:

Hypothesis

1. Mice humanized lipoprotein metabolism system will develop a more human-like diabetic dyslipidemia and cardiovascular problems.
2. Genetically determined differences in the levels of endogenous antioxidants affect the development of cardiovascular complications of diabetes.

Recent Progress and Major Accomplishments

1. We made LDLR-/- male mice expressing the human apoE isoforms (apoE2, apoE3 or apoE4) in place of mouse apoE (2KO, 3KO and 4KO respectively) diabetic with STZ injection at 2 months of age and maintained them on chow diet. Plasma glucose levels increased equally. Plasma cholesterol and triglyceride levels doubled in all groups of mice. However, as in mice carrying apoE4 and wild type LDLR, the increase of plasma cholesterol (a) was more pronounced in 4KO mice than in 3KO mice ($P<0.02$). Three months after the STZ treatment, the mean atherosclerotic plaque size in the aortic sinus of the individual animals (b) was directly correlated with their plasma cholesterol levels in the order 2KO>4KO>3KO ($R^2=0.6$, $P<0.002$). Neither 3KO nor 4KO males without diabetes developed significant atherosclerosis at this age. These results suggest that the contribution of apoE4 to the enhancement of atherosclerosis by diabetes is greater than that of apoE3.

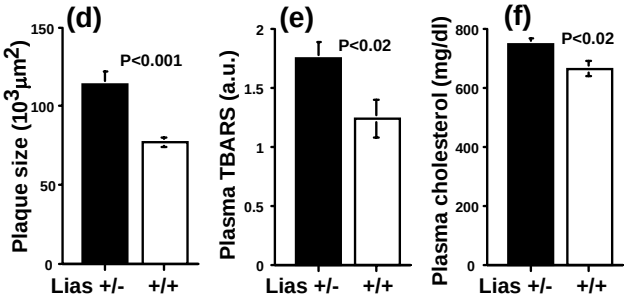


Interestingly, the body weight of the diabetic mice was larger in the order of 2KO>3KO>4KO, but the kidney weight was in an inverse order. Consequently, kidney/body weight (c) was significantly larger in 4KO>3KO>2KO. This suggests a possibility that apoE4 also enhances diabetic kidney damage.

Despite the clear demonstration of an increased risk of cardiovascular diseases in patients with diabetes, the results of studies looking for any influences of the human apoE isoforms on the risk of diabetic complications have been inconclusive, with some suggesting that individuals with apoE4 have an increased risk with others suggesting that apoE2 confers an increased risk. Our finding that LDLR-/- mice expressing apoE4 are more susceptible to diabetes-enhanced dyslipidemia and atherosclerosis than LDLR-/- mice with apoE3 is therefore significant. We will test the hypothesis that less effective clearance and lower antioxidant capacity of apoE4-VLDL than apoE3-VLDL contribute to the enhancement of diabetic complication.

2. We have previously shown that atherosclerosis in Apoe-/- mice is enhanced by diabetes, but a dietary supplement of lipoic acid (LA), a strong natural antioxidant, effectively prevents this enhancement. We also have found that the mean atherosclerotic plaque sizes were smaller by about 40% in non-diabetic ApoE-/- mice fed for four months with LA in diet (1.65 g/Kg). Plasma lipid levels were not affected by the LA in diet, but generalized oxidative stress, measured by the plasma TBARS and erythrocyte GSH, was significantly reduced. While studies addressing the effects of dietary antioxidants on atherosclerosis have given mixed results, LA clearly has protective effects.

LA is naturally synthesized in all cells of the body and is essential for metabolism. Mice lacking the key enzyme for LA synthesis (lipoic acid synthase, Lias) die in utero.



To investigate whether a 50% reduction in *Lias* gene expression accelerates the development of atherosclerosis, we produced Lias+/-Apoe-/- mice on a pure 129/SvEv background. The average size of atherosclerotic plaques in the aortic sinus (d) of the 6 months old Lias+/-Apoe-/- mice (114±8 x$10^3\mu m^2$, n=25) was about 50% larger than that in Lias+/+Apoe-/- mice (77±3 x$10^3\mu m^2$, n=25, P<0.01), indicating that the level of endogenous LA production is an important factor for athero-protection. TBARS in the plasma (e) as well as TBARS in the VLDL fraction of Lias+/-ApoE-/- mice were also higher by about 40% (p=0.02), although the distribution of plasma lipoproteins was not different. Plasma cholesterol levels in the Lias+/-Apoe-/- mice (f) were not different at 4 months of age, but were elevated at 6 months of age. The difference was small but significant (745±20 mg/dl, n=25 vs 658±15 mg/dl, n=25, P<0.02). Interestingly, we found that the levels of mRNA for LDLR in the liver of Lias+/-Apoe-/- mice were 20% those of the Lias+/+Apoe-/- mice (P<0.03). mRNA for CYP7α was not different between the two groups. STZ treatment of Lias+/-ApoE-/- mice has been initiated.

3. We have mated Lias+/- mice on 129/SvEv genetic background with Akita mice on B6 background to examine the effects of a 50% reduction in *Lias* gene expression on the complication of diabetes. At 6 months of age, body weight, organ weight, urine volume, food intake, and plasma lipids were not significantly different between Lias+/+Akita and Lias +/-Akita mice. Plasma glucose levels, in contrast, were significantly higher in Lias+/-Akita mice (596±19mg/dl, n=12) than in Lias+/+Akita mice (524±20 mg/dl, n=5, P<0.05). Urinary albumin excretion was also tended to be higher in Lias+/-Akita mice (114±16 μg/day, n=12 vs 61±16 μg/day, n=5, P<0.06). As in non-diabetic mice, generalized oxidative stress measured by GSH levels and GSSG/GSH ratios in red cells was slightly higher in Lias+/- Akita mice than Lias+/+Akita mice, although the differences were not statistically significant. Under the light microscopy, kidneys of Akita mice exhibited mesangial expansion, but *Lias* genotype effects were not evident. Clearly we need to increase the number of animals, but these preliminary data suggest that a small change in *Lias* expression may not influence the diabetic kidney pathology, because increase in ROS production and down-regulation of the *Lias* gene expression (to 20% normal) caused by diabetes are both overwhelmingly large factors.

Plans for the Upcoming Year

1. We will continue to study the diabetes-induced dyslipidemia and atherosclerosis in STZ treated LDLR-/- mice. We have crossed Akita mice with mice carrying human apoE isoforms. These mice will be used to address the role of human apoE isoflrms in diabetic dyslipidemia and nephropathy.
2. We will complete the experiments assessing the effects of a 50% reduction of the *Lias* gene expression on diabetes-induced atherosclerosis using the STZ-treated Lias+/-Apoe-/- mice.
3. We expect to obtain the Lias-H mice carrying a modified Lias gene. The first experiment is to test whether our overall scheme has worked as planned by crossing the mice with mice that express Cre-recombinase in germ-line and consequently will produce Lias-L mice. We expect that unstable transcript from the Lias-Low allele reduces steady state levels of mRNA to about 10%-50% normal. We will then generate a series of mice with Lias genotypes, L/-, L/L, L/+, -/+, +/+. H/+, H/H, that have graded expressions of Lias, and likely of endogenous LA synthesis, and examine the effects of the titrated

change in the Lias gene. Although homozygous Lias-KO mice die early during the embryonic development, we expect that the Lias-L mice will survive but will be highly susceptible to oxidative tissue damage. These mice will be crossed with Akita mice to make them diabetic.

2. Collaboration:

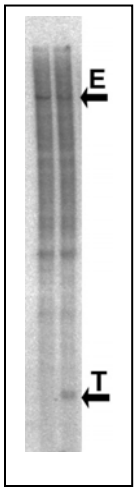
With other AMDCC PIs

None at this moment.

With Jax

4. In collaboration with JAX we have begun the generation of a new model that combines diabetes with conditionally decreased antioxidant defense: mice with more than 50% reduction of Lias.

During the past year, we successfully modified the *Lias* gene in the B6 ES-cell line so that the targeted *Lias* gene will initially produce stabilized transcripts of the gene using the 3'UTR sequence of bovine growth hormone, but will change to produce unstable transcripts using 3'UTR from the cFos gene after Cre-mediated recombination has been induced. Southern blotting confirmed the targeted modification of the Lias gene in the ES cells by the presence of 1.8kb BamH1 fragment (T) accompanied with the reduction of the intensity of 13kb endogenous band (E).



Several chimeric mice have been made with three of the modified ES cell lines. The chimeras have begun to produce offspring but so far no transmission of the ES cell genome has been attained. Nevertheless, considering the strong coat color chimerism of these males, we are confident that the mouse line carrying the modified gene (Lias-H) can be obtained in the reasonably near future.

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With the MMPCs

Not at this moment.

With other non-AMDCC Pis

none

3. Address previous EAC comments:

a. *Good progress. What level of conditional expression are you aiming for?*

Based on the experiments in cultured cells, we expect the steady state levels of Lias-Low transcripts will be less than 10% normal. However, due probably to homeostatic responses, the reduction will be less in vivo in animals, and we expect the mRNA levels in L/L homozygotes will be somewhere 30-50% normal. Further 2-fold reduction will be expected in L/- heterozygotes.

b. *The work on a humanized lipid profile is one of the essential ingredients to developing a good mouse model for diabetic atherosclerosis.*

We are currently increasing the number of diabetic mice carrying human apoE isoforms, and collecting data from them.

c. *Interesting finding regarding the Gulo-/-ApoE-/- mice not presented at the meeting. Offers some explanation for some of the negative results in clinical trials regarding antioxidants such as vitamin C. These mice will be a valuable tool when studying combinations of other endogenous antioxidant systems.*

We are maintaining the *Gulo-/-Apoe-/-* mice in our colony. Because of the complexities of introducing other mutations, we have not planned to make further crosses. However, we agree that atherosclerosis in *Lias+/-Gulo-/-Apoe-/-* mice, for example, will be worthy of being characterized.

d. *Since Lias-/- mice die in utero, the group has produced the construct for a conditional knockout of Lias and this has been sent to JAX. This will be an important model for all aspects of CV phenotyping (as well as other complications associated with oxidative stress - renal, neurological, urological). This is especially true given the observations that Lias+/-ApoE-/- develop more atherosclerotic lesions than Lias+/+ animals.*

We agree. We have begun investigating the enhancement of atherosclerosis by STZ- induced diabetes in these mice.

e. *Preliminary results were obtained with mice expressing human apoE isoforms. Appropriately, experiments are being repeated with larger animal numbers to clarify the role in atherogenesis. In addition to lesion size, measures should include complexity and extent (distribution) of lesions. Also, it may be instructive to evaluate coronary lesions.*

All our atherosclerosis study will evaluate the size, complexities and distribution of plaques in comparison to non-diabetic controls. Most of our study is in relatively young mice or short time with uncontrolled diabetes (3-5 months), and their plaque development is often limited to the aortic sinus. However, as we increase the durations, plaque distributions throughout the aorta become more important. Quantifying the development of atherosclerosis in coronary vessels in mouse models will be tedious and not reliable at present. If we detect a sign of acceleration in any of our models, however, we agree with the Advisory Committee that the effort to develop a proper procedure is important.

4. Publications: none

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Part B:

Update by Individual Project Leaders
(if applicable)

Project 1 (if applicable): "Title"

Responsible Investigator: Name

1. Project Accomplishments:

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Plans for the Upcoming Year

Preliminary Milestones for 2009 and Beyond

2. Collaboration:

With other AMDCC PIs

With Jax

With the MMPCs

With other non-AMDCC PIs

3. Publications:

Please list

Project 2 (if applicable): "Title"

Responsible Investigator: Name

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Hypothesis

Recent Progress and Major Accomplishments

Plans for the Upcoming Year

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3. Publications:

Please list

Project 3 (if applicable): "Title"

Responsible Investigator: Name

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