CBI

Atherosclerosis Models

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COMPARATIVE BIOSCIENCES, INC.
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

COMPARATIVE BIOSCIENCES, INC.

Premier Preclinical Contract Research Organization

- **Over 20 years** of experience
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- **Highly experienced staff**
- **GLP, OECD, FDA, USDA, OLAW**
- **AAALAC Accreditation**

Atherosclerosis Models

- Comparative Biosciences is a leading expert in atherosclerosis modeling in ApoE mice, rats, hamsters, watanabe rabbits and minipigs.

- CBI provides a number of well characterized animal models in the area of atherosclerosis:
  - Progression Models in ApoE Mice
  - Regression Models in ApoE Mice
  - Hyperlipidemia in rabbits
  - Hyperlipidemia in guinea pigs
  - Hyperlipidemia in hamsters

Short Term Atherosclerosis Models

- For **assessment of dyslipidemia using a hamster model**
  - 10 days of 0.25 % cholesterol diet
  - followed by test compound dosing for 1, and 3-5 days

- For **assessment of early atherosclerosis progression using a mouse ApoE knockout model:**
  - 2 weeks of an atherogenic diet to initiate the disease
  - then treatment with the atherogenic diet plus compound for 2 weeks to see if the compound has any therapeutic effect

Long Term Atherosclerosis Models

- For the **assessment of treating progression of atherosclerosis using a mouse LDL knockout model**
  - 8 weeks of a HF diet to establish the disease
  - then treatment of a high fat diet plus compound for 8 weeks to see if the compound has any therapeutic effect
- For the **assessment of treating progression of atherosclerosis using a mouse ApoE knockout model:**
  - 6 weeks of a HF diet to establish the disease
  - then treatment of a high fat diet plus compound for 6 weeks to see if the compound has any therapeutic effect

Typical Study Read-Outs

- Clinical observations, body weight, food consumption
- Plasma, serum and/or urine analysis for total cholesterol, LDL, HDL and triglyceride levels
- Plasma PK assessment
- Plasma/Serum enzymatic activities
- Liver weights/pathology
- Histology
  - sectioning of the aortic sinus
  - longitudinally opened whole mounts of the aorta from arch to iliac bifurcation to measure lesion area and determine % of total aortic atherosclerosis, respectively)
- Digital Image Analysis utilizing MOVATS or oil red O.

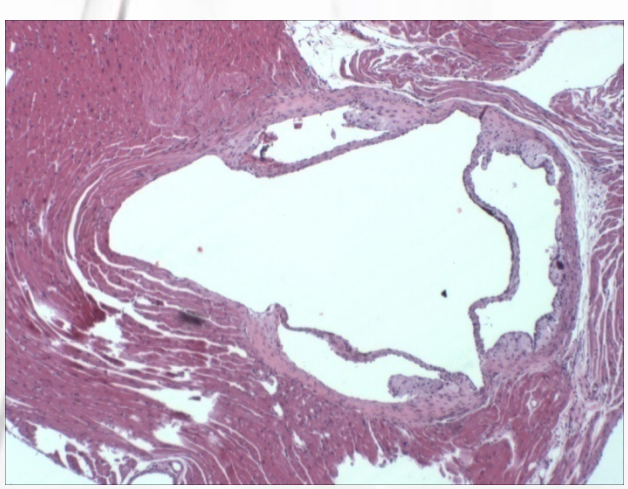
Histopathology

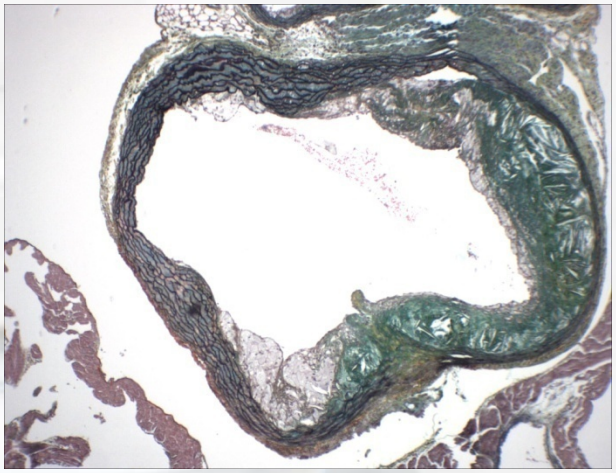
- Histopathology of aortic sinus is the key parameter in assessment of success in this model
  - Modified Paigen Technique
  - Following trimming, dehydration, and processing, the heart and the attached aorta oriented, embedded in paraffin and stained with HE, Movat's or Trichrome.
  - Aortic sinus serial sectioned from sinus root (base of valves)
    - serially sectioned in 5 mm sections from the beginning of the sinus (reference point) where the prominent valves were first visible in cloverleaf-shaped pattern
  - Analysis of plaque formation and comparison between groups

Analytical Assessments

- Specified levels measured either with an eyepiece reticle or by digital image analysis.
- For screening or pilot studies, plaque may be semi-quantified using a 0-4 scale
- Plaque area, lumen area, area of internal elastic lamina entered on spread sheet and relevant calculations performed
- Calculations – not limited to:
  - Plaque Area Ratios
  - Lumen: IEL ratio
  - Percent occlusion
  - “Plaque Burden” Calculations

Sequence of Plaque Formation

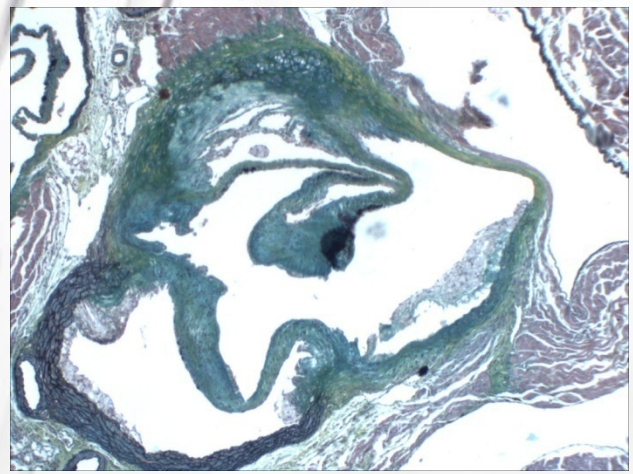


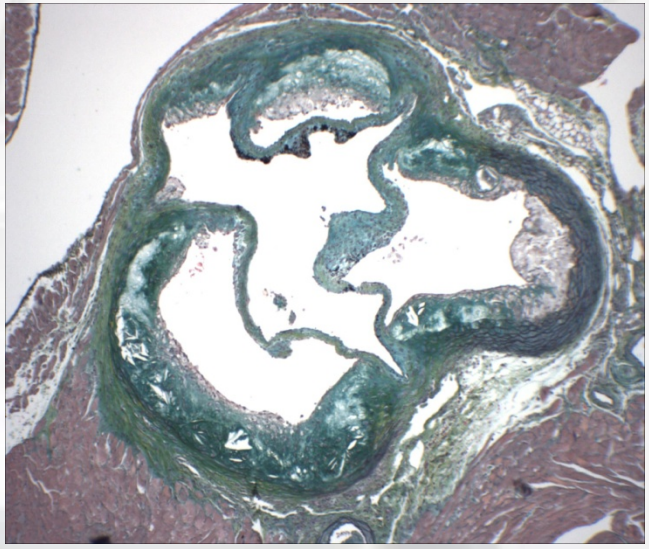


- Top: Plaque formation at 3 weeks. Small accumulations of foamy macrophages multifocally on endothelium (HE)

- Bottom: Plaque formation at 16 weeks. Abundant plaque formation over most o f the lumen surface. There is smooth muscle hypertrophy, large foamy macrophages, cholesterol clefts and mineralization (MP)

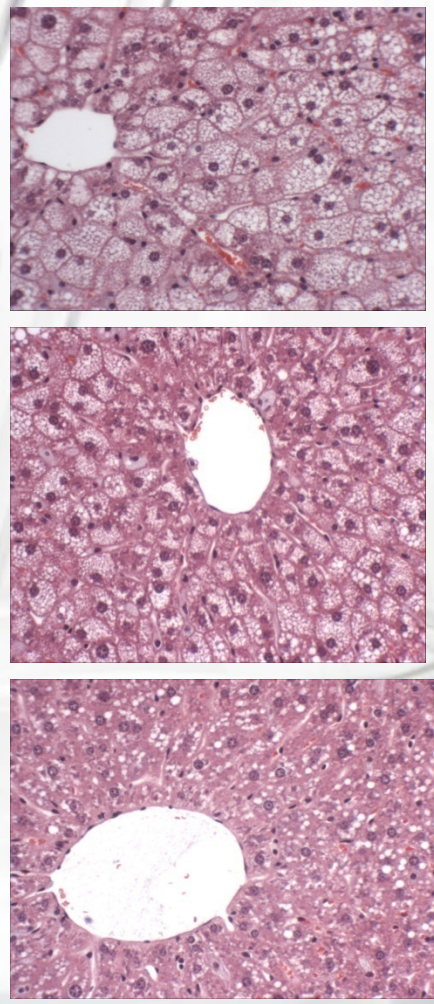
Typical Results: Histopathology – Plaque Formation





- Top: Plaque at 16 weeks, no treatment demonstrating abundant plaque formation
- Bottom: Plaque at 16 weeks, Simvastatin showing slight reduction in plaque formation

Typical Results: Histopathology –Hepatic Lipidosis



- Top: Vehicle Treated: Marked hepatic lipidosis in centrilobular area
- Middle: Low Dose Treated Moderate to marked hepatic lipidosis in centrilobular area
- Lower: High Dose Treated Significantly reduced hepatic lipidosis in centrilobular area

Typical Results: Data

SAMPLE NUMBER	CHOL (MG/DL)	TRIG (MG/DL)	HDL (mg/dL)	LDL (mg/L)	Histologic Hepatic Lipidosis	Plaqye Burden	Aortic Severity
101	2019	210	655.8	1965.6	4.0	403836.0	4.0
102	1890	200	701.0	1698.0	4.0	443725.2	4.0
103	2210	200	782.0	2033.0	4.0	502761.6	4.0
104	2160	190	812.0	2028.0	4.0	493239.6	4.0
105	2222	180	814.0	2099.0	4.0	395163.0	4.0
106	1146	110	235.0	1381.0	3.0	403836.0	3.0
107	2410	220	891.0	2236.0	4.0	443725.2	4.0
108	2260	200	785.0	2126.0	4.0	403836.0	4.0
109	2140	110	859.0	2214.0	4.0	443725.2	4.0
110	2089	210	794.0	1999.0	4.0	403836.0	4.0
	2054.6	183.0	732.9	1978.0	3.9	433768.4	3.9
	348.7	40.0	187.8	258.1	0.3	38970.8	0.3
201	1590	110	540.0	1518.0	4.0	326604.6	4.0
202	2160	250	754.0	1952.0	4.0	421824.6	4.0
203	2210	210	786.0	908.0	3.0	326604.6	3.0
204	2170	230	695.0	2047.0	4.0	421824.6	4.0
205	1920	280	685.0	1762.0	4.0	326604.6	4.0
207	2410	320	748.0	2197.0	4.0	421824.6	4.0
208	2130	230	728.0	1925.0	4.0	326604.6	4.0
209	2245	240	768.0	1825.0	4.0	421824.6	4.0
210	2311	211	734.0	1100.0	3.0	326604.6	3.0
	2127.3	231.2	715.3	1692.7	3.8	368924.6	3.8
	242.1	57.3	73.2	436.0	0.4	50185.3	0.4
301	467	190	867.0	798.0	2.0	326604.6	2.0
302	1356	150	804.0	1863.0	3.0	421824.6	3.0
303	786	113	770.0	1332.0	3.0	326604.6	3.0
304	1789	310	947.0	533.0	2.0	421824.6	2.0
305	1340	220	199.0	1150.0	3.0	326604.6	3.0
306	1400	300	349.0	1446.0	4.0	421824.6	4.0
307	1920	102	565.0	1715.0	3.0	326604.6	3.0
308	570	100	222.0	523.0	2.0	421824.6	2.0
309	456	90	224.0	335.0	2.0	326604.6	2.0
310	356	89	190.0	467.0	2.0	421824.6	2.0
	1044.0	166.4	513.7	1016.2	2.6	374214.6	2.6
	584.7	85.2	310.0	557.5	0.7	50185.3	0.7

Sample Study Data

ApoE Progression Studies

Typical Results: ApoE Progression Study

- Weight Gain
- Greasy coat
- Increased cholesterol and HDL, LDL, triglycerides
- Increased liver weights
- Marked hepatic lipidosis
- Progressive plaque formation in the aortic sinus area

ApoE Progression Study with Simvastatin

- Study Parameters:
  - ApoE female mice, on high fat diet for 8 weeks
  - 2 Groups: vehicle (saline) and Simvastatin, 20 mice/group
  - Animals dosed by oral gavage for 8 weeks starting same day as high fat diet
- Assays
  - Histopath: heart, aorta, aortic sinus, brachiocephalic artery
- Results
  - Treatment with Simvastatin for 8 weeks resulted in ~12% decrease in aortic plaque formation.

Group	Animal ID	Test Article	% Occlusion of Vessel Lumen
1	101-110	Vehicle	**43+/-6.6**
2	201-210	Simvastin	**35+/-2.1**

ApoE Progression Study with TA

- Study Parameters
  - ApoE male mice, on high fat diet for 8 weeks
  - 4 groups, 10 mice/group
  - Groups: vehicle, low dose, high dose, positive control drug (not identified by Sponsor)
- Assays
  - Daily clin obs, weekly body weights
  - Measure cholesterol, triglycerides
  - Histopath: liver, heart, aorta, aortic sinus, brachiocephalic artery
- Results
  - The positive control article (supplied by the Sponsor and the high dose test article caused a slight reduction in liver lipidosis, serum cholesterol and triglycerides, and aortic plaque formation

Group	Animal ID	Test Article	Lipidosis	Triglycerides	Cholesterol	Vascular Plaque (Combined Score)	% Occlusion of Vessel Lumen
1	101-110	None	4+	3+	3+	4+	**41+/-2.8**
2	201-210	Low	4+	3+	3+	4+	**44+/-3.2**
3	301-310	High	3+	3+	3+	3+	**33+/-4.6**
4	401-410	Positive Control	2+	2+	2+	2+	**29+/-5.8**

Summary

- CBI is experienced in conducting atherosclerosis models including ApoE progression and regression models:
  - Models have been run consistently for over 13 years
  - An experienced SD is assigned
  - Protocol Driven
  - Histopathology-ACVP pathologist
  - High quality reports
  - Prompt time lines

Service and Quality

- ***Thoroughness in planning and execution is key to a successful study***. All protocols are vetted and approved by multiple personnel. Our QAU has a rigorous training program. All non-GLP studies are conducted in the spirit of GLP.
- ***We believe in sound science.*** Our ratio of scientists to non-scientists is one of the highest in the industry. Every study director is a PhD-level scientist.
- ***We believe in communication.*** Timely responses to your inquiries and frequent updates on your study are mandatory.
- ***We welcome visitors.*** You are always welcome at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.