Version 1

UC Davis - Microvascular Permeability and Lipoprotien Flux V.1

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Abstract

Summary:

One of the three indices of arterial function that are compromised to a varying degree in individuals with cardiovascular disease is vascular permeability. This assay measures vascular permeability (as flux of labeled large molecular weight molecules: i.e. albumin or dextran) and lipid permeability (as flux of labeled lipid) in coronary or carotid arteries.

Materials

MATERIALS

- Krebs-Henseleit Solution **Catalog #**See Below
- FITC- Dextran **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**FD4, FD40S, or FD70
- TRITC- Dextran **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T1037 or T1162
- FITC- Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9771
- TRITC- Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A2289
- Alexa-546 label **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**10237
- DiL labeled Lipid **Catalog #**See protocol
- pentobarbital **Cardinal Health**
- DMEM **Invitrogen - Thermo Fisher Catalog #**11885
- DPBS **Invitrogen - Thermo Fisher Catalog #**14190
- formaldehyde **Fisher Scientific Catalog #**F79

Reagent Preparation:

Reagent 1: 10 % formaldehyde
Formaldehyde (Fisher) is diluted to 10% in DPBS (Invitrogen)

Reagent 2: Krebs-Henseleit Solution
116 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2*H_2O$, 1.2 mM $MgCl_2$, 1.2 mM NH_2PO_4, and 11mM glucose

Troubleshooting

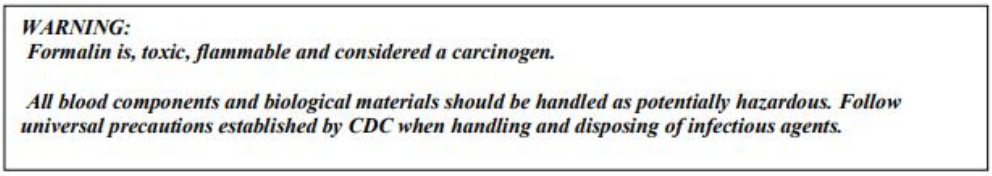
Safety warnings

WARNING:

Formalin is, toxic, flammable and considered a carcinogen.

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

Before start



1 Mice are anesthetized with an intraperitoneal injection with 50 mg pentobarbital/kg weight.

2 All treatments are administered into the left femoral vein by bolus injection. FITC-albumin (40 mg/mL) in 100 µL:
   a. PBS
   b. VLDL (150 mg/dL)
   c. VLDL (150mg/dL) + LpL (2 U/mL)
   d. LpL (2 U/mL) in PBS

3 Alternatively, the mouse is then infused at with 100 uL fluorescently labeled compound alone (FLC, see above) (40 mg/mL)

4 Excess FLC was removed from the vasculature by infusion with DMEM media for 20 min by infusion into the left ventricle of heart and followed by infusion of 10% formaldehyde for 20 min.

5 The microvascular rich tissues interest are immediately removed and fixed in 10% formaldehyde for two days.
   a. microvascular tissues = brain, heart, and mesentery ect.
   b. macrovascular tissues= common carotid arteries or aorta

6 The tissue is embedded in paraffin and sectioned to 5 µm thickness.

7 Tissues sections are deparaffinized, rehydrated, and imaged using fluorescent microscopy.