Thrombin-induced Increases in Vascular Permeability in Pressurised Human Arteries: a Novel Optical Method for Isolated Tissues

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Background

Measurements of vascular permeability have typically been conducted using in vivo animal models such as the Evans blue dye (EBD) model, where extravasation of the dye into the wall of blood vessels is used as a marker of vascular permeability. Such models suffer from disadvantages including potential species differences and an inability to track changes in real-time. In addition currently available cell-based assays, even if using human cells, might not reflect the true 3-D architecture of human blood vessels and are not therefore entirely satisfactory. The use of fresh isolated human blood vessels overcomes many of these problems. Biopta’s PM-1 instrument allows continuous monitoring of EBD extravasation in isolated human or animal blood vessels.

Methods

Human small subcutaneous arteries (intraluminal diameter 439±47 µm) were obtained with informed consent from patients undergoing cosmetic procedures. Isolated arteries were dissected free from surrounding tissue, cut into rings of 2-4 mm lengths and mounted on the PM-1 perfusion myograph (Biopta Ltd) bathed in physiological saline solution (PSS) gassed with 95% O₂/5% CO₂, and heated to 37°C. After a 30 minute warm up period, vessels were pressurised to an intraluminal pressure of 60 mmHg at a flow rate of approximately 0.05 ml/min. Changes in vessel diameter (intraluminal and outer diameter) and wall thickness in response to intraluminal perfusion of 62.5 mM KCl or 10-5 M ACh were continuously recorded using Per-Exion software (Biopta Ltd). Changes in vascular permeability in response to 0.5 units/ml of thrombin were assessed through the movement of bovine serum albumin-conjugated Evans blue dye from the intraluminal perfusate into the wall of the vessel using image analysis methods.

Results

Intraluminal perfusion with KCl (62.5 mM) caused a reduction in diameter of 152±8 µm corresponding to a 42±17% constriction in arteries pressurised to 60 mmHg. Perfusion intraluminally with ACh resulted in 75±31% relaxation of all KCl-constricted arteries, demonstrating that the arteries were functional and had an intact endothelium. Washing the vessels with PSS resulted in a further relaxation back to pre-constricted baseline levels.

The small molecule Evans Blue Dye (EBD) was conjugated to bovine serum albumin (BSA) at a ratio of 10:1 (as used in previous studies). At this ratio Evans blue dye does not readily pass through the endothelial layer of unstimulated blood vessels and in the absence of BSA there is only minimal uptake of the free dye.

Conclusions

Vascular permeability was quantified by indirectly measuring the extravasation of Evans blue dye (EBD) into the wall of human small subcutaneous arteries. Thrombin is known to induce vascular leakage in arteries and here we observed that EBD uptake into the artery was increased in the presence of 0.5 Units/ml thrombin.

Changes in vascular permeability are typically measured in vivo in animals following injection of EBD into the bloodstream (where only one time-point per animal is feasible). This new in vitro method provides continuous real-time measurement of vascular permeability in isolated human or animal tissues and represents a novel alternative to in vivo methods.

References