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Fluorescent Microscopy Imaging with DAPI stain to assess the endothelial Integrity in Goat Artery Strip

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Abstract

Isolated blood vessel experiments are commonly done to understand the dynamic functions of vascular smooth muscle, pharmacology of various receptor subtypes and influence of the endothelium on vascular biology. Experiments can be done either with endothelium-intact or with denuded vessel strip, as required. Since vascular biology can be modulated by the endothelium, it is necessary to confirm the presence or absence of endothelium in an endothelium-intact or denuded vessel respectively. To achieve the above strategy, isolated arterial strips from goat (*Capra hircus*) leg was segregated into a control group with intact-endothelium & other group with endothelium removed. After fixation, paraffin blocks were made with impregnated tissue & tissue sections were made using Leica microtome, which were then stained with DAPI (4',6-diamidino-2-phenylindole), a nuclear stain and images were taken under fluorescent microscope. Results show a layer of nuclei in endothelium-intact vessel and not seen in endothelium-denuded vessel, confirming the presence and absence of endothelium respectively. Hence,fluorescent microscopy imaging of tissue section with DAPI stain will be a reliable method to assess the endothelial integrity in blood vessel.

Keywords: Fluorescent microscopy,Goat artery,DAPI stain, Endothelium

Introduction

Isolated blood vessel experiments are commonly used by both Physiologists & Pharmacologists to study about the receptor types, actions of various drugs on receptors, dose-response curve for specific drugs etc. Such isolated tissue organ bath experiments are in vogue for more than 100 years due to its simplicity and easy reproducibility of the results. Arterial strips with intact or removed endothelium can be used depending on the experimental requirements. Careful isolation of the artery with minimal manipulation is required to preserve the endothelial integrity (1).Histology of blood vessel shows three layers from inside to outside as follows: tunica intima, tunica media & tunica adventitia. Tunica intima consists of a layer of endothelial cells, supported by internal elastic lamina, tunica media has smooth muscle cells, elastic fibres, connective tissue and tunica adventitia is composed of nutrient vessels,nerves &connective tissue. The media is separated from the adventitia by external elastic lamina.(2).Results of isolated blood vessel experiments can be influenced by the endothelium, as various substances like nitric oxide are released from the endothelium. Some experiments require endothelium denuded vessel as well.Various methods are used for removing endothelium in a blood vessel such as cotton swab method and removal of endothelium is confirmed by the lack of relaxant response to acetylcholine.

Aim of the current study is to confirm the presence or absence of endothelium in endothelium-intact or denuded vessels respectively, by fluorescent microscopy with DAPI stain.

Objective of the study is to have a control group with intact endothelium and a test group with denuded endothelium and to assess the endothelial integrity using fluorescent microscopy with DAPI (4',6-diamidino-2-phenylindole) stain.

Materials and Methods

A medium-sized artery was isolated from fresh goat (*Capra hircus*) leg, procured from the slaughter house. Endothelium was removed, when required, by rubbing the lumen with a polished dried stalk of the coconut leaves (*Cocos nucifera*) and then the lumen was flushed with distilled water for two minutes. Small pieces are made from both endothelium-intact & removed arterial strip, which was then separately preserved in 10% buffered formalin for 48 hours at room temperature for fixation.Further processing of tissue was same for both endothelium-intact & removed vessel.The tissues were then dehydrated with ascending grades of alcohol and cleared with xylene, which was then embedded in liquid paraffin wax. Paraffin blocks were made with the tissue impregnated within it.

Tissue sections of 5µM size were made from the paraffin blocks using Leica microtome, which was then picked up using a forceps and made to float on the surface of clean glass slide in a water bath at 55°C. The paraffin blocks & glass slides were labeled separately as endothelium-intact & removed tissue for identification. The slides with paraffin tissue sections were then kept at 65°C for about 30 minutes, so as to melt the wax and to fix the tissue on the glass slide. Tissue sections were dewaxinated with xylene and then hydrated with descending grades of alcohol, washed with distilled water, followed by phosphate buffered saline (PBS).The slides were then stained with DAPI for 3 to 5 minutes and then washed with PBS for 2 minutes. The slides were mounted with 90% glycerol and images were taken under fluorescent microscope.

Results

Fluorescent microscopy images with 20X magnification show a layer of nucleus in endothelium-intact tissue and are not seen in endothelium-denuded tissue (Fig: 1 & 2).

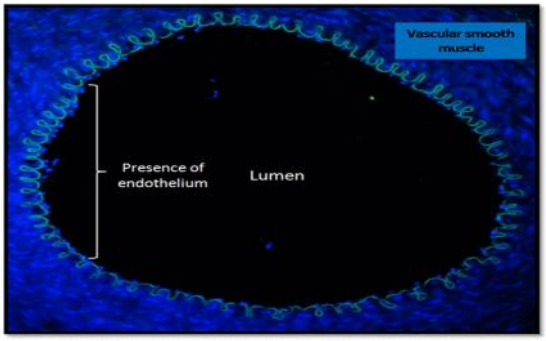

Fig1:Fluorescent microscopy image of an endothelium-intact blood vessel showing a layer of nucleus lining the internal elastic lamina (n = 3)

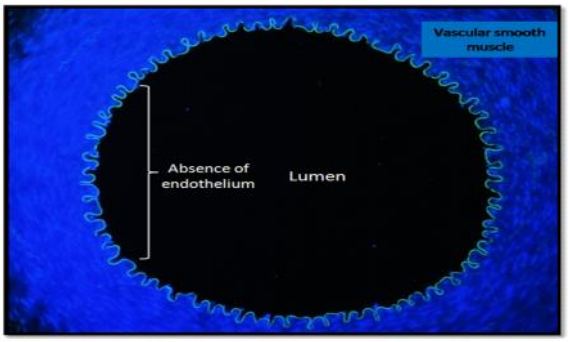

Fig 2:Fluorescent microscopy image of an endothelium-denuded blood vessel showing the absence of nucleus (n = 3)

Role of endothelium on a particular outcome can be studied with both endothelium-intact and denuded vessel in an isolated tissue organ bath experimental setup. As various chemical substances released from the endothelium might influence the results of experiments, it is necessary to confirm the absence of endothelium in a denuded vessel.

DAPI is a fluorescent stain which has a high affinity to A-T rich sequences of DNA, which stains both live and fixed cells. It is used to determine the number of nuclei and to look for the gross morphology of cell (4,5).Results of the current experiment with DAPI stain clearly shows the absence of endothelial cells in the endothelium-denuded group, when compared to the control group. The result also shows the efficacy of the method for removing endothelium used in the current study.

Conclusion

Endothelial integrity of the blood vessel can be confirmed by fluorescent microscopy imaging with DAPI stain.

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