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Detection of thrombosis in microvessels with indocyanine green videoangiography

T. Mücke [a,*], C. Wolff [b], A.M. Fichter [b], M. von Düring [c], A. Kanatas [d], L.M. Ritschl [b]

[a] *Department of Oral and Maxillofacial Surgery, Malteser Kliniken Rhein-Ruhr, Krefeld-Uerdingen, Germany*
[b] *Department of Oral and Maxillofacial Surgery, Klinikum rechts der Isar, Technische Universität München, Germany*
[c] *Department of Neuroanatomy, Ruhr University, Bochum, Germany*
[d] *Leeds Teaching Hospitals, St James Institute of Oncology and Leeds Dental Institute*

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Abstract

Atherosclerosis is a systemic condition that is responsible for many diseases, and becomes a problem in cases where plaques form at several sites. The formation of a thrombotic embolus may jeopardise vascular operations, including microvascular anastomoses in replantation procedures or free tissue transfers. A mobile imaging tool for the detection of thrombosis preoperatively or intraoperatively would be valuable. An intimal injury, simulating removal of atherosclerotic plaques, was made microsurgically in 60 rat aortas, and results were analysed macroscopically, histologically, and with intraoperative indocyanine green (ICG) videoangiography immediately postoperatively. The Spearman and Pearson correlation tests were used to compare the three techniques. The sensitivity and specificity of ICG videoangiography was calculated in relation to both macroscopic and histological results. Detection of thrombosis was possible in 25 cases, and in 18 cases no thrombosis was correctly diagnosed by all methods used. In 31 of 60 specimens formation of thrombus was detected histologically, and in 29 of 60 examinations it was detected clinically, which yielded a correlation of 93.5% between the two examinations. Macroscopic analysis correlated better with ICG videoangiography (sensitivity 86.2% and specificity 64.5%) than histological observations (sensitivity 80.6% and specificity 62.1%). There was a significant correlation among all comparisons (each $p \leq 0.001$) with correlation indexes of 0.94, 0.52, and 0.44 for macroscopic/histological, clinical/ICG videoangiographic, and ICG videoangiographic/histological results, respectively. Our results show that ICG videoangiography is an important method for the detection of formation of acute thrombi and may be an important tool in vascular procedures.

Keywords: Indocyanine green videoangiography; prediction of thrombi; endothelial defects

Introduction

Microvascular techniques have become routine in reconstructive surgery,[1] but despite their universal integration into clinical practice there remain some limitations and drawbacks. Systemic vascular disease, which can affect both the donor and recipient vessels in microvascular tissue transfers,[2,3] is often regarded as a relative contraindication to microvascular surgery because of the presence of atherosclerosis. Typically, plaque is seen as a focal deformation of the intima with the accumulation of lipids, carbohydrates, blood products, fibrous tissue, and calcium.[4] These lesions increase in size and tend to spread with age, and they need to be factored into the choice of techniques, planning, and expected complications.[2,3,5] Selection of patients, microsurgical technique used, choice of an adequate flap, and postoperative care are also important in these patients.[2] Injuries to the arte-

* Corresponding author.
E-mail addresses: th.mucke@gmx.de (T. Mücke), constantin.wolff@mytum.de (C. Wolff), andreas.fichter@tum.de (A.M. Fichter), monika.u.duering@ruhr-uni-bochum.de (M. von Düring), a.kanatas@doctors.org.uk (A. Kanatas), lucas.ritschl@tum.de (L.M. Ritschl).

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rial wall are known to be associated with an increased risk of formation of thrombi in vertical lesions, which raises the possibility of endarterectomy of plaques in microvessels without an increased thromboembolic risk in selected cases.[6]

The indocyanine green (ICG) dye that is injected binds strongly to plasma globulins, remains intravascular, and has been used in several studies as a fluorescent marker for operative assessment of arterial and venous perfusion.[7,8] The technique has also been used for the immediate analysis of patency after microvascular anastomoses as an integrated system in the operating microscope, and showed promise.[9,10] A further development is the FLOW® 800 tool which is software integrated into the operating microscope (OPMI® Pentero®; Carl Zeiss Meditec AG) with a near-infrared videoangiography detection system (INFRARED 800; Carl Zeiss Meditec AG). This allows immediate quantitative measurements of flow based on intraoperative ICG videoangiography.[11]

The aim of this study was to compare three different diagnostic instruments for the detection of adherent thromboses after intimal injury in a model of the rat aorta, which simulates removal of atherosclerotic plaques. We have compared the macroscopic and histological assessments of formation of thrombi (the gold standard) with intraoperative ICG videoangiography.

Material and methods

Ethics statement

All animals were cared for and housed in accordance with EU guidelines. The study was approved by the regional government (Regierung von Oberbayern, AZ 55.2-1-54-2532-3-35-08) and was organised in accordance with the German Animal Welfare Act. A total of 60 male Wistar rats (280–320 g, Fa.Charles River Laboratories) was used, and given free access to food and water. All procedures were done under aseptic conditions and general anaesthesia that comprised ketamine 100 mg/kg (Narketan®, Fa. Vétoquinol GmbH) and xylazine 5 mg/kg (Rompun®, Fa. Bayer Vital GmbH) was given through the femoral vein as previously decribed.[12]

Surgical technique

After induction of anaesthesia, the animals were placed supine on a work pad and a ventral, median abdominal incision 4 cm long was made. After the abdominal aorta between the renal arteries and the aortic bifurcation had been freed from perivascular tissue, all aortic branches in this section were ligated and cut. As described in detail previously, the infrarenal aorta was temporarily clipped proximally and distally, after which a longitudinal incision 10 mm long was made.[6] After standard preparation of the exposed lumen, including rinsing with physiological saline solution, endothelial defects of varying sizes were made by removing the endothelium surgically under the operating microscope on the opposite site of the longitudinal incision. Perforation of the vessel wall was prevented by meticulous preparation. After preparation of the endothelial defect the lumen was rinsed; the infrarenal longitudinal incision was closed with interrupted sutures of 11-0 monofilament polyamide (Ethilon®; Ethicon Ltd); and the temporary clips were removed for re-establishment of blood flow for one hour under continued anaesthesia for final ICG videoangiography.

ICG videoangiography

Blood flow was assessed after one hour's continued anaesthesia using the OPMI® Pentero® integrated near-infrared videoangiography detection system with the FLOW® 800 tool (INFRARED 800; Carl Zeiss Meditec AG).[9,13] As previously described, the ICG dye (ICG-PULSION; Pulsion Medical System AG) was injected intravenously (in a dose of 0.3 mg/kg body weight, 25 mg dissolved in 5 ml sterile water) as a bolus into the femoral vein using a microcatheter (Premicath; VYGON GmbH & Co. KG).[14] The ICG videoangiography began immediately after injection and was recorded in real time over a period of 120 seconds at a fixed working distance of 300 mm and with 15-fold magnification. All data were immediately analysed and stored. The detection and FLOW® 800 analyses using a integrated mathematical software tool (FLOW® 800; Carl Zeiss AG) were described earlier.[14,15] The resulting fluorescence intensity was recorded as arbitrary units (AU) and both colour-encoded figures as well as angiograms were analysed for the detection of thrombi by two independent investigators (TM and CW) who were unaware of the treatment given.

Postoperative analyses

Immediately after the ICG videoangiography, the rats were killed while still under deep anaesthesia with a lethal injection of pentobarbital (200 mg/kg, Narcoren®, Rhone–Merieux, Laupheim, Germany) and exsanguination after suprarenal dissection and explantation of the abdominal aorta. All aortas were macroscopically inspected and any thrombus seen was recorded.

The excised part of the abdominal aorta was then prepared for further histological analyses using a standardised and previously reported protocol, with the main focus being the incidence of formed thrombus.[16] Areas 500 μm proximal and distal to the longitudinal incision were specifically analysed. All histological observations were independently evaluated by two investigators who were unaware of the macroscopic and ICG videoangiogaphic results (AMF and LMR). The results were documented with an integrated CCD camera (CAMEDIA C5050; Olympus) on a Zeiss Axioskop with magnification lenses of 1.25, 10, 20, 40 and 63 Oil.

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