Noninvasive analysis of conjunctival microcirculation during carotid artery surgery reveals microvascular evidence of collateral compensation and stenosis-dependent adaptation

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Objective: **Hemodynamically relevant internal carotid artery (ICA) stenosis is a major cause of ischemic stroke. Despite its long-term benefit, carotid endarterectomy may also be associated with severe neurologic deficits. Intraoperative and early recognition of ischemia in the region of the ICA may reduce this risk. To date, direct imaging and quantitative analysis of microvascular structures and function in the human ICA region have not been possible. We purposed to visualize and quantify ischemia/reperfusion–induced microcirculatory changes in the terminal vascular bed of the ICA in patients undergoing unilateral ICA endarterectomy.**
Methods: **Sequential analysis of the ipsilateral and contralateral conjunctival microcirculation was performed with orthogonal polarized spectral imaging in 33 patients undergoing unilateral ICA endarterectomy because of moderate or severe ICA stenosis (North American Symptomatic Carotid Endarterectomy Trial score, 75% ± 13%), before clamping the ICA (baseline), during clamping of the external carotid artery and ICA, during reperfusion of the ICA (intraluminal shunt), during the second clamping of the ICA (shunt removal), after declamping (reperfusion) of the external carotid artery and ICA, and 15 to 20 minutes after the second ICA reperfusion.**
Results: **During ICA clamping for shunt placement, ipsilateral and contralateral conjunctival capillary perfusion was significantly decreased, but it was completely restored after reperfusion with carotid shunting. Reclamping of the ICA for shunt removal caused microvascular dysfunction, which was significantly less pronounced than that observed during the first clamping. The individual degree of ICA stenosis was inversely correlated with the ipsilateral and contralateral decrease in conjunctival functional capillary density during the first ICA clamping.**
Conclusions: **These results suggest adaptive mechanisms of capillary perfusion with increasing stenosis and development of collateral compensatory circulation in the vascular region of the human ICA. Conjunctival orthogonal polarized spectral imaging during unilateral ICA reconstruction enables continuous noninvasive analysis of bilateral conjunctival microcirculation in the terminal region of the ICA and enables monitoring for efficient carotid shunt perfusion during and after endarterectomy. (J Vasc Surg 2003;37:789-97.)**

Severe atherosclerotic stenosis of the internal carotid artery (ICA) is predicted to increase the risk for ischemic cerebrovascular accident (stroke). Several randomized multicenter studies demonstrated the benefit of carotid endarterectomy in patients with symptomatic severe ICA stenosis.[1-3] Despite substantial long-term benefit, carotid endarterectomy may also carry serious risk, ranging from transient and nondisabling neurologic deficits to permanent ischemic stroke.[4-6] Therefore it remains a challenge to identify measures to reduce this intraoperative risk. Consequently, early and accurate recognition of impending cerebral ischemia is the prerequisite for rapid induction of corrective therapeutic interventions to inhibit development of postoperative neurologic deficits.

Over the last two decades a variety of methods have been introduced for intraoperative risk management, including electroencephalography,[7,8] evoked potential analysis,[9] transcranial doppler scanning,[10] and carotid stump pressure monitoring.[11] These methods, however, can only assess late post-ischemic changes secondary to cessation of microvascular blood flow. Because the human cerebral microcirculation is inaccessible to direct in vivo observation, much attention has been directed toward investigation of the conjunctiva, because it is an accessible terminal vascular bed of the human ICA. The conjunctival arteries in human beings are nourished from two sources: the palpebral branches of the nasal and lacrimal arteries of the lid and the anterior ciliary artery. Both vessels are derived from the

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Characteristics of patients undergoing orthogonal polarized spectral imaging of ipsilateral and contralateral conjunctival microhemodynamics

	Ipsilateral (n = 21)	*Contralateral (n = 12)*
Age (y)	63 ± 9	62 ± 10
Degree of ICA stenosis (% NASCET)	71 ± 14	79 ± 11
Patients without/with symptoms	6/15	3/9
Mean arterial blood pressure (mm Hg)		
Baseline	98 ± 13	99 ± 13
ICA clamped	92 ± 6	93 ± 6
ICA reperfused	96 ± 12	97 ± 13
End tidal carbon dioxide (vol%)		
Baseline	4.4 ± 0.4	4.5 ± 0.4
ICA clamped	4.5 ± 0.3	4.7 ± 0.4
ICA reperfused	4.5 ± 0.3	4.5 ± 0.3
pH	7.44 ± 0.06	7.41 ± 0.07
Arterial oxygen tension (mm Hg)	199 ± 87	194 ± 79
Arterial carbon dioxide tension (mm Hg)	39 ± 5	40 ± 5
Arterial oxygen saturation (%)	98.6 ± 1.2	98.4 ± 1.1
Concentration of hemoglobin (g/dL)	12.9 ± 2.0	12.5 ± 1.8

Data are means ± SD. There were no significant differences in age, mean degree of ICA stenosis, ratio of symptomatic to asymptomatic patients, intraoperative macrohemodynamics, and arterial blood gases between patients for ipsilateral and contralateral conjunctival OPS imaging during unilateral ICA endarterectomy.

ophthalmic artery, originating from the intracranial portion of the ICA.[12] In severe carotid occlusion, variable blood flow from the external carotid artery (ECA) seems to contribute in part to the intracranial blood supply through reverse flow in the ophthalmic artery. However, because the ICA is the primary source of oxygenated blood to both the conjunctiva and cerebrum, the conjunctiva is a convenient and diagnostically important window to one capillary bed of the ICA. Furthermore, it offers a unique microvascular evaluation site because of its easy and noninvasive accessibility for continuous and serial investigations.

Previous studies in which the conjunctiva was used for intraoperative monitoring by measuring oxygen tension during carotid artery surgery did not directly visualize the microcirculation and yielded conflicting results.[13-15] Recently a new technology, ie, orthogonal polarized spectral (OPS) imaging, has been introduced, enabling noninvasive investigation and quantification of the human microcirculation in vivo[16] by placing a special camera at a distance of 0.5-1 mm to the surface of the target organ. Cross-polarization of the reflected green light results in hemoglobin-dependent contrast enhancement of vascular structures.

We evaluated the feasibility and usefulness of intraoperative OPS imaging of the conjunctival microcirculation as a new method for determining microvascular responses to ischemia-reperfusion in patients undergoing ICA endarterectomy. OPS imaging of the human conjunctival microcirculation adequately detects surgery-induced changes in ICA nutritional blood flow, and clamping-induced microvascular dysfunction within the conjunctival capillary bed of both ICAs depends on the degree of ICA stenosis. In addition, we provide in vivo evidence that previous ICA ischemia positively influences microvascular adaptation and collateral microcirculatory compensation in the human conjunctival capillary bed of both ipsilateral and contralateral ICAs within minutes of unilateral ICA clamping.

METHODS

Subjects and inclusion criteria. The study was approved by the local ethics committee of the Humboldt-University of Berlin. Informed consent was obtained from all 33 patients. ICA stenosis was symptomatic in 24 patients, based on history of transient ischemic attack (n = 12), prolonged reversible ischemic neurologic deficits (n = 3), and completed stroke (n = 9). In the remaining 9 patients, ICA stenosis was asymptomatic but hemodynamically relevant. Patients were included when scheduled for ICA endarterectomy on either side because of hemodynamically relevant stenosis. The diagnosis of hemodynamically relevant stenosis was based on angiographic and duplex ultrasound scanning criteria, which define severe carotid stenosis (70%-99%) or at least high-risk stenosis, eg, moderate stenosis (50%-69%) but angiographic plaque surface irregularity, which is associated with increased risk for ischemic stroke at all degrees of stenosis. With the exception of four symptomatic patients, patients with North American Symptomatic Carotid Endarterectomy Trial (NASCET) score less than 60% (3 ipsilateral, 1 contralateral) who had angiographic and duplex sonographic scan features of plaque ulceration with fissures and hemorrhage, and disruption of the plaque surface, all patients had significant acceleration of blood flow velocity (>200 cm/s) and high-grade stenosis (NASCET) (Table). Patients were excluded if there was hemodynamically relevant contralateral stenosis of the ICA or if an additional intracranial vessel lesion was more significant than the extracranial ICA stenosis. Additional exclusion criteria included signs of conjunctival inflammation, small vessel disease, use of contact

lenses, recent surgery, and congenital abnormalities of the anterior eye segments, because these disorders profoundly affect microvascular structure and function, thereby masking or interfering with microvascular changes caused by carotid ischemia and reperfusion. Furthermore, to reliably assess contralateral effects of ICA ischemia, the mutual interaction of clamping-induced disturbances with alterations related to preexisting contralateral ICA or vertebral stenosis should be avoided. Antithrombotic medication (eg, aspirin, warfarin sodium derivatives) was stopped in all patients 10 days before admission to the hospital.

Study design. Changes in ipsilateral (n = 21) and contralateral (n = 12) conjunctival microcirculation were analyzed in different patients because the ischemic periods during ECA and ICA clamping were too short to perform measurements of both the ipsilateral and contralateral conjunctiva in the same patient. The two groups did not differ significantly in age, degree of ICA stenosis, and ratio of patients with and without symptoms (Table). Sequential analysis of the conjunctival microcirculation was performed in accord with the various steps of the surgical procedure: (1) after induction of anesthesia and before clamping of the ICA (baseline); (2) during clamping of the ECA, which remained clamped during ICA shunting; (3) during first clamping of the ICA for insertion of the temporary intraluminal shunt; (4) during reperfusion of the ICA through the intraluminal shunt; (5) during second clamping of the ICA (shunt removal); (6) after unclamping (reperfusion) of the ECA; (7) after unclamping (reperfusion) of the ICA; and (8) before wound closure, ie, 15 to 20 minutes after onset of the second ICA reperfusion.

Conjunctival microcirculatory images were continuously recorded for at least 2 minutes per time point of analysis except for observation periods during ECA and ICA clamping. Analysis during ECA clamping was limited to 20 seconds, after which the ICA was subsequently clamped and reperfused. During ICA ischemia, changes in conjunctival microcirculation were continuously recorded during the entire clamping period.

Surgery. All surgery was performed with the patient under balanced volatile anesthesia (desflurane, 3.5-4.5 vol%, nitrous oxide- oxygen mixture). In all patients intraluminal shunts were used. Intraoperative anticoagulation with 300 IE of heparin per kilogram of body weight was carried out before the first ICA clamping and was reversed with protamine during shunt removal. Mean duration of anesthesia and surgery was 125 ± 29 minutes and 86 ± 22 minutes, respectively. Neither surgical nor systemic postoperative complications occurred. No patients had postoperative hyperperfusion syndrome or transient hypertensive encephalopathy.

Angiographic assessment of stenosis. Within 2 weeks before surgery, each patient underwent supraaortal and cerebrovascular arteriography, with selective carotid injections and multiple views in different planes, with digital substraction techniques. The degree of ICA stenosis was determined in the projection demonstrating maximal narrowing on arteriograms with the NASCET measurement technique.[3,17,18] A transparent acrylic ruler marked in millimeters was used to measure the inner and outer diameters of the ICA to the nearest half millimeter.

OPS imaging. Intraoperative OPS imaging was performed with the CYTOSCAN A/R (Cytometrics Inc, Philadelphia, Pa) equipped with a 5× lens and a charge-coupled device camera, resulting in a final 264-fold magnification on the video screen. The technique of OPS imaging consists of illuminating the tissue with linearly polarized light and processing the reflected light through an orthogonally placed polarized analyzer.[16] The conjunctiva was epi-illuminated with light of a specific wavelength (548 nm) at which oxyhemoglobin and desoxyhemoglobin absorb equally (isobestic point), focusing on a region of approximately 1 mm diameter and a penetration depth of 1 mm. Light remitted from the conjunctiva forms an image of the illuminated region within a target of the video camera. The polarization analyzer selectively allows depolarized photons scattered within the tissue to pass the analyzer and contribute to generation of the image. Generation of the OPS image with reflected light requires scattered light for illumination and absorbed light for contrast enhancement. Thus hemoglobin-containing structures such as the conjunctival microvasculature are visualized as dark areas, and the surrounding tissue void of hemoglobin appears as a light area.

With disposable sterile plastic probe covers, the camera probe was manually positioned under 0.9% saline solution immersion gently to the perilimbal surface of the bulbar conjunctiva. Special care was taken not to exert pressure on the capillary bed, thus preventing visible collapse of the microvessels. Movement artifacts were prevented by attaching the OPS camera to a custom-built C-frame consisting of several individually adjustable and securable links mounted to the surgical table. Microvascular images were transferred to a SVHS videorecorder (AG 7350-E; Panasonic, Matsushita Electric Industries, Osaka, Japan) for off-line analysis. For each patient microvascular parameters from at least 6 different windows within the ipsilateral or contralateral perilimbal region were recorded and avaraged per time point of analysis.

Microcirculatory analysis. Microhemodynamic analysis included quantitative analysis of capillary diameter, functional capillary density (FCD), and red blood cell (RBC) velocity. Parameters were quantitatively determined off-line with a computer-assisted image analysis system.[19] The physician (L.Z.) responsible for computer-assisted analysis of the videotaped images was blinded to the degree of underlying ICA stenosis. FCD was defined as the length of RBC-perfused capillaries per observation area (cm^{-1}), directly reflecting nutritive tissue perfusion.[20] The degree of capillary perfusion inhomogeneity was determined by calculating the coefficient of variation (relative dispersion) for the ipsilateral and contralateral FCD as SD/FCD_{mean}, where SD is standard deviation and FCD_{mean} is mean value obtained from all observations within the conjunctiva of one patient at the different time points. By expressing SD relative to the mean of the FCD, extent of ischemia-

induced or reperfusion-induced heterogeneities can be assessed.

RBC velocity (in millimeters per second) was measured in the centerline of capillaries with frame-to-frame analysis of the videotaped images. The individual capillary volumetric flow rate (capillary blood flow [CBF], in picoliters per second) was calculated from RBC velocity and diameter (D), based on the assumption of cylindrical capillary geometry: CBF = $\pi(D/2)^2 \times$ RBC velocity.

Statistical analysis. Data are expressed as mean ± SD. After passing the normality test (Kolmogorov-Smirnov), differences between time points were calculated with analysis of variance for repeated measures followed by post hoc Bonferroni correction. To assess the individual response of nutritive perfusion to ICA clamping, the decline (d) in FCD during the first ICA clamping was assessed in each patient as dFCD = FCD (baseline) − FCD (first ICA clamping). The strength of association between dFCD and severity of angiographically measured ICA stenosis was analyzed with Pearson product moment correlation. $P < .05$ was considered significant.

RESULTS

General characteristics. Mean degree of ICA stenosis did not differ between patients undergoing ipsilateral and contralateral conjunctival OPS imaging (Table), being 75% ± 13% in all patients, 72% ± 14% in patients with symptoms, and 77% ± 13% in patients without symptoms. During the study period, mean arterial blood pressure and arterial blood gas levels remained within normal limits and did not change significantly in response to ICA ischemia or reperfusion (Table).

OPS imaging of ipsilateral conjunctival microhemodynamics detects surgery-induced changes in ICA perfusion. OPS imaging resulted in high-quality images, enabling clear visualization and differentiation of RBC-perfused individual segments of human conjunctival microcirculation, ie, arterioles, capillaries, and venules (Fig 1, *A-C*). Baseline recordings of conjunctival microvascular perfusion showed a homogeneous perfusion pattern of nutritive capillaries arranged in an irregular network (Fig 1, *A*). Comparison of overall baseline values between patients with and without symptoms revealed no significant difference in diameter (8.6 ± 0.6 mm vs 8.2 ± 0.8 mm), FCD (104.0 ± 10.3 cm vs 102.7 ± 6.8 cm), RBC velocity (0.60 ± 0.05 mm/s vs 0.58 ± 0.06 mm/s), and CBF (34.9 pL/s ± 6.8 vs 31.2 ± 7.1 pL/s). Microvascular response of the terminal ICA vascular bed to short clamping was characterized by instant and rapid onset of capillary dysfunction, reflected by cessation of blood flow, decrease in capillary diameter, increase in intercapillary distance, and reduction in RBC velocity (Fig 1, *B*). These microcirculatory deteriorations were further accompanied by the appearance of microvessels, which displayed intermittent perfusion or oscillating flow. Sequential post-ischemic reperfusion of the ECA and the ICA, however, resulted in rapid and effective reversal of these ischemia-induced microvascular disturbances, partly exceeding baseline conditions (Fig 1, *C*).

There was a rapid and marked decrease in capillary diameter from baseline in response to clamping of the ECA. Capillary constriction was most pronounced during the first short ICA ischemia for insertion of the intraluminal shunt. Release of the clamp with reperfusion of the ICA via the shunt was associated with complete recovery of microvessel diameter to baseline values. The second ICA ischemia for shunt removal was characterized by capillary constriction, similar to the first ICA ischemia. Unclamping of the ECA and ICA, followed by 20 minutes of reperfusion, resulted in increased capillary diameter, significantly exceeding baseline values (Fig 2).

The FCD, defined as the length of RBC-perfused capillaries per observation area (cm/cm^2), directly reflects the state of nutritive tissue perfusion.[20] Sequential clamping of the ECA and ICA caused stepwise reduction ($P < .05$) of FCD by 40% and 50% compared with baseline. ICA reperfusion through the shunt effectively restored FCD to baseline levels. Sequential unclamping and reperfusion of the ECA and the ICA after the second ICA ischemia resulted in gradual and complete recovery of capillary perfusion (Fig 3, *A*).

RBC velocity is a microcirculatory variable that contributes to the efficiency of oxygen delivery to tissue. In response to carotid artery ischemia-reperfusion, RBC velocity in conjunctival capillaries revealed stepwise reduction during ECA and ICA ischemia and complete restoration during reperfusion via the shunt. Postischemic ICA reperfusion after shunt removal was associated with a significant increase in contralateral capillary RBC velocity beyond baseline values (Fig 4).

To further determine differences in microvascular volumetric flow rate, we calculated the individual capillary blood flow on the basis of RBC velocity and diameter of the analyzed vessel segments. During ECA and ICA clamping, conjunctival capillary blood flow significantly decreased to 45% and 30% of pre-ischemic baseline level. Again we found that shunt insertion and unclamping of the ICA resulted in complete recovery of CBF. Reclamping the ICA for shunt removal rapidly induced a second significant reduction in (volumetric) CBF by 60%. As a consequence of restored RBC velocity and capillary dilation after final ICA unclamping, CBF was entirely reestablished, exceeding ipsilateral and contralateral baseline values after 20 minutes of reperfusion by 15% and 12%, respectively (Fig 5).

Carotid clamping adversely affects contralateral conjunctival microcirculation. To evaluate the effect of ICA ischemia-reperfusion on microhemodynamics within the vascular territory of the contralateral ICA, we studied contralateral conjunctival microcirculation in another 12 patients undergoing surgery because of hemodynamically relevant ICA stenosis (Table). Microvascular baseline recordings in these patients were normally distributed and did not differ from ipsilateral conjunctival microhemodynamics.

During carotid artery ischemia-reperfusion a remarkably similar course of all microvascular parameters emerged

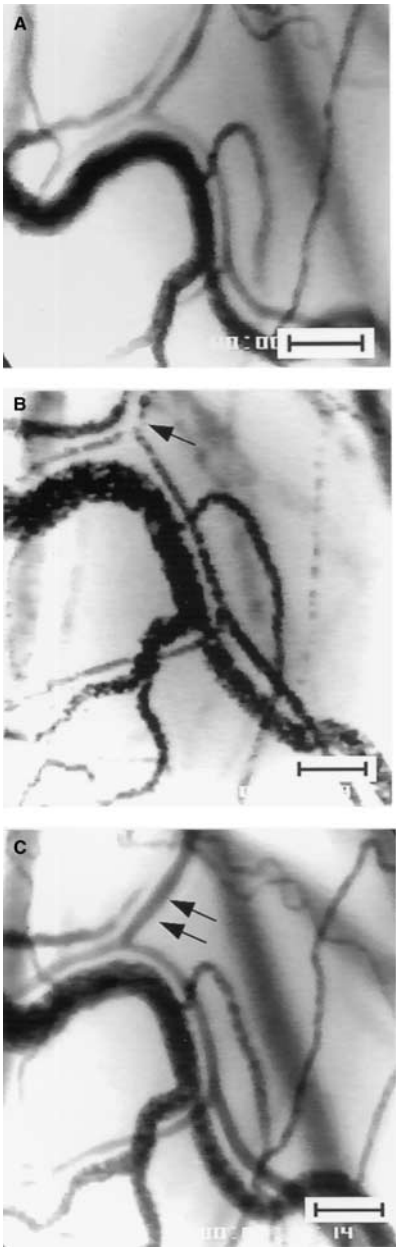

Fig 1. Microvascular response of ipsilateral conjunctival microcirculation to ICA ischemia-reperfusion. Microcirculation in the identical microvascular network within the perilimbal area of the ipsilateral bulbar conjunctiva was visualized before, during, and after unilateral ICA clamping with OPS imaging, recorded with Cytoscan A/R. **A,** Ipsilateral conjunctival microcirculation under baseline conditions, ie, before ICA ischemia, demonstrating homogeneous microvascular perfusion. **B,** During short ICA clamping ipsilateral conjunctival microcirculation exhibits typical features of ischemic microvascular dysfunction, characterized by marked vasoconstriction, microvascular stasis, and cessation of nutritive blood flow *(arrow)*. **C,** After ICA reperfusion through the intraluminal shunt, ischemia-induced microcirculatory deterioration was completely reversed, as reflected by vasodilated and hyperperfused conjunctival microvasculature *(arrows)*. Original magnification, ×264. *Scale bar* represents 50 mm.

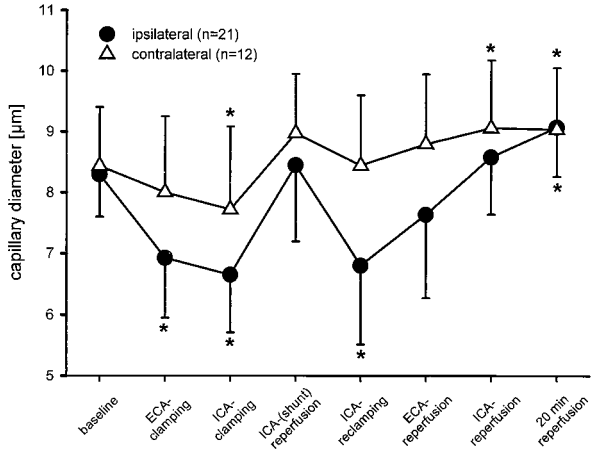

Fig 2. ICA reperfusion reverses clamping-induced constriction of conjunctival capillaries. Changes in capillary diameter in the ipsilateral (n = 21) and contralateral (n = 12) conjunctival microvascular network were analyzed during unilateral ICA endarterectomy. Note that increased diameter of capillaries in the ipsilateral and contralateral conjunctiva after final ICA reperfusion significantly exceeds pre-ischemic baseline values, indicative of substantial vasodilation in the conjunctival capillary bed of both ICAs. Data represent mean ± SD. *ECA,* External carotid artery; *ICA,* internal carotid artery. $*P < 0.05$ versus corresponding baseline.

from OPS imaging of the contralateral conjunctival microcirculation. Of importance, ipsilateral ICA ischemia was also associated with a significant decrease in nutritional blood flow in the contralateral conjunctiva relative to baseline values. When compared with ipsilateral microvascular dysfunction during ICA ischemia, analysis of FCD in the contralateral conjunctiva revealed a less pronounced but also significant reduction in response to first ICA clamping and complete restitution during shunt perfusion and after final ECA and ICA reperfusion (Fig 3, *A*). Furthermore, ICA clamping caused marked vasoconstriction (Fig 2) and a decline in RBC velocity in the contralateral conjunctival capillaries, which were reversed after shunt insertion and final ICA reperfusion (Fig 4). In parallel, volumetric CBF in the contralateral conjunctiva displayed a gradual and significant decrease after ECA and ICA clamping, complete restoration during shunt perfusion, slight reduction during the second ICA ischemia, and substantial increase exceeding baseline levels after final ECA and ICA reperfusion (Fig 5).

Clamping-induced decline in capillary perfusion inversely correlates with the degree of ICA stenosis. Clamping-induced ipsilateral and contralateral reduction in FCD varied markedly among individual patients (Fig 3, SD), implying that the responsiveness of the terminal vascular bed of both ICAs to ipsilateral ICA clamping is not equally pronounced and may depend on the degree of the underlying ICA stenosis. Therefore we assessed the individual degree of ICA stenosis from arteriograms (NASCET technique) for each patient.[3,18] To pursue this combinato-

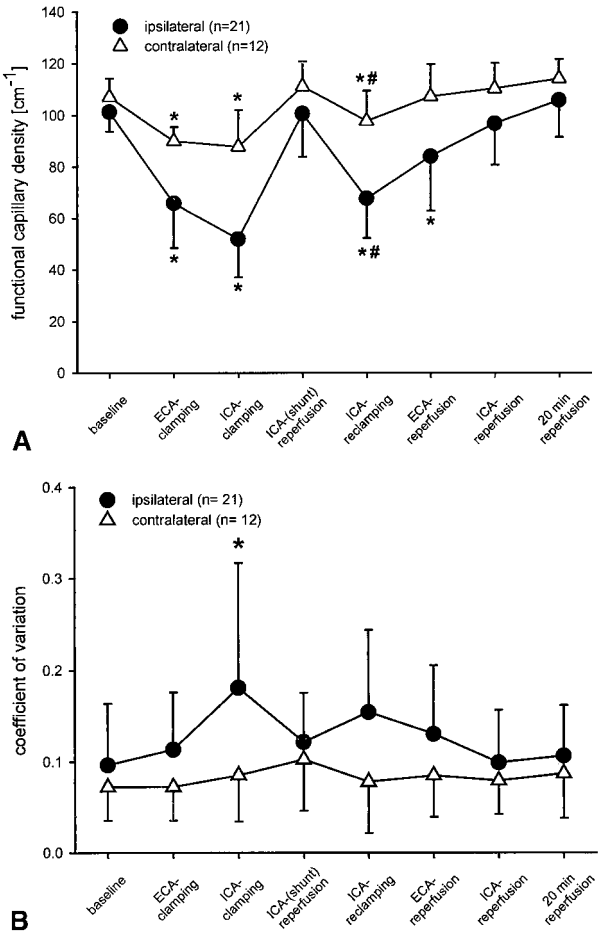

Fig 3. Decreased conjunctival functional capillary density *(FCD)* during first ICA clamping acts as protective mechanism during second ICA ischemia. **A,** Changes in FCD, ie, length of RBC-perfused capillaries per area, in the ipsilateral (n = 21) and contralateral (n = 12) conjunctival microvascular network sequentially assessed during ICA endarterectomy reflects ICA blood flow and efficiency of ICA shunting. Clamping and reperfusion of the unilateral ICA also affected the vascular region of the contralateral ICA, as reflected by ischemic decrease and post-ischemic recovery of FCD within the contralateral conjunctival microcirculation. The disturbances in nutritive perfusion in the ipsilateral and contralateral ICA conjunctival capillary bed during the second ICA clamping were substantially less pronounced compared with the first ICA ischemia, indicating rapid development of compensatory capillary adaptation in the terminal vascular bed of both ICAs initiated by preceding ICA ischemia. **B,** Changes in coefficient of variation (relative dispersion) of FCD in the ipsilateral (n = 21) and contralateral (n = 12) conjunctival microvascular network as a measure of capillary perfusion inhomogenities, yielding increased ipsilateral values throughout the entire study. The maximum increase during first ICA clamping indicates marked ischemia-induced heterogeneity of nutritive blood flow. During ICA reclamping the increase in heterogeneity was slightly attenuated, indicating improved capillary perfusion homogeneity. Data represent mean ± SD. *ECA,* External carotid artery; *ICA,* internal carotid artery. $*P < .05$ versus corresponding baseline. $^{\#}P < .05$ versus first ICA clamping.

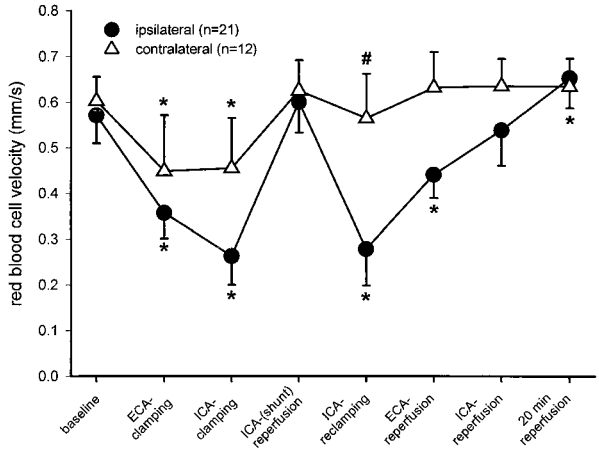

Fig 4. Carotid ischemia reduces RBC velocity in the ipsilateral and contralateral conjunctiva. Changes in RBC velocity in the ipsilateral (n = 21) and contralateral (n = 12) conjunctival microvascular network were sequentially analyzed during unilateral ICA endarterectomy. Data represent mean ± SD. *ECA,* External carotid artery; ICA, internal carotid artery. $*P < .05$ versus corresponding baseline. $^{\#}P < .05$ versus first ICA clamping.

rial approach of stenosis-dependent decrease in capillary perfusion, we correlated the individual decline of FCD after the first ICA clamping with the degree of underlying ICA stenosis. This analysis showed a significant negative relationship ($P < .02$) between the individual degree of stenosis (%NASCET score) and the corresponding decrease in ipsilateral ($r = -0.52$) and contralateral ($r = -0.67$) FCD, demonstrating that ICA clamping-induced microvascular dysfunction decreases with increasing ICA stenosis.

Preceding ICA clamping reduces microvascular dysfunction in conjunctival capillary bed of both ICAs during second ICA ischemia. Carotid artery surgery with temporary intraluminal shunting inevitably involves two short ICA clamping periods. This resulted in an unshunted ICA clamping time for shunt insertion (first ICA ischemia) and removal (second ICA ischemia) of 116 ± 28 seconds and 156 ± 43 seconds, respectively ($P < .05$; *t* test). Mean duration of ICA shunting (inter-ischemic interval) was 29 ± 12 minutes. No occlusion, kinking, or dislocation of the intraluminal shunt with additional ischemia was noted. We compared the manifestation of microcirculatory disturbances during the first and second ICA ischemic insults. Although the second clamping of the ICA was also associated with marked decline of ipsilateral FCD, this decline was significantly less pronounced than that during the first ischemia (Fig 3, *A*). Furthermore, the extent of reduction in ipsilateral and contralateral FCD and in contralateral RBC velocity after the second ICA clamping was significantly less compared with the first ICA clamping (Fig 3, *A* and Fig 4). In addition, ipsilateral CBF after the first ICA clamping was significantly different from corresponding baseline levels. By comparison, however, this significant difference was lost after the second ICA ischemia (Fig 5).

Concomitant with the ICA clamping-induced decrease in conjunctival FCD, ipsilateral capillary perfusion (FCD) showed marked heterogeneity, reflected by the nearly two-fold increase in coefficient of variation (relative dispersion) (Fig 3, *B*). The maximum increase during the first ICA clamping indicates marked ischemia-induced heterogeneity of nutritive blood flow. However, during ICA reclamping the increase in heterogeneity was slightly attenuated, indicating improved capillary perfusion homogeneity.

Together these results indicate that first ICA ischemia of less than 3 minutes decreases microvascular susceptibility to a second ischemic insult.

DISCUSSION

OPS imaging is suitable for serial and continuous in vivo assessment of microvascular structure and function of the human conjunctiva during unilateral ICA endarterectomy. Surgically induced transient ICA ischemia leads to immediate conjunctival microvascular dysfunction, which is completely restored by reperfusion of the ICA through the intraluminal shunt. Thus OPS imaging of the conjunctival microcirculation may offer a new approach to evaluation of the efficiency of shunt perfusion. However, a major restraint to routine clinical use of OPS imaging is the current inability to evaluate the microcirculatory video recordings on-line and in real time. Thus further improvement of OPS technology is needed to directly allow intraoperative quantitative on-line analysis.

In animal studies, OPS imaging of the microcirculation has been validated against in vivo fluorescent microscopy, revealing good agreement between the two techniques.[16] However, there are no quantitative data on human conjunctival microcirculation during carotid endarterectomy or clinical trials that evaluated feasibility of intraoperative OPS imaging to study the conjunctival microcirculation. Our findings strengthen earlier studies that demonstrated similar values for microvessel diameter and RBC velocity gathered in human conjunctival microcirculation with the slit-lamp technique or in vivo microscopy,[21,22] indicating that OPS imaging can be used for noninvasive and quantitative assessment of human conjunctival microcirculation. This is substantiated under both pre-ischemic and ischemic conditions, as the sequence of clamping and unclamping of the ICA during surgery was continuously analyzed. Our findings are in accord with those of Kram et al,[13] who reported that ICA clamping for endarterectomy significantly reduces conjunctival oxygen tension, which is completely restored with ICA reperfusion.[13]

The immediate responsiveness of the conjunctival microcirculation to ICA clamping and reperfusion enables valid analysis and direct evaluation of the efficiency of shunt perfusion. Therefore, given the possibility of on-line and real-time analysis, OPS imaging may be a complementary diagnostic tool for detection of reduced ICA blood flow during ICA shunting and possibly unmasking a defective shunt. Nutritive perfusion during endarterectomy can be preserved by restoring ICA perfusion, because ischemic capillary dysfunction was effectively reversed with intraluminal shunting of the ICA. Thus shunt insertion may indeed minimize the probability of generating permanent ischemic tissue damage in the carotid region during surgery.

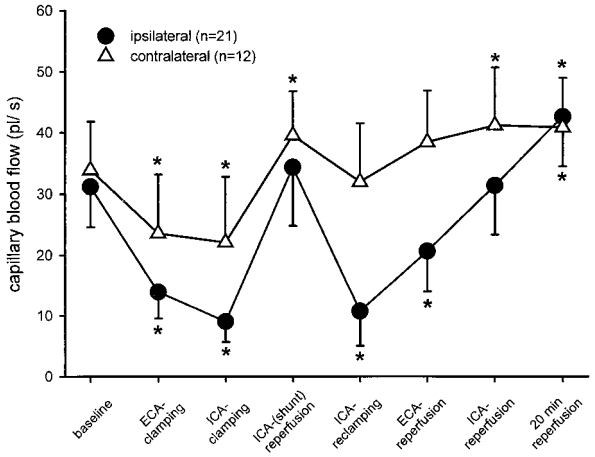

Fig 5. Repeated ischemia-reperfusion of the internal carotid artery induces hyperperfusion in the conjunctival microvasculature. Volumetric capillary blood flow, calculated from the diameter and RBC velocity in the ipsilateral (n = 21) and contralateral (n = 12) conjunctival microvascular network, was sequentially analyzed during ICA endarterectomy with intraluminal shunt insertion. In contrast to the first ICA reperfusion through the shunt, the second postischemic ICA reperfusion revealed a significant increase in ipsilateral and contralateral volumetric capillary blood flow compared with the preischemic baseline level, demonstrating capillary hyperperfusion (reactive hyperemia) in the region of both ICAs. Relative to baseline level, contralateral capillary blood flow during the second ICA clamping was considerably less impaired compared with the first clamping, possibly indicating hyperperfusion-mediated increase in resistance to second ICA ischemia. Data represent mean ± SD. *ECA*, External carotid artery; *ICA*, internal carotid artery. $^{*}P < .05$ versus corresponding baseline. $^{\#}P < .05$ versus first ICA clamping.

The significant, though less pronounced, microvascular dysfunction after ECA clamping before subsequent ICA ischemia underlines a noteworthy contribution of the ECA to the conjunctival capillary network, presumably through extracranial collateral vessels. According to the surgical procedure, the ipsilateral ECA remained clamped during ICA shunting, and it was reperfused shortly before final ICA reperfusion, ie, after completion of endarterectomy. Therefore microcirculatory changes during repeated ICA clamping and reperfusion result solely from changes in perfusion via the ICA. Complete restitution of capillary perfusion during ICA shunting while the ECA remained clamped implies that ICA perfusion has the potential to sufficiently compensate for the lack in ECA blood supply to the conjunctival microcirculation in both eyes. Although the relative contribution of the ECA and ICA cannot be assessed exactly, this type of overlapping conjunctival blood supply may also account for the substantial improvement in

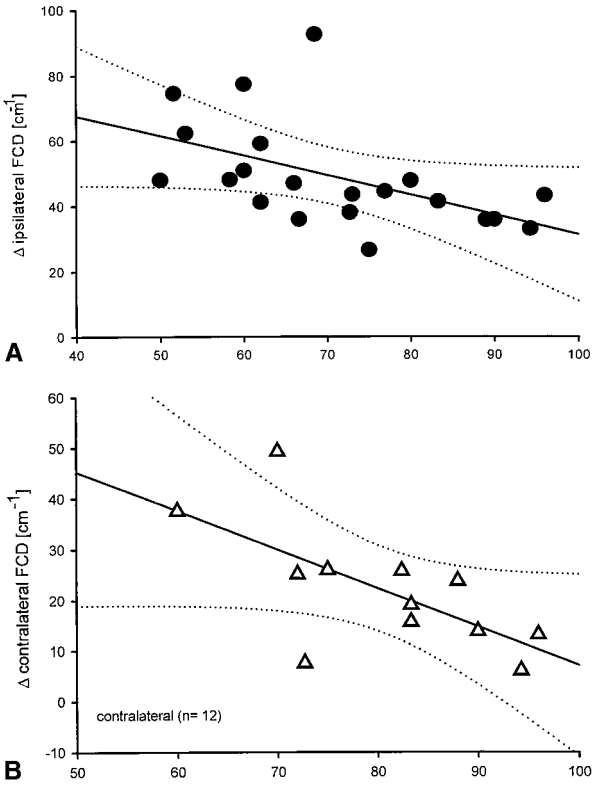

Fig 6. Clamping-induced decrease in functional capillary density *(FCD)* is inversely correlated with degree of ICA stenosis. Pearson product moment correlation between the angiographically measured degree of ICA stenosis (%NASCET) and the individual decrease in ipsilateral FCD ($r = -0.52$; $P < .02$)**(A)** and contralateral FCD ($r = -0.67$; $P < .02$)**(B)**. *Dotted lines* represent 95% confidence interval.

conjunctival microcirculation during ECA reperfusion before the second ICA reperfusion. An alternative possibility is that, in addition to the ipsilateral ECA, there may be additional leptomeningeal collateralization derived from contralateral ECA and via anastomoses with the temporal, middle meningeal, and external maxillary arteries, participating in a rich network of extracranial collateral vessels.

The substantially impaired microcirculation in the contralateral conjunctiva suggests acute redistribution of the intracranial arterial blood supply in response to ipsilateral ICA ischemia. The compromise of the contralateral conjunctival perfusion together with the residual ipsilateral conjunctival perfusion indicate steal of blood supply toward the ipsilateral eye with ICA clamping via the anterior and possibly the posterior communicating arteries of the circle of Willis. Alternatively, the contralateral conjunctival microcirculation might depend in part on the ipsilateral ICA during clamping. It is also tempting to speculate that the microcirculatory impairment in the contralateral conjunctiva after ICA clamping may be part of cerebral autoregulation, acutely responding to a sudden decrease in blood pressure within the circle of Willis. Preservation of the ipsilateral conjunctival microcirculation during ICA and ECA clamping confirms the collateral compensatory potential of the circle of Willis. This might be of particular clinical interest in that this individual contralateral carotid and vertebrobasilar perfusion reserve may now be quantified with OPS imaging of the conjunctival microcirculation.

ICA clamping-induced reduction of ipsilateral and contralateral conjunctival perfusion inversely correlates with the degree of ICA stenosis. This may be explained in part by a compensatory adaptation of capillary perfusion in both ICA regions, evolving over time with increased ipsilateral ICA stenosis. The inverse correlation may further indicate that the extent of ICA clamping- induced impairment of conjunctival microcirculatory impairment is quantitatively predictable by the degree of ICA stenosis. On the basis of this functional relationship, patients who react to carotid clamping with an acute decline in conjunctival microvascular perfusion may be identified preoperatively. In addition, the benefit of microcirculatory blood supply to one terminal vascular bed of the ICA, ie, the conjunctiva, provided by intraluminal ICA shunting could be noninvasively estimated. However, although the ICA is the primary source of oxygenated blood to both the conjunctiva and the cerebrum, changes in one terminal bed do not necessarily reflect changes in the other. Thus findings in the conjunctival microcirculation cannot be extrapolated to the cerebral microcirculation. Future studies designed to investigate similar changes in the cerebrum may have the potential to evaluate the benefit of microcirculatory blood supply to the cerebrum provided by intraluminal ICA shunting.

Although the duration of the second ICA clamping was significantly longer than the first clamping, the decrease in ipsilateral and contralateral FCD and the contralateral RBC velocity during the second ICA ischemia was significantly less pronounced. In addition, while the ipsilateral CBF after the first ICA clamping revealed a significant reduction from baseline, there was no substantial difference after the second ICA clamping. Furthermore, the ischemia-induced increase in heterogeneity (coefficient of variation of FCD) during ICA reclamping was slightly attenuated. Contrary to the first ICA clamping, there was no significant difference relative to baseline during the second clamping, possibly because of improved capillary perfusion homogeneity. The relatively large SD of this coefficient during the first ICA clamping reflects the entire range of individual responses to ICA ischemia, mainly dependent on the degree of underlying ICA stenosis (inverse correlation). That there was no significant difference in CBF and ipsilateral RBC velocity during the second ICA clamping may reflect that the different conjunctival capillary parameters do not react equally to short ICA clamping and could act as independent determinants within the evolving microvascular response after repeated ICA clamping.

The present study shows for the first time direct imaging and quantitative assessment of attenuated microvascular dysfunction in the ipsilateral and contralateral conjunctival microcirculation, the terminal beds of both ICAs after

a second unilateral ICA clamping. This suggests activation of regulatory mechanisms resulting in transient adaptation of the conjunctival microvasculature to abrupt changes in perfusion, ie, clamping-induced ischemia. The rapid induction and onset of counterregulatory mechanisms support a microvascular or metabolic pathway for the decreased microcirculatory disturbances after a second ischemic insult. The significantly increased CBF after the first and the second unilateral ICA reperfusion may indicate post-ischemic hyperperfusion (reactive hyperemia) as a possible intrinsic component of this pathway. In this context, capillary hyperperfusion induced by the first ICA clamping may represent a shift in baseline perfusion to a higher level. Therefore the level of critical microvascular blood flow may be reached much later than after the initial clamping. Consequently post-ischemic improvement in ipsilateral and contralateral capillary blood flow may contribute to increased microcirculatory perfusion reserve and less pronounced capillary dysfunction during the second unilateral ICA clamping. Thus the reduction of ischemic microvascular injury after the second ICA clamping may be reduced and possibly protect the terminal vascular bed in both ICA areas against further acute ischemic challenges. Other putative pathways leading to these diminished microvascular deficits might involve different factors, including development of compensatory collateral circulation by the circle of Willis or even possible induction of preconditioning-induced ischemic tolerance after repetitive ICA clamping. Whether these pathways are indeed responsible for these assumed protective effects exceeds the scope of the present study and merits further investigation.

Our findings provide important details of a new method for intraoperatively analyzing microvascular changes during carotid endarterectomy, which may be combined with already established monitoring systems to assist in further reducing perioperative morbidity and mortality. OPS imaging of the conjunctival microcirculation may also be used to characterize and possibly manage other vascular disorders in which microvascular integrity of the ICA region is challenged with ischemia-reperfusion injury. Ongoing studies will determine whether successful experimental application and validation of this method in patients with contralateral stenosis as well as correlation with other monitoring systems may integrate this complementary technique into contemporary clinical practice.

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