Direct Ischemic Postconditioning After Carotid Endarterectomy in the Prevention of Postoperative Cerebral Ischemic Complications—Observational Case–Control Study

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Abstract

Introduction: Ischemic postconditioning (IPCT) represents one of the several therapeutic strategies to attenuate ischemic reperfusion injury (IR) after carotid endarterectomy (CEA). We here present the first in-human study of IPCT in carotid surgery.

Methods: The study represents an observational case-control study, with the data collected in our Institution carotid database. From December 2015 to December 2020, a total of 300 patients were included in our study; IPCT group consisted of 148 patients in whom ischemic postconditioning was performed while control group consisted of 152 patients in whom IPCT was not performed. Indications for IPCT technique were: severe unilateral internal carotid artery (ICA) stenosis (>90%), severe bilateral ICA stenosis (>80%), severe ICA stenosis (>80%) with contralateral ICA occlusion and ICA subocclusion. IPCT was performed by applying 6 cycles of 30 sec reperfusion (clamping of ICA)/30 sec ischemia (clamping of ICA) after finishing the procedure and initial declamping. Two groups of patients were compared in terms of occurrence of intrahospital and early postoperative stroke, TIA (transient ischemic attack) and neurologic morbidity.

Results: Cumulative incidence of intrahospital postoperative stroke or TIA was significantly higher in the control group (5.3% vs 0.7%, \(P = .036\)). According to carotid plaque characteristics, patients in the IPCT group had significantly more frequent presence of heterogenous plaque, as well as ulcerated plaque, which was associated with the absence of postoperative stroke and significantly lower cumulative rate of TIA/stroke when compared to the control group (43.9% vs 8% and 47.3% vs 1.5%). During the follow-up period of 1 month after the surgery, there were no cases of stroke, TIA and deaths due to neurological causes in both groups of patients. Conclusion: Our results showed that IPCT significantly reduced the incidence of postoperative cerebral ischemic complications after CEA in high-risk patients for IR injury when compared to the control group.

Keywords

ischemic postconditioning, ischemic-reperfusion injury, carotid endarterectomy, stroke, TIA

Introduction

Carotid endarterectomy (CEA) has been proved to be a safe procedure for the treatment of the patients with significant symptomatic or asymptomatic carotid stenosis.1–3 Most vascular investigators has been discussing the role of CEA and carotid angioplasty and stenting (CAS) in the treatment of patients with significant carotid stenosis for the past 20 years.4,5 Moreover, technological advances in carotid angioplasty are evident—there are new types of stents, improved cerebral embolic protection devices, along with new technologies.
constantly emerging. On the other hand, there were no significant changes in carotid endarterectomy technique.

Ischemic-reperfusion (IR) injury is thought to have a significant role in adverse neurological events following CEA. In recent years, many papers have been published regarding ischemic-reperfusion injury after carotid endarterectomy. This phenomenon is characterized by a cascade of events, starting with an overproduction of free radicals during ischemic phase, which is further aggravated by reperfusion, leading to cell damage and death through mitochondrial signaling pathways. Afterward, the process is followed by inclusion of the innate and adaptive immune response, which finally leads to tissue injury. Postoperative cerebral hyperperfusion associated with IR injury could also lead to neurological complications after CEA. It is known that postoperative hyperperfusion after CEA occurs due to preoperatively reduced cerebrovascular reactivity on the basis of chronic cerebral ischemia, however, reduced cerebral oxygen saturation caused by acute cerebral ischemia during carotid cross-clamping within CEA is described to be a significant risk factor for postoperative cerebral hyperperfusion syndrome as well.

Ischemic postconditioning (IPCT) represents one of the several therapeutic strategies for IR injury, evaluated in relation with cardiac surgery and coronary interventions, while it has never been clinically described in the settings of carotid revascularization in humans. In recent years, IPCT has only been evaluated in several animal studies, defined as a repeated series of intermittent interruptions of blood flow in the early phase of reperfusion and thus altering the hydrodynamics of blood flow and organ injury. In these studies conducted on rats, IPCT was directed straight to the brain tissue after releasing the artificially made internal carotid artery occlusion. The effects of IPCT on cerebral IR injury were evaluated by assessing the neurological scores and infarct volumes, as well as by measuring different enzymes which reflect the level of oxidative stress.

We here present the first in-human study of ischemic postconditioning (IPCT) in carotid surgery that we hope will change current understandings of cerebral ischemia/reperfusion phenomenon during and after CEA.

Methods

Patients and Design of the Study

The study represents an observational case–control study, with the data collected in our Institution carotid database. From December 2015 to December 2020, a total of 300 patients were included in our study; IPCT group consisted of 148 patients in whom IPCT was performed while control group consisted of 152 patients in whom IPCT was not performed. In all patients, carotid arteries were evaluated by duplex ultrasound, and findings were confirmed by multidetector computed tomography angiography (MDCT), which was also used for the evaluation of intracranial arteries and competence of circle of Willis. Incompetent circle of Willis was defined as any interruption of continuity of circle that could affect the equalization of blood flow between the two sides of the brain and anastomotic circulation as well.

Inclusion criteria for IPCT technique were conditions in which ischemic-reperfusion injury might be expected after CEA: severe unilateral internal carotid artery (ICA) stenosis (>90%), severe bilateral ICA stenosis (>80%), severe ICA stenosis (>80%) with contralateral ICA occlusion and subocclusion of the ICA. Patients in the control group were selected from our carotid database according to the same criteria of degree of carotid stenosis as patients in the IPCT group. Exclusion criteria for IPCT technique were recent disabling ipsilateral stroke with large ischemic zone and recent intracranial hemorrhage.

All patients were examined before and after the procedure by an institutional neurologist, who was blinded to the patients’ IPCT treatment. Symptomatic patients were considered those who had a positive history for retinal or focal cerebral ischemia (transient ischemic attack (TIA) or stroke) within the 6 months of the study period. Brain computed tomography (CT) was performed in all patients, either prior to or upon admission. For ultrasound assessment, peak systolic velocities (PSV), end diastolic velocities (EDV), and internal carotid artery (ICA)/common carotid artery (CCA) ratio were used to assess the degree of carotid stenosis/restenosis prior to CEA, according to European Carotid Study Trial (ECST) study criteria. The characteristics and the structure of carotid plaque were also analyzed by the means of Color-flow duplex scan (CDS). Plaques were characterized as fibromatous, atherosclerotic or as plaques within vasculitis. Embolic potential was determined by a detailed assessment of plaque surface and echogenicity. Ulcerated or irregular surface echolucent plaques were considered as those with high embolic potential.

Institutional Practice and Terminology

For carotid stenosis ultrasound assessment, we used the following criteria: <50%—PSV <125 cm/sec and EDV <40 cm/sec; 50%-<69%—PSV 125-230 cm/sec and EDV 40-100 cm/sec; 70%-90%—PSV 230-500 cm/sec and EDV 100-300 cm/sec; 90%-99%—unpredictable PSV and EDV, from very high to very low values; occlusion—flow not detectable in the ICA. All measurements regarding the preoperative duplex ultrasound assessment of carotid arteries were done in our institutional vascular laboratory.

Primary Endpoint of the Study

Our primary objective was to investigate the effect of IPCT on the neurological outcome in selected patients with high risk of IR injury after CEA. The 2 groups of the patients were compared in terms of occurrence of intrahospital and early postoperative stroke, TIA and neurological mortality. The degree of carotid arteries stenosis, the competence of circle of Willis, characteristics of carotid plaques, clamping time and other intraoperative data, were also analyzed in the groups which
could have a significant impact on the postoperative neurological outcome. Patients were monitored for 1 month after the surgery, and follow-up data consisted of stroke, TIA and mortality due to neurological causes. Ethics Committee of our Institution approved of this study, while all patients have provided an informed consent.

**Surgical Technique**

Our eversion CEA technique has already been described elsewhere and all patients were operated on under general anesthesia. Induction of anesthesia was accomplished with fentanyl, propofol and rocuronium. The superficial cervical plexus block was used with infiltration of 2% lidocaine. Anesthesia was maintained with sevoflurane and patients received intravenous manitol infusion immediately before and during the carotid cross clamping. The same anesthesia protocol was used in both groups of patients. After the exposure of carotid arteries, systemic heparinization has been administered and arteries clamped. The mean arterial pressure was raised and maintained at values 20% above the baseline during the clamping time. CEA was performed in an eversion fashion, the plaque was removed and anastomosis finished. Following the initial declamping, IPCT procedure was then performed according to the technique described earlier in the published studies. In the IPCT group we have applied 6 cycles of ischemia (clamping of ICA) before final declamping. During the IPCT technique, ICA was clamped and declamped 1.5 cm above the end point of endarterectomy, which was assessed by external visual inspection. Furthermore, that was the same clamping site as at the beginning of the procedure, on an unaltered segment of the artery above the end of the plaque. Cerebral oximetry was used for cerebral monitoring. Whenever the postoperative course was uneventful, patients were discharged from our hospital on the third postoperative day.

**Statistical Analysis**

Data sets for continuous numerical variables were described by arithmetic mean and standard deviation while attribute variables were described by outcome frequency and percentage. Univariate analysis of the significance of differences between groups was performed using Pearson’s chi-square test or Fisher’s exact probability test for attributive variables and Student’s t test for continuous variables. The binary logistic regression model with stepwise method was used for multivariate analysis of confounding variables, in order to perform an adjustment model for estimating the relationship between stroke, TIA, stroke or TIA with the implemented IPCT procedure. All variables that had \( P < .2 \) in the previously performed univariate analyses were considered for inclusion in the final multivariate model. The evaluation of the validity of the logistic regression model included the evaluation of its measure of adequacy and its accuracy. The best-fitting model was made by estimating the Nagelkerke R square parameter. The accuracy of the logistic regression model was assessed by discriminatory measurements with an assessment of its adequacy. An assessment of c-statistics was done to demonstrate how adequately the model can differentiate IPCT-treated patients from non-IPCT-treated patients. The adequacy analysis of logistics models and the assessment of variable retention or their interactions were performed by the Hosmer-Lemeshow method. The accepted significance level was .05. Data processing was done using the IBM SPSS Statistics 22 (NY) statistical package.

**Results**

Preoperative patients’ characteristics and comorbidities are presented in Table 1. Patients in the IPCT group were significantly older (\( P = .02 \)), had higher proportion of smoking (83% vs 58.9%, \( P = .0001 \)) and positive family history for cardiovascular diseases (81.8% vs 44.7%, \( P < .001 \)). Additionally, patients in the IPCT group had higher rate of hypertension (98.6% vs 90.3%, \( P = .02 \)), hyperlipidemia (97.3% vs 88.1%, \( P = .004 \)) and previous coronary artery bypass grafting procedure (17.6% vs 8.6%, \( P = .02 \)). In regard to the competence of
circle of Willis, a significantly higher percentage of patients in the IPCT group had an incompetent circle of Willis (31.1% vs 20.4%, \( P = .002 \)), while there was a significantly higher percentage of patients in the control group with previous stroke (26.3% vs 16.2%, \( P = .03 \)). However, there was an equal distribution of patients who had a stroke or TIA between the IPCT and control group (38.5% vs 40%, \( P = .8 \)). Regarding previous aspirin and clopidogrel therapy there were no significant differences between the IPCT and control group.

The data presenting the carotid plaque characteristics are presented in Table 2. Patients in the IPCT group had significantly more heterogenous plaques (43.9% vs 8.8%), ulcerated plaques (47.3% vs 1.5%) as well as fibrocalcified plaques (\( P = .04 \)), while they had less frequently homogenous plaques. Also, a significantly higher proportion of patients in the IPCT group had plaques with high or intermediate embolic potential (70.3%; 29.7% vs 10.2%; 16.1%).

The mean clamping time, without additional clamping time due to IPCT technique, was significantly longer in the IPCT group than in the control group (19.4 ± 5.3 min vs 17.2 ± 3.8 min, \( P = .002; \) Table 3). There were no statistically significant differences in terms of primary graft interposition and abbreviation of internal carotid artery among the groups, as well as postoperative wound complications and ICA thrombosis.

Regarding the incidence of stroke postoperatively, there was no significant difference between the IPCT group and the control group (0% vs 3.3%, \( P = .06 \)). Also, no significant difference between the two groups was found in the incidence of postoperative TIA (0.7% vs 2%, \( P = .6 \)). However, cumulative incidence of postoperative TIA or stroke (TIA/stroke) was significantly higher in the control group (5.3% vs 0.7%, \( P = .04 \)). Likewise, there was no significant difference between the IPCT group and the control group regarding the interaction of a previous stroke and postoperative stroke (0.0% vs 2.0%, \( P = .2 \)). Interestingly, patients in the IPCT group had significantly more frequent ulcerated and heterogenous plaques (43.9% vs 8% and 47.3% vs 1.5%), which was associated with the lower rate of postoperative TIA and the absence stroke as well as lower cumulative TIA/stroke rate. No significant differences between the groups were found regarding reperfusion syndrome and neurological mortality (Table 4).

Multivariate analysis identified a significant interaction between postoperative TIA/stroke and plaque characteristics, as well as 5 confounding variables (COPD, family history of CVD, previous statin therapy, previous clopidogrel therapy and plaque type), all of which were significantly independently associated with the IPCT group, with a significant constant of the logistic regression model. The IPCT group was significantly more associated with a lower probability of absence of COPD (OR = 0.01), lower probability of absence of family history of CVD (OR = 0.03), as well as a lower probability of

### Table 2. Univariate Analysis of Ultrasound Characteristics of Carotid Plaques.

| Carotid plaques       | IPCT group, \( n = 148 \) | Control group, \( n = 152 \) | \( P \) |
|-----------------------|---------------------------|-----------------------------|------|
| Plaque characteristics|                           |                             |      |
| Homogeneous (%)       | 0.0                       | 73.7                        | .000 |
| Heterogeneous (%)     | 43.9                      | 8.8                         | .001 |
| Ulcerated (%)         | 47.3                      | 1.5                         | .001 |
| Irregular surface (%) | 8.8                       | 16.1                        | .06  |
| Plaque type           |                           |                             |      |
| Lipid (%)             | 0.7                       | 2.2                         | .3   |
| Fibrolipid (%)        | 26.4                      | 33.6                        | .2   |
| Fibrocalcified (%)    | 38.5                      | 26.9                        | .04  |
| Calcified (%)         | 4.1                       | 5.2                         | .8   |
| Mixed (%)             | 29.1                      | 32.1                        | .6   |
| Embolic potential     |                           |                             |      |
| High (%)              | 70.3                      | 10.2                        | .001 |
| Intermediate (%)      | 29.7                      | 16.1                        | .006 |
| Low (%)               | 0                         | 73.7                        | .001 |

Abbreviation: IPCT, ischemic postconditioning.

### Table 3. Univariate Analysis of Operative Characteristics and Postoperative Complications.

|                             | IPCT group, \( n = 148 \) | Control group, \( n = 152 \) | \( P \) |
|-----------------------------|---------------------------|-----------------------------|------|
| Rate of operations per year |                           |                             |      |
| 2016 (%)                    | 38 (25.7)                 | 42 (27.6)                   | .8   |
| 2017 (%)                    | 36 (24.3)                 | 35 (23.0)                   | .9   |
| 2018 (%)                    | 31 (20.9)                 | 33 (21.7)                   | .9   |
| 2019 (%)                    | 33 (22.2)                 | 32 (21.0)                   | .9   |
| 2020 (%)                    | 10 (6.7)                  | 10 (6.6)                    | .8   |
| Operative data              |                           |                             |      |
| Clamping time in minutes (Mean ± SD) | 19.4 ± 5.3 | 17.2 ± 3.8 | .002 |
| Primary graft interposition | 4.1                       | 1.5                         | .2   |
| Abbreviation of carotid artery (%) | 23.6                    | 21                          | .06  |
| Postoperative complications |                           |                             |      |
| Wound hematoma (%)          | 2.7                       | 4.6                         | .5   |
| Wound infection (%)         | 0                         | 0                           |      |
| Thrombosis of internal carotid artery (%) | 0                      | 0.7                         | .3   |

Abbreviations: IPCT, ischemic postconditioning; SD, standard deviation.
absence of previous statin therapy (OR = 0.07). In contrast to this, the IPCT group was associated with a 50 times greater probability of the absence of clopidogrel therapy and a 567 times greater probability of the presence of fibrocalcified plaque, taking into account the probability of the presence of the reference category for plaque type (fibrolipid plaque) (Table 5).

Multivariate logistic regression analysis demonstrated significant association between the absence of postoperative TIA/stroke and plaque characteristics (the reference category was irregular surface plaque) in interaction with IPCT group. The probability of the absence of postoperative TIA/stroke in the group of patients with heterogeneous plaque was 63 times more associated with the IPCT group, while a 153 times greater chance of the absence of postoperative TIA/stroke was demonstrated in patients with ulcerated plaque in the same group (Table 5).

Logistic regression model has shown excellent validity (Nagelkerke R square = 0.908) and adequacy by the Hosmer-Lemeshow method ($\chi^2 = 4.595; P = .800$), as well as excellent discriminatory measurements (c-statistics = 0.989; $P = .000$).

During the follow-up period of 1 month after the surgery, there were no TIAs, strokes or neurological mortality in both groups of patients.

Table 4. Univariate Analysis of Primary Outcomes and Other Clinical Outcomes.

|                        | IPCT group, n = 148 | Control group, n = 152 | $P$ |
|-----------------------|---------------------|------------------------|-----|
| Postoperative stroke (%) | 0.0                 | 3.3                    | .06 |
| Postoperative TIA (%)  | 0.7                 | 2.0                    | .6  |
| Postoperative stroke/TIA (%) | 0.7               | 5.3                    | .04 |
| Absence of postoperative stroke/TIA with homogenous plaque (%) | 0.0 | 68.6 | .000 |
| Absence of postoperative stroke/TIA with heterogeneous plaque (%) | 43.9 | 6.0 | .000 |
| Absence of postoperative stroke/TIA with ulcerated plaque (%) | 46.6 | 1.5 | .000 |
| Absence of postoperative stroke/TIA with irregular surface plaque (%) | 8.8 | 16.1 | .06 |
| Reperfusion syndrome (%) | 0                  | 1.3                    | .2  |
| In-hospital mortality—neurological causes (%) | 0 | 1.3 | .2 |
| Postoperative MI (%) | 0                  | 0                      | /   |
| Overall in-hospital mortality (%) | 0 | 0  | /   |

Abbreviations: IPCT, ischemic postconditioning; TIA, transient ischemic attack; MI, myocardial infarction.

Table 5. Parameters of Final Logistic Regression Model for Predictors Associated With the IPCT Group.

|                        | B   | SE   | Wald  | df  | $P$  | Odds ratio | 95% CI for odds ratio |
|-----------------------|-----|------|-------|-----|------|------------|-----------------------|
| COPD (No)             | −4.5| 1.4  | 10.9  | 1   | .001 | 0.01       | 0.001 0.2             |
| Family history of CVD (No) | −1.7| 0.8  | 4.7   | 1   | .03  | 0.2        | 0.04 0.9             |
| Previous clopidogrel therapy (No) | 3.9 | 1.5  | 6.8   | 1   | .009 | 50.3       | 2.6 959.9            |
| Previous statin therapy (No) | −2.7| 0.9  | 9.6   | 1   | .002 | 0.07       | 0.01 0.4             |
| Plaque characteristics (Irregular surface plaque) by postoperative stroke/TIA (Yes)—Reference | 21.9 | 3 | - | .000 |
| Homogenous plaque by postoperative stroke/TIA (No) | −21.4 | 4.199 | 0.000 | 1 | .91 | 0.000 | 0.000 |
| Heterogeneous plaque by stroke/TIA (No) | 4.1  | 1.1  | 15.7  | 1   | .000 | 63.1       | 8.1 490.6            |
| Ulcerated plaque by postoperative stroke/TIA (No) | 5    | 1.2  | 18.1  | 1   | .000 | 153        | 15.1 1549.5          |
| Plaque type (lipid)—Reference | 11.5 | 5 | .04 |
| Fibrolipid            | 4.1  | 1.9  | 4.7   | 1   | .03  | 59.3       | 1.5 2391.3           |
| Fibrocalcified        | 6.3  | 2.1  | 9.3   | 1   | .002 | 567.1      | 9.6 33603            |
| Calcified             | 4.7  | 2.1  | 4.9   | 1   | .03  | 114.8      | 1.7 7575.8           |
| Mixed                 | 5.8  | 2    | 8.1   | 1   | .004 | 332.6      | 6.2 17934.6          |
| Constant              | −7.7 | 2.6  | 9.1   | 1   | .003 | 0.000      |                      |

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; TIA, transient ischemic attack.

Discussion

The concept of ischemic conditioning (IC) is thoroughly explored, since it has been demonstrated that it can affect several different levels of complex IR injury mechanism. In addition, different forms of IC enable its application in a number of clinical settings. Our study results demonstrate that ischemic postconditioning (IPCT) could be safely performed after carotid endarterectomy in high-risk patients for IR injury with a significantly reduced incidence of postoperative cerebral ischemic complications.
Remote ischemic preconditioning (RIPC) has been previously described in the settings of carotid revascularization with beneficial effects. \(^{24,25}\) It was demonstrated that repetitive preoperative cycles of brief nonlethal limb ischemia in RIPC procedure could be protective for severe promote ischemia in distant organs during and after carotid procedures. The mechanism of ischemia protection is quite complex, related to inflammatory response, and described to be the result of a tolerated stimulus created through neuronal and humoral pathways generating an environment of greater resistance to subsequent ischemia. \(^{25}\) In addition, RIPC and IPC share common intracellular signaling pathways, thus supporting the fact that IPC could be as effective in carotid procedures and easier to perform. \(^{26,27}\) However, these studies did not demonstrate significant effect of RIPC on clinical outcomes, which is in contrast with our study in which IPC was directed straight to the target organ that needed to be protected against reperfusion injury. Application of IC has also been evaluated in the setting of acute ischemic stroke. \(^{28}\) In patients treated with repeated remote ischemic postconditioning, median infarct volume was lower. However, the difference was not statistically significant when compared with controls. \(^{28}\) Another study described association between IC and prevention of postinterventional thrombosis. It has been shown that techniques of IC favor thrombolysis through the release of adenosin and its interaction with platelet surface receptors. \(^{29}\)

Published animal studies have directly demonstrated potential of ischemic postconditioning (IPCT) to protect the brain from deleterious effects of IR injury. \(^{16-19}\) The majority of studies have unquestionably showed that IPCT suppresses the brain edema, decreases the infarct volume and improves neurological function. \(^{16-19,30}\) This is achieved by maintaining the blood brain barrier intact, inhibition of the oxidative stress, increasing the level of anti-apoptotic proteins and most importantly by modulating the mitochondrial signaling pathway. All these previously mentioned mechanisms of brain protection were highlighted in the study of Xing et al. \(^{16}\) They have applied 6 cycles of 30 sec ischemia/30 sec reperfusion, following the initial reliving of artificial middle cerebral artery occlusion in a rat. In the IPCT group, infarct volumes, 24 and 72 hours after reperfusion, were significantly decreased. Also, neurological score was significantly improved both 24 and 72 hours after reperfusion. Feng et al reported that 3 cycles of 30 sec reperfusion/30 sec ischemia, applied after relief of artificial carotid stenosis, significantly increased the percent of live neurons after 3 and 27 days compared with group without IPCT. \(^{17}\) They found that IPCT maintained the activity of antioxidative enzymes, which is of utmost importance, since their activity is very low in the brain tissue, which makes it especially vulnerable to damage by free radicals. Additionally, in the IPCT group, levels of neurotoxic cytokines (TNF-\(\alpha\), IL-1\(\beta\)) were significantly decreased. Furthermore, another study confirmed beneficial effects of IPCT in the embolic model of cerebral ischemia in rats, which reflects the real conditions even better. \(^{30}\) In the IPCT group, brain edema was significantly decreased, infarct volumes reduced, as well as neurological function improved.

It has been shown that type of anesthesia may interfere with mechanism of ischemic conditioning. \(^{31-34}\) In a study of cardiac surgery patients cardioprotective effect of remote ischemic preconditioning was attenuated under propofol or sevoflurane anesthesia. \(^{32}\) Another study showed that neuroprotective effect of remote ischemic postconditioning in ischemic stroke was independent of type of used anesthetic. \(^{33}\) Likewise, it has been described that many anesthetics such as propofol, isoflurane, sevoflurane may have neuroprotective effect in cerebral IR injury. \(^{34}\) In our study the same anesthetic protocol was used in both group of patients, and we consider that anesthetics did not have negative impact on the neuroprotective effect of IPCT.

Several recent studies demonstrated that previous antiplatelet therapy may also interfere with the effect of ischemic conditioning. \(^{35,36}\) It has been described that in the setting of myocardial IR injury, aspirin may block or attenuate cardioprotective effect of remote ischemic conditioning. \(^{35}\) Taking into account that there were no differences between the groups regarding previous aspirin therapy in our study and that IPCT was directed straight to the target organ, we consider that aspirin did not interfere with neuroprotective effect of IPCT.

Our study results confirmed the beneficial effect of IPCT performed in the early phase of reperfusion after CEA. There were no strokes and mortality in IPCT group, only one patient had transient arm weakness that completely recovered within several hours after CEA, and cumulative TIA/stroke rate was significantly lower in the IPCT group than in the control group. Although there was the higher percentage of a previous stroke in the control group, the distribution of patients who had a previous stroke and a postoperative stroke was equal between the groups. Also, an important finding in multivariate analysis is the association of the IPCT group with the absence of postoperative stroke/TIA in patients with heterogeneous and ulcerated plaques. This indicates that the IPCT is most effective in patients with these types of plaques, which otherwise significantly increase the risk of TIA and stroke. \(^{37}\) Furthermore, patients in the IPCT group had significantly longer mean clamping time, without additional clamping time due to IPCT technique, compared to patients in the control group. This could possibly be explained by a large number of patients in the IPCT group with complicated plaques, which could make the procedures technically more demanding. Finally, the fact that prolonged ischemic time during the procedure is considered a risk factor for postoperative adverse neurologic events, point to the IPCT as a potentially useful protective measure in high risk patients.

**Limitations**

It was a single-center study, which included a small number of patients in which IPCT was performed. However, this was a pilot study that clearly demonstrated the benefits of IPCT after CEA that should be further investigated in larger clinical trials. Also, the study was not prospectively designed and patients
who had met the inclusion criteria for IPCT were not randomized. Furthermore, a relatively small sample size in both groups of patients, with a consequently small proportion of patients with postoperative TIA and/or stroke, as well as additionally identified confounding variables allowed the identification of patients in whom the ICPT is most effective, however in broad confidence intervals. We are aware that a number of factors could affect the outcome of carotid endarterectomy such as previous neurological symptoms, the competence of circle of Willis, preoperative finding of brain CT, clamping time and carotid plaque characteristics. Nevertheless, the results of our study undoubtedly indicate a beneficial effect of the IPCT procedure, which should be investigated in future randomized trials.

Conclusion
This is a first-in-human study of IPCT performed in patients undergoing eversion CEA. The study has indicated that IPCT significantly reduced the incidence of postoperative cerebral ischemic complications after CEA in high-risk patients for IR injury when compared to the control group. Our first results are encouraging and we believe that IPCT should be further examined in future clinical trials. We believe there could be a prospective benefit of IPCT in prevention of postoperative ischemic complications in high-risk patients for IR injury after CEA. Multiple issues in carotid surgery involving discussions on clamping time, use of a shunt, postoperative hyperperfusion, ischemia and hemorrhage could be solved by applying the IPCT procedure and a new step in carotid surgery could be made.

Author Contributions
Conception and design: NI, ST, IA, DS, DUS, BL, and PM. Data collection and/or processing: NI, IA, ST, and SB. Analysis and/or interpretation: NI, ST, IA, BL, PG, PM, SB, and DS. Drafting the work: NI, IA, and ST. Critical revision: NI, BL, PG, PM, SB, DS, DUS, and ST. All authors approved the final version of the manuscript.

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