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Tissue Kallikrein Prevents Restenosis After Stenting of Severe Atherosclerotic Stenosis of the Middle Cerebral Artery

A Randomized Controlled Trial

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Abstract: In-stent restenosis (ISR) following intracranial artery stenting affects long-term clinical outcome. This randomized controlled trial sought to identify the long-term efficacy of exogenous tissue kallikrein (TK) for preventing ISR after intracranial stenting of symptomatic middle cerebral artery (MCA) atherosclerotic stenosis.

Sixty-one patients successfully treated with intracranial stenting for symptomatic MCA M1 segment stenosis (>70%) were enrolled and randomized into 2 groups: control group and TK group. Patients in the TK group received human urinary kallidinogenase for 7 days, followed by maintenance therapy of pancreatic kallikrein for 6 months. The primary end point was angiographically verified ISR at 6 months, and secondary end points included vascular events and death within 12 months. Endogenous TK plasma concentrations of patients were measured before stenting and at the 6-month follow-up time-point.

Patients in the TK group had lower occurrence rates of ISR and vascular events than patients in the control group. There was no difference in endogenous TK levels in plasma at 6 months postoperatively between the TK and control groups. Further subgroup analysis revealed that patients without ISR had higher endogenous TK levels at baseline and lower concentrations at 6 months postoperatively compared with patients who underwent ISR.

Exogenous TK is effective for the prevention of ISR after intracranial stenting.

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Abbreviations: CI = confidence interval, ISR = in-stent restenosis, MCA = middle cerebral artery, MI = myocardial ischemia, TIA = transient ischemic attack, TK = tissue kallikrein, VSMCs = vascular smooth muscle cells.

INTRODUCTION

Atherosclerotic intracranial arterial stenosis causes 30% to 50% of cerebral ischemic events in Asia every year.[1] Patients with >50% intracranial stenosis are at high risk of subsequent stroke despite antiplatelet therapy, especially for those with severe stenosis.[2] Stenting is a promising technology for severely symptomatic intracranial stenosis.[3,4] However, owing to the lack of experience of interventionalists,[5] less rigorous inclusion criteria of patients,[6] small vascular diameter, and thin wall and numerous perforator branches, the Stenting and Aggressive Medical Management for Preventing Recurrent stroke in Intracranial Stenosis (SAMMPRIS) trial supported the notion that medical therapy was superior to stenting for intracranial arterial stenosis.[7] However, some studies have suggested that a combined therapy of stenting and medical treatment benefits patients more than a single medical therapy. For instance, Jiang et al[8] demonstrated that medical therapy plus Wingspan stenting was superior to medical therapy alone in the reduction of 1-year clinical adverse outcomes in patients with >70% symptomatic intracranial stenosis after stenting. Despite this achievement, in-stent restenosis (ISR) remains a critical issue after intracranial stenting. ISR occurred in 30.8% of patients at the 6-month follow-up visit,[9] and induced a high recurrence of ischemic events and poor long-term prognosis.[10] Therefore, drug interventions that decrease the occurrence of ISR are of significant clinical relevance.

Kallidinogenase is a kind of tissue kallikrein (TK) extracted from fresh human urine, and TK is the essential component of the pancreatic kallikrein enteric-coated tablet. Previous studies have documented the efficacy of human urinary kallidinogenase during the acute phase of ischemic stroke.[11,12] TK possesses therapeutic potential for inhibiting inflammation[13,14] and suppressing the proliferation of vascular smooth muscle cells (VSMCs).[15–17] These effects interfere with neointimal hyperplasia after stenting; and thus, decrease the morbidity of ISR. Our recent research has demonstrated that

kininogenase attenuated balloon-induced intimal hyperplasia in rabbit carotid arteries.[18] However, how TK performs in patients with intracranial stenting of the symptomatic middle cerebral artery (MCA) atherosclerotic stenosis remains uninvestigated. This randomized controlled trial aimed to evaluate the efficacy of TK in preventing restenosis after MCA stenting and survey the prognostic value of endogenous levels of circulating TK in the occurrence of ISR.

METHODS

Patient Population

From December 2011 and June 2015, a total of 62 consecutive patients were screened at the inpatient stroke service of the Nanjing and Fuzhou People's Liberation Army General Hospitals (Figure 1). Statistical analysis of data obtained from the 62 patients revealed that TK treatment significantly reduced the occurrence rate of restenosis. Hence, the trial was discontinued according to ethical requirements. Detailed inclusion and exclusion criteria were described in a previously published protocol.[19] Briefly, inclusion criteria were listed as follows: patients who presented with ischemic symptoms with MCA M1 segment stenosis >70% despite antithrombotic and statin therapy for 3 months, and patients successfully treated with percutaneous transluminal angioplasty and stenting (PTAS) without acute surgical complications in 12 h after the operation. Patients were excluded if they met 1 of the following exclusion criteria: history of cerebral hemorrhage, brain tumors, brain trauma, cerebral embolism, or other brain lesions; current users of angiotensin-converting enzyme inhibitors; presence of severely cardiopulmonary dysfunction, renal insufficiency (serum creatinine >1.5 times normal), or chronic liver disease (A/G inversion, alanine aminotransferase increased 2-fold greater than normal); allergic history in medicine; and pregnant or lactating women. This randomized controlled clinical trial was approved by the Ethical Committee of Jinling Hospital, and all subjects signed informed consent forms. Two different types of stents, balloon-expendable (Apollo) stent and self-expanding (Wingspan) stent, were administered depending on vasculature anatomy. Eligible patients

FIGURE 1. Flow chart protocol of trial patients.

were randomly assigned into the control group and TK group, as previously described.[19] Detailed neurological and angiographic evaluations, as well as other clinical events, were performed by blinded and experienced raters.

Medical Management

All patients were pretreated with antithrombotic (100 mg of aspirin and 75 mg of clopidogrel) and atorvastatin (20 mg) therapy daily for 3 months prior to the endovascular procedure. After the procedure, an intravenous infusion of 0.15 para nitroaniline unit daily of human urinary kallidinogenase was administrated consecutively for 7 days, followed by the pancreatic kallikrein enteric-coated tablet (240 U, 3 times/d) for 6 months in patients in the TK group. All patients received 100 mg of aspirin, 75 mg of clopidogrel, and 20 mg of atorvastatin daily for the first 6 months. Thereafter, patients only took aspirin and atorvastatin at the same doses. Guideline-recommended risk factor control and lifestyle modifications were administrated in all patients.

Angiographic Evaluation

Angiography was performed before and after the stenting. The degree of stenosis was calculated based on the Warfarin–Aspirin Symptomatic Intracranial Disease (WASID) method.[20] Restenosis was defined as a lesion of >50% stenosis within or at the edge of the stent,[8] or with an absolute luminal loss >20% at angiographic follow-up when residual stenosis ranged from 30% to 50% after initial treatment.[9]

Follow-Up and End Points Assessment

Patients were scheduled for angiographic follow-up at 6 months postoperatively, and were further evaluated at the 12-month follow-up time-point at clinic or by telephone contact. Patients with cerebrovascular symptoms reported by telephone were advised to perform a clinical and computed tomography angiography examination in an outpatient clinic.

The primary end point was the angiographically verified ISR at the 6-month follow-up time-point. Secondary end points included vascular events and death within the 12-month follow-up period.

Biochemical Assays

Complete blood counts (red and white blood cells and thrombocytes) and biochemical analyses (cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, and creatinine) were measured at both baseline and 6 months postoperatively to assess their fluctuations during the study.

Additionally, endogenous levels of circulating TK were assessed before the procedure and at the 6-month follow-up time-point postoperatively. Blood was collected in EDTA and centrifuged at 1000g for 15 min at 4°C. The supernatant was collected and stored immediately at −80°C for subsequent assays. Endogenous TK was detected with an enzyme-linked immunosorbent assay kit (Abcam, 119598, Cambridge, England.) specific for human TK, according to manufacturer's instructions.

Statistical Analysis

Discrete variables were presented as count or percentages, and compared using chi-squared tests. Continuity correction or Fisher exact test was used when appropriate. Continuous variables verified with nonparametric tests (1-sample Kolmogorov–Smirnov test) as normal distribution were expressed as
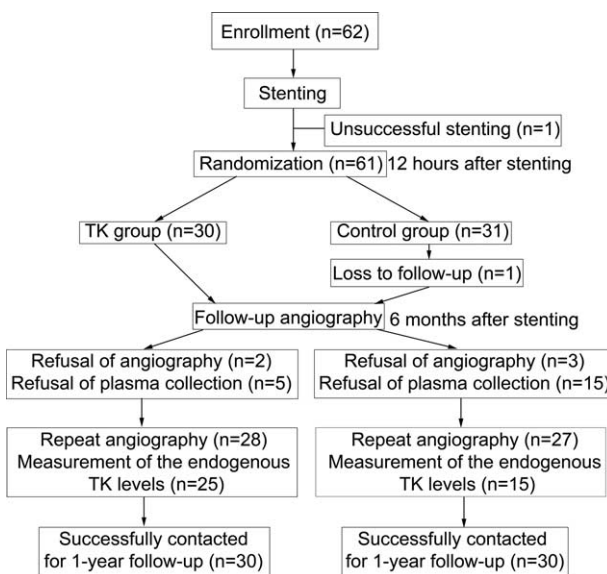
mean ± standard deviation and compared by Student *t* test. Nonparametric test was used when continuous variables had skewed distributions. *P* < 0.05 was considered statistically significant. All statistical analyses were performed with IBM SPSS statistics software (version 21.0).

RESULTS

Patient Characteristics

One patient was excluded due to cerebral hemorrhage in the temporal lobe within 12 h after stenting. Hence, 30 patients were assigned to the TK group and 31 patients were assigned to the control group randomly. One patient in the control group was lost during the 6-month follow-up period. Two patients in the TK group and 3 patients in the control group refused to have a follow-up angiography. Baseline characteristics were not significantly different between the 2 groups (Table 1).

Primary End Points

In this cohort, 55 patients underwent angiography at 6 months postoperatively. The incidence of ISR was 10.7% (95% confidence interval [CI], 3.7–27.2) in the TK group and 37.0% (95% CI, 21.5–51.8) in the control group (*P* = 0.022, Table 2). Patients with ISR in the TK group were all asymptomatic. One patient in the TK group took pancreatic kallikrein enteric-coated tablets for only 3 months after the procedure, and another patient received thrombectomy before stenting, who was diagnosed with moyamoya disease at the 1-year follow-up visit. In the control group, 2 patients with ISR were symptomatic: 1 patient had recurrence with blurring vision, and the other patient had recurrent transient ischemic attacks (TIAs). The degree of restenosis in the TK group was lower than in the control group (*P* = 0.045, Figure 2). Examples of digital subtraction angiography images obtained from these 2 patients are shown in Figure 3.

Secondary End Points and Other Adverse Events

The occurrence rate of vascular events in the TK group was obviously lower than in the control group (*P* = 0.038, Table 2). One patient who refused to have angiography had recurrent ischemic stroke in the TK group. One patient in the TK group had myocardial infarction within 30 days after the procedure. Two target vessel revascularization and 2 ischemic stroke events due to the territory of the stented artery occurred in the control group. Additionally, there were 2 TIAs, 1 ischemic stroke, and 1 unstable angina pectoris identified in the control group. No death was recorded in both groups. Hypotension (72/44 mm Hg) occurred in 1 patient in the TK group after PTAS. Blood pressure returned to normal after administration of dopamine.

Laboratory Characteristics

Laboratory characteristics of participants at both baseline and the 6-month follow-up time-point are listed in a table (see Table, Supplemental Content, http://links.lww.com/MD/A686). None of the laboratory characteristics differed significantly between the 2 groups.

Endogenous TK Concentrations

Plasma was collected from 40 patients (15 in the control group and 25 in the TK group) before the procedure and at the 6-month follow-up time-point on a voluntary basis (Table 3). At baseline, endogenous levels of circulating TK before revascularization were comparable between these 2 groups (148.8 ± 54.6 pmol/L vs 149.1 ± 96.7 pmol/L, *P* = 0.991). However, 9 patients who developed ISR had significantly lower levels of TK than the 31 patients without ISR at baseline (113.6 ± 32.0 pmol/L vs 159.1 ± 77.4 pmol/L, *P* = 0.014). In control group, patients with ISR had relatively low TK concentrations compared with patients without ISR, although no significant difference was revealed due to the small sample size

TABLE 1. Baseline Characteristics of Patients

Variable	TK Group (n = 30)	Control Group (n = 31)
Clinical characteristics		
Age	61.3 ± 7.0	59.2 ± 8.6
Male (%)	17 (56.7)	23 (74.2)
Hypertension (%)	22 (73.3)	18 (58.1)
DM (%)	12 (40.0)	8 (25.8)
Hyperlipidemia (%)	5 (16.7)	10 (32.3)
Smoking (%)	10 (33.3)	12 (38.7)
QE		
Ischemic stroke (%)	26 (86.7)	23 (74.2)
TIA (%)	4 (13.3)	8 (25.8)
Angiographic characteristics		
Location		
Left lateral (%)	14 (46.7)	21 (67.7)
Right lateral (%)	16 (53.3)	10 (32.3)
Degree of preoperation stenosis, %	85.9 ± 8.2	84.3 ± 8.2
Residual stenosis, %	5.8 ± 4.9	9.5 ± 12.1
Brand of stent		
Balloon-expendable (Apollo) (%)	24 (80)	21 (67.7)
Self-expanding (Wingspan) (%)	6 (20)	10 (32.3)

DM = diabetes mellitus, QE = qualifying event, TIA = transient ischemic attack, TK = tissue kallikrein. Values are expressed as means ± standard deviation or n (%).

TABLE 2. End-Point Events

Variable	TK Group	Control Group	*P* Value
Primary end points	(n = 28)	(n = 27)	
No. of patients with restenosis (%)	3 (10.7)	10 (37.0)	0.022
Confidence interval (CI)	3.7–27.2	21.5–51.8	
Secondary end points	(n = 30)	(n = 30)	
Vascular events (%)	2 (6.7)	8 (26.7)	0.038
Death (%)	0	0	

Values are expressed as n (%). TK = tissue kallikrein.

(111.1 ± 36.4 pmol/L vs 182.2 ± 122.0 pmol/L, $P = 0.153$). In addition, due to the limited number of patients with ISR in the TK group, plasma TK concentrations between ISR and non-ISR patients were not statistically compared. The average TK value of patients without ISR before stenting was 151.1 ± 56.4 pmol/L. Endogenous TK concentrations of the 2 patients with ISR were 118.0 and 126.3 pmol/L, respectively.

At 6 months, the levels of circulating TK did not significantly differ between the 2 groups after drug intervention (control group vs TK group: 122.4 ± 54.9 pmol/L vs 145.0 ± 60.7 pmol/L, $P = 0.245$). Among all 40 patients, 9 patients with ISR revealed higher TK levels than the 31 patients without ISR at follow up (174.8 ± 61.4 pmol/L vs 125.4 ± 54.2 pmol/L, $P = 0.025$). The difference was particularly evident between patients with and without ISR within the control group (157.5 ± 57.8 pmol/L vs 91.7 ± 29.1 pmol/L, $P = 0.014$). Serum TK concentrations in patients without ISR in the TK group averaged at 137.2 ± 56.4 pmol/L, while serum TK concentrations for the 2 patients with ISR were 215.2 and 255.2 pmol/L, respectively.

Further analysis revealed that serum TK levels in patients with ISR strikingly increased at postoperative 6 months compared with preoperative records (174.8 ± 61.4 pmol/L vs 113.6 ± 32.0 pmol/L, $P = 0.012$), while serum TK levels in patients without ISR declined at 6 months compared with preoperative records (125.4 ± 54.2 pmol/L vs 159.1 ± 77.4 pmol/L, $P = 0.034$). Additionally, the fluctuation of TK concentrations from baseline to the 6-month follow-up time-point was different between patients with and without ISR (-61.2 ± 56.9 pmol/L vs 33.7 ± 84.4 pmol/L, $P = 0.003$).

DISCUSSION

Our study demonstrates that exogenous TK was efficient in reducing the incidence of restenosis. In addition, we found that
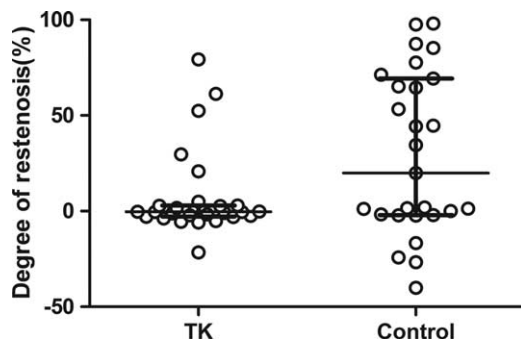

FIGURE 2. Degree of restenosis in the TK group was lower than in the control group. TK = tissue kallikrein.

the concentrations of endogenous TK in plasma did not significantly differ between the TK and control groups. However, endogenous TK levels in the patients with ISR were lower before stenting, but were higher at the 6-month follow-up time-point after stenting, compared with patients without ISR.

Stenting may cause foreign metal struts, endothelial denudation, and subintimal hemorrhage; thus, triggering the cascade of inflammation.[21–24] Inflammatory response, the main contributor to restenosis, exacerbates endothelial dysfunction, which leads to VSMC migration and proliferation, neointimal hyperplasia, and extracellular matrix formation.[25] TK plays a prominent part in anti-inflammation, anti-proliferation, as well as regulation of the contraction and relaxation of vascular and smooth muscles,[26,27] which contributes significantly to the prevention of ISR.

In our study, the administration of exogenous TK did not influence the endogenous levels of TK measured in plasma. This could be due to the fact that the ingredients of the pancreatic kallikrein enteric-coated tablet were extracted from pig pancreas, while the enzyme-linked immunosorbent assay kit could only detect human endogenous TK. Thus, we could not conclude that the risk of ISR was independent of total TK concentration, which comprised both endogenous and exogenous TK.

In the 31 patients without ISR, endogenous TK levels at follow-up time-points notably decreased compared with preoperative levels. On the contrary, in all 9 patients with ISR, there was a significant increase in endogenous TK concentrations at the 6-month follow-up time-point compared with preoperative levels. Therefore, the concentration of endogenous TK decreased in the event of successful revascularization, but elevated with the occurrence of ISR. Porcu et al[28] demonstrated that the degree of carotid artery stenosis was positively associated with TK levels in blood, and that the concentration decreased after revascularization. Another study indicated that patients with ISR after peripheral percutaneous angioplasty had a significant increase of TK levels than patients without restenosis.[29] Thus, our observations were consistent with the findings of these studies.

Our study suggests that a lower endogenous TK level before the procedure may be a potential predicator of the development of ISR after stent implantation. Accumulating evidence indicates that the inhibition of inflammation and intimal hyperplasia of TK is protective against restenosis,[26,27] which may benefit patients in the acute phase after stenting. It is reasonable to hypothesize that patients without sufficient amount of endogenous TK are at higher risk of ISR, because the ability to inhibit inflammation and intimal hyperplasia is weak. Accordingly, patients with a higher amount of endogenous TK before the procedure exhibited lower risk of ISR. This finding was consistent with an observation from a previous
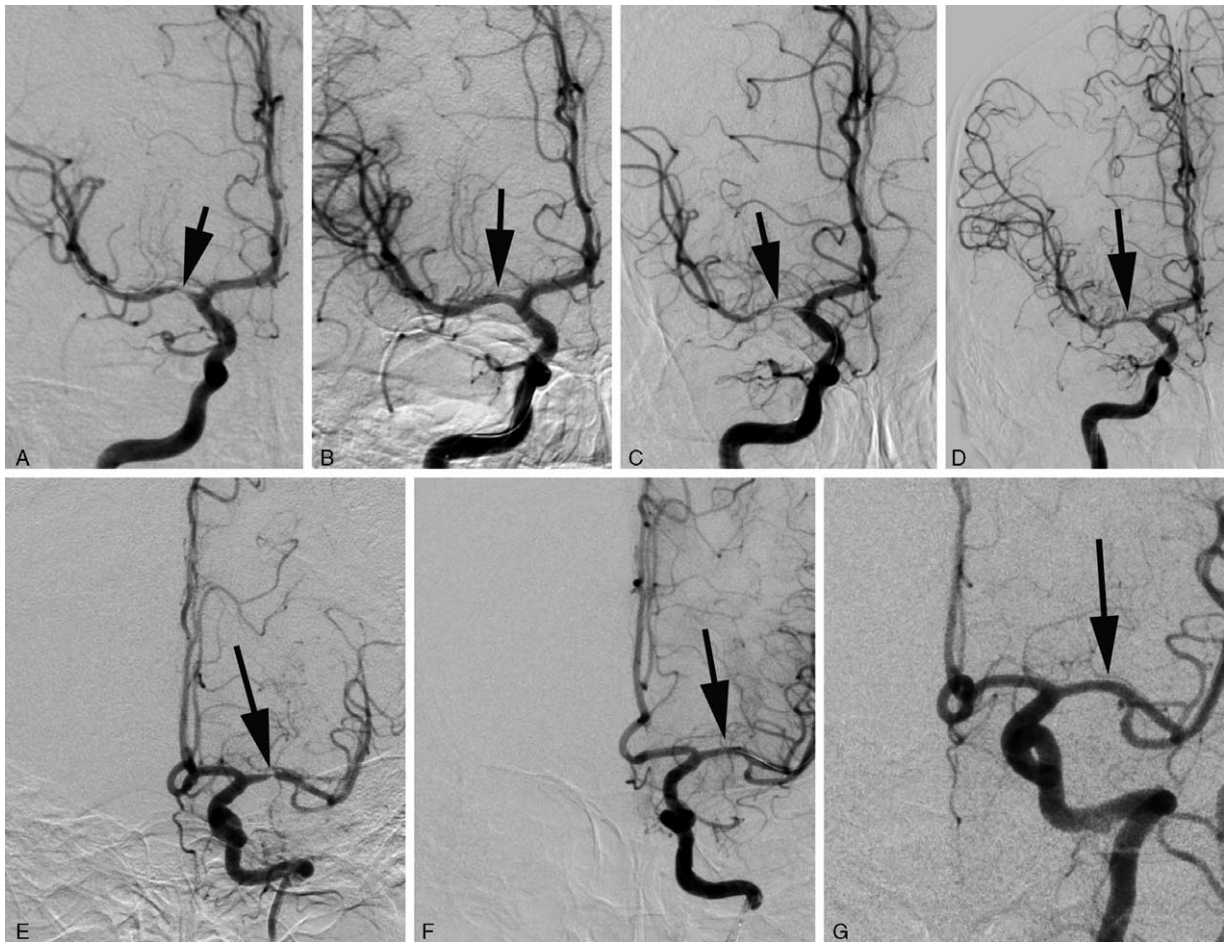
FIGURE 3. (A) Right middle cerebral artery stenosis (approximately 84%) of a patient in the control group is shown. (B) Angiogram after placement with a 2.5 × 8-mm Apollo stent. (C) Follow-up angiograph at 6 months after the procedure shows that the degree of restenosis was approximately 80%. (D) Angiograph after repeated balloon dilation therapy. (E) Preprocedural angiography of a patient in the TK group shows a stenosis of 85% in the proximal left middle cerebral artery. (F) Postprocedural angiography was performed after the placement of a 2.5 × 8-mm Apollo stent. (G) Follow-up angiogram at 6 months after stenting reveals that the degree of restenosis was 7%. Arrows appearing in the figure present the stenosis location. TK = tissue kallikrein.

report, revealing that elevated levels of TK in plasma was a protective predictor against both initial and recurrent stroke.[30]

Higher endogenous TK concentrations at 6 months were correlated with higher incidences of ISR after stenting. One possible explanation was that stenting procedure triggers the development of inflammation response, which could be attenuated by TK. Once the inflammation is suppressed, the endogenous TK concentration returns to normal. However, ISR patients with persistent inflammation may have a continuous rise of endogenous TK concentrations. Therefore, the presence of persistent high levels of endogenous TK after stenting is potentially a sign of the occurrence of ISR.

In our cohort, our overall successful stenting rate was 98.4% (61/62), which was close to the 97.6% rate reported by Jiang et al.[31] Multiple intracranial stenosis or tandem extracranial lesions occurred in 48.3% patients (29/60). Patients with multiple lesions may be at higher risk of recurrent stroke compared with patients with single intracranial artery stenosis.[32] In our study, only 18.8% of patients (3/16) in the TK group and 23.1% of patients (3/13) in the control group experienced the primary end point events after the procedure. The risk of a

recurrent stroke event in our cohort was 6.7% (4/60) within 12 months, which was similar to our previous report.[9]

TK treatment did not cause any obvious side effects regarding biochemical analyses and blood cell counts. LDL cholesterol levels in both groups significantly declined at the 6-month time-point postoperatively, owing to the administration of atorvastatin. There was no significant difference in the occurrence of major adverse events between these 2 groups during the whole follow-up period. Additionally, TK administration did not enhance the risk of bleeding in this study.

In the present study, some limitations need to be acknowledged. First, exogenous TK concentrations resulting from drug administration could not be measured, because enzyme-linked immunosorbent assay kits specific for exogenous TK are not commercially available. Thus, we were not able to evaluate whether the occurrence of ISR was associated with the total concentration of circulating TK. Second, our study is not a double-blinded study, but a small open-label multicenter study with a relative small size. A larger sample size of studies is required to confirm these outcomes. Third, our departments are large centers of neural intervention in East China. Most of our

TABLE 3. Endogenous TK Levels of Patients

	ISR	NISR	Total
TK group	(n = 2)	(n = 23)	(n = 25)
Baseline	118.0/126.3	151.1 ± 56.4	148.8 ± 54.6
The 6th month	215.2/255.2	137.2 ± 56.4	145.0 ± 60.7
Control group	(n = 7)	(n = 8)	(n = 15)
Baseline	111.1 ± 36.4	182.2 ± 122.0	149.1 ± 96.7
The 6th month	157.5 ± 57.8	91.7 ± 29.1	122.4 ± 54.9
Total patients (N = 40)	(N = 9)	(N = 31)	
Baseline	113.6 ± 32.0	159.1 ± 77.4[*]	
The 6th month	174.8 ± 61.4[†]	125.4 ± 54.2[‡,§]	
Fluctuation	−61.2 ± 56.9	33.7 ± 84.4[¶]	

Values are expressed as means ± standard deviation. ISR = patients with in-stent restenosis, NISR = patients without in-stent restenosis, TK = tissue kallikrein.

[*] $P = 0.014$, compared with patients with ISR at baseline.
[†] $P = 0.012$, compared with patients with ISR at baseline.
[‡] $P = 0.025$, compared with patients with ISR at 6 months after stenting.
[§] $P = 0.034$, compared with patients without restenosis at baseline.
[¶] $P = 0.003$, compared with the fluctuation of TK concentrations of patients with ISR.

participants came from other cities that are far away from our centers. Thus, patients failed to observe the 1-month follow-up requirement, as reported in a previously published protocol. Fourth, most of our participants could not tolerate the follow-up invasive examination at 1 year, therefore a 1-year follow-up angiography was lacking. Hence, a large multi-center clinical trial with more patient population is warranted to confirm our observations in the future.

In conclusion, our clinical trial demonstrates that exogenous TK treatment significantly reduces the incidence of ISR and greatly improves clinical outcomes in patients after MCA stenting. Therefore, TK holds a therapeutic potential for the prevention of late lumen loss without attendant risk for patients with intracranial stenting. Additionally, preoperative low concentrations of endogenous TK that increases at 6 months after the stenting procedure might serve as a simple and noninvasive predictor for late ISR. However, a large sample size study would be required to further confirm our findings in the future.

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