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DERLEME **REVIEW**

THE MYSTERIOUS PHENOMENON OF ISCHEMIC STROKE: NO-REFLOW

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ABSTRACT

Recanalization therapies based on reopening of the occluded great vessel after ischemic stroke are the only treatment options available today and have made significant advances in improving clinical function. However, providing recanalization does not always result in tissue survival and positive functional recovery. One of the various reasons underlying this incompatibility is the "no-reflow" phenomenon. This term was first used in the cardiology literature to describe the situation in which parenchymal tissue reperfusion was not achieved despite recanalization. In the following period, the existence of a similar process in experimental ischemic stroke models, in which the blood flow did not improve at the microcirculation level despite the opening of the occluded great vessels, was proven. Some of the causes of this phenomenon, which has been known in experimental studies for many years but whose pathophysiology has not been fully elucidated, are the aggregation of blood elements, increase in blood viscosity, contraction of pericytes -a component of neurovascular unit, narrowing of microvessels, and activation of inflammatory processes. Since this phenomenon causes the success of recanalization treatments not to be at the desired level, its pathophysiology should be fully elucidated, especially through clinical studies. Cocktail therapies to improve reperfusion besides recanalization therapy in ischemic stroke can prevent the "no-reflow" phenomenon.
Key Words: No-reflow, ischemic stroke, microcirculation, reperfusion.

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İSKEMİK İNMENİN GİZEMLİ FENOMENİ: NO-REFLOW

ÖZET

İskemik inme sonrası tıkanan büyük damarın geri açılmasına dayanan rekanalizasyon tedavileri günümüzdeki tek tedavi seçeneğidir ve klinik fonksiyonu iyileştirmede önemli ilerlemeler sağlamıştır. Ancak rekanalizasyon sağlanması her zaman doku sağ kalımı ve olumlu fonksiyonel iyileşme ile sonlanmamaktadır. Bu uyumsuzluğun altında yatan çeşitli nedenlerden bir tanesi "no-reflow" fenomenidir. Bu terim ilk olarak kardiyoloji literatüründe rekanalizasyon sağlanmasına rağmen parankimal dokuda reperfüzyon sağlanamaması durumunu tarif etmek için kullanılmıştır. Takip eden dönemde deneysel iskemik inme modellerinde, tıkalı büyük damarların açılmasına karşın kan akımının mikrodolaşım düzeyinde düzelmemesi ile seyreden benzer bir sürecin varlığı kanıtlanmıştır. Uzun yıllardır deneysel çalışmalarda varlığı bilinen ancak patofizyolojisi tam olarak aydınlatılamayan bu fenomenin nedenlerinden bazıları kan elemanlarının aggregasyonu, kan vizkositesinde artma, nörovasküler ünite hücrelerinden perisitlerin kasılarak mikrodamarları daraltması, inflamatuar süreçlerin aktifleşmesidir. Bu fenomen, rekanalizasyon tedavilerinin başarısının istenen düzeyde olamamasına neden olduğundan özellikle klinik çalışmalarla patofizyolojisinin tam olarak aydınlatılması gereklidir. İskemik inmede rekanalizayon tedavisi yanında reperfüzyonu iyileştirmeye yönelik kokteyl tedavileri "no-reflow'' fenomenini önleyebilir.
Anahtar Sözcükler: No-reflow, iskemik inme, mikrodolaşım, reperfüzyon.

INTRODUCTION

Stroke, the leading cause of disability worldwide and the second leading cause of death, is a significant health problem, and its incidence is increasing (1). After a stroke, recanalization treatments based on reopening the occluded vessel have substantially improved clinical outcomes and lowered functional loss. However, the treatment window is short, and the bleeding side effect is an essential factor in reducing the benefit-harm ratio of treatment. Furthermore, despite the recanalization achieved with intravenous thrombolysis and mechanical thrombectomy, the expected clinical improvement does not occur in a considerable number of patients. One of the reasons for this process, also known as futile recanalization, is a factor highlighted in both clinical and experimental studies: Although recanalization is achieved in the cerebral vessels, blood flow at the level of the microcirculation does not improve, and reperfusion cannot be achieved in the parenchymal tissue supplied by the microvessels. This image briefly referred to as the "no-reflow" phenomenon (2), is hypothesized to be mechanistically linked to occlusions in microvessels (2-6). However, in addition to aggregation of blood elements and increase in blood viscosity, dysfunction in the cells of neurovascular unit has been found to play a role (4-7). This phenomenon should be considered among the molecular targets of treatment in stroke because it decreases the success of recanalization treatments, the only treatment option available today, and raises the risk of infarct enlargement by preventing the improvement of perfusion in viable tissue. The pathophysiology must first be fully elucidated to determine these treatment goals.

In this review, the history of the no-reflow phenomenon in the brain, the experimental models used to understand its mechanisms, its clinical importance, effects on healing and its necessity of becoming a new treatment target will be emphasized.

HISTORICAL PERSPECTIVE

Prior to the 1960s, the survival of a cell when blood supply to tissue was interrupted was thought to depend on the functional capacity of that cell (8, 9). Subsequent research has suggested that ischemic injury may play a role in the pathogenesis of permanent ischemic injury by damaging blood or vessels, inhibiting blood flow return and perfusion (2). Although complete recanalization of the occluded vessel was achieved, the first observations of inadequate perfusion in ischemic tissue were made in 1959 (10), first in the kidney and then in the heart of cats and dogs. In these experimental studies, it was observed that myocardial perfusion disturbance did not wholly improve and persisted even though coronary arteries recanalized after ischemia (11). Another myocardial ischemia study in dogs confirmed perfusion impairment, and histopathologic studies revealed capillary damage, endothelial cell changes, and intravascular fibrin deposition (12).

In a 1963 study, Neely and Youmans demonstrated the existence of this phenomenon in the brain, in addition to heart. In this study, dogs experienced less brain damage after 25 minutes of bloodless ischemia after exchanging blood with saline solution before ischemia. It was suggested that the absence of microthrombi and lactic acid production in the brain deprived of blood had allowed longer survival in the state of cerebral anoxia (9).

The global cerebral ischemia model study in albino rabbits by Ames et al. was the first study investigating vascular factors in ischemic stroke in the brain and referring to the "no-reflow" phenomenon (2). In this study, the bilateral carotid arteries were clamped, and collateral circulation from the Willis polygon was prevented by raising the cuff of the blood pressure monitor to 350 mmHg in the neck. The brains were removed following the ischemia and recanalization model and fixed after perfusion with colloidal carbon or Ringer's lactate. Colloidal carbon distributed throughout the vascular lumen showed that perfusion defects in arterioles and capillaries appeared as early as 5 minutes after ischemia, and this defect became much more widespread and severe after 10-15 minutes of ischemia (2). Moreover, the occurrence of this phenomenon at ischemia times as short as 5 minutes has demonstrated that this phenomenon occurs much faster in the brain than in the heart. Administration of heparin before ischemia reduced perfusion defects but did not prevent them. This suggests that fibrin clots play a role in developing perfusion defects, but other contributing factors exist. After intravenous colloidal carbon administration, the vessels were filled to a large extent at macro-and micro-level in the bloodless ischemia model created by perfusion of bilateral carotid arteries with Ringer's solution, indicating that non-fibrin factors from blood play an important role in perfusion defects in ischemia. A widespread capillary filling defect during applying an erythrocyte suspension after bloodless ischemia revealed that vascular factors play a role in the pathophysiology of "no-reflow" in the same study.

In rats in which only cerebral ischemia was performed in some groups and cardiopulmonary insufficiency was produced by thoracotomy in some groups during cerebral ischemia, no problem was observed in vascular filling after recanalization in the ischemia and control groups without thoracotomy, whereas vascular filling was impaired in the ischemia and control groups that received thoracotomy. And this led to the wrong conception that the no reflow phenomenon could be an artifact due to the experimental conditions or observed in postmortem tissue (13). In subsequent years, the use of methods to better study microcirculation and findings from experimental models have demonstrated the importance of the role of this phenomenon in tissue survival after ischemia (14-17).

Similar microvessel occlusions were observed in a study in which focal cerebral ischemia was performed in monkeys with middle cerebral artery occlusion, and the brains were perfused with carbon black. Occlusions in microvessels were seen 3 hours after ischemia. Electron microscopic examination of these brains revealed that swelling of the glial processes and the endothelium surrounding the microvessels developed, as well as cerebral edema (4). In studies in which microvessels were pathologically examined, it was observed that 1 to 4 hours after occlusion of the middle cerebral artery, fibrin deposits appear in the microvessels and erythrocytes neutrophils, and platelets form blockages in the microvessels together with this fibrin (5). Although cellular elements remain confined within the microvessels, it was observed in further studies that partial plasma perfusion continues (6). Microvessel constriction was thought to be due to swelling of the glia around the vessel, swelling of the endothelium, and edema in the tissue in previous studies (3, 4). However, other studies have suggested that the main culprits of microvessel occlusion are erythrocyte aggregation and increased blood viscosity, and the role of blood elements has been emphasized, based on the observation that perfusion deterioration can be prevented mainly by diluting the blood with saline solution before ischemia (7).

WHAT EXPERİMENTAL MODELS TELL US ABOUT THE MECHANİSMS INVOLVED İN THE "NO-REFLOW" PHENOMENON

For many years, the mechanisms causing the "no-reflow" phenomenon have been studied in the literature, with studies focusing on various possible main mechanisms (Table 1).

1. Contraction of Pericytes and Constriction of Microvessels

Vasoconstriction was thought to play a role in the pathophysiology of "no-reflow" when it was observed that erythrocytes, when reintroduced into the experimental model of "bloodless ischemia," could not pass through small vessels. The presence of vasoconstriction in asphyxia was used to support this hypothesis, but it was argued that this was not the primary mechanism because lesions were only found in the sections of capillaries that were supposed to be incapable of contraction in early asphyxia (18).

Yemişci et al. proposed contraction of pericytes, constriction of microvessels, and entrapment of erythrocytes as a mechanism for the "no-reflow" phenomenon in ischemic stroke (6). According to this study, pericytes in microvessels contracted after two hours of the middle cerebral artery occlusion, and this contraction persisted after recanalization. Pericyte contraction has been found to constrict capillaries and impair perfusion by preventing the passage of erythrocytes and other blood elements. The factors causing pericyte contraction were found as nitrosative, oxidative stress, and peroxynitrite formation in the same study, and it was demonstrated that pericyte contraction could be prevented by suppressing these factors (6). Later, in our laboratory studies in which only the microvessels were separated from the brain, it was discovered that the pericytes in the capillaries in the core and peri-infarct areas exhibited ischemia-mediated contraction at different ischemia and recanalization times and constricted the microvessels compared with those on the opposite side (Figure).

Pericytes are located in the neurovascular unit (NVU). The concept of NVU is a comprehensive concept introduced in Neuroscience in 2001. It is at the capillary level between brain parenchyma and blood vessels having structural and dynamic functions. NVU consists of endothelium, mural cells (pericytes and vascular smooth muscle cells), glial cells (astrocytes, microglia), and neurons. Pericytes surround the endothelial cells lining the microvessels with a 4-10 microns diameter and enter into a close functional and metabolic relationship by sharing the same basal lamina with the endothelial cells (19, 20).

Pericytes perform various tasks such as stabilizing the blood-brain barrier composed of endothelial cells and tight junctions, regulating transporters, angiogenesis, and immunity (21, 22). The idea that pericytes play a role in the regulation of blood flow in central nervous system based on their location and their ability to contract was first expressed in the literature in 1923 (23). Later, the presence of contractile proteins like actin, myosin, and tropomyosin in the cytoplasm of pericytes further supported this hypothesis (24, 25). Furthermore, alpha-actin was detected immunohistochemically in pericytes in the capillary branching regions of the feline spinal cord (26). Pericytes cultured during the same period were observed to contract in vitro when exposed to some vasoconstrictive agents such as ET-1, thromboxane A2, and angiotensin 2 (27, 28). In subsequent years, animal experiments demonstrated that pericytes could contract and relax in response to various external factors, such as oxidative-nitrative stress (6), ATP (29), various neurotransmitters, amyloid-beta (Aβ)-oligomer (30), and pH, when exposed to various agents. In recent years, optogenetic manipulation has allowed brain pericytes to be sensitized to light and the contraction response to be controlled (31).

Although there is a consensus that pericytes at capillary branches contain alpha-smooth muscle actin and can contract, there is disagreement in the literature about the contractility of pericytes and their contractile mechanisms because alpha-smooth muscle actin cannot be detected in the middle segments of capillaries by immunohistochemical methods (32). Demonstrating that the small amount of alpha-smooth muscle actin in the middle capillary segments could not be observed due to rapid depolymerization during tissue capture and therefore quick fixation methods were required, a technical error frequently repeated in the literature was eliminated, and the term "contractile cell" was re-given to pericytes (20, 33). In the ischemia model performed in the retina, the tissue where pericytes are most concentrated, the pericyte-mediated contraction has been shown to produce the no-reflow phenomenon, and alpha-smooth muscle actin is responsible for contraction here (34).

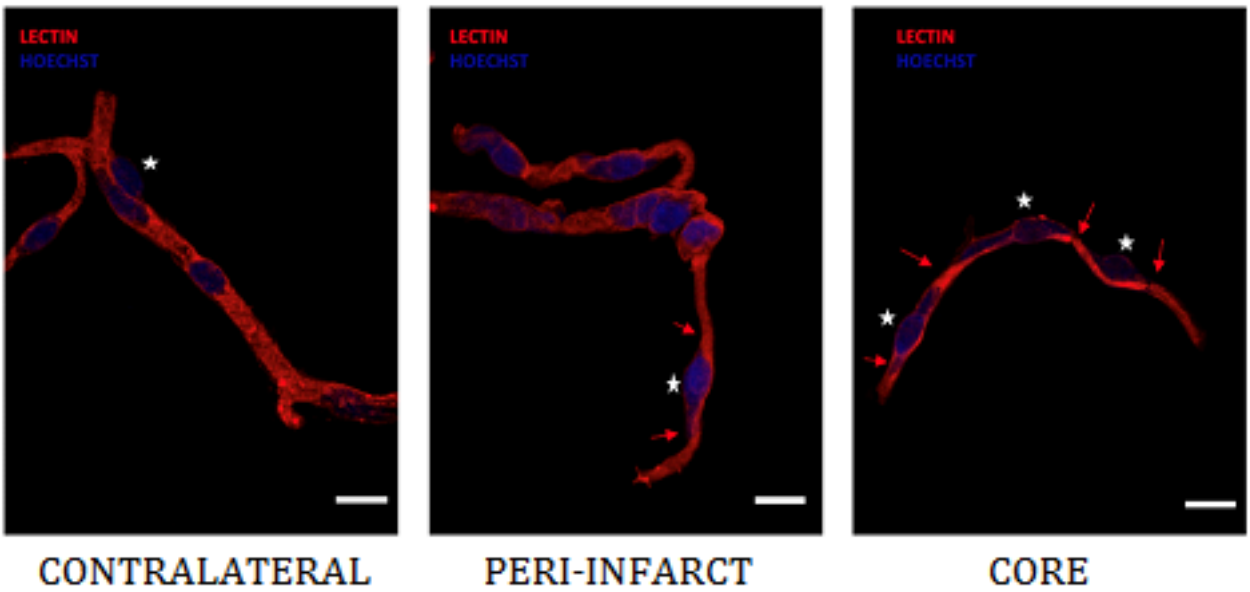

Figure. Recanalization was performed for 48 hours after a 90-minute proximal, middle cerebral artery ischemia model in mice, and microvessel isolation was performed by separating the core, peri-infarct, and contralateral regions from fresh brains. Lectin (red) is used to mark microvessels, and Hoechst is used to mark nuclei (blue). Pericyte bodies are shown with a white star, contractions with red arrows. It is observed that despite recanalization following ischemia, microvessels isolated from the core and peri-infarct areas are constricted by pericytes (Scale bar = 10 micron).

2. The Effect of Reactive Oxygen Species and the Inflammatory Response

The inflammatory response is triggered very early in ischemia. The metabolism of the tissue is disturbed due to the hypoxia caused by the arterial occlusion, reactive oxygen species (ROS) are formed, and an inflammatory reaction begins (35). In various ways, this contributes to the "no-reflow" phenomenon.

ROS formation causes intravascular damage and rapidly activates the coagulation and complement cascade. Thrombin, formed from the coagulation cascade and activation, converts fibrinogen to fibrin, and fibrin forms secondary microvascular irreversible plugs by retaining the formed blood elements erythrocytes, platelets, and polymorphonuclear blood elements (5). Furthermore, the formation of ROS decreases the level of nitric oxide (NO), which is an important vasodilator, increasing vascular tone and diminishing the ability to reverse microvascular contraction. As a potent vasodilator and inhibitor of platelet aggregation, NO also contributes to the "no-reflow" phenomena by decreasing for various reasons (36, 37). Pericytes contract and the microvessel lumen narrows due to ROS (6). Since the polymorphonuclear cells in the blood in the physiological state are more voluminous and rigid concerning the cytoskeleton than the erythrocytes, they occasionally block the entrances of the microvessels and make it impossible for the flexible, small erythrocytes to penetrate these segments (38).

Immune cells are summoned into the environment when ROS activates the complement cascade, and the main leukocyte attack occurs 12-24 hours after ischemia (39). Because the "no-reflow" phenomenon occurs within the first hour after ischemia, it can be assumed that the leukocytes already present in the blood contribute before the attack of the immune cells begins. In the later hours of ischemia, the increase in adhesion molecules (such as vascular cell adhesion molecule 1 (VCAM) and P-selectin) results in greater adhesion of leukocytes to the capillary wall (40, 41).

Later, apoptotic cell death in endothelial cells decreases the possibility of adaptive responses in the microcirculation. Neurological damage may increase at this stage, with the possibility of hemorrhagic transformation and edema formation (42, 43).

3. Swelling of Astrocyte Endfeet, Endothelial Cells in Ischemia

The fact that perivascular cell swelling is responsible for the "no-reflow" phenomenon dates back to early research on this phenomenon. In historical experimental studies, the swelling of the endothelial and astrocyte endfeet and narrowing of the vessel lumen have been discussed as probable mechanisms after global ischemia. Because the asphyxia damage begins at the level of the capillaries, the possible pathogenesis is based on perivascular edema rather than vasospasm. Some studies disagree with this conclusion, highlighting vasoconstriction and thrombus formation as reasons (44). Ultrastructural studies further revealed that the astrocyte endfeet and endothelial cells swell together with their nuclei and mitochondria starting 30 min after ischemia (45, 46).

One of the mechanisms suspected in the pathophysiology of astrocyte endfeet swelling in ischemia is the formation of lactic acid by switching metabolism to anaerobic respiration (47). Neurotransmitter-dependent cell swelling and glutamate-dependent excitotoxicity have been suggested as additional mechanisms, as the volume of astrocytes exposed to glutamate also increased in vitro studies (48). Glial fibrillary acidic protein (GFAP) reactivity increased in astrocytes adjacent to microvessel segments where FITC-dextran perfusion decreased, and edematous changes were observed in these astrocytes located around microvessels with fibrin accumulation after intravascular administration of FITC-dextran (5). In recent studies, the increase in calcium in the astrocyte endfeet in the brain and in the Müller cells in the retina is thought to be responsible for the changes in capillary diameter (34, 49, 50).

4. Increase in Blood Viscosity and Erythrocyte Aggregation

Studies have shown that the increase in blood viscosity and aggregation of erythrocytes play a role in the perfusion disorder in the microcirculation after ischemia, dating back to Neely and Youmans' revelation in 1963 that ischemic damage can be reduced by administering the saline solution to the cerebral vessels during ischemia (9). Ischemia-induced blood flow slowing and impaired ion transport due to a lack of energy may increase blood viscosity, contributing to circulatory disturbances in microvessels. This is supported by significant improvements in tissue perfusion following ischemia through diluting the blood by administering the saline solution to rabbits prior to ischemia (7). Stasis and increased blood viscosity can also increase erythrocyte aggregation, which can lead to erythrocyte occlusion of microvessels. In a study allowing direct visualization of erythrocytes after ischemia by autofluorescence, it was observed that erythrocyte plugs formed in the capillaries after cleaning the brain with cardiac perfusion using saline, and these plugs were particularly located in the capillaries in the penumbra region (16). Because of the narrowing of the capillaries caused by pericyte contraction after ischemia, erythrocytes become trapped in the capillaries and occlude the microvessels (6). As a result, the erythrocytes clogging the capillaries also block the flow of other blood elements, and the coagulation cascade is more activated (51).

5. Other Mechanisms That May Play a Role: Cortical Spreading Depolarization

Cortical Spreading Depolarization (CSD) is a type of neuroglial depolarization wave that spreads slowly in the cerebral cortex (52, 53). CSD can be seen in the brain in pathological conditions such as ischemic stroke, subarachnoid hemorrhage, and traumatic brain injury. In addition, it is thought to be the electrophysiological correlate of migraine aura, which is seen in some migraineurs, and plays a role in triggering the migraine pain (54, 55). Hemodynamic changes in cerebral blood flow after CSD in healthy tissue have been reported. Following the depolarization wave, these changes occur at various times. Initially, a hypoperfusion state is noticed, occurring concurrently with the depolarization wave and lasting less than 1 minute (56, 57). This hypoperfusion state has been shown to be due to vasoconstriction in the meningeal arteries (58, 59). This is followed by a phase of hyperemia and vasodilation that lasts a few minutes and can drastically alter cerebral blood flow, depending on the technical characteristics and the species studied (60, 61). This hyperemia phase is followed by a prolonged phase of hypoperfusion and vasoconstriction that may last for several hours (56, 57).

CSD waves have been shown to occur in the ischemic brain region (62). The duration and

frequency of CSD waves were found to be related to the growth of infarcts in the ischemic region as a result of metabolic disturbances (63). In ischemic tissue, hemodynamic changes caused by CSD show differences. Hypoperfusion, which occurs simultaneously with depolarization in ischemic tissue, becomes more prominent, and the hyperemic phase recedes to the background (64, 65). An increase in vasoconstrictive response is associated with increased hypoperfusion. This could be due to a rise in extracellular potassium after CSD (66, 67). The drop in perfusion associated with CSD after ischemia may contribute to the "no-reflow" phenomenon. Pericytes may play a role in this CSD-related vasoconstrictor response (68). On the other hand, CSD-related vasoconstriction has been reported in large-diameter vessels such as the meningeal arteries but not in capillaries (69, 70). Extracellular potassium elevated in the ischemic region by both ischemia and CSD may cause a repetition of CSD waves and enter a cycle (66, 71). The enlargement of the infarct area is caused by the deepening of hypoperfusion caused by continued CSDs in the ischemic region and the additional metabolic burden imposed by CSD waves on a tissue that is already under metabolic stress (63, 71, 72). Agents that inhibit CSD have been shown to alleviate CSD-induced hypoperfusion and increase cerebral blood flow (67).

CSD was also detected in people after ischemic stroke. Multiple CSD waves were seen in almost all patients in the days following a stroke in subdural electrocorticography studies of patients who underwent decompressive hemicraniectomy for large middle cerebral artery infarction. These CSD waves occur in clusters of high frequency (73). In humans, CSD waves formed in the ischemic region have also been shown to increase infarct size (71). These results indicate that CSD could also be an important therapeutic target for outcomes and recovery after ischemia.

Table 1: Mechanisms involved in the "no-reflow" phenomenon.

Mechanism	Description	References
Contraction of pericytes and constriction of microvessels	Ischemia induced oxidative-nitrative stress constricts pericytes, and they remain constricted despite recanalization. This contraction causes the microvessels to constrict and become clogged with blood components.	(6, 19, 20, 28, 34)
Activation of the inflammatory response and coagulation cascade	Depending on the inflammatory pathways and ROS *, the coagulation cascade is activated, and fibrin forms a plug in the vessels with other blood elements. Leukocytes summoned to the environment by the inflammatory reaction block the entrance of microvessels.	(5, 36, 38, 40, 41)
Swelling of astrocyte endfeet and endothelial cells	As a result of ischemia, the shift in the metabolism to the anaerobic side due to lack of energy, excitotoxicity and calcium accumulations in cells cause swelling in the perivascular cells and narrow the vessel diameter.	(4, 45-47, 50)
Increase in blood viscosity and erythrocyte aggregation	Because of stasis and the deterioration in ion transports blood viscosity rises. Erythrocyte aggregation increases as a result and erythrocytes clog microvessels.	(6, 7, 16, 51)
Cortical Spreading Depolarization	CSD† that forms in the ischemic tissue leads to an increase in vasoconstriction and hypoperfusion and further imposes metabolic burden on the tissue.	(62-64, 66, 68, 71, 72)

*Reactive Oxygen Species; † Cortical Spreading Depolarization.

6. Interventions to Prevent the No-Reflow Phenomenon

Since perfusion in the microvessels was thought to be critical for tissue survival and a good prognosis following recanalization, various pharmacological and genetic interventions have been tried to prevent the no-reflow phenomenon. In animal models, pharmacological inhibition of neutrophil adhesion after middle cerebral artery occlusion (15, 74), inhibition of platelet aggregation with antiplatelet agents (75), and inhibition of inflammatory and thrombogenic signals in CD40 and CD40L knockout mice and subsequent middle cerebral artery occlusion (76) improved cerebral blood flow and reduced infarct volume after ischemia. A later study showed that suppressing glutamate receptors and removing extracellular calcium reduced pericyte death after ischemia and improved microvessel perfusion (77). Clinical studies using inhibition of leukocyte adhesion (78), inhibition of platelet aggregation (79), and suppression of inflammation (51), despite promising results in experimental models, have not achieved satisfactory results in humans, and in some cases, have even produced worse results.

CLINICAL DATA

Clinical treatment of ischemic stroke is currently based on two basic approaches aimed at recanalization. In the literature, it has been reported that intravenous (IV) tissue-plasminogen activator (t-PA) application and mechanical thrombectomy had a success rate of 59-78% and 72-97%, respectively, in providing complete or partial recanalization (80). Despite the high success rates of recanalization, it is disappointing that the patients' clinical improvement is not at the desired level. The mismatch between recanalization and reperfusion caused by the failure of tissue reperfusion despite successful recanalization has been referred to in the literature as futile recanalization because it failed to produce clinical improvement and was cited as one of the reasons for the lack of clinical improvement (81, 82). Impaired cerebral autoregulation, hypoperfusion volume, collateral insufficiency, arterial reocclusion, and the "no-reflow" phenomenon are discussed in the literature as causes of futile recanalization.

The "no-reflow" phenomenon refers to the microvascular network's irreversible impairment of reperfusion. Unlike secondary occlusions caused by microthrombus migration to distal arterioles due to arterial thrombus lysis with tPA, it is a much more complex pathophysiological process involving pericyte contraction at the microvessel level, inflammatory mediators, ROS and astrocyte-endothelial cell swellings, as described above. Because the possibility of postmortem histopathologic examination with high temporal and spatial resolution, which is possible in preclinical studies, is not available in the clinic, the study of the "no-reflow" phenomenon in humans consists of approaches that indirectly focus on the mismatch between recanalization and perfusion after acute ischemic stroke (19, 83). The availability of computerized tomography (CT) perfusion, magnetic resonance (MR) perfusion, or positron emission tomography (PET) procedures in the clinic, particularly in the last 25 years, has enabled these studies.

Two studies investigating the mismatch between recanalization and perfusion in patients with ischemic stroke evaluated, for the first time in the clinic, complete or partial recanalization with streptokinase application by digital subtraction angiography (DSA) or transcranial Doppler and perfusion by single-photon emission computed tomography (SPECT). The rates of patients with hypoperfusion despite recanalization were 25% and 50% (84). Subsequent studies have evaluated the success of partial or complete recanalization with magnetic resonance angiography (MRA) or computed tomography angiography (CTA) and perfusion with perfusion-weighted imaging (PWI), computed tomography perfusion (CTP), or arterial spin labeling (ASL). In these studies, perfusion rates were found to vary between 0-80% despite recanalization (85).

Although current recanalization-reperfusion investigations and their association with clinical improvement have been demonstrated, the existence and impact of the "no-reflow" phenomenon in humans are still controversial. As mentioned in many studies attempting to understand this based on recanalization-reperfusion studies, the type of treatment (IV tPA/intra-arterial (IA) tPA/endovascular thrombectomy (EVT), the time of treatment initiation (< 3 hours/3-6 hours/4-24 hours), the methods used to assess recanalization (DSA, transcranial Doppler, MRA, CTA) and reperfusion (SPECT, DSA, PWI, ASL, Brain Tissue Pulsality (BTP)) and the timing of assessment of reperfusion (24 hours after treatment/3-6 hours/3-5 days after treatment/immediately after recanalization) vary between stuides (86, 87). Due to the nature of clinical investigations and even the retrospective nature of the studies, evaluating "no-reflow" is challenging due to differences in age, gender, sociodemographic distributions, collateral differences, comorbidities, and previous ischemic events. A significant part of this difficulty stems from the fact that there is no consensus on the definition. For example, in one study, "no-reflow" was defined as delayed contrast clearance on angiography in patients with acute ischemic stroke in whom successful recanalization was achieved (88). When patients were separated into delayed and non-delayed contrast enhancement groups in this study, there was no difference in stroke severity (based on NIHSS score) or clinical improvement at discharge (mRS 0-2). They attributed this to the fact that the "no-reflow" phenomenon only had a transient effect in the acute period and had no influence on chronic recovery. In another study, the "no-reflow"

phenomenon was defined based on preclinical studies as severe hypoperfusion leading to tissue infarction following ischemic stroke. In the case of middle cerebral artery territory, it was assessed as greater than 40% reduction in cerebral blood flow at 24-hour follow-up MR after treatment in 10 anatomic regions chosen based on the Alberta Stroke Program Early CT Score (ASPECTS). This study criticized the assessment of previous studies that classified partial recanalization as successful recanalization and said that the observed hypoperfusion may have been due to partial recanalization (88). It was also emphasized that the MRA and CTA methods used in previous studies to assess recanalization might not be sufficient to define distal occlusions and that the DSA method, which has been agreed upon regarding it's scoring, should be used instead. In addition, arterial spin-labeling MRI was used in this study to assess perfusion, rather than MRA or CTA used in previous studies. In summary, only 1 of the 33 patients enrolled in the study had a reduction in perfusion in the caudate and putamen area of more than 40% at 24-hour intervals ASL MR, and this patient surprisingly showed excellent clinical improvement at 3-month follow-up. Poor clinical improvement was not associated with the presence of no-reflow in this study but with the large infarct area observed on baseline MRI. As a result, the authors came to the conclusion that this phenomenon does not occur commonly in clinics. However, when the 40% threshold for hypoperfusion (Tmax greater than 6 penumbra threshold) was lowered in this study, 11 of 33 patients had hypoperfusion of less than 40%. However, only one of these patients experienced infarcts in these hypoperfusion areas. Therefore, considerations that the "no-reflow" phenomenon may not have clinical significance have increased. However, the authors also noted that the spatial resolution of current MR technology is low and that selective and diffuse neuron loss after ischemic stroke, demonstrated by 1C-flumazenil PET method in previous preclinical and clinical studies, cannot be detected by MRI method. Therefore, they also discussed that selective neuron loss due to widespread microvascular functional damage caused by the "no-reflow" phenomenon cannot be observed with the MR method (86).

At present, the "no-reflow" phenomenon in acute ischemic stroke in humans needs to be redefined and studied in more detail. This requires the use of MRIs with high spatial resolution, the use of methods such as 1C-flumazenil PET, transcranial Doppler USG, technically sufficient repeat imaging for temporal resolution, expansion of patient populations, and histopathologic correlation through the primacy of postmortem studies. In estimating infarct volume after recanalization, reperfusion success has been demonstrated to be a better predictor than recanalization success (89). Studies should be continued with the inclusion of reperfusion-enhancing and neuroprotective therapies in the form of cocktails in the clinical treatment strategy, in addition to successful recanalization.

CONCLUSION

The existence of the "no-reflow" phenomenon in acute cerebral ischemia has been established in experimental studies for many years, and the mechanisms involved have been studied in detail, but its pathophysiology is still not fully understood. New methods, especially imaging with high spatial resolution, are needed for the presence and detailed evaluation of this phenomenon in humans. The use of recanalization following ischemic stroke and additional treatments to improve reperfusion will improve the prognosis of patients and increase the possibility of success, in light of the information we have obtained from experimental studies.

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Ethics

Ethical Approvel: Since this study is a review article, Ethics Committee approval is not required.

Informed Consent: Since this study is a review article, consent is not required.

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