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Photochemicallly Induced Thrombosis as a Model of Ischemic Stroke

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Summary

Better understanding of ischemic brain injury mechanisms is important for the development and improvement of diagnostic and therapeutic modalities for management of ischemic stroke. As experimental studies are on demand, there's a need for relevant models of focal brain lesions. Photochemically induced thrombosis remains one of the most popular models of ischemic stroke.

The purpose of the review is to consider the pathogenesis and applicational relevance of the photochemical thrombosis in ischemic stroke modeling.

Material and methods. The information was searched using PubMed and Google Scholar databases and keywords «photothrombotic stroke» without language restrictions. 74 papers out of more than 600 sources were found the most relevant for the purpose of this review and selected for the analysis. Of these, more than 50% have been published in the last five years. The criterion for excluding a source was an inconsistency with the objectives of the review and low information content.

Results. We outlined a variety of features in modeling photothrombotic stroke, analyzed the advantages and disadvantages of the model, presented data on current method's modifications, as well as approaches to evaluation of brain lesions in ischemic stroke induced by photothrombosis, and summarized information about the mechanisms of brain damage induced in this model.

Conclusion. Several advantages of the photothrombotic stroke model, such as low invasiveness, high reproducibility, inherent control of brain infarction volume and low mortality, determine its active use in experimental studies of ischemic stroke. Pathological processes in the brain modeled by photochemical thrombosis are similar to the processes occurring in acute ischemic cerebral circulation events. Therefore, this model provides insights into cellular and molecular mechanisms of ischemic brain damage, and can be used for developing novel therapeutic approaches for management of ischemic stroke.

Keywords: focal ischemia; photothrombosis; photothrombotic stroke; mechanisms; brain damage

Conflict of interest. The authors declare no conflict of interest.

Introduction

Stroke is a leading cause of death and disability worldwide [1]. Despite an enormous number of studies, the therapeutic options for stroke patients remain very limited. This prompts further research into the intricate pathophysiological mechanisms of stroke development in order to develop new effective ways to prevent and treat stroke.

The pathogenesis of acute ischemic stroke is best mimicked by models of focal brain lesions, most commonly caused by occlusion of the middle cerebral artery (MCA) [2]. Arterial occlusion is usually achieved by the use of small-diameter synthetic filaments, blood clots, or prothrombotic drugs [3]. One such experimental model is the photochemical thrombosis model. This model allows the simulation of the events triggered by the occlusion of cerebral arteries in human stroke as closely as possible to natural conditions [4].

Most studies simulate neocortical stroke by photothrombotic occlusion of microvessels. Although this method induces a thrombotic stroke, it does not have a direct clinical analogy because it mainly involves occlusion of small cortical vessels (less than 40 μm) rather than a large artery or its branches [3, 5]. Nevertheless, it is a relatively simple, noninvasive way to induce a local infarct in any preselected region of the neocortex in the rat or mouse, and is therefore actively used in experiments on modeling, diagnosis, and therapy of ischemic stroke [6].

The aim of this review is to consider the pathogenesis and practical significance of photochemical thrombosis in ischemic stroke modeling.

Materials and Methods

Information was searched in PubMed and Google Scholar databases using the keywords «photothrombotic stroke» without language restrictions. From more than 600 sources for analysis, 74 were selected as most relevant to the objective of the review. Of these, more than 50% were published within the last five years. Criteria for the exclusion

of sources were their inappropriateness to the aim of the review and their low informative value.

Modeling photothrombotic stroke (PTS). To induce photothrombosis, a solution of a photosensitive dye (most commonly rose Bengal dye) is injected into the circulatory system (intravenously in rats or intraperitoneally in mice) [7]. It does not penetrate cells and remains in the cerebral vasculature. The animal's head is fixed in a stereotactic unit, a longitudinal skin incision is made, and the periosteum is removed. In rats, it may be necessary to perform a craniotomy at the desired site using a special drill [8, 9]. The skull is irradiated with light from a laser placed at a certain distance with a wavelength of 520–560 nm for 10–30 min, after which the surgical wound is sutured. The photosensitizer, when exposed to intense light, produces reactive oxygen species that damage the membranes of vascular endothelial cells, causing platelet adhesion and aggregation and ultimately thrombus formation in the irradiated area (Fig. 1).

Stroke localization is determined by the site of laser irradiation, whereas its severity is related to the dosage of photosensitizer and light [10, 12].

Advantages and limitations of the photothrombosis model. The pathophysiology of the PTS model is based on thrombosis due to disruption of endothelial integrity with rapidly progressing ischemic infarction and cell death in a relatively small cortical volume. In contrast, other stroke models require more invasive surgical techniques, such as middle cerebral artery (MCA) occlusion. In this case, the cortex and subcortical regions are damaged simultaneously, and the area of ischemic penumbra is well defined [5]. The PTS is a well-established model for the study of focal ischemic brain lesions [12]. The pathological processes in the brain simulated by photochemical thrombosis are similar to those occurring in acute cerebrovascular accidents of ischemic type (atherothrombotic or cardioembolic stroke). This model is characterized by high reproducibility, the ability to control cerebral infarct size, and low mortality [10, 13–15]. It has been shown that the infarct volume depends on the intensity of the laser radiation [12, 16], as well as on the duration of light exposure: increasing the exposure time from 15 to 20 minutes leads to an increase in infarct volume without worsening functional deficits [17]. The PTS model allows the study of the changes in sensorimotor pathways without the influence of subcortical areas [18]. The use of this model makes it possible to obtain statistically reliable quantitative data on the degree of brain damage and changes in pathophysiology and regeneration, as well as to assess the neuroprotective effect of pharmacological drugs [13].

Thus, the advantages of this model include minimal invasiveness, good reproducibility of cortical

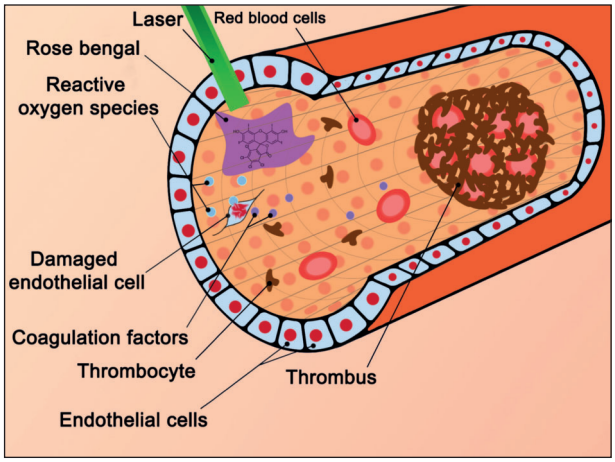

Fig. 1. Schematic illustration of the mechanism of blood vessel occlusion in photo-induced thrombosis.

stroke in both rats and mice, low animal mortality, ability to select the area of exposure, accuracy of localization of ischemic site, and potential control of the size and depth of ischemic damage [15, 19, 20].

The limitations of the model include its permanent occlusive nature, which does not allow using this model to study the mechanisms of ischemia-reperfusion cell damage, as well as reperfusion therapy [10]. Another problem is that photothrombosis causes both vascular and cytotoxic edema equally, whereas human ischemic stroke causes mainly cytotoxic edema, which does not immediately result in blood-brain barrier disruption, a major limitation that hinders extrapolation of data obtained with this model [5, 10, 21]. The rapid and intense development of tissue edema and necrosis results in a relatively small penumbra zone. This is one of the limitations of the photothrombotic model that should be considered when evaluating reperfusion efficacy [5, 22]. The ischemic penumbra was first defined by Astrup and colleagues [23] based on electrophysiological findings as the area where the reduction in cerebral blood flow causes electrical dysfunction but not membrane insufficiency. The penumbra is a spatially dynamic region of the brain with limited viability, characterized by sophisticated pathophysiological changes affecting neuronal and glial functions [24]. Today, the penumbra is defined more broadly as an area of ischemic tissue that is functionally damaged and at risk of infarction, but potentially viable [20].

Another drawback of the photothrombosis model is that experimental animals usually do not have neurological deficits or have deficits that are difficult to diagnose [18].

To address these and other problems, modifications of the PTS model and new modern methods of lesion diagnosis are being developed. In early versions of the model, photothrombosis caused a

severe stroke with a rapidly growing ischemic zone and no penumbra zone, but later modifications of the model using different laser parameters made it possible to obtain a wider penumbra zone [11]. For example, a less intense but longer photodynamic exposure of the rat cerebral cortex (diode laser; 532 nm, 60 mW/cm^2, 30 min) resulted in a 1.5–2 mm wide penumbra around a 3 mm diameter infarct core, which was confirmed by histological and ultrastructural examination [11]. Tuor et al. (2016) showed that irradiation of the rat cerebral cortex with light at 555 nm and an intensity of approximately 40 mW/cm^2 for 5 min after administration of 10 mg/kg rose bengal induced a small infarct with moderate to diffuse penumbra [25]. Clark et al. (2019) proposed a modification of the photothrombosis model in mice using a digital micromirror device [20]. Occlusion of multiple branches of the MCA was performed on the surface of the motor cortex, while limiting collateral cerebral blood flow and blocking the branches of the anterior cerebral artery. This technique made it possible to expand the penumbra zone and delay spontaneous reperfusion of the target arteries, similar to what happens in humans. This was different from the traditional photothrombotic model, which usually results in permanent arterial occlusion and relatively limited collateral blood flow. In this context, the proposed modification may serve as a potential model of cerebral ischemia-reperfusion [20].

The resistance of the PTS model to fibrinolytic therapy is thought to be related to the formation of a platelet-rich but fibrin-poor clot as a result of the photochemical reaction. Recently, a model of murine photothrombosis was proposed in which a combination of rose bengal dye (50 mg/kg) and a subthrombotic dose of thrombin (80 U/kg) was used to induce thrombosis in the proximal branch of the MCA and produce a fibrin-rich and tPA-sensitive clot. Meanwhile, infarct size and localization were constant, and intravenous injection of tPA (Alteplase, 10 mg/kg) for 2 h after photoactivation significantly reduced infarct size. Thus, the thrombin-enhanced PTS model may be useful for testing thrombolytic therapies [26].

Kim et al. (2021) developed a system of photochemical induction of thrombosis that can reproduce the damage of a specific brain region in the rabbit. The main advantage of this system is the ability to locally induce ischemic stroke in the brain region responsible for specific functions [27]. This model has shown that the volume of damage increases 24–48 hours after induction of photothrombosis and tends to decrease 72 hours after induction.

Qian et al. (2016) developed a modification of the murine PTS model in which both the cortex and basal ganglia were damaged [9]. The model is based on occlusion of the proximal MCA using a convenient laser system with an optical fiber. Other advantages of this technique include high reproducibility of results, significant penumbra, and low animal mortality. In another study, optical fibers stereotactically implanted into the surgically isolated proximal MCA were used to create infarcts in subcortical regions of the brain in rats [28]. In this model, magnetic resonance imaging (MRI) demonstrated signs of penumbra as well as the feasibility of thrombolysis with tissue plasminogen activator rt-PA [28].

More recently, Hosseinic et al. (2018) proposed a method to induce selective unilateral hippocampal ischemia in rats using a modified photothrombotic model [29]. Twenty-four hours after exposure, histological examination of the hippocampus revealed shrunken nuclei and pyknotic neurons in the ischemic zone. The average infarct volume was 6.5%, and its size did not differ significantly between experimental animals.

A disadvantage of the photothrombosis model is that a procedure of skull thinning with a special drill is used to gain access to the cortical vessels of the rat. This procedure may cause changes in intracranial pressure and may also result in bleeding during surgery or inflammation postoperatively. The use of optical tissue imaging techniques can help to avoid these problems [12]. One such technique is optical tissue clearing. Optical clearing refers to the temporary reduction of light scattering in biological tissues, and is one of the simplest and most effective methods for increasing the depth and quality of images of deep tissue structures, as well as improving the accuracy of spectroscopic information from deep layers of tissue and blood. Optical immersion clearing is based on the impregnation (immersion) of tissue with a biocompatible chemical agent (an optical clearing agent) that has a sufficiently high refractive index so that it can match the refractive indices of the scatterers and their surroundings by penetrating into the tissue fluid. In particular, glycerol, propylene glycol, ethanol, thiazone, etc. are used as tissue permeability enhancers for optical clearing of skull bone [30].

Recently, a technique of optical skull clearing in mice without craniotomy has been proposed, in which an «optical window» is created through which a light beam can pass [31]. Based on this technique, a controlled model of ischemic stroke was created by combining an in vivo optical skull clearing technique with a photothrombosis procedure. In this case, the degree of thrombotic occlusion and infarction severity can be effectively controlled by changing the light dose. The optical window can also be used for continuous blood analysis and flow mapping. This model represents a valuable asset for ischemic stroke studies [12].

Methods of Brain Research in Stroke Modeling with Photothrombosis

Imaging techniques to determine the size and volume of the lesion. Various MRI techniques are currently used to determine the volume of ischemic injury and to identify the penumbra area in both clinical and experimental settings [9, 21, 27, 32, 33]. The volume of the lesion is most commonly determined using T2-weighted MRI images [9, 18]. It is an effective non-invasive method to assess infarct size during the first 2 weeks after the onset of ischemia. Whole-brain volumetric microscopy techniques, such as serial two-photon tomography (STPT), can provide detailed information about damage and regeneration in the brain after stroke [34]. Automated mapping, connectivity, and histologic analysis using atlases are also used to compare the size and location of the lesion area [35, 36]. In contrast to other stroke models, in PTS the vasogenic edema, which corresponds to a strong hyperintense signal on T2WI, resolves within the first 2 weeks after stroke and transforms into a hypointense cavity [37].

Remodeling of the vessels surrounding the infarcted area of the brain is known to occur after stroke. A method was developed to monitor changes in vascular structure and blood flow with high spatiotemporal accuracy after photothrombotic infarction in the murine motor cortex using longitudinal two-photon and multi-exposure speckle imaging. Vascular remodeling in the peri-infarct cortex developed during the first 2 weeks after stroke, with old vessels being replaced by new ones and selectively stabilized. This vascular structural plasticity coincided with temporal activation of transcriptional programs relevant to vascular remodeling, restoration of peri-infarct blood flow, and significant improvements in motor activity. The results confirmed that vascular remodeling contributes to behavioral recovery after stroke by restoring blood flow to the peri-infarct cortex [39].

Electrophysiological methods to detect ischemic damage. Other methods for quantitative assessment of structural brain damage in the rat PTS model are being developed, particularly based on electroencephalography (EEG). Spectral analysis revealed a significant correlation of the relative power of alpha, theta, delta, delta/alpha ratio, (delta + theta) / (alpha + beta) ratio with stroke size. Analysis of auditory evoked potentials revealed a significant relationship of amplitude and latency with stroke size. These results demonstrate the usefulness of EEG in monitoring brain damage after stroke [16].

Histochemical and immunohistochemical techniques to assess brain damage in photothrombotic stroke.

Serial brain sections stained with triphenyltetrazolium chloride (TTC) solution are also used to determine the volume of damage [7–9, 27, 39].

To analyze and confirm brain damage at the cellular level, classical histological methods, such as morphometry of fixed brain sections stained with toluidine blue or Nissl cresyl violet or hematoxylin and eosin, are used [8, 26, 40–42]. A correlation has been demonstrated between the volume of the lesion detected by MRI and by histological methods [9, 18, 27].

For more detailed analysis, immunohistochemical staining techniques can be used to identify neurons and glial cells and their death or proliferation.

For example, NeuN protein is a marker of mature neurons and is used to visualize and analyze the infarct zone and assess neuronal death [7, 17, 40, 42]. Markers such as c-fos and heart shock protein 90 are used to detect the penumbra zone [9]. The astroglial marker, glial fibrillary acidic protein (GFAP), reveals the interface between ischemic and intact areas and is used to visualize activated astrocytes surrounding the stroke core as a glial scar [18, 37, 41].

Impairment of blood-brain barrier (BBB) permeability is assessed using histochemical dyes, particularly Evans Blue [17, 40]. It is known that dyes bound to serum albumin can cross the BBB after ischemia. Evans Blue dye is commonly used to assess BBB damage due to its rapid binding to serum albumin. Indocyanine green (ICG), a clinically available dye that binds to serum proteins, can also be used. More recently, a new dye, the zwitterionic NIR fluorophore (ZW800-1), has been proposed. Its advantage is that it does not bind to serum, has an extremely low nonspecific tissue uptake and is rapidly eliminated from the body by renal filtration, while allowing successful visualization of ischemic lesions in brain tissue, as demonstrated in the PTS model [43].

The response of microglia in PTS-induced brain injury can be assessed by immunohistochemical staining. In particular, antibodies against CD68 and Iba1, which are markers of macrophages and microglial cells, are used to assess the activation of microglia, which are resident macrophages of the brain [7, 17, 41, 44, 45].

Molecular methods of investigation. After ischemic stroke, cellular damage extends from the infarct site to the surrounding tissue (penumbra). To identify the proteins involved in the mechanisms of neuronal alteration and neuroprotection in the penumbra, changes in protein expression are studied using antibody microarrays [46], NanoString technologies [44], etc. For example, changes in the expression of more than 200 neuronal proteins in the penumbra 4 or 24 hours after focal photothrombotic infarction were studied using antibody microarrays. The largest changes

were detected 4 hours after injury [46] and were recorded in proteins of signal transduction pathways, proteins responsible for axonal growth and direction, vesicular transport, neurotransmitter biosynthesis, intercellular interactions, cytoskeletal proteins, and others. These proteins are known to be involved in both neuronal injury and neuroprotection.

Choi et al. (2019) examined changes in the expression of specific genes during the period from the acute to the chronic phase (up to 8 weeks) of stroke in a rat photothrombosis model. One week after stroke, there was a significant decrease in the expression of genes for neurotransmitter synaptic and signaling pathways, as well as genes for neurotrophic factors, while an activation of apoptosis-associated molecules was observed. In the first 4 and 8 weeks after stroke, proliferation of cellular adhesion and inflammatory cells increased [47].

A study of protein expression 3 days after photothrombotic MCA occlusion in mice using NanoString technologies revealed distinct regulatory proteomic profiles in the damaged hemisphere according to regions of interest, including the ischemic core, peri-infarct tissue, and peri-infarct normal tissue. The core border profile showed apoptosis, autophagy, neuronal death and immunoreactivity for early degenerative proteins. Specifically, the core border showed decreased neuronal proteins Map2 and NeuN; increased autophagy proteins BAG3 and CTSD; increased microglial and peripheral immune invasion proteins Iba1, CD45, CD11b, and CD39; and increased neurodegenerative proteins BACE1, APP, amyloid β 1-42, ApoE, and tau protein S-199. Increased apoptotic and altered proteomic profiles with increases in BAG3, GFAP, and hyperphosphorylated tau protein S-199 were detected in the peri-infarct area [44].

X-ray fluorescence analysis allows the identification of metabolically distinct areas of neuronal tissue, such as the infarct core and the intermediate area surrounding the infarct core, the so-called metabolic penumbra in the early period or the peri-infarct zone in the later post-stroke period. Studies have shown that as early as 1 hour after PTS in mice, the levels of phosphorus, sulfur, and potassium were significantly reduced in the infarct focus, with the level of potassium remaining below normal for 1 month after injury. At the same time, the concentration of chlorine and calcium increases and exceeds the physiological parameters throughout the period studied. Elemental concentrations in the penumbra or peri-infarct zone appear to be intermediate between those in the infarct core and in normal tissue. Responding glial cells alter the average elemental composition of the stroke focus, so that elemental levels 1 week after stroke and beyond are a combination of elemental levels in these cells and in the surrounding tissue. The results of the study showed that the therapeutic window for survival of a significant portion of the penumbra is within the first 24 hours, after which the penumbra expands to include previously unaffected tissue. The change in K^+ and Ca^{2+} levels is an early sign of significant neuronal tissue dysfunction and irreversible damage. It has been found that the total area of tissue affected in the acute phase (including infarct core and penumbra) reaches its maximum by the 2nd day after stroke. The method of tissue metabolic analysis is useful for monitoring stroke severity in the presence of stroke risk factors, as well as for quantifying the efficacy of stroke treatment in animal models [48].

Morphologic Changes in the Brain after PTS

Early morphological changes. Morphological studies of rat cerebral cortex showed that 4 h after PTS, neurons, glial cells, and capillaries in the infarct core were damaged, the neuropil was altered, and significant intracellular and vasogenic edema developed with cyst formation [11, 49]. In mice, one hour after proximal MCA photothrombosis (532 nm, 35 mW, 2 min), karyolysis and pyknosis were observed in the injury zone [9]. In rats with photothrombosis of sensorimotor cortical vessels caused by prolonged laser irradiation (532 nm, 64 mW/cm^2, 30 min), initial necrotic changes within the stroke core were observed, such as an increased proportion of hyperchromic neurons and the appearance of pyknotic neurons at 1 hour. At 24 hours, morphologic changes intensified. Typical ischemic changes such as massive vacuolization of neuropil, edema, and degeneration of neurons, glia, and blood vessels were observed. Microscopically, edema and destruction of mitochondria, endoplasmic reticulum and dictyosomes of the Golgi apparatus, degradation of synapses, disorganization of myelin, swelling of neurons and glial cells, edema and destruction of capillary components were evident. The morphological changes in the penumbra region were similar to those in the necrotic core, but gradually decreased toward the penumbra border [11].

While necrosis is the underlying mechanism of cell damage in the stroke core, apoptosis plays a more important role in the penumbra [11]. In the areas of the penumbra adjacent to the infarct zone, the highest percentage of apoptotic cells was observed 24 hours after PTS, and necrotic cells predominated 48 hours later [50]. Repair of damaged brain tissue begins at 72 hours and ends approximately 28 days after PTS [51].

The decrease in neuronal density at the infarct border is accompanied by an early response of glial cells. Generalized microglial activation in the ipsilateral cortex can be detected as early as 3 hours after occlusion. Astrocyte activation is observed in intact parts of the ischemic hemisphere 6 hours after occlusion [24].

Activation of microglial cells is associated with changes in their morphology and number. Signs of microglial activation include an increase in cell number and soma area, a decrease in cell area and diameter, a reduction in primary processes and their length, and an increase in cell density [41]. Thus, the immunohistochemistry studies in a rat photothrombosis model revealed an increase in the circularity index of Iba1+ cells. This index peaked at 24 hours in peri-infarct tissue and remained elevated for 3 days [42]. The proportion of Iba1-positive material in the peri-infarct zone increased significantly by day 3 and remained elevated until day 7, mainly due to an increase in the number of microglial cells [37, 42]. In a study of the PTS model in mice, microglial activation persisted up to 84 days after stroke [41].

Secondary damage. Local brain damage causes distant structural and functional abnormalities that contribute to behavioral deficits and impair functional recovery of the brain. Secondary brain cell damage is one of the major mechanisms that initiates additional selective cell death in non-ischemic brain regions with synaptic connections to the site of primary damage and correlates with functional deficit and outcome [52–54]. Thus, a murine model of photothrombosis showed secondary neurodegeneration in ipsilateral brain regions, particularly in the sensorimotor area of the thalamus [187, 54].

Focal ischemic cortical lesions can also cause distant white matter damage, but this is limited to fibers associated with the area of the primary lesion. Thus, in a model of focal unilateral PTS of the rat sensorimotor cortex, severe axonal changes were observed in the ipsilateral external capsule as well as in remote regions, including the contralateral external capsule and the corpus callosum. Further analysis of fiber tractography showed that only fibers with direct axonal connections to the primary lesion area were significantly damaged. These fibers mostly represented perilesional, interhemispheric and subcortical axonal connections. The size of the primary lesion was found to be a determinant of motor deficit [32].

In mice with photothrombotic cortical stroke, damage to hippocampal CA1 field neurons was also observed 28 days after exposure compared to sham-operated animals [40].

In addition, secondary death of midbrain dopaminergic neurons has been reported in a rat primary motor cortex model of PTS [55]. Secondary dopaminergic degeneration after stroke is associated with adverse outcomes such as a post-stroke depression or parkinsonism [45].

Mechanisms of Photothrombotic Brain Injury

The neural tissue damage in the PTS core is similar to that in other stroke models and in human stroke. However, there are some differences (Fig. 2).

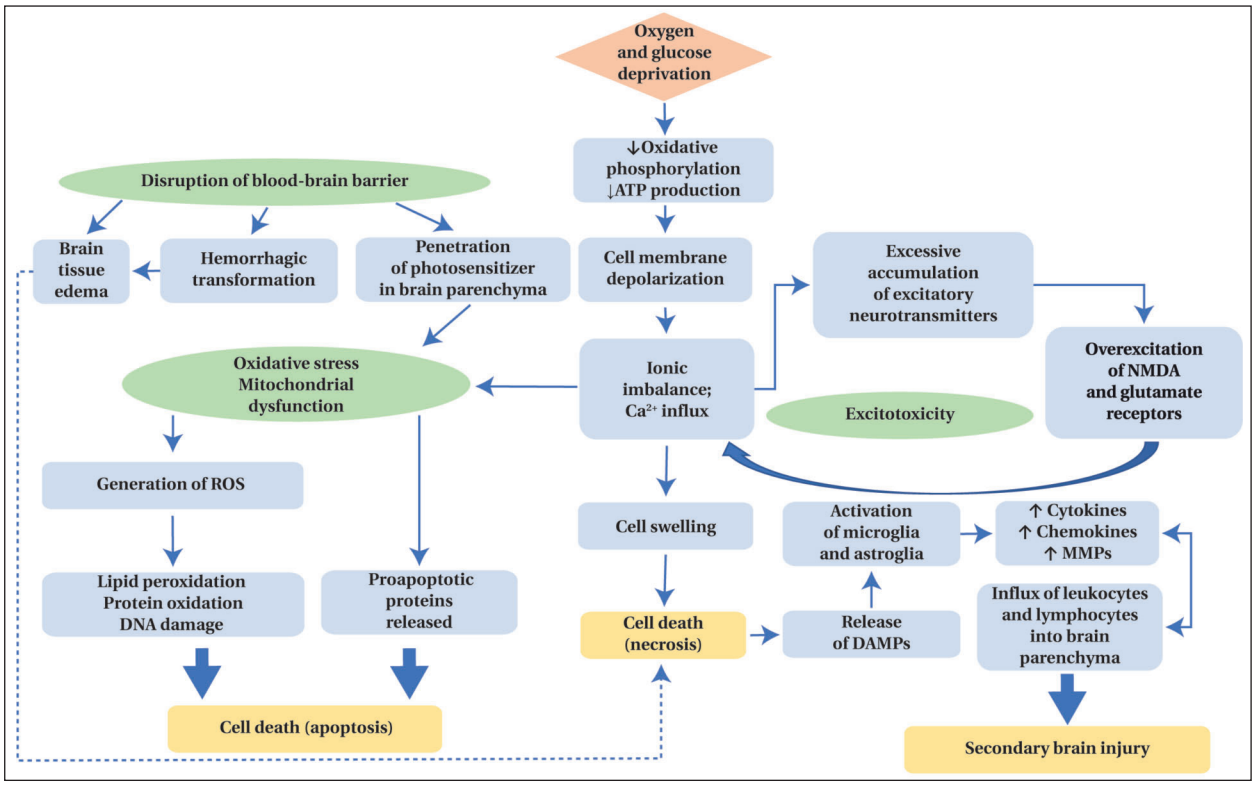

Fig. 2. Signal pathways of cell damage in photothrombotic stroke.
Note. DAMPs — damage-associated molecular patterns; NMDA — N-methyl-D-aspartate; MMPs — matrix metalloproteinases; DNA — deoxyribonucleic acid.

The PTS model is characterized by rapidly developing ischemic brain cell damage. However, in contrast to the MCA occlusion model, where platelet aggregation and coagulation abnormalities are the main pathogenic factors, neuronal damage in photothrombosis may have other causes [11]. Alterations in the PTS core include several interrelated events, such as direct photodynamic damage to cells, signaling and metabolic pathways leading to cell death, similar to other types of ischemic stroke, and the sequelae of tissue edema. As in other types of stroke, vascular occlusion and decreased blood flow result in reduced and interrupted delivery of oxygen and glucose to the infarct core, inhibition of oxidative phosphorylation, and cessation of ATP production [56]. ATP deficiency leads to rapid failure of energy-dependent ion pumps and channels, loss of membrane potential and depolarization of neurons and glia, and Ca^{2+} influx into cells [57]. As a result, the concentration of potentially toxic excitatory neurotransmitters in brain tissue increases. Massive release of glutamate and aspartate from damaged neurons causes overexcitation of cell receptors, leading to opening of calcium channels and influx of calcium and sodium ions into neurons. This causes passive water entry into the cells and their edema. Cell lysis occurs, especially in the ischemic core [5]. In addition, large amounts of Ca^{2+} ions activate hydrolytic enzymes such as nucleases, lipases, and proteinases, promoting cell destruction in the stroke core [1]. This is known as excitotoxicity.

The integrity of the BBB is very rapidly compromised in stroke due to oxidative stress, increased levels of matrix metalloproteinases, cytokines, disruption of dense contacts and proteins of integrins (transmembrane glycoprotein receptors). This leads to vasogenic edema and hemorrhagic transformation [58]. In the experiments of Kuroiwa et al. (2013), BBB disruption was observed in rat brain 4 h after basal ganglia PTS. It peaked on day 1 and completely disappeared 6 days after PTS [28]. Four to six hours after stroke, there is an influx of serum proteins, which also leads to vasogenic edema [21]. It has been shown that in PTS, during the first hour after exposure to light, there is a massive leakage of blood plasma through the vascular walls into the brain tissue. This process almost stops after 4 hours, but persists in the penumbra up to 24 hours after photothrombosis. Thus, both clot formation and blood plasma leakage through the damaged vascular walls play an important role in the pathogenesis of PTS [11, 59].

Due to the disrupted BBB, photosensitizer molecules penetrate into glia and neurons, contributing to direct photodynamic damage to brain cells [60].

Increased intracellular calcium leads to enhanced production of free oxygen radicals, causing lipid peroxidation, protein oxidation, and nucleic acid damage [61]. Calcium ions cause mitochondrial pore opening and release of pro-apoptotic proteins into the cytosol. Disruption of mitochondrial membrane integrity and mitochondrial dysfunction leads to the production of reactive oxygen species and nitrogen [11]. Mitochondria play a central role in the development of oxidative stress, which leads to cellular and structural brain damage [62]. In general, intense oxidative stress in the infarct core leads to cell necrosis, whereas moderate stress in the penumbra mainly results in apoptosis [63].

Neuronal death triggers several cascades of responses, including the release of damage-associated molecular patterns (DAMPs), which initiate the activation of microglial and astroglial cells and the production of bioactive substances, cytokines, chemokines and other factors that can affect surrounding tissues [64, 65]. In the site of injury, cytokines and chemokines cause recruitment of leukocytes and lymphocytes, which permeate brain tissue through the disrupted BBB [66].

Microglial cells can become activated within minutes of ischemia and produce biologically active substances such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α). In the murine PTS model, neurons at the core of the injury have been shown to die as early as 2 hours, accompanied by activation of microglia and astrocytes [67]. Peak microglial activity is observed two to three days after injury and persists for several weeks [21, 40].

Ischemia also causes activation of astrocytes. After PTS in rats, the astroglial response is initiated 4 h to 1 day later, peaks at 4 days, and persists for up to 28 days [15, 40, 68]. Cytokines released by neurons and glial cells after ischemia induce reactive hyperplasia of astrocytes. Activated astrocytes begin to produce monocytic chemotoxic protein-1, IL-1β, GFAP, vimentin, and nestin, resulting in reactive gliosis and glial scarring [69, 70]. In addition, astrocytes produce metalloproteinases (MMPs) that degrade basement membrane proteins and tight junctions of the BBB, increasing its permeability and leukocyte penetration into brain tissue [66]. However, astrocytosis may play a positive role in healing. Recently, in a murine PTS model, reactive astrocytes were shown to be critical mediators of vascular remodeling, which is important for functional repair [48].

Leukocytes and lymphocytes produce neurotoxic proteins such as inducible nitric oxide synthase (iNOS) and MMPs, reactive oxygen species, and proinflammatory factors [71, 72], which causes secondary brain damage [66].

The role of lymphocytes, as well as microglia and astroglia, in post-ischemic brain injury is far from clear and requires further study, which is important for the development of future immunomodulatory therapeutic strategies [8, 72–74].

The progression of secondary brain damage is believed to be associated with the activation of glial cells, the production of biologically active substances, while the severity and outcome of stroke depend on its severity [75, 76]. A difference has been found in the nature of the changes that occur in the regions adjacent to the area of necrosis (cortex) and in the subcortical structures of the brain (hippocampus) [40]. Neuronal damage in the peri-infarct area develops earlier and subsides with time, which explains the recovery of motor functions. In the hippocampus, these processes last for a very long time (3 months), which explains the persistence of cognitive dysfunction [40].

Conclusion

The advantages of the photothrombotic stroke model, such as low invasiveness, high reproducibility, ability to control the infarct volume, and low mortality, allow its active use in experimental studies of ischemic stroke. Brain abnormalities simulated by photochemical thrombosis are similar to those seen in acute cerebrovascular disorders of ischemic type (atherothrombotic or cardioembolic stroke). Consequently, this model helps to study the cellular and molecular mechanisms of ischemic brain injury and may be useful in the search for therapeutic options for stroke.

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