The Ischemic Penumbra: Correlates in Imaging and Implications for Treatment of Ischemic Stroke

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Abstract

The concept of the ischemic penumbra was formulated 30 years ago based on experiments in animal models showing functional impairment and electrophysiological disturbances with decreasing flow to the brain below defined values (the threshold for function) and irreversible tissue damage with the blood supply further decreased (the threshold for infarction). The perfusion range between these thresholds was termed 'penumbra', and restitution of flow above the functional threshold was able to reverse the deficits without permanent damage. However, in further experiments, the dependency of the development of irreversible lesions on the interaction of the severity and duration of critically reduced blood flow was established – proving that the lower the flow, the shorter the time for efficient reperfusion. Therefore, infarction develops from the core of ischemia to the areas of less severe hypoperfusion. The propagation of irreversible tissue damage is characterized by a complex cascade of interconnected electrophysiological, molecular, metabolic and perfusional disturbances. Waves of depolarizations, the peri-infarct spreading depression-like depolarizations, inducing activation of ion pumps and liberation of excitatory transmitters, have dramatic consequences as drastically increased metabolic demand cannot be satisfied in regions with critically reduced blood supply. The translation of experimental concept into the basis for efficient treatment of stroke requires non-invasive methods by which regional flow and energy metabolism can be repeatedly investigated to demonstrate penumbra tissue that can benefit from therapeutic interventions. Positron emission tomography (PET) allows the quantification of regional cerebral blood flow, the regional metabolic rate for oxygen and the regional oxygen extraction fraction. From these variables, clear definitions of irreversible tissue damage and critically perfused but potentially salvageable tissue (i.e. the penumbra) can be achieved in animal models and stroke patients. Additionally, further tracers can be used for early detection of irreversible tissue damage, e.g. by the central benzodiazepine receptor ligand flumazenil. However, PET is a research tool and its complex logistics limit clinical routine applications. As a widely applicable clinical tool, perfusion/diffusion-weighted (PW/DW) MRI is used, and the 'mismatch' between the PW and the DW abnormalities serve as an indicator of the penumbra. However, comparative studies

of PW/DW-MRI and PET have pointed to an overestimation of the core of irreversible infarction as well as of the penumbra by MRI modalities. Some of these discrepancies can be explained by unselective application of relative perfusion thresholds, which might be improved by more complex analytical procedures. Heterogeneity of the MRI signatures used for the definition of the mismatch are also responsible for disappointing results in the application of PW/DW-MRI for the selection of patients for clinical trials. As long as a validation of the mismatch selection paradigm is lacking, its use as a surrogate marker of outcome is limited.

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Energy Requirements of Brain Tissue

The energy demands of nervous tissue are very high, and therefore sufficient blood supply to the brain must be maintained consistently. A normal adult male's brain containing approximately 130 billion neurons (21.5 billion in the neocortex) [1] comprises only 2% of total body mass, yet consumes at rest approximately 20% of the body's total basal oxygen consumption supplied by 16% of the cardiac blood output. The brain's oxygen consumption is almost entirely for the oxidative metabolism of glucose, which in normal physiological conditions is the almost exclusive substrate for the brain's energy metabolism [2]. It must be kept in mind that the glucose metabolized in neuronal cell bodies is mainly to support cellular vegetative and house-keeping functions, e.g. axonal transport, biosynthesis of nucleic acids, proteins, lipids, as well as other energy-consuming processes not related directly to action potentials. Therefore, the rate of glucose consumption of neuronal cell bodies is essentially unaffected by neuronal functional activation. Increases in glucose consumption (and regional blood flow) evoked by functional activation are confined to synapse-rich regions, i.e. neuropil which contains axonal terminals, dendritic processes, and also the astrocytic processes that envelop the synapses. The magnitudes of these increases are linearly related to the frequency of action potentials in the afferent pathways, and increases in the projection zones occur regardless of whether the pathway is excitatory or inhibitory. Energy metabolism by functional activation is due mostly to stimulation of the Na^+K^+ -ATPase activity to restore the ionic gradients across the cell membrane and the membrane potentials that were degraded by the spike activity, and is rather high compared to the demands of neuronal cell bodies [3].

Overall, 87% of the total energy consumed is required by signaling, mainly action potential propagation and postsynaptic ion fluxes, and only 13% is expended in maintaining membrane resting potential [4].

Flow Thresholds for Preservation of Function and Morphological Integrity

The different energy requirements for the maintenance of membrane function and for propagation of information (signals) lead to different thresholds of energy consumption and consequently blood flow required for supply of sufficient biochemical substrates as well as for preservation of neuronal function and morphological integrity.

Experimental work on the ischemic flow thresholds of brain tissue has demonstrated the existence of two critical levels of decreased perfusion: first, a level representing the flow threshold for reversible functional failure (functional threshold); second, a lower threshold below which irreversible membrane failure and morphological damage occur [5]. The range of perfusion values between those limits was called the 'ischemic penumbra' [6], which is characterized by the potential for functional recovery without morphological damage, provided that local blood flow can be reestablished at a sufficient level and within a certain time window. The functional threshold was demonstrated in ischemic monkeys gradually developing a neurological deficit progressing from mild paresis at 22 ml/100 g/min to complete paralysis at 8 ml/100 g/min [7]. Concurrently, the electrocorticogram and evoked potentials (EPs) were abolished at 15–20 ml/100 g/min [8, 9] and the spontaneous activity of cortical neurons disappeared at approximately 18 ml/100 g/min (fig. 1a). The large variability of the functional thresholds of individual neurons (6–22 ml/100 g/min) [10] indicates selective vulnerability even within small cortical sectors. This interpretation is commensurate with the observed gradual development of neurological deficits. Furthermore, although single-cell activity is already altered at blood flow levels above the threshold, with grouped or regular discharges occurring at a high rate, clear threshold relations could be demonstrated; after short ischemic episodes, spontaneous cellular activity as well as EPs were restored when blood flow was normalized.

Whereas neuronal function is impaired immediately when blood flow drops below the threshold, the development of irreversible morphological damage is time dependent. Of course, once morphological damage be-

comes apparent, the initially reversible functional deficit turns into a persistent defect. Therefore, numerous studies were performed to investigate for how long brain tissue or individual cells tolerate ischemia of a given density. EPs are reliable markers of cortical function and its disturbance, as long as the respective sensory cortex is included in the core of ischemia. In areas outside the middle cerebral artery (MCA) territory, EPs are abolished despite nearly normal cortical blood flow values, thus indicating an ischemic effect on afferent pathways in the white matter [11]. If EP blockade was caused by ischemia of 6.4–7.6 ml/100 g/min in the afferent pathways, the reversibility of that blockade attests not only to the fact that the white matter can tolerate longer periods of severe ischemia than the cortex, but it also suggests that functional impairment due to deafferentation has a better prognosis than cortical damage caused by direct cortical ischemia.

The interaction of severity and duration of ischemia in the development of irreversible cell damage can be studied by simultaneous recordings of cortical neuronal activity and local blood flow [10]. The ischemic tolerance of neurons is obviously quite variable, as could be demonstrated with simultaneous recordings using up to eight closely spaced microelectrodes [12]. On the basis of a large number of neurons assessed during and after ischemia of varying degrees and durations, it was possible to construct a discriminant curve representing the worst possible constellations of residual blood flow and duration of ischemia still permitting neuronal recovery (fig. 1b). Typical points on this curve are blood flow rates of almost 0, 10, or 15 ml/100 g/min maintained for periods of 25, 40 and 80 min, respectively. Between 17 and 18 ml/100 g/min, the duration of ischemia tends to infinity; thus, indicating that this low-flow state can lead to morphological damage when maintained for very long, as yet undefined, periods of time. These results broaden the concept of ischemic penumbra: the potential for postischemic recovery of functionally impaired cells is determined not only by the level of residual flow in the ischemic phase, but also by the duration of the flow disturbance. Furthermore, a number of biological factors differing from neuron to neuron obviously govern the specific ischemic vulnerability of each cell. While residual flow rates of 12 ml/100 g/min invariably lead to large infarcts, if that ischemia lasts for 2–3 h [7, 13], individual cells may become necrotic after shorter periods of time and at higher levels of residual blood flow [14]. In some instances, critical, but primarily not detrimental, flow disturbances may trigger a dynamic process eventually leading to delayed neuronal death resulting from selective vulnerability [15].

Fig. 1. **a** Activity of a single neuron during graded ischemia before, during and after reversible MCA occlusion. rCBF = Regional cerebral blood flow. **b** Recovery of neuronal function after a limited period of ischemia. **c** Diagram of CBF thresholds required for the preservation of function and morphology of brain tissue. The activity of individual neurons is blocked when flow decreases below a certain threshold (dashed line) and returns when flow

is raised again above this threshold. The fate of a single cell depends on the duration for which CBF is impaired below a certain level. The solid line separates structurally damaged from functionally impaired but morphologically intact tissue, the 'penumbra'. The dashed line distinguishes viable from functionally impaired tissue. Modified from Heiss and Rosner [10].

Many interrelated biochemical mechanisms are involved in or contribute to ischemic cell damage, and therefore various markers can be used as indicators of ischemic thresholds and of reversible or irreversible functional deficits [reviews in 16, 17]. In accordance with the critical role that the supply of energy-rich substrates plays in brain function, cerebral ATP content and cerebral metabolic rate of glucose exhibit a threshold dependency

similar to the electrophysiological variables. Actually, the mean thresholds for ATP depletion (18.5 ml/100 g/min) and spontaneous neuronal activity (18 ml/100 g/min) are identical. However, it should be noted that the decrease in ATP content sets in at a slightly higher flow value, at which point glucose consumption is increased in an attempt to compensate for the lack of oxygen. At that flow level, local hypoxia induces anaerobic glycolysis, result-

ing in excessive lactate production, and increased lactate concentration is an indicator of progressive tissue destruction. Protein synthesis does not follow the usual thresholding pattern [18]. Its function of flow dependence rather indicates that normal protein synthesis requires a significantly higher level of blood flow (mean value, 55 ml/100 g/min), which is considered to be without effect on regular neuronal function and morphology. At this flow level, blockade of translation and protein synthesis are observed. Within the core region of ischemic injury protein synthesis decreases early and is associated with ATP loss and irreversible translation blockade, while in the 'penumbra' protein synthesis is initially depressed, ATP remains normal, and protein synthesis may recover over time [17]. Reduced protein synthesis may be important for mechanisms of neuroplasticity and might be a causal factor for the deactivation of large portions of the brain outside the ischemic region proper. In the core of ischemia, decrease in protein synthesis appears to be mediated by the unfolded protein response within the cell endoplasmic reticulum which leads to permanent translation blockade and cell death [19]. In the surrounding regions with decreased protein synthesis but preserved ATP supply, heat shock protein (Hsp) 70 is expressed [20] and this Hsp 70 expression is dependent on the degree and duration of ischemia [21]. In this area, flow is 18–20% of normal, and infarction would develop if reperfusion did not occur.

Ionic homeostasis and tissue water content are heavily affected by ischemia, with different mechanisms responsible for immediate and delayed destruction of cells. Irreversible neuronal damage is indicated by increases in extracellular potassium and intracellular sodium concentrations and by the water content of the tissue. Ca^{2+} influx into cells was also found to be one of the central mechanisms of immediate as well as of delayed cell damage, and the intracellular Ca^{2+} concentration again follows a flow threshold relation similar to the electrophysiological variables. Another mechanism has attracted much attention and research effort: the release of excitatory amino acids and the activation of the respective receptors and receptor-operated ionic channels during ischemia. During global as well as during focal ischemia, large increases in glutamate and aspartate concentrations in the cerebral cortex were found. Those changes as well as the increased concentration of the inhibitory amino acid GABA (γ -aminobutyric acid) followed the typical flow threshold relation. Blood-flow-dependent changes were observed mainly for transmitter and, to a lesser extent, for modulator amino acids, but not for essential

amino acids without synaptic action – thus indicating that amino acids are released by depolarization of excitable membranes and not by an unspecific effect on cell metabolism. The observed reversibility of the increase in glutamate concentration after transient ischemia emphasizes the importance of early intervention to counteract the neurotoxicity of excitatory amino acids. Another mechanism of counteracting excitotoxins may be of some import. The increases in adenosine and purine catabolites exhibit threshold characteristics similar to those of glutamate, but their blood flow threshold (25 ml/100 g/min) is slightly higher than that for amino acids. The higher flow threshold for the induction of adenosine release, which exerts an inhibitory action and therefore has the potential to ameliorate glutamate release and its receptor-mediated effects, may represent an inherent but time-limited protective mechanism against excitotoxicity.

Based on the threshold concept of brain ischemia, the penumbra can be localized on quantitative flow images using established flow thresholds. A more direct approach is the imaging of threshold-dependent biochemical disturbances on brain slices and to demarcate the mismatch between disturbances that occur only in the infarct core and others that also affect the penumbra. Under experimental conditions, the most reliable way to localize the infarct core is the loss of ATP on bioluminescent images of tissue ATP content. A biochemical marker of core plus penumbra is tissue acidosis. The penumbra is the difference between the respective lesion areas. The inhibition of protein synthesis starts at a higher flow level [17].

Progression of Ischemic Tissue Damage

Infarct Progression Can Be Differentiated into Three Phases

During the acute phase at flows below the threshold of energy metabolism required for maintenance of basic housekeeping (~20% of pre-occlusion values) in the core tissue, injury is a direct consequence of the ischemia-induced energy failure and the resulting terminal depolarization of cell membranes and is established within a few minutes after the onset of ischemia. During the subsequent subacute phase, the irreversible damage expands into the areas around the core where flow ranges between 25 and 50% of pre-occlusion values (i.e. below the value required for function due to axonal and synaptic activity, defined as the penumbra) until after several hours (usually approx. 6 h) the lesion has extended over all the area

with critically reduced blood supply. Finally, a delayed phase of tissue injury evolves, which may last for several days or even weeks, in which secondary phenomena – vasogenic edema, inflammation, programmed cell death – may contribute to further progression of tissue damage. In animal experiments, applying multiparametric imaging the penumbra area detected 1 h after complete occlusion of the MCA approximately predicted the size of the final infarct. After 3 h more than 50% and after 6–8 h almost the entire penumbra had disappeared and was converted into the irreversibly damaged core. However, some small regions with preserved oxygen consumption but reduced flow could be observed around the lesion at the border zone of the ischemia for up to 24 h or even more [20, 22].

In the early phases of ischemia, only reperfusion is a successful treatment which can prevent infarction if initiated in a phase when nerve cells are not irreversibly damaged. If residual flow is low or close to zero, the time for effective reperfusion is short and often treatment cannot be initiated early enough. In the subacute phase efficient reperfusion can be attained in many cases, and this is the basis of the up-to-now only successful treatment of stroke by thrombolysis or interventional thrombectomy.

However, only a limited number of patients benefit from this reperfusion therapy, and therefore great efforts have been undertaken to prolong the condition of the penumbra, i.e. of potentially salvageable tissue, and to better understand the mechanisms by which this tissue is irreversibly damaged, which might serve as targets for supportive therapeutic measures. A multitude of electrical and biological disturbances interact in the progression of irreversible cell damage in ischemia [review in 23]. It is a widely accepted hypothesis that peri-infarct spreading depression-like depolarisations (PIDs) play an important role in triggering and continuously stimulating this molecular/biochemical cascade of cell injury [reviews in 24–26]. These waves of depolarization appeared spontaneously in the surrounding of developing infarcts in tissue that was functionally and metabolically compromised, but not yet irreversibly damaged – i.e. the ‘penumbra’ [27]. PIDs have quite similar features to cortical spreading depressions with cortical DC shifts of around 20 mV, propagation velocities of 3–5 mm/min and massive disturbance of membrane ion homeostasis.

During spreading depression, the metabolic rate of the tissue markedly increases in response to the considerably enhanced energy demands of the activated ion exchange pumps [28]. In the healthy brain, the associated increase in glucose and oxygen demands is coupled to a parallel

increase in blood flow, but in the peri-infarct penumbra this flow response is suppressed or even reversed due to loss of vasoreactivity. As a result, a misrelationship arises between the increased metabolic workload and the low oxygen supply, leading to transient episodes of hypoxia and a stepwise increase in lactate during the passage of each depolarization. With each PID, the EEG is suppressed, and EEG is progressively altered over the course and becomes finally flat at the point of terminal depolarization [29]. These PIDs often appear in clusters, and have been observed for long periods after experimental MCA occlusion. The higher the rate of PIDs, the earlier the cortical DC potential converted into terminal depolarization as an indicator of functional deteriorations and infarct growths [30]. Their pathogenetic importance for the progression of irreversible injury is supported by the linear relationship between the number of depolarizations and the ATP depletion volume as a surrogate of infarction [27]. Correlation analysis of this relationship suggests that during the initial 3 h of vascular occlusion each depolarization increases the infarct volume by >20%. This is probably one of the reasons why glutamate antagonists, potent inhibitors of spreading depression, reduce the volume of brain infarcts if combined with reperfusion. In the gyrencephalic brain of cats the PIDs lead to changes in perfusion in the surrounding brain [31], which are related to the propagation of infarction. The PIDs often start off at the emerging core region, propagating radially over large portions of the neighboring cortex. Subsequent waves of PIDs preferentially circled around the ischemic core and enlarged the ischemic lesions [32]. These PIDs occurred spontaneously with high frequency over several days in patients with large MCA infarction, and might indicate a malignant course with large-space-occupying lesions [33].

PIDs play a central role in the cascade of molecular mechanisms involved in the propagation of ischemic damage, which may last independently of impairment of blood flow or energy metabolism. The events in the molecular cascade of injuries are interconnected in a complex way, which makes it difficult to predict their relative pathogenetic importance in different ischemic models [23]. Most of the biochemical and molecular processes suspected as potential key factors in the propagation of ischemic damage in special experimental models (release of excitatory and inhibitory neurotransmitters, activation of receptors and receptor-operated ion channels, influx of calcium, free radicals formation, nitric oxide generation, dysfunction of endoplasmic reticulum, mitochondrial disturbances, and others [reviews in 23, 34,

35]) still cannot be assessed in humans, despite the fact, for example, that Ca^{+} channels can be labeled [36]. However, all therapeutic interventions targeting these mechanisms [37] were without clinical efficacy [reviews in 38, 39].

Identification of the Penumbra by Imaging

It must be stressed that the concept of the penumbra is based on neurophysiological and functional studies in experimental models of focal ischemia. The transfer of this concept into imaging modalities is difficult as most markers used in experimental studies necessitate invasive procedures, e.g. the detection of the mismatch between tissue ATP depletion (for the definition of the infarction) and any biochemical disturbance that evolves at flow values in the penumbral range (such as lactacidosis, inhibition of protein synthesis or expression of the stress protein hsp 72) [23, 40]. These invasive autoradiographical procedures can also not be applied to demonstrating the gradual disappearance of penumbra with increasing times of ischemia, the progression of the irreversible damage, or the recovery of functionally impaired tissue after reperfusion. In order to follow these pathophysiological changes, non-invasive imaging modalities are required that provide quantitative maps of several important physiological variables, including regional cerebral blood flow (rCBF), regional cerebral blood volume, regional cerebral metabolic rate of oxygen (rCMRO₂) and of glucose (rCMRGlc), and up to now only positron emission tomography (PET) has been able to measure these variables repeatedly.

Early PET studies in stroke have identified various tissue compartments within a brain territory compromised by ischemia [41–44]. Tissue with rCBF <12 ml/100 g/min or rCMRO₂ <65 $\mu\text{mol}/100 \text{ g/min}$ at the time of measurement (usually several hours after stroke) was found to be infarcted on late CTs. Relatively preserved rCMRO₂ was an indicator of maintained neuronal integrity in regions with severely reduced CBF. This pattern, coined misery perfusion [45], served as a definition of the penumbra that is characterized as the area of an increased oxygen extraction fraction (up to >80% from the normal value of approximately 40%). Regions with CBF between 12 and 22 ml/100 g/min have an unstable metabolic situation; infarction might develop if low flow values persist. These PET studies allow the classification of three regions within the disturbed vascular territory: the core of ischemia with a flow <12 ml/100 g/min usually showing a transition into necrosis; a penumbra region with a flow between 12 and 22 ml/100 g/min of still viable tissue but

Fig. 2. Sequential PET images of CBF, CMRO₂ and oxygen extraction fraction (OEF) of MCA occlusion in cats and stroke patients. In the cat, the progressive decrease in CMRO₂ and the reduction in OEF predict infarction. In the patient, the area with preserved OEF is not infarcted (outside infarcted region on late MRI). If reperfusion occurs before OEF is reduced, tissue can be salvaged (left lower part of the figure for the cat, and some tissue compartments in the patient). If reperfusion is achieved too late, tissue cannot be salvaged (right cat column and infarcts in patients in lower part of the figure).

with uncertain chances for infarction or recovery; and a hypoperfused area (>22 ml/100 g/min) not primarily damaged by the lack of blood supply. It has to be kept in mind that the condition of the tissue is changing with time; the extent of the penumbra and its conversion into infarction is a dynamic process, and irreversible damage spreads from the core of ischemia to its border. This can be followed directly with advanced PET equipment, by which changes in the physiological variables were studied after occlusion of the MCA in baboons and cats (review in [46]). A description of the experimental results therefore, may help to understand findings in humans (fig. 2).

In the cat, changes after MCA occlusion are immediate and severe. Sequential studies of rCBF, rCMRO₂ and rCMRGlc from a control before to the endpoint 24 h after MCA occlusion recorded an immediate decrease in CBF within the MCA territory to <30% of control upon arterial occlusion. rCMRO₂ was less diminished and was preserved at an intermediate level. Consequently, OEF

was increased, indicating misery perfusion. In most instances, the misery perfusion condition was followed by a marked decrease in OEF, reflecting progressive impairment of metabolism and suggesting a transition to necrosis spreading from the core to the periphery of the ischemic territory. The infarcts were more or less complete 18–24 h after MCA occlusion. Occasionally, spontaneous collateral reperfusion resolved the penumbra condition and the morphological integrity of the cortex was preserved.

Reversible MCA occlusion was studied in cats by reopening the MCA after 60 min. If OEF remained elevated throughout the ischemic episode, reperfusion prevented large infarcts involving cortical areas. In contrast, if the initial OEF increase disappeared during ischemia, extended postischemic hyperperfusion accompanied large reductions in CMRO₂ and rCMRGluc, large infarcts developed and intracranial pressure increased fatally. These experimental findings from sequential studies and anecdotal clinical investigations at different time-points after the attack [47, 48] imply that the extent of the penumbra, i.e. of morphologically intact but functionally impaired tissue, depends on the time of measurement, relative to the onset of ischemia. The volume is large and the flow values are low if the penumbra is defined in the first hours of ischemia; at this point of time reperfusion strategies are most effective. The volume is small if defined later, limiting the efficacy of treatment.

The determination of absolute flow values for thresholds in patients, however, is difficult since the necessary calculation requires arterial blood sampling. Additionally, measurements of tracer concentrations are affected by considerable variability causing especially high statistical errors at low count rates. As a consequence, the reported values for the threshold of morphological damage and of the upper limit of penumbra (i.e. the functional threshold) given by different authors vary considerably [review in 46]. The values are also affected by the time of determination after the vascular attack since the variability in flow over the course and its effect on outcome cannot be assessed. For the preservation of morphology, the flow threshold ranged between 5 and 12 ml/100 g/min, for the upper limit of penumbra flow values between 14 and 22 ml/100 g/min were reported. These values corresponded to those assessed by single photon emission computed tomography (SPECT) and Xe-enhanced CT (Xe-CT).

Flow measurements in the first hours after a stroke permitted identification of various compartments of the tissue and their contribution to the final infarct on CT/

MRI. If the threshold for probable infarction was set to the conventional value of 12 ml/100 g/min and that for the upper limit of penumbra to 18 ml/100 g/min, a large compartment of the final infarct (70%) was perfused below 12 ml/100 g/min, i.e. at the level predicting necrosis; a smaller portion (18%) had flow values in the penumbra range (12–18 ml/100 g/min) and a fairly small compartment (12%) had perfusion at a higher level [49].

Measurement of blood flow values and determination of oxygen extraction fraction require arterial blood sampling and the clinical applicability is further limited by the complex logistics and instrumentation involved; isolated flow determinations at a single time-point might be confusing as long as the pattern over time is not known. A marker of neuronal integrity is needed that can identify irreversibly damaged tissue irrespective of the time elapsed since the vascular attack and irrespective of the variations in the blood flow over time, which also does not require arterial blood sampling. The central benzodiazepine receptor ligand flumazenil (FMZ) binds to the GABA receptor abundant in the cerebral cortex. These receptors are sensitive to ischemic damage and can therefore identify early neuronal loss. In transient MCA occlusion in cats, irrespective of the level of reperfusion, deficits in FMZ binding 2–3 h after MCA occlusion were closely related to areas with severely depressed oxygen consumption and predicted the size of the final infarcts, whereas preserved FMZ binding indicated an intact cortex [50]. Using FMZ as a marker of neuronal integrity and H₂¹⁵O for flow determinations, the pathophysiological changes early after ischemic stroke could be more accurately specified: 55% of the volume of the final infarct had FMZ uptake decreased below the limit of 95% probability for infarction in the first hours after stroke; 21% of the final infarct had flow below 14 ml/100 g/min, the 95% probability threshold for survival in this study, but FMZ uptake above the critical value, thereby indicating penumbra tissue. In only 13% of the final infarct was neuronal integrity indicated by FMZ, and CBF values were above the penumbra range. These results indicate the potential and the limits of therapy in acute stroke: early reperfusion cannot reverse already developed infarction, but is crucial for salvaging the penumbra; the small compartment which is sufficiently perfused might be damaged by delayed mechanisms and might benefit from neuroprotective measures.

MR-Mismatch as a Surrogate for the Penumbra

Due to the complexity of the methodology, the limited access, the invasive and complicated procedures, and the

exposure to radioactivity, PET cannot be applied in the clinical routine. Although PET remains the imaging gold standard for identification of the penumbra in stroke patients, MR studies using diffusion and perfusion imaging might provide a differentiation between the core and the penumbra: the early diffusion-weighted imaging (DWI) lesion might define the ischemic core, while the adjacent critically hypoperfused tissue might be identified with perfusion-weighted imaging (PWI) [51, 52]. Therefore, brain regions with hypoperfusion assessed by PWI but without restricted diffusion (PWI/DWI mismatch) were assumed to represent the penumbra. This surrogate definition of the penumbra has several uncertainties [53]: the initial diffusion lesion does not only consist of irreversibly infarcted tissue, and diffusion lesions may be reversed if blood flow is restored at an early time-point. Critical perfused tissue (i.e. penumbra) cannot be clearly differentiated from tissue experiencing oligemia; the PWI abnormality often overestimates the amount of tissue at risk. These facts are further accentuated by methodological limitations, because perfusion techniques and data evaluation are not quantitative and vary among centers [54, 55]. Despite the attempts to correct the mismatch area by subtracting the region of benign oligemia and adding the portion of reversible diffusion abnormality [53], the definition of the penumbra remains controversial and is suffering from lack of standardization of methodological approaches to imaging, post-processing and analysis, which restricts pooling of data and cross-comparison of results across studies. Absolute or relative thresholds derived from PW-/DW-MRI are still not reliable in predicting the fate of ischemic tissue [review in 56], and this is especially due to the limitations to quantify perfusion by MRI [57]. With the applied tracer bolus tracking, it is difficult to measure accurately the delay of tracer arrival, dispersion and transit in pathologically perfused tissue and to determine the arterial input function. These uncertainties are accentuated when collateral perfusion to areas beyond occluded arteries should be assessed.

In a recent study on 145 stroke patients, no combination to calculate maps or thresholds from PWI was able to give reliable estimates of tissue fate [58].

Recent studies have applied delay-insensitive deconvolution methods and corrected for the lag between the arterial input and the tissue response as well as for dispersion and mean transit time [57, 59–62] and have used more relevant definitions of critical hypoperfusion [63]; the obtained sizes and severities of PWI lesions were better predictors of outcome [64–66].

Validation of MRI Signatures on PET Measurements

A comparison of PW-DWI imaging results on quantitative measurements of flow values and oxygen consumption or FMZ uptake in the same patients early after stroke is necessary for the assessment of the accuracy of the applied signatures for predicting tissue outcome. Several studies were performed in order to validate mismatch as a surrogate of penumbra on PET-derived discrimination of irreversibly damaged critically perfused ‘at risk’ and oligemic ‘not at risk’ tissue. The studies demonstrated that the DWI lesion predicts more or less the finally infarcted tissue [67], but contains up to 25% false-positive, i.e. surviving, tissue. The DWI lesion indicated impairment of energy metabolism [68], and the degree of disturbance of oxygen consumption was variable within individual DWI lesions suggesting variable potential for recovery [69].

Perfusion values determined by MR bolus tracking were comparable to flow rates measured by H_2^{15}O PET in normal volunteers [70], but tracer delay caused errors in CBF estimates even in healthy persons and should be corrected [71].

The inaccuracy in defining the penumbra with PW/DWI mismatch is thought to be mainly related to PW data acquisition, which is a complex process, and the parameters used to estimate perfusion are variable and somewhat arbitrary [59]. As a consequence, perfusion lesion size differs markedly depending on the parameters calculated [54] and usually is overestimated and extends into considerable areas with non-critical oligemia, especially when short delays are used [63]. Overall, PWI is unable to provide a reliable quantitative estimation of cerebral perfusion when compared to gold standards such as PET, SPECT or Xe-CT [61, 71–74]. Time to peak delays of 4 and 6 s reliably identified hypoperfused and excluded normoperfused tissue (threshold arbitrarily set to 20 ml/100 g/min), but still overestimated the size of the critically perfused tissue [75], and therefore overestimated the volume of critically perfused but salvageable tissue, i.e. the penumbra [76] (fig. 3). Of 13 patients showing considerable PW-DWI mismatch only 8 had areas with elevated OEF typical for penumbra tissue, and these areas were always smaller on PW/DWI than on PET. This overestimation of the rCBF by relative distribution maps was confirmed in another comparative study [72]. It must be kept in mind that all PW-MRI methods assess perfusion indirectly from tracer transit through the vascular bed, and therefore correspond inconsistently to tissue flow measured by PET, and additionally lack values of OEF important to define viable tissue [77]. The mismatch pattern additionally fragments, making predictions of tissue outcome even

Fig. 3. a Volumetric comparison of time to peak (TTP) MRI and OEF PET images in 2 patients measured in the chronic phase of stroke. In both patients, a TTP delay of >4 s indicates a considerable mismatch volume (red contour on TTP images). The mismatch volumes were 473 cm^3 for patient A and 199.7 cm^3 for patient

B. However, only patient B had a corresponding volume of penumbra (260 cm^3). **b** Volumes of penumbra (black) and mismatch defined by $\text{TTP} > 4$ (gray) in 13 patients: all 13 patients showed mismatch, only 8 patients showed penumbra, which comprised 1–75% of the mismatch volume. Modified from Sobesky et al. [76].

more vague [78]. Overall, the mismatch volume in PW/DWI as conventionally calculated does not reliably reflect misery perfusion, i.e. the penumbra as defined by PET.

Recently, several methods have been proposed to improve the reliability of assessment of perfusion using MR methods [60, 63, 65, 79–81], but they all need to be validated by quantitative measures. Also, by using receiver operating characteristics analysis, improved validity of PET-derived mean transit time for predicting the penumbra threshold could be achieved [82]. More advanced analytical procedures may also help to identify more reliably the threshold between critical and non-critical hypoperfusion and to reduce variance in determined values [83].

The Penumbra as a Surrogate Marker for Treatment Efficiency

The efficacy of treatment in ischemic stroke can only be proven by controlled randomized double-blind clinical trials, as successfully performed for thrombolysis with i.v. rtPA [review in 84]. Since such controlled trials require large patient populations collected in many stroke centers and therefore usually take a long time and considerable funds, surrogate markers are applied to predict po-

tential therapeutic effects in small groups of patients. It has to be kept in mind that proven effects on surrogate markers always must be confirmed in controlled trials based on sufficient patient populations. In recent years, identification of salvageable tissue by neuroimaging has gained much interest as a surrogate marker for treatment efficiency in stroke.

The effect of the only approved therapy for acute ischemic stroke was also established in imaging studies, in which reperfusion to penumbral tissue was followed by improvement in neurological deficits: reperfusion was significantly increased in rtPA-treated patients compared to controls [85]. The volume of tissue salvaged by reperfusion was established in a study in which CBF, as determined by H_2^{15}O -PET within 3 h of stroke onset, was compared with the volume of infarction determined on MRI 3 weeks after the ictus [86] (fig. 4). The percentage of initially critically ischemic voxels (i.e. with a flow below the threshold of $12 \text{ ml}/100 \text{ g}/\text{min}$) that became reperfused at almost normal levels clearly predicted the degree of clinical improvement achieved within 3 weeks. Overall, only 22.7% of the grey matter that was initially perfused at rates below the conventional threshold of critical ischemia be-

Fig. 4. **a** MRI brain surface reconstruction (slightly rotated right lateral view) and superimposed target regions (red = infarct; blue = initially hypoperfused at levels below threshold of critical ischemia). Image triplets represent orthogonal T₁-weighted MRI and coregistered segmented PET sections across the center of infarction. **b** Scatter diagram relating improvement from initial assessment to 3 weeks of neurological deficit (NIHSS) and percent-

age decrease in size of gray matter region perfused at levels below threshold of dense ischemia in patients on the day of acute thrombolytic treatment ($p = 0.82$, $p < 0.01$). Two observations hidden at value 0/0. Modified from Heiss et al. [86]. **c** Carotis stenting and i.v. rTPA 2 h after stroke can reverse PWI deficit and limit permanent lesion to the initial DWI defect.

came necrotic after thrombolytic therapy in this small sample of 12 patients. This means that a considerable portion of the critically hypoperfused tissue was probably salvaged by the reperfusion therapy. Another PET study on 11 patients [87] indicated that hypoperfused tissue could benefit from reperfusion only as long as cortical FMZ binding was not reduced to or below 3.4 times the mean uptake in white matter. This marker of neuronal integrity can therefore serve as an indicator for irreversibly damaged tissue that is not amenable to treatment.

The PW/DWI mismatch as the estimated zone of the penumbra has been proposed as a surrogate marker of ef-

ficacy of stroke treatment [88, 89]. Several groups reported results of serial PW/DWI in patients after intravenous or intra-arterial thrombolysis. Inhibition of lesion growth [90] and even normalization of PWI [91] were seen with reperfusion after thrombolytic therapy and PW/DWI mismatch was proposed as an effective selection criterion for rTPA treatment of patients admitted more than 3 h after onset of symptoms [92]. In some cases, perfusion deficits can be resolved [93] and DWI signatures of early ischemic injury can be reversed by prompt vessel recanalization [94]. If a mismatch was still present 3–6 h after stroke onset, thrombolysis started beyond the accepted

therapeutic window was followed by favorable outcome [95, 96]. As a consequence of the beneficial effect of thrombolysis observed in patients with PW/DWI mismatch this signature was used for selections of patients in several clinical trials [reviews in 65, 89].

Three studies have included selection of patients with PW/DWI mismatch. In DEFUSE and EPITHET, DWI and PWI volumes were calculated after patient enrolment and outcomes were based on MRI profiles. The Desmoteplase in Acute Stroke (DIAS 2) study included only patients with visually assessed mismatch.

In DEFUSE [97] with open-label use of tPA 3–6 h after symptoms onset (n = 74), 40 patients had a mismatch (defined as a $T_{\max}$ delay of more than 2 s) 1.2 times larger than the DWI lesion. Reperfusion and recanalization were associated with favorable outcome in mismatch patients, and this effect was more apparent in the subgroup of patients who did not have a malignant profile.

EPITHET [98] was a randomized double-blind placebo-controlled trial of tPA within 3–6 h after symptom onset (n = 101). With the same mismatch definition as in DEFUSE, the study failed to demonstrate a statistically significant attenuation of infarct growth in the tPA group. However, reperfusion was strongly associated with good clinical outcome.

Desmoteplase, a newer thrombolytic agent, was administered 3–9 h after symptom onset in a multicenter

placebo-controlled double-blind dose-ranging study (DIAS-2, n = 186 [99]) of patients selected by PW/DWI mismatch (20%) or CT-perfusion-based mismatch. This study did not show any benefit of desmoteplase. However, the results might have been affected by the selection criterion (visual assessment of mismatch might be inadequate for patient selection [100]) and the low rate of patients with vessel occlusion.

Controlled trials selecting patients on the basis of mismatch did not result in a statistically significant effect of neuroprotective measures on outcome after ischemic stroke. In studies with gavestinel [101], citicoline [102], NXY-059 [103], normobaric oxygen [104] and hypothermia [105], infarct growth was not significantly reduced and clinical outcome not improved.

The up-till-now rather disappointing results in the selection of patients for treatment by MRI profiles might be related to inappropriate definition of critically perfused and salvageable tissue [106]. Additionally, more complex analysis of data might be required, including baseline DWI and PWI lesion volumes [64] and coregistration of mismatch and infarct location [107]. However, as long as a validation of the mismatch selection paradigm in a phase III trial is lacking, selection of patients for delayed treatment based on mismatch cannot be recommended in routine care [108], and this surrogate marker of outcome must be used with caution [109].

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