REWIEW

Imaging the ischemic penumbra and treatment effects by PET

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Abstract. Active treatment of acute ischemic stroke can only be successful as long as tissue in the area of ischemic compromise is still viable. Therefore, the identification of the area of irreversible damage, and its distinction from the penumbral zone, *i.e.*, tissue with impaired function but preserved morphology, may improve the estimation of the potential efficacy of various therapeutic strategies. This can be achieved by multi-tracer positron emission tomography (PET), perfusion-weighted and diffusion-weighted magnetic resonance imaging in experimental models. Neuroimaging modalities applied in patients with acute ischemic stroke cannot reliably identify penumbra tissue and detect irreversible damage in the first hours after stroke, when treatment must be initiated to have the potential for success: multitracer studies for the assessment of flow and irreversible metabolic damage usually are limited in the clinical setting, and arterial blood sampling necessary for quantitative determinations is prohibited under certain circumstances, *e.g.*, when thrombolysis is planned. The range of the penumbra can be assessed by combining determinations of flow and benzodiazepine receptor binding by PET of $H_2{}^{15}O$ and ^{11}C-flumazenil (FMZ) and relating flow values and FMZ binding to the final state of the tissue. By this approach, cumulative probability curves can be computed to predict eventual infarction or non-infarction and to define the penumbral range. The computed values are in good agreement with results from other studies proving the validity of the concept of the penumbra which was also demonstrated in several therapeutic studies in which thrombolytic treatment reversed critical ischemia and decreased the volume of the final infarcts. Such neuroimaging findings might serve as surrogate targets in the selection of other therapeutic strategies for large clinical trials. (Keio J Med 50 (4): 249–256, December 2001)

Key words: ischemic stroke, irreversible damage, penumbra, therapeutic window, positron emission tomography

Introduction

In the treatment of acute ischemic stroke two primary strategies can be followed: limitation of the ischemic insult by early reperfusion and interference with the pathobiochemical cascade leading to ischemic neuronal damage. A necessary prerequisite for either strategy is the existence of functionally impaired but viable and potentially salvageable tissue; irreversibly damaged tissue should be excluded or limited in its extent, since such tissue could not benefit from any strategy and forced reperfusion could even be detrimental by causing hemorrhage and/or edema. Severity and extent of the ischemic compromise can be clearly assessed in experimental models for which flow thresholds for preservation of function and morphology were defined.[1] Tissue perfused at a value between the thresholds for preservation of morphological integrity and of function, *i.e.*, penumbra tissue, might regain function with improvement of flow, but the time periods tolerated depend on flow levels and on severity of metabolic disturbances. In consequence, therapeutic windows are postulated for effective restoration of blood flow and for successful intervention with biochemical alterations,[2] but the durations of these windows are dependent on the residual perfusion in the tissue and therefore are different for the core and the periphery of the ischemic territory.

In the clinical setting of acute stroke management the assessment of the condition of the ischemically

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affected tissue is extremely difficult and necessitates logistically complex and expensive investigative procedures, which usually cannot be performed. However, such studies in selected cases have broadened the knowledge of pathophysiologic mechanisms leading to ischemic infarction. The results of these investigations additionally may help to explain the success or lack of efficacy of different therapeutic strategies.

Clinical Evidence for Viable Tissue as an Equivalent to Ischemic Penumbra

The most pertinent results of pathophysiologic changes during the early course after ischemic stroke have been obtained by multitracer positron emission tomography which provides quantitative maps of several important physiologic variables including regional cerebral blood flow (rCBF), regional blood volume, regional cerebral metabolic rate of oxygen ($rCMRO_2$), and regional cerebral metabolic rate of glucose ($rCMR_{glc}$) (Fig. 1). These studies conclude that tissue with $rCMRO_2$ below 65 μmol/100 g/min and/or rCBF below 12 ml/100 g/min over time turns into necrosis.[3,4]

Regions with rCBF between 12 and 22 ml/100 g/min have unstable function, and they must be considered as the penumbra zone, because infarction will occur in such a region if those low-flow values persist.[5,6] Uncoupled changes of flow and metabolism indicate the existence of viable but insufficiently supplied tissue in an ischemic region: the early perfusion failure manifests itself as a decrease in rCBF with $rCMRO_2$ and $rCMR_{glc}$ remaining relatively preserved. This condition, called misery perfusion,[3] implies that blood flow is inadequate relative to the metabolic energy demand for oxygen and substrate for still viable tissue (Fig. 1).

In repeat multitracer positron emission tomography studies of acute ischemic stroke[6–8] with differentiation between the ischemic core and periphery by identified and matched small regions of interest, it could be demonstrated that viable but misery perfused tissue exists in the border zone of ischemia up to 17–48 hours after stroke. Only in a few regions or in special cases with increased oxygen extraction fraction and slightly impaired $rCMRO_2$, was metabolism preserved close to normal values, and tissue remained morphologically intact. Most tissue compartments showing misery perfusion in the first day however suffer progressive metabolic derangement and turn into necrosis during the following two weeks. Whereas the viable peri-infarct penumbra tissue exhibits some potential for effective treatment, therapeutic routine available today in most cases cannot prevent subsequent progression to necrosis.

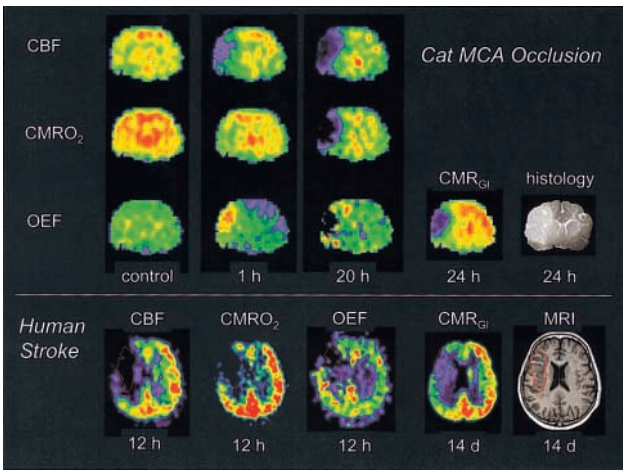

Fig. 1 Upper trace: sequential PET images from an individual cat representing $CMRO_2$, CBF and OEF before (control) and at 2 time points (1 and 20 h) after left MCAO. Progressive deterioration of oxygen consumption in the MCA territory corresponds with the spreading of the area with increased OEF and finally leads to hemodynamic and metabolic derangement, seen in the final CMR_{glc} image and in the corresponding histological cross section showing the area of infarction at the same time point. Lower trace: patient study demonstrating correspondence of regions exhibiting early (12 h) severe perfusional and metabolic deficits and decreased OEF in the anterior ischemic territory with final (14 days) deficit in CMR_{glc} and infarction observed by MRI and of regions showing a moderate perfusional deficit and increased OEF in the posterior ischemic territory with better preserved CMR_{glc} and no infarction.

Correlative PET Studies in Animal Models

In the clinical setting, only a few of the many factors contributing to the complex process of ischemic tissue damage, like blood flow, blood volume, oxygen consumption, and glucose metabolism can be assessed by functional imaging techniques. Additionally, the clinical studies are restricted to incidental time points in the course of the disease, and the complicated logistics involved in the regional determination of the physiologic variables prevent their repeated evaluation early after an ischemic attack, which is when the fate of the patient is decided. In order to better understand the results of multitracer PET studies in early stroke reproducible animal experiments are necessary in which regional changes in physiologic variables can be followed from the vascular attack to the permanent defective state and then be related to histologic alterations. With advanced PET equipment it became feasible to study pathophysiologic changes in brain perfusion and metabolism after MCA occlusion in baboons [9,10] and cats.[11]

In cats in whom CBF, $CMRO_2$, OEF, and CMR_{glc} were followed from control values before ischemia to the endpoint of infarction 24 h after MCAO, CBF within the MCA territory fell immediately upon arterial occlusion to below 30% of control, whereas $CMRO_2$ was less diminished and consequently OEF was increased, thus indicating ''misery perfusion''. This ischemic penumbra spread with time from the center to the borders

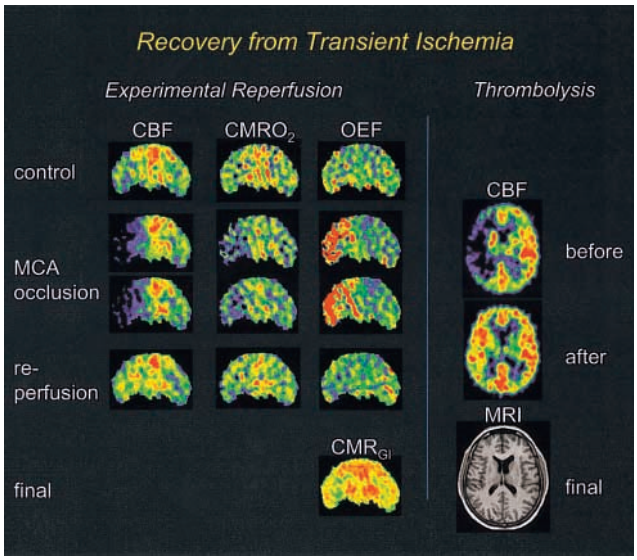

Fig. 2 Left side: sequential PET images from an individual cat undergoing 60-min MCAO and reperfusion resulting in a favorable outcome. Images represent CBF, $CMRO_2$ and OEF before (control), immediately after and at the end of MCAO and after reperfusion. During the ischemic episode, $CMRO_2$ did not deteriorate further and OEF remained increased. Hyperperfusion was not pronounced in this particular cat, and final CMR_{glc} did not show major deficits. Right side: patient study demonstrating successful reperfusion after thrombolysis. Structural damage as determined by MRI did not occur in this particular case.

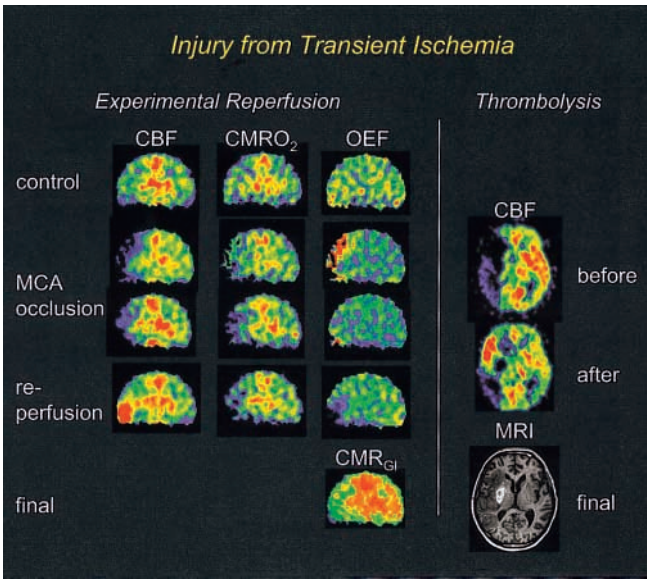

Fig. 3 Left side: sequential PET images from an individual cat undergoing 60-min MCAO and reperfusion resulting in an unfavorable outcome. Images represent CBF, $CMRO_2$ and OEF before (control), immediately after and 30 min after MCAO and after reperfusion. During the ischemic episode, $CMRO_2$ deteriorated further and the initially increased OEF decreased already during ischemia. After reperfusion, pronounced hyperperfusion occurred in this particular cat, and final CMR_{glc} showed major deficits. Right side: patient study demonstrating only partially successful reperfusion after thrombolysis (frontal part of ischemic focus), resulting, however, in pronounced hyperperfusion. Structural damage as determined by MRI occurred in this particular case in both nonreperfused and hyperperfused portions of the ischemic territory.

of the MCA territory. In most instances the misery perfusion condition was followed by a marked OEF drop reflecting progressive impairment of blood flow and metabolism and suggesting transition to necrosis, and the infarcts were more or less complete 18–24 h after MCAO (Fig. 1). Occasionally, spontaneous collateral reperfusion resolves the penumbra condition and morphologic integrity of the cortex is preserved.

Reversible MCAO was studied in cats[12] with reopening of the MCA after 60 min. Whereas in the animals surviving 24 hours of reperfusion OEF remained elevated throughout the ischemic episode (Fig. 2), the initial OEF increase disappeared already during 60 min ischemia in those cats that died during the reperfusion period (Fig. 3). In cats dying during the observation period, extended postischemic hyperperfusion accompanied large defects of $CMRO_2$ and CMR_{glc}, large infarcts developed and intracranial pressure increased fatally. These results further stress the importance of the severity of ischemia in relation to its duration for the further course after reperfusion. They also show that the penumbra is a dynamic process. The time window for eventual recovery of tissue – important as the therapeutic window – is therefore different for the core of dense ischemia (where it is short, probably below 1 h in the cat) and the vicinity with graded perfusional disturbance, where it is certainly several h and might extend to 24 h in the cat or to several days in the monkey.[13]

PET studies in animal models simulate the course of perfusional and metabolic disturbances characteristic for ischemic stroke in humans: permanent MCAO resembles the natural course after vascular occlusion leading to large infarcts in most cases (Fig. 1), with a chance of collateral reperfusion resolving misery perfusion and improving outcome. Reopening of the MCA resembles the (spontaneous) dissolution of vascular occlusions in transient ischemic attacks, spontaneous lysis of emboli after a period beyond the tolerable time period, and therapeutic thrombolysis. During MCAO of longer duration two patterns can be distinguished: decrease of the OEF during the time of MCAO reflects fast irreversible damage of tissue (Fig. 3), whereas the persistence of raised OEF indicates preserved viability of tissue over the ischemic period (Fig. 2). Forced reperfusion by reopening of the MCA cannot salvage already irreversibly damaged tissue, but may cause additional damage by inducing edema via leaking vascular endothelium as also observed with thrombolysis initiated too late.

Flow Compartments within Final Infarcts

Reperfusion induced by thrombolysis has been shown to be an effective therapeutic strategy in acute ischemic stroke when initiated within 3 hours of onset of symptoms.[14] In contrast, neuroprotective strategies thus far have been disappointing clinically and were unsuccessful with respect to improving stroke outcome[15] although significant reductions of infarct size were demonstrated in animal models with the use of strategies to antagonize the various steps in the excitotoxic cascade,[16] or free radical toxicity,[17] to inhibit harmful secondary inflammatory mechanisms[18] or to attenuate cell death by apoptosis.[19] This discrepancy is in part due to the limits of animal models concerning the complexity of clinical stroke, but it is also attributed to differences in outcome endpoints chosen for evaluation of therapeutic effects: reductions of infarct size in a particular experimental setting may in fact be poor predictions of functional outcome of stroke patients, especially since the relative impact of the various mechanisms contributing to the final infarct may be different in human stroke.

In order to determine various subcompartments of infarcts with respect to severity of initial ischemia, rCBF was assessed by $H_2{}^{15}O$ and PET in 10 patients with ischemic hemispheric stroke within 3 hours of symptoms onset. Within the boundaries of the final infarcts outlined on 3D-coregistered magnetic resonance images 2–3 weeks after the stroke, the following compartments of gray matter were identified according to their regional tracer uptake relative to the contralateral hemispheric mean,[20] (a) below 50%, representing the critical threshold of 12 ml/100 g/min,[3,4] (b) between 50 and 70%, representing the penumbra of 12–18 ml/100 g/min,[5] (c) above 70%, representing tissue outside the territory with markedly impaired perfusion.

The final gray matter infarcts varied considerably in size (median 27.7, range 1.5–138.4 cm^3). All of the three predefined ranges of initial flow were found in each of the infarcts. However, despite large variability among individuals, the largest proportion by far was the sub-compartment of critical hypoperfusion (51–92% of the final infarct), followed by penumbral tissue (8–34%), whereas the subcompartment initially perfused at a sufficient level was relatively small (2–25%). Only in one case, with a final infarct of 15.6 ml, the subcompartment of sufficient initial flow was large (41%) and the critically hypoperfused volume relative small (31%).

These data indicate that the final infarcts were caused mainly by severe initial ischemia leading to immediate tissue damage, while other mechanisms played only a minor role. Since the subcompartment of the infarct caused by delayed damage is too small to be a promising target of therapy, the benefit from treatment directed against those post-primary mechanisms is necessarily limited – and the disappointing results of corresponding clinical trials are not surprising. The penumbral tissue, another rather small fraction of the final infarct, can profit from reperfusion in due time, while neuroprotective drugs alone probably do not salvage enough tissue to improve clinical outcome.

Follow-up of Treatment Effects

The effectiveness of reperfusion to ischemically compromised tissue can be assessed by flow studies before and after the treatment and by relating the induced flow changes to the outcome. Of 100 patients receiving thrombolytic therapy (according to NINDS study protocol),[14] 15 patients were admitted during the working hours of the PET unit, and in 12 of them CBF could be studied repeatedly and the flow changes could be related to the clinical course (followed by the NIH Stroke Scale) and to the morphological damage demarcated on MRI 3 weeks after the stroke.[21] The first CBF study was performed right before or during the first 10 min of rt-PA infusion, CBF was re-assessed approximately 2 h and again 18–28 h after the end of that infusion. Since the contraindication for arterial blood sampling prevented quantitative determinations relative regional tracer uptake was used to estimate the level of residual blood flow in the affected hemisphere. The threshold of severe hypoperfusion in gray matter was operationally set to 50% (^{15}O)-H_2O uptake relative to the mean of the contralateral hemisphere, which corresponded to a gray matter blood flow of approx. 12 ml/100 g/min[20] which represents a widely accepted viability threshold.[3,4] Hence, gray matter voxels showing only 50% or less uptake on first measurement were considered critically hypoperfused and threatened by infarction. The tissue compartments defined at first measurement were followed in the subsequent measurements and compared to the MRI at 3 weeks on which the extent of the final infarct was outlined.

In this small sample patients with a severely hypoperfused (<12 ml/100 g/min) gray-matter region measuring less than 15 cm^3 on first PET showed full morphological and clinical recovery ($n = 5$), while those with ischemic areas larger than 20 cm^3 developed infarction and suffered persistent neurologic deficits of varying degree (Fig. 3). Infarct sizes, however, were smaller than expected from previous correlative PET and morphological studies of patients with acute stroke: only 22.7% of the gray matter initially perfused at rates below the conventional threshold of critical ischemia became necrotic. Actually, the percentage of initially ischemic voxels that became reperfused at almost normal levels clearly predicted the degree of clinical improvement achieved within three weeks.

These sequential blood flow studies demonstrate that critically hypoperfused tissue can be preserved by early reperfusion eventually induced by thrombolytic therapy (Fig. 2). The data support the results of the clinical trials and extend the recent observation by Grotta and Alexandrov[22] by identifying the portions of compromised tissue that become reperfused and do not proceed to infarction.

Markers for Early Detection of Irreversibly Damaged Tissue

For planning treatment of acute ischemic stroke a single marker of neuronal integrity would be extremely helpful, which could distinguish permanently damaged from functionally impaired tissue. Central benzodiazepine receptor (BZR) ligands were suggested by Sette, *et al.*[23] for that purpose, since they mark intact cortical neurons and therefore could delineate early neuronal damage. Labeled BZR ligands were successfully used for the separation between infarcted and deactivated tissue after stroke.[24,25] In transient MCA occlusion in 11 cats the development of the defects in energy metabolism was compared to the defects of the binding of ^{11}C-flumazenil (FMZ) and to the size of the infarcts determined 15 hours after MCAO.[12] Irrespective of the level of reperfusion, defects in FMZ binding 2–3 h after MCAO were closely related to areas with severely depressed oxygen consumption and predicted the size of the final infarcts whereas preserved FMZ binding indicated intact cortex. Depressed glucose metabolism outside the areas of reduced FMZ binding – and outside the final infarcts – indicated functional inactivation. Additionally, FMZ distribution within 2 min after injection was significantly correlated to flow and therefore can be used as a relative flow tracer yielding reliable perfusion images.

These strategies were applied in 10 patients suffering from acute ischemic attacks within 3–16 h after onset of symptoms.[26] The early changes in flow, oxygen consumption, and FMZ binding were compared to permanent disturbances in glucose metabolism and the size of the final infarcts determined on MRI or CT 12–22 days after the stroke. In the 9 patients with focally disturbed blood flow cortical regions with reduced FMZ binding predicted the final infarcts or areas with severely depressed glucose metabolism indicative of marked neuronal loss. The predictive value of reduced FMZ binding was comparable to that of a focal $CMRO_2$ reduction below 60 μmol/100 g/min, but the BZR ligand study does not necessitate arterial puncture and cooperation of patients, has the advantage of superior image quality and the potential of SPECT application. Additionally, perfusion can be assessed by following the early distribution of the same tracer. Overall, these results demonstrate that BZR radioligands – FMZ for PET, ^{123}I-iomazenil for SPECT – have a potential as clinically useful tracers in patients with acute ischemic stroke.

In another study[27] the multitracer approach was utilized to identify the area of irreversible damage and to distinguish it from the penumbral zone. Ten patients (seven male, three female, aged 52–75 years) with acute ischemic stroke, in whom MRI delineated an infarct involving the cortex 3 weeks after the attack, were studied by [^{11}C]flumazenil (FMZ) PET to assess their neuronal integrity, and regional CBF was measured by $H_2{}^{15}O$ PET 2–12 h (median interval 6 h) after onset of symptoms. Cortical volumes of interest (3 mm radius) were placed on co-registered CBF, FMZ and on late MRI scans. Using initial CBF and FMZ binding data from volumes of interest finally located within or outside the cortical infarct, cumulative probability curves were computed to predict eventual infarction or non-infarction. Positive (at least 95% chance of infarct) and negative (at least 95% chance of non-infarct) prediction limits for CBF (4.8 and 14.1 ml/100 g/min, respectively) and for FMZ binding (3.4 and 5.5 times the mean of normal white matter, respectively) were determined to define the penumbral range (Fig. 4). Using the lower FMZ binding threshold of 3.4 for irreversible tissue damage and the upper CBF value of 14.1 ml/100 g/min for the threshold of critical perfusion at or above which tissue

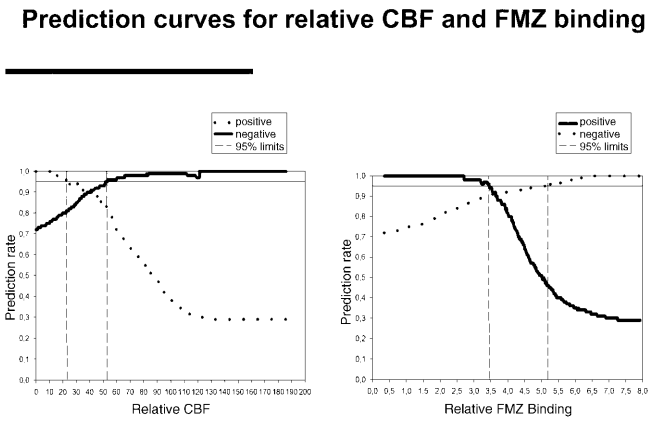

Fig. 4 Left: weighted mean curves across all patients' volumes of interest and corresponding 95% probability limits, predicting cortical infarction (positive prediction curve) or non-infarction (negative prediction curve) from early relative CBF: lower endpoints of curves denote proportion of non-infarcted and infarcted tissue, respectively, as present in the analyzed sample. Right: weighted mean curves across all patients' volumes of interest, and corresponding 95% probability limits, predicting cortical infarction (positive prediction curve) or non-infarction (negative prediction curve) from early relative FMZ binding. Lower endpoints of curves denote proportion on non-infarcted and infarcted tissue, respectively, as present in the analyzed sample.

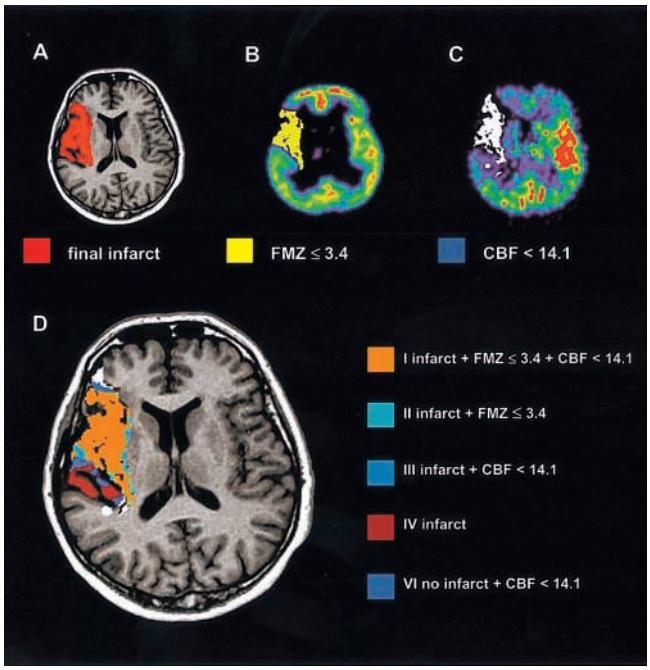

Fig. 5 Various subcompartments in and outside the final infarct of patient 2. (**A**) Extension of final infarct. (**B**) Compartment with initial FMZ binding decreased to <3.4 times the contralateral oval centre value. (**C**) Compartment with initial flow decreased to <14.1 ml/100 g/min. (**D**) Combination of **A**, **B** and **C**, showing subcompartments of decreased FMZ binding and decreased flow in relation to final infarct.

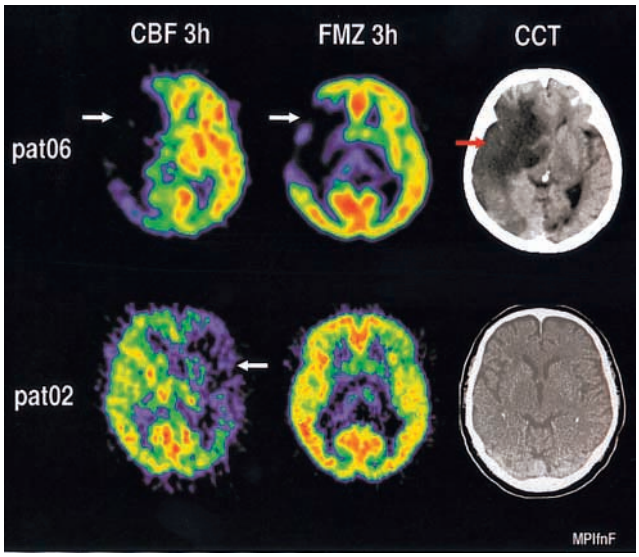

Fig. 6 Effect of rtPA treatment in two patients with large ischemic areas (white arrows): patient 6 (pat06) with area of decreased FMZ binding (blue arrow) and corresponding large infarction on late cranial CT (red arrow), and patient 2 (pat02) with no defect in FMZ binding and no infarcted cortex on late cranial CT.

will likely be preserved, various cortical subcompartments were identified (Fig. 5): of the final cortical infarct (median size 25.7 cm^3) a major portion comprising, on average, 55.1% showed FMZ binding critically decreased, thus predicting necrosis. In 20.5% of the final infarct, on average, CBF was in the penumbral range (< 14.1 ml/100 g/min) and FMZ binding was above the critical threshold of irreversible damage. Only 12.9% of the final infarct exhibited neuronal integrity and CBF values above the penumbral range. Therefore, most of the final infarct is irreversibly damaged already at the time of the initial evaluation, when studied several hours after stroke onset. A much smaller portion is still viable but suffers from insufficient blood supply: this tissue may be salvaged by effective reperfusion. Only an even smaller compartment is viable and sufficiently perfused, but eventually becomes necrotic, mainly owing to delayed mechanisms, and may benefit from neuroprotective or other measures targeted at secondary damage. Therefore, early reperfusion is crucial in acute ischemic stroke.

That BZR radioligands actually can be used to select patients who might benefit from a therapeutic intervention was shown in another study of 10 patients with acute hemispheric stroke in whom thrombolysis[14] was started within 3 hours of symptoms onset.[28] At the beginning of thrombolysis flow and FMZ binding were assessed by PET, and the early PET findings were related to change in neurologic deficits and to the extent of morphologic damage on MRI or CT 3 weeks after the stroke. Hypoperfusion was observed in all cases and in 7 patients the values were below critical thresholds estimated at 12 ml/100 g/min in variable volumes of 1–174 ml. Reperfusion was seen in most of these regions 24 hours after thrombolysis. In 3 cases, distinct areas with decreased FMZ binding were detected. These 3 patients suffered from permanent lesions in cortical areas corresponding to the FMZ defects (112 vs 146, 3 vs 3, and 1.7 vs 0.7 ml) (Fig. 6). In the other patients no morphological defects were detected on MRI or CT, although flow was critically decreased before thrombolysis in large areas (53 and 78 ml) in 2 patients and in smaller regions (1–7 ml) in 3 patients (Fig. 6). These findings suggest that imaging of benzodiazepine receptors by FMZ-PET distinguishes between irreversibly damaged and viable penumbra tissue early after acute stroke and can be used to identify patients who will benefit from interventional therapy.

Consequences for Stroke Management

The importance of the early perfusional deficit for the development of infarction and the short window of opportunity for reperfusion therapy stress the need of efficient and fast management of patients with acute

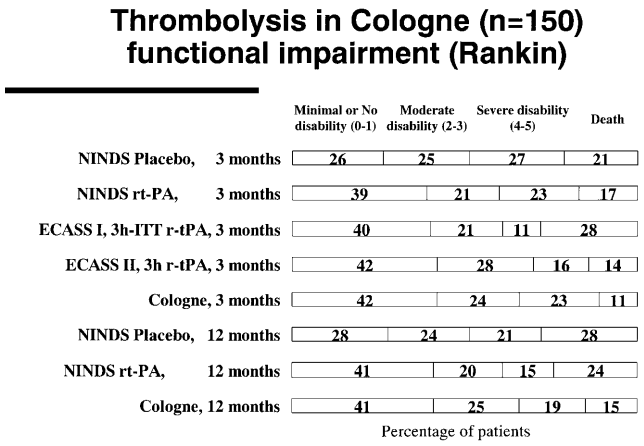

Fig. 7 Modified Rankin Scale scores at 3 and 12 months in patients treated in Cologne compared with patients from the NINDS rtPA Stroke Trial placebo and treatment groups (3 and 12 months) and with the ECASS I and ECASS II 3 h rtPA cohorts (3 months).

ischemic stroke. A cooperative initiative in the City of Cologne combining the task forces of 2 Neurological Departments, 14 Medical Departments in City Hospitals and the Emergency System made it possible to submit close to 40% of patients with suspected stroke to hospital treatment within 3 h of symptoms onset and to apply systemic rt-PA treatment to a rather large patient group (approx. 300 patients treated within 5.5 years). The results obtained are similar to those of multicenter trials[14,29,30] with 39% of patients with no or minimal deficit after 3 months[31] and 40% with no or minimal deficit after 1 year[32] (comparable to the NINDS follow-up study by Kwiatkowski, *et al.*)[33] (Fig. 7). These data impressively support the impact of the penumbra for potentially effective treatment of stroke and the importance of fast and effective management of stroke victims.

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