Hypoxic tissue in ischaemic stroke: persistence and clinical consequences of spontaneous survival

R. Markus,1,2,4 D. C. Reutens,1,2,3,4 S. Kazui,2,4 S. Read,2,4 P. Wright,1,2,4 D. C. Pearce,4 H. J. Tochon-Danguy,3 J. I. Sachinidis3 and G. A. Donnan1,2,4

1National Stroke Research Institute, 2Department of Medicine, The University of Melbourne, 3Department of Neurology and 4Centre for Positron Emission Tomography, Austin Hospital, Melbourne, Australia

Correspondence to: Geoffrey A. Donnan, MD, FRACP, National Stroke Research Institute, 300 Waterdale Road, Heidelberg Heights, Victoria 3084, Australia
E-mail: gdonnan@unimelb.edu.au

Summary

In ischaemic stroke, expansion of the infarct core occurs at the expense of surrounding hypoxic, metabolically compromised tissue over a period of 24 h or more in a considerable proportion of patients. It is uncertain whether hypoxic tissue observed at later times after stroke onset retains the potential for survival or whether such survival has an impact on functional outcome. These factors may determine the effectiveness of therapeutic strategies aimed at salvaging this tissue. We tested the hypotheses that metabolically compromised hypoxic tissue observed within 48 h after onset of ischaemic stroke retains the potential for spontaneous survival and that the impact of such survival on functional outcome is time dependent. Consecutive patients presenting within 48 h of ischaemic stroke were studied with [18F]fluoromisonidazole, a ligand binding to hypoxic but viable tissue, and PET. Subjects were grouped into two time epochs, <12 and >12 h, based on the interval from stroke onset to the time of tracer injection, and had infarct volumes measured on CT/MRI at 7 days (n = 60). The total ischaemic volume (TIV) and the proportion of the TIV that spontaneously survived (surviving hypoxic volume ratio, SHVR) were defined from the co-registered CT/MRI images. These volumetric measures were correlated with neurological outcome assessed at day 7–10 by percentage change in the National Institutes of Health Stroke Scale (ΔNIHSS), and at 3 months by Barthel Index (BI) and modified Rankin Score (mRS). Of 66 patients investigated, hypoxic tissue occurred in 33 and outcome data was available in 27. Hypoxic tissue constituted >20% of the TIV in 60% of studies <12 h and 16% >12 h. The spontaneously surviving proportion of the TIV (median 6.9%) or hypoxic tissue (median 45.9%) was not significantly different in patient subgroups studied <12 or >12 h after stroke onset. Spontaneous survival of hypoxic tissue (surviving hypoxic volume ratio) was associated with improved neurological outcome in both time epochs: <12 h, ΔNIHSS (r = 0.85, P < 0.01), day 90 BI (r = 0.86, P < 0.01) and day 90 mRS (r = -0.89, P < 0.01); >12 h, ΔNIHSS (r = 0.59, P < 0.01) and day 90 mRS (r = -0.46, P < 0.05). The finding that similar proportions of hypoxic tissue survived spontaneously within each time epoch suggests that its fate is not predetermined. The favourable neurological outcome associated with spontaneous survival of hypoxic tissue, even 12–48 h after stroke onset, suggests that the volume of hypoxic tissue that progressed to infarction may represent a valuable target for therapeutic intervention.

Keywords: [18F]fluoromisonidazole; hypoxia; ischaemic stroke; penumbra; PET

Abbreviations: BI = Barthel Index; FMISO = [18F]fluoromisonidazole; HV = hypoxic volume; IV = infarct volume; mRS = modified Rankin Score; ΔNIHSS = percentage change in the National Institutes of Health Stroke Scale; SHVR = surviving hypoxic volume ratio; TIV = total ischaemic volume

Advance Access publication May 6, 2004

Introduction

In focal cerebral ischaemia, reduced delivery of oxygen and glucose results in energy failure that induces a time-dependent cascade of functional and metabolic changes in the hypoperfused region (Hossmann, 1994). Infarction occurs rapidly in the region of most severe ischaemia (Baron, 1999; Heiss, 2000) and the infarct core expands at the expense of
the surrounding hypoxic tissue from the centre to the periphery of the hypoperfused region over a variable period of time (Markus et al., 2003). The functionally impaired region that surrounds the infarct core and is threatened by necrosis has been termed the ischaemic penumbra (Astrup et al., 1981; Touzani et al., 2001). Survival of the ischaemic penumbra is one mechanism that underlies spontaneous neurological improvement after stroke (Furlan et al., 1996). Therapeutic strategies in acute stroke are based on the concept of arresting the transition of the penumbral region into infarction, thereby limiting ultimate infarct size and improving neurological and functional outcome (Fisher, 1997).

In one-third of patients studied with serial MRI after ischaemic stroke, substantial enlargement of the infarct continued beyond the first 24 h (Baird et al., 1997). Metabolically compromised but viable penumbral tissue has been observed in regions that ultimately infarct as late as 48 h after stroke onset in patients studied with multitracer PET (Heiss et al., 1992). However, it has not been established whether the bulk of metabolically compromised tissue that is demonstrated at late time points represents mainly dying brain tissue or mainly viable brain tissue, the survival of which is associated with functional improvement. It is also uncertain whether this balance is time dependent. This has important implications for the likelihood of success of therapeutic strategies aimed at salvaging this tissue.

The ligand $^{18}$F]fluoromisonidazole (FMISO), a PET marker of hypoxic but viable tissue, identifies metabolically compromised tissue at risk of infarction following ischaemic stroke (Read et al., 1998, 2000). We prospectively evaluated patients presenting within 48 h of ischaemic stroke with FMISO PET and longitudinal clinical assessments to assess the effect of time since stroke onset on the prevalence, fate and functional outcome of this tissue. Specifically, we hypothesized that time since stroke onset has an effect on: (i) the proportion of hypoxic tissue that survives spontaneously; and (ii) the functional impact associated with spontaneous survival of hypoxic tissue.

### Subjects and methods

#### Subjects

Consecutive patients aged over 18 years presenting to the Austin and Repatriation Medical Centre with an acute hemispheric ischaemic stroke in whom PET imaging with FMISO was possible were included in this study. Exclusion criteria included brainstem or cerebellar stroke, cerebral haemorrhage on admission CT head scan, medical instability and contraindications to PET scanning. The time of stroke onset was determined from the patient or witnesses. In the case of waking deficit the midpoint between the time of wakening and the last time that the patient was known to be neurologically normal (e.g. time of going to bed) was used. The interval from stroke onset to the time of tracer injection was used to group subjects into two time epochs: ≤12 and >12 h.

The control group comprised approximately age-matched normal subjects with no prior history of stroke or transient ischaemic attack (TIA) and with normal brain CT scans. Written informed consent was obtained from the subjects or their next of kin. The Human Research Ethics Committee of the Austin and Repatriation Medical Center approved the study protocol.

The first 24 patients of this cohort have been reported previously testing different hypotheses (Read et al., 2000). At the time, the PET and CT scans were co-registered manually and regions of significant FMISO uptake were identified by comparison with the ‘normal’ contralateral hemisphere of the patients. Since then we have studied the distribution of this tracer in normal age-matched control subjects, described the application of automated image registration algorithms to align PET and CT images into standard coordinate space (Talairach and Tournoux, 1988) and validated a method of using statistical parametric mapping to identify regions of increased FMISO uptake by comparison of each patients with the group of normal control subjects (Markus et al., 2002). The cohort of 66 patients reported in this paper includes the initial 24 subjects who were re-analysed using this objective, reproducible and validated method of image analysis that is described in more detail below.

#### Imaging protocol

The FMISO PET scans were performed on an ECAT 951/31R PET scanner (Siemens/CTI Inc., Knoxville, TN, USA), 2 h after the intravenous administration of FMISO at a dose of 0.05 mCi/kg. Acquisition in 3D mode yielded 31 image slices 3.37 mm apart, with pixel dimensions of 2.34 mm in the x and y planes, a transverse image resolution of 6.5 mm and an axial resolution of 4.5 mm. Tissue attenuation of 511 KeV γ-radiation was measured with a 10-min 2D transmission scan acquired with retractable $^{68}$Ga/$^{68}$Ge sources.

The final infarct volume was assessed by CT or MRI, performed 7–10 days after stroke onset. All clinical assessments were performed by a neurologist or clinical nurse specialist certified in the administration of the stroke scales and blinded to the PET data. In patients who died, the worst score on the relevant scale was used for that assessment (i.e. 0 on BI and 6 on mRS).

#### Patient assessment and neurological scores

All patients had standard clinical management at the discretion of the treating neurologist. None of the patients was treated with thrombolysis, as it had not been approved for use in Australia at the time of the study. The National Institutes of Health Stroke Scale (NIHSS) (Lyden et al., 1994) was measured at the time of PET tracer injection (initial NIHSS, NIHSS$_{ini}$) and repeated at the time of the repeat CT or MRI performed to evaluate final infarct volume 7–10 days later (NIHSS$_{day7}$). Early neurological outcome was assessed by the change in the NIHSS ($\Delta$NIHSS), defined as:

$$\Delta$NIHSS = \frac{NIHSS_{day7} - NIHSS_{ini}}{NIHSS_{ini}}$$

Late neurological outcome was evaluated by measuring the Barthel Index (BI) (Mahoney and Barthel, 1965) and modified Rankin Score (mRS) (Rankin, 1957; Bamford et al., 1989) performed ~90 days after stroke onset. All clinical assessments were performed by a neurologist or clinical nurse specialist certified in the administration of the stroke scales and blinded to the PET data. In patients who died, the worst score on the relevant scale was used for that assessment (i.e. 0 on BI and 6 on mRS).
weighted fast spin double echo sequences [repetition time (TR)/echo time (TE)/TE 3500/10/60 ms].

**Image analysis**

Image analysis was performed on in-house software implemented for MATLAB (The Mathworks, Inc., Natick, MA, USA) utilizing previously validated methodology (Markus et al., 2002). In brief, all image data sets were transformed into standard coordinate space (Talairach and Tournoux, 1988) using the Automated Image Registration software package (AIR 3.0; Woods et al., 1998). The FMISO PET and CT templates comprised the average of 15 healthy subjects previously registered to standard stereotaxic coordinate space (Markus et al., 2002). The MRI template used was that distributed with the SPM software package (SPM 96; Wellcome Department of Cognitive Neurology, London, UK).

The FMISO images were normalized by the mean activity in the contralateral hemisphere and filtered with a Gaussian kernel of 6 mm FWHM (full width half maximum) to remove high-frequency noise. Statistical parametric maps of FMISO tracer uptake were generated for the voxel-wise comparison of each patient with the group of control subjects. Voxels with significant FMISO uptake (P < 0.05) were identified without reference to the infarct using a previously validated method of statistical inference based on the theory of Gaussian random fields (Worsley et al., 1996; Markus et al., 2002). The final infarct was manually outlined on the late CT or T2-weighted MRI by an observer blinded to the results of the FMISO PET.

**Definitions of tissue volumes**

The hypoxic volume (HV) was defined as the region of statistically significant FMISO uptake on the acute PET scan. This region was identified by setting the threshold at P < 0.05 on the statistical parametric map. The infarct volume (IV) was manually segmented on the late CT scan or the late T2-weighted MRI scan, and comprised voxels within the hypodense lesion on the former or the hyperintense lesion on the latter. Co-registration of the PET and late CT/MRI allowed the HV to be subdivided into a region that spontaneously survived, the surviving HV (HVₘ), and a region that underwent infarction, the infarcting HV (HVᵢ). The total ischaemic volume (TIV) was defined by voxels within either the final infarct or the HV, and was taken to represent tissue affected by the ischaemic process. These data from the CT/MRI and PET were used to calculate the spontaneously surviving hypoxic volume ratio (SHVR) defined as:

\[ SHVR = \frac{HV_m}{TIV} \]

Figure 1 illustrates these definitions. The proportion of hypoxic tissue in the TIV (≤20 or >20%) was used to group subjects into two categories. Hypoxic tissue comprising >20% of the TIV was used as a threshold to provide a conservative measure of those at risk of substantial infarct expansion, and was chosen because a similar value has been used in MRI studies of acute stroke to represent significant infarct expansion (Baird et al., 1997).

**Statistical analysis**

Statistical analysis was performed on SPSS version 11.5 (SPSS, Chicago, IL, USA). Demographic data, clinical scores and volumes are presented as mean values with standard deviation (SD) or median values and range, as appropriate. Non-parametric statistical methods were used to compare dependent variables, unless they were normally distributed, in which case parametric tests were preferred. Spearman’s rank correlation was calculated to assess the strength of the association between volumetric measures and clinical outcome (NIHSS, ANIHS, BI and mRS). The Mann–Whitney U-test was used to determine significant differences in lesion volumes and clinical measures between patient subgroups. Fisher’s exact test was used to assess differences between categorized groups. Multivariate analysis of covariance (ANCOVA) with the initial NIHSS score as the dependent variable, TIV subgroup as the main effect and TIV (ml) as the covariate was used to assess the effect of the volume of hypoxic tissue on the clinical deficit. A P value of <0.05 (two-tailed) was considered statistically significant.

**Effect of time since stroke onset on the fate of hypoxic tissue**

The effect of time on the fate of hypoxic tissue was examined by examining by two methods. First, we assessed the relationship between time since stroke onset and the proportion of the TIV and HV that survived spontaneously. Secondly, we compared the proportion of the TIV and HV that survived spontaneously in the patient subgroups studied ≤12 and >12 h after stroke onset.

**Effect of time since stroke onset on the functional impact of spontaneous survival of hypoxic tissue**

The effect of time on the functional impact of spontaneous survival of hypoxic tissue was assessed by ANCOVA with ANIHS as the dependent variable, time subgroup as the main effect and SHVR as the covariate in patients studied ≤12 or >12 h after stroke onset.

**Results**

Sixty-six patients (44 male and 22 female; mean age ± SD, 74.4 ± 11.6 years) presenting with acute ischaemic stroke were studied with FMISO PET. Fifteen subjects (eight males; mean age ± SD, 67.4 ± 10.7 years) with no prior history of stroke or TIA and with normal brain CT scans formed the control group. There was no significant difference in age between the patients and control subjects (P > 0.05, Student’s t-test).

Hypoxic potentially viable tissue was identified on acute PET in 33 patients (22 male and 11 female; Table 1) at a median of 16.5 h (range 3.9–47.5 h) after stroke onset. Of the patients who exhibited hypoxic tissue, 53% had hypertension, 9% had diabetes, 19% had hyperlipidaemia, 25% had ischaemic heart disease and 50% had atrial fibrillation. There was no significant difference in the incidence of these vascular risk factors between those with and without hypoxic tissue identified on acute PET (P > 0.05, Student’s t-test). Patient 4 had a second stroke on day 2 and was excluded from the clinical analysis. Patients 6, 11, 12 and 17 died, and patient 28 withdrew from the study before day 7. Results from these six patients are not included in the clinical outcome analysis but are included in all other analyses when possible.
Hence, 27 patients (19 male and eight female; mean age 73 years) had hypoxic tissue identified on acute PET, and had late CT/MRI and clinical outcome data. In these patients the median volume of hypoxic tissue was 22.3 ml (range 0.6–164 ml). A median of 27% (range 1–100%) of the TIV comprised hypoxic tissue. The median IV was 88 ml (range 0–341 ml). A significant correlation ($r = 0.6$, $P < 0.01$) was observed between the TIV and the severity of the initial clinical deficit measured by the NIHSS init. ANCOVA showed that there was no interaction between the patient subgroups with hypoxic tissue comprising <20 or >20% of the TIV and the strength of the association between the TIV and NIHSS init ($P = 0.87$).

Spontaneous survival of hypoxic tissue was associated with improved early and late clinical outcome measures. The correlations between SHVR and $\Delta$NIHSS ($r = 0.7$, $P < 0.01$), day 90 BI ($r = 0.5$, $P < 0.01$) and day 90 mRS ($r = -0.6$, $P < 0.01$) were significant. As expected, the volume of hypoxic tissue that survived spontaneously was also correlated with the absolute change in NIHSS scores ($r = 0.4$, $P < 0.05$).

**Effect of time since stroke onset on the fate of hypoxic tissue**

Overall, in the 27 patients with complete outcome data, a median of 6.9% of the TIV (interquartile range 1.3–31.7%) and a median of 45.9% of the HV (interquartile range 23.7–74.8%) survived spontaneously. The relationship between time and the fate of hypoxic tissue was analysed by two methods. First, in the whole group there was no significant correlation between time since stroke onset and the proportion of the TIV ($r = 0.01$, $P = 0.98$) and HV ($r = 0.26$, $P = 0.2$) that survived spontaneously. Secondly, the proportion of the TIV ($P = 0.56$, Mann–Whitney $U$-test) and HV ($P = 0.2$, Mann–Whitney $U$-test) that survived spontaneously was not significantly different in the patient subgroups studied ≤12 and >12 h after stroke onset. These findings indicate that hypoxic tissue has an extremely variable outcome that is not predetermined even when it is observed at later times after stroke onset.

![Fig. 1 Schematic representation of the operational assumptions underlying definition of tissue volumes in this study and a representative image from a patient. The colour code for the tissue volumes is in the right lower corner. The HV was identified without reference to the infarct as the region of statistically significant FMISO uptake on the acute PET scan by setting the threshold at $P < 0.05$ on the statistical parametric map. The IV was manually traced on the late CT scan or the late T2-weighted MRI scan. We assumed that the tissue comprising the HV was severely ischaemic but metabolically active and potentially viable at the time of tracer injection, and that all voxels within this region were at risk of infarction. The outcome of these voxels was based on their fate on the anatomically co-registered late CT/MRI, on the basis of which the HV was subdivided into HV subregions. The TIV was defined by voxels within either the final infarct or the HV, and is taken to represent tissue initially affected by the ischaemic process. Voxels that did not have significant tracer uptake that were external to the final infarct were assumed to have sufficient perfusion to have normal oxygenation and function. Voxels within the final infarct that did not have significant FMISO uptake were assumed to have undergone irreversible injury by the time of tracer injection.](image-url)
Effect of time since stroke onset on the functional impact of spontaneous survival of hypoxic tissue

The subgroups studied ≤12 and >12 h after stroke onset by PET were similar with respect to initial NIHSS, TIV and IV (Table 2). Strong correlations were observed between SHVR and ΔNIHSS \((r = 0.85, P < 0.01)\), day 90 BI \((r = 0.86, P < 0.01)\) and day 90 mRS \((r = -0.89, P < 0.01)\) in the subgroup of patients studied with PET within 12 h of stroke onset (Table 3). In comparison, for the subgroup of patients studied >12 h after stroke onset, weaker, but still significant, correlations were observed between SHVR and ΔNIHSS \((r = 0.59, P < 0.01)\) and day 90 mRS \((r = -0.46, P < 0.05)\), but there was no significant correlation between SHVR and day 90 BI \((r = 0.37, P = 0.12)\). The relationship between SHVR and ΔNIHSS for patients studied in each time epoch is shown in Fig. 2. ANCOVA showed that there was no significant interaction between time since stroke onset and the association between SHVR and ΔNIHSS. The results for the clinical characteristics, volumetric measures, prevalence of hypoxic tissue within the TIV, proportions of the TIV and HV volumes that spontaneously survived, and the correlations between SHVR and clinical outcome measures were similar when we used the median time from onset to PET study (16.5 h) to dichotomize patients into time epochs.

Table 1: Summary of patient data

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*Denotes patient died before day 90 clinical assessment. The score assigned is the worst possible score for that scale (0 for BI, 6 for mRS). †Patient 4 had a second stroke on day 2 and was excluded from the clinical analysis. ‡Patient 28 declined outcome studies. §Outcome clinical assessment and imaging performed at day 5 after stroke onset. M = male; F = female; NIHSS = National Institutes of Health Stroke Scale; BI = Barthel Index; mRS = modified Rankin Score; HV = hypoxic volume; IV = infarct volume; HV_s = spontaneously surviving hypoxic tissue volume; TIV = total ischaemic volume (IV + HV_s); SHVR = spontaneously surviving hypoxic tissue ratio (HV_s/TIV).
Effect of time since stroke onset on prevalence of hypoxic tissue

Hypoxic tissue was observed in 11 out of 13 (84%) studies performed within 12 h of stroke onset and 22 out of 53 (41%) studied beyond 12 h after onset ($P < 0.05$, Fisher’s exact test). All patients with waking deficits were in the 12–48 h category. In the 60 patients where late CT or MRI were obtained, the number of studies where >20% of the TIV comprised hypoxic tissue was six out of 10 (60%) within 12 h of stroke onset and eight out of 50 (16%) performed after 12 h ($P < 0.05$, Fisher’s exact test). Figure 3 shows the prevalence of hypoxic tissue related to time of study.

Discussion

The principal findings of this study were two-fold. First, metabolically compromised hypoxic tissue retained the capacity for spontaneous survival up to 48 h after the onset of ischaemic stroke. Secondly, its spontaneous survival was associated with improved early ($\Delta$NIHSS) and late (BI and mRS) neurological outcome measures. This association was observed for both $\leq 12$ and $>12$ h post-stroke epochs. Together with our previous report of the spatial evolution of hypoxic tissue from the centre to the periphery of the ischaemic region (Markus et al., 2003), these findings indicate that irreversible injury following ischaemic stroke is a dynamic process, spatially and temporally, that may continue for up to 48 h after stroke onset. Most importantly, interruption of this process is associated with functional improvement. These findings have important implications for therapeutic strategies in ischaemic stroke.

Our findings add to growing evidence from clinical studies in humans and experimental research that infarct expansion occurs even beyond 24 h after onset of ischaemic stroke. The results indicate that this expansion is at the expense of hypoxic but potentially viable tissue and that even at late time points, the transition of hypoxic tissue to infarction is not inevitable. Investigators using sequential multitracer PET scans to document the evolution of hypometabolic tissue following middle cerebral artery (MCA) occlusion in baboons showed that the volume of this tissue increased progressively until it reached a maximum $>24$ h after onset and equated with the volume of infarction on histology (Touzani et al., 1995). Similar observations were reported after experimental MCA occlusion in cats studied with sequential multitracer PET (Heiss et al., 1994) and rats studied with multimodal MRI (Quast et al., 1993). In humans studied with sequential MRI, Baird et al. (1997) reported an increase in infarct volume of $>20\%$ in one-third of patients with ischaemic stroke beyond 24 h after onset. Heiss et al. (1992) observed metabolically active tissue in the peri-infarct region in patients studied with multitracer PET up to 48 h of
stroke onset. Infarction of this tissue had occurred in nine out of 16 patients when the study was repeated 13–25 days later.

We observed that spontaneous survival of hypoxic but viable tissue correlated significantly with functional improvement on a number of widely used clinical outcome measures at both 1 week and 3 months in patients studied both before 12 h and between 12 and 48 h from stroke onset. There was a trend towards greater benefit in patients studied earlier. Our study thus provides evidence that spontaneous survival of hypoxic tissue confers functional benefit and may be an important mechanism underlying recovery from stroke. Furlan et al. (1996) have previously shown that spontaneous survival of penumbral tissue identified by multitracer PET within 7–16 h (mean 10 h) of stroke onset was associated with significant functional improvement measured by relative change in the Mathew and Orgogoza stroke scales at 2 months (both scales) and at 21 days (Mathew scale only), but not at 1 week. In our study, spontaneous survival of metabolically compromised tissue improved both early and late functional outcome irrespective of the clinical rating scale used and this association was observed even up to 48 h after stroke onset. In both studies the proportion of threatened tissue that survived spontaneously was similar, with a mean of 49% of hypoxic tissue surviving in our study and a mean survival rate of 52% of non-infarcted penumbral voxels being reported by Furlan et al. (1996). Four patients in the group had small total ischaemic volumes (<6 ml) but had substantial neurological impairment, with initial NIHSS scores of 7, 13, 12 and 3. Interestingly, even in this group of patients with small total ischaemic volumes the degree of clinical improvement was closely related to the proportion of the TIV that spontaneously survived. In particular, patient 2, who had 3 ml of hypoxic tissue and an initial NIHSS of 7 when examined 4.5 h after stroke onset, had no infarct on delayed CT and an NIHSS of 1 at day 7. This emphasizes that even small lesions in the eloquent areas of the brain can result in substantial deficits, and that salvage of small volumes of potentially viable tissue may confer benefit in this situation.

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**Fig. 2** Relationship between neurological improvement and spontaneous hypoxic tissue survival for patients studied ≤12 h (filled triangles) and >12 h (open diamonds) after stroke onset. The proportion of the TIV that survived spontaneously (SHVR) was significantly correlated with neurological improvement measured by the ΔNIHSS. A least-squares fit linear trendline has been inserted to highlight the correlation between SHVR and ΔNIHSS for each post-stroke time epoch.

**Fig. 3** Prevalence of hypoxic tissue related to time of study in patients studied with FMISO PET after presentation with acute hemispheric ischaemic stroke.
Misonidazole is a 2-nitroimidazole derivative that is selectively retained in hypoxic tissue (Chapman et al., 1983). Following diffusion into cells it undergoes an enzyme-mediated single electron reduction of the nitro group to a free radical. This free radical anion is rapidly reoxidized back to its parent compound by intracellular oxygen. In cells that have inadequate intracellular oxygen, reoxidation to the original compound is slowed and the compound undergoes further reduction to more reactive products that bind to intracellular cell components. This sequence of steps provides the retention that is necessary to differentiate hypoxic from normoxic cells. Because accumulation of bound metabolites of nitroimidazoles requires functional nitroreductase activity, it does not occur in necrotic cells (Rasey et al., 1987). Convergent data from studies in animal stroke models suggest that tracer retention occurs in the functionally impaired ischaemic region at risk of infarction. In gerbils following MCA occlusion, increased tracer uptake in the ipsilateral hemisphere was observed only in animals with a clinical deficit, with the extent of uptake being correlated with the severity of the deficit (Hoffman et al., 1987). In rats following carotid occlusion, autoradiograms revealed selective retention of 2-nitroimidazoles in the ischaemic region with cerebral blood flow below the flow threshold for infarction (Di Rocco et al., 1993). Lythgoe et al. (1999) studied the relationship between diffusion MRI, and autoradiographic markers of hypoxic tissue ([125]iodoazomycin arabinoside, a 2-nitroimidazole derivative with binding characteristics in hypoxic tissue similar to FMISO; Nunn et al., 1995) and blood flow ([99mTc]hexamethylpropylene amine oxide) in a rat model of stroke. The area of hypoxic tracer retention and diffusion MRI abnormality corresponded to regions where blood flow was <34% of normal. The region with mild hypoperfusion (66 but >34% of normal) that was not recruited to infarction had normal tracer uptake and diffusion. This finding suggests that hypoxic tracer uptake may discriminate between the ischaemic penumbra where tissue is at risk of infarction and ‘oligemia’ where it is not at risk.

The primary effect of cerebral ischaemia is reduced delivery of oxygen and glucose insufficient to maintain cellular energy metabolism. The ability to maintain ionic homeostasis at the expense of electrical function in the face of severe tissue hypoxia separates the penumbra, the potentially reversible region, from the infarct core. The viability of this tissue is influenced by the severity and duration of ischaemia as well as the adequacy of collateral circulation. Multitracer PET has been used to define the penumbra in terms of reduced perfusion and preserved oxygen metabolism, but the complexity of these studies has limited their application to small numbers of patients. Furthermore, the range of blood flow and metabolic values that define the penumbral region on multitracer PET is still a matter of controversy (Baron, 1999), may differ in grey and white matter compartments (Marchal et al., 1992), and the exact relationship between these thresholds and elapsed time is uncertain (Baron, 2001). Markers of tissue hypoxia have the advantage of being able to distinguish viable but metabolically compromised ischaemic regions independent of time and variations in blood flow since the vascular insult. Baron (1999) proposed three operational criteria to define penumbral tissue in the living human brain, which have been modified recently to be applicable to other modalities of functional neuroimaging (Doman and Davis, 2002): (i) the tissue should have physiological characteristics consistent with cellular dysfunction but not death; (ii) it should have an undetermined outcome; and (iii) the initial neurological deficit should be proportional to the volume of the tissue and the volume of surviving tissue should correlate with clinical outcome. These criteria were previously applied to multitracer PET (Furlan et al., 1996; Baron, 1999), and now it has been shown that tissue with FMISO uptake also fulfils these criteria. Findings in animal stroke models indicate that tracer retention only occurs in tissue that is at risk of infarction (Hoffman et al., 1987; Di Rocco et al., 1993; Lythgoe et al., 1997, 1999). Initial human studies showed that FMISO uptake identified peri-infarct hypoxic tissue that had an undetermined outcome (Read et al., 1998) and contributed to the initial neurological deficit (Read et al., 2000). The finding of the present study confirms these results in a larger sample of patients, and shows a significant correlation between survival of tissue with tracer uptake and improved clinical outcomes. The observation that infarct expansion at the expense of hypoxic tissue progresses from the centre to the periphery of the ischaemic region (Markus et al., 2003), in a manner similar to that seen in animal stroke models (Heiss et al., 1994; Touzani et al., 1995), lends further support. The relative inaccessibility of PET, however, limits the use of this tracer to research studies.

A number of methodological issues should be considered. Clinical outcome was assessed prospectively at 1 week and 3 months post-stroke. We observed an improvement in the NIHSS score at 1 week, which was comparable to the results of an earlier study of serial NIHSS change in patients with acute stroke who did not undergo thrombolysis (Wityk et al., 1994). The percentage change in NIHSS score has been used to monitor neurological outcome in other studies (Wintermark et al., 2002). We used manual identification of the infarct volume on CT, a method previously shown to have low intra-observer and inter-observer variability (van der Worp et al., 2001). Although CT scans performed 7–10 days after stroke onset maybe susceptible to the ‘fogging’ effect, the infarct contour outlined on day 7–10 CT was similar to that on later CT performed at 30 days in a subset that survived, suggesting that the effect was minimal. We used a 12 h threshold to dichotomize patients. Similar results, however, were obtained when the median time from onset to PET study was used to dichotomize patients into time epochs.

In combination, the observations that infarct expansion occurs at the expense of hypoxic tissue and that spontaneous survival of hypoxic tissue is associated with functional improvement have important clinical implications. Hypoxic tissue that spontaneously underwent transition to infarction is
likely to be a valuable target for therapeutic intervention. However, the relationship between therapy-related salvage of hypoxic tissue and outcome was not directly examined in this study. The volume of hypoxic tissue was significantly higher in the subgroup studied within 12 h of stroke onset, and is presumably greatest immediately after stroke onset, suggesting that intervention is likely to be most efficacious when initiated early. However, the observation of substantial volumes of threatened, potentially viable tissue at later times, and that its spontaneous survival was associated with improved functional outcome, suggests that some patients may benefit even at later stages. With modern neuroimaging tools, it may be possible to achieve the goal of individualized stroke therapy based on an assessment of the pathophysiological state and the potential for neurological recovery of each patient.

Acknowledgements
We wish to thank Lichun Quang for her invaluable help with preparing the figures, Indra Lim for computer support, and Gordon Chan, Kenneth Young, Kunthi Pathmaraj, Michelle Cuneen and Fiona Roberts for their assistance in scanning the subjects. This study was supported by the National Stroke Foundation, Australia. R.M. is supported by a postgraduate medical research scholarship awarded by the National Health and Medical Research Council, Australia.

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