Limitations of CT Angiography in Patient Selection for Thrombolytic Therapy

To the Editor:

We have read with great interest the article by Wildermuth et al1 on the role of CT angiography (CTA) in patient selection for thrombolytic therapy. The authors report that selection of patients with little potential for benefit from thrombolytic therapy is feasible with the use of CTA. The CTA findings, such as no vascular occlusion (ie, identification of patients with autolyzed thrombi), occlusion of internal carotid artery bifurcation, and poor leptomeningeal collaterals, indicate little potential for benefit from thrombolytic therapy. I am sure that these 3 CTA findings are useful in patient exclusion from thrombolytic therapy. However, there are 3 major problems with the use of CTA in patient selection for thrombolytic therapy.

First, CTA has diagnostic limitations of precise information about lenticulostriate artery (LSA) involvement. When deciding whether to perform thrombolytic therapy, one of the most important issues is confirmation of whether the LSAs are involved in ischemia. Because LSAs are terminal vessels with poor collaterals, thrombolytic therapy for patients with middle cerebral artery (MCA) trunk occlusion involving the LSAs may be associated with a high risk of hemorrhagic complications. In our previous study2 we found that when the LSAs are involved in ischemia and early ischemic change is present on the initial CT scan, thrombolytic therapy may result in unfavorable outcome with hemorrhagic complications. Furthermore, an arteriovenous shunt from the LSAs to the thalamostriate vein has been also reported3 to be a predictive sign for hemorrhagic complication. Therefore, in patients with MCA trunk occlusion, it may be dangerous to initiate thrombolytic therapy without precise information on LSA involvement.

Second, embolic occlusion of small peripheral arteries, which is often associated with internal carotid artery (ICA) occlusive disease, may be misdiagnosed as no vascular occlusion or mere ICA occlusion by CTA alone. Even if there were ICA occlusion, clinical symptoms might be ameliorated by recanalization of peripheral embolic occlusion. It is very important to make a correct diagnosis of artery-to-artery embolic occlusion in patients with ICA occlusion. (We wonder that CTA cannot provide precise information on peripheral small arteries.)

Third, because CTA requires a large amount of contrast medium, additional use of contrast medium is restricted and subsequent intra-arterial thrombolytic therapy is difficult to perform. Large emboli located in the MCA trunk may be resistant to intravenous thrombolysis, and intra-arterial thrombolytic therapy or direct percutaneous transluminal angioplasty may be required.4

From these considerations, although the 3 CTA findings mentioned above are useful in patient exclusion from thrombolytic therapy, CTA has some limitations in patient selection for thrombolytic therapy.

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Response

We appreciated the letter of Dr Nakano. There is obvious agreement with the main conclusion of our study,1 that certain CTA findings (ie, autolyzed thrombi, occlusion of internal carotid artery, and poor leptomeningeal collaterals) can be used to exclude patients from thrombolytic therapy. It is furthermore obvious that there is an ongoing discussion on which patients to include in thrombolytic therapy. Two large multicenter studies, NINDS2 and ECASS,3 applied systemic thrombolytic therapy without the requirement of a dedicated vascular study, especially without a time-consuming digital subtraction angiography (DSA) study. The basic idea behind this concept was a rapid initiation of thrombolysis and the lack of a randomized study showing the superiority of local thrombolysis.

We are well aware of the shortcomings of CTA, especially in depicting small-vessel involvement like the LSAs mentioned by Dr Nakano, and therefore did not attempt to study these potential risk factors for hemorrhagic complications. However, it remains to be clarified in further studies using larger numbers of patients whether it is more beneficial to initiate thrombolytic therapy as soon as possible or whether it is necessary to perform additional studies to exclude certain patients at risk. And finally, as stated in our article,1 CTA using 130 mL of nonionic contrast agent is no contraindication for a subsequent diagnostic or therapeutic DSA, and we did not experience adverse effects in our patients.

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Letters to the Editor 1149

Carotid Dissection: Pathophysiology of Stroke and Treatment Implications

To the Editor:

The article by Lucas et al1 in the December 1998 issue of Stroke analyzed neuroradiological data in an attempt to provide a reasonable pathophysiological approach to the treatment of carotid artery dissection. It is suggested that documentation of an embolic etiology for ischemia and infarction would provide a reasonable background from which to use antithrombotic medication in an acute setting. On the other hand, a hemodynamic cause of ischemia or infarct could be considered refractory to the effects to agents such as standard heparin or its low-molecular-weight relatives.

Is the pathophysiology of cerebral infarction in carotid artery dissection fundamentally different from that occurring in carotid and middle cerebral artery stroke in general? Is the presence of cortical or subcortical ischemia in carotid artery dissection a fundamentally different pathoanatomic sequence than would occur in the more common ischemic thromboembolic stroke syndromes? If embolic events as well as hemodynamic factors cause brain injury in either condition, the fundamental answer to both questions must be “no.” Each initiates a cascade of ischemic injury and potential tissue destruction.

A number of authors have described the lack of benefit from heparin in acute or evolving/progressing stroke.2–4 Whether such studies are general considerations about the use of heparinization is an ongoing area of interest, with specificity concerns similar to those considered for use of tissue plasminogen activator. Treatment response groups have not been defined to this point.

Neurologists have been unsure about the effects and use of heparin in acute stroke since the drug was introduced. Studies in the last 10 to 15 years have revealed heparin to be singularly unimpressive as a primary or ancillary treatment in acute stroke. Low-molecular-weight heparin trials have been disappointing, at best.3 The September 1994 American Heart Association guidelines for the treatment of acute and progressing stroke2 were ambiguous, not based on firm data for heparin benefits, but partly based on the legal concern for standard of care. Although ambiguity in these statements may help to diffuse inappropriate expert witness testimony against those who treat and those who don’t, as well as the good and bad outcome case, the guideline regarding heparin in acute stroke does not provide an accurate reflection of the data.

Considering the long and complex background of heparin use in acute and progressive stroke, the therapeutic implication in authors’ article is that treatment of carotid artery dissection rests on documentation of pathophysiology, not on the basic usefulness (or uselessness) of antithrombotic therapy in the acute stroke setting. This is misleading, and the position could be used to support treatment dogma when it does not exist.

Currently, the overwhelming weight of evidence rules against the role of heparin as a fundamental treatment in acute stroke. Response of carotid dissection to antithrombotic therapy remains equivocal. Low-molecular-weight heparin treatment in dissection has not been evaluated.

Emboli in the setting of acute carotid artery disease or dissection should not be compared with cardiac emboli when considering the effects of heparin. Emboli are not “created equal.” Therapeutic and management options should not be expanded by implication but instead need firm support from scientific studies.

The article’s conclusions regarding the relative contributions of embolism and hemodynamic factors are interesting and provoke thought regarding management options. The practicing neurologist may feel compelled to treat carotid artery dissection with heparin because of experts’ statements, based on insufficient data, combined with pathological implications, as suggested by the authors. A large, conclusive study may never be possible because of these general treatment recommendations. However, the determination that a particular method of treatment is valid or even reasonable does not automatically follow and should not be casually accepted in view of the potential for complications and the singular lack of supportive data.

There are those that will read more than what is written. Some “experts” are willing to suspend scientific analysis in court and testify that therapeutic options for the use of heparin are instead firm standards of care. Anecdotal experience and unsupported recommendations cannot overcome the extensive contrary evidence.

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Response

We totally agree with the first two thirds of Dr Bound’s letter, which are general considerations about the pathophysiology of ischemic stroke and the use of heparin at the acute stage. We also totally agree with the uncertainty of usefulness of heparin at the acute stage of ischemic stroke. As many other authors, we think that when possible, treatments should be given according to the rules of evidence-based medicine.

The author consider that artery-to-artery emboli and cardiac emboli are not “created equal.” Although we agree with this statement, this is just a widely accepted opinion without any proof. In many centers in Europe, most neurologists treat cerebral artery dissections by heparin at the acute stage in patients with a low risk of hemorrhagic complication, although the benefit of such a potentially dangerous treatment has never been proved by a randomized trial. The reason to prescribe heparin is that most infarcts are of embolic origin in patients with cerebral artery dissection.1 From a theoretical point of view, heparin may lead to an increased size of the mural hematoma. However, in clinical practice this is not confirmed.

The low rate of recurrent stroke2 makes any therapeutic trial in patients with cervical-artery dissection difficult. On the basis of a recurrent rate of disabling stroke or death of 2% a year,2 the number of patients necessary for a clinical trial to detect a 50% reduction of disabling stroke or death with α and β risks of 0.05 and 0.20, respectively, would be about 5000. According to the prevalence of cervical artery dissections,3-4 there are approximately 9000 cervical artery dissections per year in the European Union. These figures explain why drug trials will probably never be conducted in such disorder.

Biousse et al5 found that in dissections of the extracranial internal carotid artery, completed stroke usually occurs during the first few days after onset of the dissection and sometimes occurs as long as 1 month later. This finding suggests that any potential preventive treatment should be initiated as soon as possible after the onset of the first symptoms but might also be

worth initiating even 1 month later.5 Concerning hemodynamic failure, which is sometimes incriminated in cervical artery dissections, it may be prevented by strict bed rest during the first days, particularly when transcranial Doppler suggests a hemodynamic failure or when transient ischemic attacks occur when the patient is standing. All these arguments lead to the opinion that heparin is probably useful at the acute stage of cervical artery dissections despite the lack of scientific evidence, which will probably never be demonstrated in such a disorder because of its rarity in the community (2 cases per 100 000 persons a year)1,4 and the low rate of stroke recurrence (2% a year).2

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Microemboli in Cerebral Circulation and Alteration of Cognitive Abilities in Patients With Mechanical Prosthetic Heart Valves

To the Editor:

We have read with great interest the article by Deklunder and coworkers1 in which the authors so elegantly demonstrate the high incidence of microemboli through the brain circulation of patients with mechanical prosthetic heart valves. They also speculate on the relationship of such episodes to cognitive impairment. However, after reading the method carried out to detect high-intensity transient signal (HITS), we entertain several doubts.

In the first place, we would like to know why the authors have used the duplex scan, a procedure that carries the disadvantage of a high incidence of inadequate acoustic windows.2 The equipment used, an Acuson 128XP, has a large and heavy 2-MHz transducer that is difficult to hold upright at a given point during long periods of time, which would explain why the authors monitored their population during very brief time spans.3 The loss of the window and the appearance of artifacts may be high when a transducer of this type is used. Because of the latter, monitoring with transducers held by halos would seem more reasonable to us.

Finally, we would like to comment that for studies of this kind, multigated equipment appears to provide better information.4 These devices assess the presence of HITS at 2 different depths, which allows for measurement of HITS velocity and further adds to the differential diagnosis with artifacts.

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Response

In their letter, Drs Lagos and Cabezas address the question of the pertinence of the use of an Acuson 128XP for HITS detection in our study on the cognitive effects of microemboli in patients with mechanical prosthetic heart valves. Use of this class of equipment imposes handling constraints, as pointed out by Lagos and Cabezas. The reason this machine has been used in our study lies in the fact that it was the only TCD equipment available with an accurate spectral analyzer in our unit at the outset of the study. Nevertheless, the method we used to assess the HITS rate was the one commonly accepted.1 Under these conditions of HITS detection, we could have underestimated the number of HITS in the middle cerebral artery. Obviously, a more recent model of equipment could lead to greater accuracy in HITS counting, especially through increase of the monitoring duration, which is all the more important because HITS production appears to be an irregular phenomenon over time. However, the main objective of the study was to demonstrate the reality of cognitive impairments in patients with MHV; the accuracy of HITS counting was thus less crucial than had we sought to correlate HITS rate and impairment degree, for example.

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Ischemic Core and Penumbra in Human Stroke

To the Editor:

We would like to dispute the conclusion by Kaufmann et al1 that very little penumbra is to be found as early as 1 hour after onset of middle cerebral artery (MCA) territory ischemic stroke, a conclusion which, if true, would have obvious negative implications for acute stroke management. These authors
mapped cerebral blood flow by means of the stable xenon-enhanced CT (XeCT) method in 20 patients studied 60 to 360 minutes after clinical onset of stroke due to MCA occlusion. In support of their conclusion, they offer the following pieces of evidence: (1) a one-to-one correlation between measured volumes of final infarction (estimated by the lucency in late CT scans) and of tissue with flow of <6 (or 10) mL/100 g per minute, taken therefore to represent the core of irreversible damage; and (2) a lack of rim of tissue with flow between 11 and 20 mL/100 g per minute (ie, the classical penumbra range) surrounding the core. Their conclusion conflicts with considerable evidence from earlier positron emission tomography (PET) studies by several groups for a prolonged persistence of substantial amounts of penumbral tissue in a subgroup of patients. 2–7 For instance, in a series of patients studied 7 to 16 hours after onset, brain tissue with characteristics suggestive of penumbra (ie, with both flow in the 7 to 22 mL/100 g per minute range and very high oxygen extraction fraction) occupied up to 52% of the final infarct volume and represented a volume of up to 25 mL in the border of the ultimate infarct. 3 Recently, tissue with perfusional characteristics also compatible with penumbra was shown to be salvaged from necrosis by intravenous thrombolysis performed within 3 hours of clinical onset; furthermore, the amount of tissue so salvaged correlated with measured neurological recovery, 4 consistent with earlier findings by Furlan et al 5 that spontaneous neurological outcome is partly explained by the volume of penumbral tissue that escapes infarction. Also, it would be difficult to explain the results of the National Institute of Neurological Disorders and Stroke study 6 showing beneficial effects of thrombolysis up to 3 hours after onset (ie, in the 90- to 180-minute patient subgroup) if, as stated by Kaufmann et al based on their findings, “strategies to improve the outcome of many patients with acute MCA occlusion must ... include interventions to reverse the ischemic process within a few minutes of onset.”

We believe several limitations due to both their retrospective study and the methodology used, some of which rightly acknowledged, may account for their surprising findings. First, regarding the correlation observed with infarct volume, the latter was not optimally assessed, because it was estimated from CT scans and of tissue with flow of <6 (or 10) mL/100 g per minute, taken therefore to represent the core of irreversible damage; but—at variance with these authors—underestimated that volume by a factor of 2 on average, consistent with earlier evidence that the penumbra partly evolves to necrosis after the PET study. 2 Second, Kaufmann et al analyzed their flow maps on a voxel-by-voxel basis, which is well advised in studies of the penumbra, 2,3,7 but the accuracy of the XeCT method for measuring cerebral blood flow at the voxel level is questionable because of poor signal-to-noise ratio 11,12; thus, the error in flow estimate in a single 1X1X10 mm3 voxel may be as high as 100%. 12 In voxel-based PET studies of the penumbra, a procedure of voxel averaging was advocated to improve the signal-to-noise ratio. 2,3 To our knowledge, the XeCT method has not been validated against a gold standard at the voxel level and was found to deviate from 133Xe single-photon emission tomography—determined cerebral blood flow already with large regions-of-interest. 13 In addition, the duration of stable xenon inhalation in the study of Kaufmann et al was only 4.3 minutes, which may result in erroneous estimates of the blood-tissue partition coefficient, especially in small voxels, and, as a result, potentially also of flow. 11 Thus, it is possible that the method is insensitive or unreliable to detect voxels with flow in the penumbral range or that it underestimates flow values such that voxels with values of <10 mL/100 g per minute might effectively be penumbral (see patient 6 of their Figure 2). All the above methodological issues need to be acknowledged before the conclusions of Kaufmann et al may be considered valid.

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Response

We thank Drs Baron and Marchal for their thoughtful and appropriately skeptical letter in response to our article that examined cerebral blood flow levels within and about acute MCA territorial infarctions. We acknowledge that the response was appropriately skeptical because the conclusions have far-reaching implications and because at first examination they appear to conflict with an established body of work that has placed the penumbra as the goal of modern stroke therapy.

We agree that this was a preliminary and limited examination of a very complex question. The conclusion that many patients with acute MCA occlusion have a severe ischemic insult that for the most part is irreversible within a few hours of onset is, however, probably valid. The concept proposed by Simone of a cortical infarction surrounded by a broad band of penumbral tissue has not been consistently validated in subsequent histological or many physiological studies. Although Baron and Marchal appropriately discuss some of the articles that have examined the complex metabolic changes that occur in the 5 to 18 hours subsequent to cortical infarction, other studies have supported the hypothesis that for many patients the tissue volume at risk is irreversibly injured within a few hours of onset. If the volume enlarges it may be due to many mechanisms, only one of which is inclusion of the penumbra into the core. One mechanism that has not been adequately discussed is fluid accumulation within the core that causes secondary compression of surrounding tissues and is an acknowledged cause of secondary insult after massive MCA infarctions.

In response to the concerns raised by Baron and Marchal concerning the methodology used, we agree that 30-day follow-up CT studies would have been a preferable comparator. More levels of study would have been desirable as well. We are currently beginning a far larger prospective acute stroke study that should be able to address the above issues. In regard to the concern raised by a voxel-based analysis of the stable xenon cerebral blood flow database, although Baron and Marchal are correct in noting that there may exist an error of 100% in the measurement of flow within 1 voxel measuring 1×1×10 mm3, the error in measuring 120 contiguous voxels of this size is closer to 10%. The data presented in our article did not present single voxel data but instead analyzed volumes of tissue that contained contiguous voxels that fell within defined flow ranges. A stable xenon cerebral blood flow study appears to provide access to larger numbers of contiguous voxels that fell within defined flow ranges. A stable xenon cerebral blood flow study appears to provide access to larger numbers of contiguous voxels that fell within defined flow ranges.

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Body Temperature and Outcome of Asphyxiated Neonate

To the Editor:

In reference to the articles by Castillo et al and Schwab et al about the role of body temperature on asphyxiated brain, I would like to share the findings of our study on asphyxiated neonates, which had contradictory results. We reviewed 8 asphyxiated neonates (1-minute Apgar score of 5) with respect to birth weight, sex, Apgar score, and acid base temperature at birth and their outcome. The data were collected from the nursing sheet. Outcome variables were the mortality in normothermic asphyxiated neonates (birth temperature between 32°C and 36°C) were noted to have increased mortality (25%) compared with the 0% mortality in normothermic asphyxiated neonates (birth temperature >36°C). The mean duration of stay for hypothermic neonates was 12 days, compared with 4 days for asphyxiated neonates (1-minute Apgar score of <5) with respect to their temperature at birth and their outcome. The data were collected with respect to birth weight, sex, Apgar score, and acid base status (blood pH and base excess) at birth. The temperature was noted from the nursing sheet. Outcome variables were the mortality and duration of stay (in days) in the hospital. The mean birth weight of the cohort was noted to be 3149 ± 439 grams, with a male-to-female ratio of 1:1. The hypothermic asphyxiated neonates (birth temperature between 32°C and 36°C) were noted to have increased mortality (25%) compared with the 0% mortality in normothermic asphyxiated neonates (birth temperature >36°C). The mean duration of stay for hypothermic asphyxiated neonates was 12 days, compared with 4 days for normothermic asphyxiated neonates. Statistical tests were not applied in view of the small sample size. Our findings of increased mortality and prolonged stay in the hospital in hypothermic asphyxiated neonates is contradictory to those in previous reports. Increased mortality in hypothermic neonates can be explained by the decreased metabolic activity and energy failure (decreased generation of ATPs) secondary to hypothermia. Another possibility is the decreased cardiac contractility associated with hypothermia, as shown in one animal model study, leading to decreased perfusion of the organs and causing multiorgan failure. Also, previous studies have shown increased incidence of complications in neonates with hypothermia. In this era of evidence-based medicine, more work will be needed before hypothermia can be implied as a therapeutic measure in the management of brain asphyxia.

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Response

We appreciate Dr Manzar’s interest in our study.1 However, medicine based on evidence is supported by clinical experiences in homogeneous populations suffering from the same disease. Therefore, the results obtained in asphyxiated newborns must not be used to recommend therapeutic modifications in adults with acute ischemic stroke.

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Serum S-100 Protein in Stroke and Cardiac Surgery

To the Editor:

We have followed with interest the discussion regarding S-100 proteins in stroke and cardiac surgery between Drs Wong and Bonser and Drs Missler and Wiesmann.

We noticed that Drs Missler and Wiesmann questioned the specificity of our Sangtec 100 LIA assay. It is correct that the validation of the Sangtec 100 LIA assay has yet to be published. A complete validation regarding specificity has, however, been performed at Sangtec Medical during development of the assay. The monoclonal antibodies used in the Sangtec 100 LIA assay are the same as in the Sangtec 100 IRMA assay, and they have been shown to be S-100B specific using the biosensor-based BIAcore system (L. Nyberg, A. Ullén, K. Haglid, E. Sandström, T. Stigbrand, and J. Brundell, unpublished data, 1998). In collaboration with DAKO, we have shown that recombinant human A1, A2, A4, and A6 are not detected in the Sangtec 100 LIA assay (see Figure). Furthermore, calmodulin and troponin C are not measured in the Sangtec 100 LIA assay.

I hope that this information is valuable, and I agree with the authors that an assay for measuring cerebral damage must be S100B specific.

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Response

Several authors have shown that S-100 protein levels in serum are a quantitative marker of the extent of damage to the central nervous system (CNS). Therefore, use of this method has been proposed to detect possible cerebral injury after procedures such as coronary artery bypass.

The nomenclature used in studies of S-100 proteins has undergone some changes, which should be kept in mind to avoid possible misinterpretation of data. According to our present knowledge, the group of S-100 proteins now comprises 16 members, of which S-100A1 and S-100B are the most prominent and correspond to the previously used terms S100A1 and S-100B (or beta chain). In the biologically active form, A1 and B form dimeric proteins, which had previously been named S-100a (formed by the monomeric proteins A1 and B), S-100b (B-B), and S-100a1 (A1-A1). The initial studies, which demonstrated the usefulness of S-100 in the diagnosis of CNS pathology, described the dimeric 21-kD protein S-100b to be specific for the CNS. However, the S-100B subunit has been found in several tissues outside the CNS, including heart and aorta.

Most researchers have used two commercial assays produced by Byk Sangtec (Sangtec 100 IRMA, and Sangtec 100 LIA), which the company claims are strictly specific for S-100B, although the validation data has not yet been published. Martens et al. who measured S-100 levels after cardiac arrest with the Sangtec 100 IRMA, state that the assay detects both the dimer B-B and A1-B.

In a letter, Wong and Bonser reported that they found high levels of S-100 in 10 patients who underwent coronary artery bypass grafting and 30 patients who underwent cardiothoracic surgery using cardiopulmonary bypass and cardiac arrest. None of the patients sustained a focal neurological deficit.

In an ongoing prospective study, we have measured S-100 blood levels in patients who underwent cardiothoracic surgery using cardiopulmonary bypass and cardiac arrest. None of the patients sustained a focal neurological deficit.

The articles by Kitagawa et al. and Pulera et al. in the same issue of Stroke permit one to make some generalizations about publica-

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Two concentrations, 5 and 10 µg/L, of recombinant human S100 A1, A2, A4, and A6 were tested in the Sangtec 100 LIA assay. Relative light unit (RLU) values, generated by the S-100 proteins, are compared with the RLU background level generated by the assay’s standard diluent.

Apoptosis and Stroke Pathogenesis

To the Editor:

The articles by Kitagawa et al. and Pulera et al. in the same issue of Stroke permit one to make some generalizations about publica-


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tions implicating apoptosis in the pathogenesis of “stroke” or the “response” to cerebral ischemia. Numerous publications fail to make a clear distinction between infarction, which is total necrosis of the affected area, and delayed neuronal necrosis in areas of selective vulnerability. In the latter there is frequently no pan necrosis of tissue, only loss of neurons with, perhaps, reactive astrocytosis. These two different consequences of ischemic hypoxia, and also of hypoxic hypoxia, may overlap; however, this fact should not obscure the fact that different as well as identical pathogenetic (ie, biochemical) factors may be involved in each outcome, so that therapies reported to be of value in models of delayed neuronal death in restricted brain area might not, in fact, be useful in treating or preventing infarction. I would suggest that the potential limitations of an experimental report could best be “flagged” by insisting that all reports concerned with delayed necrosis in selectively vulnerable areas include the words “selective necrosis” or “selectively vulnerable neurons” in their title. This could then be paralleled with the word “infarction” in the title of articles dealing with that subject. I believe this would be of particular importance for neurologists and other nonneuropathologists who may not be attuned to the distinction.

The article by Kitagawa et al1 clearly deals with selective death of neurons in a limited area of temporal lobe even though global ischemia was used. The brains were examined 7 days later, and the findings correspond to delayed neuronal cell death.3 In the study of Pulera et al,2 there was apparently both infarction in the frontotemporal brain and also selective, delayed neuronal death in areas of the hippocampus. After describing both findings, the results concentrate only on the latter and report morphological evidence for a nonnecrotic, probably apoptotic, mode of delayed death in the neurons of the dentate gyrus. It is unclear from the text whether evidence for apoptosis was also found in the CA1 region where “ischemic” neurons were also found. Nor can I find a clear statement concerning the presence or absence of evidence for apoptosis in the truly infarcted portion of the brain.

A lack of clarity in terminology, at least in my mind, is also illustrated by the legend to Figure 3.1 At the bottom of the figure we are told that we are looking at the difference in time courses of “ischemic” cell death in two brain regions. Do the authors mean death “caused” by ischemia, whether or not the death is delayed, or do they mean death as indicated by the appearance of “ischemic” neurons in the two areas? This distinction is important because eosinophilia of neuronal cytoplasm was used as an important criterion for ischemic cell death in this article. This is the classic hallmark of what has been called “acute ischemic cell change.” The word “acute” implied that the ischemic/hypoxic event occurred less than 24 hours before death. Only recently has a review3 appeared which clearly demonstrates that the “red,” “eosinophilic,” or “acidophilic” neuron can also appear days after the ischemic event. Thus, these neurons may signify not only recent ischemia but also a delayed ischemia, which might be a second episode of ischemia or “something else.” This is not merely important for clinical neuropathologists who are trying to date lesions and their time of onset, but also for experimentalists, because the “something else” means that the neuron can respond in at least a superficially identical manner to very different challenges—eg, ischemia itself versus excitotoxic damage. Moreover, the red neuron has conventionally been thought to die by necrosis, not by apoptosis or some related mechanism with orderly rather than random breakdown of DNA. If at least some red neurons are destined for the latter “apoptotic”-like pathway and other red neurons are not, this is important information. The question appears to have been first explicitly raised in the review cited above.3 The data in the study of Pulera et al1 clearly suggest that at least some red neurons are destined not for necrosis but for a death that at least shares characteristics with apoptosis, and that their appearance is “delayed.”

A recent study by Ni et al4 clearly shows this. Like Kitagawa et al,1 Ni et al used caspase activity as one marker of the apoptotic path. Ni et al found that in an area where virtually all neurons appear to be marked for apoptotic death, as indicated by caspase activity, the same neurons go through the red stage. This point was not made by Ni and his coworkers.

The literature cited by both Kitagawa et al1 and Pulera et al2 notes that apoptosis can occur in or around areas of frank infarction. Are these neurons red but undergoing death with a caspase-driven orderly degradation of DNA? What about the actual mode of cell death for the red neurons in the heart of an infarct?

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Response
Dr Rosenblum has proposed that two different types of ischemic lesions in experimental animals, selective neuronal death and infarction, should be separately investigated when the involvement of apoptosis or necrosis in ischemic brain damage is examined. We agree with his notion and have focused on the effect of BCL-2 overexpression in selective neuronal death.1

Certainly, there exist several differences underlying between infarction and selective neuronal death.

One of them is the astrogial reaction. In infarction, astrocytes as well as neurons are destroyed, but in selective neuronal death they are viable and reactive after ischemia. If apoptosis is the critical determinant for infarction, the question of whether both neurons and astrocytes are killed by the same mechanism, apoptosis, will arise. To the best of our knowledge, there is no clear explanation. However, the mechanism underlying neuronal apoptosis may cause secondary damage, including energy depletion and acidosis in astrocytes in infarcted tissue.

Another difference is recruitment of inflammatory cells into tissue. Granulocytes are dominant in infarction, but they are scanty in the area with selective neuronal death. Several studies, including our recent article,2 have shown the importance of microcirculatory disturbance and the pathogenic role of granulocytes in the expansion of infarction after focal cerebral ischemia. In contrast, microcirculatory disturbance is unlikely to be involved in the course of delayed neuronal death.

In spite of the differences mentioned above, however, several important findings have been first discovered with the model of delayed neuronal death3 and then confirmed in focal cerebral ischemia producing infarction.4–6 Therefore, there seems to be no doubt that the model of delayed selective neuronal death is valuable when investigating the molecular mechanism underlying ischemic neuronal damage. Although the mechanism of selective neuronal death was examined mostly in the hippocampus, we would like to emphasize that delayed neuronal death was observed not only in the hippocampus but also in the whole brain after brief periods of ischemia.7
Letters to the Editor

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Response

We thank Dr Rosenblum for his interest in our report and thank the editor for the opportunity to provide the following clarifications.

First, if apparent that there is a lack of consensus regarding the terminology of apoptosis. Fundamental concepts such as the definition of apoptosis are not agreed upon. Consequently, the literature is full of inconsistencies, and experimental results are difficult to interpret. In our recent article,1 we attempted to minimize these inconsistencies.

The stated purpose of our study was to determine whether evidence of apoptosis was present in the cerebral cortex and dentate gyrus in our model of cerebral hypoxia-ischemia. In both the dentate gyrus and frontotemporal cortex, we described evidence of apoptosis, such as a DNA laddering pattern on agarose gel electrophoresis, the detection of apoptotic bodies by electron microscopy and ethidium bromide staining, and tdt-mediated dUTP-biotin nick-end labeling. The stated purpose of the study did not include examining the CA1 region for apoptosis, and our data indicate a relative paucity of cell death in the CA1 in our model. Consequently, we did not investigate the CA1 in this article. In addition, we had no data regarding cerebral blood flow or a physiological definition of the “penumbra” or “core” of the infarction area in our model, and the precise definition of these concepts is under debate. Therefore, we could not comment on the presence of apoptosis in the ischemic penumbra or core. Developing experimental techniques to explore these concepts is of paramount importance.

Furthermore, we believe we have used the term “ischemic cell change” appropriately. In our article, we described ischemic cell change as “eosinophilic cytoplasm with pyknotic nuclei.” We derived the definition of ischemic cell change from the classic description of Brown and Brierley.2 They described ischemic cell change by stating “the nucleus is shrunken, dark-staining and often triangular” and “the cytoplasm is acidophilic, staining pink with eosin.” Brierley et al3 go on to say that ischemic cell change is “not, as the term suggests, a neuronal alteration due to anoxia-ischemia alone” and “is the neuropathologic common denominator in all types of hypoxia.” These authors emphasize that ischemic cell change implies a process that transforms a normal cell into an essentially naked, shrunken nucleus and then results in cell loss. Regions of excessive cell loss result in infarction area. This process occurs after hypoxia-ischemia in regions of selective vulnerability. Therefore, it seems appropriate to describe cells in our study with pyknotic nuclei and eosinophilic cytoplasm generated from a hypoxic-ischemic insult as having ischemic cell change. Brierley et al3 indicate that the process of ischemic cell change occurring in regions of selective vulnerability “is most readily seen when survival after the hypoxic stress is 48 hours or more.” This concept is further supported in the literature cited in the review of Rosenblum.4

Our article provides evidence that apoptosis occurs in regions of ischemic damage defined by the criteria described above. Since techniques such as double-labeling of neurons undergoing ischemic cell change were not performed, we cannot make definitive statements regarding the fate of these neurons. However, we can speculate that perhaps some of these neurons die by apoptosis as well as necrosis. Clearly, additional investigation into these areas could provide useful information, especially regarding the development of new therapies for stroke.

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Apoptosis and Stroke Pathogenesis
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