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EDITORIAL

How to repair an ischemic brain injury? Value of experimental models in search of answers

R. Prieto-Arribas,[a,*] J.M. Pascual-Garvi,[b] F. González-Llanos,[c] J.M. Roda[d]

[a] *Servicio de Neurocirugía, Hospital Clínico San Carlos, Madrid, Spain*
[b] *Servicio de Neurocirugía, Hospital de La Princesa, Madrid, Spain*
[c] *Servicio de Neurocirugía, Hospital Virgen de La Salud, Toledo, Spain*
[d] *Servicio de Neurocirugía, Hospital La Paz, Madrid, Spain*

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Abstract The major aim of experimental models of cerebral ischemia is to study the cerebral ischemic damage under controlled and reproducible conditions. Experimental studies have been fundamental in the establishment of new concepts regarding the mechanisms underlying the ischemic brain injury, such as the ischemic penumbra, the reperfusion injury, the cell death or the importance of the damage induced on mitochondria, glial cells and white matter. Disagreement between experimental and clinical studies regarding the benefit of drugs to reduce or restore the cerebral ischemic damage has created a growing controversy about the clinical value of the experimental models of cerebral ischemia. One of the major explanations for the failure of the clinical trials is the reductionist approach of most therapies, which are focused on the known effect of a single molecule within a specific pathway of ischemic damage. This philosophy contrasts to the complex morphological design of the cerebral tissue and the complex cellular and molecular physiopathology underlying the ischemic brain injury. We believe that the main objective of studies carried out in experimental models of cerebral ischemic injury must be a better understanding of the fundamental mechanisms underlying progression of the ischemic injury. Clinical trials should not be considered if the benefit obtained in experimental studies is limited or weak.
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* Corresponding author.
E-mail: rprieto29@hotmail.com (R. Prieto-Arribas).

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PALABRAS CLAVE
Infarto cerebral;
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¿Cómo reparar el daño cerebral isquémico? Utilidad de los modelos experimentales en la búsqueda de respuestas

Resumen El objetivo principal de los modelos experimentales de isquemia cerebral es el estudio del daño isquémico cerebral en condiciones fisiológicamente controladas y reproducibles. Los estudios realizados han sido esenciales para establecer nuevos conceptos sobre los mecanismos subyacentes al daño cerebral isquémico tales como la penumbra isquémica, el daño por reperfusión, los mecanismos de muerte celular o la importancia del daño sufrido por las mitocondrias, las células gliales y la sustancia blanca. Sin embargo, debido a la discrepancia entre los estudios experimentales y clínicos respecto a la eficacia de las terapias que tratan de aminorar o revertir el daño isquémico cerebral, existe una polémica creciente en torno a la utilidad clínica de los modelos experimentales de isquemia cerebral. Uno de los principales motivos del fracaso de las diversas estrategias terapéuticas ensayadas en el ámbito clínico es el enfoque teórico reduccionista de la mayoría de los ensayos farmacológicos, que analizan el efecto de una molécula con un mecanismo de acción conocido dentro de una ruta concreta de progresión del daño isquémico. Este abordaje contrasta con la complejidad estructural y funcional del tejido cerebral y la intricada fisiopatología de las alteraciones celulares y moleculares inducidas por la isquemia. Creemos que el objetivo fundamental de los estudios realizados en modelos experimentales de isquemia cerebral debe ser la obtención de conocimientos básicos acerca de los procesos patobiológicos subyacentes al daño isquémico y que los ensayos clínicos no deberían iniciarse con agentes terapéuticos cuyos beneficios hayan sido escasos o inconsistentes en los estudios experimentales.
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[Neurons are] the mysterious butterflies of the soul, and who is to know whether the flutter of their wings may some day shed light on the secrets of life in the mind.
Santiago Ramón y Cajal (1852-1934)

Controversy is growing regarding the clinical usefulness of the experimental models for cerebral ischaemia, encouraged by the discrepancy observed between experimental studies and clinical trials with respect to the efficacy of the therapies striving to reduce or reverse the damage caused by cerebral ischaemia.[1] More than two decades of intense effort covering thousands of experimental studies and hundreds of clinical studies with an overall cost of billions of dollars seem to have been barren, as no effective therapy has been found to be capable of reducing the damage caused by cerebral ischaemia, except thrombolytic therapy with plasminogen activating factor, which is considered to be applicable to only 5% of patients admitted to hospital emergency departments with a diagnosis of ictus.[2,3] Must we therefore conclude that the experimental models of cerebral ischaemia are irrelevant for the design and testing of pharmacological therapies that can be successfully applied to patients with cerebral ischaemic damage? Although for the time being, we cannot yet give a definitive answer, it does not seem logical that the mere failure in the application of pharmacological therapies in the clinical setting should be the rationale for rejecting models that have furnished us with a huge volume of data about the mechanisms for the progression of cerebral ischaemic damage.

The animal models of cerebral ischaemia began to be developed in the nineteen seventies with the goal of studying damage due to cerebral ischaemia under physiologically controlled and reproducible conditions. Thanks to the work carried out with these models, we have been able to glimpse the complexity of the brain's response to ischaemic damage. The term "*cerebral ischaemia*" usually brings to mind the image of a messy algorithm in which multiple cascades of cellular events are activated in sequence in the cerebral parenchyma exposed to reduced blood flow. All the cell damage processes initiated by the deficit in the provision of oxygen and glucose to the nerve tissue contribute to the massive depolarization of the neurons, the uncontrolled or "excitotoxic" release of excitatory neurotransmitters and the subsequent uncontrolled activation of calcium-dependent enzymes such as phospholipases and proteases that irreversibly degrade the proteins and phospholipids in the cell membranes ultimately determining cell death.[4,5] These are the classic concepts regarding the pathophysiology of ischaemic cell death that we have learnt over the last three decades. Nonetheless, an enormous amount of information has been accumulated about the neurophysiological, biochemical and genetic alterations caused by cerebral ischaemia. In just the last 5 years, the biomedical information portal PUBMED has included over 20,000 articles under the key words "*cerebral ischemia*". This enormous influx of information seems to contradict the acceptance of simplistic pathophysiological schemes, such as those used in most clinical trials, and may be one of the main reasons

for the failure of the various therapeutic strategies tests in the clinical setting. Versus the reductionist theoretical approach of most pharmacological trials, which attempt to analyze the effect of a molecule with a known mechanism of action within a particular pathway for the progression of ischaemic damage, the structural and functional complexity of brain tissue and the intricate pathophysiology of the cellular and molecular alterations induced by ischaemia pose an enormous challenge for obtaining reproducible therapeutic effects. In principle, the absence of clinical benefit in a patient who has suffered a stroke and has received treatment focusing on a single therapeutic target should not be attributed solely and exclusively to the apparent differences between the brains of two species so far apart as a rodent and a human being. It is evident that experimental conditions cannot be considered anything more than an approximation to the human situation but, in addition, it must not be forgotten that most experimental pharmacological studies are trying to demonstrate a quantitative reduction in the volume of ischaemic infarction, assuming structural and functional homogeneity of brain tissue that is ineluctably going to limit the reproducibility of the therapeutic outcomes in the clinical arena. We should not restrict ourselves to blaming the experimental model when it does not allow the extrapolation of results to our hypotheses "as expected"; rather we should initially take on board the limitations of any therapeutic approach vis-à-vis the complexity of the variables implicated in the progression of damage due to cerebral ischaemia. Indeed, experimental models have been essential in establishing new concepts regarding the mechanisms underlying cerebral ischaemic damage and we should like to set out briefly some of the most relevant ones here:

The concept of flow thresholds and ischaemic penumbra

The development of therapies against cerebral ischaemia is based on the construct of the possibility of early action on a volume of brain tissue subjected to a reduction in blood flow without any irreversible harm to its cells, and where the biochemical and neurophysiological homeostasis can potentially be re-established.[6] The problem is how to identify, in terms of time and space, the volume of tissue subjected to conditions of ischaemic penumbra, the precise definition of which is currently still under discussion. The measurement of cerebral blood flow (CBF) in nerve tissue is insufficient in itself to identify the tissue area in penumbra conditions. Studies carried out in primates have shown that the blood flow threshold value capable of causing a critical metabolic and functional alteration in the neurons lies in a range between 10 and 15 mL/ 100g/ min, and that the tissue damage depends not only on the intensity of the reduction in CBF but also on its duration and other variables ranging from anatomy (collateral circulation pattern) to physiology (tissue temperature, glucose level).[7,8] The metabolic studies performed on experimental models for cerebral ischaemia have established a dynamic definition of ischaemic penumbra, i.e. that tissue area with a reduced CBF and an increased consumption of glucose that is metabolized anaerobically.[9] Histological studies have always shown a precise limit between the tissue with irreversible structural damage (infarcted or core area) and the cerebral tissue without structural alterations, but have not identified any intermediate strip corresponding to the penumbra area.[10] However, identifying the tissue volume with the potential to be saved in patients who have suffered ischaemic damage is very difficult to carry out accurately. Use has been made of specific magnetic resonance (MR) sequences to differentiate the penumbra area from the infarcted area.[11,12] Considering that a failure in the trans-membrane ion gradients leads to speedy cellular *swelling* and the subsequent reduction in the extra-cellular space and the mobility of the water molecules occupying it, the infarcted area has been defined as the part showing reduced perfusion and an alteration of the signal in the diffusion-weighted sequences.[13] The penumbra area has been differentiated from the infarcted tissue by the mismatch between the two MR sequences, in other words because it shows reduced perfusion but without association with signal alterations in the diffusion-weighted images.[14] This "neuro-radiological" concept of penumbra cannot be directly mapped to the metabolic concept defined using marked glucose autoradiography techniques in experimental models and, on the other hand, the cell pathophysiology correlate of a signal alteration in diffusion-weighted MR studies is not precisely known. In some reported cases, the area identified as infarcted in MR studies may revert partly or entirely hours after the occlusion following induced or spontaneous reperfusion. This suggests that early alterations in the apparent diffusion coefficient measured in the diffusion sequences do not unequivocally allow the definition of an outcome with an ischaemic area.[15-18] On the other hand, it has been seen that a reduction in flow observed on MR perfusion images does not always imply an alteration in the provision of oxygen to the tissue.[19] For this reason, the definition of the penumbra area in experimental studies cannot be directly extrapolated to clinical trials; in addition, the time and space delimitation of the tissue volume potentially recoverable following ictus may vary greatly in each patient due to multiple anatomical, physiological and biomolecular factors, which prevents the immediate reproducibility of the therapeutic outcomes obtained in laboratory conditions.

Cell death mechanisms and the importance of mitochondria

Versus the traditional concept that neurons subjected to a deficit in the provision of oxygen and energy substrates die solely through necrosis, it is currently known that death through necrosis and death through apoptosis co-exist in cerebral ischaemia, with apoptosis in addition being the most dominant in the penumbra area.[20] The discovery that death by apoptosis occurs mainly between 24 and 48 h after ischaemic damage[21] opened up the possibility of new therapeutic targets. Experimental studies performed on mice genetically modified to be deficient in caspase 3 (a kind of enzyme involved in the final stage of apoptosis) or in tumour necrosis factor receptors (a superfamily of receptors

regulating the activation of caspases) have shown that these animals are more resistant to ischaemic damage. These findings have not, however, been corroborated in other ischaemia models, probably due to the contribution in the apoptosis process of other caspase-independent biochemical cascades such as those activated by mitochondrial nucleases[22,23] or by certain non-caspase proteases.[24] In fact, the role of mitochondrial endonucleases in apoptosis is taking on ever greater importance and mitochondrial targets are being sought to block the mitochondrial swelling that precedes the rupture of these organelles and the release of their apoptotic enzymes into the cytosol. The main focus of attention is the transition pore of the mitochondrial membrane, a channel assembled between the internal and external membranes of the mitochondrion and which can be prevented from opening by using drugs such as cyclosporin A, which has given very promising results in both traumatic and ischaemic brain damage.[25,26]

The importance of glial cells in ischaemic damage

Cerebral ischaemia studies have classically focused on the effect of ischaemia on neurons. However, over the last two decades, recognition has gradually been given to the essential role played by glial cells for both the progression and the recovery of cerebral ischaemic damage.[27] Questions are also now being raised about the classical dogma of the greater resistance of glial cells to ischaemic conditions. Although the initial studies performed *in vitro* on cultures of glial cells showed that the astrocytes were comparatively more resistant to ischaemic damage than neurons,[28] studies conducted under *in vivo* conditions have revealed that the death of the astrocytes may even precede neuronal death.[10] On the other hand, after the identification, in the 1970s, of the complex three-dimensional organization of the astrocytes through gap-type intercellular bonds, it is still not clear whether the communication between glial cells through these bonds is beneficial or harmful following ischaemic damage. Some researchers have suggested that gap-type bonds may propagate and extend the damage, and so propose that this mechanism might explain the neuronal death seen in areas far from the vascular territory occluded.[29,30] Nonetheless, other recent studies carried out *in vivo* support the beneficial effect of gap-type bonds in the survival of neurons following ischaemic damage. Mice genetically deficient in connexin43 (an elementary protein in the structure of gap bonds) suffer infarctions of greater volume than in normal mice.[31-33] On the other hand, the most frequent cause of death in patients who have suffered a malignant infarction is cytotoxic or cellular oedema, which basically takes place in the astrocytes as these are the cells in charge of the clearance of K^+ and glutamate from the extracellular medium.[34,35] Great expectations have recently been placed in the role played by aquaporin 4 (AQP4), the main channel for water exchange in astrocytes, following the observation that the degree of oedema associated with a stroke is significantly lower in both AQP4-deficient mice[36] and in those lacking ·-syntropin, the protein in charge of anchoring AQP4 to the cell membrane.[37] The possible genetic or pharmacological manipulation of AQP4 expression is, however, a hard-to-achieve therapeutic target as the physiological role played by this water channel varies depending on the specific phase of progress in the damage to the brain. While an increase in AQP4 expression seems to be harmful in the initial phases of the development of ischaemic oedema, contributing to an increase in oedema during this phase, it might however be beneficial in the subsequent phases by helping in its resolution.[38] This is a paradigmatic example of the complexity of a molecule's action (in this case a channel), in which its activation or blockade may be beneficial or harmful depending on the timing of the pathological process.

On the other hand, there is great controversy over the role of reactive astrocytes in cerebral ischaemia and particularly on the consequences of the development of glial scarring following ischaemic damage. The current trend is to believe that astrogliosis has positive and negative aspects depending on the time stage the cerebral damage is going through.[39] In the initial stages, the glial scarring may provide multiple benefits, such as stabilizing the fragile nerve tissue that has suffered ischaemic damage by preventing the initially unharmed neurons from being exposed to a context with a high concentration of glutamate and free radicals.[40] In addition, the physical barrier inhibiting axonal growth represented by glial scarring represents, rather than an obstacle, an essential property to preserve the overall cytoarchitecture of the brain tissue, as allowing axonal growth inside a chaotic and metabolically unstable context may give rise to more harm than good.[41] Alternatively, several recent studies have shown that reactive astrocytes express receptors of endothelins or endothelial vascular growth factor implicated in the modulation of neuronal development, axonal growth and vasculogenesis,[42,43] which might facilitate the recovery of the damaged area of the brain.

The concept of reperfusion damage

The additional damaged caused to ischaemic tissue when it recovers its normal flow, particularly, at the expense of the formation of free radicals and the opening of the blood-brain barrier (BBB), has been an outstanding research subject since the introduction of thrombolytic therapies.[44] In fact, the synergic use of neuroprotective and thrombolytic agents has recently been put forward to try to diminish the harmful effects of reperfusion.[45-48] Among the various effects attributed to free radicals, particular importance is being given to the activation of the poly-ADP-ribose-polymerase (PARP1) enzyme in response to the oxidative damage of DNA. This enzyme, in charge of repairing the damaged DNA, is consumed when the NAD^+ co-enzyme (needed to produce ATP) is activated, something which aggravates even more the critical bio-energy situation of the nerve tissue subjected to ischaemia.[49] In fact, very promising results have been obtained with inhibitors of the PARP enzyme in mice subjected to ischaemic damage in the brain.[50]

Another of the most important effects of reperfusion is the breakage of the BBB and its associated vasogenic oedema, a very interesting focus for study in view of its

possible therapeutic implications. In animals with a transient occlusion of the middle cerebral artery for 90 minutes, the BBB opens up just when reperfusion begins and then remains closed for more than 24 to 48 hours.[51] The opening of the BBB has been seen to be due mostly to the effect of the extracellular matrix metalloproteinases (MMP), which degrade the proteins forming the structure of the narrow bonds between the endothelial cells and the proteins on the basal lamina. Animals genetically modified to be deficient in a particular type of MMP (MMP9 isoform) show less damage to the BBB and present smaller-sized infarctions in experimental models of ischaemia.[52] Once more, the pharmacological modulation of MMP is not simple as, despite their initial harmful effect, these enzymes make a major contribution in the recovery phase, facilitating angiogenesis and neurogenesis.[53]

Damage to the white matter and axonal regeneration in ischaemia

Another fundamental factor to be taken into consideration in cerebral ischaemic damage are the different pathophysiological responses of the grey and white matter, whose structural and functional characteristics are radically different.[29] Although both are essential for the 'proper operation of the brain, most studies into cerebral ischaemia have focused solely on the grey matter. Nonetheless, damage to the white matter is a factor that probably make a significant contribution to the poor results obtained in most clinical trials. The paucity of the collateral vascular provision at the level of the white matter makes it particularly vulnerable to a reduction in CBF. At the level of the white matter, the two basic structural elements are the axons in the neurons and the oligodendrocytes, glial cells responsible for myelinization and the normal operation of the axons. Energy deficit leads to the release of calcium from the axonal organelles, which may lead to a loss of ion homeostasis and the physiological axonal transportation function.[54]

With respect to oligodendrocytes, these cells have been seen to be very vulnerable to *in vivo* ischaemia, and damage to them is basically mediated by AMPA-type glutamate receptors.[55,56] The alteration of myelin synthesis by oligodendrocytes is a fundamental factor in ischaemic damage and, even in the absence of cell death, the metabolic dysfunction of oligodendrocytes prevents the re-myelinization of the axons. The hindrance of axonal growth is not only due to glial scarring but to the inhibitory effect of certain components in myelin.[57] It is very hard to achieve in any near future the stimulation of axonal regeneration in damaged tissue, as growing axons require a three-dimensional myelin structure in the form of a tunnel capable of guiding the longitudinal progress of the axonal growth cones.[58,59] Axonal growth has only been observed in partially denervated stretches where the myelinated tunnels and the associated astroglial skeleton have survived.[41] A better understanding of the mechanisms causing oligodendrocytes to die and their interaction with axons may help in the search for new therapies in the treatment of cerebral ischaemia.

The impact of cerebral plasticity on functional recovery following ischaemic damage

Most of the genes and proteins implicated in neuronal growth, synaptogenesis and proliferation of dendritic branches have their maximum expression during the early stages of the brain's development and significantly diminish with age.[60] Recent data suggest a strong parallelism between the plasticity mechanisms in developing nerve tissue those occurring in adult brains that have suffered a stroke.[61,62] Cerebral plasticity in adults who have suffered an infarction is based on varying degrees of compensation provided by two basic mechanisms: a) the surprising amount of diffuse and redundant connectivity that exists in the brain and that allows new circuits to be carved out depending on the activity required; b) the formation of new functionally-active circuits between cortical regions free of structural damage, a phenomenon known as "re-mapping".[63] However, the brain's ability to remodel after suffering a stroke in an adult, normally hypertensive patient is lower than that of a growing brain, due to the damage to microvascularization, chronic inflammation and other processes hindering plasticity. Despite the limitations in adult brains, there have been reports of a specific period of time following a stroke in which the expression of the genes involved in cerebral plasticity increases.[64] Following a small-sized infarction, functional recovery basically depends on the peri-lesional tissue, which takes over functions similar to those of the damaged tissue[62] whereas, after a large-scale stroke, the tissue with a similar functional capacity may be located only in distant areas or even in the contralateral hemisphere.[65] Studies performed on rats have shown the existence of a critical period for recovery and, if rehabilitation is delayed excessively, the improvement is significantly lower.[66] There is currently great interest in extending the time window during which the neuroplasticity processes characterizing the semi-acute phase following a stroke[63] are enhanced.

Factors hindering the clinical reproducibility of the therapeutic results obtained in the experimental arena

Despite the important considerations about cerebral ischaemia provided by the studies conducted on experimental models, the failure of the various neuroprotective therapies shown to be effective in experimental studies has caused widespread disappointment.[67] A plethora of factors might contribute to the disparity in the results between the experimental and the clinical studies. First of all, at a gross level, it is striking that rats and mice have a lissencephalic brain. Consideration should be given to the possibility of investigating the effect on therapies in higher animals with a gyrencephalic brain before taking the leap into clinical trials. Another evident difference is that the animals used tend to be young and free from co-morbidities. The last decade has seen proposals for the use of older animals with associated medical conditions (obesity, hypertension or diabetes) so as to reproduce human illness more accurately.[19,68] Another factor to be analyzed is the

difference in how the effect of the therapies is evaluated as, while experimental studies usually quantify cellular necrosis after the ischaemia, clinical trials assess the functional prognosis for patients. Finally, we wish to call attention to the large number of drugs that have been tested in clinical trials without having shown sufficiently solid results in the experimental models. Many of the agents tested in the clinical arena had shown, at best, only a modest effect in experimental studies, without any emphasis being placed on the significant variability present. By way of example, we can take what happened with nimodipine, a neuroprotective agent blocking the calcium channels on which a large number of trials have been conducted. On the one hand, of the over 250 experimental studies only 20 were controlled trials in which the drug was administered after the induction of ischaemia. Of those 20, only half the papers showed that nimodipine had a positive effect, following administration of the drug, in most cases, during the first 15 minutes after inducing ischaemia.[69] However, despite the limited benefit and the inconsistency of the results, more than ten clinical trials have been undertaken, five of them randomized and double-blind.[70-74] Moreover, it is striking that the patients received the drug between 24 and 48 hours after the start of their symptoms, in other words after a much longer period of time had elapsed than the therapeutic window identified in the experimental tests. It seems that the pressure of the pharmaceutical companies to start clinical trials without solid experimental results may be a factor implicated in the therapeutic failures seen with patients. There is also a major bias in the fact that there is no publication of experimental studies with negative results or these are published in brief formats. Overlooking the complexity of the brain is probably the most common error when designing therapy trials aimed at the various pathologies affecting this extraordinary organ.

Stem-cell therapy is one of the fields advancing most rapidly in recent years and it has been touted as the great hope for repairing ischaemic damage to the brain.[75,76] Stem-cell therapy was initially conceived as a cell replacement therapy in order to rebuild the damaged brain circuits and be able to recover lost functions. However, neither the implanting of stem-cells nor the stimulation of neurogenesis in mammals offers any guarantee of an increase in the number of functional neurons, as this requires their synaptic integration within a circuit in the brain. Perhaps excessive expectations have been created because the ultimate goal of these therapies is extremely ambitious. Repairing tissues with stem-cells requires two independent processes: a) the dead cells must be replaced by other newly-generated ones; b) the new cells must differentiate and organize themselves in a complex pattern that will ideally restore the original tissue structure. It is obvious that, in view of adult nerve tissue's limited endogenous ability to replace cells and the difficulty in re-establishing organized neuronal connections from far away, it is still premature to create expectations about cell therapy as a panacea for the recovery of ischaemia-related cerebral lesions.[77] So far, a wide variety of cell types have been implanted directly in the brain or administered systemically, including neuronal stem-cells, foetal transplants, immortalized cell lines or bone marrow cells.[78] The use of exogenous cells is complex and the factors controlling, at the molecular level, the migration, differentiation and connectivity of these cells are unknown. That is why the use of endogenous precursors is arousing more and more interest. The 1970s saw the abolition of the dogma that an adult mammal's brain cannot repair itself thanks to the discovery of neurogenesis, a phenomenon consisting in the birth of new neurons in adult brains. Nonetheless, the degree of post-natal neurogenesis diminishes as the brain's complexity increases.[79] Neurogenesis has only been seen to persist at the level of the sub-granular zone of the gyrus dentatus in the hippocampus and in the sub-ventricular zone in adult mammals.[80] Experimental studies have shown that neurogenesis is activated in both the hippocampus of rats and primates[81,82] as well as at the cortical level in adult rodents after induction of cerebral ischaemic damage.[83,84] However, most of the new neurons die and only some are able to migrate to the damaged tissue. The resulting micro-repair of the brain does not seem to be sufficient to reverse a functional deficit. For this reason, more recent projects are trying to stimulate the physiological phenomenon of neurogenesis with growth factors, a procedure not without its risks. On the one hand, it has been seen that some forms of epilepsy are due to neuronal stem-cells making more divisions than they should and giving rise to circuits with an aberrant functionality.[85] On the other hand, the administration of growth factors above the levels normal in the brain may induce the formation of brain tumours.[86] Finally, although it is still not clear which mechanisms best explain the benefits observed at the experimental level with the use of cell-based therapies, the idea that their effects might be due to these cells' ability to release certain neuroprotective or immunomodulating substances is gaining more and more acceptance.[87] The elegant study recently published by Kolb et al.,[88] consisting in the pharmacological stimulation of physiological neurogenesis after the induction of a cortical infarction and subsequent surgical removal of the regenerated cortical area, was the first to show the modulatory effect of stem-cells. This paper showed that rats worsened functionally on the seventh day after removal of the regenerated cortex but curiously not on the first day after surgery. The fact that several days had to elapse before a neurological deterioration was observed suggests that the regenerated nerve tissue acts as a promoter of the plasticity, or by maintaining homeostasis in adjacent cortical areas, and not through an effective neurophysiological integration in the damaged area.

In conclusion, we believe that the fundamental goal of the studies conducted on experimental models of cerebral ischaemia must be to obtain a basic understanding of the pathobiological processes underlying the ischaemic damage. Their use should not be restricted to the mere demonstration of a therapeutic benefit as a prelude to the execution of clinical trials. Such trials should not be begun with therapeutic agents showing scant or inconsistent benefits in the experimental studies. Cerebral ischaemia implies the parallel activation of multiple pathophysiological processes taking place in the most complex organ of the human body and interacting with each other over time so as to make the functional prognosis for the area affected in each individual practically unpredictable. It seems evident that the

adoption a reductionist style of approach, focusing on the specific biochemical or molecular processes unambiguously considered to be beneficial or harmful throughout the entire evolution of ischaemic damage over time, is insufficient. The design of new therapeutic approaches for cerebral ischaemia must be based on a prior integration of the enormous volume of knowledge provided by experimental models on the pathophysiology of ischaemic damage in the brain, accepting the structural and functional complexity of the organ suffering such damage.

References

1. García-Bonilla L, Rosell A, Torregrosa G, Salom JB, Alborch E, Gutiérrez M, et al. Guía de recomendaciones en la aplicación de modelos animales para el estudio del ictus. Neurologia. 2011;26:105-10.
2. National Institute of Neurological Disorder.s, Strok.e, rt-PA study group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med. 1995;333:1581-7.
3. Bandera E, Botteri M, Minelli C, Sutton A, Abrams KR, Latronico N. Infarct core in acute ischemic stroke. A systematic review. Stroke. 2006;37:1334-9.
4. Choi D.W. Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1988;1:623-34.
5. Siesjö B.K. Historical overview. Calcium, ischemia and death of brain cells. Ann NY Acad Sci. 1988;522:638-61.
6. Hossman K.-A. Viability thresholds and the penumbra of focal ischemia. Ann Neurol. 1994;36:557-65.
7. Astrup J, Siesjö B, Symon L. Threshold in cerebral ischemia – the ischemic penumbra. Stroke. 1981;12:723-5.
8. Jones TH, Morawetz RB, Crowell RM, Marcoux FW, FitzGibbon SJ, DeGirolami U, et al. Thresholds of focal cerebral ischemia in awake monkeys. J Neurosurg. 1981;54:773-82.
9. Back T, Zhao W, Ginsberg M.D. Three-dimensional image-analysis of brain glucose metabolism/blood flow uncoupling and its electrophysiological correlates in the acute ischemic penumbra following middle cerebral artery occlusion. J Cereb Blood Flow Metab. 1995;15:566-77.
10. Garcia JH, Yoshida Y, Chen H, Li Y, Zhang ZG, Lian J, et al. Progression from ischemic injury to infarct following middle cerebral artery occlusion in the rat. Am J Pathol. 1993;142:623-35.
11. Kidwell CS, Alger JR, Saver J.L. Beyond mismatch. Evolving paradigms in imaging the ischemic penumbra with multimodal magnetic resonance imaging. Stroke. 2003;34:2729-35.
12. Wardlaw J.M. Neuroimaging in acute ischaemic stroke: insights into unanswered questions of pathophysiology. J Intern Med. 2010;267:172-90.
13. Moseley ME, de Crespingny A, Spielman D.M. Magnetic resonance imaging of human brain function. Surg Neurol. 1996; 45:385-91.
14. Kloska SP, Wintermark M, Engelhorn T, Fiebach J.B. Acute stroke magnetic resonance imaging: current status and future perspective. Neuroradiology. 2010;52:189-201.
15. Oppenheim C, Stanescu R, Dormont D, Crozier S, Marro B, Samson Y, et al. False-negative diffuse diffusion-weighted MR findings in acute ischemic stroke. Am J Neuroradiol. 2000; 21:1434-40.
16. Li F, Silva MD, Liu KF, Helmer KG, Omae T, Fenstermacher JD, et al. Secondary decline in apparent diffusion coefficient and neurological outcomes after a short period of focal brain ischemia in rats. Ann Neurol. 2000;48:236-44.
17. Kidwell CS, Saver JL, Mattiello J, Starkman S, Vinuela F, Duckwiler G, et al. Thrombolytic reversal of acute human cerebral ischemic injury shown by diffusion/perfusion magnetic resonance imaging. Ann Neurol. 2000;47:269-462.
18. Fiehler J, Kucinski T, Knudsen K, Rosenkranz M, Thomalla G, Weiller C, et al. Are there time-dependent differences in diffusion and perfusion within the first 6hours after stroke onset?. Stroke. 2004;35:2099-104.
19. Hossman K.-A. Cerebral ischemia: Models, methods and outcomes. Neuropharmacology. 2008;55:257-70.
20. Ginsberg M.D. The new language of cerebral ischemia. AJNR. 1997;18:1435-45.
21. Graham SH, Chen J. Programmed cell death in cerebral ischemia. J Cereb Blood Flow Metab. 2001;21:99-109.
22. Yu SW, Wang H, Poitras MF, Coombas C, Bowers WJ, Federoff HJ, et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science. 2002;297:259-63.
23. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiol Rev. 2007;87:99-163.
24. Blomgren K, Zhu C, Wang X, Karlsson JO, Leverin AL, Bahr BA, et al. Synergistic activation of caspase-3 by m-calpain after neonatal hypoxia-ischemia: a mechanism of "pathological apoptosis"?. J Biol Chem. 2001;276:10191-8.
25. Matsumoto S, Friberg H, Ferrand-Drake M, Wieloch T. Blockade of the mitochondrial permeability transition pore disminishes infarct size in the rat after transient middle cerebral artery occlusion. Cereb Blood Flow Metab. 1999;19:736-41.
26. Galluzzi L, Blomgren K, Kroemer G. Mitochondrial membrane permeabilization in neuronal injury. Nat Rev Neurosci. 2009;10:481-94.
27. Perea TD, Coplan JD, Lisanby SH, Lipira CM, Arif M, Carpio C, et al. Antidepressant-induced neurogenesis in the hippocampus of adult nonhuman primates. J Neurosci. 2007;27:4894-901.
28. Juurlink BH, Hertz L, Yager J.Y. Astrocyte maturation and susceptibility to ischaemia or substrate deprivation. NeuroReport. 1992;2:1135-7.
29. In: Siesjö B.K., Wieloch T., editors. Cellular and molecular mechanisms of ischemic brain damage. Vol 71, Advances in Neurology. Philadelphia: Lippincott-Raven; 1996.
30. Anderson MF, Blomstrand F, Blomstrand C, Eriksson PS, Nilsson M. Astrocytes and stroke: networking for survival?. Neurochem Res. 2003;28:293-305.
31. Siushansian R, Bechberger JF, Cechetto DF, Hachinski VC, Naus C.C. Connexin 43 null mutation increases infarct size after stroke. J Comp Neurol. 2001;440:387-94.
32. Nakase T, Söhl G, Theis M, Willecke K, Naus C.C. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexion 43 in astrocytes. Am J Pathol. 2004;164:2067-75.
33. Perez Velazquez JL, Kokarovtesa L, Sarbaziha R, Jeyapalan Z, Leshchenko Y. Role of gap junctional coupling in astrocytic networks in the determination of global ischaemia-induced oxidative stress and hippocampal damage. Eur J Neurosci. 2006;23:1-10.
34. Garcia JH, Kalimo H, Kamijyo Y, Trump B.F. Cellular events during partial cerebral ischemia. I. Electron microscopy of feline cerebral cortex after middle-cerebral-artery occlusion. Virchows Arch B Cell Pathol. 1977;25:191-206.
35. Liang D, Bhatta S, Gerzanich V, Simard J.M. Cytotoxic edema: mechanisms of pathological cell swelling. Neurosurg Focus. 2007;22:E5.
36. Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med. 2000;6:159-63.
37. Amiry-Moghaddam M, Xue R, Haug FM, Neely JD, Bhardwaj A, Agre P, et al. Alpha–Syntrophin deletion removes the perivascular but no endothelial pool of aquaporin-4 at the blood-brain

barrier and delays the development of brain edema in an experimental model of acute hyponatremia. FASEB J. 2004;18:542-4.
38. Bloch O, Manley G.T. The role of aquaporin-4 in cerebral water transport and edema. Neurosurg Focus. 2007;15:E3.
39. Rolls A, Shechter R, Schwartz M. The bright side of the glial scar in CNS repair. Nat Rev Neurosci. 2009;10:235-41.
40. Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew M.W. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci. 2004;24:2143-55.
41. Raisman G. Myelin inhibitors: does NO mean GO?. Nature Rev Neurosci. 2004;5:157-61.
42. Fagan SC, Hess DC, Hohnadel EJ, Pollock DM, Ergul A. Targets for vascular protection after acute ischemic stroke. Stroke. 2004;35:2220-5.
43. Eichmann A, Makinen T, Alitalo K. Neural guidance molecules regulate vascular remodelling and vessel navigation. Genes Dev. 2005;19:1013-21.
44. Chan P.H. Reactive oxygen radicals in signalling and damage in the ischemic brain. J Cereb Blood Flow Metab. 2001;21:2-14.
45. Andersen M, Overgaard K, Meden P, Boysen G, Choi S.C. Effects of citicoline combined with thrombolytic therapy in a rat embolic stroke model. Stroke. 1999;30:1464-71.
46. Asahi M, Asahi K, Wang X, Lo E.H. Reduction of tissue plasminogen activator-induced hemorrhage and brain injury by free radical spin trapping after embolic focal cerebral ischemia in rats. J Cereb Blood Flow Meta. 2000;20:452-7.
47. Lyden P, Jacoby M, Schim J, Albers G, Mazzeo P, Ashwood T, et-a.l, for the CLASS IHT Investigators. The Clomethiazole Acute Stroke Study in Tissue-Type Plasminogen Activator –Treated Strokek (CLASS-T): final results. Neurology. 2001;57:1199-205.
48. Grotta J. Combination Therapy Stroke Trial: recombinant tissue-type plasminogen activator with/without lubezole. Cerebrovas Dis. 2001;12:258-63.
49. Prieto R, Pascual JM, Tavazzi B, Taya K, Barrios L, Amorini AM, et al. Early metabolic alterations following a severe diffuse traumatic brain injury in rats as detected by HPLC: Importance of PARP-1 overactivation. World Congress of Neurlogical Surgeons. 2009.
50. Eliasson MJ, Sampei K, Mandir AS, Hurn PD, Traystman RJ, Bao J, et al. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nature Med. 1997;3:1089-95.
51. Yang GY, Betz A.L. Reperfusion-induced injury to the blood brain barrier after middle cerebral artery occlusion in rats. Stroke. 1994;25:1658-65.
52. Asahi M, Asahi K, Jung JC, del Zoppo GJ, Fini ME, Lo E.H. Role of matriz metalloproinase 9 after focal cerebral ischemia: effects of gene knockout and exime inhibition with BB-94. J Cereb Blood Flow Metab. 2000;20:1681-9.
53. Rosenberg GA, Yang Y. Vasogenic edema due to tight junction disruption by matrix metalloprotinases in cerebral ischemia. Neurosurg Focus. 2007;22:E4.
54. Coleman M. Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci. 2005;6:889-98.
55. Pantoni L, Garcia JH, Gutierrez J.A. Cerebral White matter is highly vulnerable to ischemia. Stroke. 1996;27:1641-6.
56. McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg M.P. Oligodendrocytes from forebrain are highly vulnerable to AMPA/Kainate receptor-mediated excitotoxicity. Nat Med. 1998;4:291-7.
57. Xie F, Zheng B. White matter inhibitors in CNS axon regeneration failure. Exp Neurol. 2008;209:302-12.
58. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med. 2002;8:963-70.
59. SIver J, Miller J.H. Regeneration beyond the glial scar. Nat Rev Neurosci. 2004;5:146-56.
60. Hattiangady B, Rao MS, Shetty GA, Shetty A.K. Brain-derived neurotrophic factor phosphorylated cyclic AMP response element binding protein and neuropeptide Y decline as early as middle age in the dentate gyrus and CA1 and CA3 subfields of the hippocampus. Exp Neurol. 2005;195:353-71.
61. Brown CE, Li P, Boyd JD, Delaney KR, Murphy T.H. Extensive turnover of dendritic spines and vascular remodelling in cortical tissues recovering from stroke. J Neurosci. 2007;27:4101-9.
62. Brown CE, Aminoltejari K, Erb H, Winship IR, Murphy T.H. In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J Neurosci. 2009;29:1719-34.
63. Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci. 2009;10:861-72.
64. Carmichael ST, Archibeque I, Luke L, Nolan T, Momiy J, Li S. Growth-associated gene expression after stroke: evidence for a growth-promoting region in peri-infarct cortex. Exp Neurol. 2005;193:291-311.
65. Takatsuru Y, Fukumoto D, Yoshitomo M, Nemoto T, Tsukada H, Nabekura J. Neuronal circuit remodelling in the contralateral cortical hemisphere during functional recovery from cerebral infarction. J Neurosci. 2009;29:10081-6.
66. Biernaskie J, Chernenko G, Corbett D. Efficacy of rehabilitative experience declines with time after focal ischemic brain injury. J Neurosci. 2004;24:1245-54.
67. Dirnagl U. Bench to bedside: the quest for quality in experimental stroke research. J Cereb Blood Flow Metab. 2006;26:1465-78.
68. Crossley NA, Sena E, Goehler J, Horn J, van der Worp B, Bath PM, et al. Empirical evidence for bias in the design of experimental stroke studies. A metaepidemiologic approach. Stroke. 2008;39:929-34.
69. Horn J, de Haan RJ, Vermeulen M, Luiten PG, Limburg M. Nimodipine in animal model experiments of focal cerebral ischemia: a systematic review. Stroke. 2001;32:2433-8.
70. TRUST Study Group. Ramdomised, Double-blind, placebo-controlled Trial of nimodipine in acute stroke. Trust Study Group. Lancet. 1990;336:1205-9.
71. American Nimodipine Study Group. Clinical trial of nimodipine in acute ischemic stroke. The American Nimodipine Study Group. Stroke. 1992;23:3-8.
72. Kaste M, Fogelholm R, Erila T, Palomaki H, Murros K, Rissanen A, et al. A randomized. Double-blind, placebo-controlled Trial of nimodipine in acute ischemic hemispheric stroke. Stroke. 1994;25:1348-53.
73. Wahlgren NG, MacMahon DG, De Keyser J, Indredavik B, Ryman T. Intravenous Nimodipine West European Stroke Trial (INWEST) of nimodipine in the treatment of acute ischaemic. Stroke. 1994;4:204-10.
74. Horn J, de Haan RJ, Vermeulen M, Limburg M. Very Early Nimodipine Use in Stroke (VENUS): a randomized, double-blind, placebo-controlled trial. Stroke. 2001;32:461-5.
75. Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, et al. Transplantation of cultured human neuronal cells for patients with stroke. Neurology. 2000;55:565-9.
76. Scharff C, Kim JR, Grossman M, Macklis JD, Nottebohm F. Targeted neuronal death affects neuronal replacement and vocal behaviour in adult songbirds. Neuron. 2000;25:481-92.
77. Rossi F, Cattaneo E. Neural stem cell therapy for neurological diseases: dreams and reality. Nat Rev Neurosci. 2002;3:401-9.
78. Burns TC, Verfaillie CM, Low W.C. Stem cells for ischemic brain injury: a critical review. J Comp Neurol. 2009;515:125-1444.
79. Rakic P. Neurogenesis in adult primate neocortex: an evaluation of the evidence. Nat Rev Neurosci. 2002;3:65-71.
80. Alvarez-Buylla A, Garcia-Verdugo J.M. Neurogenesis in adult subventricular zone. J Neurosci. 2002;22:629-34.

81. Koketsu D, Furuichi Y, Maeda M, Matsuoka N, Miyamoto Y, Hisatsune T. Increased number of new neurons in the olfactory bulb and hippocampus of adult non-human primates after focal ischemia. Exp Neurol. 2006;199:92-102.
82. Zhang B, Wang RZ, Lian ZG, Song Y, Yao Y. Neurogenesis by activation of inherent neural stem cells in the rat hippocampus after cerebral infarction. Chin Med. Sci J. 2009;24:41-5.
83. Jiang W, Gu W, Brannstrom T, Rosqvist R, Wester P. Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. Stroke. 2001;32:1201-7.
84. Ohira K, Furuta T, Hiroki H, Nakamura KC, Kuramoto E, Tanaka Y, et al. Ischemia–induced neurogenesis of neocortical layer 1 pogenitor cells. Nat Neurosci. 2010;13:173-9.
85. Scharfman HE, Goodman JH, Sollas AL, Cross S.D. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. Exp Neurol. 2002;174:201-14.
86. Assanah M, Lochhead R, Ogden A, Bruce J, Goldman J, Canoll P. Glial progenitors in adult white matter are driven to dorm malignant gliomas by platelet-derived growth factor-expressing retroviruses. J Neurosci. 2006;26:6790-871.
87. Mattson B, Sorensen JC, Zimmer J, Johansson B.B. Neural grafting to experimental neocortical infarcts improves behavioural outcome and reduces thalamic atrophy in rats housed in enriched but not in standard environment. Stroke. 1997;28:1225-31.
88. Kolb B, Morshead C, Gonzalez C, Kim M, Gregg C, Shingo T, et al. Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats. J Cereb Blood Flow Metab. 2007;27:983-97.