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New Goals in Ischemic Stroke Therapy: The Experimental Approach – Harmonizing Science with Practice

María Alonso de Leciñana[a] Exuperio Díez-Tejedor[b] María Gutierrez[c]
Sandra Guerrero[c] Fernando Carceller[d] Jose María Roda[d]

[a]Department of Neurology, University Hospital Ramon y Cajal; Departments of [b]Neurology, [c]Cerebrovascular Experimental Laboratory and [d]Neurosurgery, Cerebrovascular Research Unit, La Paz University Hospital, Universidad Autónoma, Madrid, Spain

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Stroke therapy · Experimental brain ischemia · Animal models · Neuroprotection · Neurorestoration

Abstract
Undeniable advances have been made in clinical and experimental investigation into the pathophysiology, diagnosis, and treatment of cerebral ischemia. However, with the exception of intravenous thrombolysis and some neuroprotectors, such as citicoline, the majority of the drugs successfully tested in experimental studies have failed in clinical trials. Valuable lessons for the improvement of research methodology and appropriate coordination of experimental and clinical research can be learnt from the analysis of discrepancies between the laboratory and clinic, which will allow us to increase the power and cost-effectiveness of the studies. In addition, this progress has opened the way for the investigation of very promising new therapeutic strategies, such as combined pharmacological and mechanical thrombolysis, thrombolysis and neuroprotection, or the combination of various neuroprotectors, antiapoptotic therapies, and neurorestoration therapies, such as stem cell transplants.

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Thanks to important developments in experimental and clinical investigation which have taken place in the pathophysiology of cerebral ischemia in recent decades, we now have a very extensive and increasingly precise understanding of this area. This has allowed the identification of a large proportion of the changes which take place in the cells during ischemia and the determination of their chronological profile, leading to the detection of the existence of potentially salvable tissue (ischemic penumbra) [1–5] and, secondarily, to the investigation of therapeutic strategies and drugs (neuroprotectors) designed to inhibit the mediators of ischemic damage, with the aim of protecting the cell and avoiding the development of an irreversible infarction [6–9]. In addition to the various thrombolytic reperfusion therapies, a large number of potentially neuroprotective agents directed at different harmful factors in the ischemic cascade have been investigated (table 1) [8–10]. Some have not shown any efficacy, or have produced an excess of adverse effects and have not passed the animal research phase, but the majority of the substances which were successful in these experimental studies have failed in clinical trials. Currently, only stroke units [11, 12] and intravenous (IV) thrombolysis with rtPA in selected patients during the first 3 h after the start of symptoms [13–16] have shown beneficial effect in the treatment of acute cerebral infarction with

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María Alonso de Leciñana
Department of Neurology, University Hospital Ramón y Cajal
Ctra de Colmenar Km 9,100
ES–28034 Madrid (Spain)
Tel./Fax +34 670754255, E-Mail mariaalonsoleci@telefonica.net

Table 1. Neuroprotective drugs tested in the clinic

Calcium channel blockers	Nimodipine Flunarizine Isradipine
Calcium chelator	DP-b99
Sodium channel blockers	Fosphenytoin Lubeluzole 619C89
Potassium channel opener	BMS-204352 (Maxipost)
Glutamate antagonists	AMPA antagonists GYKI 52466 NBQX YM90K YM872 (Zonampanel) ZK-200775(MPQX) Kainate antagonist SYM 2081 NMDA antagonists Competitive NMDA antagonists CGS 19755 (Selfotel) NMDA channel blockers Aptiganel (Cerestat) CP-101,606 (Troxoprodil) Dextrorphan Dextromethorphan Magnesium Memantine MK-801 (Dizolcipine) NPS 1506 Remacemide Glycine site antagonists ACEA 1021 (Licostinel) GV150526 (Gavestinel) Polyamine site antagonists Eliprodil Ifenprodil
GABA agonists	Clomethiazole
Serotonin agonist	Bay x 3072 (Repinotan)
Free radical scavengers – antioxidants	Ebselen NXY-059 (Cerovive) Tirilazad
Leukocyte adhesion inhibitor	Anti-ICAM antibody (Enlimomab) Hu23F2G (Rovelizumab)
Nitric oxide inhibitor	Lubeluzole
Opioid antagonists	Naloxone Nalmefene
Membrane protectors	Citicoline Piracetam
Growth factors	Fibroblast growth factor (bFGF)

evidence level I. Many of the neuroprotective drugs which have shown some efficacy in clinical trials have side effects which outweigh the benefit (calcium antagonists [10, 17, 18], lubeluzole [19, 20], magnesium [21]), while in others the results are inconsistent (ebselen [22], piracetam [23], clomethiazole [24, 25]) or need to be confirmed with more trials (cerovive [10]). Some neuroprotectors, such as citicoline, have reproduced the favorable results from experimental studies in phase III clinical trials [26–30]. The causes of discrepancies between the experimental and clinical studies are diverse and depend both on the drugs studied and on the design of the experimental models and the clinical studies [31–33]. Valuable lessons have been learnt from the analysis of these discrepancies, which will allow the systematics of research into the treatment of cerebral ischemia to be improved and much more satisfactory results to be obtained in the near future [34, 35]. Developments in the understanding of the mechanisms involved in the ischemic damage, as well as the important biotechnological advances which are currently available for research, will contribute to this process.

Reasons for Discrepancies between the Results of Clinical and Experimental Studies

Physiological Differences between Rodents and Humans

Neuroanatomical, pathophysiological, and metabolic differences exist between the rat, which is the animal most often used in preclinical studies of neuroprotective therapies, and humans, and these may explain in part why the results of experimental studies are generally more favorable. The thresholds of regional cerebral blood flow below which certain cellular functions are lost and death due to necrosis eventually occurs are different, as is the chronological development of cell damage in the penumbra zone, which implies differences in the duration of the 'window of opportunity' for certain therapeutic options [5]. The great efficacy of the collateral circulation in the rat [31] provides a natural defense against focal ischemia in these animals, and this may allow the more effective contribution of systemically administered neuroprotective drugs in the penumbra zone. Although the metabolic differences between rodents and large mammals are well known, there are very few studies comparing the pharmacokinetics of any compound in experimental animals and humans, or the dosing equivalents for obtaining a certain effect. It is not uncommon for drugs under investigation in cerebral ischemia to present different tissue distribu-

tion, clearance, or hepatic metabolism among species, so that the concentrations achieved in the brain tissue of humans may be different from those achieved in animals, and as such, less effective. In most cases, dose-response curves are not obtained before the drugs are studied in clinical trials. Comparatively, much higher doses are used in smaller animals [36] than those used in clinical trials.

Differences between Experimental Cerebral Infarction and Spontaneous Brain Infarction in Patients Included in Clinical Trials

Although animal models try to reproduce ischemic stroke as closely as possible, there are inevitable differences derived from the experimental model itself. The objective of an experimental model is to achieve homogeneous and reproducible lesions with minimum variability, with the aim of maximizing reliability and providing results. To achieve this, all variables which can influence the extent and progression of the lesion, such as physiological parameters (cranial and body temperature, blood pressure, glycemia, blood gases, pH), age, sex of the animals, location of arterial occlusion, and exact time of administration of treatments, are controlled. In clinical practice it is impossible to obtain similarly homogeneous groups of patients with cerebral infarction which are large enough for the performance of reliable studies, so clinical trials group together patients with infarctions in different localizations, of different extent and etiologies, as well as patients with advanced age and multiple concomitant diseases and therapies. In these patients administration of the drug is carried out over a less precise range of time than in animal studies, and it is not possible to control the physiological parameters with such precision. This heterogeneity reduces the power of the studies and makes it difficult to obtain results with a sufficient level of significance. In addition, in the majority of the animal models of focal ischemia, infarctions are produced in the brain cortex and, in any case, the evaluation of any neuroprotector is usually limited to its effect on the gray matter. Since the pathophysiology of ischemia in the white matter differs in some aspects [37], it may be possible that an effective neuroprotector in cortical infarctions would be ineffective in lacunar or subcortical infarctions, which are very common in humans and widely represented in clinical study populations.

The target of the therapeutic effect of a neuroprotector drug is the potentially recoverable tissue in the ischemic penumbra zone. The maintenance of homeostasis and homogeneity of the lesion which is obtained in animal models are ideal conditions for the presence of an area of penumbra. However, in patients in clinical trials it is not known whether recoverable tissue is present or not, and the clinical heterogeneity of the subjects suggests that cases with different amounts of tissue in penumbra and with different chances of recovery are included. If there is no penumbra tissue and the whole area of brain affected by the focal ischemia is irreversibly damaged, it is unlikely that the neuroprotectors will provide any benefit. If the presence and amount of recoverable tissue in candidates for clinical trials of neuroprotectors could be determined, it would allow the selection of optimal cases for receiving the drug, excluding those who, due to an irreversible lesion, would not benefit from the treatment. In this way, it would be easier to demonstrate the action of a determined drug, and the conclusions drawn with regard to its efficacy would be more reliable.

Reasons Attributable to the Methodology of Experimental Studies and Clinical Trials

One of the most important aspects of experimental and clinical research is the study design. This must be appropriate for the objective under investigation so the cost-effectiveness of the study can be optimized. Although both types of investigation represent two closely related phases in the development of therapies for cerebral ischemia, they are not carried out in a coordinated fashion, and experimental studies are not often designed so that their results can later be extrapolated to the clinic. Similarly, when clinical trials are planned to test the benefit of a certain drug, the methodology used in experimental studies which showed benefit is rarely resembled. Most often clinical trials are not adapted to conditions in which the drug has previously been studied (with regard to type of infarction treated, time of administration of the drug, duration of treatment, dosing, endpoints, timing of evaluations), giving way to completely different studies for which the prior experimental phase is not valid.

In the phase of experimental investigation, the most appropriate model must be chosen. There are various models of focal cerebral ischemia [31]. The ones most frequently used at present are the model of middle cerebral artery ligation after craniotomy [38, 39], the intraluminal occlusion model, inserting a filament via the internal carotid artery [40] and the model of occlusion with autologous blood clot emboli [41, 42]. The former produces very homogeneous cortical lesions but it is traumatic and not very 'physiological', as it does not resemble human stroke. It is very useful in pathophysiological studies of ischemia, thanks to its lack of variability, and can be very useful in the study of neuroprotective agents for

demonstrating a certain effect on the lesion, but its results will be hard to reproduce in clinical trial for all the reasons mentioned above. On the other hand, the intraluminal occlusion models, particularly the model with clot embolism, are more similar to cerebral infarction due to arterial embolism in humans. It gives way to very extensive lesions of widely varying size which affect basal ganglia and the cortex and cause high mortality. This model is very attractive for the study of neuroprotectors, particularly in combination with pharmacological thrombolysis, but it has the inconvenience of being much less cost-effective due to the variability of the lesions and high mortality.

In experimental studies, drugs are administered very early, in some cases even before the infarction is produced, while in clinical studies treatment may be delayed by up to 24 h, and in any case is administered over a much more variable time range [8–10]. Although the therapeutic window for different drugs varies according to their mechanism of action, and individual differences also exist depending on factors such as the efficacy of collateral circulation or physiological parameters (blood pressure, glycemia, temperature), in general, the earlier a neuroprotective agent is administered, the greater the probability that it will be effective [43, 44]. For the results to be superposable, the drug administration protocols in the clinic and laboratory should be more similar and better adapted to the presence or absence of penumbra and to the mechanism of action of the drug.

It is also very important to choose carefully the parameters which will be used for the evaluation of the lesion and action of the study drug on this lesion, as well as the moment in the process in which these markers are measured. In most animal studies, histological parameters (size of lesion, neuronal death) [45] or biochemical parameters (quantification of markers, such as glutamate and caspases [46] and other mediators of ischemia-induced damage) in plasma, cerebrospinal fluid, or tissue are used [31, 32, 47]. Motor, cognitive or behavior deficits, evaluated using validated scales, and mortality due to brain damage have been used only recently as efficacy endpoints [48–51]. On the other hand, the results of clinical studies are just based on scales for the evaluation of neurological or functional deficits and on mortality. In addition, the evaluation is made after periods of evolution which are short in experimental studies (days) and long in clinical studies (months). The experimental studies should evaluate combined endpoints which can be extrapolated to clinical trials, since the results will be much more robust and more easily reproducible and reliability of the experiments will be increased. One single marker may not be sensitive to the effect of a neuroprotector even though it is effective (false-negative result), or on the contrary, the marker may be affected by the mechanism of action of the drug, without this being translated into clinical effect (false-positive result). In the case of experiments based only on histological criteria, particularly if they are measured at the early stages, the conclusions must be considered with caution, due to the fact that the tissue appears morphologically intact; this does not signify that it is functioning or that it will not develop a lesion later on [39, 52]. For this reason it is important to include neurological and functional evaluation using scales in the animal models and to carry out the evaluation later than is currently done, to ensure that the evolution of the process is complete and that a result will not change over time [53].

Reasons Attributable to Pharmacology, Mechanism of Action, and Administration Protocol of the Compounds Studied

The efficacy of a neuroprotector depends on its capacity to inhibit the mediators of the ischemic cascade and the processes which lead to different forms of neuronal death [1, 2, 54]. The demonstration of this efficacy in any study, whether experimental or clinical, depends, among other factors mentioned, on whether the administration protocol (treatment initiation, administration regimen, duration of treatment, dosing) is appropriate for the mechanism of action and the pharmacological properties of the drug under investigation [32, 55]. In practice, these aspects are not taken sufficiently into consideration. For example, the activation of excitatory amino acids and cytosolic calcium overload are changes which occur very early on in the ischemic cascade and their damaging effects take place very quickly. For this reason, excitatory amino acid antagonists and calcium antagonists should be administered very early for them to have any beneficial effect, but it would not be necessary to maintain treatment for long. This is done in experimental studies carried out with these types of drugs, but not in clinical trials which have studied them. This explains in part the beneficial effects demonstrated in animals which have not been obtained in humans [8, 9, 18, 19, 41, 56]. On the other hand, other disorders, such as inflammation or different proapoptotic mechanisms, are responsible for cell damage later on, and as such, an anti-inflammatory or anti-apoptotic drug would have a longer therapeutic window and would probably require a longer period of administration [57, 58]. In addition, acute treatment could

delay, but not avoid, cell death unless continued for a sufficient period of time (depending on the drug used) [55], but in many clinical trials treatment is only continued for a few hours or days after the stroke. In animals, although the treatment is not lengthy, the evaluation of the lesion is made much earlier than in humans, which favors a positive result, since the possibility of a subsequent deterioration is avoided.

In the ischemic cascade, the pathophysiological disorders responsible for the progression of the cerebral injury are related in such a way that they all participate in the production and action of the others. This suggests that the isolated inhibition of one of them, particularly one of the earlier ones, would not be particularly useful, since other lesion-producing mechanisms would be strengthened. In addition, depending on the characteristics of the cerebral infarction which we want to treat (presence or absence of penumbra, time of evolution, presence or absence of reperfusion, collateral circulation efficacy), a drug with a certain mechanism of action may or may not be useful. Consequently, it seems logical that it would be more effective to act by simultaneously inhibiting several steps in the ischemic cascade, or else by protecting the target organ more specifically (cell membrane, organelle membrane, nucleus, DNA, etc.).

Finally, the pharmacological interactions of a compound are not considered in animals, as they are studied in isolation (while patients in clinical trials are usually on multiple medications), and neither is drug toxicity considered, which may overcome the neuroprotective effect in humans.

New Goals in the Development of Neuroprotective Therapies against Cerebral Ischemia

In consideration of the discussions in the previous sections, it is easy to understand the discrepancies between experimental studies and clinical studies, and that in some cases the absence of positive results in the clinic does not indicate lack of efficacy, rather the inability of studies to demonstrate it. For this reason we should not give up investigation for the development of effective neuroprotective agents. Research into the pathophysiology, diagnosis, and therapeutics of cerebral ischemia has never been better and new advances are constantly being produced to smooth the path. The absence of success does not necessarily imply failure; rather it contributes to the advance of science, in such a way that very valuable lessons can be learned from the analysis of the lack of concordance between the results of experimental and clinical investigation, which can be used to continue this advance successfully. For this it is necessary to establish two fundamental goals: the improvement of the investigational methodology and the study of new therapeutic strategies.

Improvements in Investigational Methodology and Clinical Trial Design

The objectives to be achieved summarize the different aspects discussed throughout this article. Despite the known differences between humans and animals, the laboratory must be considered as a phase prior to the clinic in the development of neuroprotective drugs and experimental and clinical studies must be carried out in a coordinated fashion. This implies that reproducible animal models of focal cerebral ischemia, which mimic as far as possible cerebral infarction in humans, must be selected and designed in such a way that results can later be extrapolated to facilitate their scrutiny in clinical trials. The parameters for measuring the seriousness of the experimental infarction and the action of the drug under investigation must be combined to increase sensitivity and specificity, and to make them more similar to the clinic. Scales for performance status must be included in addition to histological or biochemical markers. For the same reason, the evaluation must be made later in experimental studies, with longer survival times than those usually considered. The clinical trials should study more homogeneous patient groups, particularly with regard to the localization and size of infarction, concomitant disease and physiological variables at the time of treatment, and it would be desirable to study larger sample sizes. The early phase clinical trials studying drugs with proven efficacy in animal models should reproduce the experimental conditions in which the drug was studied, including strictly only those subjects who fulfilled these conditions. The demonstration of the presence of recoverable tissue, by the identification of an area of salvageable penumbra before administration of the drug and exclusion of patients with no evidence of this, would improve the cost-effectiveness of clinical trials. Sensitive techniques, such as perfusion-diffusion mismatch detection on MRI or CT, are available for this [59]. However, some authors maintain that this is only useful if it does not involve a delay in the administration of treatment, since this may reduce its efficacy [34]. Drugs must be administered as

soon as possible, although the demonstration of the presence of an area of penumbra would justify later administration. The currently ongoing DEFUSE (Diffusion-Weighted Imaging Evaluation for Understanding Stroke Evolution) [60] and EPITHET (Echoplanar Imaging Thrombolysis Evaluation Trial) [61] studies have the objective of evaluating the usefulness of diffusion-perfusion resonance for the identification of predictive patterns of favorable evolution after thrombolysis with rtPA between 3 and 6 h after the onset of symptoms. The recently completed DIAS (Desmoteplase in Acute Stroke) and DEDAS (Dose Escalation of Desmoteplase in Acute Stroke) studies use the presence of mismatch as criteria for the selection of patients as candidates for treatment with desmoteplase up to 9 h after stroke [62, 63].

The regime of administration of the drug and the duration of treatment should be adapted to its mechanism of action. The therapeutic window should be determined before phase III trials are begun. As far as possible, the clinical study should select patients whose characteristics allow us to assume that they will benefit from the mechanism of action of the study drug. The doses administered in humans should be the equivalent to those studied in animals. For this we must know the pharmacokinetic characteristics and the dose-response curve of the compounds studied in humans. The toxic effects of the drugs in animals must be evaluated before they are tested in the clinic.

New Therapeutic Strategies under Development: Combined Therapies

Combination of IV Thrombolysis with Techniques Aimed at Increasing the Efficacy and Speed of Reperfusion

The efficacy of IV thrombolysis, when the recommendations for administration are followed, has been sufficiently well proven [13–16]. Nevertheless, its use is limited by its short therapeutic window and by the complications derived fundamentally from the risk of bleeding and possible injury from reperfusion. On the other hand, in some cases, IV thrombolysis is not effective for reperfusion and, in addition, post-rtPA rethrombosis has been described [64]. The problem of the short therapeutic window could be resolved as described above, by the identification of patients with persistent diffusion/perfusion mismatch later than 3 h after the onset of symptoms and in any case, aiming for reperfusion as fast as possible. The risk of bleeding depends, among other factors such as drug dose, patient age, blood pressure or glycemia [65], fundamentally on the extent of the lesion, which in turn depends on the duration of the occlusion and on the efficacy of the collateral circulation [66]. For all these reasons, to improve the benefit of IV thrombolysis, it appears to be necessary to improve the speed of taking actions for initiation of treatment and to increase the reperfusion ability of the drugs. In addition to the combination of IV and intra-arterial thrombolysis [67, 68], the combination of pharmacological thrombolysis together with different forms of mechanical thrombolysis, such as the transcranial application of ultrasound in the CLOTBUST trial [69] or the disruption and extraction of the clot using intra-arterial devices in the MERCI studies [70], seems to be useful. Studies are being carried out in phase I and II to see if it is possible to improve the thrombolytic capacity and to avoid rethrombosis by combining IV rtPA with other antithrombotics, such as the GIIb/IIIa inhibitors, abciximab [71], and eptifibatide [72], and the direct thrombin inhibitor, argatroban [73]. Experimental studies show that it is possible to inhibit the matrix metalloproteases to reduce the bleeding complications associated with thrombolysis [74].

Combination of Thrombolysis and Neuroprotection

According to the pathophysiology of cerebral ischemia, the treatment regime which theoretically would allow the extent and seriousness of cerebral infarction to be reduced to the maximum would be the combination of thrombolysis for restoring the blood flow as soon as possible, together with effective neuroprotection for the inhibition of injury-causing mediators due to ischemia-reperfusion. The demonstration of this hypothesis necessarily requires the identification of an effective neuroprotector, proven in clinical trials, which can be administered concomitantly with thrombolysis, investigation of the efficacy in preclinical experimental studies, identification of the optimal treatment regime, and assessment of the clinical utility of the combination. Various experimental studies show that the combination of thrombolysis with neuroprotectors (citicoline, MK-801, tirilazad, NBQX, anti-CD18) produces beneficial effects superior to those obtained with monotherapy [75–80]. There are also clinical trials in combined therapy (lubeluzole, clomethiazole) [81, 82] which have not shown efficacy. The reasons for this may be those discussed throughout this paper. The currently ongoing ARTIST+ study investigates the combination of the glutamate AMPA receptor antagonist, YM872, with IV rtPA [83].

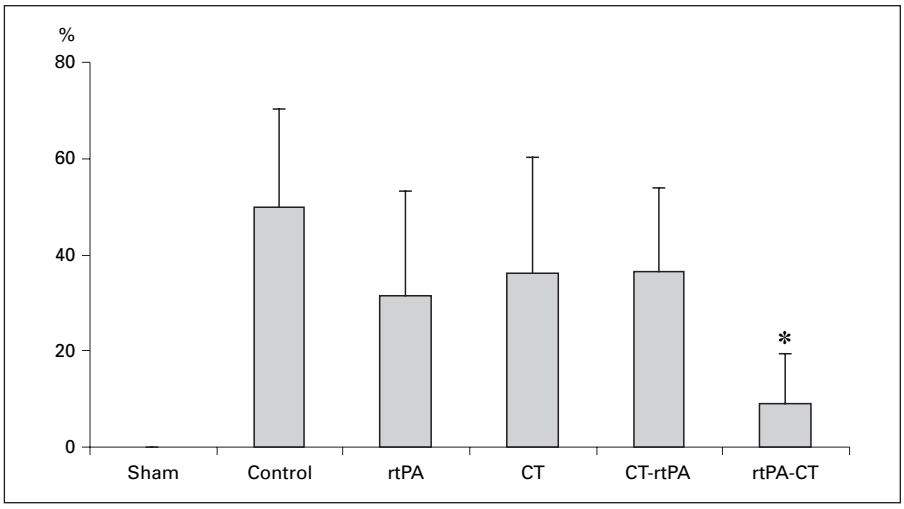

Fig. 1. Size of lesion, expressed as percentage, in an experimental model of embolic cerebral infarction in rats. CT = Citicoline; CT-rtPA = combination of citicoline before rtPA; rtPA-CT = combination of citicoline after rtPA. * Significant difference ($p = 0.027$, Mann-Whitney test).

To obtain the desired efficacy of combined therapy, it is necessary to identify the most effective administration regime. Two possibilities are proposed: (1) administering the neuroprotector before reperfusion to delay progression to irreversible infarction in the penumbra zone and to prolong the therapeutic window for thrombolysis; (2) administering the neuroprotector, once reperfusion has been carried out, to improve the penetration of the neuroprotector in the penumbra zone, and to inhibit ischemia-perfusion damage. Once again, we must say that the best regimen in each case will depend on the mechanism of action of the neuroprotective drug. In our laboratory we have shown that the combination of citicoline (250 mg/24 h for 3 days by the intraperitoneal route) started after thrombolysis with rtPA (5 mg/kg IV) reduces the size of the lesion in an embolic cerebral infarction model in rats more effectively than citicoline or rtPA alone, or than the combination of citicoline before rtPA at the same doses (final results in publication) [84, 85] (fig. 1).

Combination of Neuroprotectors with Different Mechanisms of Action

Taking into account the hypothesis that the simultaneous inhibition of various steps in the ischemic cascade may protect the brain tissue more efficiently against ischemia, animal studies have been carried out with combinations of neuroprotective drugs with different mechanisms of action [45, 86–89] and with hypothermia [90]. These experimental studies offer promising results, which should be tested in the clinic.

Combination therapy offers new treatment possibilities. In addition to possibly being useful for widening the therapeutic window, it would allow the design of 'a la carte' treatment, with the sequential administration of drugs using a protocol adapted to the clinical picture and progress of each patient. However, there remains much to be investigated before this goal can be achieved.

Antiapoptotic Treatments

The understanding of mechanisms of delayed neuronal death after focal ischemia and the possibility that the cells in the penumbra zone which escape necrosis finally die due to apoptosis [58, 91] leads inevitably to the investigation of antiapoptotic therapies such as cycloheximide or caspase inhibitors. These could be particularly useful in subjects in advanced stages of evolution, since theoretically their window of opportunity would be wider, and could be used in association with other neuroprotectors to avoid delayed cell death after inhibiting necrosis. Selective gene expression, which would contribute to apoptosis programming, may even possibly be modified. Although its utility has been demonstrated in animals [92, 93], there remains a lot to be learnt about the role of apoptosis in human stroke and about the possibility and utility of manipulating it for therapeutic objectives.

'Neurorestauration' or 'Neurorepair' Therapies

The traditional concept of stroke as a dramatic disease about which nothing could be done changed radically with improvements in prevention and later with the development of effective treatment in the acute phase. However, taking into account that stroke causes disabling

sequelae in a high percentage of cases, it is necessary to go further and find restorative therapies which can be combined with the different techniques of 'traditional' rehabilitation, for improving the possibilities of functional recovery in the subacute and chronic stages. It has been shown that growth factors are expressed after stroke and that neuroplasticity is possible. This can make way for the formation of new dendritic ramifications in viable neurons, new synapses, and even neurogenesis [94, 95]. The objective of the new neurorestoration therapies is to strengthen this plasticity.

Stem cell transplant represents an interesting therapeutic perspective. Transplants of stem cells of different origin may contribute to repair by transforming into neural tissue or else by the liberation in situ of growth factors which facilitate the local formation of specific stem cells, as well as neurogenesis and synaptogenesis. There is evidence in animal models that stem cells administered intravenously and intraarterially or else locally by intraventricular or intraparenchymatous injection can implant themselves in the area of the lesion and give way to the repair mechanisms, improving functional recovery in animals undergoing cerebral ischemia [96–99]. There is also a phase I study in humans [100], but a wider experimental basis is required for developing the clinical research phase.

In conclusion, we can confirm that, despite the apparent lack of success of the various neuroprotective drugs tested in acute stroke, important advances have been made in the understanding and improvements in methodology which offer a good overall panorama for the future of research in the treatment of cerebral ischemia.

References

1 Siesjö BK: Pathophysiology and treatment of focal cerebral ischemia. I. Pathophysiology. J Neurosurg 1992;77:169–184.
2 Siesjö BK: Pathophysiology and treatment of focal cerebral ischemia. II. Mechanisms of damage and treatment. J Neurosurg 1992;77: 337–354.
3 García JH, Wagner S, Liu KF, Hu XJ: Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995;26: 627–635.
4 Astrup J, Siesjö BK, Symon L: Threshold in cerebral ischemia. The ischemic penumbra. Stroke 1981;12:723–727.
5 Hossmann KA: Viability thresholds and the penumbra of focal ischemia. Ann Neurol 1994; 36:557–565.
6 Baron JC: Perfusion thresholds in human cerebral ischemia: historical perspective and therapeutic implications. Cerebrovasc Dis 2001;11(suppl 1):2–8.
7 Ginsberg MD: Injury mechanisms in the ischemic penumbra. Approaches to neuroprotection in acute ischemic stroke. Cerebrovasc Dis 1997;7(suppl 2):7–12.
8 Castillo J, Álvarez-Sabín J, Dávalos A, Díez-Tejedor E: Lizasoain I, Martínez-Vila E, Vivancos J, Zarranz JJ: Consensus review. Pharmacological neuroprotection in cerebral ischemia: is it still a therapeutic option? Neurología 2003;18:368–384.
9 Martínez-Vila E, Sieira PI: Current status and perspectives of neuroprotection in ischemic stroke treatment. Cerebrovasc Dis 2001;11 (suppl 1):60–70.
10 The Internet Stroke Center: Washington University in St Louis. Clinical Trials Directory. http://www.strokecenter.org/trials/index_cats.htm.
11 Indredavik B, Bakke F, Solberg R, Rokseth R, Haaheim LL, Holme I: Benefit of a stroke unit: a randomized controlled trial. Stroke 1991;22: 1026–1031.
12 Stroke Unit Trialist's Collaboration: Organised inpatient (stroke unit) care for stroke. The Cochrane Library, issue 2, 2003. Oxford: Update Software.
13 The National Institute of Neurological Disorders and Stroke rtPA Stroke Study Group: Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995;333:1581–1587.
14 Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, Larrue V, Bluhmki E, Davis S, Donnan G, Schneider D, Diez-Tejedor E, Trouillas P: Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Lancet 1998;352: 1245–1251.
15 Marler JR, Tilley BC, Lu M, Brott TG, Lyden PC, Grotta JC, Broderick JP, et al: Early stroke treatment associated with better outcome: the NINDS rt-PA Stroke Study. Neurology 2000; 55:1649–1655.
16 Hacke W, Donnan G, Fieschi C, Kaste M, von Kummer R, Broderick JP, Brott T, et al, ATLANTIS Trials Investigators, ECASS Trials Investigators, NINDS rt-PA Study Group Investigators: Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. Lancet 2004;363:768–774.
17 Mohr JP, Orgogozo JM, Hennerici M: Meta-analysis of oral nimodipine trials in acute ischemic stroke. Cererbovasc Dis 1994;4:197–203.
18 Horn J, Limburg M, Vermeulen M: VENUS-Very Early Nimodipine Use in Stroke: final results from a randomized placebo-controlled trial. Cerebrovasc Dis 1999;9(suppl 1):127.
19 Muir KW, Lees KR: Excitatory amino acid antagonists for acute stroke. Cochrane Database Syst Rev 2003;(3):CD001244.
20 Gandolfo C, Sandercock P, Conti M: Lubeluzole for acute ischaemic stroke. Cochrane Database Syst Rev 2002;(1):CD001924.
21 Muir KW, Lees KR, Ford I, Davis S, Intravenous Magnesium Efficacy in Stroke (IMAGES) Study Investigators: Magnesium for acute stroke (Intravenous Magnesium Efficacy in Stroke trial): randomised controlled trial. Lancet 2004;363:439–445.
22 Ogawa A, Yoshimoto T, Kikuchi H, Sano K, Saito I, Yamaguchi T, Yasuhara H: EBSELEN in acute middle cerebral artery occlusion: a placebo-controlled, double-blind clinical trial. Cerebrovasc Dis 1999;9:112–118.
23 Ricci S, Celani MG, Cantisani AT, Righetti E: Piracetam for acute ischaemic stroke. Cochrane Database Syst Rev 2002;(4):CD000419.
24 Wahlgren NG, Ranasinha KW, Rosolacci T, Franke CL, vanErven PM, Ahswood T, for the CLASS Study Group: Clomethiazole Acute Stroke Study (CLASS). Results of a randomized, controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. Stroke 1999;30:21–28.
25 Lyden P, Shuaib A, Lerin K and the Clomethiazole Acute Stroke Study Collaborative Group: The Clomethiazole Acute Stroke Study in ischemic stroke (CLASS I). Final results. Stroke 2002;35:122–129.
26 Clark WM, Warach SJ, Pettigrew LC, Gammans RE, Sabounjian LA: A randomized dose-response trial of citicoline in acute ischemic stroke patients. Neurology 1997;49:671–678.

27 Clark WM, Williams BJ, Selzer KA, Zweifler RM, Sabounjian LA, Gammans RE: A randomized efficacy trial of citicoline in patients with acute ischemic stroke. Citicoline Stroke Study Group. Stroke 1999;30:2592–2597.
28 Clark WM, Weschler LR, Sabounjian LA, Schwiderski UE, for the Citicoline Stroke Study Group: A phase III randomized efficacy trial of 2000 mg citicoline in acute ischemic stroke patients. Neurology 2001;57:1595–1602.
29 Warach S, Pettigrew LC, Dashe JF, Pullicino P, Lefkowitz DM, Sabounjian LA, et al: Effect of citicoline on ischemic lesions as measured by diffusion-weighted magnetic resonance imaging. Citicoline 010 Investigators. Ann Neurol 2000;48:713–722.
30 Dávalos A, Castillo J, Alvarez-Sabin J, Secades JJ, Mercader J, López S, Cobo E, Warach S, Sherman D, Clark WM, Lozano R: Oral citicoline in acute ischemic stroke. An individual patient data pooling analysis of clinical trials. Stroke 2002;33:2850–2857.
31 Alonso de Leciñana M, Díez-Tejedor E, Carceller F: Cerebral ischemia: from animal studies to clinical practice. Should the methods be reviewed? Cerebrovasc Dis 2001;11(suppl 1):20–30.
32 Gladstone DJ, Black SE, Hakim AM, for the Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery: Toward wisdom from failure. Lessons from neuroprotective stroke trials and new therapeutic directions. Stroke 2002;33:2123–2136.
33 Fisher M, Brott TG: Emerging therapies for acute ischemic stroke. New therapies on trial. Stroke 2003;34:359–361.
34 Grotta JC: Acute stroke therapy at the millennium: consummating the marriage between the laboratory and the bedside. The Feinberg lecture. Stroke 1999;30:1722–1728.
35 Fisher M, for the Stroke Therapy Academis Industry Roundtable: Recommendations for advancing development of acute stroke therapies. Stroke Therapy Academic Industry Roundtable 3. Stroke 2003;34:1539–1546.
36 McCann UD, Ricaurte GA: Caveat emptor: Editors beware. Neuropsycopharmacology 2001;24:333–334.
37 Ransom BR, Acharya AB, Goldberg MP: Molecular pathophysiology of white matter anoxic-ischemic injury; in Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA (eds): Stroke: Pathophysiology, Diagnosis and Management. Philadelphia, Churchill Livingstone, 2004, pp 867–881.
38 Tamura A, Graham DI, McCulloch J, Teasdale GM: Focal cerebral ischemia in the rat. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1981;1:53–60.
39 Roda JM, Carceller F, Díez-Tejedor E, Avendaño C: Reduction of infarct size by intra-arterial nimodipine administered at reperfusion in a rat model of partially reversible brain focal ischemia. Stroke 1995;26:1888–1892.
40 Koizumi J, Yoshida Y, Nakazawa T, Oneda G: Experimental studies of ischemic brain edema 1. A new experimental model of cerebral embolism in rats in which recirculation can be reintroduced in the ischemic area. Jpn J Stroke 1986;8:1–8.
41 Overgaard K, Sereghy T, Boysen G, Pedersen H, Hoyer S, Diemer NH: A rat model of reproducible cerebral infarction using thrombotic blood clot emboli. J Cereb Blood Flow Metab 1992;12:484–490.
42 Alonso de Leciñana M, Díez-Tejedor E, Carceller F, Vega A, Roda JM: Advantages of associate inhibitors of ischemia reperfusion injury to intravenous thrombolysis for treatment of focal cerebral ischemia. Description of an experimental model. Rev Neurol 1996;24:207–213.
43 Jonas S, Aiyagari V, Vieira D, Figueroa M: The failure of neuronal protective agents versus the success of thrombolysis for the treatment of ischemic stroke. The predictive value of animal models. Ann NY Acad Sci 2001;939:257–267.
44 Heiss WD, Thiel A, Ground M, Graf R: Which targets are relevant for therapy of acute ischemic stroke? Stroke 1999;30:1486–1489.
45 Sobrado M, López M, Carceller F, García A, Roda JM: Combined nimodipine and citicoline reduce infarct size, attenuate apoptosis and increase bcl-2 expression after focal cerebral ischemia. Neuroscience 2003;118:107–113.
46 Krupinski J, Ferrer I, Barrachina M, Secades JJ, Mercadal J, Lozano R: CDP-choline reduces pro-caspase and cleaved caspase-3 expression, nuclear fragmentation, and specific PARP-cleaved products of caspase activation following middle cerebral artery occlusion in the rat. Neuropharmacology 2002;42:846–854.
47 Corbett D, Nurse S: The problem of assessing affective neuroprotection in experimental cerebral ischemia. Prog Neurobiol 1998;54:531–548.
48 Rogers DC, Campbell CA, Stretton JL, Mackay KB: Correlation between motor impairment and infarct volume after permanent and transient middle cerebral artery occlusion in the rat. Stroke 1997;28:2060–2066.
49 Yonemori F, Yamaguchi T, Yamada H, Tamura A: Evaluation of a motor deficit after chronic focal cerebral ischemia in rats. J Cereb Blood Flow Metab 1998;18:1099–1106.
50 Virley D, Beech JS, Smart SC, Williams SCR, Hodges H, Hunter AJ: A temporal MRI assessment of neuropathology after transient middle cerebral artery occlusion in the rat: correlation with behaviour. J Cereb Blood Flow Metab 2000;20:563–582.
51 Zhang L, Chen J, Li Y, Zhang ZG, Chopp M: Quantitative measurement of motor and somatosensory impairments in mild (30 min) and severe (2 h) transient middle cerebral artery occlusion in rats. J Neuol Sci 2000;174:141–146.
52 García JH, Liu KF, Ho KL: Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. Stroke 1995;26:636–643.
53 Colbourne F, Li H, Buchan AM, Clemens JA: Continuing postischemic neuronal death in CA1: influence of ischemic duration and cytoprotective doses of NBQX and SNX-111 in rats. Stroke 1999;30:662–668.
54 Zukin RS, Jover T, Yokota H, Calderone A, Simonescu M, Lau G: Molecular and cellular mechanisms of ischemia induced neuronal death; in Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA (eds): Stroke: Pathophysiology, Diagnosis and Management. Philadelphia, Churchill Livingstone, 2004, pp 829–854.
55 Dyker AG, Lees KR: Duration of neuroprotective treatment for ischemic stroke. Stroke 1998;29:593–603.
56 Albers GW, Golberg MP, Choi DW: Do NMDA antagonists prevent neuronal injury? Yes. Arch Neurol 1992;49:418–420.
57 Iadecola C, Cho S, Feuerstein GZ, Hallenbeck J: Cerebral ischemia and inflammation; in Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA (eds): Stroke: Pathophysiology, Diagnosis and Management. Philadelphia, Churchill Livingstone, 2004, pp 883–893.
58 Dalkara T, Moskowitz MA: Apoptosis in cerebral ischemia; in Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA (eds): Stroke: Pathophysiology, Diagnosis and Management. Philadelphia, Churchill Livingstone, 2004, pp 855–866.
59 Schramm P, Schellinger PD, Klotz E, Kallenberg K, Fiebach JB, Külkens S, Heiland S, Knauth M, Sartor K: Comparison of perfusion computed tomography and computed tomography angiography source images with perfusion weighted imaging and diffusion weighted imaging in patients with acute stroke of less than 6 h duration. Stroke 2004;35:1652–1658.
60 DEFUSE Study: Diffusion-weighted imaging Evaluation For Understanding Stroke Evolution. http://www.strokecenter.org/trials/updates.aspx.
61 Butcher KS, for the EPITHET Investigators: EPITHET: Echoplanar Imaging Thrombolysis Evaluation Trial. Presented at Clinical Trials session, 28th International Stroke Conference. Phoenix, Arizona, February 2003. http://www.astn.org.au/epithet/.
62 Hacke W, Albers G, Al-Rawi Y, Bogousslavsky J, Davalos A, Eliasziw M, Fischer M, Furlan A, Kaste M, Lees KR, Soehngen M, Warach S, DIAS Study Group: The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. Stroke 2005;36:66–73.
63 Furlan A, for the DEDAS study group: Dose escalation study of desmoteplase in acute ischemic stroke (DEDAS). Presented at the 2005 International Stroke Conference. February 2005. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=470.

64 Alexandrov AV, Grotta JC: Arterial reocclusion in stroke patients treated with intravenous tissue plasminogen activator. Neurology 2002; 59:862–867.
65 Adams HP, Brott T, Furlan AJ, Gómez CR, Grotta J, Helgason CM, Kwiatowski T, Lyden PD, Marler JR, Torner J, Feinberg W, Mayberg M, Thies W: Guidelines for thrombolytic therapy for acute stroke: a supplement to the guidelines for the management of patients with acute ischemic stroke. A statement for healthcare professionals from a special writing group of the stroke council, AHA. Stroke 1996;27: 1711–1718.
66 Levy DE, Brott TG, Haley EC Jr, Marler JR, Sheppard GL, Barsan W, Broderick JP: Factors related to intracranial hematoma formation in patients receiving tissue-type plasminogen activator for acute ischemic stroke. Stroke 1994;25:291–297.
67 Lewandowsky CA, Frankel M, Tomsick TA, Broderick J, Frey J, Clark W, Starkman S, Grotta J, Spilker J, Khoury J, Brott T and the EMS Brinding Trial Investigators: Combined intravenous and intra-arterial r-TPA versus intra-arterial therapy of acute ischemic stroke. Emergency Management of Stroke (EMS) Bridging Trial. Stroke 1999;30:2598–2605.
68 Tomsick T, Broderick P, Pancioli AM, Jauch E, Woo D, Kissela BM, Kanter D, Spilker J, Carrozzella J, Ernst T, Gaskill-Shipley M, Cornelius R: Combined IV-IA treatment in major acute ischemic stroke. Stroke 2002;35:359.
69 Alexandrov AV, Molina CA, Grotta JC, Garami Z, Ford SR, Alvarez-Sabin J, Montaner J, Saqqur M, Demchuk AM, Moye LA, Hill MD, Wojner AW, CLOTBUST Investigators: Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. N Engl J Med 2004; 351:2170–2178.
70 Starkman S, on behalf of the MERCI investigators: Results of the combined MERCI I-II (Mechanical Embolus Removal in Cerebral Ischemia) Trials. Stroke 2004;35:240.
71 Morris DC, Mitsias P, Silver B, Zhang Li, Daley S, Lewandowski C, Patel S, Lu M, Ford H: Abciximab and rt-PA in Acute Ischemic Stroke Treatment. Presented at the Ongoing Clinical Trials session, 28th International Stroke Conference, Phoenix, Arizona, February 2003. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=481.
72 The Clear Stroke Trial Investigators, Pancioli AM, Brodericl JP: Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke: The CLEAR Stroke Trial. Presented at the Ongoing Clinical Trials session, 29th International Stroke Conference. San Diego, California, 2004. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=478.
73 Ireland JK, Uchino K, Andrei AV, Ford SR, Shaw SG, Matherne DE, Grotta JC: TPA Argatroban Stroke Study (TARTS). Presented at the Ongoing Clinical Trials session, 29th International Stroke Conference. San Diego, California, 2004. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=566.
74 Lapchak PA, Chapman DF, Zivin JA: Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. Stroke 2000;31:3034–3040.
75 Overgaard K, Sereghy T, Pedersen H, Boysen G: Neuroprotection with NBQX and thrombolysis with rt-PA in rat embolic stroke model. Neurol Res 1993;15:344–349.
76 Sereghy T, Overgaard K, Boysen G: Neuroprotection by excitatory aminoacid antagonist augments the benefit of thrombolysis in embolic stroke in rats. Stroke 1993;24:1702–1708.
77 Meden P, Overgaard K, Pedersen H, Boysen G: Effect of early treatment with tirilazad (U7400F) combined with delayed thrombolytic therapy in rat embolic stroke. Cerebrovasc Dis 1996;6:141–148.
78 Andersen M, Overgaard K, Meden P, Boysen G: Effects of citicoline combined with thrombolytic therapy in a rat embolic stroke model. Stroke 1999;30:1464–1471.
79 Zivin J, Mazzarella V: Tissue plasminogen activator plus glutamate antagonists improves outcome after embolic stroke. Arch Neurol 1991;48:1235–1238.
80 Zhang RL, Zhang ZG, Chopp M: Increased therapeutic efficacy with rt-PA and anti-CD18 antibody treatment of stroke in the rtA. Neurology 1999;52:273–279.
81 Grotta J, for the Combination Therapy Stroke Trial Investigators: Combination therapy stroke trial: recombinant tissue plasminogen activator with/without lubeluzole. Cerebrovasc Dis 2001;12:258–263.
82 Lyden P, Jacoby M, Schim J, Albers G, Mazzeo P, Ashwood T, Nordlund A, Odergren T: The Clomethiazole Acute Stroke Study in tissue-type plasminogen activator-treated stroke (CLASS-T): final results. Neurology 2001;57: 1199–1205.
83 Peterson C, Prince HC, Williams B, Tempel D, Grotta JC: ARTIST+: AMPA Receptor Antagonist Treatment in Ischemic Stroke Trial, YM872 + Alteplase. Presented at the Ongoing Clinical Trials Session, 27th International Stroke Conference. San Antonio, Texas, February 2002. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=253.
84 Gutierrez M, Alonso de Leciñana M, Roda JM, Carceller F, Diez Tejedor E: Effect of citicoline, iv thrombolysis (rtPA) and its combination in an experimental model of embolic ischemic stroke in rat. Neurología 2003;18:534.
85 Díez Tejedor E, Gutierrez M, Carceller F, Roda JM, Alonso de Leciñana M: Treatment with reperfusion and neuroprotection with low and high dose of citicoline in an experimental model focal cerebral ischemia. Which is the best? Cerebrovasc Dis 2004;17(suppl 5):67.
86 Onal MZ, Tatlisumak T, Sandage BW, Fisher M: Synergistic effects of citicoline and MK 801 in temporary experimental focal ischemia in rats. Stroke 1997;28:1065.
87 Uematsu D, Araki N, Greenberg JH, Sladky J, Reivich M: Combined therapy with MK-801 and nimodipine for protection of ischemic brain damage. Neurology 1991;41:88–94.
88 Schmid-Elsaesser R, Zausinger S, Hungerhuber E, Baethmann A, Releu HJ: Neuroprotective effects of combination therapy with tirilazad and magnesium in rats subjected to reversible focal cerebral ischemia. Neurosurgery 1999;44:163–171.
89 Schabitz WR, Li F, Irie K, Sandage BW, Locke KW, Fisher M: Synergistic effects of a combination of low dose bFGF and citicoline after temporary experimental focal ischemia. Stroke 1999;30:427–432.
90 Schmid-Elsaesser R, Zausinger S, Hungerhuber E, Baethmann A, Releu HJ: Combination drug therapy and mild hypothermia: a promising treatment strategy for reversible focal cerebral ischemia. Stroke 1999;30:1891–1899.
91 Linnik MD, Zobrist RH, Hatfield MD: Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. Stroke 1993;24:2004–2008.
92 Ma J, Qiu J, Hirt L, Dalkara T, Moskowitz MA: Synergistic protective effect of caspase inhibitors and bFGF against brain injury induced by transient focal ischemia. Br J Pharmacol 2001;133:345–350.
93 Du C, Hu R, Csernansky CA, Liu XZ, Hsu CY, Choi DW: Additive neuroprotective effects of dextrorfan and cycloheximide in rats subjected to transient focal cerebral ischemia. Brain Res 1996;718:233–236.
94 Lee RG, van Donkelaar P: Mechanisms underlying functional recovery following stroke. Can J Neurol Sci 1995;22:257–263.
95 Li Y, Chen J, Chopp M: Cell proliferation and differentiation from ependymal, subependymal and choroid plexus in response to stroke in rats. J Neurol Sci 2002;193:137–146.
96 Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M: Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 2001; 32:1005–1011.
97 Li Y, Chen J, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M: Human marrow stromal cell therapy for stroke in rat. Neurotrophins and functional recovery. Neurology 2002;59: 514–523.
98 Li Y, Chen J, Wang L, Lu M, Chopp M: Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology 2001;56:1666–1672.
99 Modo M, Stroemer RP, Tang E, Patel S, Hodges H: Effects of implantation site of stem cell grafts on behavioral recovery from stroke damage. Stroke 2002;33:2270–2278.
100 Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitman MA, Bynum L: Transplantation of cultured human neuronal cells for patients with stroke. Neurology 2000;55:565–569.