Improving Outcome after Stroke: Overcoming the Translational Roadblock

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Abstract

Stroke poses a massive burden of disease, yet we have few effective therapies. The paucity of therapeutic options stands contrary to intensive research efforts. The failure of these past investments demands a thorough re-examination of the pathophysiology of ischaemic brain injury. Several critical areas hold the key to overcoming the translational roadblock: (1) vascular occlusion: current recanalization strategies have limited effectiveness and may have serious side effects; (2) complexity of stroke pathobiology: therapy must acknowledge the 'Janus-faced' nature of many stroke targets and must identify endogenous neuroprotective and repair mechanisms; (3) inflammation and brain-immune-system interaction: inflammation contributes to lesion expansion, but is also instrumental in lesion containment and repair; stroke outcome is modulated by the interaction of the injured brain with the immune system; (4) regeneration: the potential of the brain for reorganization, plasticity and repair after injury is much greater than previously thought; (5) confounding factors, long-term outcome and predictive modelling. These 5 areas are linked on all levels and therefore need to be tackled by an integrative approach and innovative therapeutic strategies.

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Is There a Future for Neuroprotection after Stroke?

Stroke poses a massive clinical, social and economic burden, yet we have very limited effective therapies. This inadequacy exists in spite of intensive research efforts and numerous failed clinical trials [1]. The latest of these, SAINT-II (Stroke Acute Ischaemic NXY Treatment, testing the free radical spin trap agent NXY-059)
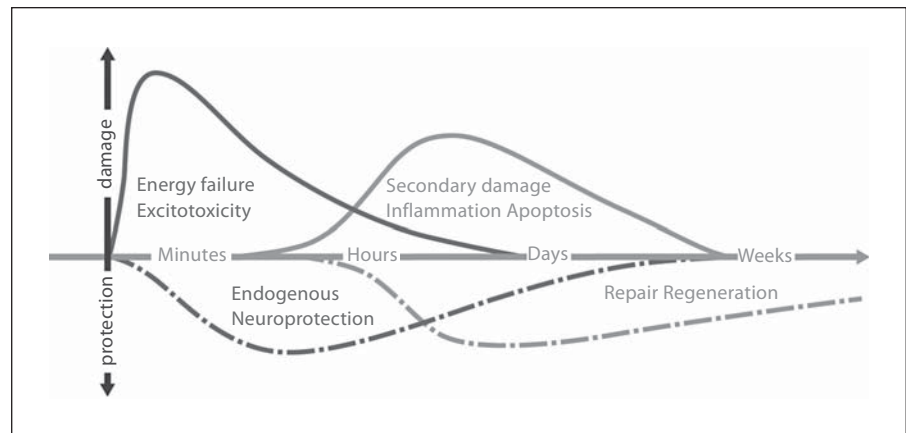
Fig. 1. Simplified pathobiology of stroke-induced damage, endogenous repair and regeneration.

and DIAS-2 (Desmoteplase in Acute Ischaemic Stroke, testing the vampire bat plasminogen activator desmoteplase), showed no efficacy even though there were extensive and convincing preclinical as well as phase IIb or early phase III data available [2–7]. Therefore, in our opinion, the apparent failure of bench-to-bedside translation demands a reappraisal of the pathophysiology of ischaemic brain injury and subsequent repair processes. In this article, we propose potential new approaches to what remains a common and devastating disorder.

Once a brain vessel has become occluded, a complex series of cellular and molecular events is rapidly set in motion. Cells depolarize and swell, excitatory amino acids and K^+ ions are released while intracellular Ca^{2+} levels soar ('excitotoxicity', fig. 1). It is this acute phase of focal cerebral ischaemia which was at the focus of numerous failed neuroprotection trials of the recent decades [8–10]. However, after a rather dramatic phase of early damage the lesion may indeed continue to grow many hours and even days after the onset of ischaemia. Targeting the underlying mechanisms may widen the time window for treatment. We have learnt that the brain mounts a potent, but only partially successful, defensive response against many of the deleterious secondary mechanisms (fig. 1). 'Learning from nature' by inducing such mechanisms or treating with the effector molecules may result in more effective treatment with fewer unwanted side effects. In addition, only recently have we learnt that the brain, albeit with incomplete success, attempts to repair itself [11, 12].

The pathomechanisms of cerebral ischaemia and their interactions are exceedingly complex, and many act as a 'double-edged sword', with both beneficial and deleterious actions (fig. 1): the dual effects of reperfusion were mentioned above. Glutamate is a major player in excitotoxicity [13]; however, it is essential for normal brain function and a key driving force of reorganization and synaptogenesis after injury [14]. Nitric oxide (NO) derived from endothelia may increase blood flow while neuronal and inducible NO synthase (NOS) may contribute to the formation of peroxynitrite and hydroxyl anions [15, 16]. Inflammation exacerbates ischaemic injury, but also provides the necessary environment for regeneration and repair [17]. Formation of a glial scar may contain the lesion and impede its progression, but at the same time produces a barrier for axonal sprouting [18]. Apoptosis contributes to lesion growth, but also inhibits inflammation [19]. Stroke induces immunodepression but at the price of an increased susceptibility to infection [20]. This list of 'Janus-faced' mechanisms could be further extended. Unfortunately, there appears to be no simple good-versus-bad dichotomy: time, cellular context and stimulus intensity are important in determining whether the same molecule, signalling pathway or cell will partake in destruction or repair.

Improving the Safety of Reperfusion

Thrombolysis is the only means of reversing vessel obstruction and inducing reperfusion. Yet, thrombolysis carries risks, particularly of intracerebral haemorrhage [21]. At present, recombinant tissue plasminogen activator (rtPA) is the only approved agent. A promising approach is ultrasound-mediated thrombolysis (sonothrombolysis) alone or in combination with rtPA and sonothrombolysis using targeted abciximab immuno-bubbles [22].
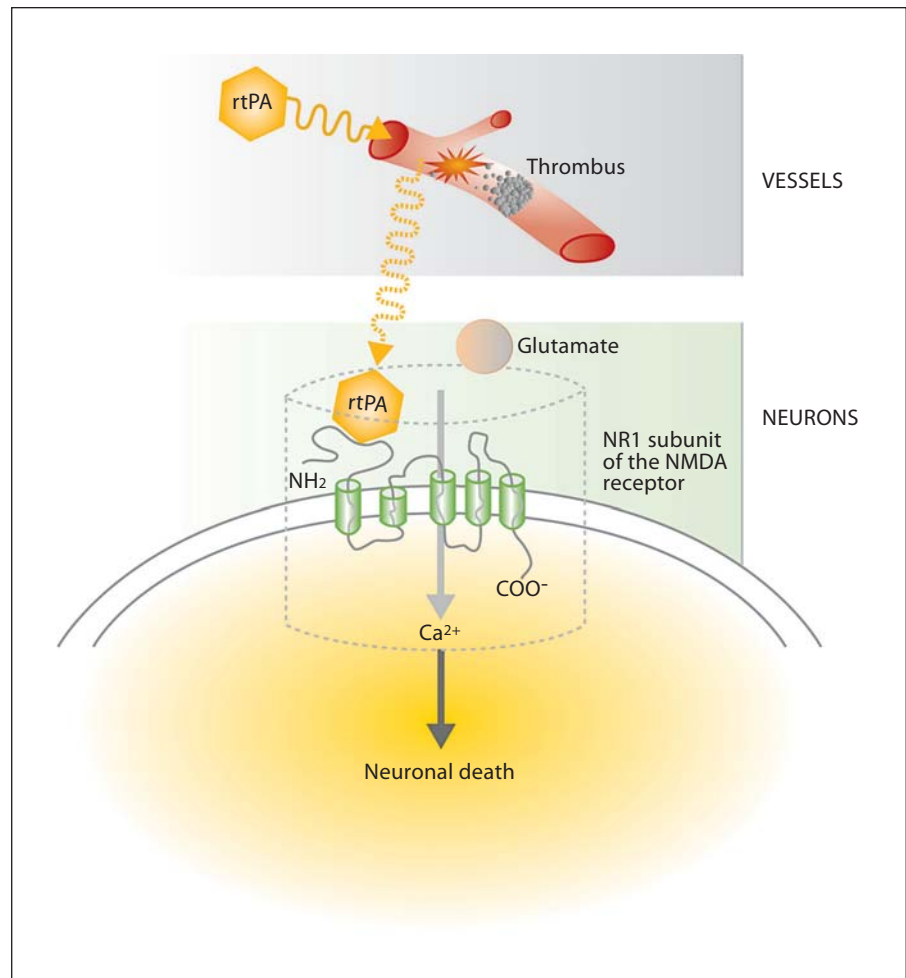
Fig. 2. Beneficial thrombolytic and deleterious neurotoxic effects of tPA.

Paradoxically, the benefit of intravenous tPA may be limited by neurotoxicity [23–25]. Excitotoxic effects of rtPA occur through the binding to the cell surface receptor low-density-lipoprotein-receptor-related protein [26] or annexin II, or by cleaving the NR1 subunit of the N-methyl-D-aspartate receptor which leads to increased calcium influx and subsequent neuronal death [27, 28]. Hence, tPA is a molecule with two faces, one which is clearly beneficial based on its thrombolytic activity and a second which could be deleterious based on its neurotoxic effect (fig. 2).

Reperfusion: Challenges and Opportunities
– Research is needed to increase the efficacy and applicability of thrombolysis, for example by extending the time window, use of new thrombolytic compounds, sonothrombolysis and combination treatment.

– Studies should be undertaken to explore alternative strategies avoiding possible negative actions of rtPA in the brain. The idea is to prevent the deleterious neurotoxic effects of tPA without affecting its benefical thrombolytic activity.
– Combination treatment should be explored combining thrombolysis and neuroprotective, anti-inflammatory agents, or brain cooling.

The Neurovascular Unit in Stroke: Trigger and Target

An important site of inflammation after stroke and thus target for therapy is the neurovascular unit, which consists of a complex cellular system including circulating blood elements, endothelial cells, pericytes, perivascular antigen-presenting cells, astrocytic end-feet and
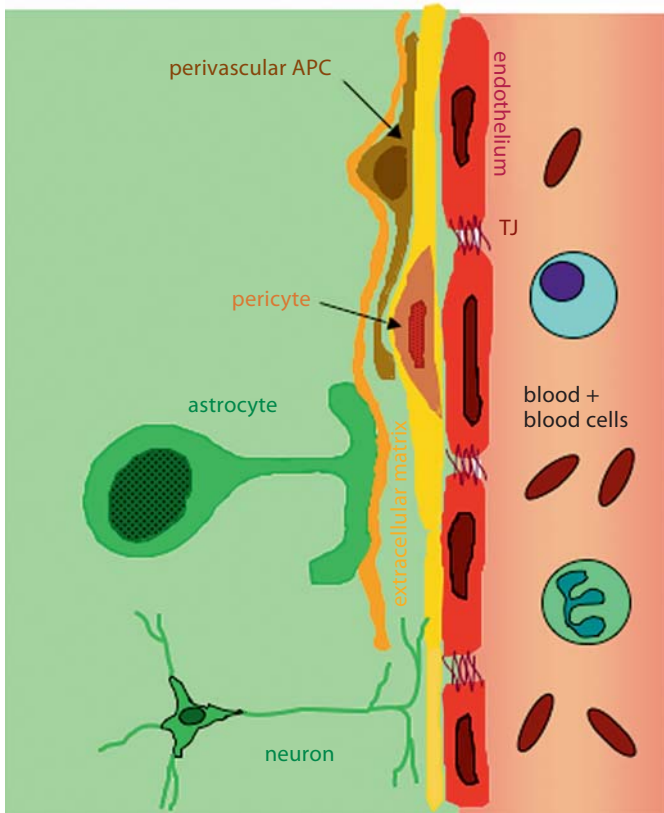
Fig. 3. The neurovascular unit. BBB endothelial cells (red) maintain homeostasis in the CNS (green) by forming a barrier against the changing milieu of the bloodstream. TJ = Tight junctions; APC = antigen-presenting cell.

neurones (fig. 3) [29]. While the endothelial cells form the blood-brain barrier (BBB) proper, the interaction with the adjacent cells and the extracellular matrix is a prerequisite for barrier function.

Maintenance of the BBB relies on a continuous, yet poorly characterized cross-talk between the cells of the neurovascular unit. Polarized astrocytes covering central nervous system (CNS) microvessels by their end-feet contribute to the proper BBB function. Compromising their normal distribution on the astrocyte surface will lead to oedema, a hallmark of ischaemic brain damage (fig. 3). Cellular infiltration and oedema formation during auto-immune CNS inflammation lead to cleavage of extracellular matrix proteins like α-dystroglycan, a transmembrane receptor in the astroglial end-foot [30], suggesting that polarization of astrocytes within the neurovascular unit is central to maintenance of the BBB (fig. 3).

It is well established that recruitment of circulating immune cells across the BBB after stroke causes BBB breakdown and reperfusion injury. In order to understand which leucocyte subpopulations confer beneficial rather than detrimental effects, it is mandatory to understand first the multi-step recruitment cascade of leucocyte subpopulations across the BBB [31]. To date, these processes have been investigated mostly during auto-immune CNS inflammation [32]. In this context, recruitment of pathogenic T lymphocytes across the BBB is unique, relying mainly on α_4-integrins [33] and occurs through the endothelial cells of the BBB. In contrast to preclinical evidence, stroke trials inhibiting leucocyte recruitment across the BBB by blocking ICAM-1/LFA-1 interaction have been disappointing, supporting the notion of insufficient knowledge on the sequence of events involved in leucocyte recruitment across the BBB during and after stroke. After ischaemia, the integrity of the neurovascular unit is compromised by mechanisms that include cellular interactions with the activated endothelium (see below), oxidative stress, up-regulation of proteases such as matrix metalloproteinases and plasminogen activators (including tPA) leading to matrix degeneration and leakage of the BBB. The neurovascular unit is a logical target for stroke therapy; at the same time it provides important mechanistic links between tPA, matrix metalloproteinases, inflammation, oedema formation and haemorrhage after stroke.

The Neurovascular Unit: Challenges and Opportunities
– Understanding the molecular and cellular mechanisms of BBB maintenance is a prerequisite to understand the BBB response to focal cerebral ischaemia.
– Elucidating the sequence of molecular traffic signals involved in the multi-step recruitment of different leucocyte populations across the BBB during stoke is essential in order to target the pathogenic leucocytes while maintaining the recruitment of repair cells.

Stroke and Inflammation: Beyond a Simple 'Good-versus-Bad' Dichotomy

Inflammation is the basic mechanism by which tissues of multicellular organisms respond to injury. The inflammatory response in stroke involves not only leucocytes, endothelial and glial cells but also neurones. At the molecular level, inflammation involves a complex cascade of mediators. On the basis of current knowledge we propose the following mediators or processes to qualify as key components of the inflammatory reaction (fig. 4).
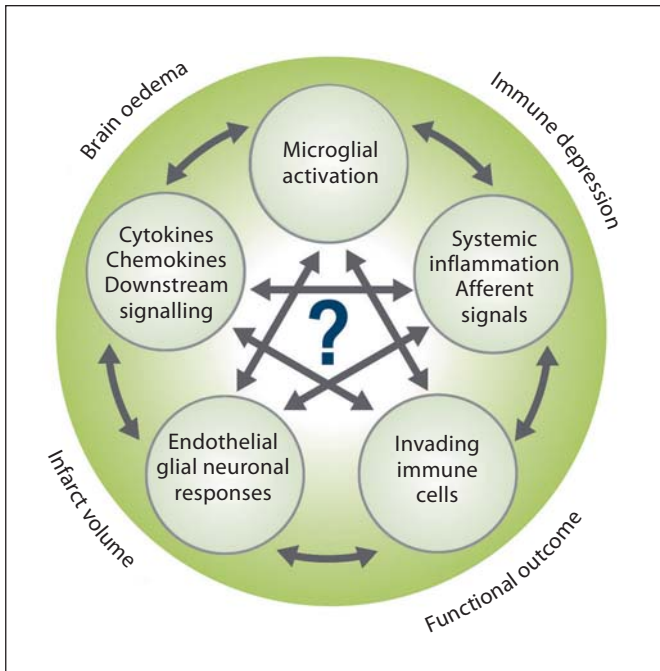
Fig. 4. Five key players of the inflammatory response in stroke have been identified, but their interrelation is unclear. Do they act in series or in parallel? Is there a target with outstanding efficacy? Do they modulate brain oedema, functional outcome, infarct volume and immune depression in a differential manner?

(1) Activated microglia/macrophages are characterized by their ability to migrate, differentiate, phagocytose and secrete a wide variety of molecules involved in inflammation [34, 35]. In fact, microglia/blood-derived monocytic cells play a key role in the development of ischaemic lesions [36].

(2) Postischaemic inflammation unfolds and exerts its influences through a complex network of cytokines. Among other cytokines IL-1, IL-6, TNF and TWEAK have been implicated in stroke [37, 38]. IL-1 appears to mediate injury at least during the acute phase, and the endogenous IL-1 receptor antagonist is potently neuroprotective in diverse animal models [37]. A recent phase II trial of IL-1 receptor antagonist versus placebo in acute stroke showed marked reductions in systemic inflammation (which is associated with poor outcome), no adverse effects and an indication of potential benefit [38].

(3) NO produced by different NOS isoforms plays an important role following stroke. Brain ischaemia activates constitutively expressed neuronal NOS along with

inducible NOS in leucocytes and macrophages leading to concentrations of NO that are toxic and contribute to secondary late-phase damage partly via formation of peroxynitrite, induction of DNA damage and activation of poly(ADP-ribose) polymerase [15]. In contrast, NO generated by endothelial NOS is crucial for maintaining cerebral blood flow and reducing infarct volume [16].

(4) Neuroinflammation in stroke causes BBB breakdown and neuronal damage, but at the same time confers neuroprotection. In order to manipulate this process, it is mandatory to understand the molecular events involved in BBB breakdown and the multi-step recruitment cascade of leucocyte subpopulations across the highly specialized endothelium of the BBB (see above).

(5) Inflammation in cerebral ischaemia relies on the regulation of gene expression. Signal transduction pathways include oestrogen receptor, AP-1 and NF-κB [39]. Binding to response elements in the promoter region of multiple genes they are master switches of the inflammatory response. Thus, transcription factor signalling may provide defined molecular targets to interfere with diverse mechanisms of ischaemic brain damage [40].

Inflammation after Stroke: Challenges and Opportunities
– We need to know how different cellular and molecular components interact to contribute to inflammatory responses in the CNS and impact on cerebrovascular disease.
– There is a need for benchmarking of accepted anti-inflammatory strategies in stroke. Is there a therapeutic principle with outstanding efficacy?
– Inflammation interacts with several other processes in stroke pathophysiology. Therefore, it is essential to use a broad definition of efficacy incorporating several clinically relevant parameters beyond infarct volume.

Brain-Immune-System Interactions after Stroke

Stroke affects the normally well-balanced interplay of the two supersystems – the nervous and the immune system [20]. Reciprocally, the course and outcome of stroke are strongly affected by the immune status of the stroke victim at the time of stroke. Brain-immune-system interactions are highly relevant for functional outcome after stroke. Stroke induces immunodepression and increases the susceptibility to infection. However, immunodepression after stroke may also have beneficial effects, for ex-

ample by suppressing immune-mediated responses during injury-induced exposure of CNS-specific antigens [41]. In the normal brain, co-stimulatory molecules are expressed only at low levels but become up-regulated upon brain damage such as stroke. In addition, systemic infection induces the up-regulation of co-stimulatory as well as major histocompatibility complex class I and II molecules in the periphery and the brain, thus sensitizing T and B cells to brain antigens. As noted above, stroke induces the production of pro-inflammatory cytokines. As a result of systemic inflammation, additional cytokines are produced outside and within the brain, which mediate aspects of sickness behaviour and lead to an exaggerated pro-inflammatory phenotype. Taken together, interactions between the brain and adaptive as well as innate immunity are highly relevant for tissue damage, regeneration as well as systemic infection after stroke, and present a target for protecting the brain, fostering its regeneration and preventing systemic complications (fig. 5).

Infection and Immunity in Stroke: Challenges and Opportunities
– Infections are the most important complications after stroke. Understanding the susceptibility of stroke patients to infections may result in their prevention and improved outcomes.
– Systemic inflammation and infections modulate the susceptibility to stroke and alter its pathobiology. Understanding the consequences of systemic inflammation for stroke will lead to novel strategies for the prevention and treatment of stroke.

Inducing Endogenous Neuroprotection – Learning from Nature?

Tissue damage and functional impairment after cerebral ischaemia are the result of the interaction of endogenous protective mechanisms and those events that ultimately lead to cell death (fig. 1). Recently, endogenous protective mechanisms have entered the centre stage in stroke research, and 'ischaemic preconditioning' (or 'ischaemic tolerance') is widely used to study such endogenous neuroprotection [42]. Practically any stimulus capable of causing damage, when applied close to the threshold of damage, but below it, can protect the brain against subsequent ischaemia. Recent studies found evidence for ischaemic preconditioning/tolerance in the human brain [43, 44], which can guide investigators to targets for acute therapy.
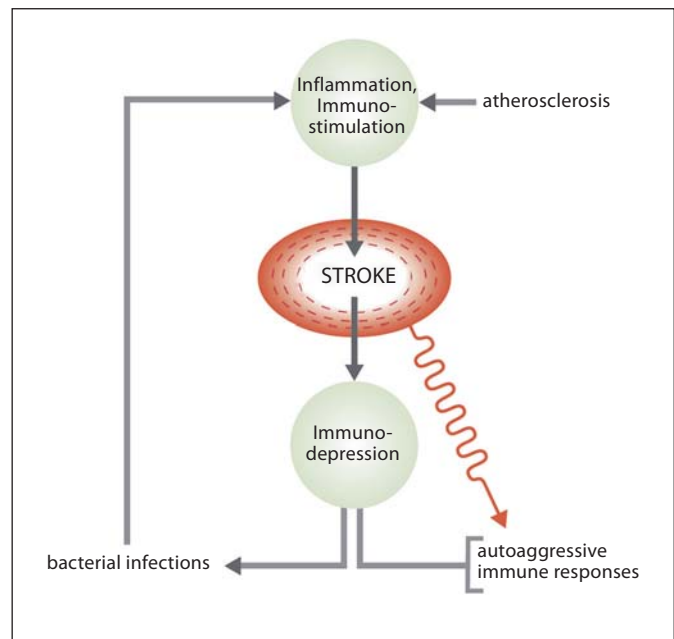
Fig. 5. Mechanisms of stroke-induced immunosuppression.

Several promising endogenous brain protectants have been identified so far, among them erythropoietin, IL-1 receptor antagonist and granulocyte colony-stimulating factor, all of which are in clinical testing at present. In addition, there is evidence that many of the endogenous brain protectants also have regenerative activity, for example by inducing neuro-, angio- and arteriogenesis (see below).

Endogenous Neuroprotection in Stroke: Challenges and Opportunities
– We propose that research on endogenous brain protection can guide investigators to targets for acute therapy against the consequences of brain ischaemia.
– Future research needs to identify brain-specific variants of these molecules, and elucidate the signalling pathways.
– Clinical trials need to test the most promising candidates. Trials in high-risk collectives (e.g. heart or brain surgery) should investigate the possibility to induce ischaemia tolerance (e.g. by inducing hypoxia-inducing factor 1) to preventively protect the brain against cerebrovascular complications.
– The mechanisms of mild hypothermic neuroprotection (in combination with the above) need to be elucidated.

Regeneration and Repair: The Next Frontier

Neurogenesis: Epiphenomenon or Causative for Regeneration?

In the adult brain, neural stem/progenitor cells are localized along the lateral ventricle in the subventricular zone. Stroke triggers increased cell proliferation in the subventricular zone in rodents [45]. Newly formed neuroblasts migrate into the damaged striatum [46], where they mature and express markers characteristic of striatal medium spiny neurones [47, 48]. It is now important to establish whether neurogenesis can contribute to functional recovery after stroke, a causal relationship has yet to be established. It is also important to determine if neurogenesis from endogenous stem/progenitor cells occurs after stroke in humans [49]. Postischaemic inflammation (see above) may be detrimental for the survival of the newly born neurones [50, 51], but the interplay between neurogenesis and inflammation in CNS pathologies remains controversial [52]. Clarification of the role of inflammatory processes for the different steps of stroke-induced neurogenesis might explain the lack of efficacy of this potential self-repair mechanism and lead to the identification of novel therapeutic targets.

Promoting Regeneration via the Formation of New Vessels?

Brain ischaemia promotes the formation of new vessels with altered morphology and microvascular structure [53]. Stem and progenitor cells from the bone marrow, in particular so-called endothelial progenitor cells, may promote vascular repair, neovascularization and improve endothelial function [54, 55]. Only recently has it become apparent that poststroke angiogenesis can indeed promote regeneration [56, 57]. How the formation of new vessels improves recovery even at time points when neuronal and axonal cell death is already completed remains to be elucidated.

Plasticity and Synaptogenesis

It is well documented that housing rodents in an enriched environment even after damage has fully matured can enhance plasticity and improve functional recovery by stimulating axonal outgrowth, and that constraint-induced movement therapy stimulates recovery of motor functions after stroke in humans. The recovery process following ischaemia is a complex and highly dynamic process [58]. In a later phase dendritic arborization, axonal growth and cell genesis enhance formation of new neural networks. Details of how these processes develop over time and how they are regulated are largely unknown and open new venues for the pharmacology of stroke. Only recently has a number of pharmacological strategies been established in preclinical models by which poststroke re-organization of the adult brain can be enhanced, e.g. by inactivation of the Nogo-A/NgR signalling system [59], the tophic factors erythropoietin and granulocyte colony-stimulating factor [60, 61], or potentially activators of the basal forebrain cholinergic system, such as cholinesterase inhibitors [62].

Neurorepair after Stroke: Challenges and Opportunities

- Long-term studies and complex end points including a number of functional tests along with hallmarks of regeneration may be better suited to determine stroke outcome.
- Research is needed to demonstrate whether neurogenesis causally contributes to functional recovery. Do newly generated neurones make synaptic contacts? Does ablation of newly generated neurones have an impact on outcome?
- Does new vessel formation contribute to improved long-term outcome? Is this via enhanced neurogenesis?
- We propose to explore in more detail the role of inflammation in neurogenesis, angiogenesis and repair after stroke.
- We propose to explore the factors regulating axonal outgrowth and spine remodelling, in order to develop novel therapeutic strategies that stimulate functional recovery.
- We propose to explore factors that are involved in the enhancing recovery of function by excercise and enriched environment.

Modelling of Stroke: Crossing the 'Valley of Death'

More than 150 clinical trials (for an overview, see www.strokecenter.org) have aimed at establishing an effective brain-protective therapy after stroke, but with very few exceptions most have failed. So how do we explain the failure of bench-to-bedside translation in stroke, and how can we improve on it? Two key concepts which successfully found their way into clinical practice are: the concept of the ischaemic penumbra [63–65] and thrombolysis in acute ischaemic stroke were both developed in animal models of cerebral ischaemia [66, 67]. The Stroke Therapy Academic Industry Roundtable

(STAIR) [1] has published 'recommendations for standards regarding preclinical neuroprotective and restorative drug development'. With the failure of SAINT-II, which fulfilled many of these recommendations, including testing in subhuman primates, are the STAIR recommendations a failure? A critical analysis of the preclinical NXY-059 data, however, and of a number of shortcomings argues that SAINT-II does not refute the utility of the STAIR criteria [3, 4]. A number of recent systematic reviews of experimental stroke research [68–70] have clearly demonstrated that practically all treatments that were tested clinically were not evaluated preclinically in comparable populations. Also, the vast majority of clinical trials used treatment windows to the very edge of efficacy in rodent models, i.e. 5–6 h after stroke onset. In contrast, almost all of the few studies that have compared the effect of atherosclerosis, diabetes, age or gender have found that these confounders drastically affect not only the pathobiology of stroke, but also the potential benefit of treatment. In addition, O'Collins et al. [10] have recently systematically evaluated experimental studies on 1,026 putative neuroprotectants and concluded that the drugs taken forward to clinical trial have not been distinguished by superior efficacy. It is therefore clearly premature to pronounce the end of the neuroprotection era or to denounce the relevance of preclinical testing in models faithful to human pathology. There is an urgent need for academic investigators and the pharmaceutical industry to detect promising treatments and the possibility of failure at the earliest stage as possible. We propose that an academic, public and industry multi-disciplinary collaboration focuses on the following measures.

Modelling of Stroke: Challenges and Opportunities
– Use of clinically relevant end-points (functional outcome, behaviour) at clinically relevant end-points (weeks or months instead of hours or days after stroke) in preclinical research.
– Modelling of confounding diseases, in particular hypertension, arteriosclerosis, diabetes.
– Modelling of the most important risk factor for stroke: age.
– Investigating gender-specific effects.
– Development of models for lacunar stroke and white-matter injury.
– Developing preclinical surrogate markers of effectiveness using surrogate markers already established in humans (bed-to-bench translation).

– Development and validation of brain imaging approaches (structural, functional, molecular) applicable preclinically as well as clinically to demonstrate CNS effects and predict effectiveness.
– Observation of quality standards, establishment of a trial register and funding of replication studies in preclinical stroke research.

Clinical Stroke Trial Design

Clearly, the apparent failure of neuroprotection in human stroke cannot be blamed on deficiencies of experimental research alone. While this review was focused on the problems and future challenges of translational stroke research, a few comments on clinical stroke trials appear warranted.

Clinical trials continue to reflect the hope that one or more single variables could reflect the disease process and show the benefits of therapy. Examples include the early complex clinical scales like the Toronto, Mathew, NIH (modified or not), Canadian, Frithz-Werner, Orgogozo and Scandinavian. Recently the Rankin scale (also modified or not) has become more popular, seems to many simpler than the Barthel index and is more readily applied by a wide range of investigators. Of interest, the Rankin scale has proved more useful interventionally than in drug trials for ischaemic stroke. The most recent disappointment has been with NXY-059 [3]. Hopes that imaging methods might provide useful surrogates have also been blunted by the just-completed phase III desmoteplase study DIAS-2 [7].

That stroke is not a unitary disease state is obvious to all, but to date there has been a great reluctance to follow the infectious disease model. Here, symptoms and signs can reflect a wide variety of different, identifiable causes, many having unique treatment sensitivities, each of which must be discovered and pursued for the gratifying therapeutic outcome. As proposed in the current review, why not apply such an approach to stroke?

Admittedly, sponsors of clinical trials find it tiresome to spend huge resources with the likelihood their agent will be approved for but a narrow subset of cases. They understandably keep hoping for a global favourable result, only to have the subset of responders lost, buried or arguably not discoverable in the broad array of cases tested in a trial. Reeling from successive failures, the combatants may be becoming exhausted and could abandon the field entirely. Many have already. If too many do so, we may have a long road ahead in shaping new test methods

to demonstrate the utility of treatments already on hand which have yet to have made the translational step from animal model to human.

Conclusion

The past two decades have witnessed an enormous expansion in our understanding of events that determine the fate of brain cells following vessel occlusion. Cell death following brain ischaemia is mediated by a complex interplay of a number of pathophysiologically distinct mechanisms. Both, blood vessels and parenchyma have been implicated in this interplay, and their complex interactions define the fate of compromised tissues and cells. Improvement of reperfusion, inhibition of secondary damage as well as induction of endogenous protection and repair deserve increased efforts to overcome the 'translational roadblock' that appears to exist in the stroke field. Importantly, new strategies will have to widen the time window for therapeutic interventions and may lead to the regeneration of lost function after stroke. Focussing on a limited number of key mechanisms, we will be able to establish an orderly sequence of events, to investigate the causal relation of individual mechanisms and to identify novel and promising therapeutic targets. To achieve this, however, we will need to observe the complexity and heterogeneity of stroke pathobiology and the important impact of age and co-morbidity. Certainly, there will be no single agent or strategy, no 'one size fits all' approach for effective stroke treatment. Given the close link and interrelation of the different damage mechanisms, it is essential not (only) to use infarct size to assess outcome but to consider also other clinically relevant parameters in the preclinical phase such as peripheral immune state, brain water content and long-term functional outcome. Moreover, biomarkers (e.g. measured in blood) and novel imaging approaches may serve as surrogate parameters and help to identify on an individual basis those patients suited for a particular therapy. Hence, despite the complexity and drawbacks, there is reason for optimism for a better translation of discoveries from bench to bedside.

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