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Review

Drug development for stroke: importance of protecting cerebral white matter

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Abstract

Multiple pharmacological mechanisms have been identified over the last decade which can protect grey matter from ischaemic damage in experimental models. A large number of drugs targeted at neurotransmitter receptors and related mechanisms involved in ischaemic damage have advanced to clinical trials in stroke and head injury based on their proven ability to reduce grey matter damage in animal models. The outcome to date of the clinical trials of neuroprotective drugs has been disappointing. Although the failure to translate preclinical pharmacological insight into therapy is multifactorial, we propose that the failure to ameliorate ischaemic damage to white matter has been a major factor. The recent development of quantitative techniques to assess ischaemic damage to cellular elements in white matter, both axons and oligodendrocytes, allows rigorous evaluation of pharmacologic mechanisms which may protect white matter in ischaemia. Such pharmacological approaches provide therapeutic opportunities which are both additional or alternatives to those currently being evaluated in man.

Keywords: Cerebral ischemia; Axon; Oligodendrocyte; Stroke

1. Introduction

Cerebrovascular disease ranks third, after cancer and heart disease, as the cause of death in Western Europe and North America. Moreover, mortality over the first year after first stroke is approximately 20% and a large proportion of surviving patients are left disabled and dependent, with attendant social, personal and economic costs. Ischaemic damage to the central nervous system (CNS) is also a feature of a number of other conditions, notably head trauma; in more than 85% of fatal cases of head injury, ischaemic damage is prominent at postmortem examination (Graham et al., 1989). In the USA, 2,000,000 patients attend hospital each year after head injury with 75,000 deaths and an equal number left permanently disabled while in the UK, one in 300 families has a member permanently disabled by head injury. In view of the magnitude of the clinical problem, amelioration of ischaemic damage in stroke and head injury has become a major pharmacological goal (see McCulloch et al., 1991).

A decade ago, there was little compelling evidence that pharmacological intervention could radically alter outcome after cerebral ischaemia even in experimental animals. By 1996, the pace of advance was such that a large number of drugs targeted at neurotransmitter receptors, and related mechanisms involved in ischaemic damage, had advanced to clinical trials in stroke and head injury (Koroshetz and Moskowitz, 1996). The transformation of the pharmacology of cerebral ischaemia had been achieved for two major reasons: firstly, the elucidation of neurochemical cascades initiated by ischaemia which revealed potential targets for intervention (Fig. 1) and secondly, due to the systematic assessment of drug efficacy using robust endpoints (quantitative histopathology) in the most pertinent animal models. Since the elucidation of the excitotoxic cascade (Fig. 1) numerous other pathological mechanisms have been identified via which neuroprotection can be achieved in ischaemia. However, excitotoxicity remains central to current concepts of neuronal cell death and provides the

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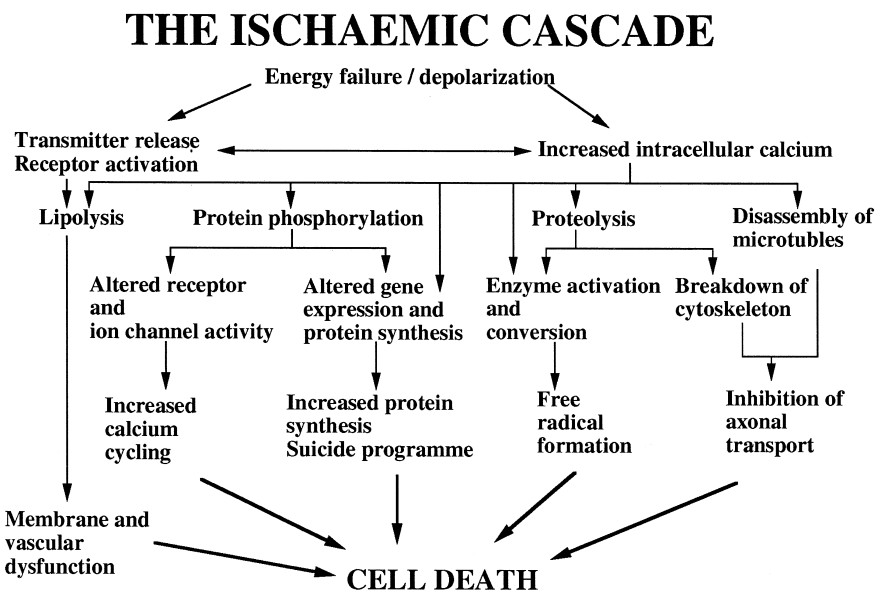

Fig. 1. The neurochemical cascades initiated by a cerebral ischaemic challenge. Elucidation of these cascades has underpinned the development of pharmacological agents as anti-ischaemic drugs and their complexity indicates the multiplicity of potential pharmacological targets. Both in vitro studies using cell culture systems and in vivo investigations using animal models have contributed to this knowledge. However, in the main, such investigations have focused on events induced by ischaemia in neuronal perikarya (in vitro) or grey matter (in vivo). The most clinically advanced of the anti-ischaemic drugs are targeted at glutamate receptor activation. However, this strategy is not appropriate for myelinated fibre tracts which are essentially devoid of such receptors. New classes of anti-ischaemic drugs should be targeted at parts of the ischaemic cascade which are shared by both grey and white matter. For example, cytoskeletal breakdown and altered ion channel activity occur during ischaemia in both grey and white matter and thus inhibition of these parts of the cascade may ameliorate damage in both cerebral grey and white matter. Modified from Siesjö et al. (1992).

prototype for anti-ischaemic drug development for new pharmacological targets.

2. Preclinical methodology

Animal models of focal cerebral ischaemia are generally recognised as the most pertinent in relation to human stroke. The most widely employed models of focal cerebral ischaemia employ a permanent or temporary occlusion of a major cerebral artery, normally the middle cerebral artery. Occlusion of the middle cerebral artery produces pannecrosis of the territory supplied by the artery with the final lesion size being established after approximately 3 h of ischaemia (Jones et al., 1981), although enlargement of the lesion, as distinct from oedema development may occur at later times particularly with mild ischaemia (Du et al., 1996; Touzani et al., 1997). Middle cerebral artery occlusion has been used in large animals such as cats, dogs and primates for 25 years or more and these species have particular advantages with respect to cardiovascular stability under anaesthesia and in possessing, like man, a gyrencephalic brain with extensive white matter. However, due to financial considerations and ease of use, rodent models of middle cerebral artery occlusion (Tamura et al., 1981a; Zea Longa et al., 1989) have become increasingly dominant in anti-ischaemic drug development. Middle cerebral artery occlusion in the rat results in a range of cerebral blood flow from moderate, the ischaemic (penumbra), to severely reduced, the ischaemic core (Tamura et al., 1981b; Tyson et al., 1984; Mies et al., 1991). This pattern of ischaemia results in a sharp boundary between viable and damaged tissue at postmortem examination, and this lends itself to volumetric assessment of the lesion in grey matter (Osborne et al., 1987). While the volume of the ischaemic lesion is generally well defined when putative anti-ischaemic drugs are evaluated, histological definition of the lesion is often rudimentary. At best, mapping the distribution of eosinophilic neuronal perikarya; at worst, simply mapping the area of tissue pallor using triphenyltetrazolium staining. This limited histological definition of ischaemic pathology in many pharmacological investigations has contributed to a neglect of ischaemic white matter pathology where definition of ischaemic damage is difficult. The neglect of white matter pathology has been exacerbated by the excessive use of rodents in which white matter is proportionately less of the total brain volume than in larger gyrencephalic species.

3. Clinical trials

Based on their proven ability to reduce grey matter damage in animal models, a large number of drugs targeted at neurotransmitter receptors and related mechanisms involved in ischaemia damage have advanced to clinical trials in stroke and head injury (McCulloch et al., 1991; McCulloch, 1992; Koroshetz and Moskowitz, 1996). The outcome, to date, of the clinical trials of neuroprotective

drugs has been disappointing. Numerous trials of neuroprotective drugs have not advanced to phase 3 and those which have (aptiganel, selfotel, D-CPPene, lubeluzole, enlimolab, trilazad inter alia) have been suspended before completion of patient recruitment or failed to provide compelling evidence of clinical efficacy (Dyker and Lees, 1998). The notable exception in clinical trials of stroke is the benefit demonstrated with tissue plasminogen activator (TPA; The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). The mechanism of action of TPA is not the direct protection of neurones, and its clinical development was based on the concept of clot dissolution rather than preclinical efficacy in cerebral ischaemia models. The failure to translate insight of ischaemic damage into effective stroke therapy is due to multiple factors. For some agents, efficacy in animal models of ischaemia was not compelling and many of the issues outlined in Table 1 had not been addressed adequately prior to the trial. In some instances, definition of the effective dose for use in clinical trials was complicated by concerns over dose limiting adverse effects, for example, in the case of NMDA receptor antagonists. A number of agents, with at best modest brain entry (SNXIII, bFGF and GV 150526) have advanced to clinical trials but unlike the situation in animal models where artefactual blood–brain barrier breakdown may facilitate entry (Tyson et al., 1982), definition of effective dose has been difficult. There has been concern that the entry window in clinical trials (typically within 6 h of stroke) has been too long in view of the few agents with proven efficacy in focal models at this time point. The successful rt-PA trial in human stroke had an entry window of 3 h (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). In animal models, neuroprotective efficacy is confined to ischaemic penumbra with its special neurochemical characteristics (McCulloch et al., 1991; Mies et al., 1991). The anatomical and temporal extent of the 'penumbra' in man is not as consistent and nor as large as the penumbra in animals even when assessed with similar technology (e.g., diffusion weighted imaging) (Baird and Warach, 1998).

In addition to the issues discussed above, a more fundamental biological issue may be a major reason why the current generation of neuroprotective drugs do not display marked clinical benefits. That is, their failure to protect white matter, specifically axons and oligodendrocytes, from ischaemic damage. Many drugs (e.g., NMDA receptor antagonists) are targeted at receptors which are not present to any extent in axons or oligodendrocytes. The protection of myelinated fibre tracts by drugs has hitherto been largely neglected in preclinical investigation of drug action. However, in man, it is obvious that improved *functional* outcome after drug treatment depends not only on the protection of cortical grey matter but also the simultaneous protection of associated white matter. In other words, preserving neuronal perikarya from ischaemic damage is of little consequence if those same perikarya are non-functional because their axons are not protected from ischaemia. The refocusing of ischaemia research away from selective protection of grey matter towards the protection of all cellular elements in the CNS is timely. Mechanisms of therapeutic potential have been identified in myelinated fibres (vide infra) and more importantly, quantitative techniques have been developed which permit ischaemic insults to oligodendrocytes and axons to be measured and the effect of drugs evaluated.

Table 1
Anti-ischaemic drug development

Define salvageable zone
Establish quantitative outcome measures
Demonstrate improved histological outcome acutely
Demonstrate improved histological outcome chronically
Define window of therapeutic opportunity
Examine models with post-ischaemic reperfusion
Demonstrate improved outcome in gyrencephalic species
Assess functional outcome after drug treatment

4. White matter is vulnerable to ischaemic damage

Both components of the functional unit comprising the axon and its associated oligodendrocytes exhibit structural damage rapidly in response to an anoxic or ischaemic challenge. Structural damage to axons occurs in the form of cytoskeletal breakdown and consequent to this, disruption of fast axonal transport. In the isolated optic nerve model developed by Waxman et al., 60 min of anoxia was sufficient to induce loss of both microtubule and neurofilament components of the axonal cytoskeleton (Waxman et al., 1992). Short periods (2–4 h) of focal cerebral ischaemia in vivo were also associated with cytoskeletal breakdown and disturbances of fast axonal transport in myelinated fibre tracts (Fig. 2). Structural damage to the cytoskeleton was indicated by marked disruption in the patterns of immunostaining for a variety of microtubule proteins, including tau and microtubule-associated proteins 1 and 5, as well as the neurofilament protein 68 kDa subunit (Dewar and Dawson, 1995, 1996; Yam et al., 1998). The functional consequences of cytoskeletal disruption were indicated by the accumulation at later times of proteins such as SNAP 25 and amyloid precursor protein (APP) in damaged axons which are normally transported by fast axonal transport (Stephenson et al., 1992; Yam et al., 1997; Dietrich et al., 1998; Yam et al., 1998). The time course of the cytoskeletal dysfunction in these studies was consistent with axonal damage per se and not as a consequence of secondary degenerative processes in perikarya.

Acute structural changes in oligodendrocytes also occurred after short periods of focal cerebral ischaemia. Swelling of oligodendroglia occurred within 30 min to 3 h

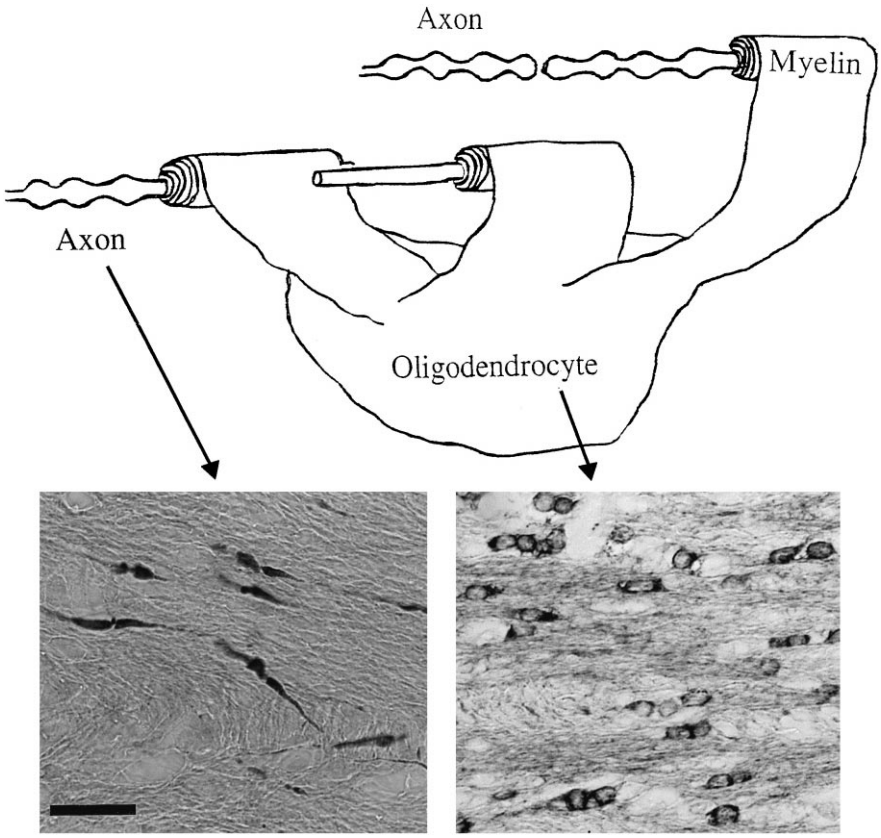

Fig. 2. Axons and oligodendrocytes comprise the functional unit of the myelinated fibre. (Modified from Compston, 1993.) Each oligodendrocyte myelinates an internodal segment of several axons. Oligodendrocytes are highly vulnerable to ischaemia in vivo and exhibit abnormalities of the cytoskeletal protein, tau. The right photograph shows numerous tau-positive oligodendrocytes in the subcortical white matter 1 h after induction of focal cerebral ischaemia in the rat. In non-ischaemic white matter oligodendrocytes are not immunostained with tau antibodies. Quantitation of tau-positive glia in white matter can be used to assess the effects of potential anti-ischaemic drugs on oligodendrocyte pathology (see Fig. 4). The sensitivity of oligodendrocytes to ischaemia and the numeric relationship which exists between one oligodendrocyte and the internodal segments of its associated axons could mean that damage to one oligodendrocyte may affect multiple axons and thus have a marked functional effect within a given myelinated fibre tract. Axons themselves are also vulnerable to ischaemic damage in vivo. The left photograph shows axonal damage detected with APP immunostaining 6 h after middle cerebral artery occlusion in the cat. Damaged axons exhibit a characteristic 'string of sausages' or bulb-like appearance indicative of cytoskeletal breakdown and obstruction of fast axonal transport. Scale bar = 10 μm. Quantitation of APP accumulation in white matter can be used to determine the effects of anti-ischaemic agents on axonal pathology (see Fig. 3).

after induction of ischaemia in the rat (Pantoni et al., 1996) while abnormalities of microtubules were also detected within affected cells (Petito, 1986). Cytoskeletal pathology in ischaemic oligodendrocytes was also indicated by the alterations in the immunostaining of the microtubule-associated protein, tau which we have demonstrated after focal cerebral ischaemia in experimental animals (Dewar and Dawson, 1995; Irving et al., 1997; Dewar et al., 1999). Increased tau immunostaining in oligodendrocytes in subcortical white matter after permanent middle cerebral artery occlusion in rats occurred rapidly after induction of ischaemia (Fig. 2B, Fig. 4) (Irving et al., 1997) and highlights the particular sensitivity of oligodendrocytes to experimental ischaemia in vivo. Moreover, the presence of tau-positive oligodendrocytes in human brain after either stroke or head injury (Irving et al., 1996) demonstrates that this pathological response in oligodendrocytes is not peculiar to animal models of ischaemia and thus validates the utility of the response in assessing the effects of pharmacological intervention strategies on oligodendrocyte ischaemic damage (vide infra). Thus, there is now compelling evidence that both components of the functional unit comprising the axon and its oligodendrocytes are susceptible per se to ischaemic damage and therefore that neuronal perikarya should not constitute the sole target for protection by anti-ischaemic agents.

The challenge of future anti-ischaemic drug development is to identify mechanisms which provide common targets for both grey and white matter. The need for this new strategy is highlighted by the failure of the classic 'neuroprotective' agent, MK-801, to reduce the amount of myelinated fibre tract damage as assessed by accumulation of APP in animals where the agent predictably attenuated the amount of grey matter damage (Fig. 3; Yam et al., 1999). Similarly, the tau pathology in oligodendrocytes after focal cerebral ischaemia in the rat was not attenuated

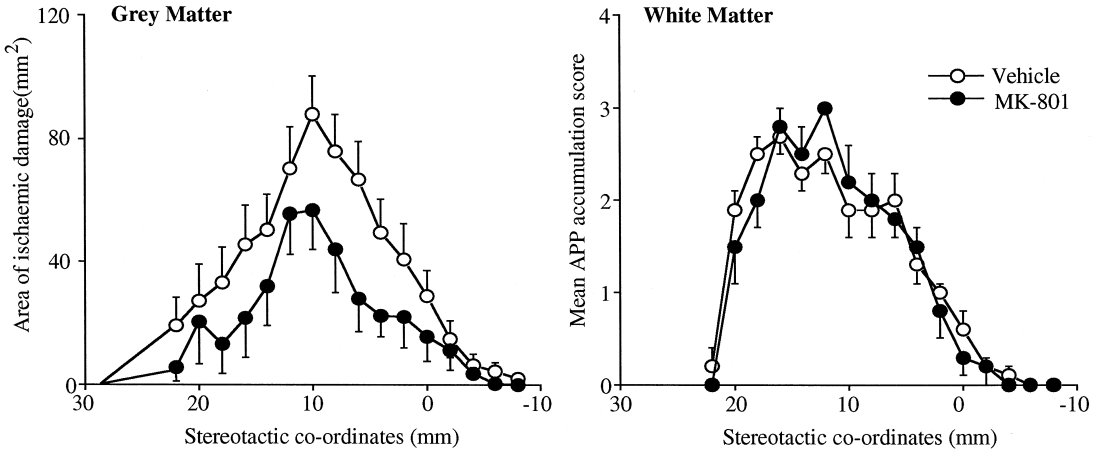

Fig. 3. Effect of the NMDA receptor antagonist, MK-801 on both grey matter and axonal pathology after focal cerebral ischaemia in the cat. Grey matter pathology after middle cerebral artery occlusion was determined using quantitative histopathology as described previously (Ozyurt et al., 1988). MK-801 reduced the amount of ischaemic grey matter damage in the cerebral cortex. White matter pathology was assessed by semi-quantitative determination of the amount of APP immunostaining in damaged axons at the same coronal levels as for grey matter pathology. Despite the reduction of grey matter damage by MK-801 treatment, the drug failed to reduce in the amount of axonal damage (Yam et al., 1999). These data reinforce the view that future pharmacological strategies should be targeted at pathophysiological mechanisms common to both grey and white matter in order to maximise outcome and recovery from stroke.

by MK-801 (Irving et al., 1997) at a dose which has previously been shown to reduce grey matter damage (Park et al., 1988). Thus, neither component of the myelinated fibre unit is protected from ischaemic damage by NMDA receptor blockade, a conclusion not surprising in the light of the relative absence of these receptors in cerebral white matter.

5. Mechanisms of ischaemic damage in axons

The isolated optic nerve model of anoxia has proved useful in elucidating potential mechanisms leading to ischaemic damage in axons (for review, see Stys, 1998). In brief, the initial injury phase after an anoxic period in isolated optic nerves involves a sequence of ion channel-linked events leading to loss of ionic homeostasis within axons, the major disturbances being the accumulation of Na^+ and consequently Ca^{2+} within the axoplasm. Attenuation of the anoxia-induced impairment of electrogenesis was achieved by pharmacological blockade by a wide range of Na^+ channel blockers. Not surprisingly, however, some of these compounds also have profound effects on the optic nerve compound action potential in the normoxic state. Promising candidate compounds tested in this model system which afforded protection from the effects of anoxia but which did not have a major effect on normal electrogenesis included QX-314. However, this is an ionised compound which will not penetrate the blood–brain barrier and such agents are therefore of little utility in vivo (Stys, 1998). The antiarrhythmic, use-dependent Na^+ channel blocker, mixiletine, did appear to gain entry into the optic nerve when administered systemically and afforded some protection from anoxic injury in vitro (Stys and Lesiuk, 1996).

The role of raised levels of intracellular calcium in damage to neuronal perikarya has been extensively described and raised intracellular Ca^{2+} also plays a role in axonal damage after anoxia. Thus, exclusion of Ca^{2+} from the extracellular medium during anoxia in isolated optic nerves ameliorated cytoskeletal destruction and improved functional recovery (Waxman et al., 1993). Moreover, in isolated optic nerves or in segments of spinal cord, both L- and N-type Ca^{2+} channel blockers improved functional recovery after anoxia while they had minimal effect on normoxic conduction (Stys et al., 1990; Fern et al., 1995; Imaizumi et al., 1997). The heterogeneity of Na^+ and Ca^{2+} ion channels and the possibility for state dependent blockade provides considerable opportunities for drug development for white matter ischaemia.

The pathophysiological effects of axoplasmic accumulation of Ca^{2+} include activation of the neutral proteases, calpains, and substrates for these enzymes include a number of axonal cytoskeletal proteins, such as tau, fodrin and neurofilament subunits. Pharmacological blockade of calpain activity has been shown to reduce the amount of grey matter damage after focal cerebral ischaemia in the rat, even when administered systemically up to 6 h after induction of ischaemia (Bartus et al., 1994a,b, 1995; Hong et al., 1994; Markgraf et al., 1998). The brain penetration of such agents used to date remains sub-optimal, however, calpain-mediated cytoskeletal destruction represents a potential target for pharmacological protection of both white and grey matter ischaemic damage.

In contrast to the ion channel-mediated mechanisms proposed above, a receptor-mediated mechanism which

may prove to be an appropriate target for protection of both white and grey matter involves the endogenous release of γ-aminobutyric acid (GABA) and adenosine. Using the in vitro anoxic nerve model, enhancement of extracellular levels of either or both GABA and adenosine improved functional recovery from anoxia, both substances being released from endogenous stores in response to anoxia (Fern et al., 1994). The specific involvement of $GABA_B$ receptors was indicated by the protective effect being reduced in the presence of phaclofen (Fern et al., 1995). Furthermore, the signal transduction system involving protein kinase C which transduces both $GABA_B$ and adenosine receptor activation was also implicated since protection was abolished in the presence of the protein kinase C inhibitor, staurosporine and mimicked by protein kinase C activation by 12-myristate 13-acetate (Fern et al., 1995).

Adenosine has long been recognised to protect neurones in ischaemia (see Rudolphi et al., 1992). GABA receptor agonists have been reported to be neuroprotective in vitro and in vivo but the available evidence suggests that $GABA_A$ receptors are of greater importance in this effect with $GABA_B$ receptors displaying minor effects at best (Sternau et al., 1989; Muir et al., 1996).

6. Mechanisms of ischaemic damage in oligodendrocytes

Quantitation of the density of tau-positive oligodendrocytes in subcortical white matter provides an index of oligodendroglial pathology after focal cerebral ischaemia in the rat. Tau pathology in oligodendrocytes induced by focal cerebral ischaemia in the rat was attenuated by pretreatment with the spin trap agent, PBN, at a dose previously shown to reduce the amount of grey matter damage (Fig. 4). These data implicating oxidative damage as a mediator of ischaemic damage in oligodendrocytes in vivo are consistent with in vitro investigations demonstrating the sensitivity of cultured oligodendrocytes to oxidative stress (Kim and Kim, 1991; Oka et al., 1993; Mitrovic et al., 1994, 1995; Richter-Lansberg and Vollgraf, 1998; Laskiewicz et al., 1999). The vulnerability of cultured oligodendrocytes to oxidative damage has been attributed to features of their phenotype including high metabolic activity, low intracellular concentrations of glutathione and their high iron content. The developmental stage of the cultured oligodendrocytes in vitro appears to influence their vulnerability to oxidative damage: cells with a precursor-like phenotype exhibiting greater vulnerability than of mature oligodendrocytes (Back et al., 1998). However, our data (Fig. 4) indicate that oxidative stress is involved in the expression of tau pathology in mature oligodendrocytes in vivo (Irving et al., 1997). Given the ability of antioxidants to reduce the amount of grey matter damage in focal cerebral ischaemia (Cao and Phillis, 1994; Folbergrova et al., 1995), this non-receptor mediated pharmacological strategy may hold promise for protection of both grey and white matter. Although the role of free radicals in injury to axons has not been explicitly addressed, given the high lipid content of myelinated fibres their involvement could be envisaged.

Oligodendrocytes do express glutamate receptors of the AMPA/kainate and in similarity to axons do not express the NMDA subtype (Patneau et al., 1994; Garcia-Barcina and Matute, 1996; Matute et al., 1997; McDonald et al., 1998). Recent studies have demonstrated that activation of

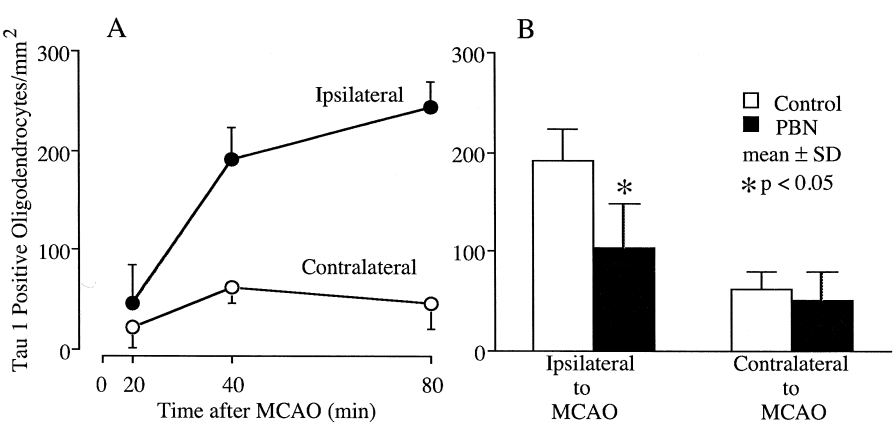

Fig. 4. Quantitation of oligodendrocyte pathology after focal cerebral ischaemia in the rat (redrawn from Irving et al., 1997). Sections of ischaemic tissue were labelled with an antibody against dephosphorylated tau protein, Tau 1, and the density of labelled oligodendrocytes was determined in ischaemic subcortical white matter. (A) The density of Tau 1-positive oligodendrocytes increased rapidly in the white matter ipsilateral to the occluded middle cerebral artery compared to the contralateral side. This method provides an index of ischaemic oligodendrocyte pathology which can be used to assess the effects of anti-ischaemic drugs. (B) The number of ischaemia-induced Tau 1-positive oligodendrocytes in subcortical white matter was attenuated by pretreatment with the spin trap agent, PBN. This agent was previously shown to reduce the amount of grey matter damage resulting from focal cerebral ischaemia (Cao and Phillis, 1994). Although the role of free radical-mediated damage in axonal pathology is not at present known, free radical scavengers may represent a class of agent which has the potential to salvage both ischaemic grey and white matter.

AMPA/kainate receptors causes oligodendrocyte damage both in vivo and in vitro (Yoshioka et al., 1995; Matute et al., 1997; McDonald et al., 1998). Stereotaxic injection of AMPA into the myelinated fibre tract of the external capsule of rats caused a reduction in the density of oligodendroglia 24 h later (McDonald et al., 1998). In keeping with the lack of NMDA receptors on oligodendrocytes, injection of NMDA into the external capsule did not cause death of these glial cells. Using mixed oligodendrocyte/astrocyte cultures the same authors also showed that oligodendrocyte injury resulting from oxygen/glucose deprivation was attenuated by co-incubation with the AMPA receptor antagonist, NBQX, the source of glutamate being suggested to be astrocytes in the co-culture. This raises the possibility that astrocytically-released glutamate may also be toxic to oligodendrocytes during ischaemia in vivo, in the manner which it is thought to be involved in neuronal perikaryal damage. Although we were not able to demonstrate a protective effect of NBQX pre-treatment on the tau pathology response after focal cerebral ischaemia in the rat (Irving et al., 1997), a more systematic investigation (different time points, greater statistical power) with AMPA antagonists with greater in vivo potency of oligodendrocyte damage may be warranted.

In view of their lack of glutamate receptors, axons are unlikely to be damaged directly by glutamate receptor activation. However, injury to their associated oligodendrocytes may severely compromise their function through the process of demyelination. The distribution of oligodendrocyte tau pathology is anatomically more widespread than axonal damage detected by APP accumulation at least, after 6 h of focal cerebral ischaemia in the cat (Dewar et al., 1999). In multiple sclerosis where the primary pathology is demyelination, it is now recognised that axonal damage is also a feature of the disease (Ferguson et al., 1997). While a causal relationship has not been established between oligodendroglial and axonal damage, in a mutant mouse model in which oligodendrocytes may be dysfunctional as a consequence of expression of an abnormal form of proteolipid protein in their myelin, axonal damage is present (Griffiths et al., 1998). These data from chronic degenerative conditions support future investigations of the concept that protection of oligodendrocytes from ischaemic damage may have a profound effect on late outcome after ischaemic injury. The applicability of this concept in ischaemic brain injury receives support from a recent investigation of the effect of the AMPA receptor antagonist on white matter damage after experimental spinal cord contusion. Focal injection of NBQX into the site of spinal cord injury reduced the loss of oligodendrocytes, attenuated the degree of axonal injury and improved neurological outcome (Rosenberg et al., 1999). The authors suggest that the ability of the AMPA receptor antagonist to reduce white matter pathology is attributable to the protection of oligodendrocytes.

7. Developing the concept of total brain protection in cerebral ischaemia

Over the last decade, a coherent strategy emerged for the preclinical development of new anti-ischaemic drugs which protected grey matter. A strategy for the preclinical development of anti-ischaemic drugs which can additionally protect white matter now needs to be developed and it is already apparent that similar issues will need to be addressed (Table 1). The preliminary phase in neuroprotective drug development was the identification of a zone (the ischaemic penumbra) in cortical grey matter whose cerebral circulatory and neurochemical characteristics highlighted its potential for salvage (Tamura et al., 1981a; Mies et al., 1991). For white matter, precise definition of the primary ischaemic insult and neurochemical heterogeneity after middle cerebral artery occlusion is presently limited; the anatomical location and quantitative extent of any salvageable zone in ischaemic white matter remains to be established.

Volumetric assessment of histological damage after middle cerebral artery occlusion (Osborne et al., 1987) provided the first compelling demonstrations of protection of grey matter with neuroprotective drugs (Ozyurt et al., 1988; Park et al., 1988) and rapidly became the predominant quantitative measure worldwide for assessing drug efficacy (McCulloch et al., 1991; McCulloch, 1992). The most appropriate methods for quantitatively assessing outcome in white matter after ischaemia in vivo remains to be established but quantitative assessment of cytoskeletal pathology in oligodendrocytes (Irving et al., 1997) and disruption of fast axonal transport (Yam et al., 1997) appear to have considerable potential particularly when employed in combination. The robustness and sensitivity of these approaches in detecting drug effects in white matter needs additional investigation.

The concept of neuroprotection in ischaemic grey matter was strengthened by demonstrations of drug efficacy after acute ischaemia (where potential artefact like blood pressure and temperature can be monitored continuously and strictly controlled) and in chronic ischaemia (where concern that the drug may merely have delayed the histopathologic process can be addressed). Similar issues need to be addressed in relation to white matter protection particularly in view of the limited data available on the temporal evolution of ischaemic damage to myelinated axons and oligodendrocytes in ischaemia. The definition of the window of therapeutic opportunity has been integral to the development of neuroprotective drugs (McCulloch et al., 1991; McCulloch, 1992) and may be of major importance and of extended duration for pharmacologic interventions for white matter ischaemia; the potential for early ischaemic insults to oligodendrocytes (Fig. 4) producing delayed demyelination is considerable.

The protection of grey matter with neuroprotective drugs could be demonstrated equally readily in permanent and in

transient focal ischaemia. The evidence of free radical involvement in the ischaemic insult to oligodendrocytes (Fig. 4) suggests that transient models (with enhanced oxidative challenge during reperfusion) may be particularly valuable in the study of white matter.

The demonstration of grey matter protection with pharmacological intervention in large gyrencephalic brain was pivotal in demonstrating that neuroprotection was not peculiar to small lesions in rodents (whose lissencephalic brains are particularly susceptible to spreading depression). The greater proportion of white matter in gyrencephalic brains suggests that large animal models may be of particular value in the study of drug efficacy in white matter ischaemia (see Fig. 3).

While neuroprotective drug development was dominated by volumetric histology as the outcome measure, the demonstration of functional benefits (EEG power, behaviour) was an essential element in neuroprotective drug development. The advances which have been made in assessing animal behaviour after ischaemia (Hunter et al., 1998) provides opportunities for assessing contribution of grey matter and white matter protection to functional outcome after drug intervention when combined with the techniques (described above) for defining ischaemic damage to myelinated fibres.

From the perspective of functional recovery (the endpoint of most clinical trials in stroke and head injury), it is axiomatic that all cell types and cellular components should be protected not solely neuronal perikarya. Irrespective of the outcome of the active neuroprotective trials which will report over the next few years, the refocusing of the pharmacology of cerebral ischaemia from selective protection of grey matter towards total brain protection is timely. Pharmacological approaches which also protect white matter in ischaemia provide therapies which would be both additional (were current clinical trials of neuroprotection to succeed) and alternative (were current trials to fail) to existing therapeutic approaches.

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