CURRENT TOPIC

Pharmacological advance in the treatment of acute brain injury

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This article discusses some of the issues related to advances in our understanding of the pharmacology of acute brain injury. My particular emphases are neuroprotective strategies, experimental models, and progress in clinical development. The primary aim is to provide a framework for assessing the literature of this important, clinically relevant field. The reader is also directed to recent reviews on the subject.

Ischaemia induced ionic derangement

The abrupt cessation of blood flow to brain tissue results in progressive pathophysiological changes which culminate in neurodegeneration. These acute, ischaemia induced processes can be divided into three important phases based on major movements in cellular ions (fig 1).¹

First, there is a phase of metabolic depression, occurring within minutes of an insult, with a rapid decrease in electrical activity and suppression of neurotransmission (phase 1). At a cellular ionic level, there is a slow increase in extracellular potassium concentration ($[K^+]_e$) from ~3 mM up to 8–10 mM. At least two

types of potassium (K^+) channel appear to be responsible for this change in K^+ conductance, one activated by an increase in intracellular calcium (Ca^{++}) concentration ($[Ca^{++}]_i$) and the other activated by a decrease in adenosine triphosphate (ATP), the ATP sensitive K^+ channel (K_{ATP}).

The second phase is characterised by almost complete energy failure and anoxic depolarisation (phase 2). It starts abruptly when a $[K^+]_e$ of 8–10 mM triggers, within seconds, a rapid transition to an $[K^+]_e$ of 50–70 mM. Once this depolarisation has occurred there follows a loss of sodium (Na^+) and Ca^{++} ion gradients and a release of various neurotransmitters into the extracellular space, including glutamate, aspartate, and dopamine. The sudden increase in membrane permeability, which has resulted in this transition, may be attributable to the opening of voltage gated K^+ , Na^+ , and Ca^{++} channels. Ca^{++} ions, however, may also enter neurons through ligand gated ion channels, such as the N-methyl-D-aspartate ionophore.^{2,3} This latter route of entry is not dependent on a net increase in endogenous concentrations of the receptor ligands, glutamate and aspartate, as either cellular energy failure or depolarisation can also open the channel in the absence of neurotransmitter. (Another mechanism whereby Ca^{++} may move into the cell is as a result of a functional reversal of the Na^+-Ca^{++} ion exchanger, which instead of moving Ca^{++} out of the cell is driven to work in reverse by a high $[Na^+]_i$, thus further increasing $[Ca^{++}]_i$.)

The third and final phase marks a period of neurodegeneration (phase 3), which may be played out over many hours, or even days.⁴ In this regard, a number of hypotheses about the mechanism of neuronal injury have been proposed, and include: excitatory amino acid neurotoxicity⁵; disturbed function in mitochondria,⁶ or endoplasmic reticulum,⁷ or both⁸; and Ca^{++} mediated toxicity.^{9,10} At an ionic level, initially, the cell membrane is fully depolarised with high $[Ca^{++}]_i$. These two derangements are able to trigger a number of degradative processes either on their own, or in combination with any of the above mechanisms (fig 2). As to which of these mechanisms predominates in a particular insult, there are a variety of factors which may be important in determining the specific process of

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Figure 1 Schematic illustration of cellular ionic changes occurring during the ischaemia induced periods of metabolic depression with bioenergetic stress (phase 1) and complete energy failure with anoxic depolarisation (phase 2). [ATP], concentration of adenosine triphosphate; $[Ca^{++}]_i$ and $[Na^+]_i$, intracellular concentrations of calcium and sodium respectively; K^+ , potassium.

Figure 2 Schematic illustration of degradative changes occurring during ischaemia induced neuronal destruction (phase 3). These mechanisms of injury may be a direct consequence of the episode of injury, or the effect of reperfusion, or both, and the time course of the cell effects may be played out over many hours, or even days. FFA, free fatty acid; LPL, lysophospholipids; PAF, platelet activating factor; NOS, nitric oxide synthase; XDH and XO, two forms of the xanthine oxidoreductase enzyme, xanthine dehydrogenase and xanthine oxidase; PARP, poly (ADP ribose) polymerase.

neurodegeneration—for example, the intensity and time course of the insult,¹¹ whether or not a period of recovery or reperfusion occurs, and whether or not irreversible cell swelling (an important process leading to necrosis starting during phase 1) has also resulted.

Experimental strategies

Interventions during each of the ischaemia induced ionic phases may have beneficial effect. For example, the first phase can be extended by specific blockers of the K_{ATP} channel, or by limiting metabolic activity with hypothermia, or by increasing substrate delivery. When considering the value of such effects, however, it is important to distinguish between two basic phenomena: first, protection because of better tolerance to the insult; and second, limitation of induced injury by inhibiting the activation of degradative processes. Agents acting in phases 1 and 2 improve the tolerance to an ischaemic insult—that is, it takes longer to develop complete energy failure or anoxic depolarisation. In fact a severe insult may never occur. Agents acting on phase 3 (and subsequent reperfusion) ameliorate against the effects of a severe insult that has already occurred. Protection in this case is not because the insult has been less severe or limited, but because some postischaemic mechanism has been interrupted or down regulated.

In this context, when evaluating the experimental literature, it is important to consider what neuroprotective approach is being influenced. This can be summarised in two questions, which should be asked of any study: “Is the protection a consequence of modifying the insult?” and “Is the protection the result of ameliorating the processes of neurodegeneration that have been induced?”.

EXPERIMENTAL MODELS

In the laboratory, two types of experimental paradigm are used as models of human hypoxia ischaemia related disease: occlusion of a major cerebral artery to induce focal ischaemia as a model of focal cerebral insults such as stroke; and brief, reversible global cerebral ischaemia as a model of the cerebral effects of resuscitation from cardiac arrest. Of relevance to neonatal and paediatric practice are the slight nuances of these experimental conditions which have been used to model the effects of in utero, repetitive global ischaemia¹²; birth related transient focal and global cerebral ischaemia^{13–15}; and hypothermic circulatory arrest and cardiopulmonary bypass.^{16–17}

In the adult experimental models, focal and global ischaemia are associated with specific neuropathological phenomena, which are respectively the ischaemic penumbra¹⁸ and selective regional vulnerability.¹⁹ In focal ischaemia, the penumbra is defined as a region close to the infarct core which has a blood flow below that needed to sustain electrical activity, but above that required to maintain cellular ionic gradients. This phenomenon is a time limited condition, with a tendency to evolve towards infarction and to propagate to adjacent viable tissue. Metabolically, its features include increased oxygen extraction, acidosis, and high glucose utilisation, but residual ATP. In other words an unstable region of partial ischaemia with, in time, evolution to sporadic neuronal necrosis or impaired function in the region. In contrast to the focal insult, if global ischaemia is kept relatively short, distinct regions of the brain turn out to be more susceptible to neuronal damage than others, even though the whole brain has undergone a similar insult. For

Table 1 Some of the pharmacological targets identified in laboratory models of ischaemia related injury

	Type of agent	Protective mechanism
Receptors		
Kappa opioid	Agonist	Lower ischaemia induced glutamate release
Adenosine	Agonists	Inhibit neurotransmitter release
	Reuptake blockers	Prolongs the effect of endogenous adenosine
Glutamate	NMDA antagonists	Blocks glutamate mediated toxicity
Serotonin 1A	Agonists	Blocks glutamate mediated toxicity
Ion channels		
Sodium	Inhibitor	Reduced sodium influx provides less drive for reverse operation of sodium/calcium exchanger
		Probably vascular
Calcium	L type channel blocker	
Oxidants		
Superoxide anion radical	Superoxide dismutase	Enzymic conversion of radical to hydrogen peroxide
	Steroids	Inhibits lipid peroxidation
	Lipoxygenase inhibitors	Inhibits lipid peroxidation
	Allopurinol	Inhibits xanthine oxidase
	Iron chelator	Inhibits the action of oxygen radicals
Nitric oxide	NOS substrate analogue	Inhibits NOS
Miscellaneous		
Hormones	TRH analogue	Hormone induced gene expression
Growth factors	NGF, TGF- β 1, FGF	Antipoptotic or promoters of cell survival
White cell adhesion	Antibodies	Blockade of inflammatory component of ischaemia
Thrombus	Thrombolytic	Vascular
	Antiplatelet	Vascular

NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; TRH, thyrotropin releasing hormone; NGF, nerve growth factor; TGF- β 1, transforming growth factor- β 1; FGF, fibroblast growth factor.

example, a brief period of cerebral ischaemia results in neuronal necrosis which preferentially affects, uniformly, the CA1 and CA4 pyramidal cells of the hippocampus, and all middle laminae of the neocortex. During the insult the tissue exhibits complete ischaemia, but on reperfusion there is a restoration of normal regional cerebral blood flow and bioenergetics. Interestingly, survival times of 24–48 hours are needed to allow for full morphological expression of neuronal death. The exact mechanism of this delayed phenomenon is unknown, but, as already discussed, several hypotheses have been proposed (see above).

Over the past 20 years, much has been learned in the laboratory by examining the mechanisms and signal transduction pathways underlying neuronal injury in each of these two adult models. Although seemingly disparate on superficial examination, the injurious processes in each of these models are, in essence, different manifestations of the same ischaemia induced ionic derangement (see above): a prolonged period of partial ischaemia with phase 1 characteristics in the case of the penumbra; and reperfusion following complete ischaemia with phase 3 characteristics in the case of global ischaemia.

In neonatal or infant cerebral ischaemia models, neuropathological phenomena similar to those described in adult models have also been reported,²⁰ albeit with some development specific patterns of reduced vulnerability.²¹ In many instances, however, the models, although effective at inducing certain forms of neuronal death which may be of clinical relevance,^{22–23} have been criticised in two main areas.²⁰ In general, they do not mimic clinical entities; rather, experimental insults are developed in order to ensure consistent pathology. Second,

there is a lack of standardisation of study design, such that manipulations may be undertaken during partial or complete energy failure, or after either. Both of these issues are particularly important when it comes to evaluating the results of interventions purporting clinically applicable neuroprotection. Hence, there are two important questions to ask: “In what way is the insult in the model similar to those seen in clinical practice?” and “Is the experimental paradigm weighted toward a particular type of ischaemia induced pathology?”.

NEUROPROTECTION STUDIES

The theory of pharmacological neuroprotection posits the existence of agents that will minimise the effects of ischaemia on the brain. The focus of much animal laboratory work in neuroprotection has been on focal ischaemia (stroke) models in mature animals, in which treatment is given during or after an artery supplying the brain has been occluded, or during both periods. To a lesser extent, studies have also been conducted in immature animals. In all such studies, the aim of the neuroprotective manoeuvre is to influence the ischaemia induced cascade so as to maximise the proportion of (previously) ischaemic brain that will survive and recover. To date, many pharmacological approaches or combination of approaches have been applied to animal models with beneficial effects, which promise efficacy in patients (table 1). Particularly important is the finding that some agents have a therapeutic window for treatment which extends beyond the episode of cerebral ischaemia, so that the drug can be given even after an insult.

In this context it is, however, also worth considering three questions related to drug efficacy, which are of critical importance in extending experimental research to clinical trials. First, do we know that the agent is getting to the site where we think its mechanism of action takes place? For example, in some animal models, irreversible brain damage may begin within minutes of complete ischaemia, with the window for partial benefit being limited to 1–3 hours after the insult. It is, therefore, crucial to minimise the time before the neuroprotective agent reaches an optimal concentration in ischaemic brain tissue. Second, do we understand the mechanism of how a drug or agent is acting in a given experimental model? Although perhaps obvious, this may not always be the case. For example, glutamate receptor mediated neurotoxicity is now firmly established as playing a major role in ischaemia induced brain injury in experimental animals. However, the mechanisms underlying the differential manner in which excitatory amino acids influence focal versus global ischaemia, as well as insults in young versus mature brain, remain unresolved. Third, does the ability to protect neurons in one animal model extrapolate to similar activity in other animal models? A corollary to this question is the possibility that data from animal models may not necessarily predict successful neuroprotective treatment in humans.

LABORATORY VERSUS CLINICAL EFFICACY

A variety of promising, strongly neuroprotective, agents have been identified by studies in animal models (table 1). Despite this advance, translation to clinical practice has not been forthcoming. Only thrombolysis with recombinant tissue plasminogen activator²⁴ and antiplatelet treatment with aspirin^{25–27} have proved to be effective for acute stroke. In a number of instances other agents, although in advanced stages of clinical development, have had to be withdrawn during clinical trials with, in some cases, results still to be published. This may, in part, be because of unwanted side effects that render their future clinical therapeutic use difficult. Another possibility, however, is simply that they fail to be of benefit in man.

Unfortunately, the process of converting a promising drug effect in the laboratory to a clinically useful test product may be fraught with difficulty. This is illustrated by some of the following points. First, man may exhibit important differences to the experimental model in relation to disease, anatomy, sex, or age specific factors. For example, there are species differences in brain structure and the ratio of grey to white matter; experimental stroke in rats is a disease of grey matter, while pathology in the white matter is more significant in humans. The question here is: “Has there been sufficient preclinical data, using appropriate species?”. Second, laboratory studies are biased towards inducing poorer outcomes, as this makes quantification easier. In this context, a commonly used experimental end point is lesion size and histology. Oedema tends to enlarge the volume of injury in brains examined within the first week of insult, whereas infarct volumes tend to shrink after the first week as fluid is resorbed. Hence early examination may overestimate the protective effect of a drug. Even discounting this acute versus chronic bias, the critical question is whether assessments based on lesion volume translate to clinically important end points. Alternatively, when considering the results of a particular study, the question is does the experimental end point translate to a clinically relevant end point? For example, functional outcome is the end point most relevant to the patient and their family. In humans, however, there is not yet a correlation between lesion size and functional outcome. Finally, and perhaps most relevant to clinical use, are the issues concerning the drug itself. What are its effects in the human being? Can it cross the blood brain barrier? How is it metabolised? What are the interactions? Is it protein bound?

Towards clinical research and development

SURROGATE MARKERS

In studies of clinical pharmacology, efficacy trials follow dose finding and toxicity studies. For example, when evaluating a drug which is postulated to have a beneficial effect on outcome of hypertensive patients, because of its novel endothelial site of action as an antihy-

pertensive agent, we can confirm its in vivo action by monitoring blood pressure. It should be possible to titrate drug dose against effect on blood pressure, and then identify the minimal dose necessary for such an effect. Other information about the profile of human toxicities and maximum tolerable dose should also be known before embarking on clinical efficacy trials.

In the above example, blood pressure is being used as a surrogate measure or marker of the more important clinical end point, such as mortality, quality of life, or myocardial and cerebral complications. The surrogate marker is a measurable response variable which is correlated with, or directly attributable to, the pathophysiological process of the clinical event of interest. In the context of our example, the advantage of a surrogate marker is twofold. First, reduction in blood pressure as a consequence of drug administration proves that the drug is acting by the proposed mechanism—reduction in blood pressure. In other words, it is a test of “proof of concept”. Second, (small) pilot studies in (carefully) selected patients using blood pressure control as an end point could be undertaken relatively quickly before deciding that it would be worthwhile to embark on larger, longer efficacy trials using the more relevant outcome measure, such as incidence of myocardial infarction by one year.

In contrast to this example of an antihypertensive drug, an agent with a theoretical neuroprotective effect—albeit well supported by experimental evidence—has the distinct clinical disadvantage of having its central mechanism of action not accessible to rigorous, non-invasive monitoring. Such a problem will hamper effective clinical trial. For example, it may not be possible to prove that the drug is getting to its site of action, or indeed that any beneficial effect is directly attributable to such a presumed mechanism of action. Consequently, determining the optimal neuroprotective dose in humans is based on extrapolation from pharmacological findings in experimental models rather than determinations of effective dose in humans. Without this information studies in humans could be flawed fundamentally. Yet a number of studies have been undertaken, and not surprisingly, the failure rate is high.

ETHICAL ISSUES

Clearly, before there can be a reasoned argument about the ethical implications of experimental studies in children, many of the above questions should be addressed initially in adults. However, irrespective of these findings, a debate about the ethics of experimental pharmacological studies in infants and children will, invariably, have the potential for being highly contentious, not least because involvement in research entails a theoretical risk of harm which may not otherwise be present. Yet, without paediatric research into neuroprotection and brain injury, sick children will remain “therapeutic orphans”²⁸ and they may even be at major clinical risk as a consequence of being

deprived of an equal and safe access to new therapeutic agents.²⁹

In the clinical setting, balancing these disparate issues is particularly difficult during the development of experimental neuroprotective treatments, especially when dealing with a critically ill population. Clinical investigators, and their supervising institution ethics committees, will have to address many concerns, some of which are discussed below.

First, in relation to the assessment of benefits and harms, is the importance of selecting the most appropriate patients who have the potential for benefiting from an intervention. For example, experimental studies in children with very severe cerebral insult would be inappropriate if a potential outcome was survival in a persistent vegetative state. In this regard, the recent clinical validation of amplitude integrated electroencephalography in neonatal hypoxia ischaemia induced encephalopathy^{30–32} may prove particularly useful in identifying and excluding young infants with a likelihood of a very poor outcome. Even with the application of these stringent clinical criteria, however, there will always remain the statistical risk of inappropriate patient selection³² and, in some instances, the theoretical possibility of even “treating” patients without the condition under investigation.

Another issue, which should also be of paramount importance to anyone involved in the research process, is the concept of informed, uncoerced, and ongoing consent, and how it must be seen to be influencing our attitudes and practice. Central to this issue is the particular vulnerability and predicament of parents placed in the position of having a critically ill child, and our professional, moral, and ethical responsibilities during all interactions with them. For example, even in less critical clinical circumstances, parental priorities may be such that they fail to comprehend, or remember, all that is explicitly described and discussed.³³

Lastly, given the above, is the necessity for a realistic approach to patient enrolment in view of present day public perceptions and expectations about medical research,³⁴ and the inevitable impact of these on study design. For example, Pierro and Spitz³⁵ reported that when parents were fully informed about an ethically approved, non-therapeutic study in critically ill children (which included being given a document describing in lay terms the study protocol and their rights) as many as 70% refused to give consent for invasive investigation, even in those patients with vascular access in situ. According to these authors, among the factors which seemed to influence the parental decision were the invasiveness of the research protocol, the lack of benefit for the individual undergoing study, the severity of illness, and the wholly understandable perception that “their child had had enough”.

Future strategies

Between 1996 and 1998 a review of neuroprotection in acute brain injury after trauma and stroke was undertaken under the aegis of the

Medical Research Council's neuroscience and mental health board.³⁶ This report recognises and supports the value of experimental models, which have, to date, provided considerable insight into how and why brain cells die. However, the challenge for the future is to “bridge the gap” between preclinical research and clinical utility. In this context, the report has made a number of important recommendations. Within these recommendations are certain principles which will be of interest to paediatricians, both those actively involved in neuroprotection research as well as those wanting to keep abreast of this fast developing field. First, it is proposed that techniques be developed so that elements of the pathophysiology of brain damage can be detected and quantified in patients. Obvious examples will be to find ways of monitoring *in vivo*, either directly or indirectly, glutamate induced neurotoxicity and oxygen free radical induced membrane and nuclear damage. Second, strategies must be devised to ensure that, in patients, there is optimal administration of appropriate pharmacological agents or physical techniques—for example, hypothermia—to ameliorate these pathophysiological processes. By necessity, this will entail the development of surrogate markers or evidence of brain penetration in man, as well as surrogate evidence of injury and drug protection in man. Last, before novel neuroprotective treatments are tested in man, preclinical studies should include responsible and ethical examination in appropriate gyrencephalic models.

Taken together, one can see the immediate relevance of dynamic imaging techniques and invasive physiological and biochemical monitoring of the human brain. However, the emphasis should now be on quantification and the detection of injury and of drug effects by using surrogate markers. Notwithstanding these aims, there is also the continued need for new ideas. These may relate to defining and augmenting endogenous homeostatic neuroprotective defence mechanisms, or even the development of better models that mimic clinical entities.

Table 2 Questions that enable the interpretation of experimental findings on neuroprotection

1. Is the protection a consequence of modifying the insult?
2. Is the protection the result of ameliorating the processes of neurodegeneration that have been induced?
3. In what way is the insult in the model similar to those seen in clinical practice?
4. Is the experimental paradigm weighted toward a particular type of ischaemia induced pathology?
5. Do we know that the agent is getting to the site where we think its mechanism of action takes place?
6. Do we understand the mechanism by which a drug or agent is acting in a given experimental model?
7. Does the ability to protect neurons in one animal model extrapolate to similar activity in other animal models?
8. Has there been sufficient preclinical data, using appropriate species?
9. Does the experimental end point translate to a clinically relevant end point?
10. What are the effects of the drug in the human being? Can it cross the blood-brain barrier? How is it metabolised? What are the interactions? Is it protein bound? etc.

Summary

In this article a series of questions has been presented as a means of addressing reports on pharmacological advances in the treatment of acute brain injury (table 2). (These questions relate to the interpretation and clinical translation of the experimental findings, and should be distinguished from one's assessment of the report's scientific quality, which is not discussed in this review.) In relation to the development of clinically useful treatments, the aim of future laboratory and clinical experimental studies has been clearly defined by recent experience. Essentially, there is a need to develop surrogate markers of drug brain penetration in man, as well as surrogate evidence of injury and drug protection in man, before advancing to large clinical trials.

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