Introduction

Treatment of brain ischaemic remains a challenging issue. During the last decade there has been much interest in the development of drugs with significant neuroprotective effects against ischaemic injury in stroke patients. However, clinical trials have been disappointing as, so far, they all have failed to provide evidence for neuroprotection. Several reasons including lack of convincing experimental data, underpowered clinical trials, heterogeneity of patients, timing of drug administration with respect to the therapeutic window, and choice of endpoint measures (for example, one year mortality) may account for these negative results [1-3]. On the other hand, there is a large body of recent experimental data showing that pharmacologic/anaesthetic agents exhibit neuroprotective properties in-vitro as well as in animals (in-vivo). In this lecture we will first present a brief summary of the main pathophysiologic changes triggered by ischaemic/anoxic injury and reperfusion that lead to neuronal cell death and repair. Secondly, we will review the recent promising advances in physiological and pharmacological neuroprotection, including anaesthetic agents. We will discuss their putative clinical impact and therapeutic perspectives for the future in the clinical setting with particular emphasis on the peri-operative context of surgery and intensive care.

Unravelling the mechanisms of ischaemia-reperfusion-induced neuronal death and repair

Considerable progress has been made in the understanding of the consequences of brain anoxia/ischaemia and reperfusion on metabolism and neuronal viability [2-5]. The major mechanisms of ischaemic cell death include excitotoxicity, oxidative stress, and peri-infarct depolarisation in the early stages and inflammation and apoptosis later. However, specific repair processes are also triggered by the anoxic/ischaemic injury in the brain.

Excitotoxicity, oxidative stress and peri-infarct depolarisation

When the blood or oxygen supply to the brain is impaired, the ATP content falls due to a decrease in its production. Subsequently, energy-dependent processes such as Na/K ATPase transporter activity decreases. Activation of ATP-dependent K+ channels (K_ATP) and opening of calcium-activated K+ channels is interrupted, which leads to neuronal hyperpolarisation and then electrical silence just after the onset of ischaemia. Loss of activity of the Na/K ATPase transporter leads to the accumulation of K+ outside neurons and subsequent slow depolarisation. Once a threshold is reached depolarisation with massive Na+ and Ca2+ entry into cells leading to a complete loss of membrane potential. This depolarisation triggers the release of excitotoxic glutamate from nerve terminals which activates both NMDA and AMPA receptors enhancing Na+ and Ca2+ entry and K+ extrusion from neurons through the glutamate receptor-coupled cationic channels. During ischaemia, Ca2+ cytosolic concentration markedly increases, due to activation of both NMDA receptors and voltage-gated Ca2+ channels and the blockade of the Na/Ca+ transporter and release of Ca2+ from intracellular stores. This significant increase in cytosolic calcium concentration plays a prominent role in the development of ischaemic injury and neuronal death by necrosis and/or apoptosis. Indeed, further detrimental effects occur during reperfusion as a result of excessive production of superoxide and generation of free radicals. Reactive oxygen species cause lipid and protein oxidation and DNA damage.
Apoptosis and inflammation

Necrosis causes disintegration of the cell which spreads to adjacent neurons. Apoptosis (programmed cell death) is a physiological process which eliminates a number of neurons during development. During brain ischaemia apoptosis is triggered initiated by the release of cytochrome C from the mitochondria, which produces ATP when oxygen is available. This leads to activation of caspase 3 and then programmed cell death. Apoptosis is tightly regulated by both anti-apoptotic factors (bcl-2) and pro-apoptotic factors (bax, bad). Tyrosine phosphorylation, triggered by brain derived nerve growth factor (BDNF), and nerve growth factor (NGF), plays a major role in the inhibition of neuronal apoptosis. At the early stage, peri-infarction depolarisation may contribute to increased neuronal damage triggered by excitotoxic injury. Inflammatory mediators such as i-NOS, Cox-2, IL-1 and monocyte chemoattractant protein-1 (MCP-1) are generated by microglia, macrophages, leukocytes, as well as astrocytes and neurons in the ischaemic area. In the minutes following initial vessel occlusion there is an increase in the expression of transcription factors such as c-fos, c-jun followed by a second wave of heat shock genes (HSP-70) that increase their expression in the 1-2 h to 1-2 day period. An increase in chemokine expression (IL-1, IL-6, IL-8, TNF-α…) is observed in the first 24 h after occlusion.

Role of gray versus white matter and the neurovascular matrix

The relative involvement of gray versus white matter in the ischaemic zone has an impact on outcome, since ischaemia in deep white matter is generally severe due to the lack of collateral blood supply in this area. In addition to neurons, stroke injury also involves endothelial and smooth vascular cells, astrocytes and microglial cells and associated tissue matrix proteins. Plasminogen activator (PA) and metalloproteinases (MMP) are the most important factors that contribute to matrix proteolysis after disruption of the neurovascular matrix by ischaemic injury.

Repair

Inflammation may promote repair via the ability of T-cells to produce neurotrophic factors such as BDNF, NGF and neurotrophins 3, 4 and 5 which facilitate neuronal cell proliferation and differentiation [4]. Macrophages also play a role in this process via secretion of cytokines (IL-1β and IL-6), chemokines and TNF-α. Cellular repair by remyelination and reorganisation of compensatory pathways, such as increased activity of contralateral or undamaged adjacent brain areas, may attenuate the long term effects of ischaemic injury on outcome.

In summary, unravelling the mechanisms whereby ischaemic injury exerts its effects on brain tissue provides potential targets for a pharmacological approach to neuroprotection. The development of sophisticated brain imaging methods such as fMRI should contribute to a better understanding of the damage and repair processes induced by ischaemic/hypoxic brain injury and guide therapy. Pharmacological neuroprotective strategies may, therefore, aim at either attenuating brain cell and tissue damage or enhancing repair processes.

Pharmacological approach to neuroprotection

Clinical trials with neuroprotective drugs in acute ischaemic stroke

The pharmacological approach to neuroprotection against ischaemic brain injury aims at blocking the biochemical, metabolic and cellular cascades leading to cell death. The primary goal is to limit the devastating consequences of no flow or low flow in the penumbra and prevent reperfusion-induced secondary insults. Almost all steps leading to cellular death represent putative targets for neuroprotective agents. The main strategies can be summarized as follows: decreasing ischaemia duration, blocking ionic Na⁺, Ca²⁺ glutamate receptor-channel-mediated flux, eliminating free oxygen radicals, inhibiting apoptosis, decreasing the inflammatory secondary phenomena, promoting tissue growth and repair. Although many drugs show promising neuroprotective effects in experimental models of focal or global ischaemia, to date not a single so-called ‘neuroprotective’ pharmacologic agent has demonstrated efficacy in a clinical phase III stroke trial [3, 5-7]. This is particularly the case for both competitive and noncompetitive NMDA receptor antagonists, sodium channel blockers, γ-aminobutyric acid agonists, calcium channel blockers, lipid peroxidation inhibitors, intercellular adhesion molecule (ICAM-1) antibodies, or CDP-choline, an intermediate in the synthesis of phosphatidylcholine. Several CT-scan based clinical trials have failed to show benefit of intravenous plasminogen activator administration in patients with ischaemic stroke beyond 3 h after the onset of symptoms. New primary endpoints such as the NIHSSS (National Institute of Health Stroke Scale Score)
score could add a realistic contribution to the concept of diffusion- and perfusion-weighted magnetic resonance mismatch [8]. The free radical-trapping agent NXY-059 showed promise as a neuroprotectant in animal models of acute ischaemic stroke. However, the SAINT I and II trials did not show any benefit for this agent in the treatment of acute ischaemic stroke within 6 h of symptom onset. This was also true for the prevention of alteplase-induced associated haemorrhage [9]. The same consideration applies to the trials of magnesium (IMAGES, FAST-MAG). The results of an ongoing study on albumin in acute stroke (ALIAS) will be available after 2010. There is an ongoing phase IB trial (NeuSTART) which is studying the optimal dose and safety of lovastatin in acute ischaemic stroke given for 3 days beginning within 24 h of the onset of symptoms [10]. Finally, two open-label trials (ESPRIT and ACTIVE W) support the use of double anti-platelet therapy with aspirin and dipyridamole in the prevention of stroke.

Recent advances in experimental pharmacological neuroprotection

Several lines of evidence indicate that some ‘novel’ pharmacological agents are effective in preventing or attenuating brain injury in experimental models. NPO31112, a thiazolidinedione compound, prevented inflammation and neurodegeneration under excitotoxic conditions. The neuroprotective effect of this agent may be mediated via the nuclear receptor peroxisome proliferator-activated receptor γ [11]. These results offer a new perspective for the treatment of neurodegenerative disorders. Intraperitoneal delivery of a transactivator of the Nogo6 antagonistic peptide (TAT-NEP1-40) immediately after a 120 min ischaemic period protected the brain in a rat model of focal ischaemia [12]. This effect was shown to be mediated via attenuation of cell apoptosis in ischaemic areas. Suppression of heat shock proteins may enhance the effects of neurotrophic factors, such as NGF, on brain recovery. Inhibition of cannabinoid B1 receptors exhibits neuroprotective properties in a mice model of ischaemia-reperfusion. Furthermore, modulating the CB1/CB2 cannabinoid receptor balance by endogenous cannabinoids may represent an important target for future neuroprotective strategies. Lithium improves neurological outcome in experimental models of global ischaemia, and has been recently show to down-regulate tau (a phosphoprotein involved in the pathophysiology of Alzheimer’s disease) in cultured cortical neurons. These actions may contribute to explain its efficacy in the treatment of neurological disorders, such as lateral amyotrophic sclerosis [13]. Many haematopoietic factors are produced locally in the brain. Among these, erythropoietin (EPO) has a prominent role for neuroprotection, neurogenesis and acting as a neurotrophic factor in the brain. It also exhibits anti-inflammatory and angiogenetic properties. These functions make EPO a good candidate for treating diseases associated with neuronal cell death, including stroke and degenerative diseases. Encouraging clinical results have been obtained for EPO as a neuroprotectant in acute stroke. This has yet to be confirmed in ongoing clinical trials [14].

Role of hypothermia and normobaric hyperoxia

Mild hypothermia has been convincingly shown to protect the brain in a large number of studies of experimental models of both global and focal ischaemia. However, the clinical impact of mild hypothermia in protecting the brain against ischaemic injury remains controversial. Non-significant or conflicting results have been obtained, most likely because of the systemic complications associated with the use of hypothermia in humans. It is recommended in comatose survivors of out-of-hospital cardiac arrest. Some data support its use in traumatic brain injury. On the other hand, mild hypothermia failed to significantly improve neurologic outcome in patients undergoing surgery for intracranial aneurysm [15]. Selective brain cooling without inducing systemic deleterious effects represents a challenge for the future.

Hyperbaric hyperoxia exhibited remarkable efficacy in limiting the consequences of brain ischaemia in experimental models. Its clinical use remains, however, difficult in routine practice. Therefore, there has been interest in the use of normobaric hyperoxia in the treatment of acute ischaemic stroke. This technique can be used safely in patients undergoing thrombolysis. Normobaric hyperoxia may increase the therapeutic window for thrombolysis and other neuroprotectant drugs. Theoretically, increasing oxygen delivery can increase oxygen free radicals, which could worsen brain injury by promoting processes, such as lipid peroxidation, inflammation, apoptosis and glutamate excitotoxicity. These harmful effects have been documented in global ischaemia, but data in focal ischaemia is scarce. For example, normobaric hyperoxia did not increase the levels of indirect markers of oxidative stress, such as MMP2 and MMP9, or markers of superoxide generation. A pilot clinical MRI-based data suggested that 8 h of normobaric hyperoxia is effective in reducing infarct volume and NIHSSS scores within 12 h of the onset of symptoms, even in the absence of recanalisation of the occluded vessel [3]. However, the beneficial effects of normobaric hyperoxia seem to be only transient.
Recent advance in the neuroprotective properties of anaesthetic agents

Since sodium influx is the first step in the ischaemic cascade, truncating Na⁺ influx represents a potential target for drugs to achieve neuroprotection. Several lines of evidence suggest that the local anaesthetic lidocaine, a blocker of voltage-gated Na⁺ channels, protects the brain against ischaemic damage. The ability of local anaesthetic agents to block the hypoxia-induced changes in Na⁺ influx rather than blocking propagation of action potential is predictive of their neuroprotective effects.

Intravenous anaesthetics also exhibit neuroprotective properties in many experimental models. However, there is no evidence that intravenous anaesthetics can protect the brain against ischaemic insult. One promising agent is dexmedetomidine, an alpha2-adrenoceptor agonist, which exhibits potent experimental neuroprotective properties and also reduces the number of delirium-free or coma-free days of mechanical ventilation in ICU patients when compared with midazolam [16].

Volatile anaesthetics, including the NMDA receptor antagonist xenon, also exhibit potent neuroprotective properties in in-vitro and in-vivo experimental models. A reduction in glutamate excitotoxicity represents the cornerstone of the neuroprotection induced by these agents. Recent attention has been focused, however, on the ability of volatile agents to open the potassium two pore channels. Increasing the activity of the TREK (Twik related K⁺ channel) channels correlates with neuroprotection induced by halogenated agents and agents like polyunsaturated fatty acids and riluzole, an anti-glutamate and anaesthetic agent which may improve the outcome of lateral amyotrophic sclerosis [17, 18]. A large body of evidence indicates that volatile anaesthetics are effective in preconditioning tissue against ischaemic injury. Of the mechanisms involved in volatile-anaesthetic induced early preconditioning, the mitochondrial K_{ATP} channels represent one of the most recognised effectors. Other cellular targets involved in this effect are adenosine receptors, PI3-AKt kinases, serine threonine and tyrosine kinases, inducible NO synthase and inhibition of reactive oxygen species formation [19]. More recently, postconditioning of brain tissue has been reported with isoflurane [20]. In that study, isoflurane (given for 30 min starting 10 min after a 15 min period of oxygen glucose deprivation) improved both cell survival and neurologic outcome of rats at 24 h. The mitochondrial K_{ATP} channels seem to play a pivotal role in this effect. These findings have great potential interest in the context of intra-operative brain ischaemia.

Conclusion and perspectives for prevention and treatment of ischaemic brain injury in the peri-operative period

Although there is a continual improvement in the understanding of the pathophysiology of brain ischaemia and reperfusion, the pharmacological approach of treating or preventing brain ischaemic injury has had limited clinical impact so far. The great majority of clinical trials testing neuroprotectants for the treatment of acute ischaemic stroke have failed to demonstrate any benefit on any major outcome endpoint. Several strategies combining physiologic (oxygen, hypothermia), pharmacologic and thrombolytic therapies may, however, be promising in the future provided a more rigorous design of clinical trials is achieved. The place of anaesthetics as clinically effective neuroprotectants in the peri-operative period remains to be established. Pre- and postconditioning by volatile anaesthetics in the brain may be theoretically interesting in specific situations such as neurosurgery, cardiac surgery, carotid surgery or surgery in the elderly. Whether the preconditioning and protective actions reported so far have only a limited duration of action will need further investigation. Lidocaine remains a safe and possibly effective drug to protect the brain in this context. Limiting factors such as the pro-apoptotic effects of some anaesthetic agents, their effects on cerebral metabolic coupling and the right therapeutic windows with respect to the uncertainty of the onset of ischaemia have yet to be considered.

Key Learning Points

- The major mechanisms of ischaemic cell death involve peri-infarct depolarisation, excitotoxicity, oxidative stress at the early stage, and inflammation and apoptosis later. Each of these phenomena represent putative therapeutic targets for neuroprotection.
- Although many drugs show promising neuroprotective effects in experimental models of focal or global ischaemia, not one so-called ‘neuroprotective’ pharmacologic agent has demonstrated efficacy in a clinical phase III stroke trial.
- New promising approaches of neuroprotective therapies include attenuation of the inflammatory cascade and enhancement of neurotrophic factors. Hypothermia and normobaric hyperoxia have proven at least transient efficacy.
- The use of anaesthetics (particularly dexmedetomidine and volatile agents) may be promising in the peri-operative context because of their neuroprotective as well as pre- and post-conditioning properties on brain tissue.
References