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Stroke, dementia, and drug delivery

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Stroke and dementia represent a major health burden for elderly subjects as they are associated with significant morbidity and mortality. The rates of stroke and dementia are progressively increasing due to the ageing population in most westernized countries. Therefore, both these conditions represent a major therapeutic target. However, the therapeutic options available for the management of stroke and dementia remain largely unsatisfactory, the main reason being the difficulty in transferring the results obtained in animal and *in vitro* studies to the clinical setting. This review focuses on the recent advances in pathophysiology and treatment of these conditions and future directions for research. Moreover, the technique of functional magnetic resonance imaging is discussed in detail as a tool to assess the effects of therapeutic agents on the central nervous system and monitor the progression of diseases. Finally, an overview of the issue of drug delivery into the central nervous system is presented.

Stroke (G. A. Ford)

Stroke is a key therapeutic target because it is the leading cause of severe disability and third highest cause of death in developed countries. Over 75% of patients are aged ≥65 years. In 85–90% of cases stroke is due to cerebral infarction. Important advances have been made in establishing benefits of lowering blood pressure and serum cholesterol as well as in antiplatelet drug therapy in primary and secondary stroke prevention [1, 2]. This review focuses on acute management within the first

2 weeks of stroke, and only briefly discusses the benefits of secondary prevention with antiplatelet, blood pressure, and cholesterol-lowering drugs. There remains a pressing need for interventions to reduce the extent of initial damage in the first few hours. Animal studies have demonstrated that the size of cerebral infarction following middle cerebral artery occlusion can be reduced by both thrombolytic and neuroprotective drugs. These drugs, respectively, establish reperfusion or prevent death of ischaemic but potentially salvageable tissue in the rim of tissue surrounding the ischaemic core (ischaemic penumbra) [3].

Thrombolysis

Interpretation of phase III clinical trials of thrombolytics in acute ischaemic stroke undertaken in the 1990s is controversial [4]. Initial studies of thrombolysis in acute ischaemic stroke with streptokinase were terminated because of increased mortality due to haemorrhage [5]. Further studies of streptokinase in acute ischaemic stroke were abandoned. A number of reasons may account for the apparent greater complication rate with streptokinase compared with the large alteplase trials. No dose-finding studies were undertaken in acute stroke, 10% of patients develop hypotension which may be detrimental in the hyperacute phase, and very few patients were treated within 3 h. Alteplase has been shown in the pivotal NINDS study to be effective when administered within 3 h to selected patients [6] and is licensed for the treatment of acute stroke in North America and now Europe, with restrictions on its use and a requirement that the outcomes of treated patients are documented in the SITS (Safe Implementation of Thrombolysis in Stroke) international database (http://www.acutestroke.org). The NINDS [7] study was a major advance and unlike the other alteplase trials, ECASS I, ECASS II, and ATLANTIS [8–10], clearly positive with a 12–14% absolute increase in the likelihood of a good outcome (no or minimal disability), despite an increase in intracerebral haemorrhage. These results are almost certainly due to the very early treatment, average time from symptom onset to treatment of 90 min in the NINDS trial compared with an average time to treatment of >4 h in the ECASS and ATLANTIS trials. The efficacy of thrombolysis is highly time dependent, with risks of cerebral haemorrhage probably outweighing benefits or tissue salvage from reperfusion in most patients at 5–6 h following symptom onset. 'Time is Brain' is a critical concept that needs to be embraced in future acute stroke trials and implementation of thrombolysis. Intra-arterial thrombolysis is effective in highly selected patients within a 6-h time window [11] but the need for skilled neuroradiological expertise suggests that intra-arterial therapy will probably be used as a second-line 'rescue' therapy for patients who fail to reperfuse following intravenous thrombolysis. Scepticism has been expressed that the results of NINDS cannot be achieved in routine clinical practice. However, multiple North American and European series have shown outcomes comparable to the treatment arm of NINDS can be achieved with a low symptomatic haemorrhage rate [12–14]. Two exceptions are studies from Cleveland [15] and Connecticut [16] that demonstrated a high level of protocol violations and poor outcome, although the Cleveland group have recently reported outcomes similar to NINDS following a programme of education [17]. Many barriers exist in the implementation of hyperacute stroke treatments such as thrombolysis [18]. Initial diagnosis by A&E and primary care physicians is frequently inaccurate [19], access to rapid computed tomography (CT) and magnetic resonance imaging (MRI) is often difficult and few hospitals have acute stroke units to enable skilled care and monitoring of patients.

Neuroprotection

To date all neuroprotective trials have failed to show any benefits in humans. A large number of therapeutic targets have been identified in acute ischaemic stroke. Initial studies focused on antagonism of excitatory amino acids, particularly glutamate, which are increased early following acute cerebral ischaemia and activate N-methyl-D-aspartate (NMDA) receptors [20]. The mechanism of action of some agents, such as lubeluzole, was not fully elucidated prior to clinical studies [21]. Subsequently, more distal mediators in the ischaemic cascade or secondary phenomena such as caspase inhibition and inflammatory response have been targeted. A number of on-going studies are examining the effects of inhibition of 'neuro-inflammation' following acute ischaemic stroke. With hindsight and closer consideration of the conditions under which beneficial effects are seen in animal models, it is perhaps unsurprising that many neuroprotective trials have been negative [22]. Issues potentially contributing to the failure of clinical trials are too long a time window, failure to achieve target neuroprotective drug concentrations, and the heterogeneous nature of ischaemic stroke. Recent recommendations [23] have attempted to ensure that appropriate animal and early phase II studies are undertaken before proceeding to large phase III clinical trials. Pre-clinical data are almost entirely generated in young

animals and data on the effect of ageing on neuroprotective drug effects in animal models are needed. In animal models considerable attention is paid to maintaining physiological stability of blood pressure, oxygenation, temperature and blood glucose in contrast to what happens in routine clinical care, although the neuroprotective effects observed with agents in animal models may have been due to secondary effects on temperature and blood pressure regulation rather than a direct neuroprotective action. The ongoing Glucose Insulin Stroke Trial (GIST) is examining the benefits of optimizing glycaemic control in the first 24 h following acute stroke.

Imaging in acute stroke trials

Considerable advances have been achieved in defining the value of CT and MRI in achieving better patient selection for acute stroke trials and better defining the effects of agents on salvaging brain tissue. Early trials frequently entered patients into acute stroke studies without prior imaging to exclude stroke-mimics and intracerebral haemorrhage, thought unlikely to benefit from most neuroprotective agents. More recently, MR diffusion/perfusion studies suggest a method to delineate potentially salvageable penumbral tissue. The intuitive elegance of this approach is, however, tempered by recent work showing the area of increased diffusion weighted imaging does not always evolve to complete infarction, the phenomenon of 'DWI reversal' [24]. Moreover, the time required to obtain MRI results in further delays in 'door to needle' times, reducing any potential neuroprotective efficacy. Despite these limitations, MRI offers many prospects for undertaking proof of concept trials before proceeding to phase III studies using clinical outcome measures. Future acute-stroke neuroprotective drug development strategies should focus on (i) demonstration of efficacy in relevant animal modes; (ii) establishment of rapid drug penetration into the central nervous system (CNS) [using positron emission tomography (PET) scanning of labelled drugs or cerebrospinal fluid (CSF) sampling in patients with head injury or stroke with ventricular drains/pressure monitors]; (iii) evidence of drug effect in early phase II studies through assessment of MR diffusion/perfusion mismatch salvage; and (iv) 'molecular imaging' utilizing PET and MR spectroscopy studies of effects on brain metabolism [25, 26]. The latter approaches potentially enable direct comparison of effects in animal models with acute stroke in humans. Given the importance of time in all animal models and with the efficacy of thrombolysis in man, phase III trials should focus on early drug administration and consider trial designs encompassing prehospital drug administration by paramedic staff who are frequently first point of contact and can identify acute stroke on clinical criteria with high accuracy [27].

Blood pressure following acute stroke

Hypertension is common following acute stroke and is associated with a worse outcome, but there are no reliable data to guide its management during the acute phase. In a minority of acute stroke patients urgent blood pressure lowering is indicated because of aortic dissection, acute renal failure, severe left ventricular failure, or stroke in the context of accelerated hypertension. Lowering blood pressure acutely might have beneficial effects, through reducing re-infarction, stroke progression, cerebral oedema and haemorrhagic transformation of established cerebral infarction. Conversely, blood pressure lowering might decrease perfusion to the ischaemic penumbra and result in further neurological damage, suggesting blood pressure elevation might be beneficial in the hyperacute (0–6 h) stage. Ongoing studies looking at either blood pressure lowering or elevation, and continuing or discontinuing blood pressure-lowering therapy taken by patients prior to the stroke are now being established. The ACCESS trial that examined the effects of candesartan commenced 36–72 h following acute ischaemic stroke in patients with elevated blood pressure has reported a large reduction in subsequent cardiovascular events at 3 months, suggesting blood pressure lowering in the subacute stage of acute stroke may be beneficial (data presented at German Hypertension Society 2002). At 2 weeks after a nondisabling stroke or Transient Ischaemic Attack (TIA) blood pressure-lowering therapy should be initiated in most patients. The PROGRESS study demonstrated that even normotensive patients have a 20–25% reduction in the risk of stroke and myocardial infarction with combined thiazide and ACE-inhibitor treatment [1].

Aspirin

Because of the 2–3% absolute reduction in myocardial infarction mortality associated with early aspirin administration, there was considerable optimism that aspirin would have similar benefits in acute ischaemic stroke. The International Stroke Trial and Chinese Aspirin Stroke Trial each examined the effects of aspirin administered within 48 h in 20 000 patients. Combined analysis of both trials reported a 1% reduction in death and disability, most probably mediated through an early secondary preventative effect on reducing recurrent stroke [28].

Anticoagulation

Although anticoagulation is commonly administered to patients with acute ischaemic stroke, particularly those with atrial fibrillation, carotid artery occlusion, basilar artery thrombosis, and stroke following myocardial infarction, multiple studies have failed to show a benefit of heparin and heparinoids in acute ischaemic stroke patients [28, 29], with any effect on reducing stroke recurrence and pulmonary embolism being negated by an increased risk of symptomatic cerebral haemorrhage. Whilst there may be subgroups of patients who would benefit from anticoagulation, no controlled trial data support the use of heparin to prevent recurrent stroke or stroke progression in the acute phase of ischaemic stroke. Patients with atrial fibrillation should be anticoagulated with warfarin, unless contraindications are present, to reduce the risk of future cardioembolic stroke, after the risk of haemorrhagic transformation has passed, generally 2–3 weeks. In patients with atrial fibrillation and TIA or stroke with minimal deficits, it is common to commence anticoagulation early on the basis that the risk of symptomatic cerebral haemorrhage is very low.

Therapies to enhance recovery during rehabilitation

Although most patients demonstrate neurological and functional recovery in the weeks and months following a stroke, the pathophysiological processes and neuronal mediators underlying recovery remain poorly characterized, therefore limiting the study of therapeutic interventions. Amphetamines may improve functional outcome and a recent study of L-dopa suggests other drugs that increase central adrenergic activity may enhance recovery [30, 31]. Drugs have usually been given intermittently in association with physiotherapy, and it is unclear whether this therapeutic strategy rather than continuous drug therapy is preferable. Given the potential adverse effects of these agents in an older population (hypotension, hypertension, hallucinations), phase II studies to optimize dose regimens are necessary before proceeding to phase III trials. Treatment of secondary complications of stroke such as depression, incontinence, and spasticity are important in optimizing recovery.

Summary

The last 10 years has seen the commencement of large clinical trials of thrombolysis, neuroprotection and secondary prevention for acute ischaemic stroke. Neuroprotection trials have been disappointing but hold many lessons for future drug development strategies. The next 10 years are likely to see further optimization of thrombolysis, identification of a successful neuroprotective agent, and increased research in agents to enhance neuronal plasticity and recovery.

Dementia (C. A. Bryant, S. H. D. Jackson)

Dementia prevalence in the UK is around 6.6% in the population aged over 65 years [32]. The number of people with cognitive impairment is increasing due to the ageing population, particularly in people aged over 85 years [33].

Epidemiological studies in the UK that have used clinical diagnostic criteria have shown that Alzheimer's disease (AD) is the commonest form of dementia, although other subtypes are not uncommon [vascular dementia (VaD) approximately 21%, dementia with Lewy bodies approximately 11% and frontal lobe dementia approximately 8%] [34]. Traditionally diagnostic criteria for VaD have focused on its distinction from AD. However, atherosclerosis is associated with both AD and VaD [35] and traditional vascular risk factors are associated not only with VaD but also with AD [36]. The presence of cerebrovascular disease appears to intensify the presence and severity of AD [37]. Newer diagnostic criteria for dementia are needed to take account of the evidence that vascular pathology is a potentially modifiable process in both AD and VaD.

The National Service Framework for Older People advocates comprehensive mental health services and good service models for dementia are emphasized [38]. Management of dementia should involve early and accurate diagnosis, good communication and involvement of carers. Drug treatment remains a relatively small part of the overall management strategy.

AD shortens life expectancy and epidemiological data (incorporating corrections for length bias) suggests a median survival of 3.1 years (95% confidence interval (CI) 1.45, 4.83] from onset, which is comparable to many other chronic illnesses such as heart failure in an elderly population [39]. The socio-economic impact of AD is enormous. The formal costs of caring for AD patients in the UK has been estimated as £5.8 billion whilst the economic costs to informal carers may be as much as £17 billion [40]. About 90% of public spending on AD in the UK is on institutional care. Traditionally drug costs in AD have represented a small proportion of the overall costs of caring in AD. Whilst clinical drug trial patients may not be representative of the normal population of patients with dementia in the community, economic evaluations of anticholinesterase therapy have

suggested savings in terms of healthcare costs and caregiver time [41]. There is also evidence that anticholinesterase therapy can reduce caregiver burden [42].

Clinical trials on the efficacy of antidementia drugs have in the past focused on cognitive abilities, with the assumption that changes in cognition will be accompanied by general changes in a patient's condition. In 1997 the European Medicine Evaluation Agency (EMEA) issued guidelines for trials of symptomatic treatments in AD and emphasized the importance of assessment of changes in functional abilities of the patient, of the use of behavioural change as a valid endpoint for trials, and that drug treatment responders should be defined as having attained a prespecified degree of improvement in cognitive abilities and an improvement or at least a stabilization in functional and global abilities [43].

Anticholinesterase therapy in AD

Three anticholinesterase drugs are licensed in the UK for treatment of cognitive decline in AD: donepezil, rivastigmine and galantamine. Current National Institute for Clinical Excellence (NICE) guidelines [44] recommend that these drugs are prescribed in mild to moderate AD in patients with a Mini Mental State Examination score (MMSE) of above 12/30. Assessment and initiation of treatment should be made by a specialist. A follow-up assessment of drug efficacy is made after 2–4 months on maintenance dose and thereafter 6-monthly. Anticholinesterase therapy is continued beyond this period only where a clinical response is suspected lack of deterioration or improvement. Beyond this, therapy is continued only if the MMSE score remains >12 and patient's global, functional and behavioural condition remains at a level where the drug is considered worthwhile. Whilst a meta-analysis of donepezil concluded that the drug did produce modest benefits in cognition and a physicians' rated global clinical assessment [45], the review also concluded that there had been no improvements in patients' self-assessed quality of life and the practical importance of these changes remained unclear. In UK clinical practice open-label prescription of donepezil to patients referred to a Memory clinic improved cognitive function in >50% patients at 3 months and almost 50% of patients showed a reduction in neuropsychiatric symptoms [46]. In this group of patients 50% of carers also showed an improvement in carer distress, maintained over time if the patient continued donepezil.

Unfortunately, there is still no reliable method of predicting response to anticholinesterase therapy, nor is it clear for how long treatment is beneficial. In a year-long study donepezil delayed functional decline by 5 months compared with placebo and the benefits of donepezil on activities of daily living remained throughout the study period [47]. The cognitive benefits of donepezil remained at 1 year compared with baseline, although patients on donepezil continued to show disease progression over the study period.

Of the three cholinesterase inhibitors currently licensed in the UK for treatment of AD, galantamine may offer a theoretical advantage as, in addition to its acetylcholinesterase activity, it also allosterically modulates nicotinic receptors, thus potentiating their response to acetylcholine [48]. Rivastigmine is also a butyrylcholinesterase inhibitor and shows acetylcholinesterase selectivity for the hippocampus and cortex [49]. However, the full results of randomized controlled trials of head-to-head comparisons of the different drugs are awaited in peer-reviewed journals. In an open study comparing rivastigmine with donepezil in AD patients, both drugs had similar effects on cognition, although donepezil was better tolerated with fewer withdrawals due to adverse events [50]. Significantly more patients remained on the maximum approved dose of donepezil compared with rivastigmine at the end of 12 weeks (87% *vs.* 47%). However, in another open-label trial in patients who had either not responded to or had not tolerated donepezil, rivastigmine was well tolerated and produced a cognitive and global response in around 50% of patients [51]. In clinical practice it would seem reasonable to consider switching to a different drug within this class if a patient does not respond to or tolerate the initial treatment.

Reducing behavioural disturbance in AD patients is an important treatment goal as neuropsychiatric disturbances are associated with carer distress and may precipitate institutionalization [52]. Cholinesterase inhibitors have psychotropic and cognition-enhancing effects. A reduction in apathy levels and visual hallucinations appears to be the most reproducible effect. Patients with neuropsychiatric improvements on therapy usually have cognitive benefit as well but further clinical trials are needed specifically designed to investigate the neuropsychiatric effects of these drugs.

Although anticholinesterase therapy is only currently licensed for mild to moderate AD, there is some evidence to suggest tolerability and some efficacy in more severe AD. Measuring clinically meaningful response to drug therapy is vital as small, albeit statistically significant, improvements in cognition may be less important. Donepezil has been assessed in patients with moderate to severe AD, some of whom were living in residential homes and around 40% of subjects were on psychoactive medication [53]. Using a clinician global measure

of change scale 63% of donepezil-treated patients compared with 42% of placebo-treated patients improved or showed no change at 24 weeks ($P < 0.0001$). Secondary outcome measures of cognition and neuropsychiatric symptoms showed significant benefit in favour of donepezil, whilst donepezil stabilized functional status compared with a decline in placebo-treated patients.

Efficacy of donepezil has also been investigated in nursing home residents with high co-morbidity and significant psychoactive drug use [54]. The primary outcome measure was neuropsychiatric disturbance. Although patients on donepezil had significant improvements in cognition and dementia severity rating, both groups declined in activities of daily living. All patients improved on a neuropsychiatric rating scale with no significant differences between placebo and donepezil. High placebo responses have been commonly noted in trials of anticholinesterase therapy. Possible explanations are the confounding effect of psychotropic drug use or the greater input from staff in managing behavioural problems. Clearly more research is needed.

Anticholinesterase therapy in other types of dementia

Cholinesterase inhibitors have also been used in dementia with Lewy bodies (DLB) where there are also deficits in cholinergic neurotransmission. Rivastigmine produced clinically and statistically significant effects on behaviour and cognition compared with placebo [36]. Treatment with neuroleptics in DLB can worsen extrapyramidal features and neuroleptic sensitivity reactions are particularly common and associated with increased mortality [55]. Cholinesterase inhibitors may therefore represent a valuable therapeutic advance in treatment of DLB, although again further trials are needed.

Galantamine has also been used in AD combined with cerebrovascular disease *vs.* patients with pure VaD. At 6 months galantamine in VaD produced an absolute improvement in cognition compared with stable cognition in the placebo-treated group [56]. Appropriately powered trials are needed to look at the effects of cholinesterase inhibitors on cognition, behaviour and global change in VaD. Results of a trial of donepezil in this condition are awaited.

Other drug classes and dementia treatment

Memantine has been licensed in the UK since October 2002 for the treatment of moderately severe to severe AD. It is due to be considered by NICE in December 2003 when the current NICE guidelines on anticholinesterase therapy in AD are due for review. Glutamate is one of the principal excitatory neurotransmitters in the brain and excessive glutamatergic overstimulation and subsequent neuronal calcium overload has been implicated in a number of neurodegenerative disorders including dementia [57]. Memantine is an uncompetitive NMDA receptor antagonist that blocks pathologically elevated levels of glutamate. Memantine has been studied in a randomized placebo-controlled 28-week trial in community-living patients with severe dementia and baseline MMSEs of 3–14 [58]. All patients showed deterioration on the cognitive and functional scales during the study and there was a high drop-out rate of around 30% (related probably to the severity of the disease as memantine was well tolerated). Patients treated with memantine did show significantly less deterioration in activities of daily living and also on a cognitive assessment designed for use in severe dementia. Also, caregiver time spent with patients was significantly reduced in the memantine patients (difference between treatment groups, 45.8 h per month, 95% CI 10.37, 81.27). Memantine has also been studied in patients with moderate cognitive impairment due to VaD and the cognitive improvements appear to be comparable to those of anticholinesterase therapy in AD patients [59], although no global or functional improvements were seen. The use of memantine in AD and VaD is still not clear in terms of efficacy in mild disease and how it will fit in with anticholinesterase therapy.

Other compounds in clinical trials of AD include monoamine oxidase inhibitors, selegiline, antioxidants, oestrogen, and anti-inflammatory drugs. Although small benefits have been measured for a variety of different drug classes, none of them was comparable to anticholinesterase therapy in robust clinical trials. Pathological and epidemiological evidence has implicated both oestrogen and the process of inflammation in the pathophysiology of AD. However, recent clinical trails of both hydroxychloroquine (an anti-inflammatory agent) and oestrogen showed negative results [60, 61].

Treatment of behavioural problems in dementia

Specific management of behavioural and psychological symptoms is often needed in dementia. Nonpharmacological treatment is the mainstay of management [62]. There is evidence for moderate efficacy of antipsychotics and newer atypical neuroleptics may offer a better side-effect profile. In a randomized placebo-controlled trial, olanzapine was well tolerated and effective in reducing behavioural disturbances and psychotic symptoms in AD patients in a nursing home setting [63]. The differential indications for anticholinesterase *vs.* atypical neuroleptics remain to be defined.

Conclusions

Cholinesterase inhibitors and memantine are the only licensed treatments for AD in the UK. There is some evidence that they may also be efficacious in other forms of dementia and more severe forms of AD. However, with increasing knowledge of the molecular and genetic pathophysiology of AD it is hoped that future therapeutic intervention may either prevent the condition or significantly alter the course of disease progression [64]. Much interest has centred on targeting amyloid-beta (A-beta) peptide [65], the constituent of plaques, which along with neurofibrillary tangles form the key pathological lesions in AD. Whilst vaccination with A-beta reduced plaque formation and produced cognitive benefits in a transgenic mouse model of AD [66], trials in humans were halted last year due to toxicity. Another area likely to attract increasing attention is modification of vascular risk factors in prevention of dementia and preventing decline in established dementia. Treatment of isolated systolic hypertension in older people in a large randomized placebo-controlled trial reduced the incidence of dementia (AD and VaD) by around 55%, equivalent to treating 1000 patients for 5 years in order to prevent 20 cases of dementia [67].

fMRI as a tool to assess drug responsiveness (C. A. Bryant, S. H. D. Jackson)

The development of functional neuroimaging over recent years has allowed the investigation of neurophysiological processes and the effect of drugs and disease on these processes. PET and single photon emission computed tomography (SPECT) are established neuroimaging techniques for examining the effects of drugs [68]. Although functional magnetic resonance imaging (fMRI) is a validated technique for brain mapping and neuropsychology, it has been recently used to explore the action of pharmacological agents on the brain.

Neuroimaging designs employing pharmacological agents can be subdivided into those measuring receptor occupancy directly using radiolabelled partial agonists and antagonists, and those measuring the effect of pharmacological challenges through changes in neuronal activity. The first experimental designs investigated receptor occupancy in a particular condition (e.g. AD). Newer designs have examined dynamic displacement of the radioligand following pharmacological or cognitive challenge [69]. The second type of study design examines changes in neural activity following pharmacological challenge. Again these studies can be subdivided into two classes: those simply looking at the effects on cerebral physiology, and those examining the effects on the brain's responses to sensorimotor or cognitive challenge [70, 71].

fMRI

The technique of fMRI is based on the principle of blood oxygen level dependent (BOLD) contrast. The concept of BOLD contrast utilizes the different paramagnetic properties of hydrogen atoms (protons) in oxygenated and deoxygenated haemoglobin [72]. Changes in neural activity will change MR signal intensity [73]. Neural activation increases oxygen consumption, causing a momentary (100–200 ms) increase in local deoxyhaemoglobin concentration. There is then a large local increase in capillary blood flow decreasing the local deoxyhaemoglobin concentrations, thereby increasing the MR signal. Signal acquisition is obtained by ultrafast scanning techniques. The local increase in BOLD contrast reflects an increase in neural activity [74].

The temporal resolution (seconds) and spatial resolution (ml) of fMRI is far superior to PET and SPECT. As a non-invasive procedure with no exposure to X-rays, fMRI offers repeatability in subjects, allowing treatment monitoring. Of course, contraindications exist for MRI and implants and prostheses are more common in an older population.

Generally, fMRI studies consist of measuring the MR signal in response to epochs of repeated stimuli, a continuous task performance or event-related responses to a single stimulus. Data analysis can incorporate corrections for movement artefact, unlike structural scanning data. Changes in MR signal are mapped directly onto a high-resolution scan of the subject's anatomy. Data from a cohort of subjects can then be combined to provide group averaged images mapped into standard neuroanatomical co-ordinates.

Functional neuroimaging (PET and fMRI) studies have shown different patterns of brain activation with increasing age. For example, most cognitive studies show that neural activity is less asymmetric in older *vs.* younger adults, especially in the prefrontal cortex, possibly as older subjects compensate for neurocognitive deficits by engaging both hemispheres for tasks [75]. We have shown reduced fMRI activation in older subjects to olfactory stimuli [76]. However, ageing may affect the BOLD response. Some authors have found reductions in the absolute number of activated voxels with a simple reaction time task, but no significant changes in the temporal pattern of the BOLD response or signal magnitude [77]. Others have documented an age-related decrease in the magnitude of the BOLD response for a given volume of brain activated [78]. Ageing could

affect the BOLD signal via a number of parameters such as neural activity, neurovascular coupling or vascular changes. The age-related cerebral atrophy may also contribute to reduced fMRI activation. However, one study has shown that activation of language regions in the brain and cerebral atrophy within those regions did not correlate in healthy volunteers over a broad age and atrophy range [79]. The mechanism of the reduced BOLD signal in older people is not clear.

A drug may potentially exert its effect on the BOLD signal by modulating neuronal activity, affecting neurovascular coupling or directly on the vasculature. Experimental design and analysis must deal with all of these aspects. Investigators have used a 'control' paradigm (passive visual stimulation) in a study examining the effects of cocaine on cerebral blood flow [80]. Although there was a reduction in cerebral blood flow with the drug, the BOLD response to visual stimulation was unaltered, suggesting that neurovascular coupling was unaffected.

Methods of analysis have been developed for the detection and analysis of brain activation patterns following acute drug administration using a waveform analysis protocol based on single-dose pharmacokinetics [81].

Examples of pharmacological fMRI studies

Serotonin stimulates motor function in animals. The effect of a selective serotonin reuptake inhibitor (SSRI) on motor function was studied in healthy subjects receiving paroxetine 20 mg, 60 mg, and placebo [82]. fMRI of subjects performing a motor task was performed to coincide with peak concentrations of paroxetine. Paroxetine decreased activation in the hemisphere ipsilateral to the moving hand and focused the activation toward the contralateral executive sensory motor areas compared with placebo. There was also bilateral cerebellar hypoactivation. A dose effect was observed with paroxetine 20 mg unexpectedly producing a maximal effect on sensorimotor activation. The effects of a single dose of fluoxetine and placebo on motor performance on a finger-tapping task in eight patients with lacunar stroke and pure motor hemiparesis have also been studied [83]. During active movement of the affected arm fluoxetine strongly and selectively activated the ipsilateral sensorimotor cortex, a pattern quite different from healthy controls. Subjects' improved motor performance with fluoxetine (tested offline with a variety of validated scales) also correlated with the increased activation. During passive movement of the affected hand fluoxetine did not affect cerebral activation patterns compared with placebo, suggesting that either the BOLD effect or neurovascular coupling was unaffected. These studies have suggested a causal effect of fluoxetine on the motor cortex mediated by serotonin. The role of SSRIs in promoting motor recovery in stroke patients remains to be elucidated.

Physostigmine, a cholinesterase inhibitor, has been used to investigate how cholinergic enhancement impacts on working memory. Young volunteers underwent fMRI during a visual memory task in a crossover trial of placebo and physostigmine [84]. Physostigmine resulted in enhanced neural processing in visual cortical areas due to enhanced encoding, with a trend towards faster reaction times and a reduction in activation in dorsal prefrontal regions subserving working memory. Failing cholinergic function correlates with failing cognition in AD. Therefore fMRI could be used as a tool to assess drug responsiveness in AD, as only about 50% of patients have a meaningful response to anticholinesterases and at present there is no way of identifying responders prior to treatment [46]. In two clinical responders to donepezil fMRI showed increased visual cortex activation during a passive visual paradigm, findings which need replication in larger numbers [85].

Conclusions

fMRI offers much promise in the field of functional neuroimaging to assess the effects of drugs. However, experimental design and sophisticated analysis techniques are necessary and are in a relatively early phase of development.

Neurodegenerative diseases: the delivery issue (A. A. Mangoni, S. H. D. Jackson)

Medications used for the treatment of neurodegenerative diseases include drugs that replace the missing neurotransmitter or inhibit the degrading enzyme [86, 87]. One of the problems encountered in the treatment of such disorders involves the inadequate penetration of therapeutic agents through the blood–brain barrier with reduced drug concentrations at the receptor site. Two potentially useful methods of enhancing drug delivery are (i) enhanced brain penetration and (ii) alternative routes of administration (i.e. intranasal drug delivery).

Enhanced brain penetration

Transport of therapeutic agents to the brain might be augmented using either enhancing agents or conjugation to transporter molecules. The bradykinin analogue RMP-7, a compound designed to increase blood–brain barrier permeability, enhances the delivery of loperamide and chemotherapeutic agents to the brain [88–90]. An alternative method of enhanced delivery involves the

conjugation to the drug delivery vector OX26 (a murine monoclonal antibody to the rat transferrin receptor) [91, 92]. Because CNS capillaries are uniquely enriched in transferrin receptors, antibodies to these receptors might serve as potential carriers to transport large molecules. Thus, the mechanism for enhanced transport across the blood–brain barrier might utilize receptor-mediated transcytosis through the endothelial cells of the brain microvessels into the brain extracellular fluid [93]. This mechanism is effective for several compounds, including vasoactive intestinal peptide analogue, nerve growth factor, and glial cell line-derived neurotrophic factor, a possible therapeutic target for Parkinson's disease [91, 94, 95]. Additional methods developed to increase the transport of proteins and peptides through the blood–brain barrier include protein transduction [96]. This entails conjugation to small peptides with membrane penetration capacities, thus allowing cellular uptake. Protein transduction occurs in a concentration-dependent manner, achieving maximum intracellular concentrations in <5 min, with nearly equal intracellular concentrations between all cells in the transduced population. In all cases of enhanced drug delivery and cellular uptake, the question of specificity has to be addressed and side-effects should be closely monitored.

Intranasal drug delivery

The mechanism of transport of nasally administered peptides entails targeting the olfactory nerves and/or the epithelium and the olfactory bulbs, followed by the retrograde transport to the olfactory bulb and penetration into the brain microcirculation and CSF [97–99]. Several peptides have been successfully administered intranasally. Vasopressin was found to improve sleep in elderly people following long-term treatment [100]. Insulin improved short-term memory functions [100]. Adrenocorticotropin/melanocyte-stimulating hormone diminished selective attention and reduced body fat [100]. It is possible that specific transport systems are in operation. However, some reports suggest that the high permeability of the nasal epithelium facilitates rapid drug absorption rates with high molecular mass cut-off (approximately 1000 Da) and plasma drug profiles which in some cases are almost identical to those resulting from intravenous injections [101]. Small peptides might use both routes depending on their biophysical characteristics and the nature of the carrier. However, problems and pitfalls still exist and need to be addressed on a case-by-case basis. Moreover, tests should be conducted for actual brain drug penetration, distribution and specificity. Thus, the degree of drug absorption through the nasal route is dependent on drug structure and size and vehicle properties. Enhanced absorption might be achieved using positively charged liposomes, which confer a bioadhesive effect on the negatively charged nasal mucosa [102]. Furthermore, intranasal peptide delivery might be markedly increased by caprylocaproyl macrogolgycerides by enhancing absorption through the nasal mucosa [103].

Conclusions

Stroke and dementia remain an important therapeutic target. Despite our better understanding of the mechanisms responsible for the acute damage in stroke and disease progression in Alzheimer's dementia, few therapeutic options are currently available. The use of modern imaging techniques in assessing drug–receptor interactions might represent a breakthrough in drug development. Alternative ways of drug delivery could enhance the penetration of therapeutic agents into the brain, thus allowing the administration of lower doses.

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