Review Article

The International Spinal Research Trust's strategic approach to the development of treatments for the repair of spinal cord injury

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The International Spinal Research Trust (ISRT) has selected a sub-set of the key molecular and cellular events underlying spinal injury and nerve regeneration, to be the focus of their funding and other means of research support. These priority targets are (i) to understand and to minimise the damage caused by spinal injury and the resulting inflammatory and fibrotic events, in order to prevent the establishment of a post-acute situation that is ill-placed for regeneration; and (ii) to understand and then to manipulate the integrated environment for regrowth that is created by the interplay of soluble and matrix- or membrane-associated factors, both trophic and inhibitory. Investigation and, ultimately, exploitation of these targets requires the development of standardised and representative animal models and the application of quantitative methods for assessing functional re-innervation. The ISRT will also sponsor the networking of different disciplines and technologies to apply the most productive skills to spinal repair.

Keywords: spinal cord injury; repair; experimental models; researches

Introduction

Since the foundation of the International Spinal Research Trust (ISRT) in 1980, progress in the neurosciences has been dramatic. Our understanding of the basic biology of the nervous system has increased to such an extent that we now have profound insights into the growth and maintenance of the nervous system and are able to influence these phenomena in a way that is relevant to treating the nervous system's responses to pathological insult and to injury. As this progress in our understanding continues to accelerate, there is thus the real prospect of effective therapies for a number of the most intractable health problems facing human society. Alzheimer's disease, stroke, Parkinson's disease and multiple sclerosis are just a handful of the conditions where realistic prospects now exist for disease-modifying therapies. Furthermore, the underlying advance of neurobiology on a broad front also offers opportunities for the amelioration and repair of spinal cord injury—an area that generally attracts fewer resources from both public and private sectors. ISRT's mission is to fund research that will exploit this newly encouraging situation to achieve the successful treatment of spinal cord injury. Recently, ISRT has

adopted a more focused prioritisation for its research awards and this article describes the purpose and content of its strategy.

Current status: justifying realistic expectations of new advances

To date, meaningful advances in treating spinal cord injury have been confined to interventions designed to limit the tissue damage immediately following the acute injury.

A study of more than 150 subjects with severely traumatised spinal columns, who died at the roadside and therefore within moments of the injury, reveals that the spinal cord was apparently intact in many cases because the series of cellular events initiated by the injury was interrupted by death.¹ If these patients had survived for even a few hours, the usual signs of necrosis would have become evident; and at some stage, 12–24 h after injury, macrophages (microglia) would have entered the lesion and become activated locally. However, the CNS has an atypical inflammatory response to injury.²

As a result of this and similar observations, various agents have been tested for their ability to interfere with the damaging series of cellular events that follow injury to the spinal cord. Clearly, corticosteroids can

moderate the inflammatory response and, after laboratory trials, the first National Acute Spinal Cord Injury Study (NASCIS 1) was initiated in the USA in 1979.³ In this study, high (1000 mg/day) and low doses (100 mg/day) of methylprednisolone were given to patients within 48–72 h of injury and continued for 10 days. 330 patients took part in this first double-blind randomised multi-centre trial. No significant differences were found between the patients treated with high or low doses of the steroid and laboratory studies subsequently suggested that too little methylprednisolone was given too late in NASCIS 1. Meanwhile, naloxone was reported to be beneficial in animal models of spinal cord injury; and NASCIS 2 was initiated in 1985 to compare very high doses of methylprednisolone (30 mg/kg bolus and maintained at 5.4 mg/kg/h for 23 h), naloxone (5.4 mg/kg bolus followed by 3.0 mg/kg/h for 23 h) and placebo.

A total of 487 patients were randomised within 12 h of spinal cord injury in this new study and patients treated within 8 h were compared with those treated between 8 and 12 h after injury. This was, once again, a multi-centre trial and care was taken to achieve uniform grading of muscle groups on the standard clinical scale of 0–5. Follow-up scores were obtained at 6 weeks, 6 months and a year after admission to hospital.⁴

Patients treated more than 8 h after injury did not differ markedly from placebo controls and may even have had a worse outcome 1 year after injury. However, patients treated with methylprednisolone within 8 h of injury had significantly greater motor and sensory recovery than their equivalents treated with placebo. The group treated with naloxone within 8 h did not significantly differ from the placebo group, apart from those who were paretic (partially paralysed), who had a slight but significantly better outcome after 1 year. Further NASCIS trials have been initiated to test the efficacy of monosialic gangliosides⁵ and the inhibitor of free radical-initiated lipid peroxidation, tirilazad mesylate⁶ (U-74006F), but, due to the publicity surrounding the success of methylprednisolone in NASCIS 2, all patients in the US have had to be treated with methylprednisolone in addition to any other compounds. Even in this community, however, the effectiveness of the steroid remains controversial.⁷

The NASCIS trials have achieved two major advances. Firstly, they demonstrated that pharmacological interventions, even those based on very familiar cell biology and well-established drugs, can yield benefits in spinal cord injury: an important encouragement. Secondly, they indicate that the neuropathology of spinal cord injury gives us a 'window of opportunity' in which to intervene at the hyper-acute stage before the nervous tissue is further damaged by a cellular reaction to the initial trauma. This window closes approximately 8 h after injury, strongly indicating the need for intervention to begin at the accident site.

Nevertheless, the success of the NASCIS trials should not encourage us to rely on direct clinical

studies alone, however appropriate this may seem to the prompt and direct introduction of beneficial new approaches into routine SCI management. Owing to the size, cost, complexity and duration of these trials, we need to develop the optimum agent for neural protection in the laboratory rather than in the clinic.⁸ Only in the laboratory setting will we be able to study agents in the many combinations that are worthy of exploration, in a sufficiently timely and cost-effective manner, using the highest resolution methods of analysis and without the restrictions inherent in choosing only from pharmacological agents already approved for human use.

Further progress in this area is likely to occur as a spin-off from efforts to develop effective therapies for stroke and head injury. A number of classes of agent are currently in clinical development including NMDA receptor antagonists, new classes of free radical scavengers and bradykinin antagonists. The NMDA antagonist GK11, for example, has shown promising neuroprotective effects on cultured human spinal cord cells.⁹ Prospects for yet greater benefit are offered by approaches still at the pre-clinical stage, such as neuronally selective calcium-channel blockers and calpain inhibitors.¹⁰ The timely application of these advances to SCI demands the generation of relevant model systems based on a detailed understanding of the molecular and cellular mechanisms shared between these various neurological diseases.

A coherent approach to further progress – towards the repair of chronic injury

However, even these advances will not alone be sufficient for SCI. While the developments described above are having a significant impact on the acute management of spinal cord injury, it is most unlikely that they will deliver complete protection from long term neurological damage, even if they are delivered sufficiently early. They will also offer nothing to the currently intractable problem of the sequelae of established SCI. ISRT's strategy therefore includes these early stages within an integrated approach to SCI that also addresses yet more challenging objectives.

The multi-component nature of the pathology underlying spinal cord injury and the complexity of modern neurobiology encourages a broad and essentially serendipitous (or, perhaps, opportunistic) attack on a wide range of research targets. Yet, ultimately, this may not be the most effective policy for a funding body such as ISRT:

- It under-exploits the major advances made in recent years in the relevant fields of molecular and cellular neurobiology, which make possible the sub-division of an overwhelmingly complex cascade of biological events into realistically resolvable sub-problems. These can be tackled essentially separately, as parts of a relentless advance towards a full reconstruction of the steps required to minimise spinal cord damage and maximise functional regeneration.

- It is susceptible to unpredictable changes in focus over time, determined predominantly by the fields within which spontaneous grant applications arrive at ISRT, and perhaps by research 'fashions' led by neurobiological foci divorced from spinal cord biology. This risks a lack of purposeful persistence in tackling key spinal cord problems, and may therefore directly retard progress.
- It is likely to lead to the dissipation of funds across too broad an area of science to allow ISRT to make any noticeable difference to the overall efforts of the international research community. This is not as irrelevant a consideration as it might at first sight appear, for a charity dependent on attracting private donations and responsible to its constituencies of SCI patients, volunteer fund-raisers and sponsors.
- By viewing the final goal as one unstructured whole, it makes it almost impossible for the management of ISRT to plan fund-raising that matches the task in hand: the budget required to deal with the full spectrum of relevant biology is both too large to be acquired by a single charitable institution and virtually impossible to estimate in a meaningful way at this point in our detailed understanding of the underlying possibilities.

These considerations, coupled with the substantive advances in understanding at the molecular and cellular levels, have led ISRT to formulate a coherent but deliberately selective strategy for future funding priorities. It is well-recognised that a strategy must remain responsive to advances in neuroscience of

relevance to spinal injury research. The adoption of a strategy by ISRT will not be allowed to create a strait-jacket, impeding innovative thinking and rejecting potentially ground-breaking ideas outside the current mainstream consensus. It is also self-evident that, as individual components of the neurobiological puzzle are 'solved', so the focus of this strategy must change if the next obstacle to successful regeneration is to be overcome. In reality, completing a reparative armamentarium is likely to require several iterative cycles, returning to previous foci of effort as their associated molecular mechanisms require further manipulation within a different pathological environment created by other, interacting factors further down the pathological cascade. The delineation of selected areas of focus for funding therefore does not imply any abandonment of the Trust's commitment to solve the whole puzzle in the end (summarised in Figure 1): it is, rather, a deliberate policy to accelerate this process by concentrating on selected elements of the puzzle that are, currently, 'rate-limiting' obstacles to successful repair. It is a truism that spinal repair will not follow a single scientific or clinical breakthrough: each of the problems delineated in Figure 1 will eventually have to be solved.

This philosophy has led to the identification of the following broad areas as core targets for future ISRT-funded research (Figure 2):

- (a) preventing early stage deleterious events that impede subsequent regenerative capacity
- (b) promoting regeneration and re-innervation

Figure 1 The conceptual framework of spinal repair. The main elements of successful repair of spinal injury are grouped in broad temporal order. Eventually, all or most of the individual targets will need to be achieved, but this presents a daunting and, as discussed in the text, unrealistically ambitious approach. The ISRT Strategy seeks to focus on a more limited range of accessible mechanistic steps, as shown in Figure 2

Figure 2 The ISRT Strategy for spinal repair. The ISRT Strategy has selected two main experimental strands for support, with the equally important underpinning technologies required to establish representative animal models and to evaluate both partial and full restoration of function in these models and in man, preferably non-invasively. Education, training, cross-disciplinary dialogue and networking between groups with different skills to apply to spinal repair provide additional, essential infrastructure

through a co-ordinated approach to stimulatory and inhibitory factors affecting both intrinsic neuronal capacity for repair and extrinsic (environmental) capabilities for supporting repair

(c) measuring the change from impaired to restored function as the most meaningful criterion of success in treating SCI

Each of these areas is delineated in more detail in the following sections. A general policy favouring research explicitly directed towards spinal cord biology will be adopted by ISRT. Where model systems other than spinal cord cells and molecules are proposed as the basis of a project because of the existence of essential background understanding or relevant research tools, applicants will be expected to delineate their approaches to confirming the explicit relevance of their results to the situation in the spinal cord. Moreover, projects extending or developing relevant new technologies for specific application to spinal cord systems are an appropriate way to focus advances made elsewhere towards the resolution of SCI, and will therefore be supported by ISRT.

Understanding and treatment of early stage trauma and inflammatory processes

Three issues merit focused attention in the early aftermath of SCI:

- preventing early-stage local neuronal and glial death (see also next Section)
- understanding the natural history and effects of the inflammatory response to injury in the CNS
- preventing secondary glial reactions, e.g. those producing regeneration-blocking scar tissue

Descriptive biology of SCI based on new molecular and cellular understanding

There are many features of SCI for which we lack detailed information, even at the descriptive level, applying recent advances in our molecular and cellular understanding to the injured human spinal cord. In particular, the relationship between the cytokines and the neurotrophic factors (see next Section) is proving a fruitful area of research and offers significant potential for integrating the various patho-physiological facets described in this review. We need to determine whether the cellular and molecular features of the inflammatory response in the immediate period following spinal cord injury are broadly similar to those in other inflammatory pathologies, because of the intervention of essentially 'peripheral' inflammatory cascades, and to what extent the atypical inflammatory response of the CNS modulates the overall picture.^{2,11} A review of models of acute experimental spinal cord injury indicates the importance of studying the early

changes.¹² It is therefore a high priority objective to describe the pathology of SCI in more detailed cellular and molecular terms. As far as the availability of suitably preserved material extends, these studies should, of course, attempt to examine SCI in man. However, this approach is unlikely to allow the most effective use of modern advances, which offer sophisticated *in vivo* imaging technologies, biochemical methods for tracking the appearance and disappearance of different cellular phenotypes and molecular factors, etc. Full pursuit of this descriptive objective, as well as subsequent experimental manipulations, requires the establishment of standardised animal models of acute SCI.

We acknowledge the major contribution being made in this regard by a multi-centre US initiative [derived from work exemplified in reference⁷] but we are also aware of its limitations. Dr Wise Young has produced a rigorously defined contusion model in the rat which is, however, neither an ideal mimic of the much larger and more complex human spinal cord, nor relevant to all clinical injury situations. The Miami Project to Cure Paralysis has undertaken a *post mortem* review of the anatomical features characterising chronic spinal cord injury in man.⁸ This suggests that the Young model is a valid representation of around 33% of human injuries: it equates to the 'contusion converting to cavity type'. The other types identified comprise 'massive compression/laceration' which describes around 50% of injuries, with 'solid core injury' comprising the remaining 17%. As well as the relevance of the model being limited to only a minority of human cord injuries, the restricted biochemical assessments routinely undertaken (sodium and potassium content of the extracellular fluid) mean that the insights to be gained from the model, at least as currently used, into the underlying pathophysiology of SCI are very limited. This is, in essence, an empirical approach to screening currently available therapeutic entities in animals prior to early clinical studies (of NASCIS trials). There is, therefore, a real risk that approaches that might be clinically used in defined patient sub-populations will be unsuccessful in this model, and therefore not pursued further.

We therefore intend to support the development of alternative animal model(s) both to investigate the pathophysiology events in the medium-term post-traumatic period (focusing on the first 10 days), and subsequently therapeutic and prophylactic interventions.

The development of this animal model may diverge from the development of a model suitable for evaluating therapies promoting the repair of chronic lesions, although there are obvious synergies, and a common model, if feasible, would have obvious advantages in analytical power. A key related consideration in our strategy is that the production of multiple models, each used by one or only a few laboratories and each only rarely

correlated with the other, often seriously hampers progress in any field. Without wishing arbitrarily to pre-define an acceptable number of models that, together, will adequately reflect human SCI, it is self-evidently more efficient if models with a broad range of acceptability to the SCI community can be developed consensually, operated according to a standardised protocol and analysed in mutually informative ways – indeed, this is one of the key strengths of the USA initiative. For these reasons, we favour the establishment of networks of laboratories with pre-existing, interlocking skills to develop such new animal models, rather than wholly new ventures with their associated heavy start-up costs and delays in reaching peak efficiency.

The choice of species will also be important. It is again inappropriate to impose inflexible requirements for the choice of species, but those other than the rat should be considered – not least because the available range of monoclonal antibodies, *in situ* hybridisation reagents, transgenics, gene 'knock-outs' and other genomic tools, and the amenability to electrophysiological investigation, etc will be prime determinants of the power with which new models can be interrogated at the molecular and cellular levels. By deliberately seeking to integrate existing knowledge from different technologies and disciplines, this strategy aims to accelerate our rate of progress and maximise our cost-effectiveness.

The model should examine:

- oedema in the injured spinal cord
- inflammation- and immune cell-mediated tissue destruction
- leukocyte adhesion and infiltration
- cytokine – NTF cascades and their interactions^{15,16}
- neurological and neurophysiological function

Clinical studies

While the pre-clinical programme described above is viewed as the most effective way of obtaining an initial, detailed understanding of the pathophysiological processes occurring in the post-traumatic period, ultimately these advances must be correlated with the situation in man, if they are to be exploited for the benefit of SCI patients. Recent developments in the field of non-invasive imaging, such as PET, SPECT, MRI and clinical neurophysiology, etc, have resulted in a situation where substantial insights may be anticipated from the study in patients of specific events defined in animal models and other laboratory systems. Moreover, explicit comparisons with clinical pathologies will, in turn, validate the new animal models.¹⁷

ISRT will therefore support a programme to evaluate the changes following acute SCI in man. ISRT sees an important role exists for the Trust in fostering productive links between groups specialising in neurophysiology, clinical imaging, human *post mortem* studies and relevant animal models.

Experimental exploitation of the new descriptive biology of SCI

The information gained from the basic but powerful descriptive biology described above should underpin trials, in the animal models, of imaginative new pharmacological manipulations. We recognise that interventions with early 'tool' reagents frequently offer the most powerful ways of probing particular descriptive hypotheses^{18,19} but we are alert to the reality that this degree of focus can often fail to generate a body of agreed, comprehensive and fundamental information on which others can build. The research community needs to recognise both that early experimental probing of individual mechanistic components of SCI and repair is likely to fail – because of our still incomplete understanding of the full range of contributing events – and that such failures cannot be allowed to discourage further exploration of the same phenomena as we learn more. As ISRT strategy changes with time, the emphasis in this first part of our strategic focus is likely to shift from a predominantly descriptive focus to one that repeatedly tests and refines the resulting picture pharmacologically and through genetic manipulations.

This development might, for example, start to examine:

- the use of alternative approaches to 'neuroprotection' to prevent cell death immediately post-trauma
- stabilisation of the axonal cytoskeleton to minimise early retraction, thereby reducing the distance over which regeneration must be promoted

In this regard, an important clarification from the descriptive phase would be to quantify the degree of axonal retraction and cell death that occurs away from the site of the acute lesion^{20–22}, a matter on which there are currently divergent reports.

Repair of chronic injury

Beyond minimising the immediate damage and the subsequent medium-term deleterious changes evoked by inflammation and axonal disconnection and retraction, the key objective of SCI research is then the induction of functional re-innervation. To achieve this, several challenging objectives must be met:

- maintained survival of the majority of affected neurons
- stimulation of axonal outgrowth in appropriate directions (without deleterious collateral sprouting)
- overcoming the undoubtedly anti-regenerative characteristics of the adult CNS environment
- converting physical outgrowth into useful (ie correctly mapped) functional re-innervation of effector tissues

Failure in any single element of this programme will preclude successful treatment of SCI. ISRT will therefore favour an integrated approach as far as it is currently feasible.

In this regard, ISRT is cognisant of the alternatives to stimulating the regrowth of the patient's own

surviving neurons and masking intrinsic inhibitory facets of the tissue environment within which axonal outgrowth must occur.²³ For example, exciting advances have been made by studying the beneficial effects of autologous peripheral nerve segments or Schwann cell implants^{24,25} as a regenerative *milieu*, the enhancement of outgrowth across artificially-created substrata more reminiscent of the peripheral nervous system, and the promising potential for using foetal cells or tissue to replace adult neurons that may be able to regrow enough to restore meaningful function after SCI.^{26,27} At the very least, this type of investigation can further dissect the molecular mechanisms involved in the control of regeneration and repair,²⁸ and they may become viable clinical strategies in the future, either alone or complemented by the manipulation of intrinsic systems.²⁹ However, they have significant logistical and even ethical, complications if they are to become routinely available to the majority of SCI patients. At this point in our understanding of spinal cord repair, however, the choice does not have to be made: all these approaches generate valuable fundamental understanding, and how this is best exploited in routine practice can better be assessed some time in the future.³⁰

Stimulation of neurite outgrowth

The central problem at the core of repairing chronic SCI remains the failure of neurons to regenerate in a CNS environment. Funding of investigations into the reasons for this and approaches directed to the stimulation and maintenance of neurite outgrowth have been and should remain at the centre of ISRT's activities. There are two principal elements to the problem:

- trophic stimulation of initial axonal outgrowth, and support of continued axonal elongation^{31–33}
- identification and blocking of inhibitory mechanisms operating in the spinal cord^{34–36}

Over the past few years, ISRT's primary focus has been on the second of these objectives; the equivalent emphasis will now be given to the first. Looking more broadly, the historical emphasis of trophic factor biology has been on soluble, diffusible agents, while the emphasis of inhibitory factor research has been on substratum effects. There is ample reason to try to complete this picture by integrating soluble inhibitory factors and substratum-derived permissive or trophic influences. Moreover, the concept of a purely passive substratum is clearly inappropriate, and studies should incorporate 'solid phase' cell-cell and substratum-cell information flows, *via* membrane receptors coupled to active signalling mechanisms. The overall 'environment for growth' may be considered as shown schematically in Figure 3. Recently, very substantial progress has been made, although not usually (yet) within the specific context of adult mammalian spinal cord damage and regeneration, in delineating a large

Figure 3 The environment for regeneration. The integration of soluble and 'solid-phase' (substratum, matrix- and membrane-associated) stimulatory and inhibitory factors is illustrated schematically. Deficiencies in the intrinsic levels of trophic agents can as readily contribute to defective repair as the presence of overtly inhibitory molecules. The cross-talk between non-neuronal cells, as producers of these agents, and neurons, as both producers and responders and the likely sophisticated balance between the levels of these molecules changing over time, all contribute to generating a highly complex environment. Only by addressing this complexity in an integrated way can the manipulations required to stimulate effective nerve regeneration and repair be created

number of new molecules – the netrins, semaphorins and related factors – regulating axonal outgrowth and the direction in which such outgrowth will occur.^{37,38} These advances begin to distinguish neurite outgrowth (distance) from orientation (direction), with the interactions between these newly discovered molecules and the neurotrophic factors³⁹ offering both increased complexity but also the increased specificity of neurobiological control that will be required if future therapeutic interventions are to avoid non-selective effects on the whole nervous systems.

For the immediate future, the primary focus will be on the development of strategies to provide trophic stimulation of initial axonal outgrowth and continued elongation. Three broad approaches are worthy of support.

Evaluation of the role of neuronal trophic factors

Over the next few years, advances in human and animal genomics are likely to result in the identification of many more neural growth and survival factors than we currently recognise.⁴⁰ The rate of current progress is already beyond the scope of this review to summarise in any detail. Characterisation of the regulation of biosynthesis and patho-physiological relevance of these factors will be an enormous task, beyond the resources of ISRT alone. The obvious involvement of multiple (changing) neurotrophic factors in varying but rigorously controlled proportions across the time frame of

spinal cord damage and repair dramatically exacerbates this problem. Successful repair is likely to need both 'de-differentiative' factors to allow neuronal outgrowth and 'differentiative' factors to restore full function. Our approach to this enormous and complex problem is for ISRT to encourage the study of growth factors in spinal cord-specific systems (specific nerve tracks or neurotransmitter-defined subsets) including, in relevant animal models, an examination of the role of non-neuronal cells in factor production as well as their responses to these neural local hormones. The recent identification of neuregulins as messages between neurons and their non-neuronal support cells⁴¹ offers the prospect of a more rigorous understanding of this still only vaguely perceived cell-cell synergistic inter-relationship.

The most feasible approach is likely to involve contributions from numerous independent laboratories seeking to apply their skills and technologies to the spinal cord. While 'merely' extending the catalogue of neurotrophic factors with explicit relevance to SCI has intrinsic merit while we have only limited understanding of this area, the critical need is to translate knowledge of this catalogue into practicable approaches to the manipulation of injury and repair.

Countering the effects of inhibitory factors

The concept of inhibitory factors in the central nervous system warrants significant further effort^{42,43} because it is likely to remain a major obstacle to successful repair

even if the most appropriate time-dependent cocktail of neurotrophic factors and growth cone guidance systems can be determined and applied to the injured spinal cord. It is already clear that the presence of growth-inhibitory molecules in the substratum is not the only level of control in this system: the expression of 'receptors' for such molecules on the regenerating neurite can add further complexity.⁴⁴

As described above in Figure 3, there is a need to integrate the biology of specifically outgrowth-inhibitory molecules in the substratum and growth-restrictive soluble factors. Furthermore, possible local deficiencies in suitable outgrowth-supporting adhesion and other substratum molecules, and the control of directionality by more recently discovered chemoattractant and chemorepulsive molecules, need to be explored. Both excessive growth inhibition and defective growth support are likely to contribute to reluctant regeneration in the spinal cord and certainly one could overwhelm the other, both deleteriously in the natural course of events but potentially beneficially, if we can understand the signals to alter the balance therapeutically. Specifically successful neurite outgrowth/axon elongation may be assumed to require temporal and topological patterns of substratum/adhesion molecule expression.

Furthermore, greater attention to sources of inhibitory factors other than glia is justified. Interactions between substrate molecules and soluble NTFs as well as links between multiple adhesion molecules *via* signal transduction coupling, further complicate the situation but promise valuable new insights that might be therapeutically manipulatable. Recent work has exemplified such integrated interventions.^{45,46}

Masking inhibitory substrata (eg with antibodies) may be inappropriately restrictive in realistic therapeutic terms, though an important proof of concept having the advantage, for a macromolecular agent, of prolonged pharmacokinetics.⁴⁷ Again, small molecule treatments (receptor antagonists or moieties interceding at the molecular expression or post-receptor signal transduction levels) would be attractive developments in this field. Progression to small molecule therapeutics (and even the production of proteins of sufficiently quality-controlled consistency and quantity), in this and most other areas considered in this review, is likely to require an industrial approach.

'Genetic' approaches

Variations in the expression of neuronal genes are relevant to the failure of adult central neurons to regenerate. Genes expressed during development are assumed to become dormant during adult life when neurons do not normally 'need' to regrow. Similarly, the developmentally silent inhibitory genes may be switched on in adulthood to prevent excessive plasticity and the risk of 'randomised' neural connectivity patterns.⁴⁸ Studying the developmental differences in the expression of key neural gene systems therefore

offers perspectives that complement the more direct study of neurotrophic factors and inhibitory molecules described above. One potentially important gain through this approach is that the impact of other biological systems of less immediately obvious regenerative importance, such as those regulating neuronal structural proteins, may be more easily recognised through such hypothesis-independent comparisons.

In addition to providing further conceptual underpinning to the previous recommendations, this approach may offer opportunities for gene therapy,⁴⁹ although the means for ensuring the necessary localisation remain to be developed. Furthermore, genetically-modified implanted cells offer the scope to stimulate regeneration in spinal neurons following SCI⁵⁰ by providing 'cell factories' generating NTFs and other agents continuously and locally.⁵¹⁻⁵³ There is no reason why, eventually, such biofactories should not produce the full range of different factors that are required for fruitful spinal cord repair, with the biosynthetic spectrum being controllable (both temporally and quantitatively) with small molecule triggers, once we fully understand the regulation of the biosynthesis of the engineered neurotropic agents. This is an area where a specific targeted initiative is appropriate as the technologies already exist in a sufficiently mature form to be exploited in a focused manner.

Practical realities in exploiting the neurotrophic system clinically

Any successful approach to the repair of SCI is likely to involve the application of protein and/or non-protein molecules (possibly many) to specific sites inside the blood-brain barrier, and conceivably within quite rigorous temporal patterns. As alluded to above, this may reflect primary therapy or secondary support to a physical approach to promoting neurite outgrowth (eg Schwann cell bridging⁵⁴). A number of issues are of potential major relevance here:

- the normal but temporary breakdown of the blood-brain barrier at the time of trauma could be artificially extended to permit prolonged treatment eg with neurotrophic factors
- localised areas of less tight blood-brain barrier such as the entry points of the spinal dorsal roots could usefully be exploited
- agents that open the blood-brain barrier non-specifically through loosening of the inter-endothelial tight junctions are normally viewed cautiously because of the risk of increased susceptibility to oedema, infiltration of the CNS by immune cells and greater access by microbial infection. However, either localised administration of such agents or strictly temporary dosing could achieve acceptable therapeutic gains
- the stimulation of angiogenesis may be a key factor in permitting successful neuronal survival and neurite outgrowth

The enhanced retrograde axonal transport of some NTFs that has been observed after axotomy¹⁸ may bypass some of the conceptual drug delivery problems involved in promoting spinal core repair. Thus, the neuronal system may allow damaged neurons to be preferentially stimulated, avoiding potentially deleterious effects from overgrowth of undamaged and functional neurons, and may also allow motor neurons to be modulated without intercranial administration of drugs.

With these perspectives in mind, it is clear that the issues surrounding the administration of putative SCI treatments will eventually become rate-limiting. ISRT regards a heavy emphasis on this area as premature at present, but recognises that turning to these issues only after solution of the intrinsic regeneration problem is also inappropriate. Research into model systems and approaches that are cognisant of the ultimate practical requirements for SCI treatment will therefore be favoured.

Functional assessment

As spinal cord biology, and neurobiology in general, has made promising advances, we feel that the time has come to impose on ourselves a more challenging criterion for success. Morphological evidence of the regeneration of a few, hesitantly-growing axons is encouraging as a first step, but falls well short of the needs of the SCI patient. In particular, such a restricted result can lead to the premature interpretation of work on growth-inhibitory molecules as indicating successful regeneration when all that might have been achieved are very local perturbations in an essentially still growth-blocked environment. Furthermore, anatomical changes are poorly representative of functional consequences: it is noteworthy that rats may show full functional recovery following experimental SCI in the presence of histologically demonstratable persistent destruction of the lateral cortico-spinal tracts. Moreover, any correlation of anatomical changes with function in animals may very well not be directly applicable to man.

In addition, regrowth that can be anatomically demonstrated may actually be functionally incompetent or even deleterious. Local, high resolution 'Darwinian' exploration of the effector cells may be required to produce an appropriate innervation pattern. Inappropriate innervation patterns at effector cell level may be compensated for by re-organisation of the central connections (implying very sophisticated plasticity and adaptive controls⁵⁵⁻⁵⁷). These are issues that extend beyond ISRT's current strategy, although they will have to be addressed in the future.

The ultimate objective for spinal cord repair is the restoration of *functional* innervation. Therefore, ISRT will favour studies that:

- apply existing technologies, particularly neurophysiology, to determine the nature of loss of function in SCI, and the reaction of the intact nervous system to damage, and to track regenerating SC systems

- develop new technologies for assessing the dysfunction and restoration of function in animal models and in patients. In both cases, higher resolution and non-invasive methods are required: resolution is important because being able to detect only *full* success does not allow progressive (iterative) improvements in the development of new treatment strategies to be assessed nor their ultimate tailoring to individual patient needs during treatment, as may be necessary given the inevitable differences in pathology between SCI cases

An *a priori* requirement for evaluating strategies for the repair of chronic SCI will be the existence of a validated, functional animal model. There is currently no generally accepted animal model of chronic spinal cord injury. In any event, as noted above, there appear to be at least three distinct patterns of anatomical change following chronic injury in humans. As discussed above, integrating animal models of acute and chronic SCI would have self-evident benefits, but it is recognised that this may be an almost impossible task.

There is considerable scope for using neurophysiology to measure damage and to detail functionally competent regeneration. Existing detailed understanding of cat neurophysiology and behaviour may usefully be exploited. The necessary neurophysiological skills can readily be accessed in most research institutions through appropriate collaborations: a consistent thread through ISRT's strategy is the concerted application of pre-existing skills to SC problems, to capture their maximum potential. Standardised neurophysiological measures are important to define the extent of lesions and the extent of recovery in models used in different laboratories world-wide. Following the development and validation of such a functional model, ISRT will require that *physiological* evidence be provided for restoration of function in research projects funded by the Trust.

Risks and opportunities

As already discussed, it is well recognised that the delineation of a focused research strategy carries both the risk that the selection is wrong and the danger that, inflexibly applied, it will exclude new developments, innovative thinking and the entry into the field of scientists experienced in other fields who could make a substantial impact on the field of spinal cord injury by the application of different thinking. ISRT self-evidently wishes to avoid these pitfalls.

It is therefore axiomatic that the strategy outlined here will remain valid only for a strictly limited time. It is the intention of ISRT regularly to evaluate and update the proposals at appropriate intervals and to disseminate the resulting documentation to all interested parties. In particular, we intend to establish close relationships with the other voluntary grant-giving bodies with a particular interest in the area of spinal cord injury.

Without wishing to blur the edges of our strategy by denying the intention to focus the majority of our research funds on the areas described in this review and the attendant consequence that other areas will receive reduced support, ISRT will remain open to the innovative, even iconoclastic, proposal that promises to break radically new ground, and which merits a short-term 'pump-priming' award.

Underpinning the whole concept of creating an integrated approach to treating SCI is the need to encourage cross-disciplinary awareness and co-operation. ISRT will, therefore, support:

- temporary secondments of scientists and clinicians between institutions to strengthen, in a directly practical way, the networks required to achieve the integrated research goals described in this review
- joint specialist-clinician workshops to promote the mutual exploitation of different perspectives on SCI. The involvement of patient groups will also promote both a focused awareness of the key issues and a sensible degree of public understanding of the scale and complexity of the task, and of progress as it is accomplished.

Abbreviations

ISRT, International Spinal Research Trust; MRI, magnetic resonance imaging; NASCIS, National Acute Spinal Cord Injury Study; NMDA, N-methyl-D-aspartate; NTF, neurotrophic factor; PET, positron emission tomography; SCI, spinal cord injury.

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