

Editor's Note: We believe our readers will benefit from knowing about the annual ASENT (American Society for Experimental Neurotherapeutics) meeting and having available the abstracts from this meeting. Emphasizing therapy in neurology is the objective of ASENT and thus this information is both timely and important.

Roger N. Rosenberg, MD

Abstracts From the Program of the Second Annual Meeting of the American Society for Experimental Neurotherapeutics, Washington, DC, March 23-25, 2000.

Some Statistical Issues in the Design of Cancer Clinical Trials With Surrogate End Points

Steve Piantadosi, MD

Surrogate end points are widely used for the development of new therapies in cancer and many other chronic diseases. Surrogate outcomes are particularly useful because they greatly increase the efficiency of developmental clinical trials. In particular, they can shorten the time required to observe treatment effects and reduce the sample size required for some trials. As a consequence, surrogate end points can reduce costs and speed up the development time for new therapies. To be useful, a surrogate end point should be strongly associated with the definitive outcome, lie in the causal pathway for the definitive outcome, should manifest early in the course of follow-up, and should be relatively easy to measure. However, the defining characteristic is that the surrogate outcome should be affected by treatment in the same way (direction and relative magnitude) as the definitive outcome. It is this last characteristic that is difficult to verify. Even when a surrogate outcome is judged to be appropriate for a particular treatment, different therapies for the same disease could potentially invalidate a given surrogate outcome. There are numerous examples of the apparently successful application of surrogate outcomes in cancer. However, there are other circumstances in which the traditionally employed surrogate (tumor shrinkage) is not appropriate. Furthermore, for other diseases, such as cardiovascular disease and AIDS (acquired immune deficiency syndrome), the putative surrogate outcome may be wholly inadequate. All of these ideas were discussed in more depth during the presentation.

When Is Surrogate Marker Clinically Useful? Statistical and Clinical Significance May Not Be the Same

Daniel F. Hayes, MD

Few molecular markers have gained widespread clinical use in the practice of clinical oncology. In therapeutic clinical investigations, standardized terminology for type of study (phases 1, 2, and 3), efficacy (complete, partial response), and toxic effects (grading scales) have been accepted. In contrast, tumor marker studies have been performed haphazardly. In 1996, the American Society of Clinical Oncology convened a Tumor Marker Expert Panel to establish practice guidelines for breast and colon cancer (*J Clin Oncol.* 1996;14:2843-2877). Several members of the panel coauthored a proposed Tumor Marker Utility Grading System (TMUGS) that was used to evaluate each marker (Hayes, et al. *J Natl Cancer Inst.* 1996;88:1456-1466). The TMUGS provides a framework to define the marker designation, how it is assayed, and the proposed clinical use (risk, screening, differential diagnosis, prognosis, and monitoring). For each use, the marker only has clinical utility if it influences a clinical decision that results in an improvement in overall survival, disease-free survival, quality of life, and/or cost. A semiquantitative scale, ranging from 0 to 3+, was generated to grade the clinical utility. Routine clinical use is recommended for tumor markers that are assigned grades of 2+ to 3+. The assigned grade is supported by a scale of levels of evidence (LOE). Grades should only be assigned in the presence of LOE I or II data, preferably the former. Levels of evidence I and II studies provide a measure not only of the reliability (statistical significance) of the observed relative prognostic (outcome independent of therapy) or predictive (outcome related to specific therapy) values but also of the magnitude of difference between groups of patients identified by marker positivity or negativity (Hayes, et al. *Br Ca Res Tr.* 1998;52:304; Hayes. *Eur J Cancer.* 2000;36:302-306). Ultimately, these considerations will help evaluate the clinical utility of novel surrogate markers more rapidly, leading to improved patient care.

Surrogate Markers: The AIDS Clinical Trials

Donna Mildvan, MD

Clinical trials during early anti-HIV (human immunodeficiency virus) drug development were designed as large-scale studies, using mortality and progression to AIDS (acquired immunodeficiency syndrome) as end points. Representing the only

end points of relevance at the time, clinical end points defined “clinical efficacy” for new drug approvals but necessitated cumbersome, prolonged, and costly trials, often requiring years to initiate and complete. Resulting delays in defining useful therapies had real and implied impact on the ever-widening AIDS epidemic and led to a concerted effort to accelerate new drug development through identification of surrogate end points, that is, biomarkers that could be used to substitute for clinical end points in trials of antiretroviral drug efficacy. With the advent of highly sensitive molecular tools for the detection and quantitation of circulating HIV-1 RNA in all infected individuals, a key advance was made. The quantitation of HIV-1 RNA together with the CD4 cell count (the end organ of HIV attack) were validated as useful biomarkers in that they were shown to predict clinical outcome in longitudinal studies of untreated HIV infection (type 0 marker). Each of these markers responded to effective antiretroviral therapy (type 1 marker) and together they accounted for a substantial proportion of the clinical benefit mediated by therapy (type 2 marker; Mildvan, et al. *Clin Infect Dis*. 1997;24:764-774). These findings have been so consistent across trials and populations that they resulted in a paradigm shift for new drug approvals based on “antiretroviral efficacy.” This has translated into an accelerated drug development/approval process for anti-HIV therapies, which ultimately resulted in dramatically improved patient outcomes. The extent to which the AIDS model may be extrapolated to other disciplines was discussed.

Neuroimaging and Genetic Markers in CNS Disorders

Linda Brady, PhD

Advances in technology and basic biology have provided opportunities to develop neuroimaging and genetic biomarkers to study CNS (central nervous system) disorders. Functional imaging of brain metabolic activity, biochemistry, and circuitry reveals normal and abnormal physiological conditions and the molecular basis for CNS disorders. The application of MRS (magnetic resonance spectroscopy) and MRI (magnetic resonance imaging), and PET (positron emission tomographic) and SPECT (single photon emission computed tomographic) radiotracers to image CNS receptors and other signaling molecules has provided new opportunities to assess the onset and progression of CNS disorders and to monitor the effectiveness of treatments. Cognitive biomarkers (eg, attention, working memory, and eye movement) have been applied to prospectively assess biological susceptibility in populations at risk for developing neuropsychiatric disorders. Cognitive markers may also be useful in assessing new treatments and long-term functional outcome in neuropsychiatric and neurodegenerative disorders.

Advances in genomic technologies (completion of the human genome and a catalog of human sequence varia-

tions) will provide important information about the identity of genes that confer risk to neuropsychiatric disorders and genes involved in their pathogenesis. The development of genetic markers will aid in understanding the developmental time and the molecular and anatomical substrates of neuropsychiatric disorders. The application of genetic markers in clinical studies will ultimately enhance the power of neuroimaging and cognitive markers as tools to understand disease pathogenesis and will aid in the development and assessment of new preventive strategies and therapeutic interventions for CNS disorders. Examples of the application of neuroimaging, cognitive, and genetic markers in clinical studies of neuropsychiatric disorders were presented.

Traumatic Brain Injury—Biochemical Markers

Bertil Romner, MD, PhD

The severity of traumatic brain injury after head injury is difficult to assess because specific measures of the presence and severity of such injuries have been unavailable. Computed tomographic scans reveal a brain injury in 8% to 22% of patients with minor head injuries. Magnetic resonance imaging is more sensitive but availability is limited. Single photon emission computed tomographic scans demonstrate abnormalities in more than 50% of patients, but this measure of regional blood flow is not necessarily related to structural damage of the brain. There is a major need for a biological marker for the severity of traumatic brain injury, especially in mild injury in the medical-legal context in which it may be desirable or necessary to prove that disability or neuropsychological impairment after a traumatic event is really due to the head injury. The need for an early prognostic indicator exists so that therapies can be selected.

Biochemical markers of brain lesions and/or ischemia quantify secondary cerebral injury, identify toxic mediators, optimize target-controlled therapy or neuroprotective agents, and possibly to facilitate economical and/or ethical decision making. Problems in relying on biochemical markers, such as S100, neuron specific enolase (NSE), aldolase C40, glial fibrillary acid protein (GFAP), creatine kinase (CK), adenylate kinase (AK), myelin basic protein (MBP), and τ , include the following: (1) No currently used markers are unique to the brain. (2) Sex, age, species variability, and possibly neurological disease influence production of a marker. (3) Type and extent of the injury and localization of the marker in the cell affect marker release. Moreover, several markers are physiologically released. (4) Concentrations of biochemical markers found are dependent on rate of diffusion and CSF (cerebrospinal fluid) volume and flow. (5) Kidney and liver function affect clearance of the biochemical markers and little is known about their clearance from the CSF. (6) Assay properties need to be standardized to avoid variations.

Functional MRI Case Study: Monitoring Stroke in Humans

Gregory Sorensen, MD

Neuroimaging already plays an important role in the diagnosis and management of acute ischemic stroke. Magnetic resonance imaging (MRI) has also established itself as a surrogate end point in animal models of ischemia. Recent developments have allowed a transition of these MRI techniques from animals to humans. Ongoing work indicates that computed tomography (CT) and MRI may be able to assess tissue status at an early stage and serve as an indicator of additional tissue at risk. Furthermore, additional statistical modeling appears to increase the power of these techniques.

The ability of these imaging techniques to help identify individual differences appears promising. Such approaches may allow for the determination of efficacy with smaller numbers of patients than that found in traditional approaches. Furthermore, these approaches may allow for the establishment of the biological effect of novel therapies.

While these techniques are still under development, they do seem to indicate that, as in other neurologic diseases, imaging has the possibility of greatly enhancing our insight into the pathophysiologic mechanism of human acute cerebral ischemia.

Clinical End Points for Stroke Trials

Justin A. Zivin, MD, PhD

The only proven acute stroke treatments act by restoring blood flow to the ischemic brain region. Numerous neuroprotective drugs are effective in animal models but have failed in clinical trials. A reason for many of these failures is that preclinical investigations optimize the treatment conditions and such protocols often differ substantially from methods that are feasible for patient management. Identification of the critical treatment variables is essential to improve the trial designs and clinical rating scales, but they are rather inefficient for this purpose. Specifically, we would like to identify surrogate end points that can be used in phase 2 trials to facilitate protocol designs for phase 3 studies. An important advantage for investigations of possible stroke treatments is that the cause of the disorder is well understood—it is simply a mechanical disruption of blood supply. Detailed mechanistic understanding at the molecular level is not required to generate useful therapies, although that would be helpful. No single abnormality has been identified that is responsible for irreversible ischemic nervous system damage.

Clinical investigation can employ empiric methods for developing valid biomarkers. Imaging techniques show some promise for providing useable surrogate end points to identify salvageable tissue. However, no such meth-

ods have been proven to be useful for clinical trials. The general problems with current imaging technology for assessment of stroke treatments were discussed as well as feasible objectives for future studies.

Signature MRI Drug Effects in MS: the Demand for Composite MRI Outcomes

Jerry S. Wolinsky, MD

Magnetic resonance imaging (MRI), an established secondary outcome for multiple sclerosis (MS) clinical trials, looms on the near horizon as a primary outcome of therapeutic efficacy. It provides a window on pathology. Gadolinium enhancements, reflecting blood-brain barrier dysfunction common in early disease, are easily quantified. Tissue involvement or disease burden is represented by increased signal intensity on T2-weighted images, and hypointense lesions on T1-weighted images capture the most extensive destruction. These are complemented by global atrophy measures, advanced imaging, and spectroscopic assessment of neuronal and myelin integrity. All reflect different aspects of the complex pathologic process that ultimately compromises function. As recent studies show, therapy may differentially affect the progression of these parameters. The interferons have early and profound effects on enhancement but lesser effects on disease burden and delayed effects on atrophy. Glatiramer has delayed and modest effects on enhancement but still affects disease burden. Linomide has dose-dependent effects on both enhancements and hypointense lesions. Cladribine has profound and long-lasting effects on enhancements not mirrored on disease burden. These varied patterns support an unweighted MRI composite incorporating different measures of the pathologic process as potentially preferable for monitoring MS trials, especially when pharmacological drug mechanisms are incompletely understood.

The Relationship Between Acute Disease Activity on MRI as Reflected by Blood-Brain Barrier Disruption and Clinical Measures of Disease Activity

Henry McFarland, MD

The last decade has seen a steady increase in interest in using magnetic resonance imaging (MRI) measures to monitor disease activity in multiple sclerosis (MS). While MRI unquestionably provides an important tool for studying the natural history of MS and is useful as a biological marker for disease in studies of disease mechanisms, the use of MRI as a surrogate outcome measure has been problematic. The stringent criteria for a validated surrogate that includes evidence that the surrogate predicts future levels of clinical disease activity and that the effect of various classes of

treatments on clinical disease can be accounted for by their effect on the surrogate outcome have not been met at any stage of MS. It is evident that MRI does reflect the underlying pathological conditions of the disease and in this regard may, under some conditions, be acceptable as an invalidated surrogate-outcome measure. In the early stages of relapsing-remitting MS, conventional imaging techniques, such as burden of disease on T2-weighted images and the number of contrast enhancing lesions, appear to have value but more advance imaging techniques will be needed to reflect the pathological changes leading to progression of the disease. The design of clinical trials using MRI as a primary outcome measure present important difficulties and possibilities for error. Future research will need to focus on how MRI can best be used to monitor disease in patients with MS.

Unraveling the Genetics of Alzheimer Disease

Rudolph Tanzi, PhD

Alzheimer disease (AD) is the most common cause of dementia in the elderly. While roughly 5% of AD occurs in people younger than 60 years of age, less than half of these cases are caused by autosomal dominant mutations in the β -amyloid protein precursor (*APP*) and presenilin (*PSEN1* and *PSEN2*) genes. For late-onset AD, a growing number of common public polymorphisms (CPP) appear to confer increased risk for AD but are neither necessary nor sufficient to cause AD. The most well-established, late-onset AD genetic risk factor is apolipoprotein E (APOE). In addition, we recently described CPPs in α_2 -macroglobulin (α_2 M) that confer increased risk for AD. While early-onset AD gene mutations increase the production of β -amyloid peptide, the principal component of senile plaques in the AD brain, CPPs in APOE and α_2 M influence the accumulation of β -amyloid peptide by modulating clearance/degradation and the rate of aggregation of the peptide into amyloid plaques. We have recently completed (with Center of Inherited Disease Research at National Institutes of Health, Bethesda, Md) a high resolution (9 cM) genome screen of more than 400 families to localize additional genetic risk factors for AD. Candidate genes localized in regions of chromosomal hits are being assessed primarily by family-based association techniques. The results of these ongoing analyses were discussed.

Biochemical Markers of Neurodegenerative Diseases: τ and Synucleins

John Trojanowski, MD, PhD

The aggregation of brain proteins into filamentous lesions is emerging as a common mechanistic theme in sporadic and hereditary neurodegenerative disorders, including Alzheimer disease (AD) and Par-

kinson disease (PD). For example, in the appropriate clinical setting, numerous telencephalic β -amyloid peptide-rich senile plaques and τ -rich neurofibrillary tangles (NFTs) are diagnostic of AD, while Lewy bodies (LBs) formed by α -synuclein (α -syn) in substantia nigra neurons are signatures of PD. However, filamentous τ lesions are the characteristic neuropathological feature of several other neurodegenerative disorders, referred to as *tauopathies*, while filamentous α -syn inclusions are hallmarks of another group of diverse neurodegenerative diseases known as *synucleinopathies*. Moreover, AD and PD commonly cooccur in the same patient, and α -syn is a major component of LBs in an AD-like cognitive disorder known as *dementia with LBs* and in the LB variant of AD. Further, α -syn positive LBs occur in more than 60% of familial AD brains and in more than 50% of Down syndrome brains, thereby linking mutations in the presenilin and β -amyloid precursor protein genes as well as trisomy 21 to the pathogenesis of α -syn lesions. Although the role of LBs and NFTs in the mechanisms of brain degeneration has been controversial for many years, the recent discovery of pathogenic α -syn gene mutations in familial PD as well as pathogenic τ -gene mutations in familial frontotemporal dementia with parkinsonism linked to chromosome 17 provides unequivocal evidence that abnormal α -syn and τ cause neurodegenerative disease. Clarification of how alterations in α -syn and τ genes and/or proteins lead to the onset/progression of synucleinopathies and tauopathies, respectively, could advance understanding of these disorders and the development of more effective therapies. This presentation reviewed recent insights into the pathobiological features of synucleinopathies and tauopathies that could be exploited for the development of diagnostic biomarkers of these 2 distinct categories of neurodegenerative disease.

Using Registration of Serial MRI to Measure Atrophy Progression in Alzheimer Disease and Related Disorders

Nick Fox, MD

The advent of therapies for Alzheimer disease (AD) and related disorders has increased interest in biological markers of early disease and in surrogate measures of disease progression. Magnetic resonance imaging (MRI) has potential in both these areas. Direct in vivo imaging of specific molecular pathological features (eg, β -amyloid plaques and neurofibrillary tangles in the AD brain) is not yet possible. However, MRI can be used to measure a surrogate marker of neuronal damage, that is, atrophy. Imaging studies have shown marked regional (eg, hippocampus and entorhinal cortex) and global (whole brain and ventricles) atrophies to be a consistent feature of AD. Because of the wide normal variation in cerebral structure, there is considerable overlap in these measures when mildly affected patients and matched controls are compared. Rates of atrophy from serial imaging allow individuals to form their own control avoiding problems of intersubject morphological variability.

Registration (positional matching) of serial MRI is now possible to subvoxel (0.2 to 0.4 mm) levels and permits direct measurement of rates of atrophy. The methods are automated and unbiased, independent of *ora priori* decisions about regions of interest, and avoid laborious manual measurements. Rates of cerebral loss derived in this way are highly reproducible (to 0.2% of brain volume), are significantly raised in AD ($2.5\% \pm 1.1\%/y$ vs controls $0.3\% \pm 0.4\%/y$), and are sensitive even in early cases. Rates of loss correlate with cognitive decline in untreated patients with AD. These techniques, together with volumetry, may prove useful both in diagnosis and in tracking disease progression.

Imaging in Parkinson Disease—a Window to Neurodegeneration, a Tool to Assess Neuroprotection?

Kenneth Marek, MD

Parkinson disease (PD) has been a model neurodegenerative disorder in which advances in neuroscience have informed, and in turn, have been informed by clinical neurology. *In vivo* imaging of the nigrostriatal dopamine system has provided a tool to bridge developments in basic neuroscience and clinical neurology and to identify markers for the chronic degenerative process in PD. During the last decade, neuroreceptor imaging has been used to monitor disease onset, severity, and progression, and the physiological conditions of the degenerative process. Several markers, most focused on the dopamine system, using positron emission tomography (PET) and single photon emission computed tomography (SPECT) technology have been used to assess PD. The 2 most mature imaging biomarkers are F-Dopa ($[^{18}\text{F}]$ -6-fluoro-L-Dopa) PET, which measures dopamine function, and $[^{123}\text{I}]$ β -CIT SPECT, which tags the dopamine transporter. In numerous studies, these ligands have demonstrated dopamine striatal deficits that identify symptomatic and presymptomatic subjects and that correlate with severity of PD. More recently, longitudinal imaging studies have shown that the dopamine loss in PD occurs at a rate of approximately 10%/y and have suggested that the preclinical phase of PD was 5 to 10 years. Ongoing studies are evaluating whether the rate of loss of F-Dopa or β -CIT differs in response to several putative neuroprotective or restorative therapies. These studies will further elucidate the use of neuroreceptor imaging as a biomarker or surrogate end point for disease progression. As genetic and environmental at-risk groups for PD are identified, imaging studies will also be crucial in establishing the onset of neurodegeneration and whether potential protective treatments are effective even prior to symptoms. In a slowly, but variably, progressive neurodegenerative disorder like PD, imaging may be particularly useful in establishing at-risk groups for rapid or slow progression of disease and assessing therapies directed at each group. As we are poised on

the brink of new protective and restorative therapies for PD, neuroimaging offers the potential to provide an objective end point for therapeutic trials on disease progression.

Can We Measure ALS?

Hiroshi Mitsumoto, MD

In dealing with a disease like amyotrophic lateral sclerosis (ALS) that has no fully known cause and no cure, clinical trials are becoming the sole vehicle of hope to identify effective treatments. The World Federation of Neurology Committee of Motor Neuron Disease has published revised diagnostic criteria for ALS and guidelines on the design and conduct of clinical trials in ALS. These criteria and guidelines are helpful since we have no diagnostic or surrogate markers in ALS. Given this, clinical trialists must rely on currently available measurement techniques, including survival rate, neuromuscular assessments, clinimetrics, and quality of life (QoL). Survival has been used in several clinical trials, such as riluzole, SR57746A, and new brain-derived neurotrophic factor (BDNF) trials, but this has inherent problems. Tufts quantitative neuromuscular evaluation (TQNE), including the maximal voluntary isometric muscle contraction (MVIC), expresses a direct clinical feature of ALS; however, it has not been able to demonstrate positive results in trials, such as ciliary neurotrophic factor (CNTF), BDNF, and gabapentin. The use of TQNE remains important, though in investigator-driven trials. Among all measurement techniques, forced or slow vital capacity and ALS functional rating scale (ALSFRS) appear the most universally accepted evaluation techniques in ALS trials. For QoL measurements, only generic QoL instruments have been available until recently. Now ALS-specific QoL tools, such as the ALS assessment questionnaire-40 (ALSAQ-40), have just been introduced.

The use of objective quantitative measurements of lower motor neuron and upper motor neuron functions are imperative to directing the focus of clinical trials in ALS. Motor unit number estimate (MUNE) has been developed by 2 fundamentally different concepts and methodologies: statistical methods and multipoint stimulation methods. Although there are pros and cons for these 2 techniques, the importance of these measurement techniques in clinical trials must be emphasized. For measuring upper motor neuron function, we introduce the use of 2 novel methods. One is magnetic resonance spectroscopy imaging (MRSI), demonstrating a relative or absolute amount of *N*-acetyl aspartate, analyzing the anatomical neuronal (neurons and neuritis) tissue in the brain; the other is transcranial magnetic stimulation (TMS), analyzing electrophysiological excitability and descending tract integrity. Further studies of these 2 techniques are absolutely essential to establish the reliability and validity in assessing the disease evolution in ALS. In the near future, we should be able to measure ALS more

objectively than before for identifying therapeutic agents for this disease.

Gene Expression Monitoring in Central and Peripheral Nervous System Injury

A. A. Cameron, W. Huang, J. Chang, L. Pham, C. R. Ill, W. Young, D. J. Carlo

Trauma to the central nervous system (CNS) and peripheral nervous system (PNS) cause an inflammatory response that is dichotomous and may support or inhibit subsequent regenerative capacity. However, very little is known about the patterns of gene expression in the injured tissues, nuclei, or ganglia. We have used complementary DNA microarrays to monitor gene expression in models of peripheral nerve regeneration and spinal cord trauma and in peripheral and central glia. The expression profile of the regenerating dorsal root ganglion (DRG) showed a number of gene groups whose function is consistent with metabolic activation and neurite elongation. The absence of cell cycle-related gene activation and tissue mitosis in the DRG suggests the existence of a modified form of the cell cycle, the "regeneration cycle." In addition, a cluster of 111 genes up-regulated in the DRG were identical to genes down-regulated in activated Schwann cells (and a subset of oligodendrocytes), indicating that components of the gene-expression program are highly conserved and can be reciprocally regulated. The gene expression profile in the injured spinal cord following methylprednisolone treatment is consistent with the reduction in messenger RNA and protein synthesis levels and the provision of neuroprotection through the up-regulation of genes encoding antioxidants, growth factors, and matrix proteins. Gene-expression monitoring is useful in characterization of the genetic response following PNS or CNS trauma and for the identification of leads for the development of novel therapeutic targets.

Electrophysiological Surrogate Markers in Clinical Development of CNS Active Compounds

F. D'Aniello, T. Lincker, R. Luthringer, M. Toussaint, P. Boeyjinga, L. Staner, J. P. Macher

Considering that to optimize drug development, it is important to anticipate go/no-go decisions. Our aim is to show the usefulness of electrophysiological markers in early clinical phase decision-making studies. In particular, our purpose is to illustrate the usefulness of wake electroencephalography (wake EEG) and polysomnography (sleep EEG) techniques in optimizing central nervous system

(CNS) drug development by giving an overview of some phase 1/2a study results in which these techniques were applied. The possibility of defining at the earliest time the potential of the CNS active compound passing the blood-brain barrier, if it does (its minimal active dose and dose/time effects), is addressed. The ways to obtain more powerful assessments of its pharmacological activity and pharmacodynamic profile are also shown as well as inputs toward potential therapeutic indications. Finally, to show the predictive power of these techniques, the link between the surrogate markers, and the clinical outcomes are examined. Examples of discriminating between electrophysiological techniques based on their relative advantages and applications are given depending on the characteristics of the compound and aims of the study. In addition to the above mentioned uses, electrophysiological markers may also provide an important warning of possible adverse effects and use in diagnosis of CNS diseases.

Generation of Human Neural Stem Cell Lines and Brain Transplantation in Animal Models of Parkinson Disease and Multiple Sclerosis

Seung U. Kim, Eiji Nakagawa, Atsushi Nagai, Evan Y. Snyder, Jai K. Ryu, Jin Kim, Myong A. Lee, In H. Mook, Byong K. Jin

Neural stem cells (NSCs) of the central nervous system (CNS) have recently aroused a great deal of interest not only because of their importance in basic research of neural development but also for their therapeutic potential for neurological diseases. Previously we have produced immortalized cell lines of human NSCs (Flax, et al. *Nat Biotech.* 1998;16:1033). Recently we have produced several more clones of human NSC lines via retrovirus-mediated v-myc transfer into embryonic human telencephalon cells. One of the NSC lines, HB 1/C4, is maintained as a stable cell line and remains uncommitted, undifferentiated, multipotent, and express nestin and vimentin, which are phenotypes specific for NSCs. Immortalized human NSCs have clinical utility for cell replacement and gene therapy for patients suffering from degenerative, developmental, and acquired neurological diseases. The HB 1/C4 cells carrying β -gal gene were stereotaxically implanted into brains of parkinsonian model rats and of myelin-mutant shiverer mice. Two to 6 weeks postoperation, implanted human NSCs were found to migrate extensively, integrate into host brain, and develop into neurons and/or glial cells as shown by immunohistochemistry. These results indicate that human NSCs are capable of survival, migration, and differentiation in animal models of Parkinson disease and multiple sclerosis. (Supported by the Canadian Myelin Research Initiative, Mississauga, Ontario, and Korea Social Engineering Foundation, South Korea.)

Transdermal Delivery of Interferon beta-1a by Transfersomes and Its Effects on Surrogate Markers

B. Scorneaux, J. Lehmann, G. Payne, G. Cevc, M. Rother

Noninvasive delivery of interferon-beta (IFN- β) may reduce adverse events at the injection site, including necrosis, reduce systemic adverse effects potentially linked to the pharmacokinetics after injection, be less immunogenic (less neutralizing antibodies), and increase patient acceptance. Transfersomes (Tfs) were used to deliver IFN- β transdermally. Transfersomes are novel self-regulating drug carriers that can deliver macromolecules across the intact skin (Cevc, et al. *J Controlled Release*. 1995;36:3-16). The main objective of this pilot study was to investigate if IFN- β associated with Tfs produces systemic levels of IFN- β . The second aim was to study surrogate markers for drug effects (serum β_2 -microglobulin and urine neopterin) and adverse effects (body temperature). Normal pigs were treated with a single epicutaneous dose of 4.6×10^6 U/kg body weight IFN- β in Tfs, or with 3.8×10^5 U/kg body weight IFN- β solution subcutaneously. This was compared to empty vehicles epicutaneously and subcutaneously. Interferon- β Tfs produced plasma levels with a Cmax (peak arterial plasma concentrations) of 6 hours as compared with 2 hours for subcutaneous injection. The area under the curve (AUC) was lower for epicutaneous application by a factor of about 3. Interferon- β Tfs produced comparably higher amounts of neopterin and β_2 -microglobulin, indicating a better biopotency; in contrast, IFN- β Tfs were less pyrogenic. The results of this study indicate that Tf-mediated transdermal IFN- β delivery could become a valuable alternative to subcutaneous injection. More recent work using IFN- α Tfs showed that the bioavailability could be substantially increased by an improved formulation.

Transplantation of Human Expanded Progenitors From Midbrain and Neocortex Into a Rat Model of Parkinson Disease

R. Sánchez-Pernaute, L. Studer, Conrad Messam, K. S. Bankiewicz, R. D. G. McKay

The use of in vitro expanded precursors of the central nervous system (CNS) might overcome some of the current limitations for neural transplantation. Rat midbrain precursors can be proliferated in vitro

and differentiated into functional dopaminergic (DA) neurons capable to compensate the motor asymmetry induced by 6-hydroxydopamine in a rat model of Parkinson disease (Studer, et al. *Nat Neurosci*. 1998;1:290-295). In this model, we have now examined the survival and differentiation of human expanded precursors transplanted into the denervated striatum. Human precursors from the mesencephalon gave rise to TH positive (+) neurons in vitro and in vivo. Six weeks after transplantation, the number of TH+ positive neurons in the graft was correlated with the degree of recovery of rotational behavior following amphetamine stimulation. Furthermore, cortical progenitors could also be differentiated into TH+ neurons in vitro, and they partly maintained this phenotype after transplantation. These results provide additional basis for future clinical applications of in vitro expanded CNS precursors.

Dose-Dependent Neuroprotection of Taigabine After 2-Hour Middle Cerebral Artery Embolization in the Rat

Y. Yang, Q. Li, C. X. Wang, A. Shuaib

γ -Aminobutyric acid (GABA) is a potent and ubiquitous inhibitor in the central neuronal system (CNS), and enhancement of its inhibitory activity may protect ischemic neurons. In a current study, we evaluate the neuroprotective effect of a novel GABA agonist, taigabine, in a reversible focal cerebral ischemia model of rats subjected to 2-hour middle cerebral artery embolization with a filament. Taigabine was given at 10, 20, and 40 mg/kg intraperitoneally at 1 hour after the onset of reperfusion. Neurobehavioral outcome was examined at 1 hour and 24 hours, respectively, after reperfusion. The percentage of brain infarction volume was calculated from the coronal brain sections, which were stained with 2,3,5-triphenyltetrazolium chloride (TTC) at 72 hours after cerebral ischemia. Significant neurological improvement was observed only in animals treated with taigabine at a dose of 20 or 40 mg/kg (both $P < .05$). Postischemic treatment of taigabine displayed a dose-dependent reduction in brain infarct size, but only taigabine given at 20 and 40 mg/kg showed significant differences when compared with that of the control group (control, 28.7 ± 11.0 ; 10 mg/kg, 19.5 ± 10.8 , $P = .07$; 20 mg/kg, 12.3 ± 9.7 , $P = .006$; and 40 mg/kg, $P < .001$). The results from this study suggest that postischemic administration of taigabine is neuroprotective in the focal cerebral ischemia model.