Biomarkers and Vasospasm After Aneurysmal Subarachnoid Hemorrhage

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Subarachnoid hemorrhage (SAH) from the rupture of saccular aneurysms (nontraumatic SAH) is a devastating and often fatal disease. Its incidence is 6 to 8 per 100,000 people years.[1] Even with the advancement and increased availability of neurosurgery and intensive care, the morbidity and mortality from aneurysmal SAH remains high. Prior to hospitalization, 12% of those with SAH die while approximately 40% of those surviving to hospitalization do not survive the first 30 days. These figures contribute to an estimated case-fatality rate of 25% to 50%.[2,3] The most common cause of death after survival of the initial hemorrhage is vasospasm. SAH is most frequently related to trauma. However, it is rarely a singular cause of fatal outcomes in traumatic brain injury and is not commonly associated with the medical complications of SAH, although this has been debated recently.[4] SAH from intracranial vascular rupture can be classified into two groups: those resulting from the rupture of a berry or saccular aneurysm, which represent 80% of nontraumatic SAH, and those associated with vascular malformations, such as arterial venous malformations, which represent 20% of all nontraumatic SAH.[2] The classical medical complications associated with SAH, such as vasospasm and cerebral salt wasting, are usually associated with the rupture of saccular aneurysms and are further discussed in this article.[5]

Vasospasm is the most common complication of SAH that leads to clinical deterioration. However, the molecular basis and pathophysiology are still poorly understood. Up to 70% of patients who survive SAH develop signs of vessel narrowing on transcranial Doppler ultrasound or angiography while clinically symptomatic vasospasm occurs in only 46% of those patients.[2,6,7] Rates of infarction after SAH are estimated to be approximately 25%.[8-11] Vasospasm can be defined both angiographically and clinically. Angiographic definition requires visualization of vessel narrowing in the affected vessel by angiography, while clinical vasospasm requires observation of the physical signs and symptoms of vasospasm. Classic symptoms of clinical vasospasm include altered levels of consciousness, confusion, focal motor symptoms, and new-onset aphasia. These symptoms typically develop over a period of hours to days and are referred to as delayed ischemic neurological deficits (DINDs).[2,12] In the early days of subarachnoid surgery, most of the delayed neurological deficits were attributed to progressive cerebral edema from the initial damage of the hemorrhage or from the surgery itself. However, as time passed, it has become clear

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that DINDs are occurring independently of the initial hemorrhage or surgery.[12]

The proximal event leading to DIND still remains unclear. However, two facts are well established: (1) DIND is the result of tissue ischemia and represents a form of ischemic stroke and (2) DIND occurs in vascular territories where vasoconstriction has been documented angiographically. These observations strongly suggest a causal relationship between vascular constriction and ischemia, although in recent studies it has become less clear that the primary event causing ischemia and cell death is the constriction of the associated blood vessel.[13] It is possible that vascular constriction may be an epiphenomena or a contributing factor to parenchymal destruction and that the final ischemic event may be caused by other factors occurring directly within the parenchyma at the level of the neurovascular unit. This can be loosely supported by the results of trials, such as the Neurosurgery Cooperative Trial, which used the calcium channel blocker nicardipine, and the CONSCIOUS-1 Trial (Clazosentan to Overcome Neurological Ischemia and Infarction Occurring after Subarachnoid Hemorrhage–1 Trial), which tested the endothelin antagonist clazosentan.[14] While both nicardipine and clazosentan prevented vasospasm, neither was shown to prevent ischemia or DIND. Mechanical intervention with angioplasty, though successful in reversing the narrowing of the vessel, also failed to prevent DIND when performed at the onset of symptoms or prophylactically before the symptoms develop.[15] These interventions demonstrated efficacy in reducing the occurrence of angiographic vasospasm. However, they did not reduce the incidence of DINDs.[14,16]

The failure of new drugs and techniques to protect against DINDs has spurred research to isolate proteins that may affect the development of vasospasm. These proteins represent potential therapeutic targets as well as markers of vasospasm onset. The use of proteomics and translational research to understand vasospasm has a long history and understanding the current techniques can aid clinicians interested in furthering vasospasm research or in applying the newest clinical techniques to aid in the care of patients with vasospasm from aneurysmal SAH.

BASICS OF THE HUMAN PROTEOME

Human proteomics is the study of human proteins in the context of three realms: drug discovery, proteome mapping, and biologic understanding.[17] A number of new scientific techniques have been developed that allow for the identification of proteins from a number of human biologic tissues.[18] This material, usually collected in a specific clinical context, gives researchers a glimpse of the activity occurring as the direct result of disease. The study of proteomics in vasospasm and DIND could potentially help identify a variety of biomarkers from diverse tissue sources, including serum, cerebrospinal fluid (CSF), and extracellular parenchymal fluid. Vasospasm is a molecularly based phenomenon that involves complex cellular signaling. The identification of proteins associated with different stages of disease progression may enable the isolation of specific protein signals. The understanding of vasospasm through proteome analysis requires data collected in the setting of well-described clinical phenotypes.

The characterization of the human genome has produced a wave of information that has made it possible to estimate the number of genes contributing to the human proteome. Thus far, 19,438 genes have been identified with an additional 2188 predicted genes based on data from the completed human genome study. Thus far, 34,124 transcripts have been isolated, contributing to more than 100,000 proteins. Each gene can code for an estimated 10 to 20 different protein species. Thus the human proteome is vast with many potential targets.[17]

TISSUE SAMPLING

During the treatment of aneurysmal SAH, various parenchymal sites can be harvested for proteomic analysis. These include solid tissue, such as brain parenchyma; tissue approximating the vascular substructure, such as adventitia; and sections of vessel affected by aneurysmal rupture. Each of these tissue types requires sacrifice in the setting of surgical intervention and is rarely obtained in normal practice. More commonly acquired and easily accessible tissue types include blood, CSF, and extracellular fluid from cerebral microdialysis. Blood is a tissue with some characteristics of fluid. However it is actually a complex conglomerate more similar to that of a colloidal suspension. It potentially contains protein or genetic material from any compartment of the human body. Its proteome has not yet been completely characterized and is probably the largest of any tissue compartment in the body. The most commonly analyzed portion of human blood is serum and within it are tens of thousands of analytic targets. Injury or changes in any compartment of the human body are usually reflected in the proteome of blood. Thus blood is the most commonly accessed source for protein analysis in any clinical scenario.[18]

CSF and extracellular fluid are the next two most commonly tested sources of body fluid for protein analysis in the setting of SAH. CSF studies of proteins in the context of vasospasm are relatively common. The protein concentration of CSF is 400 times less than blood. About 2600 proteins have been identified in CSF at this time and a proteome map of CSF is available from several sources (http://www.expasy.ch/).[19,20] Extracellular fluid is often difficult to obtain as it requires the placement of a cerebral microdialysis catheter and yields only small amounts of fluid. However, its use is more frequent now as microdialysis catheters are increasingly placed for monitoring ischemic stress in the setting of brain injury. Studies looking at extracellular fluid in the setting of SAH and vasospasm are still limited while study of the extracellular fluid proteome is incomplete. A number of peptides have been identified by mass spectroscopy and these peptides represent 27 individual proteins. All of these proteins appear to represent proteins derived from CSF.[21–23]

Proteins isolated from blood exist in a background of great protein diversity represented by vast numbers of different protein types. It is estimated that 22 proteins represent 99% of the protein content of the blood. Thus, protein concentration in blood varies extensively, spanning 10 orders of magnitude. This dynamic range makes detection of a low concentration of small proteins specifically related to a single injury quite difficult. The dynamic range of mass spectroscopy, the most common means of identifying proteins in blood, is 2 orders of magnitude, not the 10 required to encompass the proteome. The situation is further complicated in that the proteins of highest interest tend to be those occurring at lower concentrations and are not the common constituents of blood. The solution to this problem requires both protein isolation and concentration with the goal of the purification and selection of specific components of blood.

The problem of dynamic range exists with CSF and extracellular fluid to a much lesser degree. The decreased number of proteins and the relative lower total concentration of protein lead to a decrease in the dynamic range and makes for more efficient protein isolation and concentration. However, there is still a wide dynamic range and the challenge of detecting proteins of interest in small samples with very low concentrations is still a major roadblock, particularly with the present technology.[18]

PROTEIN ISOLATION

The first stage of proteome analysis requires the isolation of individual proteins. The most commonly applied technique for protein isolation is two-dimensional gel electrophoresis. This technique uses a two-stage process, including pH immobilization with isoelectric focusing, followed by standard polyacrylamide gel electrophoresis. The proteins can then be visualized using special stains or immunoblotting techniques. Other important protein isolation technologies include affinity chromatography, ion-exchange chromatography, reversed-phase liquid chromatography, and capillary electrophoresis. Chromatography using all mediums is a popular method to isolate proteins. Such techniques as high-performance liquid chromatography are common and effective. Isotope-coded affinity tagging is another technique that modifies liquid or gas chromatography to isolate proteins. Isotope-coded affinity tagging incorporates a thiol-specific reactive group, with a radioactive marker containing an affinity tag, such as biotin. These markers bind to predetermined protein moieties in specific proteins, enabling isolation with traditional methods, such as high-performance liquid chromatography. Other gel-free proteomic technologies, such as capillary liquid chromatography and capillary electrophoresis, enable the isolation and identification of extremely small protein samples in a liquid or gas state.[24]

PROTEIN IDENTIFICATION

The time and effort required to identify proteins are determined in part by the abundance of the target of interest, as well as its purity, size, and stability during the purification process. The most commonly employed technique for definitive protein identification is mass spectrometry. Mass spectrometry employs protein ionization technology to produce ionized peptide fragments, from which mass-to-charge ratios are determined. Proteins are first eluted from a reversed phase column and then placed into the mass spectrometer. This is followed by chemical ionization and a mass-to-charge ratio determination for all of the individual peptides in the mixture of proteins. The tandem mass spectrometry technique is also frequently employed in protein identification. Individual ionized particles are isolated in time or space and then further fragmented by ionization. The resulting ionized products are further analyzed for their mass-to-charge ratio, resulting in a spectra, or collection, of masses for a given sample. The resulting spectra can then be compared to data from a large library of previously identified proteins. Most mass spectrometers can process approximately 7000 spectra per hour yielding identifiable proteins 10% of the time,

enabling the identification of up to 700 to 1400 proteins an hour.[18]

Variations of quantitative mass spectroscopy methods have been developed, each with its own advantage and disadvantage.[25] The most common types are single or double mass spectroscopy, as previously discussed. However, this technique is limited in that it can isolate and identify only a few proteins from a given sample. Other mass spectroscopy techniques include electrospray ionization, which incorporates a coulombic pressure methodology to isolate single ionized columns of macromolecules in the setting of an aerosolized mixture. This enables greater protein isolation. Fourier transform ion cyclotron resonance mass spectrometry is another highly accurate mass spectroscopy technique. Especially suitable for samples with extremely low concentrations, this technique examines the cyclotron frequency of the charged macromolecule in a fixed magnetic field to determine mass-to-charge ratios.[26]

For high-throughput protein analysis, approaches to proteome analysis now combine protein isolation with protein identification via mass spectroscopy. These methods can be used in situations in which the tissue examined has a large dynamic range. Surface-enhanced laser desorption/ionization–time-of-flight (SELDI-TOF) mass spectrometry isolates proteins by using a stage that incorporates protein binding to a surface plate.[27] To selectively immobilize the target to the surface plate, this plate can be coated with antibodies or other substrates that interact with the sample. The surface is then energized with a laser and the ionized proteins are passed through an electric field and the time of flight is measured, which can be used to determine the mass-to-charge ratio. This method enables mass spectroscopy signaling and often direct protein identification.[26] This technology can be used to produce signatures that incorporate mass spectroscopy and time-of-flight ion analysis, which enables the identification of unique patterns that can be associated with specific diseases. These unique signatures have been used to identify specific disease states, such as cancer. One of the newer variants of SELDI is the matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) technique. This technique incorporates traditional SELDI technology with greater protein preparation, as the difference lies in the sample pretreatment. The protein is placed in a solid matrix instead of on a surface plate prior to ionization. This method makes it possible to more precisely control energy delivery. It also uses the characteristics of the matrix to enable better protein isolation through a high-throughput methodology that combines protein purification with precise protein identification.[26]

Other common methods of protein identification are enzyme-linked immunosorbent assay (ELISA) and luminex. These techniques use immunoprecipitation to isolate and purify proteins from various sources. ELISA is an older technique and often requires large quantities of proteins.[28,29] Each ELISA processing run requires recalibration and restandardization, which introduces variability. Furthermore, the volume of sample required is quite large and is typically an order of magnitude greater than that of other available technologies. Luminex uses newer technology and provides several benefits, including the ability to identify proteins from smaller samples, to run a larger number of samples within each batch and to reduce the variability between batches.

Protein microarrays are another method for protein isolation and identification.[30] These techniques employ a high-throughput methodology analyzing hundreds to thousands of samples at once. Arrays with specific markers for proteins or protein moieties are employed in a chip format. This technology enables the identification of thousands of proteins in a single chip and requires only microliters of sample.

Ultimately any protein isolated in sufficient quantity can be identified through protein sequencing. All methods employed in protein sequencing require highly purified samples in relatively large quantities. This approach is reserved for proteins and polypeptides that have not yet been characterized or for which precise characterization is needed for drug development or biological understanding.

BIOMARKERS AND MECHANISTIC APPROACHES TO VASOSPASM

The search for biomarkers in SAH and vasospasm has been proceeding for decades. A remarkably large literature focused on the analysis of biomarkers in the context of human tissue and SAH has been developed. At different times various techniques and theoretical perspectives have been employed. Each approach has been based on a theoretical understanding as to the underlying mechanisms of vasospasm. Different approaches have evolved with developments in molecular biology. The first and perhaps most successful mechanistic approach emphasizes moderators of inflammation.

Inflammation

Inflammation has become a focus of research in vasospasm as many observations have confirmed

this direct link. It has been demonstrated that leukocytosis and fever are associated with worse outcomes in SAH.[31–33] Increased protein levels of several of the cellular adhesion molecules, including ICAM-1, VCAM-1, and the integrins, are associated with the development of vasospasm and worse clinical outcomes. Higher levels of these molecules have been measured in both the serum and CSF of patients who develop vasospasm.[34–40] Other inflammatory markers, including the cytokine interleukin (IL) 2 (IL-2), IL-2–receptor antagonists, and soluble CD8 have been sampled in the CSF of patients with SAH and vasospasm and are elevated as compared to controls. However, they are not predictive of vasospasm.[41] Furthermore, IL-6 and monocyte chemotactic protein–1 are higher in the CSF of patients who develop vasospasm.[42] IL-8 and E-selectin are inflammatory markers that have been measured in the ventricular cisterns of patients with ruptured and nonruptured aneurysms. IL-6 levels are significantly elevated in the blood of patients with SAH in general.[41,43,44] Complement has been measured using standard radioimmuno-assays in the serum and CSF of patients with vasospasm. These studies have demonstrated increased levels of the C3 and C4a components of the complement cascade in the early stages of SAH. These levels decline after the first few days.[45] Furthermore, CH50, C3, and C4 complement levels have been measured in serum of SAH patients and their levels have been predictive of vasospasm. After 5 to 10 days, however, serum complement levels were depleted in patients who went on to develop vasospasm.[46] Other mediators of inflammation, such as arachidonic acid metabolites, have also been examined. To date, no study has documented an association with prostacycline or thromboxane in any serological compartment.[2] However, 20-hydroxyeicosatetraenic acid, a metabolite of arachidonic acid, is elevated in the CSF of humans and animals with vasospasm.[47] Chitotriosidase, a human chitinase member of family 18 glycosyl-hydrolases and selectively secreted by activated macrophages, has been measured in the CSF and serum of humans with SAH. Mean CSF chitotriosidase levels were found to be higher on days 5 and 7 of SAH. The serum levels were higher than controls at all times in SAH patients although no relationship between symptomatic vasospasm and outcome was observed.[48]

Endothelial Activation

Endothelial activation is an important consideration in the setting of ischemic disease. Concentrations of soluble ICAM-1, soluble P-selectin, soluble E-selectin, and ED1-fibronectin are traditional markers of endothelial activation. Their levels have been sequentially measured throughout the course of treatment of patients with SAH. Concentrations of ICAM-1, soluble E-selectin, soluble P-selectin, ED1-fibronectin, von Willebrand Factor (vWf), and vWf propeptide have been measured in the serum of patients within 3 days of the onset of SAH to determine if any of the markers predict outcome. Early elevated vWf concentrations have been associated with poor outcome and the occurrence of ischemia. No other marker of endothelial activation has been associated with ischemia or poor outcome.[49] This positive association of raised vWf concentrations with DINDs may reflect a predisposition to further ischemic injury via the formation of microthrombi in the cerebral circulation secondary to endothelial activation. Further studies are needed to confirm these findings and to better understand the molecular basis of this relationship.

Hemoglobin Degradation

Undoubtedly the breakdown products of red blood cells and hemoglobin induce vasospasm as it has been well demonstrated that blood volume plays a role in the risk of vasospasm in animal models and in human observation studies.[50,51] It has also been shown that removal of the blood from the cisternal space facilitates a reduced risk of vasospasm.[13] Deoxyhemoglobin is the most likely irritant triggering vasospasm. Oxyhemoglobin is the most vasoactive substance in subarachnoid blood and its concentration in CSF mirrors the time course of vasospasm.[52–54] Free radicals, formed as a result of the toxic effects of subarachnoid blood and hemoglobin degradation, have been hypothesized to mediate many aspects of the pathophysiology of vasospasm. Emerging research implicates lipid peroxidation in addition to hemoglobin degradation byproducts in triggering vasospasm.[55] The hydroxide generated in the process of lipid peroxidation acts as a vasoconstrictor and, furthermore, 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and other lipid byproducts initiate vasospastic physiology. Their CSF levels have been associated with an increased risk of vasospasm.[56] Measurements of cholesteryl ester hydroperoxides (CEOOH), which are generated during lipid peroxidation in plasma, may provide prognostic value. Plasma levels of CEOOH are elevated and peak 5 days after the onset of SAH. Furthermore, increased levels of CEOOH are associated with

an increased mortality, are associated with an increased risk of vasospasm, and correlate with clinical outcome scales.[57]

Calcium Metabolism

Vasospasm and vasoconstriction are abnormal prolonged contractions of the smooth muscles of the vasculature, resulting in the sustained narrowing of the artery. Calcium plays a central and integral role in this process.[2,58] The contraction of the vessel walls involves the release of cytosolic calcium from a cellular reservoir, which leads to the contraction of smooth muscle in a sustained manner for prolonged periods. This can occur in a calcium dependent or independent manner. Many people have suggested that vasospasm stems from continuous elevation of cytosolic calcium, which causes sustained contraction of smooth muscle cells.[59] CSF levels of intracellular species of proteins that bind calcium are lower in individuals with symptomatic vasospasm.[60] Additionally, increased phosphorylation of key enzymes regulating cytosolic calcium reserves could also contribute to vasoconstriction in the setting of vasospasm.[61–64] The roles of protein kinase C–, calponin-, and caldesmon-based mechanisms, as well as mechanisms mediated through mitogen-activated protein kinase, have been examined.[44,65,66] To date, no proteomic approach has been applied to detect changes in the serum, CSF, or extracellular fluid level of any of these proteins. Levels of such proteins as calponin and caldesmon are potential markers of increased vasoconstriction mediating the prolonged contraction of smooth muscle. Phosphorylated-phosphorylated myosin light chain kinase is another potential protein marker for prolonged and sustained constriction of smooth muscle cells and merits further study.

Nitric Oxide

Endothelial relaxing factors, such as nitric oxide (NO) or NO-containing compounds, play a central role in the mediation of vasoconstriction in vasospasm.[2] A variety of NO donors in humans and animals have demonstrated efficacy in the treatment of vasospasm and suggest that NO is a key player in vasoconstriction leading to clinical vasospasm.[67,68] NO is a diffusible free radical gas with a half-life of seconds. To date, no efficient method exists for precisely detecting and measuring NO in vivo. The findings of several studies reporting temporal changes in CSF total nitrite/nitrate (NOx) levels after SAH vary considerably. Many have focused on detection of its metabolites, yet even these are difficult to measure because of their very short half-lives. The total nitrite/nitrate concentration has been determined by a vanadium-based assay with the colorimetric Griess reaction along with CSF oxyhemoglobin level as assessed by spectrophotometry. After an initial peak within the first 24 hours after SAH, CSF NOx decreases gradually and there is a significant correlation between CSF concentrations of NOx and levels of oxyhemoglobin. It has been demonstrated that patients with very good outcome have significantly lower CSF NOx than those with a worse outcome.[69]

Endothelin

Endothelin, a 21–amino acid polypeptide, is a potent vasoconstrictor and has been implicated in the pathophysiology of vasospasm. Endothelin 1 has been found to be elevated in the CSF of patients with vasospasm.[70,71] Two clinical trials have suggested efficacy for endothelin antagonists in the reduction of the observed vasoconstriction seen on angiograms. However, there was no significant reduction of DINDs.[14,72] Endothelin has been measured in the CSF and serum using a variety of methods and its level correlates closely with the time course of vasospasm in virtually all mediums measured.[71,73–75] Endothelin acts as the counterbalance to NO. However, its effects are long-acting than those of NO. Increases in endothelin's efficacy would have a profound effect on the duration and degree of vasoconstriction. Evidence related to endothelin could indicate that the transition of the artery of normal tone to its constricted state stems from a disruption of its normal physiologic state. A promising goal for the treatment of cerebral vasospasm would be to determine effective measures to regulate these two systems. Further basic and translational research is needed.

The Coagulation Cascade

SAH is associated with a consumptive coagulopathy. D-dimers and other fibrin split products are elevated in the serum and CSF of patients with SAH. Fibrin, fibrin degradation products, fibrinopeptide A (FPA), and other coagulant byproducts have been measured in the CSF of affected patients and these proteins act as markers of activation of the coagulation cascade. FPA has been selectively studied in patients with SAH with an initial elevation on days 0 to 1. However, this is followed by a subsequent decline over the ensuing days.[76] The mean level of FPA is higher in patients with higher Fischer grade SAH and this corresponds to the risk of vasospasm. Furthermore, a significant increase in the FPA level of patients

with infarction has been observed. Patients with decreased blood clearance on computed tomography scan demonstrate a significantly higher rate of infarction and a higher level of FPA. These results suggest that the thrombin activity present in CSF correlates with increasing Fischer grade, faster clearance of blood on computed tomography scan, and the development of vasospasm.[76]

RECENT OBSERVATIONS

Recent advances in proteomics have yielded the discovery of new proteins that act as markers of brain injury in the setting of SAH and vasospasm. These approaches have identified new protein candidates and have aided in elucidating the molecular basis for vasospasm induced by SAH. Furthermore, these findings may provide new potential targets for the development of therapeutics and interventions aimed at preventing vasospasm. Research has focused on molecules that are markers for damage to different cellular components, such as neurons or axons. Recent analysis of the extracellular fluid from the frontotemporal lobes of a few SAH patients with vasospasm identified glyceraldehyde-3-phosphate as a protein candidate as a predictor of vasospasm. Several isoforms of glyceraldehyde-3-phosphate were elevated prior to the development of vasospasm while protein heat-shock cognate 71 kDa protein was decreased prior to vasospasm. These changes were observed at least 4 days prior to the onset of vasospasm.[77]

Neuronal Damage

Serum levels of S100-B and neuron-specific enolase have been examined in the setting of SAH. Levels of both of these proteins are known to correlate with outcome in head injury and stroke and are markers of neuronal injury. Oertel and colleagues[75] conducted a study to determine if the serum levels of S-100B and neuron-specific enolase could predict the development of vasospasm and outcome within the first 3 days after SAH. S-100B levels were significantly higher in those patients who did not develop vasospasm and also significantly higher in those patients who died, compared to those who survived no matter if their outcome was unfavorable or favorable. For the first 3 days after SAH, measurements of S-100B were identified as potential markers useful to predict death and the onset of vasospasm but did not distinguish favorable versus unfavorable outcomes for survivors.

Astrocytic Damage

Serum glial fibrillary acidic protein (s-GFAP) is a protein produced by the astrocytes. It is thought to be a marker of the degradation of the blood-brain barrier as well as damage to astrocytes. Knowing that s-GFAP concentrations are increased after stroke, Nylen and colleagues[78,79] prospectively collected serum samples of patients with SAH to determine if levels would correlate with brain injury and clinical outcomes. Serum samples were obtained over a 2-week interval from 116 adults after aneurysmal SAH and analyzed for s-GFAP levels. Increased s-GFAP levels were seen in 81 of these 116 patients. The maximum measured s-GFAP levels correlated with World Federation of Neurological Surgeons scale on arrival as well as on days 10 to 15. Maximum s-GFAP levels were increased in the patient group with radiological signs of focal lesions in the acute setting or at 1-year follow-up, compared with the group without focal lesions. Most importantly, maximum s-GFAP correlated with a worse clinical outcome.

Axonal Damage

Axonal damage occurs in the setting of SAH and serological protein markers specific for damage to the axons have been detected in patients with SAH. Patients with SAH have secondary axonal degeneration, which may adversely affect their outcome. Therefore, studies were aimed to determine if concentrations of axonal proteins, such as the neurofilament heavy chain (NfH) SMI35, are altered in SAH and whether these levels correlate with clinical outcome. Ventricular CSF was collected daily for up to 14 days in a longitudinal study of patients with aneurysmal SAH and the NfH SMI35 was quantified. The primary outcome measure was the Glasgow Outcome Score at 3 months. Of 148 samples from patients with SAH, pathologically high NfH levels in the CSF were found in 78 (52.7%) samples, compared with 20 (5%) of 416 samples from the reference population. A pathological increase in NfH was observed in all patients with a bad outcome. This increase typically became significant 7 days after the hemorrhage. The result was confirmed by analyzing the individual mean NfH concentrations in the CSF, and was reinforced by the inverse correlation of NfH in the CSF with the Glasgow Outcome Score.[80]

SUMMARY

Proteomics now offers an array of advanced technologies that enable the identification of small

quantities of proteins from small samples. As these technologies are further developed, a major challenge will be to address the issue of dynamic variability. Other important factors hampering protein identification are the limitations of techniques for the sequestration and concentration of proteins of interest. Such techniques as two-dimensional gel electrophoresis and chromatography of all types are now becoming the focus of efforts to improve protein concentration and separation. Other technological breakthroughs, such as SELDI, MALDI, and protein microarrays, will play roles in the proteomics of vasospasm and help to more easily isolate and identify key proteins involved in vasospasm after SAH.

Prior to the isolation and purification of proteins, one must ensure that the compartment chosen for obtaining samples is the most biologically plausible. Different proteins from different tissue and cellular compartments can help identify proteins that are strongly related to very specific pathological processes, such as vasospasm. These proteins could be nuclear proteins derived from the nucleus of damaged cells and representing signaling characteristics specifically associated with the development of vasospasm. They could be proteins from the axons of cells representing axonal damage from prolonged ischemia. Other compartments include the vascular endothelium, the subintimal zone, and the muscularis layer. All of these are sites represent potential sources of proteins of interest that may be biomarkers or therapeutic targets in the setting of vasospasm.

Although the field of proteomics has achieved the goal of enabling the isolation and identification of targets from small samples, an even more important goal is the perfection of technology to ensure the proper sample selection from a biologically precise and generalized source. To achieve this requires the optimal design of clinical and translational studies aimed specifically at identifying protein substrates in the correct clinical setting. For SAH, this has been a major effort in recent years. The most import variable in any clinical study involves appropriate phenotype identification. In vasospasm, the use of transcranial Doppler and angiography is unlikely to become the gold standard for identification of symptomatic individuals. The gold standard continues to be the sharp eye of the experienced clinician who identifies patients with clinical changes directly attributable to vasospasm and who confirms the diagnosis by other modalities, such as transcranial Doppler and angiography. The appropriate clinical classification of patients with symptomatic vasospasm is the most important feature of phenotypic identification in this disease. This can be difficult in the setting of a complex brain injury surrounding SAH where other potential causes of neurological clinical decline exist.

It is also important to consider the timing of disease when investigating tissue sources as a means of studying this disease. Comparing samples from different days and their changing patterns of protein expression can help elucidate different aspects of a multistep biological process that initiates over the first 3 days after exposure to the initial SAH and progresses to disease expression over the ensuing 14 days. Such proteins as endothelin or other factors affecting endothelial activation may not be elevated until the patient is symptomatic. Such proteins as cellular adhesion factors are often activated early on in the first 3 days prior to the onset of clinical symptoms. However, this may prepare the vessels for further pathological changes seen during the next 2 weeks.

Proteomics have already played a role in the understanding of the pathophysiology of vasospasm in the setting on nontraumatic SAH. As technology improves and clinical experience heightens the sophistication of our understanding of this disease, the scientific yields from proteomics will only expand. It is likely that, as these technologies are further developed, we will develop a series of biomarkers that signify the onset and progression of vasospasm as well as molecules that can be effective therapeutic targets for future drug discovery.

REFERENCES

1. Wardlaw JM, White PM. The detection and management of unruptured intracranial aneurysms. Brain 2000;123(Pt 2):205–21.
2. Kolias AG, Sen J, Belli A. Pathogenesis of cerebral vasospasm following aneurysmal subarachnoid hemorrhage: putative mechanisms and novel approaches. J Neurosci Res 2009;87(1):1–11.
3. Hop JW, Rinkel GJ, Algra A, et al. Case-fatality rates and functional outcome after subarachnoid hemorrhage: a systematic review [review]. Stroke 1997; 28(3):660–4.
4. Armonda RA, Bell RS, Vo AH, et al. Wartime traumatic cerebral vasospasm: recent review of combat casualties. Neurosurgery 2006;59(6):1215–25 [discussion: 1225].
5. Diringer MN. Management of aneurysmal subarachnoid hemorrhage [review]. Crit Care Med 2009; 37(2):432–40.
6. Solenski NJ, et al. Medical complications of aneurysmal subarachnoid hemorrhage: a report of the multicenter, cooperative aneurysm study. Participants of the Multicenter Cooperative Aneurysm Study. Crit Care Med 1995;23(6):1007–17.

7. Dorsch NW. Cerebral arterial spasm—a clinical review. Br J Neurosurg 1995;9(3):403–12.

8. Hijdra A, et al. Delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: clinicoanatomic correlations. Neurology 1986;36(3):329–33.

9. Hirashima Y, et al. The use of computed tomography in the prediction of delayed cerebral infarction following acute aneurysm surgery for subarachnoid haemorrhage. Acta Neurochir (Wien) 1995;132(1–3):9–13.

10. Forssell A, et al. CT assessment of subarachnoid haemorrhage. A comparison between different CT methods of grading subarachnoid haemorrhage. Br J Neurosurg 1995;9(1):21–7.

11. Rabinstein AA, et al. Patterns of cerebral infarction in aneurysmal subarachnoid hemorrhage. Stroke 2005;36(5):992–7.

12. Zambranski J, Hamilton MG. Cerebral vasospams. In: Spetzler R, Carter L, editors. Neurovascular surgery. New York: McGraw-Hill; 1994. p. 583–601.

13. Suarez JI, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. N Engl J Med 2006;354(4):387–96.

14. Macdonald RL, et al. Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial. Stroke 2008;39(11):3015–21.

15. Zwienenberg-Lee M, et al. Effect of prophylactic transluminal balloon angioplasty on cerebral vasospasm and outcome in patients with Fisher grade III subarachnoid hemorrhage: results of a phase II multicenter, randomized, clinical trial. Stroke 2008;39(6):1759–65.

16. Haley EC Jr, Kassell NF, Torner JC. A randomized trial of nicardipine in subarachnoid hemorrhage: angiographic and transcranial Doppler ultrasound results. A report of the Cooperative Aneurysm Study. J Neurosurg 1993;78(4):548–53.

17. Moseley FL, et al. The use of proteomics to identify novel therapeutic targets for the treatment of disease. J Pharm Pharmacol 2007;59(5):609–28.

18. Issaq HJ, Xiao Z, Veenstra TD. Serum and plasma proteomics. Chem Rev 2007;107(8):3601–20.

19. Hoogland C, et al. The 1999 SWISS-2DPAGE database update. Nucleic Acids Res 2000;28(1):286–8.

20. Bairoch A, et al. The Universal Protein Resource (UniProt). Nucleic Acids Res 2005;33(Database issue):D154–9.

21. Mann M, Pandey A. Use of mass spectrometry-derived data to annotate nucleotide and protein sequence databases. Trends Biochem Sci 2001;26(1):54–61.

22. Appel RD, Bairoch A. Post-translational modifications: a challenge for proteomics and bioinformatics. Proteomics 2004;4(6):1525–6.

23. Steen H, Mann M. The ABC's (and XYZ's) of peptide sequencing. Nat Rev Mol Cell Biol 2004;5(9):699–711.

24. Ramstrom M, et al. Cerebrospinal fluid protein patterns in neurodegenerative disease revealed by liquid chromatography-Fourier transform ion cyclotron resonance mass spectrometry. Proteomics 2004;4(12):4010–8.

25. Righetti PG, et al. Proteome analysis in the clinical chemistry laboratory: myth or reality? Clin Chim Acta 2005;357(2):123–39.

26. Bergquist J, et al. Peptide mapping of proteins in human body fluids using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mass Spectrom Rev 2002;21(1):2–15.

27. Merchant M, Weinberger SR. Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry. Electrophoresis 2000;21(6):1164–77.

28. Lequin RM. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin Chem 2005;51(12):2415–8.

29. Lawlor K, Nazarian A, Lacomis L, et al. Pathway-based biomarker search by high-throughput proteomics profiling of secretomes. J Proteome Res 2009;8(3):1489–503.

30. MacBeath G, Schreiber SL. Printing proteins as microarrays for high-throughput function determination. Science 2000;289(5485):1760–3.

31. McGirt MJ, et al. Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2003;98(6):1222–6.

32. Maiuri F, et al. The blood leukocyte count and its prognostic significance in subarachnoid hemorrhage. J Neurosurg Sci 1987;31(2):45–8.

33. Oliveira-Filho J, et al. Fever in subarachnoid hemorrhage: relationship to vasospasm and outcome. Neurology 2001;56(10):1299–304.

34. Bavbek M, et al. Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. Stroke 1998;29(9):1930–5 [discussion: 1935–6].

35. Polin RS, et al. Detection of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the cerebrospinal fluid of patients after subarachnoid hemorrhage. J Neurosurg 1998;89(4):559–67.

36. Nissen JJ, et al. The selectin superfamily: the role of selectin adhesion molecules in delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. Acta Neurochir Suppl 2000;76:55–60.

37. Nissen JJ, et al. Serum concentration of adhesion molecules in patients with delayed ischaemic neurological deficit after aneurysmal subarachnoid haemorrhage: the immunoglobulin and selectin superfamilies. J Neurol Neurosurg Psychiatr 2001;71(3):329–33.

38. Mack WJ, et al. Outcome prediction with serum intercellular adhesion molecule-1 levels after aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;96(1):71–5.

39. Mocco J, et al. Elevation of soluble intercellular adhesion molecule-1 levels in symptomatic and asymptomatic carotid atherosclerosis. Neurosurgery 2001;48(4):718–21 [discussion: 721–2].

40. Tanriverdi T, et al. Serum and cerebrospinal fluid concentrations of E-selectin in patients with aneurysmal subarachnoid hemorrhage. Braz J Med Biol Res 2005;38(11):1703–10.

41. Mathiesen T, et al. Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid hemorrhage. J Neurosurg 1993;78(4):562–7.

42. Gaetani P, et al. Cisternal CSF levels of cytokines after subarachnoid hemorrhage. Neurol Res 1998;20(4):337–42.

43. Gallia GL, Tamargo RJ. Leukocyte-endothelial cell interactions in chronic vasospasm after subarachnoid hemorrhage. Neurol Res 2006;28(7):750–8.

44. Laher I, Zhang JH. Protein kinase C and cerebral vasospasm. J Cereb Blood Flow Metab 2001;21(8):887–906.

45. Kasuya H, Shimizu T. Activated complement components C3a and C4a in cerebrospinal fluid and plasma following subarachnoid hemorrhage. J Neurosurg 1989;71(5 Pt 1):741–6.

46. Kawano T, Yonekawa Y. Serum complements as indicator for predicting vasospasm and its severity after aneurysmal subarachnoid hemorrhage. Nippon Geka Hokan 1990;59(3):189–97.

47. Renic M, et al. Effect of 20-HETE inhibition on infarct volume and cerebral blood flow after transient middle cerebral artery occlusion. J Cereb Blood Flow Metab 2009;29(3):629–39.

48. Isman FK, et al. Cerebrospinal fluid and serum chitotriosidase levels in patients with aneurysmal subarachnoid haemorrhage: preliminary results. Turk Neurosurg 2007;17(4):235–42.

49. Frijns CJ, et al. Endothelial cell activation markers and delayed cerebral ischaemia in patients with subarachnoid haemorrhage. J Neurol Neurosurg Psychiatr 2006;77(7):863–7.

50. Zhang ZD, et al. Delayed clot removal and experimental vasospasm. Acta Neurochir Suppl 2001;77:33–5.

51. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6(1):1–9.

52. Macdonald RL, Weir BK. A review of hemoglobin and the pathogenesis of cerebral vasospasm. Stroke 1991;22(8):971–82.

53. Nishizawa S, Laher I. Signaling mechanisms in cerebral vasospasm. Trends Cardiovasc Med 2005;15(1):24–34.

54. Pluta RM. Delayed cerebral vasospasm and nitric oxide: review, new hypothesis, and proposed treatment. Pharmacol Ther 2005;105(1):23–56.

55. Macdonald RL, Weir BK. Cerebral vasospasm and free radicals. Free Radic Biol Med 1994;16(5):633–43.

56. Cook DA, Vollrath B. Free radicals and intracellular events associated with cerebrovascular spasm. Cardiovasc Res 1995;30(4):493–500.

57. Polidori MC, et al. Increased levels of plasma cholesteryl ester hydroperoxides in patients with subarachnoid hemorrhage. Free Radic Biol Med 1997;23(5):762–7.

58. Horowitz A, et al. Mechanisms of smooth muscle contraction. Physiol Rev 1996;76(4):967–1003.

59. Tani E, Matsumoto T. Continuous elevation of intracellular Ca2+ is essential for the development of cerebral vasospasm. Curr Vasc Pharmacol 2004;2(1):13–21.

60. Alexander SA, et al. Cerebrospinal fluid apolipoprotein E, calcium and cerebral vasospasm after subarachnoid hemorrhage. Biol Res Nurs 2008;10(2):102–12.

61. Harada T, et al. The time course of myosin light-chain phosphorylation in blood-induced vasospasm. Neurosurgery 1995;36(6):1178–82 [discussion: 1182–3].

62. Sun H, et al. Myosin light chain phosphorylation and contractile proteins in a canine two-hemorrhage model of subarachnoid hemorrhage. Stroke 1998;29(10):2149–54.

63. Chrissobolis S, Sobey CG. Evidence that Rho-kinase activity contributes to cerebral vascular tone in vivo and is enhanced during chronic hypertension: comparison with protein kinase C. Circ Res 2001;88(8):774–9.

64. Chrissobolis S, et al. Role of inwardly rectifying K(+) channels in K(+)-induced cerebral vasodilatation in vivo. Am J Physiol Heart Circ Physiol 2000;279(6):H2704–12.

65. Aoki K, et al. Role of MAPK in chronic cerebral vasospasm. Acta Neurochir Suppl 2001;77:55–7.

66. Aoki K, et al. Role of MAPK in chronic cerebral vasospasm. Life Sci 2002;70(16):1901–8.

67. Pluta RM, Oldfield EH, Boock RJ. Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage. J Neurosurg 1997;87(5):746–51.

68. Pradilla G, et al. Delayed intracranial delivery of a nitric oxide donor from a controlled-release polymer prevents experimental cerebral vasospasm in rabbits. Neurosurgery 2004;55(6):1393–9 [discussion: 1399–400].

69. Rejdak K, Petzold A, Sharpe MA, et al. Cerebrospinal fluid nitrite/nitrate correlated with

oxyhemoglobin and outcome in patients with subarachnoid hemorrhage. J Neurol Sci 2004; 219(1–2):71–6.

70. Seifert V, et al. Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage. Correlation with cerebral vasospasm, delayed ischemic neurological deficits, and volume of hematoma. J Neurosurg 1995;82(1):55–62.

71. Kastner S, et al. Endothelin-1 in plasma, cisternal CSF and microdialysate following aneurysmal SAH. Acta Neurochir (Wien) 2005;147(12):1271–9 [discussion: 1279].

72. Shaw MD, et al. Efficacy and safety of the endothelin, receptor antagonist TAK-044 in treating subarachnoid hemorrhage: a report by the Steering Committee on behalf of the UK/Netherlands/Eire TAK-044 Subarachnoid Haemorrhage Study Group. J Neurosurg 2000;93(6):992–7.

73. Zimmermann M, et al. Prevention of cerebral vasospasm after experimental subarachnoid hemorrhage by RO 47-0203, a newly developed orally active endothelin receptor antagonist. Neurosurgery 1996;38(1):115–20.

74. Pluta RM, et al. Source and cause of endothelin-1 release into cerebrospinal fluid after subarachnoid hemorrhage. J Neurosurg 1997;87(2):287–93.

75. Oertel M, et al. S-100B and NSE: markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. J Clin Neurosci 2006;13(8):834–40.

76. Kasuya H, Shimizu T, Okado T, et al. Activation of the coagulation system in the subarachnoid space after subarachnoid haemorrhage: serial measurement of fibrinopeptide A and bradykinin of cerebrospinal fluid and plasma in patients with subarachnoid haemorrhage. Acta Neurochir (Wien) 1988;91:120–5.

77. Maurer MH, et al. Identification of early markers for symptomatic vasospasm in human cerebral microdialysate after subarachnoid hemorrhage: preliminary results of a proteome-wide screening. J Cereb Blood Flow Metab 2007;27(10):1675–83.

78. Nylen K, et al. CSF-neurofilament correlates with outcome after aneurysmal subarachnoid hemorrhage. Neurosci Lett 2006;404(1–2):132–6.

79. Nylen K, et al. Serum glial fibrillary acidic protein is related to focal brain injury and outcome after aneurysmal subarachnoid hemorrhage. Stroke 2007; 38(5):1489–94.

80. Petzold A, et al. Axonal pathology in subarachnoid and intracerebral hemorrhage. J Neurotrauma 2005;22(3):407–14.