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BASIC SCIENCE ISSUES IN THE FIELD OF STROKE & SUBARACHNOID HAEMORRHAGE

DIVISION OF MENTAL HEALTH
AND PREVENTION OF SUBSTANCE ABUSE

WORLD HEALTH ORGANIZATION

GENEVA

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This document arises from a WHO meeting held in Geneva on 27 and 28 June 1996. It considers fundamental basic aspects of brain injury including imaging of cellular events, energy metabolism, mitochondrial alterations and oxidative stress, as well as astrocytes function. Windows of opportunity for therapeutic interventions following ischemia are also presented.

UNIT OF NEUROSCIENCE
DIVISION OF MENTAL HEALTH
AND PREVENTION OF SUBSTANCE ABUSE
WORLD HEALTH ORGANIZATION
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This document results from a WHO meeting on Basic Science Issues in the field of Stroke & Subarachnoid Haemorrhage held at WHO, Geneva, 27 and 28 June 1996. The following experts participated:

Dr M. Chopp, Detroit, Michigan, USA (Co-Chairman)
Dr G. Fiskum, Washington DC, USA (Rapporteur)
Dr P. J. Magistretti, Lausanne, Switzerland (Chairman)
Dr W. Paschen, Cologne, Germany
Dr B. K. Siesjo, Lund, Sweden
Dr R. Swanson (Rapporteur)

WHO Secretariat

Dr L. Prilipko, Chief, Unit of Neuroscience, Programme on Mental Health, Division of Mental Health and Prevention of Substance Abuse (MSA)
Dr C.L. Bolis, Consultant, Unit of Neuroscience, Programme on Mental Health, Division of Mental Health and Prevention of Substance Abuse (MSA)

Dr L. Prilipko and Dr C.L. Bolis had the technical responsibility for the production of this document.

We would like to acknowledge all the participants who contributed the materials used in the present report.

Further copies of this document may be obtained from

Unit of Neuroscience, Programme on Mental Health
Division of Mental Health and Prevention of Substance Abuse
World Health Organization
1211 Geneva 27
Switzerland

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BASIC SCIENCE ISSUES IN THE FIELD OF STROKE AND SUBARACHNOID HAEMORRHAGE

1. INTRODUCTION

1.1 Welcome address

A WHO meeting on Basic Science Issues in the Field of Stroke and Subarachnoid Haemorrhage was held at WHO headquarters, Geneva, on 27-28 June 1996.

Dr L. Prilipko, Chief of the Neuroscience Unit of the Division of Mental Health and Prevention of Substance Abuse, opened the meeting and stressed the commitment of the World Health Organization towards the prevention, control and treatment of stroke and subarachnoid haemorrhage especially with regard to neurological involvement.

2. SCOPE OF THE MEETING

Dr Prilipko stressed the purposes of the meeting as follows:

2.1 In the framework of the project of the Neuroscience Unit on brain injury, it is important to discuss the state of the art of studies on the mechanisms underlying cerebrovascular disorders and stroke and related neurological states.

2.2 To consider the different aspects of the importance of Ca^{++} ions, mitochondria, apoptosis, glutamate, astrocytes, vascular components, and other aspects.

2.3 To consider models for further studies.

2.4 To draw conclusions and recommendations for better understanding and to apply the knowledge for clinical control and treatment approaches.

3. BASIC SCIENCE ISSUES

3.1 Imaging of cellular events

At present, there are three different experimental models at hand to study basic mechanism of ischemic cell damage: 1) transient global cerebral ischemia; 2) permanent; and 3) transient focal cerebral ischemia. In stroke patients the situation is complex due to regional inhomogeneities caused by opening of collateral vessels. We therefore need to know which pharmacological strategies can improve recovery of the tissue following an ischemic episode, and how the spread of severe disturbances from the ischemic focus into the adjacent tissue can be blocked. In animal models of focal cerebral ischemia, the ischemic focus, where blood flow is reduced below the threshold for the breakdown of the energy state and ion homeostasis, is surrounded by the penumbra, where blood flow is reduced to about 20-50% of control. One goal of therapeutic intervention in focal cerebral ischemia is to block the spread of tissue exhibiting severe metabolism disturbances from the ischemic focus to the perifocal area.

Results from experimental studies point to a role of cortical spreading depression (SD) in the spread of tissue exhibiting metabolic disturbances from the ischemic focus into the peri-focal region within the first 24 hours after vascular occlusion: the volume of the ischemic territory correlates closely with the number of SD waves elicited spontaneously, and glutamate antagonists that block the elicitation of SD waves also reduce the site of the tissue exhibiting ischemic damage.

Cortical spreading depression is a wave of cell depolarization which can be elicited by different means (e.g., glutamate application) and which propagates with a constant speed over the cortex. During the passage of the SD wave massive electrolyte shifts occur. To restore the physiological state energy is required. In the intact brain the increased energy demand is grossly matched by an increase in energy supply, and the SD-induced disturbances in electrolyte homeostasis are fully reversible. In the peri-focal region, in contrast, blood flow and oxygen pressure are already markedly reduced and the increased energy demand due to the propagation of spontaneously elicited SD waves cannot be matched by an increase in energy supply. As a result the size of the tissue exhibiting severe metabolic disturbances is increasing with each SD wave. This view is corroborated by NMR studies using diffusion-weighted imaging techniques, where a shift of water from the extra- to the intracellular compartment can be identified by an increase in signal intensity.

Spreading depression (SD) is a phenomenon which has been shown to occur in rodents, and experimental studies of permanent focal cerebral ischemia have identified SD as one of the causative factors contributing to the spread of the size of tissue exhibiting severe metabolic disturbances from the ischemic focus into the peri-focal region. It is not known at the present time whether SD can be elicited in the human brain and whether spontaneously elicited SD waves contribute to the increase in infarct size with time in stroke patients, or whether this is a phenomenon restricted to the rodent brain. SD-like phenomena have been identified in a patient suffering from head trauma implicating that elicitation of SD waves is not restricted to the rodent brain.

3.2 Energy metabolism, mitochondrial alterations and oxidative stress

Severe disturbances in energy metabolism are hallmarks of any form of cerebral ischemia, including focal ischemia (stroke), global ischemia due to cardiac arrest, subarachnoid hemorrhage, trauma, and others. Ischemic tissue must be reperfused with oxygen and glucose in order to stand any chance of recovery and cell survival. Decades of basic neurochemical research have demonstrated that the altered patterns of energy metabolism apparent during the hours to days following ischemia/reperfusion are critical determinants in neural cell survival and neurological outcome. Recent work has also demonstrated close relationships between alterations in mitochondrial energy transducing activities, including those related to oxidative phosphorylation, calcium sequestration, and free radical production, and the induction of a cascade of events that eventually lead to either necrotic or apoptotic cell death. The following summarizes what may be some of the more important aspects of relationships between altered energy metabolism and oxidative stress and provides suggestions for future avenues of research that may be

critical in the development of effective therapeutic interventions for neurodegeneration caused by global and focal cerebral ischemia.

Lactic Acidosis

Abnormally elevated tissue lactate levels and the associated abnormally low tissue pH are important determinants of postischemic cell injury and recovery. The primary cause for persistent lactic acidosis during many hours of reperfusion appears to be inhibition of one or more mitochondrial enzymes or electron transport chain components involved in aerobic energy metabolism and oxidative phosphorylation. Components that require particular attention include the pyruvate dehydrogenase complex and Complex I and IV of the electron transport chain. Although even severe acidosis (e.g. pH 6.0) that can occur following even 10-15 min of ischemia is not necessarily directly cytotoxic, even mild acidosis can potentiate other mechanisms of cell injury including oxidative damage to molecules and calcium uptake-induced damage to mitochondria. One mechanism of potentiation that requires considerable further investigation is acidosis-induced release of protein-bound iron and subsequent catalysis of hydroxyl radical production. The effects of acidosis on the cascade of events involved in apoptosis, including mitochondrial membrane permeability alterations, protease activities, and nuclear degeneration are largely unknown and also require increased attention. Ultimately, optimal postischemic therapeutic interventions will likely include agents or conditions that ameliorate brain acidosis through potentiation of aerobic energy metabolism.

Mitochondrial Alterations

Although ischemia induced mitochondrial injury has been recognized for many years, there is a resurgence in the appreciation of the critical roles that mitochondrial alterations play in many forms of neurodegeneration. Most importantly, it is now recognized that altered mitochondrial function can affect neural cell outcome in ways independent of the ability of cells to maintain normal levels of ATP. In addition to being the primary cause of lactic acidosis, mitochondrial injury probably is also responsible for at least part of the increase in free radical production apparent during the early stages of reperfusion. Impairment of the ability of mitochondria to accumulate the supraphysiological levels of cytosolic calcium present following even a few minutes of ischemia can also exacerbate calcium induced damage to membrane phospholipids, proteins and DNA. Perhaps most importantly, overloading the mitochondria with calcium can induce mitochondrial membrane and electron transport alterations, often referred to as the "membrane permeability transition", can induce a cascade leading to necrotic or apoptotic death. Recent work provides strong evidence that the anti-death gene *bcl-2* increases the capacity for mitochondria to accumulate calcium and increases the resistance of mitochondria to the transition and respiratory inhibition caused by calcium overload. Much more needs to be understood concerning the role of prelethal mitochondrial alterations in ischemic cell death and, particularly, how these alterations activate apoptotic

events such as protease activities and DNA degradation. Agents, e.g. derivatives of cylcosporin A and N-acetylcysteine, that can protect mitochondria from such alterations may prove extremely useful in postischemic treatment of neurological impairment.

Oxidative Stress

There is now considerable evidence that free radical mediated damage to lipids, proteins, and nucleic acids plays a critical role in reperfusion injury and perhaps also in injury occuring during ischemia itself. There is also good evidence that there is a close interrelationship between metabolic alterations and oxidative stress. For example, just as acidosis may potentiate free radical production, the presence of hydroxyl radicals and peroxynitrite can damage important proteins, e.g. pyruvate dehydrogenase and the electron transport chain, that will lead to further metabolic failure. Many approaches can be taken to ameliorate postischemic oxidative stress, including administration of antioxidants e.g. spin traps and iron chelators, or by simply altering the degree of tissue oxygenation. Early postischemic brain hyperoxygenation is actually detrimental following global cerebral ischemia (cardiac arrest) due to increased oxidative molecular damage and no improvement in aerobic energy metabolism. Delaying hyperoxygenation may prove more effective since brain blood flow is often severely depressed during 1-4 hr of reperfusion and until the metabolic capacity of the tissue has recovered to a certain extent. These factors should be seriously considered in light of the current guidelines for resuscitation following cardiac arrest that recommend the use of 100% ventilatory oxygen for undefined periods.

3.3 Metabolic trafficking between neurons and glia: relevance to stroke

Astrocytes play an important role in brain energy metabolism. They are the only cells (with very few exceptions) that contain glycogen. They are ideally positioned to control the entry of energy substrates within the brain, since their specialised processes, the end-feet, surround virtually all brain capilliaries. In addition, they possess receptors and reuptake sites for a variety of neurotransmitters, in particular, glutamate. These features imply that astrocytes are ideally positioned to sense neuronal activity and to couple it with energy metabolism.

A better knowledge of the various steps involved in the coupling between activity and metabolism may provide useful insights to understand that pathophysiology of stroke, a situation in which, by definition, there are major metabolic disturbances.

A direct mechanism to couple activity to metabolism was recently described (Pellerin and Magistretti, 1994). Glutamate, the principal excitatory neurotransmitter released by afferent circuits to the cortex, stimulates glucose uptake into and lactate release from astrocytes. This effect of glutamate is mediated by the Na/KATPase which is activated by Na cotransported with glutamate inside the astrocyte. In addition, a converging set of evidences indicates that lactate is an adequate substrate for neurons, as long as oxygen is available, since it can be transformed into pyruvate and then enter the TCA cycle to produce 17 ATP/lactate. Thus, a neuronal activation signal, glutamate,

triggers the entry of glucose into the brain parenchyma, specifically into astrocytes, which then release lactate as an energy substrate for neurons. What then is likely to happen to this cycle in stroke and particularly in the penumbra, the area which offers the window of opportunity for post-ischemic therapeutic intervention. In the penumbra, evidence indicates that there is a very profound hypoxia, with still some glucose available provided by the residual blood flow. Another point is that glutamate is being released in the penumbra, most likely from extra-synaptic sites, in addition to synaptic ones. It is important to note here that the cytosolic concentration of glutamate vastly exceeds that present at synapses. Work by Swanson and colleagues has shown that glutamate uptake into astrocytes is rather insensitive to anoxia, as long as glucose is available and that pH is maintained by physiological levels. Thus, in the penumbra, the excess glutamate release is likely to be "neutralised" by reuptake into astrocytes. As noted earlier, this uptake of glutamate into astrocytes triggers glucose uptake and lactate production from these cells. In the penumbra the oxygen availability is very low, implying that the lactate released by astrocytes will not be oxydised by neurons, meaning that lactate will accumulate and pH drop. This drop in pH has deleterious effects, notably it will drastically reduce the reuptake of glutamate into astrocytes given the anaerobic conditions present in the penumbra. The excess glutamate in the extracellular space will then lead to excitoxic damage to neurons. The decreased pH has numerous other deleterious effects such as change in the Ca homeostasis, generation of reactive oxygen species (ROS) and bioenergetic failure. An observation supporting this view is that in the early post-ischemic stages there is an increase in glucose uptake and lactate production in the penumbra. This situation may actually even persist after reperfusion since evidence has been provided for a decrease in PDH activity that persists for several hours after reperfusion. This decrease in PDH activity will impair the flux of lactate through the TCA cycle, thus contributing to the sustained elevation in lactate levels observed even after reperfusion.

The foregoing set of potentially occurring events is one of probably several examples which indicates the importance to consider the role of astrocytes in stroke. Other aspects to consider are the role that astrocytes play in scavenging ROS and in Ca^{++} homeostasis.

3.4 Alterations in astrocytes function during ischemia

A presentation was made regarding alterations in astrocyte function during ischemia. An expanded and annotated report of that presentation is submitted. What follows is an additional discussion of astrocyte functions and roles with respect to each of the other presented topics, in order to provide a better integration of these topics.

Paschen presented evidence supporting a contribution of repetitive spreading depression to the progressive metabolic derangement of the ischemic penumbra. It may be useful to note that spreading depression passes through the glial syncitium, and appears to require functioning astrocyte gap junctions to occur. This suggests a potential means of preventing ischemic damage: by pharmacological blockade of astrocyte gap junctions. This may be accomplished by a number of anaesthetic and other agents, and the use of such agents may in fact be an important variable among different ischemic models.

Fiskum provided evidence for a particular vulnerability of the pyruvate dehydrogenase complex following brief periods of ischemia such as occur during cardiac arrest/reperfusion and evidence that administration of L-acetyle carnitine which can enter the TCA cycle "downstream" of PDH, can ameliorate ischemic injury. In this respect it is noteworthy that data from several laboratories suggest that astrocytes may normally function to provide neurons with such substrates. For example, astrocyte export of glutamine, alpha-ketogluturate mutute and citrate have been identified. The protective effects obtained with ALCAR suggest that the normal flux of these intermediates from astrocytes to neurons is either normally low, or suppressed after ischemia. It may be of interest to explore means of promoting increased astrocyte export of such intermediates.

Magistretti illustrated metabolic communication between endothelia, astrocytes, and neurons, and the central role of astrocytes was stressed in this presentation.

Chopp spoke on the role of adhesion molecules in initiating an inflammatory response that can contribute to both apoptotic and necrotic cell death after ischemia. This interesting and exciting new approach to stroke therapy raises several issues with regard to astrocycte interactions; particularly in the light of the role of astrocytes in the formation of the blood-brain-barrier:

(1) What function, if any, do astrocyte end-feet play in permitting or excluding entry of neutophils?

(2) How do signals reach endothelin to induce I-CAM expression? Are they communicated by underlying parenchymal cells?

(3) Do astrocytes play a role in promoting or preventing neuronal apuptosis?

(4) By what mechanism do glial cells die in regions of neuronal apoptosis?

Siesjo presented a succinct re-apparaisal of the evidence for glutamate-mediated excitoxicity as a significant mechanism of neuronal death during complete and incomplete ischemia.

Acute elevations in extracellular glutamate are seen as initiating a cascade of events leading to mitichondrial dysfunction and cell death. Given that astrocytes are normally the primary site of glutamate uptake, and may also be the primary site of glutamate release during ischemia, factors affecting astrocyte function during the early stages of ischemia may have an impact on the eventual extent of cell death. As with Dr Chopp's presentation, the issue arises as to why glial cells die in regions of dying neurons.

3.5 Role of cell adhesion molecules in neuronal damage following ischemia

Secondary and progressing damage to brain occurs after stroke. From studies in the experimental animal, the ischemic lesion may take days to reach maturity. Thus, cerebral tissue, after occlusion of a large intracerebral vessel becomes infarcted and achieves its maximal extent well after onset of ictus. This delayed process of ischemic lesion maturation allows intervention to reduce the volume of dead tissue and to improve neurological function.

A major contributing factor to secondary ischemic cell damage is the post ischemic inflammatory response. Inflammatory cells, primarily neutrophils, adhere to the vascular endothelium and migrate into the parenchyma after stroke. The signalling mechanisms orchestrating the targetting of the inflammatory cell to the site of injury involve a number of finely tuned steps. Sequential expression of molecules both on the inflammatory cell and on the endothelium dictate the progression of the inflammatory response. These molecules include selectins, which are responsible for the initial rolling of the inflammatory cell within the microvasculature and integrins-ICAMS which promote adhesion and tight binding of the inflammatory cell to the endothelium. By interfering with the expression and binding of these and other (e.g., cytokine, chemokine) signalling molecules, the process of inflammation mediated damage may be abrogated. Thus, by employing antibodies against adhesion molecules or peptides and proteins that competitively bind the adhesion sites, the binding of the inflammatory cell to the vessel wall may be blocked and thereby damage reduced. Interventions are therefore readily available that can significantly reduce ischemic cell damage. Experimental studies in the animal capitalizing on these possible interventions have demonstrated great efficacy in reducing the volume of dead tissue, sometimes completely eliminating damage, and on the average reducing the volume of cerebral infarction by 50-60%. A great benefit of this therapeutic approach is that it can be applied hours after the onset of ischemia, and experimental studies to date have indicated benefit when treatment begins 4-5 hours after ictus.

The anti-adhesion molecule approach to ameliorating ischemic cell damage is only effective under conditions where reperfusion (reflow) is present. In the human, reperfusion can occur spontaneously, by endogenous thrombolysis, or exogenously by administration of recombinant tissue plasmogen activator (r-tPA) to the patient. Thus it is reasonable to administer anti-adhesion molecule therapy to the patient in combination with a thrombolytic agent, in order to protect against reperfusion injury. Administration of an anti-adhesion therapy may also extend the therapeutic window by which thrombolytic therapy may be effective. Therefore it is perfectly reasonable to consider combination therapies in the treatment of stroke in patients, particularly therapies such as antiadhesion approaches that may reduce reperfusion injury and thereby expand the utility of thrombolytic approaches to treatment.

Another major aspect to the successful management of the stroke patient is to develop noninvasive imaging modalities which can identify independently of time the status of the affected tissue. Magnetic Resonance Imaging (MRI) has the potential to

provide this noninvasive assessment of the histological status of the tissue. The primary approach to implement this technology is to combine multiparameter MRI indices into a single image. Thus, a combination of e.g., a diffusion weighted image (DWI) and a T2 weighted image may allow identification of the status of the ischemic lesion. MRI modalities exist that may also permit early identification of tissue vulnerable to hemorrhagic transformation. An important concern in thrombolytic therapy is that patients may suffer hemorrhage. Being able to identify potential hemorrhagic transformation acutely after stroke may greatly extend the utility of thrombolytic therapies, save lives and reduce neurologic impairment. The utilization of imaging technologies may also significantly reduce the costs of performing clinical trials on patients, by providing a quantitative measure of therapeutic response, however, more importantly, by identifying patients with salvageable cerebral tissue and excluding patients for which therapeutic intervention may be of no benefit, because the cerebral tissue is necrotic. Thus, the MRI technology is useful in the management of the stroke patient.

In order to further develop therapies for the treatment of ischemic stroke, better experimental modes more relevant to human stroke are needed. Ideally, models should be used in which a "clot" occludes a large intracerebral vessel. With utilization of thromboembolic models, investigations of the hemodynamics of thrombolysis and therapeutic interventions designed to increase the efficacy and safety of thrombolytic therapies could be implemented.

In summary, thrombolytic therapies are now available to treat stroke. However, it is essential to reduce the reperfusion injury associated with thrombolytic therapy. An effective approach to reduce brain damage is to implement anti-adhesion molecule therapy and thereby reduce reperfusion injury. This therapeutic approach can be optimally exploited by using imaging modalities, particularly MRI, which will allow identification and monitoring of the patient to receive therapeutic intervention. Further investigations to improve the treatment and management of the stroke patient should be pursued by experimental studies on appropriate models of ischemic stroke.

3.6 Windows of opportunity for therapeutic interventions following ischemia

New information has been gained on mechanisms of reperfusion damage in the brain. This information pertains to two types of ischemia. These will be described separately.

1. Global or forebrain ischemia of brief to intermediate duration

Results in this field have been obtained in global ischemia due to cardiac arrest, and in forebrain ischemia in gerbils and rats. Characteristic for this type is the brief duration of the ischemia, which usually results in selective damage to neurons, a type of damage which is characteristically delayed by hours or days (so called maturation period).

It has been hypothesized that the delayed damage is due to a sustained perturbation of plasma membrane function, with a resetting of the pump-leak relationship for calcium. This leads to a slow rise in the intracellular calcium concentration (Ca^{2+}) and eventually to mitochondrial calcium overload. This hypothesis has recently received support from experiments in which either Ca^{2+} or mitochondrial calcium content was measured.

New data has revealed that the immunosuppressant cyclosporine A (CsA), when allowed to pass the blood brain barrier, dramatically reduces selective neuronal necrosis, e.g., in the CA sector of the hippocampus. The results can be interpreted in terms of the calcium hypothesis of delayed neuronal death. Thus, CsA is known as a virtually specific blocker of the mitochondrial permeability transition (PT) which is observed under adverse conditions, such as mitochondrial calcium accumulation and oxidative stress. The PT involves the opening of a megapore in the inter minochondrial membrane which, by creating a leak for H+, causes mitochondrial failure. If prolonged, the pore opening leads to production of reactive oxygen species (ROS) and cell death. It can be speculated, therefore, that CsA reduces tissue damage by preventing the assembly of a mitochondrial pore.

Data obtained during recent years have shown that certain drugs ameliorate selective neuronal necrosis. These drugs either reduce presynaptic transmitter release or block postsynaptic influx of Na+ and Ca^{2+} via channels which are directly or indirectly gated by glutamate receptors. However, there is now some doubt about the mechanisms since at least two of the drugs used (both glutamate antagonists) seem to work by causing a long-lasting, moderate reduction of body (and brain) temperature. The results highlight the importance of a reduction in temperature, while other data demonstrate an aggravating effect of hyperthermia, even if incurred in the recirculation period.

2. Focal ischemia of the "stroke" type

Focal ischemia is usually much more long-lasting (if not permanent). Because of that, the pathophysiological events triggered by the ischemia has a strong inflammatory and immunological component. In fact, recent results have demonstrated that drugs or procedures which have anti-inflammatory or immunosuppressant properties can dramatically reduce the tissue damage which would otherwise result from a long period (2h) of transient middle cerebral artery occlusion (MCAO).

This represents a shift of focus in the field. Previously, much interest was directed towards the effect of glutamate antagonists and calcium channel blockers. These were assumed to act by suppressing the occurrence of repeated depolarization waves in the perifocal "penumbra" zone, assumed to trigger an extension of the infarct into the penumbral areas. However, this hypothesis is based on the unsubstantiated assumption that perifocal depolarization waves are observed in other animal species than the rat, or in man. Besides, it does not explain why post-treatment with unrelated drugs after 2h of MCAO have a similar effect as pretreatment with glutamate antagonists. An additional problem is that glutamate antagonists have a narrow therapeutic window of opportunity.

Three new approaches to treatment have emerged which allow treatment in the reperfusion period, with a window of opportunity of several hours. One involves the use of spin trap nitrones, which scavenge free radicals of several types. The second one is based on effects of immunosuppressant drugs, such as FK506. The third, finally, involves the use of antibodies directed against adhesion molecules for (or on) leucocytes. It seems likely that all three of these therapeutic principles work by curbing the inflammatory or immunological cascade which is set in motion by the ischemic transient.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Basic biomedical research has been successful at elucidating many of the mechanisms responsible for ischemic brain injury due to stroke, cardiac arrest, subarachnoid hemorrhage and head trauma. Further analysis of the pathophysiological mechanisms of delayed ischemic brain injury will lead to the development of therapeutic interventions that will reduce the mortality and permanent neurologic impairment associated with ischemic brain disorders.

4.2 Evolving concepts of mechanisms responsible for ischemic brain injury as well as improved opportunities for testing neuroprotective agents dictate the need for improved animal models of both global and focal ischemia and reperfusion. The fundamental differences between these forms of ischemic brain injury must be recognized and taken into account when conclusions are drawn from experiments performed with different models.

4.3 A re-analysis of the mechanisms involved in delayed neuronal cell death following ischemia/reperfusion is in progress.

(a) It is important to recognize that both necrotic and apoptotic forms of cell death are involved and to further characterize the cascade of molecular events involved in both processes.

(b) Increasing evidence points to the critical involvement of early, pre-lethal mitochondrial alterations and to the redistribution of intracellular calcium between the mitochondrial, cytosolic and endoplasmic reticulum compartments in these cascades.

(c) Non-neuronal death contributes to the morbidity of stroke and subarachnoid hemorrhage, and a further understanding of the mechanisms causing non-neuronal death is required.

(d) Stroke is treatable, because much of the damage to brain is a secondary injury. Inflammatory cells are a major contributing factor to the promotion of this secondary injury.

4.4 Increased knowledge of pathophysiological mechanisms and of brain imaging techniques has led to improved management and treatment of stroke victims.

(a) Magnetic Resonance Imaging should be utilized in combination with management and therapeutic approaches to the stroke patient. Technology exists to non-invasively measure important biophysical parameters, such as cerebral blood flow, blood brain barrier integrity, and status of the cerebral tissue.

(b) Thrombolytic therapy under specified conditions can improve neurologic outcome in stroke patients. It is recognized that adjunct therapies may be beneficial in reducing reperfusion injury following thrombolysis. Evidence from experimental studies indicate that such therapies may involve anti-inflammatory agents, immunosuppressants and anti-oxidants.

(c) Recent results have demonstrated a beneficial effect of even moderate reductions in body and brain temperature in the postischemic period. Conversely, it is recognized that elevated temperature should be aggressively treated in the post-ictal period.

(d) Clinical implications of these basic research findings are that there is an increasing need for individuals who suffer a stroke to reach a clinical setting as soon as possible. Therefore the WHO should provide guidelines and educational materials to recognize that stroke is an acute medical emergency and is treatable.

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