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Dear Colleagues,

It gives me great pleasure to place before you the July issue of this year, which contains the presidential address, two original articles, one review article and one case report. I hope that some more speakers at the last year’s conference also give their work for publication in the journal following the lead taken by Prof PN Tandon.

The spectacular advances in neurosciences in the last couple of decades have made it possible to not only explore but also to modify the function of human brain, even human mind and consciousness at such levels that it has aroused concerns about their ethical implications. Prof PN Tandon in his Presidential Address on Neuroethics delivered at the Silver Jubilee Conference at Varanasi last year deals with the various aspects of this.

Peroxidation of cellular membrane lipids, or circulating lipoprotein molecules generate highly reactive aldehydes among which one of the most important is 4-hydroxynonenal (HNE), which is a highly reactive molecule due to Ú, ß unsaturation. At low concentrations, HNE is involved in cell signaling whereas high concentrations of HNE are cytotoxic. Siddiqui et al. have examined the metabolism of HNE in PC-12 cells and studied its cytotoxic effects. They have also looked at the effect of nontoxic concentrations of HNE on neurotransmitter receptors. At low concentrations, HNE is primarily metabolized by glutathiolation and oxidation, whereas at higher concentrations, in addition to glutathiolation and oxidation a significant fraction of HNE is reduced in PC-12 cells.

Zidovudine is used in pregnant HIV positive women to prevent transmission of infection to the baby. Zidovudine exposure during prenatal period is associated with alterations in brain morphology and function in the baby. Rajalakshmi et al have assessed the neurobehavioral effects of one stage zidovudine exposure during pregnancy and lactation in F1 and F2 generation of mice. Their results suggest that neurobehavioural functions are affected in F1 generation and there is recovery in F2 generation. Recovery in F2 generation beyond normal profile could be due to activation of oncogenes as a result of chromosomal alterations.

The primary human brain tumours account for less than 2% of all human cancers but yet cause a disproportionate burden of cancer related morbidity and mortality. Research in the past four decades has resulted in no improvement in the survival of these patients. A variety of chromosomal alterations have been reported for various brain tumours. The p53 gene is one of the most important and intensely studied human tumour suppressor gene. Gope et al have reviewed the functional modulation of p53 gene in human brain tumour development.

Cognitive and behavioral problems are common in children with West syndrome. These behavioral disorders further impair the educational and social developmental of such children. Agarwal et al report a case of treatment resistant Attention Deficit Hyperactivity Disorder as an outcome of West syndrome.

Dr Rakesh Shukla
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Introduction
It is a unique privilege to deliver a Presidential Address for a second year in a row, that too at the Silver Jubilee Conference of the Academy. It further adds to the sanctity of the occasion when it happens to be at this venerable institution of learning established by a great visionary, Mahamana Shri Madan Mohan Malviyaji. I am doubly blessed to deliver this oration in the holy city of Varanasi with the longest uninterrupted tradition of education longer than any other city in the World. Let me once again thank the Academy for this rare honour.

As you all know Neurosciences research is a continuum of study from the molecular to the behavioral level. It encompasses the body of research directed towards understanding the molecular, cellular, intercellular processes-mediated through electrochemical signals, in the nervous system, integrated to sub serve behavior. The spectacular advances in neurosciences in the last couple of decades have made it possible not only explore but also to modify the function of human brain, even human mind and consciousness at such levels that it has aroused concerns about their ethical implications. It is generally recognized that recent advances in neurogenetics, brain mapping, imaging, neural implants, transplants and emerging technologies of brain – machine interface, deep brain stimulation, psychosurgery for aggressive behavior, pharmacological or genetic attempts for enhancing human mental capacity have already alarmed ethicists. Some thinkers consider these to pose far more threat to human dignity and autonomy than cloning (1). The profound accomplishments in the neurosciences in 1990s have also given rise “to thorny moral and ethical quandaries not previously faced by our discipline” (2).

To meet the ethical challenges of these investigations has led to the evolution of the new discipline i.e. Neuroethics (3). While most of the ethical concerns related to neurosciences could be covered under the broad discipline of Bioethics, but as I will elaborate later, there are unique ethical aspects specific to the professional conduct and research related to Neurosciences. Let me first define the term Neuroethics as it is viewed by different experts.

Definition
The term Neuroethics was coined by William Safire to describe, “the field of philosophy that discuss the rights and wrongs of the treatment of, or enhancement of, the human brain” (4).

Gazzaniga defined it as, “the examination of how we want to deal with the social issues of diseases, normality, mortality, life style, and the philosophy of living informed by our understanding of underlying brain mechanisms”. “It is – or should be – an effort to come up with a brain based philosophy of life” (5).

More simply, “Neuroethics is concerned with ethical, legal and social implications of neuroscience research findings and with the nature of neuroscience research itself” (6).

To Illes and Bird definition I would like to add the professional conduct while dealing with the unfortunate victims of neuro-psychological disorders.

Historical Perspectives
While the term Neuroethics might have been coined recently ethical concerns have been expressed for many years especially in respect to psycho-surgery, consent for treatment of psychiatric patients, the inhuman practices in mental asylum, maintenance of confidentially of personal information gathered during professional interaction with the patients etc. Already in 1959, a Commission set up by UNESCO observed that progress in the physical sciences has far outrun our understanding of even the most elementary brain mechanisms, upon which such progress is based and stressed that the “universe of the brain was relatively unexplored”. We have no doubt this has advanced a lot since then. However, the recent developments in the field of neurosciences research have added new dimensions to the ethical concerns. These have aroused global interest. Thus in 1995, UNESCO Commissioned a study on Ethics and Neurosciences, Prof. Jean Didier Vincent, as its Rapporteur, submitted its report to UNESCO. A conference organized by DANA Foundation along with UCSF & Stanford University in the USA in 2002, primarily devoted to “Neuroethics-Mapping the field including neuroscience and law and neuroscience and religion”(7). In addition to devoting special attention to the subject in the American Journal of Bioethics, a new journal on Neuroethics has been initiated. Farah summarized some of the emerging issues in Neuroscience(8).

Scope of Neuroethics
- Neuroethics will overlap substantially with traditional issues of bioethics and genetics and extend beyond to concerns related to brain, behavior, self and consciousness.
- The intimate connection between our brain and behaviors, as well as the peculiar relationship between our brain and ourselves, generate distinctive question that beg for the interplay between ethical and neuroscientific thinking.

Thus Neuroethics has two major subdivisions,
- Ethics of Neuroscience
- Neuroscience of Ethics
Ethics of Neuroscience

- The ethical issues concerned with pursuit of neuroscience research.
- Evaluation of ethical, social and legal impact of these studies.
- Ethics of practice of neurosciences-neurology, neurosurgery, psychiatry and cognitive sciences.

Neuroscience of Ethics

Exploring the neural basis of ethical, moral, spiritual traits, human nature and self.

Interpretation of philosophical notions of free-will, self control, personal identity, intention, empathy, altruism, value judgment on the basis of emerging knowledge of brain function.

Recognizing that human behavior is controlled by our brain and that ethics constitutes a part of our behavior, it is not surprising that neuroscientists have been looking for neural basis of ethical and moral behavior. The nearly 150 years old examples of Phineas Gage, who following an injury to the frontal lobe lived with his physical capacity intact and his cognitive faculties unimpaired – became incapable of making moral choices, supports this view(9). This has been further strengthened by Antonio Damasio in his studies. (Descartes’ Error: Emotion, Reason and Human Brain)”(10).

However, it must be emphasized, “There are no moral “centers” of the brain- though extensive neural systems are indeed involved- and that although genes impel our ethical behaviors they do not compel such behaviors which varies with our culture, our living situation, and the health of our brain”.

Patricia Churchland of University of California San Diego- “Neuroscience, still does not know the neural basis of morality”.

Time won’t permit me to elaborate this any further. This is not to say that damage to the brain will not result in moral impairment.

Why is there a need for specific discipline?

Notwithstanding some overlap with the existing UNESCO Declarations (Human Genome, Human Genetic Data, Bioethic and Human Rights), ethical issues related to Neurosciences have several unique concerns not covered in to in these earlier declarations.

Farah pointed out that, “At the start of the 21st Century, neuroscience has developed to a point where (like genetics) it, too, may have profound effects on society, extending far beyond the research laboratory or medical clinic . . . . . . . . . . . . . (it) concerns the biological foundations of who we are, of our essence . . . . . . . . . . . . . . . . . . . . Yet until recently there has been little awareness of the ethical issues arising from neurosciences”(12).

Neuroscience research and its applications involve a much larger group of scientific community than biologists and biomedical scientists. It concerns psychologists, cognitive scientists, other natural sciences, technologists, robotics experts, engineers, information & computer scientists and ethicologists. There are emerging new disciplines of Neuroeconomics, Neuromarketing, Neuroaesthetics not covered by the commonly accepted disciplines of Bioethics.

Understanding the historical foundations of ethics in neuroscience, its connection to other areas of modern science and bioethics, and the vital role that neuroethics must have, are key to illuminating its future(6).

With an ever-increasing understanding of the brain mechanisms associated with core human attributes and values. There is an increasing public interest in the results of neuroscience research and the ways in which that new knowledge will be used. “Neuroethics has surfaced, and is here to stay” (13).

While the neurosciences do bring hope, notably in the area of mental health, they are also a particularly dangerous terrain for genetic manipulation and for the use of pharmacology and computer science for behavioral ends. As a possible instrument of encroachment on human liberty and dignity, the neurosciences may also turn out to be a poisoned chalice on which the worst form of ideology may thrive (14).

Some Areas of Special Ethical Concerns

Clinical Practice: In addition to the general principles of biomedical ethics, there are some special areas of ethical concerns:

a) Consent for participation in research or treatment of brain compromised individuals e.g., demented, psychotic
b) Misuse of Psychoactive drugs: Prozac, Ritalin, modafinil
c) Risks associated with implantable devices; Transcranial Magnetic stimulation for altering behavior; Revival of ethical concerns of psychosurgery.
d) Use of predictive diagnostic techniques for conditions for which currently no prophylaxis or therapy is available e.g. genetic or imaging procedures which predict the possibility of disease at some future date.
e) Genetic or pharmacological intervention to:
ii) alter behavior
ii) neuroaugmentation of healthy, normal individuals e.g. memory-a form of eugenics. Use of drugs for memory enhancement by students, army personnel, drugs for interfering with sleep by long distance truck drivers, and their possible addiction and long term toxic effects. The ethical concerns related to chemical or technological enhancements would these make us less human or “post human” (15).

Informed Consent

In clinical practice besides the usual ethical considerations as recently summarized in the Universal Declaration on Bioethics and Human Rights by UNESCO e.g. respect for human dignity and human rights, to maximize benefits and minimize any harm, ensuring the autonomy and individual responsibility, respect for human vulnerability and personal integrity, privacy and confidentiality, non-discrimination and non-stigmatization etc, special attention is required to the question of informed consent.
Any preventive, diagnostic and therapeutic medical intervention and even more so scientific research, on human subjects requires prior free and informed consent of the person concerned. This poses ethical concerns specially in case of infants, children, psychiatric challenged individuals or patients with dementia. Resort may have to be taken to obtain consent of the legal representative and in certain cultures of the community leader. For this purpose some legislation may be required.

**Definition of Death:** Till recently death implied complete cessation of cardio-pulmonary function. However, with advances in critical care medicine life can be artificially prolonged with intensive care management. In addition the need for organ harvesting for transplantation death has now universally been equated with brain-death. This raises important questions regarding definition of brain-death-whether it implies irreversible loss of whole brain function or the brainstem function alone. While in most countries, including India, legally acceptable procedures to establish brain death have been laid down, the debate on the subject is not over yet as will be evident from a recent round-table discussion organized by the Pontifical Academy. (16).

**Ethical Issues Related to Beginning and End of Consciousness:** Even more difficult than the determination of brain-death and under certain circumstances even related to it is the question of determining the beginning (in case of embryos) and end (in cases of chronic vegetative state) of consciousness. This has acquired emotional, religious and legal implications when deciding issues related to medical termination of pregnancy, embryonic stem cell research, in vitro fertilization, biomedical cloning. As Gazzaniga recently commented, central to many of the ethical issues of our time is the question, “When should society confer moral status on an embryo” (5)? It is interesting to note that at an interactive meeting of the International Bioethics Committee of UNESCO, leaders of diverse religious expressed very differing views. It may be mentioned from a purely neuroscience perspective we could argue that life begins with a sentient being. Thus fourteen days of foetal life is the age many neuroscientists accept as the beginning of human life worthy of moral status because it marks the beginning of brain function. While it may sound irreverent I am persuaded to argue that if we all agree, as we do now, that brain death is death, then how can an embryo constituted by a mass of cells but without any brain be considered as living person. Be as it may, for the time being it is obvious that currently this ethical dilemma remains unresolved.

Similarly ethical dilemma, is created in respect of decisions to withdraw life support system particularly in persons in persistent vegetative state etc. A recent report by Nicholas Schiff has heightened this debate (17). Using the new imaging techniques called diffusion tensor imaging Nicholas Schiff and Colleagues from the Weill Medical College of Cornell University N.Y., on a patient in a persistent vegetative state demonstrated the astounding growth of new axons, rewiring the brain nineteen years after the insult resulting in this state, in ways never thought possible before. They suggested that there is a compelling evidence that some patients in a supposedly permanent vegetative state may actually show signs of awareness, a finding that could force a review of how such patients are treated.

**Ethical Issues related to Neuroimaging & Brain Mapping: Brain Finger Printing**

Imaging for lie-detection: The greatest advances in the field of neurosciences in recent years have been in the field of the neuroimaging. While these are of immense value for disease diagnosis and therapy, these are also the source for major ethical concerns when used for exploring human thought, behavior, personality and decision making. Illes and Bird pointed out that, “Recent fMRI studies demonstrated the possibility of obtaining measurements of biological correlates of complex human processes such as existential thought and decision making, moral and non moral social judgment, love and altruism, personality and human complications. Do these studies demonstrate a definite neural basis of morality or consciousness? Certainly not”. (see below) (6,18).

**Issues of Personal Responsibility for Criminal Offence**

“Issues of free-will: Did the defendant carry out the crime freely and by choice or was it inevitable because of the nature of his brain or past experience. Already lawyers have starting to defend their client by attributing responsibility for crime to brain disorder or past damage. Any abnormality revealed by brain imaging comes in handy for them to support their argument. Defense lawyers are looking for that one pixel in their clients brain scan that shows abnormality, a predisposition to crime or malfunction, thereby arguing, “Harry didn’t do it. His brain did it”. It has been suggested that a large proportion of inmates on death row may have damaged or injured brain. If this is confirmed how would it affect our views about moral and legal responsibility and in fact judicial system.

Diagnosis of behavioral dispositions, motivations or beliefs with the help of new imaging, genetic and electrophysiological techniques. In what cases can such information be used ethically? What are the consequences of reliable but not perfect, diagnosis techniques of exploring human thought and intent. Stephen Morse warns that the colourful images of the brain such as those produced by functional magnetic resonance imaging (fMRI) might blind people to the fundamental legal assumption that “people are conscious, intentional and potentially rational agents” and therefore responsible for their actions(19). He cautions about the use of neuroscientific evidence either in assessing responsibility or in determining punishment for criminal acts. Immense human benefit has accrued as a result of introduction of new neuron-imaging techniques specially MRI and fMRI, but the same could be utilized for brain mapping in which case they raise ethical issues.

Wolpe et al argued that recent advances in neuron-imaging can gain access to the seat of a person thoughts, feelings, intention and knowledge(20). These have been used to develop reliable brain-imaging lie-detection technologies. These raise difficult
Ethical and legal questions. They proposed that “we would need to define the parameters of a person’s right to ‘cognitive liberty’, the limits of the state’s right to peer into individual’s thought process with or without his or her consent, and the proper use of such information in civil, forensic and security setting”. Daniel Langleben found differences in brain activations when people were lying versus when they were telling the truth(21). Farwell who developed the technology of the Computerized Knowledge Assessment (CKA) utilizing P300 response in an EEG and called his method “brain fingerprinting”. While some investigators have questioned the absolute reliability of these data, others warned that we may be entering the era of brain incrimination, many have raised the questions about its implications for our constitutional rights to privacy and to freedom of speech and thought. According to Helen Phillips “brain imaging has already delved into our personal lives. Among other things, it has been used to investigate love, personality traits, political leanings, racial prejudice, tendency to violence, deception, moral reasoning.............

Studies are even beginning to encroach on legal issues such as whether we are responsible for our actions, whether it is possible to predict who is likely to commit a crime and whether people are lying or have false memories”(22).

Greene and Cohen argued that neuroscience will probably have a transforming effect on the law……………… new neuroscience will change the law, not by undermining its current assumptions, but by transforming people’s moral intuitions about free-will and responsibility (23). In their view, “neuroscience will challenge and ultimately reshape our intuitive sense(s) of justice. They concluded, “Neuroscience is unlikely to tell us anything that will challenge the law’s stated assumptions. However, we maintain that advances in neuroscience are likely to change the way people think about human action, and the criminal responsibility………………”. This will further complicate the debate on free will and determinism in respect to human behaviour and responsibility for crime. Those interested in the subject of law and the brain are referred to a recent volume of the Philosophical Transactions of the Royal Society London (Vol 359, 2004).

**Ethical Issues Related to “Brain Enhancement”**

Already a number of techniques exist that have their objective the enhancement of the intellect of the off springs. It is claimed that the stunning advances in genetics will make enhancement possible relatively soon. This raises the spectra of a new form of eugenics. If selecting for intelligence, temperament and other psychological factors is on the horizon, we should begin now to think about the social implications of having such power. Should parents be allowed to genetically engineer their children? No less a person than James Watson believes that “genetic enhancement should be a matter of choice”.

Leave aside the issue of possibility of genetic manipulations for brain enhancement in future there is already a more immediate concern in respect to pharmacological attempts to achieve the same. As pointed out by Gazzaniga in the chapter on “Shaping the Smart Brain with drugs : Memory Enhancers, Nootropes, Noos-mind and ‘Tropin’, many ‘smart drugs’ are in clinical trials and could be in market in less than five years(5). Nobel Laureate Eric Kandel’s company Memory Pharmaceuticals is working to produce a drug based on enhancing a protein CREB. The drug MEM1414 is already in clinical trial. Some drugs currently available to patients with memory disorders may increase intelligence in the healthy population. Should this be permitted? Is their use for normal individuals ethical?

There is enough evidence already about the gross misuse of psychoactive drugs, Ritalin, Modafinil. The pharmacological advances that will surely stem for the ongoing researchers will present the following question: When can drugs be ethically used to enhance normal capacities, rather than just to treat deficits?(3). Fukuyama believes that, “chemical and technological enhancement make us less human or ‘post human’(14). Farah questions, “How does the enhancement of the individual affect society, and what kinds of policy might we adopt to best manage these society-wide effects? She adds, we have ambitious college students turbo charging their attention with dopaminergic drugs and military-aimed at creating “enhanced war fighters” (24). Today athletes using steroid are disqualified from international competition. In future, would the students using “memory enhancers” be disqualified from the examination, on the grounds of unfair competition?

**Neuroscience in Ethics**

In the end let me briefly present the less understood but an area of profound implications for neuroscientists to explore i.e. the role brain plays in human behavior, implies that it may be responsible for our ethical behavior. As mentioned earlier this view finds support in the 150 years old example of one Phineas Gage who following an injury to the frontal lobe lived with his physical capacity intact and his cognitive faculties unimpaired but incapable of making moral choices (8). This line of thinking can also be gleamed from the work of Antonio Damasio in his book Descarte’s Error: Emotion, Reason and Human Brain he described one of his patients-Elliott- (and other similar ones) with similar problems in respect to “understanding moral situation to making a moral choice”. He proclaimed that, “unsurprisingly, I believe that what we call ethics today depends on the working of brain systems” (10). Damasio goes on to clarify, “Although certain systems in the brain are clearly related to moral behavior, they are not set by genes to operate for the purposes of morality and ethics. These systems are indeed dedicated making or to creativity”. However, let it be categorically stated that there are no moral “centers” of the brain. Anderson et al have described the long-term consequences of early prefrontal cortex lesion (25). The patients manifested defective social and moral reasoning in adult life.

There is voluminous literature on “behavioral brain” which is directly or indirectly implicated in ethical behavior. Plaff discussed some of the ethical aspects of such studies in his book, “Ethical Questions in Brain and Behavior: Problems and Opportunities”
(26). Without going into the details, the recent imaging techniques have shown “lighting up” of insula when people crave for drugs, feel pain, anticipate pain, empathise with others, listen to jokes, see disgust on some one’s face, are shunned in a social settings, see someone cheat and decide to punish them. The neural underpinnings of responsible behavior, moral reasoning, consciousness, and spiritual experience have all been the subject of neuroscience research. Behavioral traits have not only be studied by psychiatrists, psychologists, cognitive scientists and specialists in neuroimaging, but more recently increasingly by geneticists (27). These researchers have also apparent implications for such metaphysical concepts as morality (24). Illes and Raffin pointed out that fMRI studies that probe into our deepest thoughts, define or engagement in complex cognitive behaviors across the life span and provide measures of our ability to make judgments that invoke phenomenon like rational decision making and consciousness(2). These reflect the neural basis of cognitive profiling. The well known neurological syndrome-Kluver-Bucy due to bilateral amygdaloid lesions, results in abnormal sexual behavior, which could be considered unethical. Lesion of the hypothalamus results in ‘rage’-obviously an antisocial and hence unethical behavior. Patients with Asperger’s syndrome do not recognize others as having feelings and minds. This raises the question if they have neuroconscience. These are just a few examples which prompt us to presume that certain regions of the brain, or as Damasio says systems in the brain, are intricately linked to ethical behavior. Emotions, decision making, responsible behavior all involved in ethical practice have been neuroscientifically explored. We believe that emotions play an important role in moral cognition (28). The questions like how values are represented and what system in the brain promotes morality are yet to be answered. Damasio proclaimed, “Unsurprisingly I believe that what we call ethics today depends on the working of certain systems of the brain(10). But the very fact that these are being explored and some may even be modifiable genetically, or technologically if not today, but in near future. This may some day result in a better understanding of the biological basis of moral cognition (3).

Futuristic Issues

Issues related to brain-machine interaction, neural basis of spirituality, beliefs and religion-its impact on society (we are still debating the relative significance of evolution and intelligent design) (29). Imaging studies suggested that our brain responds selectively to race. Do these changes reflect social or merely perceptual judgments? Will the biologizing of the moral undermine its status as moral? How will a better understanding of the biological basis of moral cognition and behavior modify our philosophical ethical frame work? (3,30) can brain scans of a racist, liar or psychopath accurately tell whether that person will persecute fob or kill? While most neuroscientists answer these in the negative there is an increasing concern that some images may be used to make dangerous legal or social judgment about people’s behavior (30).

References


**Addendum to Paper on Neuroethics**

The author will be obliged if the members of Indian Academy of Neurosciences could spare a few minutes of their busy time to answer the following question and E-mail the same to tandon@nbro.ac.in

1. Are you interested in this subject?
2. Is there a specific aspect of it which interests you?
3. Is there a specific issue about which you would like to know more?
METABOLISM OF 4-HYDROXY TRANS 2- NONENAL (HNE) IN CULTURED PC-12 CELLS

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Introduction:
Oxidative stress has been implicated in the etiology of several disease processes including atherosclerosis, ischemia-reperfusion, neurological disorders, rheumatic arthritis, diabetes and cancer. Amongst the cerebro-vascular disorders, increasing levels of lipid peroxidation products have been detected in Alzheimer’s disease (AD), multiple sclerosis, Parkinson’s disease, cerebral ischemia and stroke (1-5).

Radical-mediated peroxidative reactions of unsaturated lipids leads to the generation of the reactive alkoxy radical, which upon spontaneous radical elimination (β-scission) generates several saturated and unsaturated aldehydes. With ω-6 polyunsaturated fatty acids (arachidonate, linolenate, linoleate), the most abundant aldehydes generated are the 4-hydroxyalkenals. Of the various hydroxyalkenals, 4-hydroxy, trans-2-enal (HNE), generated mainly from ω-6 polyunsaturated fatty acids such as arachidonic and linoleic acids, has received considerable attention due to its high bioactivity. The electrophilic nature of α, β unsaturation in HNE renders it highly reactive with cellular nucleophiles such as glutathione, cysteine, lysine, and histidine of proteins, and with nucleic acids. HNE is generated in high concentrations during lipid oxidation and accounts for up to 95% of the total enals generated during lipid peroxidation. High concentrations of HNE are cytotoxic, whereas lower concentrations of HNE modulate cell proliferation and gene expression and cell signaling (1, 2,3, 6, 7).

Because these aldehydes are highly reactive and are generated in high concentrations, it has been suggested that they mediate and amplify the cellular effects of their radical precursors. Significant quantities of these aldehydes may also be formed by other metabolic processes such as myeloperoxidase-catalyzed oxidation of amino acids, oxidative modification of nucleosides and polyamine metabolism. Furthermore, α, β-unsaturated aldehydes are produced during metabolism of several drugs, toxins and foods, and are ubiquitous components of pollutants(1,2,3,6,7).

Accumulation of unsaturated aldehydes or their products has been observed under several pathological conditions, e.g. proteins modified by these aldehydes have been immunochemically localized to lesions and neurons of patients with Alzheimer’s(8) and Parkinson diseases (9). The tissue content of HNE is elevated approximately 3-fold over controls in diseased regions of brain and cerebrospinal fluid of patients with AD and in patients with mild cognitive impairment (6). Multiple studies show that, in diseased tissue derived from patients with AD, HNE alkylates many proteins (creatine kinase, tau, neurofilament proteins, and the glutamate transporter) whose dysfunction have neurotoxic consequences. In the cerebral cortex of patients with AD and dementia with Lewy bodies, neurons and astrocytes are immunopositive for the presence of HNE-proteins adducts (10,11,12). In Creutzfeldt-Jacob disease, the highest accumulation of HNE is in astrocytes, but not in neurons and microglial cells (5,13,14).

While high concentrations of HNE illicit toxic responses, low levels of HNE and structurally related aldehydes are involved in cell signaling. HNE exposure modulates intracellular signaling by

Abstract
One of the most reactive aldehyde generated from the lipid peroxidation reactions is α, β-unsaturated aldehyde, 4,hydroxy trans-2-nonenal (HNE). Due to α, β-unsaturation, HNE is extremely reactive molecule. At low concentrations, HNE is involved in cell signaling whereas higher concentrations of HNE are cytotoxic (1). The biological effects of HNE will be dependent on the metabolism of HNE. The present investigations were carried out to examine the metabolism of HNE in PC-12 cells and to study the cytotoxic effects of HNE as well as the effect of non-toxic concentrations of HNE on neurotransmitter receptors.

Our data shows that in PC-12 cells, at low (physiological) concentrations HNE is primarily metabolized by glutathiolysis and oxidation, whereas at higher (pathological) concentrations, in addition to glutathiolation and oxidation, a significant fraction of HNE is reduced in PC-12 cells. Moreover, at higher concentrations, HNE also shows the abundance of two hydrophobic peaks, the structural identities of which has yet not been established. Mass spectroscopic analysis also shows that glutathionyl adduct of HNE is present as two forms-GS-HNE and its reduced metabolite GS-DHN. However, unlike the cardiovascular cells, reduction of GS-HNE is not catalyzed by aldose reductase, since inhibition of aldose reductase, did not abolish the reduction of GS-HNE in PC-12 cells. However, similar to other cells, oxidation of HNE in PC-12 cells was significantly inhibited by aldehyde dehydrogenase inhibitor, benomyl, suggesting that aldehyde dehydrogenase-mediated oxidation of HNE is an important route for the elimination of HNE in neuronal cells.

Key Words: PC-12 cells, HNE metabolism, oxidoreductases
activating the MAPK, stress-activated protein kinase and c-Jun
N-terminal protein kinase cascades (15,16) while inhibiting nuclear
factor κ-B activity (3,17,18). HNE affects cell surface receptor
function. Studies have shown that HNE inhibits G\textsubscript{\textalpha}s signaling of
muscarnic and dopamine receptors but can cause activation of
the epidermal growth factor receptor (19).

To assess the contribution of HNE and structurally related
aldehydes to specific pathological states and redox signaling, it is
essential to identify the metabolism of these aldehydes.
Metabolism of HNE is well studies in liver and cardiovascular
tissues, but the biochemical mechanisms for the metabolism of
α, β -unsaturated aldehydes such as HNE is not well understood
in the brain. In the present study, we have examined the
metabolism of HNE in PC-12 cells.

Material and Methods

Cell culture: PC-12 Cells were cultured in 5% CO\textsubscript{2} - 95%
atmosphere in high humidity at 37°C in Nutrient Mixture, F-12
Hams cell culture medium supplemented with 2.5% fetal bovine
serum (FBS), 15% horse serum, 0.2% sodium bicarbonate and
antibiotic cocktail from Gibco. For all the metabolism studies,
passage 6-10 cells were used. Viability of the cells was measured
by trypan blue dye exclusion.

Reagents and consumables: All the specified chemicals,
reagents, diagnostic kits, were purchased from Sigma Chemical
Company Pvt. Ltd. St. Louis, MO, USA, unless otherwise stated.
Nutrient mixture F-12 Hams culture medium, antibiotics, fetal
bovine serum and horse serum were purchased from Gibco BRL,
USA. Culture wares and other plastic consumables used in the
study were procured commercially from Nunc, USA. Milli Q water
(double distilled deionized water) was used in all the experiments.
HNE was purchased from Caymen Chemicals, U.S.A.

Chemical Synthesis of reagent HNE and its putative
metabolites: The radio labeled [4-\textsuperscript{3}H] HNE was synthesized from
the dimethylacetel of HNE, which was oxidized to the 4-keto
derivative using polymer-supported chronic acid as an oxidizing
agent (1). The resulting ketone was further reduced to the
dimethylacetel of HNE by using tritiated NaBH\textsubscript{3}. The [4-\textsuperscript{3}H] HNE
obtained by acid hydrolysis was purified on HPLC. Structural
identity of HNE was established by gas chromatography-mass
spectroscopy (GC-MS).

1,4-Dihydroxy-2-nonenone (DHN) was synthesized enzymatically by
incubating 60 nmol of [4-\textsuperscript{3}H] HNE with 300 milliliters of human
placenta recombinant aldose reductase (AR) and 0.1 mM NADPH
in 0.05 M potassium phosphate, pH 6.0, containing 0.4 M
Li\textsubscript{2}SO\textsubscript{4}. The reaction was monitored by following the decrease in
absorbance at 224 nm. The enzyme was removed by
ultrafiltration using an Amicon Centriprep-10, and DHN in the
filtrate was purified on HPLC as described above. Structural
identity of DHN was established by gas chromatography-mass
spectroscopy (GC-MS).

4-Hydroxy-trans-2-nonenic acid (HNA) was synthesized by
incubating 100 nmol of HNE ([\textsuperscript{3}H]-HNE) with 1.0 unit of aldehyde
dehydrogenase, and 1.5 mM NAD\textsubscript{H} in 0.1 M potassium phosphate,
pH 7.4, at 25 °C. The reaction was monitored by following the
increase in absorbance at 340 nm. The enzyme was removed by
ultrafiltration and HNA in the filtrate was purified by HPLC.
Structural identity of HNA was established by gas
chromatography-mass spectroscopy (GC-MS).

The conjugate of reduced glutathione (GSH) with HNE (GSH-
HNE) was prepared by incubating 1 μmol of [4-\textsuperscript{3}H]-HNE (55,000
cpm/nmol) with 5 μmol of GSH in 0.1 M potassium phosphate,
pH 7.0, for 1 h at room temperature. The reaction was monitored
by following the consumption of HNE at 224 nm. The GS-HNE
conjugate was purified by reverse phase HPLC as described below.
For the synthesis of the reduced form of the glutathione-HNE
conjugate (GS-DHN), 100 nmol of GS-HNE was incubated with
300 nmol of NADPH and 100 μg of aldose reductase in 0.1 M
potassium phosphate, pH 6.0, for 3 h at 37°C. The reaction
was monitored by following the consumption of NADPH at 340 nm.
Structural identity of HNA was established by electrospray
ionization-mass spectroscopy (GC-MS).

HPLC Analysis: Synthesized standards and metabolites of GS-
HNE and GS-DHN were separated by HPLC using a Varian
reverse phase ODS C\textsubscript{18} column pre-equilibrated with 0.1% aqueous trifluoroacetic acid. The compounds were eluted using a
gradient consisting of solvent A (0.1% aqueous trifluoroacetic acid)
and solvent B (100% acetonitrile) at a flow rate of 1 ml/
min. The gradient was established such that solvent B reached
24% in 20 min, 26% in 30 min, and was held at this value for 10
min. Furthermore, in the next 10 min solvent B reached 60%,
and in an additional 5 min it reached 100% and was held at this
value for 10 min.

Gas Chromatography-Mass Spectrometry (GC-MS): For
GC-MS analyses of DHN and HNA, the samples were derivatized
in 20 μl of acetonitrile with 20 μl of N,O-bis(trimethylsilyl)-
trifluoroacetamide (BSTFA) for 1 h at 60°C. The mixture was
cooled to room temperature and 2 μl aliquots were used for
analysis. The GC-Cl/MS analysis was performed using a HP5890/
HP5973 GC/MS system (Hewlett Packard; Palo Alto, CA, USA)
under 70 eV electron ionization conditions. The compounds were
separated on a bonded phase capillary column (DB-5MS,
30 m × 0.25 mm ID × 0.25μm film thickness from J7W Scientific
Folsom, CA, USA). The GC injection port and interface
temperature were set to 280°C, with helium gas (carrier)
maintained at 14 psi. Injections were made in the split less mode
with the inlet port purged for 1 min following injection. The GC
oven temperature was held initially at 100°C for 1 min and then
increased to a rate of 10°C min\textsuperscript{-1} to 280°C, which was held for 5
min. Under these conditions, the retention time for DHN and
HNA derivatives was 8.08 and 9.52 min respectively (1,2,20).

For the GC-MS analysis of HNE, 0.4 ml of a 0.05 M solution of
O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride
(PFBHA) was added to the samples and the mixtures were
vortexed for 1 min and incubated for 30 min at room temperature.
After incubation the samples were extracted in 4 ml hexane
containing (12 drops of concentrated sulfuric acid. The mixtures
were centrifuged at 2000 rpm and the upper hexane layer was
aspirated and dried under nitrogen. The dried samples were
derivatized with BSTFA as described above and analyzed by GC-MS (17,21).

**Electrospray Ionization Mass Spectrometry:** Chemical identities of the GS-HNE and GS-DHN were established by electrospray ionization mass spectrometry (ESI/MS). The samples were analyzed on a single quadrupole Micromass LC2 instrument as described (20). The ESI+/MS operating parameters were as follows: capillary voltage, 3.0 kV; cone voltage, 13 V; extractor voltage, 9 V; source block temperature, 100 °C; and dissolution temperature, 200 °C. Nitrogen at 3 p.s.i. was used as nebulizer gas. Samples were reconstituted in 50 μl of acetonitrile/water/ acetic acid (50/50/0.1) (v/v/v), and applied to the mass spectrophotometer using a Harvard syringe pump at a rate of 5 μl/min. Spectra were acquired at the rate of 200 atomic mass units/s over the range of 20–2000 atomic mass units.

**Metabolic studies:** Initial experiments described the time course of HNE metabolism. PC-12 Cells cultured in 10 cm dish were incubated in pre-warmed (37°C) modified Hank’s Balanced Salt Solution (HBSS). Pre-warmed (37°C) HBSS had no observable effect on the viability of PC12 cells for the duration of the experiment. The incubation was started with the addition of 1-20 μM [3H]-HNE in 2.5 ml HBSS. Aliquots were withdrawn at indicated times, centrifuged at 10 000 x g for 10 min at 4°C, and the supernatant was ultra filtered and applied to Varian ODS C18 reverse-phase column. The metabolites of [3H]-HNE were determined by quantifying the radioactivity in each fraction. Individual peaks were analyzed by ESI/MS or GC–Cl/MS.

**Inhibition of oxidoreductases:** PC-12 cells were incubated in 2.5 ml HBSS without or with 0.05 mM sorbinil, 0.01 mM tolrestat, 0.5 mM miconazole or 5.0 μM benomyl for 30 min at 37°C. [3H]-HNE, 5 μM was then added in the incubation media of each sample and the incubation was carried out for an additional 30 min at 37°C. After the incubation, cells were separated from the incubation medium and the radio labeled metabolites extracted in the medium were resolved by reverse phase HPLC and analyzed by ESI/MS or GC/MS as described above.

**Results**

**HPLC method for quantification and characterization of HNE and its metabolites:** In order to characterize and quantify radio labeled HNE and its putative metabolites, we established a reverse phase HPLC method as described in Materials and Methods. As shown in Figure 1, HNE and most of its putative metabolites nicely separated under our chromatographic conditions. However, glutathionyl conjugates of HNE (GS-HNE) and its reduced product (GS-DHN) co-eluted with the retention time of 23 min. DHN, the reduced metabolite of HNE eluted with the retention time of 31 min, whereas HNA, the oxidized metabolite of HNE eluted with the retention time of 37 min. HNE was eluted at 43 min. For the quantification and characterization of HNE and its metabolites, one ml fractions were collected and an aliquot of each fraction (100-250 μl) was used for quantification of radioactivity whereas the remaining aliquot was used for mass spectrometric analysis as described below.

**Characterization of glutathionyl conjugates by ESI-MS:** Glutathionyl conjugate of HNE (GS-HNE) and reduced metabolite (GS-DHN) were characterized by ESI-MS. Samples corresponding to the glutathionyl conjugates (HPLC Peak I) were dried under nitrogen on speed vac and analyzed by ESI-MS as described under Materials and Methods. ESI-MS chromatogram of reagent GS-HNE and GS-DHN is illustrated in Fig. 2. The ESI mass spectrum of the reagent GS-HNE showed a molecular ion [M+H]+ with a m/z of 464. An additional prominent ion with m/z 446 was identified and ascribed to daughter ion of GS-HNE arising from the loss of a single water molecule from the parent 464 ion, because the 446 species could be completely converted at lower cone voltages to the 464 species. The ESI mass spectrum of reagent GS-DHN displayed a single pseudo-molecular ion [M+H]+ with a m/z value of 466. No daughter ions due to dehydration of the parent ion were observed.

**Characterization of DHN, HNA and HNE by GC-MS:** For the characterization of DHN, HPLC Peak II, was subjected to GC-MS analysis. The samples were silled as described under Materials and Methods and one micro liter of the derivatized sample was injected into the GC. As shown in Fig 3A, on GC, DHN eluted with the retention time of 8.08 min. Fig. 3B shows the fragmentation pattern of DHN. The signature ions with m/z values of 199, 231 and 287 were due to M–CH2OTMS (cleavage between C1–C2), M–C5H11 (saturated carbon chain where D is labeled) and M–CH3 respectively. Structural identity of HNA, HPLC Peak III, was established by GC-MS analysis. The samples...
were silicated and analyzed as described for DHN. As shown in Fig. 4A, on GC, HNA eluted with the retention time of 9.52 min. Fig. 4B shows the fragmentation pattern of HNA. The signature ions with m/z values of 245 and 301 were due to M-C5H11 and M-CH3 respectively. Structural identity of HNE (HPLC Peak IV) was established by GC-MS under chemical ionization conditions. Figure 5A shows the GC profile of HNE (21.99 min) and Fig. 5B shows its fragmentation pattern.

**HNE consumption:** To examine HNE metabolism in PC-12 cells, cells were cultured in 10cm dishes. The culture medium was removed and the cells were incubated with 1-20 μM [3H]-HNE in 2.5 ml HBSS. Incubation of the cells with 1-20 μM HNE for 3 h did not cause a significant change in cell viability as determined by the MTT assay. For measuring the rate of HNE metabolism, aliquots were withdrawn at various times and the radioactivity in the medium was separated by HPLC. HNE remaining in the medium was determined by measuring radioactivity in the peak eluting with a retention time (T_R) of the HNE. As shown in Fig. 6, PC-12 cells efficiently metabolized HNE in a time and concentration dependent manner. When the cells were incubated with 1 μM [3H]-HNE, 90% of HNE was metabolized in 5 min whereas incubation of the cells with 20 μM [3H]-HNE, resulted in the metabolism of ~ 50% HNE in 5 min. Under these conditions, after 1h of incubation, only ~ 50% unmetabolized HNE remained in the medium. The total radioactivity recovered from the incubation medium was 84±4%; ~1% radioactivity was recovered from the acid-insoluble fraction after 1h of incubation.

**Quantification and characterization of HNE-derived metabolites in PC-12 cells:** For the quantification and characterization of HNE-derived metabolites, PC-12 cells were incubated with 5 μM [3H]-HNE for 1h. Incubation medium was separated from the cells and the radio labeled metabolites extracted in the incubation medium were separated on HPLC. Upon HPLC separation of the medium, individual peaks were assigned to specific HNE metabolites (Fig. 7) on the basis of the retention time (T_R) of synthesized standards. As shown in Fig. 7, after 1h of incubation with 5 μM [3H]-HNE, 46% of the radioactivity in
the medium was present as glutathione conjugates. Thus, conjugation with glutathione appears to be a rapid, high-affinity route of HNE elimination in PC-12 cells. Because the \( T_\text{g} \) of Peak I of the HNE-treated PC-12 cells was identical to reagent glutathionyl conjugates, fractions corresponding to this peak were subjected to ESI\(^+\)/MS in order to characterize the metabolite(s) present in that peak. The ESI\(^+\) mass spectrum of HPLC Peak I showed a predominant peak at \( m/z \) 466.2, which was assigned to the +1 charge state of GS-DHN (Fig. 8). Ions with \( m/z \) values of 464.2 and 446.2 respectively, represent GS-HNE and its daughter ion originating from the in source dehydration of GS-HNE. Quantification of the signals for the ions for GS-HNE and GS-DHN showed that 62% of the conjugate was in the reduced form (GS-DHN), whereas 38% of the conjugate was present as GS-HNE.

The \( T_\text{g} \) of HPLC Peak II, which accounted for 4% of the total radioactivity, corresponded to the \( T_\text{g} \) of reagent DHN (Fig. 7). Structural identity of this peak was established by GC-MS. As shown in Fig. 9, the retention time and fragmentation pattern of this peak was identical to reagent DHN, indicating that peak II is due to DHN.

The \( T_\text{g} \) of HPLC Peak III, which represents 40% of the total radioactivity, was identical to reagent HNA (Fig. 7). To characterize this peak further, fractions corresponding to this peak were pooled, silylated and subjected to GC-CI/MS. The gas chromatograph (Fig. 10A) shows a prominent solvent-independent peak with a \( T_\text{g} \) value identical to reagent HNA. This peak was further subjected to MS analysis. As shown in Fig. 10B, a molecular ion with a \( m/z \) value of 301, corresponding to derivatized HNA, was observed. The fragmentation pattern of this ion was found to be identical to that of synthetic HNA, indicating that peak III is due to HNA.

The HPLC, Peak IV, representing 1% radioactivity, displayed high absorbance at 224 nm and appears to be due to the unmetabolized HNE, since it eluted with a \( T_\text{g} \) value identical to reagent HNE (43 min). GC-CIMS of this peak (Fig. 11) showed that the retention time and fragmentation pattern of this peak is identical to reagent HNE, suggesting this peak is due to HNE.

Two other peaks were observed on HPLC, eluting with the retention time of 53 (Peak V) and 57 min (Peak VI) respectively. These peaks represented 5 and 4 % radioactivity, Structural identity of these peaks has yet not been established.

**Concentration dependent metabolism of HNE in PC-12 Cells:** Next we examined concentration dependent metabolism of HNE in PC12 cells. As described above when PC-12 cells were incubated with 1 or 5 \( \mu \text{M} \) [\(^3\text{H}\)-HNE for 1h, ~HNE was metabolized and glutathionyl conjugates and HNA were the major metabolites accounting for 90% of the metabolism. Incubation of the cells with 10 \( \mu \text{M} \) [\(^3\text{H}\)-HNE for 1h, resulted in a concentration dependent increase in glutathione conjugates formation whereas...
no further increase was observed in the oxidation of HNE to HNA (Fig. 12). Under these conditions, a robust increase was observed in the reduction of HNE to DHN (Fig. 12). More significant increases were also observed in the conversion of HNE to Peak V and Peak VI. Incubation of the cells with 20 μM [3H]-HNE for 1 h did not cause a significant change in the formation of glutathione conjugates, DHN, HNA and Peak V. However, metabolism of HNE to Peak V was increased by three-fold under these conditions. Moreover, 25% of HNE remained non-metabolized under these conditions.

The metabolism of HNE in PC-12 cells was, however, significantly inhibited by the ALDH inhibitor, benomyl. (Fig. 14) The radioactivity in Peak III corresponding to HNA decreased by >75% in cells pre-incubated with benomyl as compared with those treated with [3H]-HNE alone. In addition, the abundance of GS-HNE and Peak V was significantly increased in benomyl treated samples as compared to controls. Based on these observations, we infer that the formation of HNA is catalyzed by ALDH, and that inhibition of HNA formation leads to a corresponding increase in the glutathione-linked metabolism and abundance of Peak V.

Incubation of PC-12 cells with miconazole, decreased the rate of HNE metabolism. As shown in Fig. 15 there was a >6 fold increase in the abundance of unmetabolized HNE. Miconazole also slightly inhibited the glutathione conjugates formation. A significant decrease (>40 %) was evident in the formation of HNA in miconazole-treated cells as compared to control. A corresponding increase was also observed in Peak V formation in miconazole-treated cells.

Identification of the metabolic pathways for the metabolism of HNE in PC-12 Cells: The HPLC and mass spectroscopic analyses already described clearly demonstrate that the major metabolic products of low concentrations of HNE in PC-12 cells are GS-HNE, GS-DHN and HNA. To identify the biochemical pathways involved in the formation of these metabolites, the PC-12 cells were incubated for 1 h at 37°C with inhibitors of AR (sorbinil, 50 μM or tolrestat, 10 μM), ALDH (benomyl, 5 μM) and Cytochrome P450 (miconazole 500 μM) in 2.5 ml HBSS, after which 5 mM [3H]-HNE was added to the medium, and the incubation was continued for an additional 30 min. Cells incubated with HBSS under identical conditions served as control. Incubation of the PC-12 cells with 5.0 μM HNE, with or without these inhibitors for 3 h, did not significantly affect cell viability as determined by the MTT assay.

Radioactivity in the incubation medium of sorbinil or tolrestat-treated PC-12 cells separated on HPLC as described above. No change was observed in the amount of radioactivity recovered in Peaks I-VI. The ESI+ mass spectrum of peak I obtained from sorbinil or tolrestat-treated cells also did not show an difference in the abundance ratio of GS-HNE:GS-DHN (Fig. 13).
Discussion

The development of oxidative stress, in which production of highly reactive oxygen species (ROS) overwhelms antioxidant defenses, is a feature of many neurological diseases: ischemic, inflammatory, metabolic and degenerative. Oxidative stress is increasingly implicated in a number of neurodegenerative disorders characterized by abnormal filament accumulation or deposition of abnormal forms of specific proteins in affected neurons, like Alzheimer’s disease (AD), Pick’s disease, Lewy bodies related diseases, amyotrophic lateral sclerosis (ALS), and Huntington disease (5,10-14).

The effects of oxidative stress on “post-mitotic cells”, such as neurons may be cumulative, hence, it is often unclear whether oxidative damage is a cause or consequence of neurodegeneration. Peroxidation of cellular membrane lipids, or circulating lipoprotein molecules generates highly reactive aldehydes among which one of most important is 4-hydroxynonenal (HNE). The presence of HNE is increased in brain tissue and cerebrospinal fluid of AD patients, and in spinal cord of ALS patients. Immunohistochemical studies show presence of HNE in neurofibrillary tangles and in senile plaques in AD, in the cytoplasm of the residual motor neurons in sporadic ALS, in Lewy bodies in neocortical and brain stem neurons in Parkinson’s disease (PD) and in diffuse Lewy bodies disease (DLBD). Thus, increased levels of HNE in neurodegenerative disorders and immunohistochemical distribution of HNE in brain tissue indicate pathophysiological role of oxidative stress in these diseases, and especially HNE in formation of abnormal filament deposits.

Most of the studies thus far have only established that lipid derived aldehydes such as HNE, MDA and acrolein are associated with neurological disorders. However, little is known about whether HNE is casually involved in the pathogenesis of the disease process. The toxicological/pathological effect of these aldehydes would be dependent on their resident time in the cells. We have therefore examined the metabolism of HNE in PC-12 cells. Identification of the biological pathways which are involved in aldehyde metabolism would enable us to further investigate whether inhibition or over-expression of aldehyde metabolizing enzymes would alter their pathological and toxicological affects.

HNE was used as a model α,β -unsaturated aldehyde since it represents up to 95% of the total unsaturated aldehydes produced during in lipid oxidation (Estebauer, et al., 1991). During oxidation of arachidonic acid for example, HNE is generated in 100-fold excess over malonaldehyde and glyoxal. Since it is a potent electrophile, HNE is one of the most toxic aldehydes generated during lipid peroxidation. It combines spontaneously with glutathione, and with cysteine, histidine and lysine residues of proteins, and displays a variety of cytotoxic and genotoxic effects (3).

The results of our study show that several enzymatic pathways contribute to the metabolism of HNE in neuronal cells. A distinctive feature of HNE metabolism in PC-12 cells was that most of the HNE was rapidly transformed and extruded into the incubation medium. Only a small percent (<1 %) of the radioactivity was retained by the cells. These data suggest that endogenous aldehydes generated by lipid peroxidation or other metabolic processes also may be metabolized similarly and extruded from the neuronal cells.

One of the major pathways of HNE metabolism in PC12 cells appears to be oxidation to HNA. In the liver, heart and vascular cells, HNA is generated by aldehyde dehydrogenase-catalyzed oxidation of HNE (1,2,22-24), and a similar enzymatic pathway may be responsible for HNA formation in neuronal cells. The brain contains several aldehyde dehydrogenases, including the mitochondrial aldehyde dehydrogenase, which may be responsible for catalyzing HNE oxidation. This view is strengthened by the observation that in the presence of the mitochondrial aldehyde dehydrogenase inhibitor benomyl, the formation of HNA was significantly attenuated. In addition of the mitochondrial aldehyde dehydrogenase(s), results originating from Dr. Picklo’s laboratory (6,10,25) also show that in the brain enal can also be oxidized by succinic semialdehyde dehydrogenase (aldehyde dehydrogenase V). Further studies are required to examine the contribution of specific isozymes of aldehyde dehydrogenases in the metabolism of HNE and structurally related aldehydes in neuronal cells. Oxidation of HNE has also been suggested to be catalyzed by cytochrome P450s (26). Our data shows that inhibition of cytochrome P450s with miconazole, a non-selective P45 inhibitor, not only partially inhibited the oxidation of HNE, but also decreased the glutathiolation of HNE and slowed down HNE metabolizing capacity of the cells. It is yet not clear whether this is a drug specific response or indeed P450 inhibitors slow down the aldehyde metabolizing ability of the cells, in which case inhibition of P450 would be deleterious rather than advantageous.

Similar to other metabolites, HNA was also rapidly extruded from the cells. Although additional investigations will be required to identify the specific transporter(s) involved in this process, it is likely that the efflux of HNA (a long chain fatty acid analog) may be mediated in part by the fatty acid transporter, which interestingly, is up-regulated during oxidative stress.
In addition to oxidation, conjugation with glutathione (GSH) also appears to be a major route of HNE metabolism in PC12 cells. We found that in these cells >45% of the metabolized HNE was in the form of GSH conjugates. The extent to which HNE is conjugated with GSH in these cells is comparable to that observed in liver (22-24), heart (1) and vascular cells (2). While HNE spontaneously reacts with GSH to form a Michael adduct, it has been proposed that intracellular formation of GS-HNE is due to glutathione S-transferase-mediated catalysis, which enhances adduct formation 600 times over the spontaneous rate (27). Several glutathione S-transferases have been localized to the brain. "HNE-specific" glutathione S-transferase (4-4 in rats and 5-8 in humans), suggested to have evolved specifically to metabolize lipid peroxidation products, is also present in the brain, although the localization and the kinetic properties of the neuronal enzyme have not been well characterized.

ESI-MS of glutathionyl conjugates showed that, in addition to GS-HNE, significant amounts of the reduced form of the conjugate GS-DHN, were also recovered in the incubation medium of PC-12 cells incubated with HNE. The amount of GS-DHN recovered from the incubation of PC-12 cells with HNE is comparable to that formed in the cardiovascular cells. The reduced conjugate, GS-DHN, could arise either from catalytic reduction of GS-HNE or conjugation of DHN with GSH. However, due to loss of the conjugated aldehyde following reduction, it is unlikely that DHN could spontaneously react with GSH, and for the same reason, enzymatic catalysis of the adduct formation between GSH and DHN may also be inefficient. Thus, it appears unlikely that GS-DHN arises from the formation of adduct between DHN and GSH. This view is further supported by our observation that incubation of PC-12 cells with [3H]DHN did not result in the formation of GS-DHN (data not shown).

Thus, the most likely route of GS-DHN formation appears to be enzyme-catalyzed reduction of GS-HNE. This reductive transformation in the heart and in vascular cells is catalyzed by the polyol pathway enzyme AR. However, our observations show that in PC-12 cells, formation of GS-DHN is not catalyzed by aldose reductase since pre-incubation of PC-12 cells with aldose reductase inhibitors sorbinol and tolrestat did not inhibit GS-DHN formation. Further studies are required to investigate the biochemical pathways involved in the formation of GS-DHN in neuronal cells. Moreover, although a large fraction of the conjugate was recovered in the reduced form, the metabolic significance of this reductive transformation remains to be established. No GS-HNA adducts were recovered from PC-12 cells treated with HNE. However, mercapturic acids of HNA and its lactone cysteine have been recovered from the urine of HNE-treated rats (3,28), but these could arise from oxidation of Cys-Gly-HNE and N-acetyl-Cys-HNE rather than GS-HNE. Our data do not distinguish between these possibilities, but nevertheless, indicate that, at least in these cells oxidation of GS-HNE (in contrast to free HNE) is not catalyzed by an aldehyde dehydrogenase, and that the conjugation of HNA with GSH does not efficiently compete with HNA.

Our data also show that incubation of PC-12 cells with higher concentrations of HNE as would be expected under pathological conditions associated with enhanced oxidative stress, resulted in the increased formation of new metabolites (Peak V and VI). Although, structural identities of these compounds have not been established, Peak V co-elutes with reagent HNA-lactone. This could be an important route for the elimination of HNE when the GSTs and oxido-reductase metabolizing capacity is exhausted, particularly under the conditions of acute and severe oxidative stress.

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References


NEUROBEHAVIORAL PROFILE OF F₁ AND F₂ GENERATION MICE FOLLOWING ONE STAGE ZIDOVUDINE EXPOSURE THROUGH PREGNANCY AND LACTATION

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Abstract
Zidovudine is administered to pregnant women with HIV to prevent the spread of infection to their fetuses. Several animal studies reported behavioural alteration in zidovudine prenatally exposed offspring, possibly resulting from an action of this drug on CNS targets. The aim of the present study was to assess the neurobehavioural effects of one-stage zidovudine exposure during pregnancy and lactation in F₁ and F₂ generation of mice. Zidovudine (50 mg / kg / day) was administered to female Swiss mice given orally from day 8 of gestation through pregnancy and up to day 10 of postnatal life. Control mice were given equal volume of distilled water. F₁ generation mice so produced, on attainment of 60-90 days age, underwent neurobehavioural testing for propensity towards anxiety and disturbances of learning and memory in the open field test, elevated plus-maze and Morris water-maze test. F₁ generation mice were crossed to produce F₂ mice. On attainment of 60-90 days, F₂ mice were also subjected to same neurobehavioural tests. Perinatal Zidovudine exposure caused significant decreased mobility suggesting certain degree of anxiety in F₁ mice whereas the contrary is true in F₂ mice. No derangement of motor activity was detected at maturity in the zidovudine exposed generation of mice in F₂ mice. F₁ mice, in the elevated plus maze test showed reduced entry in open arm whereas the entries are increased in F₂ mice. Similar increase in the number of entries in the closed arm lay F₂ mice as compared to F₁ mice was observed. Result of Morris water maze test showed decrease in learning capabilities in F₁ mice and regained learning ability in F₂. Overall results suggest that neurobehavioral functions are affected in F₁ generation and recovery in F₂ generation. Recovery in F₂ generation beyond normal profiles could be due to activation of oncogenes as a result of chromosomal alterations.

Key words: Perinatal, CNS, Elevated plus maze, Morris water maze test, Oncogenes, Chromosome.

Introduction
Zidovudine has been extensively used in pregnant HIV positive women to prevent transmission of infection to the baby (1,2). Zidovudine exposure during prenatal period is reportedly associated with alterations in brain morphology and function in the baby. Earlier experimental studies in mice and monkeys and clinical studies have indicated mitochondrial dysfunction and shortening of telomere length with prenatatal exposure to various doses of zidovudine (3-8). A variety of abnormalities in neurobehavioral observations have been documented in rodents prenatally exposed to zidovudine (9-13). Other studies point to the transient nature of the functional damage in prenatal zidovudine exposed rodents (13). Rodents are altricial mammals with offspring born neurologically premature. The maturation takes place after birth over sometime. In human instances, the mother receives anti-HIV therapy during pregnancy through to the lactation period with relevant impact on the offspring. This has been imperative in designing the zidovudine schedule in mice through pregnancy to lactation (14). In the present study neurobehavioural screening of the offspring was carried out in matured offspring aged 60-90 days. The toxic profile of zidovudine on genetic mixed with development effect on fetus was studied by observing F₁ offspring of zidovudine treated animals. Any pure genetic damage of transmissible nature was assessed by study of F₂ generation of the originally exposed mice.

The present study was conducted on Swiss mice to assess the neurobehavioural effects of zidovudine exposure during pregnancy and till Post Natal Day (PND) 10. The next generation of mice produced by crossing of the perinatally exposed (F₁) mice also underwent the same neurobehavioural tests to assess the effect of drug in the next generation.

Materials and Methods

Animal breeding and drug administration
After approval of Institutional Ethics Committee, the present study was conducted on inbred Swiss mice. Male and female mice weighing 25-30g and 80-90 days old were mated in the mating cages in the ratio of 1 male to 3 female mice. A 12 hours light-dark cycle, a room temperature 25 ± 2°C, relative humidity of 45-55% was maintained. Five pregnant mice (Fo) were assigned as control and 5 pregnant mice (Fo) as treated group. The F₁ generation mice produced were reared till 60-90 days age. F₁ generation mice are crossed to produce F₂ generation mice. Similarly, F₂ mice so produced were reared up to 60-90 days age..

Only the healthy F₂ mice were chosen for the study as 60-90 days old as, many sick F₁ mice were also produced. Both the F₁ (n=10) and F₂ (n=10) along with control (n=10) mice were subjected to neurobehavioral study. F₀ mice was treated with 50mg / kg / d Zidovudine (Zidovir oral solution, Cipla Drug Company, Goa, India), orally from day 8th of gestation through delivery till postnatal day 10. Perinatal drug dosing was as per Venerosi et al, 2000. Drug dosing was standardized in our laboratory after an initial dose of 70 mg/g/day where 100% fetal resorption was observed. Equal volume of distilled water were given orally to control mice. Principles of laboratory animal care (NIH publication No 86-23, revised 1985) guidelines were followed throughout.
Tests for neurobehavioral assessment

The two generations of mice underwent neurobehavioral studies to observe locomotor activity, anxiety and effects on learning and memory. The locomotor activities observed in an open field paradigm, anxiety was assessed in an elevated plus-maze and learning and memory was assessed in Morris water-maze test. The mice were divided into 3 groups- Group A comprises of the Control mice \( n=10 \), Group B comprises of F1 mice \( n=10 \) and Group C comprises of F2 mice \( n=10 \).

Open field test

Locomotor activity was evaluated in an open field paradigm (15). Individual mice \( n=10 \) were transferred from the home cage to an open field arena made of plywood and consisted of floor \( 96\times96 \) cm with high walls. The entire apparatus was painted black. The bottom of which is subdivided by 6mm thick white lines into 16 equal size squares. The test started by placing the animal in a corner of the apparatus. The behavior of the animal was then observed under red light. After each test, the apparatus was thoroughly cleaned with cotton pad wetted with 70% ethanol. Following behavioral parameters observed, ambulation (number of crossing the square boundaries with both forepaws), frequency of rearing (including wall rearing), grooming (rubbing the body or mouth with paws and rubbing the head with paws), total period of immobility (in seconds) and fecal pellets. Observations were made during 5 minutes on each mouse in each parameter.

Elevated plus maze

Adult mice were tested in a elevated plus maze (16) to assess anxiety. Four arms \( 15\times5 \) cm of the maze were set at \( 90^\circ \) angles in a plus-shaped design. Two opposing arms had high walls \( 12 \ cm \) while the other two arms were devoid of rails of wall. The maze was elevated 25cm from the floor. The mice were individually examined in 5-min session in this apparatus. Each mouse was placed in the central platform facing one open arm. Dependent measures were recorded including, the number of times the mouse enter the closed and open arm and the time spent in open and closed arm.

Morris water maze test

Spatial learning and memory was tested in water maze (17). The maze consisted of a black circular pool \( 2.14m \) diameter \( 80cm \) filled to a depth of \( 44cm \) with water \( 25^\circ C \). A circular platform \( 9 \ cm \) diameter was kept hidden \( 2cm \) below water level in the center of one of the quadrants. The platform remained in the same position during training days (reference memory procedure). At the beginning of each session, a random sequence of four starting poles along the perimeter of the pool was generated. All animals followed this sequence for that session. Each mouse was placed in the water facing the wall at the start location and was allowed 90 seconds to find the platform. The latency to reach the platform was recorded. If the mouse was unable to locate the hidden platform, it was lifted out and placed on the platform for 20 seconds.

Two sessions of four trials each were conducted on the first day of testing separated by 4 hrs and one session of four trials was conducted on the next day. After that the platform was removed and a Probe trial (without platform) was conducted 4 hr later. Each mouse was placed in the pool at the same randomly selected starting pole and swimming path was observed and time spent in the quadrant of the pool which initially contained platform was measured.

On completion of the probe trial, a black platform that extended 1cm above the surface of water was placed in a quadrant other than that chosen for the submerged platform. Each mouse was then given four trials of 90 seconds to locate it. The latency to reach the platform was recorded (working memory).

Statistical Analysis

Analysis of the data was done by using the SPSS version 12.00 software. Data are expressed as means ± SE values. The data were first analysed by non-parametric ANOVA- Kruskal Wallis (KW) test and followed by determination of Pair-wise significance difference using Multiple Ranges Student Newman Kuel (SNK) Test. \( P \)-value <0.05 was taken as the level of significance.

Result and Discussion

Zidovudine is administered during pregnancy in HIV positive women. Studies in animals have variously reported permanent damage to mitochondria in a variety of tissues, alterations in neurobehavioural profiles and overall growth of the organism.

The present study focused on effect of zidovudine exposure through prenatal to 10th postnatal day on the neurobehavioral profile of the offspring. It is understood that murine nervous system development and maturation is optimum by 60h PND (Postnatal day). In the present study, the period of drug exposure was extended till PND 10 to include more of neurogenesis period (14). Certain past studies have also suggested the transient nature of some of the toxic influences of the zidovudine exposure. The present study has been conducted in Swiss strain of mice while earlier studies had been in CD-1 mice, rats and monkeys.

Open field test employs various observations that indicate components of anxious behaviour in tested animals. As apparent from Table-1 (a-b), the F1 generation of mice that were exposed to zidovudine during prenatal and 10th PND singularly exhibited prolonged immobility profiles in this test. This was however not seen in F2 generation offsprings. On the contrary, the F2 generation had significantly reduced immobility profiles when compared to the unexposed control group of mice. Many of the tested behaviors under open field test are assigned to dopaminergic neurotransmission in associated brain areas (18-20). It appears that zidovudine exposure leaves significant and perhaps prolonged changes in this regard on the exposed F1 generation animals. The role of counteracting mechanism may be inferred at genetic level to explain marked reduction of immobility profile in F2 offspring from the results of the study. The parameters involving motor activity were not significantly different between either of F1 or F2 offspring compared to the unexposed control. It can be
stated that no motor derangement was detectable at maturity in the zidovudine exposed generation of mice.

As seen in Table 2(a-c) the observation in elevated plus maze test gives a further examination of anxiety behaviour. It was seen that compared to the control, F1 generation did not show greater preference for entry in open arm as also the F2 generation animals. Despite this the F1 mice spent much lesser time in closed arm and more time in open arm. These durations were less differentiated in case of F2 mice which showed increased number of entries in both closed and open arm, compared to either the control or the F1 generation. The observation indicates overall increased activity rather than anxiety (21). The entries in arm are particularly enhanced in F2 generation mice even above the control levels. This points to some genetic mechanism to counter decreased entry in F1 generation following exposure to pre and perinatal zidovudine.

The results of Water maze test displayed in Table 3(a-b) show significant differences in the probe trial. This indicates memory and learning. Decreased spending of time in this security quadrant was seen both in the F1 and F2 generation of mice. There was no indication whatsoever of recovery between F1 to F2 generation. Summarily, the learning process was seen as a change in escape latency at successive sessions tested in water maze test. The controls exhibited decreased successive latency profiles. Such a change was lost in zidovudine exposed F1 generation indicating deficit of learning. On the contrary, the F2 appeared to regain learning ability suggesting some reparative phenomena through change of generation. The ability to adopt new platform (working memory) was increased in F1 generation but the working memory was diminished in F2 generation mice. The probe trial test which tells reference memory profile shows that both the generation of the zidovudine exposed mother have decline of reference memory.

The overall result from evaluation at PND 60 may represent adult situation. The differences in observation parameters as compared to other studies (11,13,21) may be assumed to be based in differences of strain and other independent variables like dose of the drug and selection of only physically healthy looking offspring. The indication is that neurobehavioural functions do get affected in F1 generation of mice exposed to zidovudine through out pregnancy and till 10th PND skin to human situation. We have not examined recovery profiles of F1 generation over different periods of lifetime. The observation in F2 generation of mice indicates operation of genetic mechanism to counter the damage suffered in F1 generation. Indication of genetic mechanisms getting activated and apparently rendering recovery beyond normal profiles as that of controls, seen in F2 generation.

Table 1a.: Effect of Zidovudine (50mg/kg x day 8 of gestation through delivery and PND 10) on open field behavior parameters (values in Mean ± SE).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ambulation (Number)</th>
<th>Rearing (Number)</th>
<th>Immobility Period (Sec)</th>
<th>Grooming (Number)</th>
<th>Fecal Pellets (Number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>118.50±15.37</td>
<td>27.60±4.04</td>
<td>67.60±21.58</td>
<td>2.2±0.55</td>
<td>1.40±0.60</td>
</tr>
<tr>
<td>F1 Generation</td>
<td>117.3±11.82</td>
<td>28.20±4.71</td>
<td>119.8±15.34</td>
<td>3.2±0.35</td>
<td>1.6±0.54</td>
</tr>
<tr>
<td>F2 Generation</td>
<td>137.3±3.0</td>
<td>26.4±50.7</td>
<td>26.4±5.09</td>
<td>3.2±0.62</td>
<td>1.2±0.59</td>
</tr>
<tr>
<td>K-value</td>
<td>1.141</td>
<td>0.412</td>
<td>15.429</td>
<td>3.57</td>
<td>0.372</td>
</tr>
<tr>
<td>p-value</td>
<td>0.565</td>
<td>0.814</td>
<td>0.000</td>
<td>0.217</td>
<td>0.830</td>
</tr>
</tbody>
</table>

Table 1b.: Group comparison study by Multiple Range SNK test for Immobility period

<table>
<thead>
<tr>
<th>Groups</th>
<th>q</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Vs F1</td>
<td>3.93</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Control Vs F2</td>
<td>4.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F1 Vs F2</td>
<td>5.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

![Fig1. Percentage of ambulation, rearing, immobility, Pd., Grooming and faecal pellets of perinatal ZDV treated F1 mice and F2 bred as compared to control mice.]
Table 2a. Effect of Zidovudine (50 mg/kg x Day 8 of gestation through delivery upto PND 10) on elevated plus maze behavior (values in Mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of entries in</th>
<th>Time spent in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open arm</td>
<td>Closed arm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Open arm (sec)</td>
</tr>
<tr>
<td>Control</td>
<td>11.20±5.75</td>
<td>7.5±1.80</td>
</tr>
<tr>
<td>F1 mice</td>
<td>8.60±1.49</td>
<td>8.40±1.24</td>
</tr>
<tr>
<td>F2 mice</td>
<td>14.20±0.96</td>
<td>14.60±0.90</td>
</tr>
<tr>
<td>K-value</td>
<td>8.418</td>
<td>11.287</td>
</tr>
<tr>
<td>p-value</td>
<td>0.015</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 2b: A posthoc Group comparison study (SNK test) of the number of entries in open arm.

<table>
<thead>
<tr>
<th>Groups</th>
<th>q</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Vs F1</td>
<td>0.91</td>
<td>NS</td>
</tr>
<tr>
<td>Control Vs F2</td>
<td>3.81</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>F1 Vs F2</td>
<td>4.25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2c: A posthoc group comparison study (SNK test) of the number of entries in closed arm

<table>
<thead>
<tr>
<th>Group</th>
<th>q</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Vs F1</td>
<td>0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Control Vs F2</td>
<td>4.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F1 Vs F2</td>
<td>5.85</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig 2a. Percentage of No. of entries in closed and open arm in elevated plus maze test

Fig 2b. Percentage of time spent in closed and open arm in elevated plus maze test
is the strong indication available in this study. Such a phenomenon is seen when free-radical stress leads to increased level of antioxidant enzyme within the cell as a restorative mechanism (22,23). We have examined only neurobehavioural parameters however; zidovudine exposure is understood to cause diverse effect throughout the organism. The consequence of genetic activity to counter or repair deranged fine process provides unlimited vistas for research and understanding. It may be naive to speculate but it seems akin to gene amplification phenomena in activation of oncogenes as well (24,25).

References
FUNCTIONAL MODULATION OF THE P53 GENE AND ITS PROTEIN IN HUMAN BRAIN TUMORS

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Abstract
The primary human brain tumors account for less than 2% of all human cancers but yet cause a disproportionate burden of cancer related morbidity and mortality. These are the second most common form of tumors in the pediatric population, next only to leukemia and hence the second leading cause of death due to cancer in children. Among the adults they rank 6th and 8th in frequency of all neoplasms and form 2nd and 5th leading cause of death in men and women respectively who belong to the age group of 20 to 39 years. The survival rates for brain tumor patients have not changed over the past several decades. The p53 gene is one of the most important and intensively studied human tumor suppressor genes. It has been shown to play a major role in cell proliferation as well as cell death. Because of its varied functions the p53 gene and its pathways have become important therapeutic target. Mutations in the p53 gene and the oncogenic function of its protein product have been well documented. However, numerous evidences indicate that based on its conformation and post translational modification the function of the wild type p53 protein can be modulated to be similar to that of the mutant form. In this paper we have reviewed the functional modulation of p53 gene in human brain tumor development.

Keywords: Brain tumors, Cell-cycle, Conformation, p53 protein, Ser-392 phosphorylation, Tumor suppressor gene

Introduction
Human brain tumors arise from various cells of the central nervous system and the nomenclature is based on the origin of cell type (Figure 1). The tumors that arise from the glial cells are called gliomas. However, most gliomas are a heterogeneous group of tumors that arise from various cell types (1). Tumors that arise from the cells resembling the primitive neuroepithelium, that is, the precursors of the nervous system are called embryonal or primitive neuroectodermal tumors (PNETs). Medulloblastomas are the most common among the PNETs (1,2). The most prevalent primary brain tumors among the pediatric population are astrocytomas, ependymomas and medulloblastomas. Some of the common adult brain tumors include diffuse astrocytic tumors such as astrocytomas, anaplastic astrocytomas and glioblastoma multiforme (GBM). The oligodendrogliomas and meningiomas are also some of the commonly found brain tumors in adults (3).

Types of Human Brain Tumors

Gliomas
Gliomas are the most common form of brain tumors accounting for more than 50% of all primary brain tumors and more than 90% of all primary malignant central nervous system (CNS) tumors (4,5). Astrocytomas account for approximately 80% of all malignant brain tumors and are the most prevalent among the glial tumors. Approximately 25% of gliomas are oligodendrogiomas or mixed oligoastrocytomas. Gliomas are

Figure 1: Diagramatic representation of the location of various human brain tumor types.
prevalent in both pediatric as well as in adult population (1, 2). In general gliomas affect males 40% more frequently than females. Male predominance is evident in adolescent population and this observed male predominance does not exist if the tumor frequency is compared between the older males and the females who have crossed the menopause. This data is suggestive of a protective role for female hormones against this tumor type (6).

**Meningiomas**

Meningiomas are the second most common form of human brain tumors. These are also the most common non-glial primary intracranial tumors constituting approximately 20% of all intracranial neoplasms. Most meningiomas occur in adults of middle and old ages. These tumors are uncommon in pediatric population. Meningiomas in a benign form show a female preponderance, with a female to male ratio of approximately 2:1. However, among the malignant meningiomas the female to male ratio is 1:1 (1.2).

**Other Brain Tumors**

The medulloblastomas are the second most common pediatric brain tumors and also the most common malignant brain tumors found in children. The age of peak incidence is between 5 and 9 years and a second peak occurs in adults from 20 to 24 years (1.2).

Hemangioblastomas are tumors of uncertain cell origin comprising approximately 2% of primary central nervous system tumors and 25% of these tumors occur in association with the von Hippel-Landau (VHL) syndrome. These are adult tumors that primarily occur between the ages of 30 and 60 years (7). Gangliogliomas are benign tumors composed of a mixture of neoplastic astrocytes and nerve cells and they predominantly affect infants or young children of both sexes equally. Craniopharyngiomas arise from the embryologic precursors of the anterior pituitary gland and occur from infancy to old age, with the predominant peak in childhood at 5 to 14 years and in adults at 65 to 74 years (1.2).

**Symptoms**

Most brain tumors present in one of the following ways: seizures, focal signs and signs of increased intracranial pressure (ICP). ICP is commonly presented in any of the following forms such as headache, typically frontal or generalised and worse on waking, nausea and vomiting and reduced conscious level. The factors that determine the signs and symptoms of brain tumors generally include the location, growth rate, histology and size of the tumor (7). Presence of papilledema and optic atrophy is suggestive of raised intracranial pressure. Early symptoms are often non-specific such as forgetfulness, word-finding difficulties, headaches and in many cases a history going back several months is obtained in retrospect. By the time of presentation, however, clinical signs are usually obvious and even basic examination of the motor or sensory systems, speech function and visual fields will reveal significant abnormalities (4).

**Etiology**

Certain inherited genes or exposure to certain physical or chemical agents are considered to be potent risk factors which could play a causal role for brain tumor development (2). Some of the genetic polymorphisms that are specific for detoxification or metabolic enzymes are implicated in the pathogenesis of certain brain tumor types. Data from some studies have suggested mutagen sensitivity to gamma radiation and exposure to therapeutics, diagnostic or industrial ionizing radiation to be significantly associated with risk of brain tumor development (8). A personal medical history of serious head trauma and also seizures have been implicated as potential risk factors. Various factors like diet containing cured food items, alcohol, tobacco, residential chemicals like N-nitroso compounds, industrial and occupational exposure to various carcinogenic and neurotoxic substances are also suspected to act as causal agents for brain tumor development (1.2).

**Classification and Grading**

In 1979, the World Health Organization (WHO) published a classification system that encompassed all the central nervous system tumors and they were classified based on the cell or tissue of origin and their patterns of differentiation. It was subsequently revised in 1993, which included tumor data from immunohistochemistry (9). The WHO classification system includes 7 major categories and more than 120 sub categories of brain tumors (3,10,11). They include tumors of the neuroepithelial tissue, meninges, peripheral nerves, lymphomas and hematopoietic neoplasms, germ cell tumors, sellar region and the metastatic tumors, to mention a few.

**Histopathology**

The histopathology of brain tumors varies widely between and within the tumor types (Figure 2). The pilocytic astrocytomas have diverse morphological spectrum which makes the histopathological diagnosis extremely difficult (2,3,9,11). The grade II astrocytomas show increased cellularity and microcystic

![Figure 2 a : H & E stained section of transitional meningioma grade III. Fibroblastic appearance, psammoma bodies and whorls of cells are seen. Magnification-100 X](image-url)
formation. The grade III anaplastic astrocytomas exhibit increasing degree of anaplasia exhibiting complex nuclear morphology with increasing cellularity. The histopathology of the GBMs is highly variable. They show a high degree of cellular and nuclear pleomorphism and numerous multinucleated giant cells (9). The classic oligodendrogial tumor is moderately cellular composed of cells with round nuclei and perinuclear halos (9,11). The typical histologic features of grade I benign meningiomas include a whirling pattern, concentric calcifications known as psammoma bodies, and nuclear pseudo-inclusions (9).

Genetic Alterations in Brain Tumors

A variety of chromosomal alterations have been reported for the various human brain tumor types(12). Allelic loss on the q and p arms of chromosome 17 including the p53 and NF1 gene loci have been shown in pilocytic astrocytomas grade I (13). Gain of chromosome 7 and 8 have also been reported in these tumors (14). Loss of heterozygosity (LOH) on chromosomes 6q, 13q and 22q has been reported in some astrocytomas. More than 60% of grade II astrocytomas have loss of alleles on 17p, including the p53 gene locus, and in these cases the remaining p53 allele was found to be mutated (15). LOH of the intron I regions of RB1 and p53 genes have been reported in brain and nervous system tumors (16-22). Amplification and over expression of PDGF and its receptor have been reported in all grades of gliomas and is considered to be an early event in glioma development (12,23). In glioblastomas mutations of the p53 tumor suppressor gene and EGF-R amplification/over expression are mutually exclusive events (24). A tumor suppressor gene frequently inactivated in astrocytic tumors is the CDKN2, which encodes the protein p16 (25). p16 protein binds to and inhibits the function of cyclin dependent kinase 4 (CDK4). CDK4 forms complex with cyclin D and inhibits pRb, the protein product of the tumor suppressor gene retinoblastoma (RB1) and results in loss of RB1-mediated growth suppression. Inactivation of either p16 or RB1 is observed in most GBMs and rarely both are inactivated in the same tumor (26). RB1 mutations have been reported in 25% of high-grade astrocytomas (27). LOH at the intron I region of the RB1 gene have been reported in brain tumors (19,20,21). Deletion or loss of an entire copy of the chromosome 10 is also a common finding in high-grade astrocytic tumors. Mutation as well as LOH of PTEN, a tumor suppressor gene located on chromosome 10q has been reported both in anaplastic astrocytoma and GBMs. Cytogenetic analysis has revealed abnormalities in the chromosomal copy number in chromosomes 1, 6, 7, 9, 10, 13, 17, 19 and 22 in many human brain tumor types (3). The most common chromosomal abnormalities associated with the meningiomas are at chromosome 22q locus. Chromosomal gains, most commonly on 20q, 12q, 15q, 1q, 9q and 17q, have been noted in high-grade meningiomas. In addition, alteration at the p53 tumor suppressor gene has been considered as a reliable marker for malignant transformation of meningiomas (2,3,21).

Diagnosis and Management

Computerized tomography (CT) and magnetic resonance imaging (MRI) are the key diagnostic modalities for identification of human brain tumors. Positron emission tomography (PET) scanning and single photon emission tomography (SPECT) have supplementary roles in the imaging of brain tumors (28). A CT head scan with contrast will usually make the diagnosis of a brain tumor with some degree of certainty. Standard T1- and T2-weighted MRIs detect brain tumors with high sensitivity (7). There is some evidence that advanced MR techniques such as spectroscopy, to look at the chemical constituents of the tumor tissue, are used to improve accuracy in noninvasive diagnosis of tumor type and grade (29). For the present, however, the key to further management of almost all patients with brain tumors remains the establishment of an exact histological diagnosis. While an operative procedure is being arranged, majority of the patients are commenced on dexamethasone either orally or intravenously for management of tumor-related ICP. Dexamethasone can very significantly reduce focal neurological deficit (30). The primary modality of treatment for most primary brain tumors is surgery. The key aim of surgery with many brain tumors is the achievement of a histological diagnosis (28). Radiation is used when the entire primary tumor cannot be surgically removed. Local radiation therapy techniques, including external focal, brachytherapy, and stereotactic radiosurgery; may be administered to patients (7). Highly focused radiotherapy has a role in the treatment of certain well-localised tumors; for example, stereotactic radiosurgery (with a Cobalt-60 “gamma knife”) is very effective with small targets (tumor size of below 3 cm diameter) and is also used in the management of tumor metastases (31). An alternative approach to very focal radiotherapy is interstitial brachytherapy with 125-Iodine (2,4,7). Patients with certain tumor types show good response to drugs like Procarbazine, CCNU and Vincristine, Temozolomide and Liposomal Daunorubicin etc (2,4,7). Alternative approaches to delivering the drug to the tumor include intra-arterial chemotherapy and the implantation of wafers impregnated with Carmustine (BCNU), one of the first and still one of the most effective anti-glioma agents, directly into the resection cavity at the time of surgery (2,4,7).

Therapeutic attempts using the wild type p53 gene or restoration of its tumor suppressor function are underway (32). Various anti-
angiogenic drugs are under development; analogues of thalidomide inhibit the angiogenic action of bFGF and these may be seen in clinical trials over the next decade (33). The potentially interesting technique of Boron Neutron Capture Therapy is undergoing evaluation for treatment of malignant gliomas in a number of centres at present. Research in this area has lead to the use of nanotechnology (34,35). Targeted multifunctional polymer nano particles that specifically destroy brain tumors in laboratory animals have already been developed and their potential as a therapeutic in human needs to be evaluated (36).

The p53 gene
The p53 gene product was originally considered as an oncogenic protein. One of the evidences to disprove p53 as an oncogene came from the Friend virus induced mouse erythroleukaemias where p53 gene was found to be a frequent target for viral integration, which resulted in its inactivation (37). However, the most important finding came from the examination of the p53 clones used in the earlier transfection studies which revealed that the p53 gene used in these studies was actually a mutant form (38,39). Subsequent studies showed that p53 was indeed a tumor suppressor gene. By the year 1990, p53 was widely accepted as a tumor suppressor gene and was recognized as one of the frequently inactivated gene in more than 50% of all human cancers (40,41) (Figure 3).

Structure of the p53 Gene and mRNA
The human p53 gene is located on chromosome 17p13.1 (42). Southern blot analysis revealed presence of a single p53 gene in the human genome (43). The gene spans 20 kilobases (kb) of genomic DNA, and the structure is conserved in all the species studied. It comprises 11 exons, first exon is a non-coding exon, followed by a large first intron 10 kb in length (45) (Figure 3). There are five highly conserved domains within the exons 5, 7 and 8. The p53 mRNA transcript is approximately 2.8 kb in length (46) (Figure 3) and can be detected in most human cells, with the exception of cells in G0 phase (47).

RFLP analysis of Genomic DNAs from several human tumor tissues with Bgl II restriction enzyme digestion followed by hybridization with p53 polymorphic probe showed the presence of a 12 kb fragment in majority of the tumor tissues. However, some of the tissues showed an additional 9 kb fragment along with the 12 kb fragment. Both the fragments represent allelic variants of the p53 gene and the additional Bgl II enzyme site was mapped in the intron 1 (48). The wild type p53 gene expression and function was not affected due to the presence of this additional Bgl II site and the p53 protein also had no differences except for the substitution of arginine for proline at the 72nd position in the proline rich region (45,48) (Figure 3,4,5).

Structure of the p53 Protein
The p53 gene encodes for a protein of 393 amino acids with a molecular weight of 53 kDa (49). Based on its structure and function the p53 protein has been divided into three distinct domains, (a) the transcriptional activation domain at the amino terminal or N-terminus, (b) the central sequence-specific DNA binding domain and (c) the multifunctional basic carboxy terminal or C-terminal domain (Figure 3).

Isoforms of p53
The human p53 gene contains alternative promoters and transcribes multiple splice variants or isoforms and these are expressed in normal human tissue in a tissue-dependent manner and they are also found in some tumor tissues (21,50). Instead of the normal pattern of splicing between exons 9 and 10 that occurs to generate full-length p53, the β and γ isoforms are devoid of amino acids encoded by exon 10, and instead are equipped with 10 and 15 novel amino acids, respectively, due to cryptic splice sites located within intron 9 that are promptly followed by premature stop codons. Thus, the β isoform terminates with the amino acids ‘DQTSFKENC’ and the γ isoform ends with the amino acids ‘MLLDRWCYFLINSS’ (50). These isoforms are speculated to have altered functions (21,50).

The p53 Family
Two members of the p53 family, p63 and p73 have been identified. The structures of the p63 and p73 genes are more similar to one another than to p53. Similar to p53, both p63 and p73 can form homo-oligomers, bind DNA, activate transcription from p53-responsive genes, and induce apoptosis (51-53) (Figure 4).

Posttranslational Modifications, Stabilization and Degradation of p53 Protein
Phosphorylation of p53 protein has been shown to increase its sequence-specific DNA binding (54). Approximately 17 phosphorylation sites have been identified in human cells following DNA damage induced by ionizing radiation or ultraviolet (UV) - light radiation. In humans these residues include serines 6, 9, 15, 20, 33, 37, 46 and threonines 18, 81 in the N-terminal region; Ser 315 and Ser 392 in the C-terminal region; and Thr 150,
Thr 155, Ser 149 in the central core (55,56). In addition, Thr 55, Ser 376 and Ser 378 are reported to be constitutively phosphorylated in unstrained cells. Phosphorylation at Ser 33, 46 and Thr 81 has been shown to increase the half-life of the p53 protein and hence stabilize the same. Acetylation of p53 is suggested to be important for p53 stability and transcriptional activation (55,56). Under normal conditions the p53 protein is a latent short-lived protein with a half-life of 6 to 20 minutes (57). Having a short half-life the p53 protein is normally maintained at low levels in unstrained cells by continuous ubiquitylation and subsequent degradation via MDM2 and the 26S proteasome (45,55,57) (Figure 5).

Role of p53 in Cell Cycle Arrest
p53 functions as a cell cycle checkpoint protein and plays an important role in the induction of cell cycle arrest in response to DNA damage (Figure 5). p53 has been implicated in both the G1-S phase and the G2-M phase checkpoints of the cell cycle (60,61). The Rb protein regulates the restriction point or master signal for cell cycle progression. In response to DNA damage p53 is activated which in turn transactivates one of its downstream genes p21 (WAF1, Cip-1) (62). p21 binds to a number of cyclin and CDK complexes and inhibits the kinase activity thus preventing the phosphorylation of Rb resulting in cell cycle arrest. Thus, through the inhibition of CDKs, p21 acting downstream of p53 arrests the cells at the G1-S transition (45,63) (Figure 6).

p53 and Apoptosis
p53 is known to promote apoptosis through transcription-dependent and independent mechanisms that act in concert to ensure that the cell death program proceeds efficiently (45,64). Two major pathways trigger the apoptosis program: the death receptor induced extrinsic pathway and the mitochondria apoptosome mediated intrinsic pathway. The link between p53-mediated transactivation and apoptosis comes from its ability to control the transcription of the pro-apoptotic members of the Bcl-2 family, Bax, as well as the BH3-only members Puma, Noxa, and Bid. This increases the ratio of pro-apoptotic to anti-apoptotic Bcl-2 proteins, thereby favoring the release of cytochrome c from mitochondria into the cytoplasm leading to caspase activation and apoptosis. Many studies have indicated that p53-mediated apoptosis proceeds primarily through the intrinsic pathway. The extrinsic pathway is also regulated by p53 but the overall contribution of this regulation to p53-mediated cell death is poorly understood (45,64) (Figure 6).

Transcription-independent activities of p53 in apoptosis
Transcription independent pro-apoptotic functions of p53 have been proposed many years ago and recently have been shown to

**Cellular Functions of p53**
The p53 pathway is composed of hundreds of genes and their products that respond to a wide variety of intrinsic and extrinsic stress signals such as DNA damage, oncogene activation, hypoxia, cellular ribonucleotide depletion, mitotic spindle damage and nitric oxide (NO) production (58). These stress signals all impact upon the cellular homeostatic mechanisms that monitor and control the fidelity of DNA replication, chromosome segregation and cell division (59). Among the stresses that activate the p53 protein is damage to the integrity of DNA in a cell (45,57) (Figure 6).
facilitate cell death by genotoxic agents. It has been suggested that the transcription-independent pro-apoptotic activities of p53 is due to its ability to modulate the functions of the proteins involved in the apoptotic machinery (45,65).

**Antiapoptotic effects of p53**
p53 has been observed to possess antiapoptotic capabilities under a variety of conditions. The cells lacking p53 are sometimes more sensitive to apoptosis than their p53-proficient counterparts. Studies have implicated the wild type p53 protein in protection against cell death. It has been suggested that this mechanism involves the ability of p53 to bring about more effective DNA repair. The ability of p53 to turn on several antiapoptotic genes in addition to many proapoptotic targets suggests a possibility that the decision of life or death of a cell is determined by p53 (45,65).

**p53 in Senescence and Aging**
p53 is a critical regulator of the senescence response to a variety of signals including short telomeres, DNA damage, oncogenes and overexpressed tumor suppressor genes. Early indications of the importance of p53 in cellular senescence came from studies using the SV-40 large-T antigen, which binds and inactivates p53. The T-antigen extended the replicative life span of cultured human fibroblasts and also stimulated postmitotic senescent cells to initiate DNA replication. Human cells that over express oncogenic ras or E2F1 or the p14ARF or PML tumor suppressors fail to undergo a senescence-arrest if p53 function is defective. These studies clearly indicate a role for wild type p53 in senescence (66).

Another important function with which p53 has been associated in the recent times is organismal aging (67). Using mice model association between increased wild type p53 activity and premature organismal aging was demonstrated. In contrast to these studies it has also been illustrated that under specific circumstances excess levels of wild type p53 can protect mice against cancer and aging. A hint that reduced level of p53 could potentially increase longevity has also been observed in p53 heterozygous mice that could evade tumor formation. These mice were observed to live longer as compared to their wild type counterparts (67).

**p53 in the Maintenance of Genetic Stability**
Consistent with the role for p53 in protecting genomic integrity, fibroblasts from p53 deficient mice have demonstrated chromosomal abnormalities that appear at early passage in homozygous null fibroblast and at later passage in heterozygous fibroblasts. Aneuploidy and chromosome instability have also been demonstrated in p53 null mice. The cellular DNA replication is shown to be error-prone and the DNA repair genes correct these errors. When these DNA repair genes are inactivated the cells accumulate errors in genes leading to genetic instability and predispose the cells to tumorigenesis. Two specific genes involved in global genomic repair DDB2 and XPC are shown to contain p53 binding consensus sequences. Products of these genes p48 and XPC respectively have been shown to increase in the presence of wild type p53 in response to UV irradiation. Under these conditions the global genomic repair of these cells were shown to be enhanced. Deficiency in base excision repair mechanism has also been observed in cells lacking wild type p53 indicating a possible role for p53 in this mechanism of DNA repair (45,59) (Figure 6).

**p53 in Differentiation and Embryonic Development**
p53 deficient mice are reported to exhibit developmental abnormalities of the nervous system at a high frequency. p53 deficient mice embryos have been shown to be defective in neural tube closure resulting in an overgrowth of neural tissue in the region of the mid-brain resulting in a condition known as exencephaly. High-frequency of developmental abnormalities including neural tube defects, ocular abnormalities and defects in upper incisor tooth formation have been reported in p53-deficient mice and these abnormalities were found predominantly in females. Studies have suggested the presence of a p53-dependent “guardian” in the embryonic or fetal tissues which plays an “embryo-protective” role by aborting cells bearing teratogenic DNA damage and hence prevent off-springs with developmental abnormalities. p53 is also suggested to play a key role in protecting embryos against diverse environmental stresses (68).

Recent studies have also implicated p53 in adult neurogenesis. Neuronal-turnover is a two-step process wherein an excess of neuronal progenitors are generated, only few of the progenitors differentiate into fully functional neurons while the excessive progenitor cells are eliminated. Alteration in the p53 activity has been shown to upset this fine balance by affecting the rate of cell proliferation but not the rate of cell death in the neurogenic regions of adult brain. Genetically engineered mice with increased p53 activity have shown premature loss of neurogenic capacity. A link between the premature loss of neurogenic capacity and accelerated organismal aging has been demonstrated (69).

**Role of p53 in Carcinogenesis**
Identification of germ-line p53 mutations in the familial Li-Fraumeni syndrome associated with an early onset of various cancers strongly suggested a role for the p53 gene in tumorigenesis. The fact that p53 null mice were highly prone to tumors further emphasized that the loss of p53 function plays an important role in tumorigenesis. p53 is the most commonly mutated gene in human malignancies, with mutations reported in more than 50% of all cancers (40,45) (Figure 6). In the latest released version of the IARC p53 mutation database there are 19,809 somatic mutations of p53 reported.

The wild type p53 protein functions optimally when it binds to DNA as a wild type p53 tetramer. One mutant p53 protein can disturb a functional tetramer and is therefore able to override the function of the wild type p53 protein. This is known as the ‘dominant negative’ means of inactivation (71). In this
mechanism mutation in only one allele of the p53 gene is sufficient for the functional inactivation of both the alleles, although loss of the second, wild type allele may further contribute to oncogenesis (72).

p53 loses its tumor suppressor function as a consequence of mutation as most p53 mutants have impaired function with respect to sequence-specific transactivation of genes. Certain types of p53 mutations exert functions that the wild type p53 does not and hence are called as the “gain-of-function” mutants (73). Mutant p53 alleles expressed in cell lines lacking p53 resulted in enhanced tumorigenic potential, metastatic capacities and a shorter survival in mice (74). Gain-of-function mutants of p53 are known to exert oncogenic effects through the induction of increased expression of a diverse group of genes either directly or indirectly (73).

Mutant p53 proteins are shown to be intensely phosphorylated and acetylated at sites that are well known to be involved in the stabilisation of wild type p53. Such altered p53 proteins have been shown to facilitate accumulation of dysfunctional, mutant p53 in the nucleus, where it can exert oncogenic functions (56). Intense acetylation of the mutant p53 proteins at the lysine residues 320, 373, 382 and significant phosphorylation at Ser 392, Ser 15, Thr 81 has been reported in tumor-derived cell lines (75). Phosphorylation at these residues is suggested to stabilise the mutant p53 protein by inhibiting its degradation and facilitating its accumulation in the nucleus. Greater level of phosphorylation at Ser 6, 15, 37 and Thr 81 of wild type p53 in human tumors has also been reported but the significance of these observations is not yet clear (75). Studies on the UV-light-induced mouse tumors have shown that mutant p53 protein in these tumors is constitutively phosphorylated at Ser 15, localized in the nucleus and is resistant to MDM2-mediated degradation (76). These data indicate that phosphorylation of mutant p53 at Ser 15 contributes to its increased stability and to its oncogenic activities (Bode and Dong, 2004). Phosphorylation of p53 at Ser 392 has been reported to stabilize the tetramer formation (77). In human transitional cell carcinomas 60% of the tumors with accumulated mutant p53, but none with wild type p53, showed phosphorylation of Ser 392 (78). Loss of phosphorylation at the Ser 392 is reported to increase the tumorigenic potential of mutant p53 and also inhibit p53-induced apoptosis suggesting its importance for the oncogenic function of mutant p53 (79). Ser 392 phosphorylation has been suggested to be an early event in the pathogenesis of squamous cell carcinoma (80). Higher levels of Ser 392 phosphorylated p53 have been associated with higher proliferation, tumor progression and poor prognosis of human esophageal squamous cell carcinoma (80). Increased Ser 392 phosphorylation of mutant p53 has been frequently reported in transitional cell carcinoma (TCC). It has been suggested to promote the dominant negative effects of the mutant p53 through hetero-oligomerization, thereby contributing to the proliferation and aggressive behavior of these tumors (78). Increased percentage of Ser 392 phosphorylated form of p53 protein was found in human high grade gliomas as compared to the lower grade astrocytomas (21). In addition, Ser 392 phosphorylation of the p53 protein was reported to occur in human vestibular schwannomas (VS) in an age dependent manner (16,17).

**Alterations of the p53 Gene in Human Brain Tumors**

Alteration of the p53 gene has been reported in all grades of gliomas and studies have suggested that p53 plays a role both in the formation of low-grade disease and in the progression towards tumors of higher histological grade (9-11,21). Mutations in the p53 gene have been reported in more than 50% of gliomas (81). The fact that patients with Li-Fraumeni syndrome, who inherit germline mutations in the p53 gene are predisposed to the development of brain tumors early in life and that gliomas are part of the tumor spectrum suggested a causal role for p53 inactivation in gliomagenesis (82). Addition of wild type p53 to the glioblastoma multiforme cell lines lead to reversal of their malignant phenotype confirming a role for p53 in the astrocytic tumor development (83). p53 mutations in astrocytomas were first described in late eighties and were followed by a more extensive analysis of the gene mutations and p53 protein alterations in adult astrocytomas. Accumulation of wild type p53 protein has been reported in astrocytomas (84). The loss of p53 gene and LOH at this locus have also been implicated in the progression of gliomas (21,85). In the anaplastic astrocytomas grade III more than 60% cases have been reported to harbor p53 gene mutations (86). p53 has been suggested to play a role in the progression of secondary glioblastomas. The p53 protein is detected in approximately 15 to 40% of low grade astrocytomas, 35 to 60% of anaplastic astrocytomas, and 45 to 70% of glioblastomas (87). Malignant progression of astrocytic neoplasms has been associated with increasing expression of p53 protein (21,87).

**p53 as a Therapeutic Target**

Mutations at p53 gene locus are present in every second human tumor and during malignant transformation p53 or p53-pathway related molecules are known to be frequently disabled. Therefore, inactivation of p53 is critical for tumorigenesis. Since the normal function of p53 is critical to the regulation of cell cycle arrest and apoptosis, restoration of the p53 pathway has been the logical strategy for the treatment of cancer (88). Molecular therapeutic strategies to normalize p53 signaling in cells with mutant p53 include pharmacologic rescue of mutant protein, gene therapy, small-molecule agonists of downstream inhibitory genes, antisense and use of oncolytic viruses. Other strategies include activation of normal p53 pathway, inhibition of mdm2-mediated degradation of p53 and blockade of p53 nuclear export (89). p53 is one of the important molecular targets in radiation oncology. It is an important molecule that determines tissue-specific radiosensitivity. As a consequence the p53 pathway can be exploited to enhance cancer therapies especially when the tumors are caused by DNA damaging agents (90). Ad-p53 has been suggested to be most effective against gliomas when combined with radiation therapy and chemotherapy. Despite these encouraging findings, use of p53 gene therapy in patients has its constraints in the efficiency
of gene delivery and therefore development of novel methods ensuring the gene distribution throughout the tumor is required. A recent study on the childhood tumor retinoblastoma has revealed deregulation of the p53 pathway due to over expression of MDMX, a negative regulator of p53. MDMX has therefore been identified as a specific chemotherapeutic target for treatment of these tumors (91).

**Current Status**

Human brain tumors arise as a consequence of accumulation of multiple genetic alterations in brain cells, these cells become tumorigenic and further progression of these tumors lead to malignant phenotype. Brain tumors are heterogeneous because of the various cell types from which they initiate and are classified accordingly (2,92) (Figure 7). Wide range of genetic alterations is acquired during human brain tumor development and also during their further progression to higher histological grades. The various genetic alterations that characterize low-grade and high-grade tumors are as shown in Figure 8 (2,10,16, 17,21,87,93).

The wild type p53 protein is known to function as a cell cycle checkpoint protein at G1 to S and G2 to M transition phases of the cell cycle. Under normal conditions p53 gene is known to be expressed at lower levels in all tissues. In response to various cellular stresses that could cause DNA damage the level of p53 protein is reported to increase in these cells. As a response to DNA damage, p53 is known to induce cell cycle arrest which could allow repair of the damaged DNA. If the damage is irreparable then the p53 protein is known to induce apoptosis and thereby eliminate cells that contain damaged DNA (45). The increased p53 protein during such a process is then rapidly degraded by binding to MDM2, a primary negative regulator of p53 protein, which maintains a controlled p53 protein level in normal cells (45,94) (Figure 9a). Mutations at the p53 gene locus result in an altered or mutant p53 protein, which is resistant to MDM2-mediated degradation and hence results in the accumulation of the same in the cells carrying these mutations (45,95,96). Wild type p53 is a tumor suppressor and it is also a well-known DNA binding protein, which is known to bind to DNA as a tetramer. Presence of one mutant p53 allele and one wild type allele results in a hetero-tetramer complex containing both mutant and wild type p53 protein. The mutant p53 protein in such a complex is known to inactivate the wild type p53 protein in a ‘dominant negative’ fashion resulting in the loss of wild type p53 functions including the tumor suppressor function, cell cycle arrest and apoptosis. Presence of such a p53 tetramer in the cell with altered function could eventually lead to tumor development (45,95,96) (Figure 9b). If both the alleles of the p53 gene are altered it would result in a mutant tetramer which could lead to tumorigenesis (96) (Figure 9c).

LOH at the p53 gene locus, increased level of p53 mRNA and protein in all the human brain tumor types and tumors of all grades have been reported (21). The increased levels of p53 mRNA and protein observed in all the human brain tumor types could be due to deregulation of p53 pathways (97). Therefore, results from these studies are suggestive of a role for the p53 gene in the overall development of human brain tumors (21) (Figure 8, 9).

The observed increase in the levels of p53 protein in all the human brain tumor samples analysed could be due to a defective MDM2-mediated p53 degradation pathway in these tumors (98). Recent studies have reported increased apoptosis in neuronal cells with increased levels of wild type p53 protein (97). Therefore, it is conceivable that the increased level of p53 protein could lead to increased apoptosis in slow growing tumors such as meningiomas. Meningiomas are reported to be mostly benign and slow growing tumors (81). The slow growing behavior of meningiomas could possibly be due to a higher rate of apoptosis induced by the increased p53 protein levels, which in turn could be a limiting
Increased percentage of Ser 392 phosphorylated form of p53 protein was found only in the high-grade gliomas of grade III and IV (GBMs). To the contrary, lower percentage of Ser 392 phosphorylated form of p53 was present in all the low-grade gliomas and meningiomas of all histological grades as well as in the other brain tumor types (21) (Figure 11). Presence of increased percentage of Ser 392 phosphorylated form of p53 protein has been reported in human vestibular schwannomas (VS) of young patients only (16, 17). The VS tumors in the young patients have been reported to be highly proliferative compared to that of the older patients (99). Therefore, the observed increase in the percentage of Ser 392 phosphorylated form of p53 protein only in the high-grade gliomas could be indicative of high proliferative potential of these tumors (21). Phosphorylation at the Ser 392 residue of the p53 protein has been reported to stabilize the p53 tetramers (77). It appears that the increased percentage of Ser 392 phosphorylated form of p53 protein could lead to the formation of highly stable tetramers, which could be resistant to MDM2-mediated degradation (Figure 11). This could result in accumulation of higher levels of Ser 392 phosphorylated form of p53 protein in these high-grade gliomas (Figure 11). The wild type p53 protein has been reported to adopt a mutant-like conformation when it binds to DNA (100) (Figure 10). It is conceivable that the presence of higher percentage of Ser 392 phosphorylated form of p53 protein in the high-grade gliomas could have acquired an altered conformation. The function of wild type p53 is suggested to be regulated via its ability to adopt distinct conformation (100) and therefore an altered conformation could result in an altered function, which in turn could aid in tumor development (Figure 11). Increased Ser 392 phosphorylation of mutant p53 protein has been suggested to promote its dominant negative effects through heterooligomerization, thereby contributing to the proliferation and the aggressive behavior of transitional cell carcinomas (TCC) (78). Wild type p53 protein is also reported to undergo phosphorylation at the Ser 392 residue (55). The high-grade gliomas, particularly the GBMs are known to be biologically one of the most aggressive tumors with a survival period of less than 1 year for the patients from the time of diagnosis (82). Therefore, presence of higher
percentage of Ser 392 phosphorylated form of p53 protein only in the high-grade gliomas could be associated with the aggressive behavior of these tumors (21) (Figure 11). Emphasizing this conclusion further is the presence of lower percentage of Ser 392 phosphorylated form of p53 protein in all the low-grade gliomas as well as meningiomas of all grades and the other human brain tumor types. Meningiomas are reported to be mostly benign, slow growing and biologically less-aggressive tumors (2) (Figure 10, 11). Presence of higher percentage of Ser 392 phosphorylated form of p53 protein in the high-grade tumors could be suggestive of highly aggressive and fast growing tumors with high proliferative potential, decreased apoptosis and enhanced metastatic potential (21) (Figure 10, 11). Moreover, presence of lower percentage of Ser 392 phosphorylated form of p53 in slow growing tumors like meningiomas is suggestive of its role in less-aggressive tumors with low proliferative potential. It is conceivable that these slow growing tumors could have increased rate of apoptosis, probably p53-mediated apoptosis. Thus, presence of increased level of p53 protein could have a role in p53-mediated apoptotic pathway in these slow-growing tumors (21) (Figure 11).

Conclusion

Human brain tumors are one of the most difficult to manage and treat. Research in the past four decades resulted in no improvement in the survival of these patients. Therefore, development of novel approaches is essential to manage and treat these tumors. The p53 gene is one of the most important human tumor suppressor gene which affects both cell growth and cell death. Experimental evidence suggests that the function of p53 could be modulated at various levels – gene structure, expression, level of mRNA and protein, protein conformation and post-translational modification. Apart from point mutations, alteration at any of these levels could also affect the function of the protein and hence could lead to tumor development. In addition, presence of p53 isoforms along with the wild type p53 could also affect the tumor suppressor functions of the latter and could lead to tumor progression. Thus, in addition to other genetic parameters it is important to evaluate the p53 gene structure and expression and the protein status, posttranslational modification in particular, and presence of isoforms, if any, in individual tumor as this information could help in the development of custom-made protocols to manage and treat various human brain tumor types.

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TREATMENT RESISTANT ATTENTION DEFICIT HYPERACTIVITY DISORDER AN OUTCOME OF WEST SYNDROME: A CASE REPORT

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Abstract
West syndrome has been associated with cognitive decline and behavioural disorders like autism and attention deficit hyperactivity disorder. These disorders further impair the development of the child. However, the studies have not focused upon the prevention or management of these disorders in the course of west syndrome. We hereby report a case of treatment resistant attention deficit hyperactivity disorder as an outcome of west syndrome.

Key words: West syndrome, Outcome, ADHD

Introduction
Large numbers of children suffering from West syndrome have cognitive and behavioral problems. About 71-80% of children with West syndrome have mental retardation, 13% have infantile autism and 15% have hyperkinetic disorder (1,2). These behavioral disorders further impair the educational and social development of such children. However, there are no studies available about the management of hyperactivity in children with West syndrome. We are reporting a case of treatment resistant Attention Deficit Hyperactivity Disorder (ADHD) as an outcome of West syndrome.

Case report

History
A 10-year-old female was brought to child psychiatry OPD with complaints of inability to sit at one place, restlessness and beating other children. Her antenatal period was uneventful. She was a full term normal delivery and was delivered in a hospital. She started having salaam attacks at 5 months of age. Her EEG was suggestive of primary generalized epilepsy. CT scan head was normal. She was diagnosed to be a case of cryptogenic west syndrome by neurologist. She did not respond well to 400 mg per day of sodium valproate given for 1 year. She responded well to prednisolone 20 mg per day given in four divided doses for 4 weeks, but it had to be tapered off as she had developed cushingoid appearance. She was again put on sodium valproate but without much benefit. However, her problems resolved spontaneously at the age of 2.5 years.

From 3 years of age her parents found her to be hyperactive and difficult to manage. She would not sit at one place and would keep moving around. She would also push other children around. She would talk a lot and would not wait for her turn in play or for any sweets given at home. She dropped out of school because of her hyperactivity. She did not sit still in the class and disturbed other children. She did not take interest in studies at home also. There was no family history of similar illness and her other siblings were normal.

Examination
Physical examination did not reveal any abnormality. On examination she was hyperactive and was difficult to control. She did not follow instructions and did not involve in tasks given. Her I.Q. was 60 on Seguin form board test.

Diagnosis and treatment
She was diagnosed to be a case of attention deficit hyperactivity disorder-combined type with mild mental retardation as per DSM IV TR criteria (3). She was put on clonidine up to 8mcg/kg/day, atomoxetine up to 1.4 mg/kg/day, modafinil up to 400 mg/day and risperidone up to 1.5 mg/day subsequently without any benefit. However, she showed improvement on 400 mg/day of carbamazepine in 4weeks. Her scores on ADHD Rating Scale decreased from 48 to 18 after carabamazepine was started. In neurologist’s opinion no active neurological management was required. She still has symptoms of ADHD but is much more manageable than before.

Discussion
There is no literature about the treatment of ADHD in patients with west syndrome though it is reported to occur in 15% of patients with this condition. ADHD was difficult to treat in our patient. She did not show any response to specific medications for ADHD while she showed good response to carbamazepine. This shows that neural mechanism involved for causation of ADHD in children with west syndrome could be different from that of ADHD per se. The behavioral problems in these children could be due to the brain damage or dysfunction caused by epilepsy or the process leading to epilepsy (4).

Epileptic syndromes are associated with large number of cognitive and behavioral problems, which should be recognized and treated. Although, Cryptogenic form of west syndrome has been associated with good outcome, our patient developed mild mental retardation as well as ADHD. Early medical and psychoeducational intervention will lead to better long-term outcome in these children. Then early, effective treatment of epileptic syndromes may be important in preventing a number of permanent cognitive and behavioral problems.
Future research should focus on the prevention as well as the treatment of behavioral problems in children with epileptic syndromes.

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