Introduction
Introduction

1.1 Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a clinical syndrome characterized by a number of neurological, neuropsychiatric and motor disturbances seen in patients with liver dysfunction. HE is classified into two major divisions, acute (fulminant) and chronic based on the type of liver dysfunction. Fulminant hepatic failure (FHF) is a clinical syndrome resulting from severe inflammatory or necrotic liver damage. FHF is rapid in its onset and the symptoms appear within a short period of time. This is associated with sudden onset of necrosis of hepatocytes and degeneration of liver tissue without any established liver disease (Katelaris et al., 1989; Sherlock et al., 1971). Chronic hepatic encephalopathy, on the other hand, is slow in its occurrence and it takes a long period of time before the symptoms appear. The development of this form is gradual and it happens over a long period of time. The present study concentrates on the former type of the two kinds of Hepatic encephalopathy mentioned above.

1.1.1 Symptoms of Fulminant Hepatic Failure (FHF)

A wide spectrum of neurological, neuropsychiatric and neuromuscular derangements are associated with various stages of FHF. In the early stages, very subtle disturbances in the sleep rhythms, personality and emotional changes are observed. As the condition deteriorates, hypothermia, hyperventilation, confusion and drowsiness are seen (Jalan, 2003; Gayed et al., 1987; Margolis et al., 1979; Strauss et al., 1998). Seizures are not uncommon and multifocal
random muscle twitching is often seen before coma (Brown et al., 1992). In addition, neuromuscular changes such as asterexis, hyperflexia, unsustained clonus, deterioration of pupillary, corneal, occulovestibular, occulocephalic and brain stem reflexes are seen. Convulsions, cortical blindness, retarded speech, deterioration of speech, deterioration of EEG, sixth nerve palsy are also seen in these patients (Hoyumpa and Schenker, 1985) which finally lead to coma and death (Kanamori et al., 1996; Dejong et al., 1993). Chronic hepatic failure is slow in its onset and the symptoms appear gradually over a period of time.

1.1.2 FHF- Etiology

An important cause associated with fulminant hepatic damage is acute viral hepatitis. Of the several types of hepatitis viruses, hepatitis B is the most common and accounts for 74% of the clinical cases (Floersheim et al., 1975; Saunders et al., 1972). Non-A, non-B type viral hepatitis accounts for 24%, while type A accounts for 2% (Papas-Venegalu et al., 1984). Because of the horizontal and vertical spread of virus, this viral infection often assumes epidemic proportions.

Next to viral infections, ingestion of hepatotoxins also result in FHF. These include indiscriminate usage of drugs such as paracetamol (acetaminophen) (Morton et al., 1999; Baudouin et al., 1995), halothane (Braude et al., 1981), tetracycline, valproic acid (Ziyeh et al., 2003), anti-tuberculosis drugs, sulfonamides, diuretics etc (Plum and Hindfelt, 1976; Papas-Venegelu, 1984). These drugs, especially
paracetamol, act as hepatotoxins when they are used indiscriminately in large doses (Braude et al., 1981). Food contaminants, such as aflotoxins (fungal contaminants on ground nut), mushroom poisons, shellfish poisons, bacterial toxins and heavy metals also cause massive necrosis of hepatocytes and FHF (Mullen et al., 1994; Hoyumpa et al., 1985; Papas-Venegalu et al., 1984; Braude et al., 1981; Delong et al., 1982; Burroughs et al., 1982). Other known causes for FHF are Wilson's disease and hepatic malignancy (DeLong and Glick, 1982; Burroughs et al., 1982). FHF may also be due to extensive accumulation of fat in the liver as in Reye's syndrome, in pregnancy, and in patients with surgically constructed jejunoileal bypass for morbid obesity (Burroughs et al., 1982; DeLong and Glick, 1982).

In hepatic failure surveillance study, a very poor survival rate (22%) was observed in the patients. Clinical outcome of the patients with FHF depends on the number of surviving hepatocytes, age, sex, etiology and the stage at which the patient is provided with medical help. There is no specific treatment or drugs to be administered to the patients with Fulminant Hepatic Failure and the outcome depends on the supportive intensive care and better patient management. Even then, management of FHF patients is less rewarding (Hoyumpa and Schenker, 1985; Ferenci, 1991; Mullen et al., 1994).
1.1.3 Management of Patients

Various therapeutic procedures are adopted to treat Fulminant Hepatic Failure with limited success. These include dietary restrictions, administration of lactulose, lactulose + neomycin Sulfate, infusion of synthetic mixtures of amino acid or a-keto acids (Iwasa et al., Butterworth, 2003), dialysis (including haemodialysis, peritoneal dialysis), providing artificial liver support (by way of extracorporeal dialysis, administration of fetal liver cell agglutinates etc), use of bioreactors with immobilized hepatocytes and finally liver transplantation. The rationale behind these therapeutic approaches, their advantages and disadvantages are not discussed here as it is out of the scope of present investigation. However, it is suffice to mention here that the outcome of these therapeutic practices is not very satisfactory and the results are equivocal. This is quite evident from the poor survival rate (22%) of these patients and the final outcome is chiefly dependent on the support and management of the patient (Gerlach et al., 1994; Sussman et al., 1993; Takabatake et al., 1991; Morgan et al., 1990; Cooper et al., 1987; Conn et al., 1979).

Multiplicity of the therapeutic practices and the uncertainties in their outcome in FHF might be due to the enigmatic nature of the pathophysiological mechanisms involved in this condition. Several mechanisms have been proposed to explain the pathophysiology of FHF. Of these, only the major ones are described below. However, it is also cautioned that
Introduction

> these are not the ultimate mechanisms
➢ there may be some other mechanism(s) to be discovered
➢ more than one mechanism may be acting synergistically for the neural dysfunction in FHF and finally
> there could even be a unified mechanism.

The uncertainty of enigmatic mechanism of the pathophysiology of the disorder is also attributed to

➢ Lack of an appropriate animal model, which closely mimics the clinical condition of the human disease.

➢ Regional cellular heterogeneity of the brain which renders the studies more difficult, if not impossible. This heterogeneity in brain is seen in structure, function, and metabolism at cellular and sub cellular levels of organization.

➢ Multiplicity of factors implicated in the etiology of cerebral dysfunction in HE has further complicated the issue. These factors include ammonia, false neurotransmitters, mercaptans and short chain fatty acids (Zieve, 1981).

1.1.4 Pathophysiological Mechanisms

1.1.4.1 Ammonia

Elevated level of blood and tissue (including brain) ammonia is the hallmark of conditions of hepatic inadequacy (Jayakumar et al., 2004; Jalan et al., 2003; Murthy et al., 2000; Norenberg et al., 1996; Albrecht 1998; Ferenci et al., 1991; Hoyumpa et al., 1985; Cooper et al., 1987). Intestine is the major site for the production of ammonia in
the body. The microbial flora of the intestine act on the dietary nitrogenous compounds and produce large quantities of ammonia. Moreover, urea, which reaches the gut through entero-hepatic circulation is used by the microbes to produce ammonia. In addition, intestinal smooth muscle uses glutamine for its energy metabolism and thus generates ammonia even in the post-prandial period. Ammonia, thus generated, enters the portal circulation and reaches liver. In normal conditions, ammonia is efficiently detoxified in the liver by incorporation of ammonia into urea (periportal hepatocytes) and glutamine (perivenous hepatocytes). However, in the absence of functional hepatocytes, ammonia directly enters the systemic circulation and floods all the extra-hepatic tissues including brain. As a result, the levels of ammonia in brain and cerebrospinal fluid are elevated in fulminant hepatic failure and other liver diseases which is known to be neurotoxic (Schenker et al., 1974; Conn et al., 1979). Elevated levels of ammonia in blood and brain have also been reported in several conditions - for example in conditions of congenital deficiencies of urea cycle enzymes (Cooper et al., 1987).

Ammonia exists in two forms - either as unprotonated NH$_3$ (non-ionized) form or a protonated (ionic) NH$_4^+$ ion. These two forms are in a state of dynamic equilibrium in a solution and their inter conversion was found to be rapid and time dependent on the pH of the medium. The unprotonated form has a capacity to freely diffuse across the cell membranes as it is lipid soluble (Cooper et al., 1987; Roos et al.,
The protonated $\text{NH}_4^+$ form, on the other hand is largely impermeable. At physiological pH (7.4) about 97-98% of ammonia is in ionic form which is impermeable across the biological membranes. When the pH is towards the alkaline side, large amount of ammonia is present in the freely diffusible form ($\text{NH}_3$) and hence considered to be potentially very toxic.

1.1.4.1.1 Ammonia-Toxicity

The mechanism of ammonia toxicity, by itself, is a subject of intense debate and controversy. Energy depletion theory of Bessman and Bessman (1955) has attracted considerable attention and has been a subject of much controversy. The main tenet of this hypothesis is that ammonia is detoxified in the brain to glutamate and glutamine in the reactions mediated by glutamate dehydrogenase and glutamine synthetase respectively. In the glutamate dehydrogenase reaction, ammonia reacts with $\alpha$-ketoglutarate to form glutamate with the concomitant conversion of NADH to NAD. This would drain $\alpha$-ketoglutarate from citric acid cycle and interfere with energy production. Moreover, oxidation of NADH to NAD in this reaction, bypassing electron transport chain, would also affect energy production. In the glutamine synthetase reaction, glutamate reacts with another molecule of ammonia resulting in the production of glutamine and one ATP molecule is used up in this process. It was postulated that a combination of these two reactions would adversely affect the cerebral energy metabolism and consequently the energy dependent metabolic
Introduction

and physiological processes (Ratnakumari and Murthy, 1993; 1992; 1990; Jessy et al., 1991; Hindfelt and Siesjo, 1971; Bessman and Bessman, 1955). An alternate hypothesis for energy depletion is the adverse effects of ammonia on the operation of malate-aspartate shuttle in the brain (Faff-Michalak et al., 1991; Ratnakumari et al., 1989; Hindfelt et al., 1977). Since glutamine synthesis occurs in the cytosol, it has been postulated that this process depletes cytosolic pool of glutamate leading to lowered cytosolic glutamate. This would enhance the production of lactate and also affect the transport of reducing equivalents across the mitochondrial inner membrane (Therrien et al., 1991; Hindfelt et al., 1977).

Both these hypotheses have been tested vigorously and the results are equivocal - some in favor and some against. However, it is now established beyond doubt that cerebral glutamate levels are decreased while glutamine levels are elevated in the presence of elevated levels of ammonia in the brain (Ratnakumari and Murthy, 1993; 1992; 1990; Jessy et al., 1991; Murthy and Hertz, 1988; Butterworth et al., 1988; Subbalakshmi and Murthy 1985; 1983).

1.1.4.2 Disturbances in Neurotransmitter Functions

Yet another hypothesis proposed for the neurotoxic effects of ammonia is its effect on the neurotransmitter functions. Ammonia, at pathological concentrations, has been shown to interfere with the synthesis, storage (in synaptic vesicles), release and post-synaptic action of major neurotransmitters (Schafer and Jones 1982). As
glutamate and GABA are the chief excitatory and inhibitory neurotransmitters in mammalian brain (Danysz et al., 1995; Fonnum et al., 1984; Ericinska et al., 1990) much of the attention was focused on these two neurotransmitters. Studies from this and other laboratories have shown that in hyperammonemic conditions, glutamate release is enhanced while the reuptake (to terminate the neurotransmitter action) of glutamate is decreased in brain (Rao et al., 1992; 1991). In addition, region specific, time dependent alterations in the binding of glutamate to the receptors, particularly to NMDA subtype, has also been reported in conditions of hyperammonemia with and without liver failure. It is particularly interesting to note that NMDA receptor binding increases in cerebral cortex and pons-medulla regions while there is a great decrease in cerebellum.

Alterations in GABA receptors (parallel and opposite to glutamate), loss of M1 subtype of muscarinic acetylcholine receptors, receptors for dopamine, serotonin and opioid peptides have also been reported in conditions of fulminant hepatic failure (Rao et al., 1992; 1991; Van der Kloot et al., 1987; Fischer and Baldessarini, 1971).

In addition the involvement of mercaptans (Gracia-Compean et al., 1995; Zeive 1980; Chen et al., 1970; Phear et al., 1956) short chain fatty acids (Butterworth, 2003; Zeive, 1980; samson et al., 1956) have been implicated in the etiology of the cerebral dysfunction in hepatic failure conditions. Reports on hepatic inefficiency conditions also reveal an elevation in the levels of short chain fatty acids. Though the reasons
for this increase in the levels of short chain fatty acids is not understood completely, few investigators report that the decrease in the ATP and creatinine phosphate levels may be the cause (Cooper and Plum 1987; Papas-Venegelelu et al., 1984). In support of this, Zeive et al (1974) have observed that the infusion of short chain fatty acids results in the induction of coma.

1.1.4.3 Gamma Amino Butyric Acid (GABA)

GABA hypothesis was proposed by Schaffer and Jones (1982) to explain the HE. Gamma-amino butyric acid (GABA), a neuro inhibitory substance produced in the gastrointestinal tract is believed to be involved in the pathogenesis of HE. About 25%-45% of all the brain nerve endings may be GABAergic. During cirrhosis and FHF an increase in GABA levels is observed (Mullen et al., 1988; Maddison et al., 1987) which is probably due to the decreased metabolism of GABA in liver. When GABA crosses the extra permeable blood brain barrier of cirrhotic patients, it interacts with supersensitive postsynaptic GABA receptors. The GABA receptor, in conjugation with receptors for benzodiazepines and barbiturates regulate a chloride ionophore. Binding of GABA to its receptors permits an influx of chloride ions into the post synaptic neuron, leading to the generation of an inhibitory postsynaptic potential. Administration of benzodiazepines and barbiturates to patients with damaged liver or cirrhosis increases GABAergic tone and predisposes to depressed consciousness. This evidence includes isolation of 1,4-benzodiazepines from brain tissue of
Introduction

patients with FHF as well as the partial response observed in some patients and experimental animals after administration of flumazenil, a benzodiazepine antagonist (Als-Nielson et al., 2004).

1.1.4.4 Plasma Amino Acids and False Neurotransmitters

Fisher and his group observed an increase in the plasma content of aromatic amino acids (phenylalanine, tyrosine and tryptophan) and a decrease in the content of branched chain amino acids (leucine, valine, isoleucine) in conditions of hepatic failure (James et al., 1979). Observed changes in the plasma content of amino acids in FHF promote the transport of aromatic amino acids from the blood to the brain. Consequently brain will be flooded with aromatic amino acids in the absence of functional liver. As a result of this the content of these aromatic amino acids in the brain will increase beyond the $K_m$ of the respective hydroxylases (phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase) which are rate limiting enzymes in the conversion of aromatic amino acids into their respective neurotransmitter monoamines (dopamine, nor epinephrine, epinephrine and serotonin). In such conditions, aromatic amino acids are decarboxylated directly to aromatic amines such as $\beta$-phenyl ethylamine, tyramine, tryptamine etc, which are called false neurotransmitters. These amines displace the resident catecholamines (dopamine, nor epinephrine, epinephrine and serotonin) from the synaptic vesicles and are released upon stimulation thus altering the neurotransmitter balance in brain and hence the cerebral function. A
decrease in dopamine and nor epinephrine levels and an accumulation of false neurotransmitters have been reported in conditions of FHF (Ferenci et al., 1984). These false neurotransmitters even bind to the post- and pre- synaptic receptors of biogenic monoamines and switch on otherwise not needed neurotransmission (Fisher and Baldessarini, 1971). This mechanism has been implicated in the etiology of the cerebral dysfunction in conditions of liver inadequacy (Butterworth et al., 1994). Similarly, cerebral levels of serotonin were reported to be increased and the number of serotonin receptors on neurons were observed to be decreased in HE and this might contribute to the neuronal inhibition in HE (Riederer et al., 1982; Cummings et al., 1976). In certain cases of hepatic insufficiency and in experimental animal models, it was observed that perfusion of branched chain amino acids or their keto analogues resulted in an improvement of the clinical condition (Iwasa et al., 2003; Herneth et al., 1998; Beaubernard et al., 1984). It has also been demonstrated in recent years that aromatic amino acids are metabolized by alternate pathways generating kynurenine and quinolinic acid. The latter is known to act as an agonist for NMDA receptors and act as a pro-oxidant (Santamaria et al., 2003a).

1.1.4.5 Benzodiazepines

Apart from the above mentioned factors several groups have reported an increase in the levels of endogenous benzodiazepines leading to their accumulation in conditions of hepatic failure (Jones,
2000; Mullen et al., 1996; Butterworth et al., 1991). Further, an increase in the levels of these compounds also correspond with the progression and severity of the HE (Butterworth 1991; Rothstein et al., 1989). Due to their increased levels, these compounds will bind with the benzodiazepine receptors and trigger the receptors to produce neurosteroids (Papadopoulos et al., 1995) which may play a role in the etiology of disorder (Norenberg, 1997; 1991). An increase in the up-regulation of the peripheral benzodiazepine receptors has been reported by many investigators (Itzhak and Norenberg, 1994; Giguere et al., 1992; Lavoie et al., 1990). This up-regulation has also been shown in hyperammonemic conditions in the cultures treated with pathological concentrations of ammonia (Itzhak and Norenberg, 1994).

The research on the pathology of the brain in conditions of the hepatic failure suggest that the astrocytes have critical role to be played in the etiology of the disorder. Observations of the affected brains have shown astroglial degeneration (Albrecht, 1999, Butterworth, 1998; Norenberg, 1991). Astrocyte swelling is thought to be the major phenomenon that leads to the edema and death of the patients (Vaquero et al., 2003; Cardoba et al., 1996; Swain et al., 1991; Norenberg, 1991; 1977).

1.1.5 Mitochondria - Role in Neurodegenerative Disorders

Mitochondrial dysfunction has been implicated in ischemic brain damage, Alzheimer's disease, Parkinson's disease, Huntington's chorea, Fredric's ataxia, amyotrophic lateral sclerosis, Wilson's
Introduction

disease, Hereditary spastic paraplegia, some forms of dystonia, Apoptosis/ necrosis, aging, traumatic brain injury and list is still growing (Hatton et al., 2004; Schols et al., 2004; Beal, 2000; Tyurin, 2000; Kroemer et al., 2000; Wallace, 1999; Kaplan, 1999). Recent literature suggests the involvement of mitochondria and oxidative stress in the pathophysiology of FHF.

1.1.5.1 Oxidative Stress

Oxidative stress is a condition in which the production of free radicals is far in excess of their rate of detoxification by endogenous mechanisms (Rao et al., 2002). Being a highly aerobic tissue, accounting for 20% of total oxygen consumed by the body, brain is highly prone to oxidative stress (Gupta et al., 2003). Free radicals are produced in normal course of respiration and are estimated to be about 1 to 2% of the total oxygen consumed by the tissue (Kowaltowski et al., 1999). Furthermore, brain is rich in polyunsaturated fatty acids (Halliwell, 1992) and possesses high content of iron in certain areas, which is supposed to promote free radical production. Added to this, brain has low levels of antioxidant enzymes, low repair mechanisms and non-replicative neuronal cells (Halliwell, 1992). All these factors play a critical role in balancing the damaging effects and the antioxidant defenses. Mitochondria are the major sites of production of free radicals especially by the respiratory electron transport chain (Koch et al., 2004; Fiskum et al., 2004; Muller et al., 2004; Chance et al., 1979). Most of the free radical production occurs in the electron
transport chain present in the mitochondria especially in complexes I and III. Usually, these are detoxified by endogenous free radical scavengers such as glutathione, ascorbic acid and vitamin E and also by the enzymes superoxide dismutase and catalase. Failure of these detoxification mechanisms results in the condition of oxidative stress. It is paradoxical that free radicals affect the function of the complexes of electron transport chain leading to the production of more free radicals (Muller et al., 2004). Moreover, free radicals initiate peroxidation of lipids of the mitochondrial and plasma membrane and also affect the iron-sulfur centers of respiratory chain and enzymes such as aconitase. All these lead to the collapse of mitochondrial membrane potential, altered permeability of mitochondrial membranes and release of cytochrome c and apoptosis initiating factor. The last two activate caspase 3 and initiate the down stream events of apoptotic pathway (Bernardi et al., 1998; Zamzami et al., 1997; Kristal and Dubinsky 1997; Zorratti and Szabo 1995; Gunter and Pfeifer 1990).

1.1.5.2 Mitochondria - Source of Free Radicals

As the power houses of the cell, mitochondria are involved in the generation of ATP through the electron transport system. The transport of electrons through the respiratory complexes is a highly regulated process leading to the flow of electrons from high redox potential to low redox potential compounds. Occasionally some of the electrons are directly transferred to oxygen leading to the production of reactive oxygen species (Cadenas, 2004). In this process instead of two...
Introduction

electrons only one electron is transferred to oxygen resulting in the production of a super oxide anion (Muller et al., 2004). This is highly reactive and unstable oxygen radical. It is postulated that under normal physiological conditions oxygen is converted to superoxide radicals. In addition to this, superoxide radicals are also produced by certain mixed function oxygenases such as xanthine oxidase, aldehyde oxidase, by auto-oxidation of hydroquinones and catecholamines.

The superoxide radicals thus produced can oxidize a variety of biological substances and render them inactive. In addition the superoxide radical also reacts with other compounds to produce other reactive oxygen species. For example super oxide radical reacts with protons to produce hydroperoxy radical which further metabolizes to produce hydrogen peroxide. Superoxide can also react with hydrogen peroxide to produce hydroxyl radicals. In addition superoxide also reacts with nitric oxide to produce peroxynitrite radicals. All these three radicals, like superoxide radical oxidize proteins and lipids and adversely affect their functions. In fact evidences are now accumulating to indicate that free radical production can trigger cell death. Though mitochondria are the primary source of free radical production some amount of free radicals are also generated in other sub-cellular components through a variety of mechanisms. For example: transition metal ions especially iron and copper are known to produce free radicals by Fenton reaction.
1.1.5.3 Antioxidant Defenses

The free radicals that are produced under physiological conditions are detoxified by a variety of antioxidant processes. The cells are endowed with an enzyme superoxide dismutase, which converts superoxide to hydrogen peroxide. Both mitochondrial as well as cytosolic SOD have been identified. The hydrogen peroxide is converted to water and molecular oxygen by the enzyme catalase. It is interesting to note that this enzyme has the highest turnover rate indicating that it can very efficiently nullify the toxic effects of hydrogen peroxide. In addition to these two major mechanisms for detoxification of free radicals, there are several minor pathways participating in this process. For example, the thiol groups of glutathione are oxidized by hydrogen peroxide, thereby neutralizing the highly reactive peroxidase. Similarly Ascorbic acid and alpha tocopherol (vitamin E) also participate as antioxidants.

1.2 Evidences for Mitochondrial Dysfunctions of Brain in FHF

As the concept of oxidative stress occurring in brain during fulminant hepatic failure is new, there is no direct evidence for this as of today. Some of the results reported earlier for fulminant hepatic failure indicate the conditions which favor increased free radical production in brain during fulminant hepatic failure.

The levels of glutamine are enhanced in the cases of acute and chronic hyperammonemia conditions. (Rao et al., 1992; Butterworth and Giguere, 1986). This increase in the levels of glutamine is due to
the enhanced release and decreased uptake or reduced deamidation of glutamine (Subbalakshmi et al., 1985; 1983; Mathenson and Vandenberg, 1975). In addition to this evidence, Yu et al., (Yu et al., 1984) reported an increase in the synthesis of glutamine in the primary cultures of astrocytes that were subjected to pathophysiological concentrations of ammonia. Decreased uptake of glutamate was observed in primary cultures of astrocytes that were exposed to pathophysiological concentrations of ammonia for four days (Norenberg et al., 1989; 1985). Due to this condition of enhanced release and decrease uptake of glutamate from the nerve terminals, it would result in excessive accumulation of glutamate in the synaptic cleft and in the intracellular spaces in the brain. Glutamate, by itself is the main excitatory neurotransmitter. Prolonged exposure of neurons to glutamate due to the enhanced glutamate release will lead to excessive activation of the receptors for glutamate (especially NMDA subtype) which is supposed to be neurotoxic that leads to neuronal degeneration and cell death (Kosenko et al., 2003). Beal and his group (Beat et al., 1992) have reported that excessive activation of NMDA receptors is involved in neuronal damage in case of ischemic brain and it is also implicated in other neurodegenerative disorders. Glutamate receptors of NMDA subtype are highly permeable to calcium ($Ca^{2+}$). Elevated NMDA receptor activity results in increased $Ca^{2+}$ influx (Kosenko et al., 1997b; White and Reynolds, 1996) which would hamper the ATP synthesis and thus mitochondrial membrane potential.
Introduction

This might further result in mitochondrial electron transport chain alterations leading to enhanced production of free radicals and thus resulting in mitochondrial dysfunctions (Nicotera et al., 1997; Choi, 1996). This is supported by the evidence that blockers of NMDA receptors do prevent the glutamate and NMDA neurotoxicity (Kosenko et al., 1999; Koroshetz et al., 1996).

Collapse of mitochondrial membrane potential, production of ROS like superoxide (O$_2^-$), NO and activation of several other degradative enzymes are all the events that are proposed to be involved in creating an imbalance of cellular homeostasis that is triggered by the unregulated levels of glutamate (Nicotera et al., 1997; Choi et al., 1996; Beal et al., 1996; Patel et al., 1996; Mattson et al., 1995). A highly reactive peroxynitrite ion may be formed due to the interaction of superoxide and nitric oxide (Hensley et al., 1997). The above discussion suggests that the ROS that are produced due to the increased Ca$^{2+}$ levels have a major role to play in the glutamate mediated toxicity. Mitochondria seem to have an unique role in this regard since it is believed to be the major source of ROS and mobilization of intracellular Ca$^{2+}$ (Ichas et al., 1997; Budd et al., 1996; Bernardi et al., 1994). As discussed above excessive Ca$^{2+}$ accumulation in mitochondria uncouples electron transfer in the electron transport chain, leading to the enhanced production of free radicals (Nicotera et al., 1997; Ichas et al., 1997; Choi et al., 1996).

Now mitochondria have emerged as a missing link between the
Introduction

elevation of Ca$^{2+}$ and ROS mediated glutamate toxicity (Shinder et al., 1996).

Besides the conversion of tryptophan to serotonin, an alternative pathway for tryptophan metabolism has been observed in brain in recent years. In its alternate pathway, tryptophan is converted to kynurenine, kynuramine and kynurenic acid, 3-hydroxy kynurenine and 3- hydroxyl kynuramine. Further conversion of 3-hydroxy kynurenine to 3-hydroxy anthranilic acid and then to quinolinic acid has been detected in brain (Ratnakumari et al., 1993). All these reactions involve the opening of indole ring of tryptophan and the enzymes and the required cofactors are found in brain (Guidetti et al., 1995; Mawal et al., 1991). In addition to the endogenous synthesis, some of these compounds are also transported by neutral amino acids such as leucine, valine, isoleucine tyrosine, tryptophan and methionine (Fukui et al., 1991).

Studies on the formation of these compounds were given importance following the observation that some of them have modulatory effect on the neurotransmission mediated by other neurotransmitters. It has been shown that kynurenine and its derivatives act as antagonists for NMDA receptors while quinolinic acid acts as an agonist (Santamaria et al., 2003a). Quinolinic acid was observed to act as an excitotoxin and prolonged exposure to this compound results in the death of neurons. Kynurenine and its metabolites have been implicated in some of the neurodegenerative
disorders such as ischemic brain damage, Huntington's disease, poliovirus infection, AIDS, septicemia and encephalopathy (Stone et al., 2003; Sardar et al., 1995; Saito et al., 1993). It has been observed that the content of tryptophan derivatives is enhanced in conditions of fulminant hepatic failure. Hence it has been postulated that kynurenine and its derivatives, especially quinolinic acid might be affecting NMDA receptor mediated functions. As the compounds, which stimulate the glutamate receptors for prolonged period act as excitotoxins, it is believed that excess of quinolinic acid produced under these conditions might be acting as an excitotoxin (santamaria et al., 2003a; 2003b). It is suspected that this might be involved in the Pathophysiology of hepatic encephalopathy in fulminant hepatic failure (Chiarugi et al., 1995). In recent years, it has been shown that quinolinic acid can cause lipid peroxidation and induce oxidative stress in brain.

An elevation in the content of glutathione has been observed in the astrocytes treated with pathological concentrations of ammonia (Murthy et al., 2000). Glutathione has multiple roles in the functioning of cell and its survival which include maintenance of mitochondrial integrity, protection against free radicals (anti oxidant), oxidative stress, metabolism of xenobiotics and has a role to play in mitochondrial permeability transition (Janaky et al., 1999; Cooper and Kristal, 1997b; Wilson, 1997). The studies of Kosenko and his group (Kosenko et al., 1998; 1997) have indicated that the production of NO and other super
Introduction

oxides in the brain in hyperammonemic conditions influence the GSH levels.

Collapse of ΔΨₘ and permeability change which is a consequence of oxidative stress has been reported in mitochondria of astrocytes exposed to pathological concentrations of ammonia and this was prevented by pretreatment of the cells with methionine sulfoximine (Bai et al., 2001).

The above said results indicated that at least in astrocytes ammonia induces the production of free radicals and affects the mitochondrial function. However, it should be mentioned that most of these studies were carried out on cultured astrocytes. Such studies do help in identifying the cellular site of action and in avoiding the complications arising out of the inter organ and inter cellular interactions in in vivo studies, other toxic/protective factors produced in the body in response to the liver damage. Moreover, in vivo metabolic response of neuronal cells is governed by various blood borne factors and the interaction between neurons and glial cells. Such interactions are eliminated in the primary cultures. Even the drugs which protect the cells in cultures need not exert the same action under in vivo conditions due to modifications by other tissues or may even prove to be toxic.

Since the mitochondria are the major sites of free radical generation they are closely associated with the oxidative stress. As of today very scanty information is available on the role of cerebral mitochondria in generation of free radicals and imposing oxidative
stress leading to its dysfunctions in conditions of hepatic encephalopathy.

Overall, the present literature suggests that the above conditions prevailing in brain during FHF or hyperammonemic conditions are thought to be highly favorable for the generation of excessive free radicals and induction of oxidative stress. However there is no direct evidence of demonstration of these conditions in brain during FHF and hence this sets the stage for a detailed investigation on this aspect.
Introduction

Scope of the Present Work

Fulminant hepatic failure is not uncommon and may even assume epidemic proportions. Viral infections and ingestion of hepatotoxins appear to be the primary reasons. A wide range of neurological, neuropsychiatric and neuromuscular changes are associated with the conditions of FHF. Clinical outcome of the patients with FHF depends on the number of surviving hepatocytes, age, sex, etiology and the stage at which the patient is provided with medical help. There is no specific treatment or drugs to be administered to the patients with Fulminant Hepatic Failure and the outcome depends on the supportive intensive care and better patient management. Even then, management of FHF patients is less rewarding.

The Present work is aimed at evaluating the changes in the functioning of cerebral mitochondria leading to the production of free radicals with a concomitant oxidative stress in conditions of hepatic encephalopathy. For this purpose an animal model of Fulminant Hepatic Failure (FHF) was used. Animal model for FHF was generated by using thioacetamide, a well-known selective hepatotoxin. The present study will contribute towards the understanding of the pathophysiology of FHF and also help in developing an unified hypothesis.