I N T R O D U C T I O N

INTRODUCTION

The liver is the largest organ in the body and acts as a well equipped biochemical laboratory where practically metabolism of all the nutritional substances takes place. It has numerous functions in relation to blood circulation, production of bile, excretion, detoxification and protein synthesis (Ganong, 1993).

It is also the major organ responsible for the clearance and degradation of foreign antigens which enter the portal circulation from the gastrointestinal tract. Moreover several workers have demonstrated that the liver is a rich source of Kupffer cells (Shearman and Finlayson, 1982; Daniel et al, 1990) which belong to the mononuclear phagocytic system and are able to remove a variety of materials from blood like aging red blood cells, bacteria, viruses, endotoxins and antigen-antibody complexes. Unlike phagocytes elsewhere in the mononuclear phagocytic system which render antigens suitable for activating the immune system, Kupffer cells inactivate antigenic material and prevent cellular and humoral immune responses (Shearman and Finalyson, 1982; Tzung et al , 1990). Indeed antigen injected directly into the portal vein or ingested orally in larger amounts can lead to specific immune suppression to that antigen (Cantor and Dumont, 1967; Callery et al, 1989). Besides this the liver is

the primary site of synthesis of most complement components (Hoohwald et al , 1961; Rother et al, 1968; Alper et al, 1969; Morris et al, 1982; Miller, 1990; Brauer et al, 1994).

The stem cells for lymphocytes are known to migrate from yolk sac to the primary lymphoid organ via fetal liver (Davis et al, 1980). There is a recent view proposed by some workers that liver could be the site of generation of extrathymic T cells in old age (Ohteki et al, 1992).

Considering the overall function of the liver and the liver's possible role in immune regulation it is necessary to account for the immune functions affected by hepatic damage.

In recent years the role of immune reactions in relation to pathogenesis and prognosis of hepatic disorders has been stressed. It has been observed (Feizi, 1968; Wilson et al, 1969; Iturriaga et al, 1977) that increased plasma immunoglobulins is characteristic of chronic liver disease. While IgG increases in chronic active hepatitis and cryptogenic cirrhosis, IgA increases in alcoholic cirrhosis (Shearman & Finlayson, 1982) and IgM in primary biliary cirrhosis (Berk and Chalmers, 1981; Jones, 1983). But these increases are much variable and overlapping. Bradfield (1974) suggested that the increased immunoglobulin production in hepatic disorder could be due to increased antigenic stimulation of the immune system in consequence of diminished hepatic phagocytic function. Chronic liver diseases has also been characterised by fall in serum albumin concentration and

rise in the serum globulin concentration which is related to the severity and duration of the disease (Rothchild et al, 1972).

The discovery that a liver specific lipoprotein from the hepatocyte cell membrane could be used to immunise rabbits to produce chronic active hepatitis and cirrhosis further suggests relation between immune response and hepatic disorder (Meyer Zum Buschenfelde et al, 1972).

In general, liver diseases such as cirrhosis and obstructive jaundice (Roughneen et al, 1986; Feduccia, Scott and Grogan,1988; Vane et al, 1988) result in depressed immune function. Liver cirrhosis results in polyclonal hypergammaglobulinaemia with enhanced concentration of IgG, IgM, IgA (Husby et al, 1977) and IgE (Van Epps et al, 1976) but cellular immunity was observed to be defective. Peripheral blood T cells (Berenyi et al, 1974; Thomas et al, 1976), delayed type hypersensitivity (Thomas ,1977), lymphocyte proliferation (Macsween and Thomas, 1973) and natural killer cell function (Nakamura et al, 1983; Charpentier et al, 1984) are all diminished in liver cirrhosis. On the other hand there are also some reports indicating significant increase in the production of soluble IL-2 receptor in patients with cirrhosis and obstructive jaundice and a significant increase in soluble CD8 in liver cirrhosis (Wagner et al, 1990). Several workers have also

observed increased production of interleukin-1 (Sakamoto et al, 1984; Yokota et al,1987), interleukin-6 (Deviere et al, 1989), tumor necrosis factor alpha (Yoshoka et al, 1989) from the cells of liver cirrhosis patients in vitro.

It has been proposed by some workers that antibody dependent cellular cytotoxicity (ADCC) (Eddleston & Williams, 1974; Cochrane et al, 1976) and T cell mediated cytotoxicity (Thomson et al, 1974; Wants & Isselbacher, 1975; Paronetto & Vernace, 1975) may play some role in the destruction of hepatocytes in chronic active hepatitis. Accumulation of $CD8^+$ lymphocytes at the sites of hepatocellular necrosis have been reported by Eggink et al (1982; 1984) and Colucci et al (1983). Moreover, there are reports regarding infiltration of mononuclear cells in the liver (Hoffman et al, 1985; Meuer et al, 1985). Heavy infiltration of $CD8^+$ cytotoxic T lymphocytes (CTL) in the liver in experimental autoimmune hepatitis has been demonstrated by Kohda et al (1990). There is also a report (Stites et al, 1984) showing decrease in the number and function of suppressor T cells in autoimmune hepatitis patients.

In hepatitis B virus infection, the virus belongs to a strain that is poorly cytopathic or noncytopathic and it is perhaps the host's immune response to the virus that cause liver damage (Chisari et al, 1984; Barnaba & Balsano, 1992). Liver biopsies of patients with active liver disease show

accumulation of inflammatory cells, mainly T lymphocytes in close proximity of damaged hepatocytes (Colucci et al, 1983). In a recent investigation a conspicuous accumulation of $CD4^+$ $CD56^+$ T cells in the liver of hepatitis B virus patients were observed by Barnaba et al (1994). Interestingly these cells exhibited cytotoxic functions.

Certain drugs and chemicals or their metabolites may induce liver damage by immunologic mechanism by altering the immune regulatory system so that reactions to self antigens are no longer suppressed or it may alter hepatocyte antigen so that they are no longer recognised as self components (Stites et al , 1984).

Alcohol is known to cause liver damage since 1793 by Matthew Baillie (Sherlock, 1985). Direct hepatotoxic effect of alcohol and its metabolites have been ovserved by several workers (Lieber, 1984; Lieber, 1988; Ishak et al, 1991). The damage caused to the liver due to alcohol abuse leads to liver diseases (International group, 1981; Lieber, 1984).

As observed in liver cirrhosis, a wide range of immunological alterations have also been reported in alcohol induced liver diseases (Kanagasundaram & Leevey, 1981; Mendenhall, 1981; Watson et al, 1986; Mufti et al, 1989; Palmer, 1989). The humoral changes include increased

production of serum immunoglobulins. One of the earliest changes in these patients is an increase in the level of serum IgA (Stites et al, 1984; Itturiaga et al, 1977). Functional B cell alterations such as degree of responsiveness to T independent antigens have also been reported (Smith et al, 1980; Mendenhall et al, 1984; Drew et al, 1984; Aldo Benson, 1989). There are contradictory reports of increase (Si et al, 1983; Watson et al, 1984; Spinozzi et al, 1986; Bagasra et al, 1987) and decrease (Fernendez et al, 1982; Jovanovic et al, 1986; Roselle et al, 1988; Ewald, 1989) of total T cells or their subsets in alcoholic patients and experimental animals. Some qualitative changes in these lymphocytes like decreased responsiveness to mitogenic stimulation have also been reported (Mutchnik and Lee, 1988; Jerrels et al, 1989; Norman et al, 1989).

It has been shown that due to alcoholic abuse with large amount for a long time there is not only liver injury but also changes in peripheral T4/T8 ratio and immune regulatory function (Baeg and Siksun, 1990). A high concentration of alcohol was found to impede killer cell capacity under certain in vitro conditions (Schnabel et al, 1990). On the other hand, increased killer cell activity was also observed in alcoholic hepatitis and alcoholic cirrhosis by Schnabel et al (1991). Furthermore, phagocytic activity of Kupffer cells decreased and changes in endothelial cell structure ensue following perfusion with hepatotoxic chemicals

such as ethyl alcohol and ethyl hexaonl (Kopelle et al, 1991). Ethanol has been shown to enhance the expression of MHC class I and class II antigen on mouse fibroblasts and teratocarcinoma cells, human neuroblastoma cells and on human peripheral blood lymphocytes (Parent et al, 1987; Kolber et al, 1988; Singer et aL, 1989). Ethanol also influence the expression of theseo antigens on human fetal islet type cell clusters (Ruhland et al, 1991). There are also reports of decrease in DNA synthesis of lymphocytes and increase in lipid peroxidation in plasma of alcoholics (Tupinka et al, 1991). Increased lipid peroxidation in alcoholics have also been reported by Jaya et al (1993).

All these reports are mainly concerned with changes in immunological parameters after extensive damage and liver disease. There is no comprehensive report to indicate the changes in the immunological parameters thet may set in, in the stage when there is no apparent alcoholic liver disease. It is important to determine whether the changes in immune status occurs only in the aggravated state of liver disease or it begins in the early stage with the onset of liver damage when there is no overt disease. **In the present investigation,** we intend to analyse the changes in immune status in chronic alcoholics in terms of serum immunoglobulin concentration, T and B cell ratio in peripheral blood, percentage of rosette forming cells, blastogenesis and DNA synthesis in proliferating T cells and the cytotoxic

capability of T lymphocytes in the stage when the liver is damaged but there is no apparent liver disease. The damage to hepatocytes was determined in terms of albumin-globulin ratio and activity of gammaglutamyl transpeptidase (GGT), in the serum.

It has been suggested recently that the hepatotoxic effect of alcohol and its metabolites is possibly mediated by free radicals (Floersheim, 1987; Roll et al, 1991). Moreover, The changes in immunological parameters may also contribute in the development of hepatic damage. In this context it may be practical to use ascorbic acid which is known to counteract and neutralize the harmful effects of many toxins in the body (Holmes et al, 1939; Marchmont, 1941;Hill, 1979) and thus playing the role of a potent detoxicant. It has been known for sometime that ascorbic acid is not only a good reducing agent but also an effecient radical scavenger (Benon, 1975). Lewin (1973) has proposed that high dietary levels of ascorbic acid might result in increased elimination of toxic elements from the body. Dietary ascorbic acid was also found to play a protective role against many of the adverse effects of heavy metals (Fox et al, 1971; Suzuki and Yoshida, 1979) and nitrites (Mirvish et al, 1972; Kam et al, 1973; Cardessa et al, 1974; Mergens et al, 1978). Recently, it has been demonstrated by Shiraish and Waalkes (1993) that ascorbic acid pretreatment decreased the toxicity of cadmium in rats. The beneficial effect of ascorbic acid in the

degradation and elimination of pesticides (Chadwick et al, 1971; Wagstaff & Street, 1971) has also been stressed. Some workers also found that ascorbic acid was capable of inactivating a number of bacterial toxins (Juneblut et al, 1935; Juneblut, 1937).

Moreover, ascorbic acid is important for maintenance of epithelial cells and the function of polymorphonuclear neutrophils (PMNS). This vitamin is present in high concentration in polymorphonuclear neutrophils and macrophages and is important for maintaining the phagocytic activity of these cells (Cottingham and Mills, 1943; Dechatelet et al, 1971; Chatterjee et al, 1975). Wilson (1975) observed that ascorbic acid has a regulatory role in maintaining the structure and number of lysosomes and their enzymatic activity. In a recent report it has been shown by Wolf (1993) that human neutrophils acquire a high level of intracellular ascorbic acid to counteract the damaging effect of oxidants produced by the neutrophils themselves for destroying bacteria. The production of interferon is dependent at least to some extent on the action of ascorbic acid (Lewin, 1974). Loh et al (1973) have suggested that ascorbic acid may play a role in the antigen-antibody reaction. This may not be totally out of context to mention here that recently an enhanced serum complement activity has been ovserved in Atlantic Salmol receiving high dose of dietary vitamin C (Hardie et al, 1991).

Intake of large dose of ascorbic acid have also been reported to be beneficial in reducing the frequency and severity of common cold (Pauling, 1970; Cook et al, 1977) and possibly in reducing the incidence of cancer (Schneider, 1954; Schlegel et al, 1969 ; Polasa, 1989; Yu et al, 1994). It is possible that ascorbic acid plays a role in prostaglandin synthesis and thereby influence the inflammatory reactions in the upper respiratory membranes (Sharma,1974). Besides this ascorbic acid may also play an important role in the detoxification of histamine (Chatterjee et al, 1975). The need for a higher intake of ascorbic acid than the conventional dose has been stressed recently by several workers for counteracting the oxidative stress in the body (Gershoff, 1993; Barja et al, 1994). Reports also indicate that mega ascorbic acid therapy would produce dramatic and rapid recovery of different cases of hepatic disorder (Baur & Staub, 1954; Calleja & Brooks, 1960) but till date its relation to immune system has not been elucidated.

Furthermore it has been reported by several workers that ascorbic acid is readily oxidised to dehydroascorbic acid and can be reversibly reduced (Seberell et al, 1954; Burns, 1975). It has also been reported that dehydroascorbic acid content of blood is negligible in normal subjects but under varying physiological and pathological conditions

dehydroascorbic acid accumulates in blood and tissues and sometimes produce toxic effect (Banerjee et al, 1953; Chakraborti & Banerjee, 1955; Bhaduri and Banerjee, 1960). In man the greatest ability to reduce dehydroascorbic acid to ascorbic acid was shown by the liver (Matusis, 1951). Therefore disturbance of liver function may be associated with change in blood dehydroascorbic acid content. Since reports regarding the ascorbic acid and dehydroascorbic acid status in alcoholics are scanty we thought of studying the level of ascorbic acid and dehydroascorbic acid in the blood of chronic alcoholics as well.

In the present study we intend to investigate whether the radical scavenging and other effects of ascorbic acid can help to arrest the hepatic damage due to alcohol abuse and the associated immunological changes in alcoholics.

Interestingly, zinc has also been gaining importance in recent years not only as a detoxicant but also as an immunostimulant. Moreover a moderate zinc deficiency has been observed in alcohol induced liver diseases by several workers (Vallee et al, 1956; McClain & Su, 1983). Thus in the present investigation we would also like to evaluate the efficacy of zinc as therapy for alcoholics, especially to boost the immunity.

Organic zinc salts have been reported to have a

protective effect against acute ethanol toxicity in mouse (Floersheim , 1987). A protective effect of zinc in the hepatotoxicity of bromobenzene and acetaminophen has also been observed by Szymanska et al (1991). In addition, zinc may function as a membrane stabilizer. This is based on the observations that zinc can stabilize lysosomes in vitro, inhibit lipid peroxidation in livers of CCl_4 -treated rats (Chvapil et al, 1972; Chvapil et al, 1974) and inhibit hemolysis of erythrocytes (Bettger et al,1978). Zinc may also play a role in portein synthesis and therefore in wound healing (Senopati & Thompson, 1985). Karcioglu et al (1980) have also proposed that zinc may have some role in the inhibition of carcinogenecity.

Furthermore, zinc is necessary for several cellular processes, such as steps in cell cycle, m RNA metabolism and function of more than 70 enzymes (Follin et al, 1941; Prasad, 1979; Bunk et al, 1989; Bhaskaram and Hemlatha, 1994) some of which (thymidine kinase, DNA polymerase) are involved in DNA synthesis (Stites et al, 1984). Thus this metal has a stimulating effect on all replicating cells, including those involved in immune response. Clinical and experimental observations have highlighted the importance of zinc status in maintaining immunological integrity (Fraker et al, 1977; Cossack, 1989; Moulder & Steward, 1989). Zinc may have a key role in regulating antibody synthesis (Cunningham-Rundles et

al, 1980), activation of T cells (Fernandez et al, 1979; Fraker et al, 1986) and natural killer cells (Chandra, 1980). In Addition, zinc has been shown to augment in vitro production of T-cell lymphokines such as IL-2, IL-4 and gamma interferon (Salas & Kirchner, 1987; Winchurch, 1987; Tanaka et al, 1990).

Zinc deficiency affects both cell mediated and antibody mediated immune responses (Malave & Benaim, 1984; Mathe et al, 1985). An increased susceptibility to a variety of diseases has also been reported in zinc deficiency (Oleske et al, 1979). King Louis et al (1991) have shown that zinc deficiency in adult mice caused significant reduction in the total number of splenic lymphocytes. It has also been demonstrated (Martin et al, 1991) that cell death can be induced by zinc deficiency in human cell lines of both lymphoid and myeloid origin. The principal cell type thought to be down-regulated by zinc deficiency, at least in animal models, is the helper subpopulation of T lymphocytes (Fraker et al, 1978). Some workers pointed out that even mild zinc deficiency may be associated with impaired interleukin-2 production (Kaplan et al, 1988).

The possible effect of zinc as an immunostimulant was evaluated by Bumb et al (1984). They supplemented some multibacillary leprosy patients with oral zinc sulphate and

observed satisfactory changes. A significant increase in DNA synthesis in proliferating lymphocytes was also observed in patients with Down's syndrome after oral administration of zinc sulphate (Stabile et al, 1991). Moreover it has been observed that supplementation of zinc also decreased the percentage of sickled cells in patients with sickle cell disease (Brewer et al, 1977; Muskiet et al, 1991).

In the present investigation an attempt has also been made to study the effect of oral zinc supplementation on alcohol induced hepatotoxicity and altered immunological parameters in chronic alcoholics without apparent liver disease. **Thus the objective of the present investigation** is to study the changes in immunological parameters that may set in, in the stage when there is no apparent alcoholic liver disease and also to evaluate the possible protective effect of ascorbic acid and zinc against the alcohol induced hepatotoxicity and altered immunological parameters.

OBJECTIVE OF THE PRESENT INVESTIGATION AT A GLANCE

1) *Liver damage in the chronic alcoholics without overt liver disease have been ascertained by estimating the serum GGT activity and albumin-globulin ratio.*

Increased GGT value and decreased albumin-globulin ratio is the index of hepatic damage.

2) *The immunological parameters such as the level of serum immunoglobulin, T:B cell ratio in peripheral circulation, percent of rosette forming T cells, blastogenesis and DNA synthesis in proliferating T cells and cytotoxic capability of peripheral T lymphocytes of the subjects with liver damage were studied to find out the immune status of a person with hepatic disorder.*

3) *The persons with hepatic disorder and changed immunological parameters were divided into two groups for therapy.*

i) *A group of subject was treated with mega ascorbic acid therapy orally at a dose of a 3 gms per person per day for 1 month.*

ii) *The 2nd group was treated with oral zinc sulphate at a dose of 660 mg per person per day for 1 month.*