CHANGES IN COMPOSITION AND PROPERTIES OF ERYTHROCYTE MEMBRANE IN CHRONIC ALCOHOLICS

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Abstract — Aims and Methods: Alterations in cholesterol and phospholipid contents as well as fluidity and lipid peroxidation in erythrocyte membranes from chronic alcoholic humans were investigated. Results: While an increase in cholesterol with no change in phospholipid content was observed in erythrocyte membranes, the phospholipid content increased with no change in cholesterol in plasma. Conclusions: An increase in microviscosity and a consequent decrease in membrane fluidity were evident from the studies of fluorescent hydrocarbon pyrene mobility in the bilayer of erythrocytes in chronic alcoholics. Also, an enhancement in the lipid peroxidation of erythrocytes from alcoholics is indicative of structural damage of membrane resulting from oxidative stress.

INTRODUCTION

Earlier studies relating to alcohol intoxication in humans and certain animals clearly indicate that ethanol affects the physicochemical properties of the cell membrane (Beauge et al., 1988; Aurfrere et al., 1988; Stibler et al., 1991). Membrane reorganization and adaptation can develop against acute disorganizing effects of ethanol during chronic intoxication (Linda et al., 1982; Hoek and Taraschi 1988; Beauge et al., 1990). In the absence of a precise mechanism explaining the alcohol intoxication, two theories, namely lipid theory and protein theory, have been proposed (Peoples et al., 1985, 1986; Wood et al., 1991). Extraction of lipids from erythrocyte membrane and the determination of cholesterol and phospholipids were done as per the method adopted by Jain et al. (1988). The plasma cholesterol was assayed according to the enzymatic kit method of Allian et al. (1974). Plasma phospholipids were assayed accordingly to the method of Connert et al. (1961).

To determine membrane fluidity, the optical method of Galla and Sackmann (1974), as adopted by Bryszewska et al. (1986), was used. This method was based on the dependence of microviscosity — the lateral diffusion rate of fluorescent aromatic hydrocarbon pyrene. A measure of lateral diffusion rate is the ratio of fluorescent intensity of dimer and monomer (D/M ratio) in the fluorescence spectrum of pyrene. Pyrene fluorescence was excited at 339 nm and the intensities were measured at 395 nm (monomer) and 469 nm (dimer). All the fluorescence measurements were performed at 37°C with an F-3010 fluorescence spectrophotometer (Hitachi, Japan). Red cell preparation was done according to the method described by Beutler (1975) and the extent of lipid peroxidation in red cells was measured by assaying the malondialdehyde (MDA) formed (Buege and Aust, 1978).

Statistical analysis

The data obtained in the present study were subjected to Student’s t-test.

MATERIALS AND METHODS

Human male volunteers aged 38–50 years with similar dietary habits were divided into two groups, namely nonalcoholic controls and chronic alcoholics. Chronic alcoholics were those who had consumed ~125 g of alcohol at least five times per week for the past 10–12 years and volunteers had a smoking status of 8–12 cigarettes/day. All volunteers were informed about the experimentation and their consent was obtained; subjects using any drugs (tranquilizers, analgesics, etc.) or with any disease or disorder were excluded.

Sampling and assay methods

Freshly drawn heparinized blood samples were used for membrane and plasma analysis. Erythrocyte membranes were prepared as per the method of Dodge et al. (1963); membrane protein was estimated following the method of Lowry et al. (1951). Extraction of lipids from erythrocyte membrane and the determination of cholesterol and phospholipids were done as per the method adopted by Jain et al. (1988). The plasma cholesterol was assayed according to the enzymatic kit method of Allian et al. (1974). Plasma phospholipids were assayed accordingly to the method of Connerty et al. (1961).

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RESULTS

An increased membrane cholesterol content and unaltered phospholipid content caused an increase in the membrane cholesterol/phospholipid (C/P) ratio in alcoholics (Table 1). There was no change in plasma cholesterol and a significant increase in the phospholipid levels of plasma leading to the decrease in plasma C/P ratio in alcoholics when compared to the nonalcoholic controls. An increase in the microviscosity of alcoholic erythrocyte membrane was observed in the present study and is expressed as dimer/monomer (D/M) ratio. A three-fold increase in the formation of MDA in red cells indicated enhanced lipid peroxidation in chronic alcoholics.

DISCUSSION

An enhanced membrane cholesterol level in the present study clearly indicates enrichment of erythrocyte membrane with cholesterol, and this might be due to ethanol-induced enhanced transfer of cholesterol from plasma (Hagerman and Gould, 1951; Chin and Goldstein, 1984). Enrichment of human erythrocyte membrane with cholesterol decreases the bulk lipid fluidity and causes surface exposure of membrane proteins (Borochov et al., 1979). In addition, cholesterol-enriched membranes have been shown to be relatively resistant to disordering effect of ethanol, and cholesterol may act by decreasing the partition coefficient of ethanol in membranes (Chin and Goldstein, 1984). Increased C/P ratio in alcoholic erythrocyte membrane indicates the decreased fluidity, influencing viscoelastic properties of the membrane which is in agreement with other reports (Beaune et al., 1985, 1988; Stibler, 1991). This result was confirmed further by the fluorescence anisotropic studies using pyrene which showed a decrease in translational mobility of pyrene due to increased intramembrane microviscosity, which is indicative of the decreased erythrocyte membrane fluidity (Bryszewska, 1986). This finding contradicts the results reported by Hrelia et al. (1986). The observed increase of lipid peroxidation corroborates the reports of others (Uysal et al., 1986; Meagher et al., 1999). Recent studies also reveal that alcohol induces oxidative stress and increases formation of various adducts (Meagher et al., 1999; Nalini et al., 1999; Adachi et al., 2001; Niemela, 2001). Punchard et al. (1994), Clemens et al. (1997) and Gatti et al. (1995) have reported decreased lipid peroxidation that was attributed to an increased resistance to lipid peroxidation in alcoholics. Such a resistance was not observed in the present study. The observed increase in LPO in the present study might be due to increased oxidative stress and by the ethanol-induced generation of free radicals and increased formation of cholesterol hydroperoxides (Adachi et al., 2003) or due to other adducts, as reported by others (Niemela, 2001; Reinke et al., 2001). Depletion of antioxidants in alcoholics may also be a contributing factor (Punchard et al., 1994; Morelli et al., 1998; Zima et al., 2001). Increased LPO is indicative of membrane damage and further sequelae (Selvam and Anuradha, 1988). Increased amounts of cholesterol in the membrane were shown to increase the apparent microviscosity, which explains the decreased pyrene mobility or diffusion in the erythrocyte bilayer. It is interesting to note that cholesterol incorporation in the hydrocarbon core of erythrocyte membrane enhances oxygen diffusiveness (Dumas et al., 1997). The present data clearly suggest that alcohol dependence in humans results in decreased erythrocyte membrane fluidity coupled with enriched membrane cholesterol and increased lipid peroxidation.

REFERENCES


