Symposium: Nutritional Factors and Oxidative Stress in Experimental Alcoholic Liver Disease

Introduction

Eduardo A. Porta

Department of Pathology, School of Medicine, University of Hawaii, Honolulu, HI 96822

This symposium is dedicated to the memory of Dr. Nicholas DiLuzio, who was the first to propose a role of oxidative stress in experimental alcoholic liver disease (ALD) (DiLuzio 1994). Although his findings also originated the controversies that are even today so highly debated, his dedicated efforts greatly stimulated the interest in this area of research. Direct in vivo evidence that chronic ethanol ingestion generated hepatic free radicals was reported by Reinke et al. (1987). In this symposium he presents the spin trapping and electron paramagnetic resonance spectroscopic studies in rats chronically fed ethanol liquid regimens containing a high or low level of fat (Reinke and McCay 1997). The possible pathogenic role of oxidative stress in ALD has been the topic of many reviews and symposia. However, the well-known modulating effects of nutritional factors on free radical reactions and antioxidant defense mechanisms (Chow 1979 and 1991, Machlin and Bendich 1987) have not been properly addressed in the context of experimental ALD. This field of research is now dominated by in vitro studies that make it difficult to relate detectable oxidative stress to the in vivo conditions in the liver during chronic excessive ingestion of ethanol. On the other hand, most of the in vivo studies primarily implicating ethanol or its metabolites in the generation of oxidative stress have been based on results obtained by the administration of totally liquid ethanol diets (high in fat and low in carbohydrate) that were intended to be nutritionally adequate (i.e., DeCarli and Lieber 1967) but which were shown to be inadequate (Frank and Baker 1980, Rao et al. 1992). Thus, a great deal of confusion exists about the relative roles of malnutrition and hepatotoxicity of ethanol on the oxidative stress eventually detected in animal models of ALD. Several reasons have contributed to this uncertainty; one of the most notorious is the highly questionable assumption originated and still maintained by some investigators (Lieber et al. 1963, Lieber and DeCarli 1991) that chronic ethanol administration to experimental animals invariably exerts hepatotoxic effects even in the absence of malnutrition and genetic, infectious or toxic factors.

In recent years, many workers in this field have frequently disregarded or ignored fundamental nutritional research on ALD reported during the middle of this century by Best et al. (1949), who showed that the fatty liver and fibrosis observed in rats chronically fed ethanol were essentially due to an induced deficiency of choline and methionine and were prevented by the dietary supplementation with these lipotropic factors. The notion that ethanol feeding increases the requirements of lipotropes in rats has been amply confirmed (Barak et al. 1973, Forbes and Duncan 1950, Klastkin et al. 1954, Thompson and Reitz 1979), although it was then argued that choline may not be an essential hepatoprotective nutrient in humans because primates have less choline oxidase than rats (Sidransky and Farber 1960). However, in view of recent findings (Buchman et al. 1995, Chawla et al. 1989, Zeisel and Blusztajn 1994), few nutritionists would now dismiss the essentiality and hepatic lipotropic action of choline in humans. Furthermore, those who most strongly denied for years the importance of lipotropes in ALD have recently recognized their hepatoprotective effects in their baboon model (Lieber et al. 1990). It was also shown some 30 years ago that the level and type of dietary fat influenced the development of fatty changes and cirrhosis in rats fed hypolipotropic regimens. High fat diets, for example, increased the severity of hepatic lesions (Zaki et al. 1963), and diets high in unsaturated fat (i.e., corn oil) aggravated the severity of cirrhosis (Gyorgy et al. 1959, Patek et al. 1963). Thus, a diet high in ethanol and unsaturated fat should provide enough lipotropes to account for these increased requirements. In relation to oxidative stress, it is worth mentioning the very old demonstrations that elevated dietary levels of unsaturated fat increased the requirements of vitamin E (Evans and Burr 1927) and rendered tissue lipids susceptible to peroxidation (Zalkin and Tappel 1960). The most commonly used ethanol liquid diets are high in unsaturated fat and contain inadequate amounts of vitamin E (Eskelson et al. 1990).

When the experimental models of ALD are based on totally liquid regimens high in fat, another frequently overlooked problem is the fact that by replacing large parts of the carbohydrate in the control diets with ethanol, the carbohydrate content becomes extremely low. Low carbohydrate, high fat regimens are obviously ketogenic, and because the metabolism of ethanol per se is also essentially ketogenic rather than glycolytic, these regimens are unbalanced and nutritionally inadequate. Additionally, as Rao discusses in this symposium (Rao and Larkin 1997), the fatty liver, high blood ethanol concentrations, subnormal food intake and growth, low hepatic levels of glycogen, and altered hepatic methionine metabolism observed in rats fed the high fat, low carbohydrate ethanol liquid regimens can all be avoided or abated by dietary manipulations (Rao et al. 1992).

1 Presented as part of the symposium “Nutritional Factors and Oxidative Stress in Experimental Alcoholic Liver Disease” given at Experimental Biology 96, April 15, 1996, Washington, DC. This symposium was sponsored by the American Society for Nutritional Sciences. Guest editor for the symposium publication was Eduardo A. Porta, University of Hawaii, Honolulu, HI.
Although malnutrition is clearly a fundamental pathogenic factor in experimental ALD, it is obviously the result of excessive ethanol ingestion first diminishing the quality of the diet and secondarily affecting the bioavailability of nutrients. Low or moderate amounts of ethanol do not produce this condition in animals or humans. Clearly, the excessive consumption of ethanol produces a series of metabolic changes that, although largely prevented or abated by dietary manipulations, have perforce to contribute in some measure to the development of the whole pathologic spectrum of ALD, from fatty liver to alcoholic hepatitis, cirrosis and even cancer, as seen in many but not all human alcoholics. It is therefore the synergic action of ethanol and malnutrition that leads to this disease. This should not be interpreted as assigning serious hepatotoxicity to ethanol chronically consumed even in large amounts.

Because nobody has yet reproduced in animals the whole pathologic spectrum of human ALD in the absence of malnutrition, and because only a minority of human alcoholics develop this spectrum, other factors may be partly responsible for the development of human ALD. It is accepted now that viral hepatitis B and C infections are important co-factors in a substantial proportion of human cases of alcoholic cirrhosis and hepatocellular carcinoma (Bréchot et al. 1982, Parés et al. 1990). It is quite probable that other pathogenic mechanisms might be involved in the human situation. For example, it has been known for some years that, depending on the diet, the chronic ingestion of ethanol by animals and humans may result in altered lipid and fatty acid profiles in hepatic membranes. French was in fact the first to report that, under defined nutritional conditions, chronic ethanol ingestion in rats altered the composition of hepatic fatty acids, so that the proportion of linoleate increased and that of arachidonate decreased in various liver organelles (French et al. 1970). In this symposium he discusses his recent findings (French et al. 1997) on the possible relations among hepatic fatty acid metabolism, activities of CYP2E1 and lipid peroxidation in the experimental model of chronic alcoholism he developed with H. Tsukamoto, UCLA School of Medicine, Los Angeles, CA.

Among other pathogenic mechanisms related to oxidative stress that have been implicated in experimental ALD are the hepatic centrilobular hypoxia as a consequence of increased oxygen extraction and decreased oxygen tension in perivenular hepatocytes (Israel et al. 1975), the formation of deleterious covariant protein adducts (Sorrell and Tuma 1987) and the effects of endotoxia (Bode et al. 1987). Thurman, who has been working for many years in different aspects of ALD using diverse experimental models, is presenting in this symposium his recent findings on the interrelations between endotoxia, Kupffer cells and free radicals (Thurman et al. 1997).

One of the most intriguing observations is the fact that a substantial proportion of liver biopsies from humans with chronic alcoholism did not show significant pathologic changes (Derr et al. 1990). It has been shown, however, that under certain nutritional conditions, experimental animals may ingest large amounts of ethanol for prolonged periods without showing any significant liver damage. This has been demonstrated in an experimental model using totally liquid diets (Porta et al. 1968, Rao et al. 1992), in the intragastric ethanol infusion model of Tsukamoto and French (Nanji et al. 1989) and in the sweetened ethanol model of Porta and Gomez-Dumm (Koch et al. 1969). Recently, we explored in rats the chronic effects of spontaneously consumed ethanol and diets with variable amounts of nutrients on several prooxidant and antioxidant hepatic factors. I will present our results and discuss my personal views on the nutritional modulation of these factors (Porta 1997).

The role of oxidative stress in the pathogenesis of ALD remains controversial, and more research is necessary to resolve this situation. In future studies, it would seem necessary to consider some of the requisites given in Table 1 to establish a role of oxidative stress in ALD.

### Table 1

| Some of the requisites to establish a causal role of oxidative stress in alcoholic liver disease |
|---------------------------------|-----------------|
| Evidence of abnormal in vivo increase in hepatic prooxidant factors and/or decrease in antioxidant defense mechanisms. |
| Evidence that increased prooxidant or decreased antioxidant factors precede hepatic damage. |
| Evidence of a correlation between the extent of oxidative insult and the magnitude of liver damage. |
| Evidence that the oxidative stress creates lesions severe enough to compromise the structure and function of the liver. |

### Literature Cited

INTRODUCTION


