Session V

THERAPEUTIC OPTIONS FOR NASH

Clofibrate and Other Fat Lowering Agents for Treatment of NASH

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The pathogenesis of NASH is unknown but may relate to excessive accumulation of lipids in the liver. Steatotic livers secondary to alcohol abuse or associated with type Il diabetes mellitus contain predominantly triglycerides and to a lesser extent cholesterol esters. Elevated hepatic free fatty acids, products of triglyceride hydrolysis, have been identified in fatty liver of pregnancy, alcohol-induced hepatitis, and morbid obesity and have been shown to cause cellular injury. There is no proven treatment for NASH. Few studies have been performed examining the role of lipid lowering agents in the treatment of NASH. Clofibrate has been demonstrated to decrease the amount of hepatic triglycerides in animal studies. In a pilot study, sixteen patients with hypertriglyceridemia and NASH based on a compatible liver biopsy with other causes of liver disease, including alcohol abuse, excluded by history, serum tests, and use of ultrasound were placed on clofibrate, 2glday for 12 months. (Laurin J et al. Hepatology 1996;23:1464-7). All obese patients were strongly encouraged to lose weight. The group comprised 11 women and 5 men with a mean age of 50 years. Obesity was present in 10 of 16 (62%) patients. Six patients were taking oral hypoglycemic agents or had a fasting glucose ~ 150 mgldL. No patients required insulin or had diabetes that was difficult to control. Among these patients, there was no significant change from baseline in mean ALT, AST, Vglutamyl transpeptidase (GGT), total serum bilirubin, cholesterol, or triglyceride level, or in histological grade of steatosis, inflammation, or fibrosis after 12 months of treatment as compared with entry. Alkaline phosphatase decreased 8igniflcanuy from baseline. The change in alkaline phosphatase did not correlate with weight loss. This group did not have significant weight loss after 12 month8 of therapy. Despite the known lipid-lowering effects of clofibrate, it did not appear to be of clinical benefit in the treatment of NASH in this one-year pilot study.

In Israel, 48 consecutive patients with chronically elevated liver enzymes and fatty liver, diagnosed by ultrasonography, were studied. (Knobler H et al. QJM I 999;92:73-9). In 16 of the 48 patients, the diagnosis of fatty liver was confirmed by a liver biopsy; all the patients had steatosis and 8 patients had inflammatory changes. Sixty4our percent of the patients were obese. Forty-four percent had diabetes mellitus, 29% impaired glucose tolerance and 17% were hyperinsulinemic. Elevation of triglycerides and/or low HDL-C was found in 86% of the patients. Seventy-nine percent of the patients achieved weight loss with diet therapy alone. Of the diabetic patients, six were treated with oral hypoglycemic agents, one patient with insulin and 14 received dietary treatment only. Eight patients received lipid-lowering drugs; of these 5 received statins and 3 fibric acid derivatives. The median follow-up period was 24 months. Mean triglyceride and cholesterol levels were significantly reduced and HDL-C levels significantly increased. During the follow-up period, liver enzyme levels were reduced to some degree in all but two patients. In 25 patients (52%), they were reduced back to the normal range. Unfortunately, interpretation of this data is difficult because of the lack of controls.

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α-Tocopherol and Antioxidants in the Treatment of Non-Alcoholic Steatohepatitis (NASH)

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Oxidative stress is central to our current understanding of the pathogenesis of NASH. Induction of enzymes that produce hydroxyl radicals such as cytochrome P450 *2E1* and excessive deposition of iron in the liver parenchyma favoring the formation of oxygen radicals, are events associated with NASH. Products of lipid peroxidation such as 4-hydroxynonenal act as activators of hepatic stellate cells and the activity of the transcription factor NF-kB is enhanced by oxidative stress. This leads to the production of cytokines to which the steatotic liver may be exquisitely sensitive. It is logical to address this pathogenic oxidative stress from a therapeutic perspective. Antioxidant compounds such a a-tocopherol (Vitamin E), which has an excellent safety profile, and powerful free radical properties may have a place in the treatment of NASH. Animal experiments support this approach. Administration of vitamin E reduces the hepatotoxicity of halothane in guinea pigs by diminishing lipid peroxidation, prevents injury due to acute intraperitoneal injection of CCl_4 and activation of NF-kB in mice and, decreases the production of fibrotic cytokines in rats chronically exposed to CCl_4. Vitamin E reduces the expression of procollagen I mRNA in human hepatic stellate cells exposed in vitro to reactive oxygen species from neutrophils. Studies have shown low hepatic levels of antioxidants which can be missed by blood levels. In patients with chronic hepatitis C, a supplement of vitamin E reduces the fibrogenic cascade in stellate cells as observed in biopsy specimens. Preliminary studies in patients with NASH suggest indeed that vitamin E could improve NASH not only in terms of serum levels of liver enzymes but also by reducing the production of the fibrogenic cytokine TGF ß. Clearly there is enough background and preliminary data to launch clinical trials studying the effect of antioxidants and of a-tocopherol in particular, in NASH.

NASH and Exercise

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NASH is increasingly accepted as of major epidemiological significance in Westernized society, frequently portrayed as a liver disease of affluence, reflecting excessive eating and insufficient exercise. Cross sectional studies suggest that fatty liver progresses via NASH to "cryptogenic" cirrhosis over a few decades, frequently in asymptomatic subjects([2.3]). Risk factors overlap with those recognized as predisposing to premature death from atheroma (diabetes , central obesity, hyperlipidaemia) but it is not known to what extent mechanistic causes and effective treatments may or may not overlap. A cohort of our NASH patients ,some lacking recognized risk factors present with symptoms of lethargy of relatively recent onset that has a major impact on their quality of life.

A prudent plan to control NASH includes reversal of the known premorbid associations. About 70% of NASH patients are obese and 35-70% have either NIDDM or raised blood sugar. Weight reduction had been shown to improve transaminase levels in adults with hepatic steatosis. Eriksson et al([7]), showed normalization of liver biochemistries and histology after weight loss of between 10 and 30% in 3 overweight patients with NASH. A later study([8]) showed similar results from a restricted diet in a Japanese cohort which involved only obese patients and included those with alcohol consumption of up to 8Og/d for males and 4Og/d for females; exercise instructions of walking and jogging were also instituted. This is the only published article to look at exercise and fatty liver, but the exercise plan was not described.

We have completed a pilot study in 27 fatty liver patients which showed that a three month period of dietary modification and regular exercise (patients were asked to exercise according to their preference) improved indices of liver dysfunction, and reduced steatosis of the liver, determined by ultrasound This regimen was associated with weight reduction in the obese, but in NASH patients with an initially normal body mass index (BMI) the response included a reduction in waist: hip ratio despite constant body weight, and symptomatic improvement in ~ The findings suggest that the regimen mobilized fat from "central "adipose tissues along with hepatic fat. It has been known that exercise increases overall body fatty acid oxidation and also lipolysis in the muscles and adipose tissues([10,11]) Through exercise-induced lowering of plasma insulin ,there is enhanced free fatty acid mobilization from the live([12]). Such studies have not been conducted, however, in patients known to have fatty liver or NASH. We wish to determine, more precisely than in our pilot study, the effect of exercise on hepatic fat and carbohydrate metabolism as well as the influence of physical fitness on measures of insulin resistance in subjects with fatty liver.

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Session VI

Histopathology and Clinical Aspects of Alcoholic Steatohepatitis

Histological Hallmarks of Alcoholic Hepatitis

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Ninety years ago, F.B. Mallory published the seminal paper describing the histopathologic lesions which characterize alcoholic hepatitis. These hallmark features included liver-cell swelling; a coarse, hyaline meshwork (Mallory bodies) found within hepatocytes; a surrounding infiltrate of neutrophils; fatty change; difficulty finding hepatic veins; and proliferation of fibroblasts resulting in an irregular fibrous dissection of the lobules. The application of light microscopic analysis, electron microscopy, immunohistochemical stains, *in vitro* studies and molecular analysis by subsequent investigators, coupled to working terminology provided by liver histopathologists, has expanded our understanding of the clinicopathologic condition which we now term "steatohepatitis".

Most of the distinctive histopathological features of alcoholic hepatitis have now been examined in light of recent advances in molecular biology. The predilection of the initial injury in alcoholic hepatitis for acinar zones 3 (centrilobular regions) and the roles of protein adduct formation, stellate cell-mediated pericellula ripe risinusoidal fibrosis and generation of lipid peroxidation products which result inneutrophil chemotaxis, have been clarified. The biochemical identity of the Mallory body, an aggregate of polyphosphorylated cytokeratin filaments, has been elucidated in humans and in experimental models. The relative roles of hepatocyte apoptosis, accumulation of hepatic iron, cholestasis, bile ductular proliferation, "directionality" of fibrosis and of macrovesicular vs. microvesicular fat in the pathogenesis of alcoholic hepatitis and cirrhosis are other important issues for future investigation.

Mallory Bodies and the Cytoskeleton in Alcoholic and Non-Alcoholic Steatohepatitis

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Chronic alcohol abuse may lead to two different types of liver injury. Most drinkers develop fatty liver, which is a reversible condition and has a low risk of progression to liver cirrhosis. Approximately 20% of heavy drinkers, however, develop a special type of alcoholic liver disease, namely alcoholic steatohepatitis (ASH), which in most cases rapidly progresses to liver cirrhosis. In ASH, besides steatosis of variable degree hepatocytes are ballooned and contain characteristic cytoplasmic inclusions, termed Mallory bodies (MBS). ASH is further characterized by necrosis and apoptosis, pericellular and perivenular fibrosis, inflammation with predominantly polymorphonuclear granulocytes, cholestasis and activation of Kupifer cells. ASH shares most, if not all, morphologic features, including development of MBs, with a variety of non-alcoholic disorders, collectively termed non-alcoholic steatohepatitis (NASH). To this group of disorders belong (I) lesions associated with morbid obesity and surgical treatment of it and/or diabetes mellitus, and (ii) lesions associated with toxic agents.

The pathogenesis of hepatocyte injury in ASH and NASH is unclear. Our studies concentrated on MBs, the morphologic hallmarks of ASH and NASH, and concomitant cytoskeletal alterations. They revealed that one of the major hepatocytic structures affected is the cytokeratin (CK) intermediate filament (IF) cytoskeleton. The CK cytoskeleton of MB-containing hepatocytes is severely deranged and there are even some hepatocytes which appear to be completely devoid of CK filaments. It is shown with antibodies directed to various CK phosphoepitopes that in the human disease and related animal models CKs become hyperphosphorylated at multiple sites, and that hyperphosphorylated CKs preferentially accumulated in MBs. In addition, there was marked overexpression of CK mRNA, most pronounced in MB-containing hepatocytes, implying a possible relationship between CK overexpression, posifranscriptional and posttranslational modifications, and MB formation. Moreover, the 1:1 CK8 to CK18 ratio, essential for spontaneous assembly of CK IFs, was disturbed with predominance of CK8. Biochemical and immunological analyses of MBs revealed that besides CKs also non-CK components, namely, the stress-inducible MM 120-1 antigen, a 62-65kDa component resembling the stress-inducible protein p62, heat shock proteins and ubiquitin, which is a common constituent of a variety of cytoplasmic inclusions occurring in different chronic degenerative diseases, were present in MBs.

The role of these different components in MB formation as well as the relevance of MBs and the associated cytoskeletal alterations in NASH was, at least in part, elucidated with the help of CK gene knockout mice. From experiments performed in these mice it became clear that CK8 is the essential nucleating MB component and that the other non-CK MB components either bind to or coassemble with CK8 in course of MB formation. It is most likely that the final trigger of MB formation is the association of CKs with non-CK proteins and impairment of proteolytic degradation.

It was further shown that MBs by themselves are not detrimental to hepatocytes but can be considered as a product of a defense response involving CK8, and that overexpression of CK enables hepatocytes to better tolerate toxic damage, which is a novel non-structural function of CK.

Oxidative stress may play a major role regarding protein alteration leading to MB formation, since several inducers of MBs, particulariy alcohol and some chemicals, initiate oxidative stress. Our results and those of other authors suggest that inclusion bodies, like MBs, apparently consisting of structurally altered and misfolded proteins only occur when the capacity of proteasomal degradation is exhausted or overstressed by high concentrations of aggregation-prone (modified) substrates. This may also be true in livers of old CKI 8 knock out mice which only produce CK8 and spontanteously develop MBs. It is well known that with age the adverse influence of reactive oxygen species on proteins, DNA and lipids exceeds the cellular defense mechanisms leading to accumulation of damaged proteins.

Clinical Spectrum of Alcoholic Steatohepatitis

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A large proportion of patients with alcoholic liver diseases are clinically asymptomatic and are detected only by routine laboratory test. Clinical examination usually reveals a marked hepatomegaly. Patients may be overweight and there is no definitive parameter which differentiates alcoholic from nonalcoholic steatohepatitis. After exclusion of other causes of liver disease diagnosis is usually made on clinical data which include the normalization of enzyme abnormalities on alcohol abstinence. Without histologic evaluation steatosis cannot be distinguished from steatohepatitis.

Symptoms of alcoholic steatohepatitis may be mild (right upper quadrant pain due to hepatomegaly) and are mostly the consequence of other diseases of the digestive tract induced by alcohol abuse (like inappetance, diarrhea, weight loss due to alcoholic gastroenteritis or pancreatitis). In addition, neuropsychiatric symptoms (alcohol withdrawal syndrome, Wemicke syndrome, alcoholic polyneuropathy) may be present. The typical laboratory abnormalities include a marked increase in the activity of gamma-glutamyl transpeptidase (GGTP), of aspartate aminotransferase (AST), and of serum levels of triglycerides and cholesterol. Diagnosis of steatohepatitis requires a liver biopsy.

Severe cases present with the full clinical picture of either acute or chronic liver failure. Symptoms include marked jaundice, fever, right upper quadrant pain, typical skin changes of advanced liver disease, easy bruising and hepatosplenomegaly. Depending on the severity tense ascites, hepatic encephalopathy, and other sign of portal hypertension may be present. The typical laboratory abnormalities are a marked increase in

serum bilirubin concentration (> 20 mgldl) and in the activity of GGTP (>1000 UIL) but also of other liver tests. Other typical findings are leucocytosis (20,000-30,000 GIL), thrombocytopenia and a prolonged prothrombin time. In addition to confirm the diagnosis, a liver biopsy may provide a useful information on the presence or absence of cirrhosis.

Prognosis of Alcoholic Hepatitis

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Alcoholic liver injury results in different pathological conditions, evolving from simple fatty changes through alcoholic hepatitis to cirrhosis. Alcoholic hepatitis, characterized by liver cell injury with ballooning and necrosis, and neutrophil infiltrate, predominantly in the perivenular areas, occurs in less than 20% of chronic alcoholics, and may coexist with cirrhosis. Progression from alcoholic hepatitis to cirrhosis is observed more commonly in women, in patients with severe histological damage and in those who continue to drink. However, the disease may progress to cirrhosis despite abstinence in women. Thirty-day mortality rates range from 2 to 25% in those patients assessed by liver biopsy. The short-term outcome of alcoholic hepatitis depends on the severity of the liver lesion: prothrombin time and high hyperbilirubinemia as well as hepatic encephalopathy and renal failure are the markers of poor prognosis. Severe malnutrition, low aminopyrine breath test, increased blood levels of tumour necrosis factor and interieukin 8, histological severity score and cholestasis are other features associated with bad prognosis. In patients with severe alcoholic hepatitis the cumulative 2-yr survival rate is approximately 40%. The long-term survival of patients with alcoholic hepatitis depends on the severity of the initial lesion, the subsequent development of cirrhosis and persistence of drinking. In a series of 126 alcoholic patients with alcoholic hepatitis and cirrhosis, gamma-globulin concentration at diagnosis and abstinence during the follow-up were the main long-term prognostic variables. In patients with less severe alcoholic hepatitis the 5-year survival rate can be as high as 80%, particularly in those who remain abstinent.

Is There a Safe Dose of Alcohol to Prevent Liver Damage?

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Although alcohol consumption has been since a long time regarded as a major cause of liver damage, many studies failed to clearly define the risk threshold dose of alcohol ingestion, and no clear information is available on the potential additional role of the type of alcoholic beverages and drinking habits. The Dionysos Study, a cohort study aimed to explore the prevalence of liver disease in the entire adult population of Northern Italy provided hints on these clinically and epidemiologically relevant questions. 6534 subjects, without HBV- or HCV-related liver disease, were examined in terms of the presence of liver disease and quantitative and qualitative alcohol intake. Multivariate analysis showed that the risk threshold for developing alcoholic liver damage was the ingestion of more that 30 g alcohol per day in both sexes. Using this threshold value, 21% of the population screened was at risk, though only 5.5% of these subjects showed persistent signs of liver damage. This finding stresses that additional factors (most probably genetic) play an important role in determining the hepatic susceptibility to alcohol. In those subjects drinking more than 30 g alcohol per day, the risk of developing liver damage increases with age and peaked after the age of 45. Multivariate analysis showed that drinking alcohol with and without meals and the use of multiple types of alcoholic beverages were significantly ($p< 0.01$) more associated with alcoholic liver disease (either non-cirrhotic or cirrhotic) than drinking only at mealtime and consuming only one type of alcoholic beverage (Odd Ratio = 5 and 20, respectively). It may be concluded that a safe dose of alcohol in preventing liver damage exists though rather small being less that 30 g per day in both sexes equal to 3-4 glasses of vine, 2-3 pints of beer or 2 drinks. Not only the amount but also the habit of drinking (consumption of alcohol with or outside meals) and the type of alcoholic beverage consumed are important factors in the development of alcohol-related liver damage.

Carbohydrate-Deficient Transferrin: Clinical Value and Pitfalls

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Carbohydrate-deficient transferrin (CDT) is a microheterogeneous form of serum transferrin poor in sialic acid residues, which steeply rises in the serum of patients with a chronic alcohol intake of at least 50-80 g/day for 10-14 days and rapidly falls into normal ranges after alcohol withdrawal. Indeed, CDT is potentially useful in detecting chronic high alcohol consumption and monitoring either abstinence or relapse during treatment. Because of its high sensitivity and specificity CDT has been proposed as the most reliable marker for detecting chronic alcoholism. However, false-positive values have been reported in rare genetic conditions like genetic D variants of transferrin and carbohydrate~deficient glycoprotein syndrome (CDG), in primary biliary cirrhosis, and in severe non-alcohol-related hepatic failure. False negative results have been related either to the very rare transferrin B variant or to errors in selection of patients. Recently, an influence of body Iron on CDT values has been also demonstrated, with iron overload reducing Its sensitivity in alcohol abusers and iron deficiency its specificity in nonabusers. In nonabusers with depleted iron stores false positive results disappear when CDT results are expressed as a percentage of serum transferrin suggesting that sensitivity and specificity can be also influenced, in different clinical conditions, by the method used for CDT measurement.