The LEC rat: a useful model for studying liver carcinogenesis related to oxidative stress and inflammation

Adriana Marquez¹,², Saul Villa-Treviño², Françoise Guéraud¹

¹Institut National de la Recherche Agronomique, UMR 1089-Xenobiotiques, Toulouse, France
²Departamento de Biología Celular, Centro de Investigacion y de Estudios Avanzados del IPN, Mexico, DF, Mexico

Growing evidence indicates oxidative stress as a mechanism of several diseases including cancer. Oxidative stress can be defined as the imbalance between cellular oxidant species production and antioxidant capability shifted towards the former. Lipid peroxidation is one of the processes that takes place during oxidative stress. Lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), are closely related to carcinogenesis as they are potent mutagens and they have been suggested as modulators of signal pathways related to proliferation and apoptosis, two processes implicated in cancer development. Mechanisms by which oxidative stress leads to tumor formation are still under investigation. The need of suitable in vivo models that could reflect that inflammation-related human carcinogenesis is evident. In this regard, the mutant strain Long Evans Cinnamon-like (LEC) rat provides a promising model for investigation of the relationship between hepatitis induced by oxidative stress and hepatocarcinogenesis because it has been demonstrated to develop spontaneous liver tumor formation related to copper accumulation and oxidative stress. In this review, the findings regarding oxidative stress and its relation with liver pathologies in LEC rats are discussed; we focus on the mechanisms proposed for HNE carcinogenesis.

Keywords: LEC rat, oxidative stress, hepatocarcinogenesis, HNE

INTRODUCTION

Oxidative stress can be defined as the imbalance between reactive oxygen species (ROS) production and the cellular antioxidant capacity shifted towards the former. ROS can interact with biomolecules such as DNA, RNA, proteins and lipids, leading to their oxidation and, as a consequence, to cellular damage, genomic instability, apoptosis and cell cycle alterations.¹ There is strong evidence for the involvement of oxidative stress in carcinogenesis. In the case of liver, it is evident that oxidative stress plays a critical role during tumor development related to chronic inflammation mechanisms. Oxidative and nitrative DNA damage are induced in humans and animals under inflammatory conditions. Patients with hepatitis C, 8-nitroguanine has been found in large amounts,² and there is a high incidence of 8-hydroxyguanine formation in peripheral leukocytes in human populations highly susceptible to hepatocarcinogenesis suggesting a relation between oxidative stress and hepatocellular carcinoma (HCC).³

Carcinogenesis is a multistep process with complex biological mechanisms that are not yet well defined. In humans, the major HCC etiologies involve chronic viral hepatitis, alcohol and metabolic disorders that lead to increased cellular turnover induced by chronic liver injury, regeneration and fibrosis.⁴ Hepatic oxidative stress is present throughout these disorders. Chronic hepatitis C leads to mitochondrial dysfunction, ROS production, lipid peroxidation and oxidatively modified proteins; alcohol consumption increases hepatic oxidative stress that is associated with fibrosis and HCC progression.⁵ In spite of the strong evidence of oxidative stress in human hepatocarcinogenesis, the mechanism of hepatocyte transformation is not yet fully understood and suitable models for studying the mechanisms of inflammation-
related hepatocarcinogenesis are necessary. In this regard, the Long Evans Cinnamon-like (LEC) rat model is of interest since it generates spontaneous acute hepatitis, fibrosis and liver tumors as a consequence of an abnormal liver copper (Cu) accumulation and subsequent oxidative stress. This rat strain shows chronic liver damage, hepatocyte death and regeneration, at the promotion stage of carcinogenesis. Such a natural history of HCC development in LEC rats is similar to that of human HCC.

In this review, current knowledge about LEC rat carcinogenesis is discussed, and the use of the LEC rat as a model in chemoprevention is highlighted.

**Copper accumulation in LEC rats**

The LEC rat is an inbred strain of a mutant rat that was originally isolated from a closed colony of Long Evans rats. LEC rats are characterized by excessive Cu accumulation in the liver and impaired biliary Cu excretion. Cu accumulation produces great quantities of reactive oxygen species (ROS), mainly the hydroxyl radical (HO·), which is believed to be the origin of the acute hepatitis and the subsequent HCC that is observed in this rat strain.

LEC rats have a deletion in the gene homologous to the Wilson’s disease gene, the ATP7B gene. The atp7b gene protein is a Cu-transporting P-type ATPase responsible not only for Cu loading into the trans-Golgi network but also for biliary Cu efflux. Wu et al. identified the LEC Atp7b gene mutation. The disease causing mutation is a deletion of 900 bp in the 3′-terminus which removes the critical ATP binding domain of the wild-type gene leading to a non-functional protein.

Introduction of the human ATP7B gene into LEC rats restores biliary Cu excretion and prevents hepatic abnormalities, showing that Atp7b gene mutation is solely responsible for LEC rat pathologies.

Hepatocyte Cu distribution in LEC rats progresses from being initially well distributed in cytoplasm, bound to metallothionein (Cu-Mt complexes) to an accumulation of the Cu-Mt complexes in the lysosome just before acute hepatitis. The acid conditions in the lysosome result in the degradation of these complexes resulting in the formation of a partially polymerized Cu-Mt containing reactive copper. This Cu initiates lysosomal lipid peroxidation, leading to hepatocyte necrosis and fulminant hepatitis.

Plasma Cu concentration in LEC rats is low because of the lack of holo-ceruloplasmin (hcp) formation in the Golgi apparatus in the liver. However, toward and after the onset of chronic hepatitis, plasma Cu concentrations increase in the form of hcp, while the liver Cu concentration is maintained at a constant level without re-occurrence of fulminant hepatitis. The mechanisms of Cu balance in chronic hepatitis are not clear. Komatsu et al. suggested that the increased hcp during acute and chronic hepatitis was explained by the delivery of Cu to ceruloplasmin outside the Golgi apparatus in the liver; however, more investigations are needed.

**Clinicopathological characteristics of LEC rats**

LEC rats present elevated hepatic Cu levels, reduced biliary Cu excretion, hemolysis, ceruloplasmin deficiency and increased hepatic iron levels. This mutant strain also possesses reduced hepatic selenium that may induce a reduction in the antioxidant capacity against copper-induced free radical damage.

Around 11–16 weeks old, LEC rats suffer from an acute hepatitis period, with symptoms of jaundice. Symptoms include decrease in body weight, yellowish skin on ears, tail and genital region, hematuria, oliguria, subcutaneous hemorrhage and sluggish movement. During this period, activities of serum enzymes, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and \( \gamma \)-glutamyltranspeptidase (GGT), as well as bilirubin levels, are increased significantly. Kasai et al. have described the clinicopathological characteristics of acute hepatitis in the LEC rat. Around 1 week from the first hepatitis signs, LEC rats suffer of a fulminant hepatitis period in which 40–50% of rats die. The remaining animals survive for more than 1 year with chronic hepatitis and develop neoplastic and neoplastic lesions of the liver such as hepatocellular carcinoma (HCC) together with cholangiofibrosis. Most of the liver cancers are histologically classified as well-differentiated HCC. Sex differences exist in hepatitis index and liver tumor formation. Male LEC rats have higher HCC formation frequency while females develop cholangiocarcinoma more frequently. Moreover, hepatitis in female LEC rats takes place earlier than in males.

The study of liver lesion incidence shows a sequential development of liver foci, nodules and HCC similar to those seen in chemical hepatocarcinogenesis models. Furthermore, the phenotype of preneoplastic and neoplastic lesions in the LEC rat is the same as that in chemical hepatocarcinogenesis – increased levels of the positive tumor markers glutathione-S-transferase type \( \pi \) (GSTP), \( \gamma \)-glutamyltranspeptidase (GGT) and \( \alpha \)-fetoprotein (AFP) and decreased levels of the negative markers glucose-6-phosphatase (G6Pase) and adenosine triphosphatase (ATPase). These results indicate that, in both models, spontaneous and chemically-induced cancer may result from similar preneoplastic processes.

**Mechanism of hepatocarcinogenesis in LEC rats**

Hepatocarcinogenesis in LEC rats is related to the liver Cu and iron accumulation, since in rats fed a low Cu and...
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Iron diet or treated with copper-chelating agents, the preneoplastic lesions almost disappear. Copper-associated liver injury is regarded as resulting ultimately from oxidative stress. The majority of the hydroxyl radical (HO•) in vivo comes from the metal catalyzed breakdown of hydrogen peroxide (H₂O₂), according to the Fenton reaction:

\[ \text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{HO}^+ + \text{HO}^- \quad \text{Eq. 1} \]

Yamamoto et al. have quantified the HO• production in plasma and liver by trapping HO• with salicylic acid. They found an increased HO• production in rats suffering from hepatitis as compared with Wistar rats and LEC rats showing no signs of hepatitis. When they treated the LEC rats with the HO• scavenger D-mannitol, they observed reduced ALT and bilirubin concentrations together with a reduction in mitochondrial lipid peroxidation. That re-inforces the idea that oxidative stress mediated lipid peroxidation is important in the pathogenesis of Cu-induced hepatotoxicity.

Lipid peroxidation refers to oxidative degradation of lipids. It most often affects polyunsaturated fatty acids (PUFAs) because they contain multiple double bonds interrupted by methylene groups that have especially reactive hydrogen atoms. The major aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). Both MDA and HNE have the ability to interact with biomolecules such as proteins and nucleic acids. MDA is mutagenic in bacterial and mammalian cells, and it reacts with DNA to form the premutagenic pyrimido[1,2-a]purin-10(3H)-one (M1G) adduct. M1G adduct is mutagenic in Escherichia coli, inducing transversions to T and transitions to A. Similarly, HNE is genotoxic for hepatocytes and cerebral endothelial cells. Treatment of hepatocytes with HNE leads to a spectrum of DNA alterations from sister chromatid exchanges to microsomal aberrations. The genotoxicity and mutagenicity of HNE and MDA may be implicated in hepatocarcinogenesis initiation in LEC rats. Increased mutagenic exocyclic DNA adducts were observed in the liver of LEC rats. These adducts come from the addition to DNA of lipid peroxidation products. Levels of etheno-DNA adducts, 1,N⁶-ethenoxyadenosine and 3,N⁶-ethenodeoxycytidine, increase with age reaching a peak at 8 weeks and 12 weeks in nuclear and mitochondrial DNA, respectively. The mechanisms by which oxidized DNA bases are repaired include the base excision repair (BER) pathway. M1G, etheno-DNA adducts, 8-hydroxyguanine and other oxidized base lesions are removed by this pathway. Acute hepatitis in LEC rats hinders the repair of oxidative DNA-base damage by altering the expression of DNA glycosylases, endonuclease II and 8-oxoguanine DNA-glycosylase which initiate the BER pathway.

Interestingly, Feng et al. have demonstrated that HNE can inhibit BER of DNA damage induced by benzo[a]pyrene diol epoxide as well as damage induced by UV light radiation in human cells. These findings suggest that during LEC rat hepatitis, HNE production may contribute to mutagenesis and carcinogenesis by both direct adduction to DNA and diminishing the DNA repair pathways.

Carcinogenesis is a process in which the balance between apoptosis and cell proliferation is altered. In normal tissues, cell proliferation and apoptosis are strictly regulated by complex mechanisms that include cell-cycle regulation by p53 and cyclins. During hepatitis, p53 expression and hepatocyte apoptosis are higher in LEC rats than in age-matched control rats. Even with the increment of p53 expression, Ba et al., using a yeast-based functional assay, demonstrated the presence of p53 mRNA mutations in LEC rat liver. The authors suggested that during hepatitis the cellular damage degrades transcriptional fidelity and that mutations in p53 may have an effect on p53 function and, hence, cell-cycle control. During chronic hepatitis, there is a continuous cellular turnover. However, the proliferation/apoptotic index ratio indicates an imbalance in favor of cellular proliferation.

The analysis of G1-phase-related cell cycle cyclins suggests that while cyclin D1 may be involved in the regeneration of hepatocytes during chronic hepatitis, cyclin-dependent kinase 4 (Cdk4) may play an important role in the development of HCC since it is significantly increased in HCC compared to precancerous and chronic hepatitis LEC rat livers. Together, these results indicate that during HCC development in LEC rats there is an increase in hepatocyte proliferation rather than a diminution in apoptosis, as is the case in human HCC.

LEC rats as a model in chemoprevention

The LEC rat is accepted as good model for Wilson’s disease. Several studies using different compounds have been made in order to test their efficiency in diminishing hepatic failure and HCC development. Cu-chelating agents like D-penicillamine, trientine dihydrochloride and N-benzyl-D-glccamine dithiocarbamate have good results in inhibiting hepatitis and HCC formation by preventing the Cu-dependent ROS production in LEC rats. D-Penicillamine is nowadays the treatment of choice for Wilson’s disease patients. D-Penicillamine not only prevents the development of hepatitis, but it can reverse the hepatitis stage by dissolving the polymerized Cu-Mt complexes and diminishing ROS production. Antioxidant treatment has successfully diminished the incidence of hepatic failure in LEC rats. N-acetylcysteine, proline, ascorbic acid, thioredoxin in combined administration have significantly delayed the appearance of jaundice and rat mortality. Treatment of LEC rats with quercetin or curcumin has not succeeded in inhibiting liver failure; contrarily, these compounds increase liver Cu...
accumulation and, hence, rat mortality. These findings highlight the deleterious effects of flavonoids in diseases where Cu or iron accumulation is involved, and the importance of testing the different antioxidants in adequate models of inflammation and oxidative stress.

From the fact that oxidative stress in LEC rats affects mitochondrial stability and function, several compounds like L-carnitine and D,L-lipoic acid have been tested. L-Carnitine protects mitochondria from ROS attack while D,L-lipoic acid is a good antioxidant and can chelate Cu and iron ions. Interestingly, dietary PUFAs prevent the hepatitis stage in female LEC rats by altering expression of genes involved in fatty acid oxidation, energy metabolism, and metal-related genes like transferrin and ceruloplasmin, indicating a better management of Cu accumulation and lipid oxidation. Furthermore the authors suggest that PUFAs could serve as scavengers rather than proliferators of free radicals produced in the liver of LEC rats.

REFERENCES


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