Chronic Hepatitis C and Fibrosis: Evidences for Possible Estrogen Benefits

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The main injury caused by hepatitis C virus is the hepatic fibrosis, as a result of a chronic inflammatory process in the liver characterized by the deposit of components from the extracellular matrix. The fibrosis development leads to the modification of the hepatic architecture, of the hepatocellular function and to irregularities in the microcirculation. The tissue remodeling process observed in fibrosis has stellate cells, located at the space of Disse, as main acting agents. These cells, in response to a harmful stimulus, undergo phenotypic changes from non-proliferating cells to proliferating cells that express a– smooth-muscle actin (α-SMA), a process called as transdifferentiation. There are evidences that the oxidative stress is involved in the chronic liver disease and serves as bond between the injury and the hepatic fibrosis. A number of studies suggest that the estrogen, at physiological levels, presents an antifibrogenic action probably through an antioxidant effect, decreasing the levels of lipid peroxidation products in the liver and blood, thus inhibiting the myofibroblastic transformation of stellate cells and contributing for gender-associated differences in relation to the fibrosis development. The aim of this paper was to describe data from literature concerning the interaction between chronic hepatitis C and estrogens, pregnancy, use of oral contraceptives, menopause and hormone reposition therapy.
<u>Key-Words</u>**: Hepatitis C, fibrosis, estrogen, benefits.**

The main injury caused by hepatitis C virus is the hepatic fibrosis, as a result of a chronic inflammatory process in the liver characterized by the deposit of components from the extracellular matrix. The fibrosis development leads to the modification of the hepatic architecture, of the hepatocellular function and to irregularities in the microcirculation [1].

Chronic hepatitis C is responsible for significant morbidity and mortality rates. The main outcomes of chronic hepatitis C virus infection are the development into cirrhosis and the appearance of its implications such as portal hypertension, hepatic insufficiency and hepatocellular carcinoma [2]. Currently, the cirrhosis resulting from chronic virus C infection is the main cause of hepatic transplantation worldwide. Thus, the fibrosis supports the vulnerability of an individual in relation to the cirrhosis appearance and its potential injuries.

The development of the chronic hepatitis C is better estimated by the fibrosis stage rather than by the necro-inflammatory activity level [3]. The fibrosis development is a dynamic process and, although a number of papers on this topic have already been published, the hepatic fibrogenesis is still object of debates. The fibrosis development is difficult to be estimated due to many reasons: difficulty in obtaining seriated hepatic biopsies; necessity to include a large number of patients in protocols and variability in the fibrosis intra-hepatic distribution.

The fibrosis development rate observed (direct) is defined as the ratio of the fibrosis stages difference between two biopsies expressed as METAVIR units and the interval between these two biopsies in years. The estimated fibrosis development rate (indirect) is defined as the ratio between fibrosis stage in METAVIR units and the estimated infection duration in years [1,3].

The natural hepatic fibrosis development history was designed in patients with chronic hepatitis C. Patients with slower and faster fibrosis have been identified; however, the genetic and environmental mechanisms that participate on this process have been only partially cleared [3].

Several factors may influence on this process, mainly host's factors such as sex, infection duration, alcohol consumption, co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), which modify the hepatic fibrosis development. Currently, metabolic diseases such as obesity, diabetes and hepatic steatosis appear as co-factors for fibrogenesis [4-6].

Clinical observations show that chronic hepatitis C seems to develop more quickly in men than in women. Fibrosis and hepatic cirrhosis are clearly diseases of men and women at the post-menopause phase, except for auto-immune diseases and primary biliary cirrhosis [7,8]. These observations suggest that the estrogens may influence the development of chronic hepatitis C.

Some european trials evaluated prospectively women who had been contaminated by hepatitis C virus when young, after anti-D immune globulin transfusion. The patients were followed within a period from 17 to 20 years and they were evaluated from the biochemical, virological and histological point of view. Most of these patients presented light to moderate hepatic inflammation evidence and half of them presented hepatic periportal fibrosis and only about 2% had cirrhosis after 17-20 years of contamination. The low risk of progressive disease in these young women also suggests that estrogens play a protective role [9,10].

Since the demonstration that the hepatic fibrosis process may be reversible [11] and considering the great necessity of alternative therapeutic procedures, researchers have

investigated anti-fibrotic agents [12]. Few studies are aimed at the relation between hormones and fibrosis. Most works demonstrating the beneficial role of estrogens are experimental, and studies evaluating the influence of this hormone in the fibrosis development in humans are scarce. However, there are evidences about a potentially favorable effect in relation to the use of the hormone reposition therapy (HRT) in women after menopause with chronic hepatitis C. Such results suggest that the HRT is safe and possibly beneficial in relation to the hepatic fibrosis [13].

In this paper, it was intended to describe data from literature concerning the interaction between chronic hepatitis C and estrogens, pregnancy, use of oral contraceptives, menopause and hormone reposition therapy.

Hepatic Fibrogenesis

The hepatic fibrosis is a result of the chronic liver injury due to several etiologies. The main causes for the hepatic fibrosis include chronic virus C infection, alcohol abuse and nonalcoholic steatohepatitis (NASH) [2]. Liver chronic diseases cause a persistent regeneration stimulus and inflammatory reaction and a cicatrisation process, what leads to the progressive replacement of the hepatocytes by abundant extracellular matrix (ECM). The accumulation of ECM proteins distorts the hepatic architecture and forms a fibrous scar with deposition of collagen, elastin and structural proteins [2,14].

In almost all forms of chronic hepatitis including viral hepatitis C, the fibrosis emerges around portal areas (zone 1) and progressively extends inside lobules towards the central vein (zone 3) with formation of septums and fibrosis bridges. The final injury stadium is the cirrhosis itself, with fibrosis connecting portal to central areas and nodular regeneration of the hepatic parenchyma.

The tissue remodeling process observed in the fibrosis has stellate cells (Ito cells), located at the space of Disse, as main acting agent. These cells, in response to a harmful stimulus, undergo phenotypic change from non-proliferating cells, reservoir for retinoids, to proliferating cells that express a–smooth muscle actin (α-SMA), a process called as transdifferentiation. This transformation of the stellate cells is considered as the key event in the fibrogenesis physiopathology, once the resulting phenotype (the myofibroblast – MFB) produces not only the fibrotic connective tissue but also a large amount of cytokines and chemokines. Other types of myofibroblasts as those derived from small portal vessels might be involved, as observed in the development of the biliary type liver fibrogenesis in patients with primary biliary cirrhosis [15]. The stellate cells and the portal myofibroblasts are different in relation to the specific cell markers and response to apoptotic stimulus [2]. Thus, hepatic fibrosis is characterized by the transformation of normal extracellular matrix (basal membrane) into a reticulated and dense matrix (fibrillar type), far more resistant to the enzymatic degradation. There are evidences that the oxidative stress is involved in the chronic liver disease and serves as bond between the injury and hepatic fibrosis [16]. The injury to the parenchymatous cells may result in the release of free radicals and other reactive oxygen species derived from the peroxidation process of lipids that occurs in inflammatory sustained response [8]. Due to these findings, studies have invested in the attempt of reducing the oxidative stress through the use of antioxidant agents in order to prevent the occurrence of hepatic fibrosis [17].

Estrogen and Hepatic Fibrosis

Sexual hormones may play an important role in the progression of chronic hepatic diseases. Estrogen and its derivatives are powerful endogenous antioxidant agents that reduce the lipid peroxide levels in liver and blood [8] and inhibit the myofibroblastic transformation of stellate cells in cell culture of rats [18]. Several experimental studies have been performed showing the benefits of this hormone in the reduction of the hepatic fibrosis.

An experimental study performed with a dimethylnitrosamine (DMN)-induced hepatic fibrosis model compared the response of male and female rats and found interesting results [18]. The fibrotic response in the liver of females was less intense than that observed in the liver of males. In males treated with DMN and anti-estradiol antibody, the inflammation, necrosis and collagen deposition were more intense than in rats treated with DMN alone and such alterations were, in turn, less important in the group that received estradiol. In females, after ovariectomy, the number of alpha-SMA positive cells in the liver increased along with the fibrosis parameters, changes that were prevented by the estrogen reposition. Estradiol reduced the number of stellate cells in a dose-dependent effect and after the interruption of the hormone administration, these stellate cells started proliferating.

Moreover, it has been proposed that the estrogen may suppress the hepatic fibrosis through an effect that depends on its hepatic tissue receptors. A study suggested that the estrogen could increase the number of receptors in the fibrotic liver of rats [19]. Other papers also demonstrated that the tamoxifen, an anti-estrogenic agent that acts by occupying the receptor connection site, increases fibrogenesis [19,20], suggesting the importance of such tissue receptors in the fibrosis suppression.

Xu et al. [20] demonstrated that the estrogen is capable of reducing the CCL-4-induced fibrosis in the liver of rats. In these animals, the estradiol administration was associated to the reduction on the aminotransferase, hyaluronic acid and type-IV collagen serum levels. The hormone also reduced the content of hepatic collagen as well as the stellate cells areas positive for lysine-actin muscle (α-SMA). The tamoxifen induced to opposite effects. Other studies performed with idoxifen, an estrogen receptor modulator developed for the treatment of breast cancer and for the osteoporosis prevention after menopause, demonstrated its effect in preventing the

hepatic fibrosis and also suggested that the estrogen receptors are involved in this process [17]. Both the idoxifen and the estrogen, besides their anti-inflammatory and antifibrotic activities, seem to have an anti-apoptotic effect due to the increase on the Bcl-2 expression [16].

All these results suggest that the estrogen, at physiological levels, presents an antifibrogenic action, probably due to an antioxidant effect, reducing the levels of lipid peroxidation products in liver and blood, contributing for differences associated to gender in relation to fibrosis progression.

The average menopause age is of 51 years (variance from 41 to 59 years), but the ovarian estrogen and progesterone production starts dropping some years before the complete discontinuance of menstruations [21]. There are evidences that such decline of the ovarian function is associated with increases on the serum levels of pro-inflammatory cytokines such as interleukins -1 (IL-1) and IL-6 and the tumoral necrosis factor (TNF-α). The production of these cytokines in peripheral mononuclear cells seems to be higher in patients with chronic hepatitis C than in healthy patients. Physiological estrogen concentrations inhibit the spontaneous secretion of such pro-inflammatory cytokines [8].

A case report demonstrated that the administration of estrogen in a male patient with chronic hepatitis C and radiation-induced testicular dysfunction resulted in the reduction on the ALT levels and the hepatic iron concentration, and this is another evidence of the protective role of the hormone against hepatic fibrogenesis [22].

The estrogen also seems to be capable of suppressing the hepatocarcinogenesis. The risk of hepatocellular carcinoma (CHC) in women is one third lower than in men. Data have demonstrated that masculine gender, age older than 49 years, reduced levels of hepatic tissue estrogen receptors and increased lipid peroxidation production are variables independently associated with the CHC development in cirrhotic patients or in patients with hepatitis C [8].

Oral Contraceptives, Pregnancy, Menopause and Chronic Hepatitis C

There are physiological and clinical effects of the estrogen in the liver already known, such as the reduction on the synthesis and secretion of biliary acids, reduction on the bile flow and participation in some clinical syndromes such as intrahepatic cholestasis of pregnancy, choletitiasis, hepatic adenomas and a questionable role in the focal nodular hyperplasia pathogenesis [23].

Despite this previous knowledge, there is not much information on the use of sexual hormones in women with chronic hepatitis C. The influence of the use of oral contraceptives, menopause and the TRH effect in chronic hepatitis C progression, mainly in relation to fibrogenesis, is not fully understood.

Usually, clinicians and gynecologists trend to contraindicate the use of hormones in women with liver chronic diseases due to the fear of hepatic side effects. However, a better knowledge on the results of the use of sexual hormones in women with chronic hepatitis will enable a better therapeutic orientation by these professionals.

Interactions between hepatitis C virus and pregnancy are still unclear and awake the interest of hepathologists and obstetricians. Some papers have demonstrated reduction or normalization of the transaminase levels during pregnancy in women with hepatitis C. However, persistence or elevation of the viremia during pregnancy and a rebound of the transaminase levels after parturition have been described in these prospective studies [24,25].

The decrease on the inflammatory activity, despite the viremia persistence, observed during pregnancy may be explained by a maternal-fetal immunotolerance phenomenon favored by the production of estrogenic and progestogenic agents. This phenomenon could be induced by hormonal alterations, particularly the elevation on the serum estrogen and progesterone levels. Possibly, estrogens modulate the cytotoxic activity of T cells with depression of the immune system and maternal protection of fetal antigens. It is speculated that the immunosuppressive effect of pregnancy also induces to the remission of other immune system-mediated diseases such as self-immune hepatitis, rheumatoid arthritis or psoriasis [26,27].

Studies on the effect of the hormone reposition therapy (HRT) among women after menopause and hepatitis C are scarce. Guattery et al. studied a heterogeneous group of 6women with average age of 60 years with hepatic inflammatory diseases (viral hepatitis B or C, self-immune and cryptogenic hepatitis). The authors reported normalization of the transaminase level (ALT) after the use of ethinylestradiol, elevation and drop of the hepatic enzymes after later suspension and hormone reintroduction [28].

Recently, Di Martino et al. [13] evaluated the impact of estrogens in the hepatic fibrosis development in women infected by the C virus. Previous pregnancies, menopause, use of oral contraceptives or hormone reposition therapy were evaluated. A total of 157 women with hepatic biopsies and estimated infection duration were evaluated. The authors demonstrated that menopause is probably associated with a worsening of the hepatic fibrosis and that HRT is possibly beneficial in relation to the fibrosis. The authors also demonstrated that the use of oral contraceptives is safe and well tolerated among this population and that previous pregnancies could have a long-term favorable impact, delaying the fibrosis development.

We prospectively evaluated 251 women with chronic hepatitis C, and the severity of liver fibrosis in function of pregnancies, previous use of oral contraceptives, menopause and hormone reposition therapy were evaluated. Women with moderate to severe liver fibrosis (F2 to F4 according to the METAVIR classification) were more frequently under menopause; however, the probability of important fibrosis was lower in the group of menopaused women who received hormone reposition therapy [OR 0.26 (95% CI 0.11-0.62),

p=0.002]. Unlike the study of Di Martino et al. [13], no association between previous pregnancies or the use of oral contraceptives with fibrosis was found. Moreover, our study suggested that the steatosis is an important fibrosis predictor among women under menopause with chronic hepatitis C [29].

Our results and those obtained by Di Martino et al. [13] corroborated data from experimental studies and verified important clinical implications in the treatment of women infected with hepatitis C. These results suggest that the HRT is safe, well tolerated and possible beneficial in relation to the hepatic fibrosis.

Conclusions

Several studies suggest that estrogens play a possible antifibrotic and anti-apoptotic role. However, prospective and randomized studies on their effects on women with chronic hepatitis C should be conducted before spreading the use of these hormones as promising therapeutics.

The advantages of the hormone reposition therapy (HRT) on liver fibrosis require confirmation and comparisons in relation to the potential risks of this treatment. The HRT brings proved benefits such as the increase on the bone density and reduction on the possibility of fractures. Nevertheless, this therapy is not free of risks. It may favour the appearance of breast cancer or cardiovascular events such as coronary diseases and thromboembolism in susceptible women [21,30]. When the hormone reposition therapy is suggested for women under menopause, an individualized risk-benefit evaluation should be conducted with the objective of verifying the presence of some menopause symptoms and risks for specific chronic diseases.

Studies with new antifibrotic agents have been conducted [12]; however, it is still necessary elucidating a number of aspects and waiting for clinical assays outcomes in order to better define how would be its use in the clinical practice.

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