Melatonin limits the expression of profibrogenic genes and ameliorates the progression of hepatic fibrosis in mice

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We investigated whether melatonin ameliorates fibrosis and limits the expression of fibrogenic genes in mice treated with carbon tetrachloride (CCl_4). Mice in treatment groups received CCl_4 5 μL/g body weight intraperitoneally twice a week for 4 or 6 weeks. Melatonin was given at 5 or 10 mg/kg/d intraperitoneally, beginning 2 weeks after the start of CCl_4 administration. Treatment with CCl_4 resulted in fibrosis evidenced by the staining of Van Gieson and α-smooth muscle actin (α-SMA) positive cells in the liver. At both 4 and 6 weeks, CCl_4 induced an increase in the messenger RNA levels of collagens I and III, transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), amphiregulin, matrix metalloproteinase (MMP)-9, and tissue inhibitor of metalloproteinase (TIMP)-1. Protein concentrations of CTGF, amphiregulin, MMP-9, TIMP-1, and phospho-Smad3 were also significantly augmented in fibrotic mice. Melatonin successfully attenuated liver injury, as shown by histopathology and decreased levels of serum transaminases. Immunohistochemical staining of α-SMA indicated an abrogation of hepatic stellate cell activation by the indol. Furthermore, melatonin treatment resulted in significant inhibition of the expression of collagens I and III, TGF-β, PDGF, CTGF, amphiregulin, and phospho-Smad3. The MMP-9 activity decreased and the expression of nuclear factor erythroid–2–related factor 2 (Nrf2) increased in mice receiving melatonin. Data obtained suggest that attenuation of multiple profibrogenic gene pathways contributes to the beneficial effects of melatonin in mice with CCl_4-induced liver fibrosis. (Translational Research 2015;165:346–357)

Abbreviations: α-SMA = α-smooth muscle actin; CCl_4 = carbon tetrachloride; CTGF = connective tissue growth factor; HSC = hepatic stellate cell; MMP-9 = matrix metalloproteinase 9; Nrf2 = nuclear factor erythroid 2–related factor 2; PDGF = platelet-derived growth factor; TGF-β = transforming growth factor β; TIMP-1 = tissue inhibitor of metalloproteinase 1

Hepatic fibrosis is a reversible wound-healing response to either acute or chronic cellular injury from a wide variety of etiologies, characterized by an excessive deposition of extracellular matrix (ECM) resulting in liver dysfunction and irreversible cirrhosis. During liver fibrogenesis, hepatic stellate cells (HSCs) undergo activation to a α-smooth muscle actin (SMA)-positive myofibroblastic phenotype and synthesize excess ECM components, particularly collagen.[1] Among the numerous

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AT A GLANCE COMMENTARY

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Background

Melatonin reduces liver damage in animal models of experimentally induced liver fibrosis such as carbon tetrachloride administration. However, changes in the expression of fibrogenic factors have not been tested, and only a preventive effect before the onset of liver toxicity has been demonstrated.

Translational Significance

Melatonin given 2 weeks after the start of chronic carbon tetrachloride treatment delays the development of fibrosis in mice through effects involving the inhibition of hepatic stellate cell activation, the suppression of various profibrogenic mediators, and the promotion of extracellular matrix degradation. Results suggest that melatonin might be an effective antifibrotic drug in the prevention of liver disease progression.

profibrogenic factors, transforming growth factor (TGF)-β is a key mediator that activates Smad2/3 to induce fibrosis. Other cytokines, such as platelet-derived growth factor (PDGF) or connective tissue growth factor (CTGF), and the epidermal growth factor receptor amphiregulin, play an important fibrogenic role.[2] Moreover, fibrogenesis is a dynamic process involving not only net accumulation of ECM but also its ongoing remodeling by proteases, including the balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs).[3]

Recent clinical and experimental evidence indicates that hepatic fibrosis may be reversed on removal of the underlying etiologic agent.[4] The prospect that fibrosis is reversible has generated great interest for researchers to develop antifibrotic therapies, although an effective therapeutic approach is still required and there is a need for searching antifibrotic strategies that can prevent, halt, or reverse hepatic fibrosis. Oxidative stress aggravates liver fibrosis.[5] Thus, inhibiting oxidative stress has been considered a potential useful strategy to prevent the development of hepatic fibrogenesis, and it has been reported that antioxidants such as epigallocatechin-3-gallate,[6] polyprenols,[7] quercetin,[8] or proanthocyanidin,[9] among others, may prevent liver injury in different animal models of fibrosis.

Melatonin is a versatile molecule endowed with an abrogated activation of HSCs induced by reactive oxygen species in vitro,[10] and different studies have shown that the pineal hormone prevents liver damage in rats with fibrosis induced by bile duct ligation,[11] dimethylnitrosamine,[12] or thioacetamide.[13] The most commonly used approach to cause experimental liver fibrosis is the periodic administration of carbon tetrachloride (CCl_4) in mice or rats.[14] CCl_4-induced liver fibrosis in rodents can be completely resolved within several weeks after withdrawal of the toxic treatment, and it resembles all important properties of human liver fibrosis, including inflammation, regeneration, fiber formation, and potentially fibrosis regression.[15] Using this toxin-mediated model, it has been found that melatonin administration, at doses ranging from 2.5 to 20 mg/kg body weight, prevents liver histopathologic changes, reduces hepatic hydroxyproline content, inhibits oxidative stress and apoptosis, increases antioxidant enzyme levels, or reduces proinflammatory cytokine production, when administered intraperitoneally to rats or mice.[16-22]

However, in these in vivo studies, effects of melatonin on the activation of HSCs and changes in the expression of fibrogenic factors or molecules involved in ECM degradation have not been tested. Moreover, because melatonin was always given before or in parallel to CCl_4 administration, only a preventive effect before the onset of liver toxicity was demonstrated. Thus, in the present research, it was decided to assess if melatonin treatment, beginning 2 weeks after the start of the toxic injection to allow initial activation of HSCs, could attenuate the development of liver fibrosis in the progression of chronic CCl_4-induced liver injury in mice. HSCs' turnover, ECM components, profibrogenic cytokines, and molecules involved in ECM degradation were evaluated. We showed that melatonin treatment impaired HSC activation, reduced the MMP-9 activity, and resulted in a significant inhibition of the expression of profibrogenic factors in a dose dependent-manner, leading to the improvement in liver function and amelioration of fibrosis.

MATERIAL AND METHODS

Animal experiments and drug treatment. Male C57BL/6J mice (Harlan Laboratories, Barcelona, Spain) weighing 20–25 g were used in this study. The animals were acclimated to the temperature (22 $\pm$ 2°C) and humidity (55 $\pm$ 5%) of controlled rooms with a 12–12 hour light-dark cycle for at least a week before experiments. They were allowed access to mice chow and water ad libitum. Mice in treatment groups received CCl_4 at a dose of 5 μL/g body weight (10% CCl_4 in corn oil) via intraperitoneal injection twice a week for 4 or