بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
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Pathological, immunohistochemical and molecular studies on the curative role of synthetic cannabinoid receptors-2 agonist (AM1241) in induced hepatic and pulmonary fibrosis in male rats

A thesis submitted by
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For the degree of the Ph.D.
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(General, Special and Postmortem)

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2021

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Abstract

The activation of cannabinoid receptor 2 (CB2) plays a pleiotropic role in the innate immunity and is considered a crucial mediator in liver and lung disease. This study aimed to explore the role of activating CB2 using synthetic agonist (AM1241) on suppressing the progress of liver fibrosis induced by two models; bile duct ligation (BDL) and TAA administration as well as lung fibrosis induced by bleomycin (BLM) instillation. The study also attempted to clarify the mechanisms of the antifibrotic effects of synthetic CB2 agonists; AM1241 on the ongoing both hepatic and pulmonary fibrosis. AM1241 was administrated to rats of the aforementioned three models in two doses (3 and 6 mg/kg) for 14 days (in BDL experiment) and 21days (for the other experiments). Liver function and oxidative stress markers, hepatic TNF-α, IL-6 and IL-10, IL-1β, TGF-β1, RT-qPCR expression of Toll like receptor 4 (TLR4), TGF-β1, α-SMA and microRNA-155 (miR-155) genes, western blot for Vimentin and E-cadherin proteins, immunohistochemical expression of CD31, CD34, α-SMA and NF-κB as well as histopathology of liver tissue were all assessed. In lung experiment, miRNA-21, Myd88 and TLR4 genes expression were detected by RT-q-PCR, while collagen I, Smad2 and Myd88 protein levels were analyzed by western blot. In addition, antioxidant activity (SOD and GSH), pro-fibrotic (TGF-β1) and pro-inflammatory (IL-4 and IL-13) cytokines were determined in lung tissue homogenate using ELISA kits. Histological examination and immunohistochemical (IHC) evaluation of CD4, α-SMA and TGF-β1 expression were also examined. In both liver models; AM1241 administration at both doses significantly ($P<0.05$) ameliorated liver function markers, pro-inflammatory cytokines (TNF-α, IL-6), significantly decreased i GSH content and TLR4 gene expression. Histologically, AM1241 limited fibroplasia extension decreased immune-expression of NF-κB in both models. While in BDL model, AM1241 showed significant increase in the immune-medulatory cytokine; IL-10, decreased immune-expression of CD31 and strongly expressed CD34. Regarding TAA induced liver fibrosis; AM1241 showed significant decrease in Vimentin level, TGF-β1, α-SMA and microRNA-155 gene expression with significant increase in E-cadherin level. While in the lung model; AM1241 group resulted in significant decrease in western blot protein levels (TLR4,

Myd88 and miR-21) as well as cytokines level with significant upgrade the anti-oxidant activity. Lung fibrosis in AM1241 administrated rats was minimal rather than BLM group as confirmed by the Ashcroft scale of fibrosis. Moreover, AM1241 groups showed significant negative immunohisto-expression of CD4, α-SMA and TGF-β1 in dissimilarity with BLM rats. In conclusion, this study points out that; CB2 receptors activation could curb both hepatic and pulmonary fibrosis via distinct mechanisms. The hepatic fibrosis in BDL model was suppressed through inhibition of TLR-4/NF- κB pathway, while in TAA model it was terminated through inhibition of TLR4/miR-155/ NFkB p65 pathway. In BLM models the pulmonary fibrosis was abolished via inhibiting TLR4/Myd88/miR-21/ TGF-β1/Smad2 signaling pathway. Also, these results suggest that CB2 agonists display potent hepatoregenrative properties, in addition to their antifibrogenic effects.

Key words:

Cannabinoid receptor 2; AM1241; TLR4; Liver fibrosis; lung fibrosis; miR-21; miR-155; Myd88

Dedication

My deep thanks to

my Mother,

my Father,

my lovely husband,

my sisters, brothers

and

my close friends

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during the whole work

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