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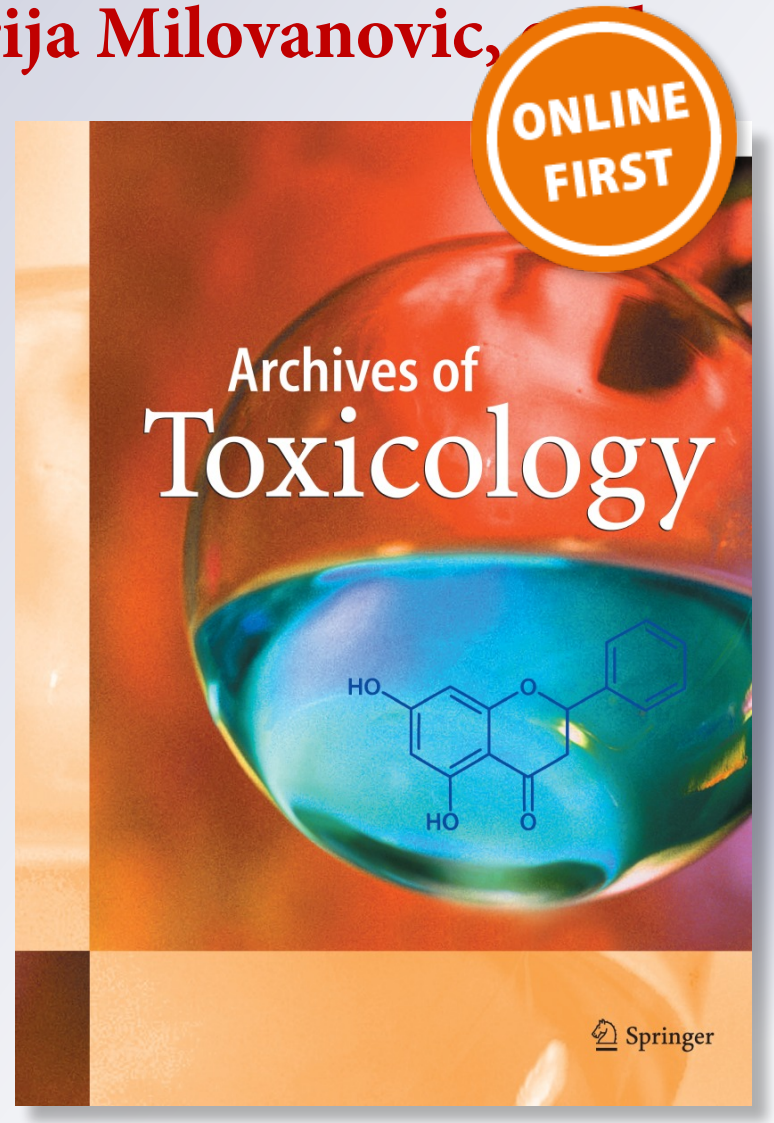

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ORGA TOXICITY AND MECHANISMS

Metformin aggravates immune-mediated liver injury in mice

Vladislav Volarevic · Maja Misirkic · Ljubica Vucicevic · Verica Paunovic · Bojana Simovic Markovic · Maja Stojanovic · Marija Milovanovic · Vladimir Jakovljevic · Dragan Micic · Nebojsa Arsenijevic · Vladimir Trajkovic · Miodrag L. Lukic

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Abstract Hepatotoxicity of the antidiabetic drug metformin has been reported, but the underlying mechanisms remain unclear. We here investigated the effect of metformin in immune-mediated liver damage. While not hepatotoxic alone, metformin (200 mg/kg) aggravated concanavalin A (Con A, 12 mg/kg)-induced hepatitis, an experimental model of T cell-mediated liver injury, in both relatively resistant BALB/c and highly susceptible C57Bl/6 mice. Metformin + Con A-treated mice had elevated serum levels of pro-inflammatory cytokines TNF-α and IFN-γ, accompanied by a massive mononuclear cell infiltration in the liver. This was associated with the higher numbers of $CD4^+$ T cells producing TNF-α, IFN-γ and IL-17, $CD4^+$ T cells expressing chemokine receptor CXCR3 and activation marker CD27, $CD4^+CD62L^-CCR7^-$ and $CD8^+CD62L^-CCR7^-$ effector memory cells, IFN-γ producing NK cells, IL-4 and IL-17 producing NKT cells and IL-12 producing macrophages/dendritic cells. The percentage of $CD4^+CXCR3^+Tbet^+IL\text{-}10^+$ and $CD4^+CD69^+CD25^-$ regulatory T cells was reduced. Metformin stimulated inducible nitric oxide synthase (iNOS) expression in the liver and spleen, and genetic deletion of iNOS attenuated the hepatotoxicity of metformin. Metformin increased the autophagic light chain 3 conversion and mRNA expression of important autophagy-inducing (beclin-1, Atg5 and GABARAP) and pro-apoptotic (p21, p27, Puma, Noxa, Bax, Bad, Bak1, Bim and Apaf1), but not anti-apoptotic molecules (Bcl-xL, survivin and XIAP), which correlated with the apoptotic caspase-3/PARP cleavage in the liver. The autophagy inhibitor chloroquine (20 mg/kg) prevented liver injury and apoptotic changes induced by metformin. Therefore, metformin aggravates immune-mediated hepatitis by promoting autophagy and activation of immune cells, affecting effector, as well as liver-specific regulatory T cells and iNOS expression.

Keywords Hepatitis · Metformin · T lymphocytes · Concanavalin A · Autophagy · Apoptosis

Introduction

Metformin is the most commonly prescribed oral antidiabetic medication (Miralles-Linares et al. 2012). The hepatic uptake mediated by organic cation transporters and the subsequent inhibition of gluconeogenesis are apparently required for its pharmacological activity (Graham et al. 2011). Although hepatotoxicity is not considered as a major side effect of metformin, several cases of metformin-induced liver injury have been reported recently (Cone et al. 2010; de la Poza et al. 2008; Miralles-Linares et al. 2012; Olivera-Gonzalez et al. 2010). Liver damage was

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