学 位 論 文 の 要 旨

氏名　SANCHEZ SILES ALVARO ADOLFO

学 位 論 文 名　Administration of PPARβ/δ Agonist Reduces Copper-Induced Liver Damage in Mice: Possible Implications in Clinical Practice

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著 者 名　Alvaro A. Sanchez-Siles, Norihisa Ishimura, Mohammad Azharul Karim Rumi, Yuji Tamagawa, Satoko Ito, Shunji Ishihara, Toru Nabika, Yoshikazu Kinoshita

論 文 内 容 の 要 旨

INTRODUCTION

Wilson's disease (WND) is an autosomal recessive disorder that leads to excess copper accumulation in several organs, particularly the liver. It has been proposed that the mechanism of copper toxicity is via generation of free radicals which are strongly linked to inflammation, fibrosis and apoptosis that eventually leads to liver failure. Peroxisome proliferator-activated receptor (PPAR) β/δ is the last member of the PPAR's family to be carefully studied. PPARβ/δ has been reported to have anti-inflammatory activities including inhibition of cytokine production, modulation of cell adhesion molecules and inhibition of nuclear factor kappa B signaling. As well, it has been described to ameliorate carbon tetrachloride (CCl_4) induced liver toxicity and an important role in oxidative stress reduction.

MATERIALS AND METHODS

BALB/c mice divided into 6 groups of 5 were treated for 3 days and sacrificed on fourth day. Groups: control, PPARβ/δ agonist (GW0742), PPARβ/δ antagonist (GSK0660),

copper-nitriloacetate (NTA), copper-NTA + GW0742 and copper-NTA + GSK0660. Body weight, serum ALT, liver histology, liver RNA and DNA studies were also performed. The oxidative stress marker, 8-hydroxy-2-deoxy guanosine (8-OH-2-dG) in DNA of liver tissue was assessed by ELISA after its purification and denaturation.

Human hepatoma cell line, HepG2 cells were exposed to copper sulfate, GW0742 and/or GSK0660; either in its wild form or transfected with full length PPARβ/δ expression vector. Chemosensitivity and cellular toxicity (MTS assay), intracellular reactive oxygen species (ROS) and caspase 3/7 activity were assessed.

RESULTS AND DISCUSSSION

Mice treated with copper had significant body weight loss, serum ALT increase, modest changes in liver histology, increase of tumor necrosis factor alpha (TNFα) and macrophage inflammatory protein 2 (MIP-2) mRNA and 8-OH-2-dG. These changes were significantly reduced in mice co-treated with GW0742 and exacerbated in mice co-treated with GSK0660. In vitro, copper induced ROS formation was lower in cells treated with GW0742 or transfected with PPARβ/δ expression vector; together, transfection and GW0742 had an additive ROS-reducing effect. Copper also upregulated Fas ligand and Caspase 3/7 activity; when these cells were treated with copper and GW0742, the upregulation was lower.

The short duration of our study and the lack of chronic copper storage and inflammation resulted in a non-florid histology; however, the type and severity of changes in the group that received copper alone and copper plus GSK0660 resemble those seen in WND patients and help us understand the protective role of PPARβ/δ activation in copper-challenged mice.

Mice treated with the GW0742 alone showed significant upregulation of 8-OH-2-dG and TNFα in liver, what translates as an increase in liver injury probably due to the reduced protection against ROS. The steep increase of TNFα and MIP-2 in mice challenged with copper and GSK0660 and the opposite effect observed with copper and GW0742, show the protective

role of PPARβ/δ.

PPARβ/δ's roles in reactive phase and pro-inflammatory cytokines, apoptosis and oxidative stress have been proven once again under the particularity of copper-induced toxicosis. Since our findings are comparable to that in which PPARβ/δ protected from CCl_4 induced hepatotoxicity, PPARβ/δ agonists have the potential of becoming an important tool in the management of some patients with acute onset of WND and probably other causes of liver damage and failure.

CONCLUSION

PPARβ/δ activation protects mice from copper-induced liver damage by reduction of TNFα, MIP-2 and oxidative stress. High presence of PPARβ/δ receptors in liver and its anti-inflammatory and anti-oxidative stress activity lead us to believe PPARβ/δ agonists could become an important tool in the management of copper-induced liver damage and probably other causes of liver damage and failure where few therapeutic options are available.