Induction of aminolevulinic acid synthase gene expression

Induction of aminolevulinic acid synthase gene expression, down-regulation ferrochelatase and enhancement of metabolite, protoporphyrin IX, excretion by co-therapy with isoniazid and rifampicin

(1. Isoniazid and rifampicin induced liver injury by regulating 5-aminolevulinate synthase and ferrochelatase and enhancing protoporphyrin IX

2. Mechanism of rifampicin and isoniazid induced cell death in L-02 cell line and mice)

Abstract

Isoniazid(INH) and rifampicin(RFP) are first-line antituberculosis drugs, co-therapy with INH and RFP is highly effective. However, the combination of these two drugs frequently cause liver injury or liver failure in humans. The risk of hepatotoxicity is considerably higher in patients receiving both RFP and INH than in those receiving either RFP or INH alone. Numerous studies have been conducted to investigate the mechanism of injury after isoniazid or rifampicin used in various animal models, however, the important mechanism for the combination of isoniazid and rifampicin in humans remains unclear. Here we investigated this combination induced hepatotoxicity using L-02 cells and mice.

Introduction

Tuberculosis remains a global public health problem whose effects have major impact in developing countries. World Health Organization estimates that there were 8. 6 million new TB cases in 2012 and 1. 3 million TB deaths. The currently recommended treatment for new cases of drug-susceptible TB

is a six-month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide. (Global tuberculosis report 2013). However, the combination of isoniazid(INH) and rifampicin(RFP) frequently cause liver injury or liver failure. The risk of hepatotoxicity is considerably higher in patients receiving the combination than in those receiving either RFP or INH alone. The mechanisms leading to liver failure in humans were poorly understood.

Recently, a new mechanism , independent of INH metabolism, is found in the RFP and INH co-therapy induced liver injury. Li et al. (Li, et al. 2013) found that co-therapy with RFP and INH targets porphyrin biosynthesis and results in hepatic protoporphyrin IX (PPIX) accumulation and liver injury . PPIX is an intermediate in porphyrin biasynthesis. Normally the concentrations of PPIX is very low in the liver. However, in some cases the concentration abnormally elevated in blood and liver, such aserythropoietic protoporphyria. High concentrations of PPIX in the liver are known to cause liver injury (Anstey and Hift 2007; Casanova-Gonzalez, et al. 2010). Using hPXR mice, Li et al. demonstrated that the accumulation of endogenous PPIX is through PXR-mediated transcriptional activations of aminolevulinic synthase-1(ALAS1) genes. ALAS1 is the rate-limiting enzyme of heme synthesis in the liver and is drug-responsive, providing heme for CYPs and other hemoproteinsis. Activation of PXR can upregulate ALAS1 expression in liver (Fraser, et al. 2003). RFP upregulate ALAs1 increasing heme-biosynthesis in the liver and overproducing PPIX through activating PXR signalling pathway. However, PPIX accumulation strongly suggests that ferrochelatase became a ratelimiting enzyme during INH-RFP treatment (Lyoumi, et al. 2013).

Ferrochelatase (FECH) , the final enzyme in the heme biosynthetic pathway, catalyses ferrous iron inserted into precursor porphyrin protoporphyrin IX to form heme, and when defective or deficient, causing accumulation of protoporphyrin IX. Ferrochelatase is active in cells that produce 80% heme in the bone marrow (Bloomer, et al. 1991) and the rest in hepatocytes (Bonkowsky, et al. 1975).

The excess protoporphyrinIX becomes insoluble in bile and exerts cholestatic effects leading to architectural changes in the hepatobiliary system ranging from mild inflammation to fibrosis and cirrhosis (Anstey and Hift 2007).

MATERIALS AND METHODS

PI staining

L-02 were allowed to adhere on glass bottom dishs for 4h, followed by INH, RFP or INH/RFP. The medium was removed after h and cells were stained with for 30 min. Nuclei were stained with 4’, 6-diamidino-2-phenylindole (DAPI) and images were recorded with a fluorescence microscope.

Western blotting

L-02 cells cultured in flask were harvested using 0. 25% trypsin (Hyclone, Thermo Scientific, Waltham, Mass). After centrifugation at 1000r and lysis using buffer for Western blotting (), total proteins were collected by following the kit instructions. Protein concentrations were determined using the BCA Protein Assay Kit (). After heating at 95°C for 5 minutes in sample buffer, proteins were separated on SDS-PAGE using 10% polyacrylamide gels before electroblotting onto PVDFmembrane(). Nonspecific binding was blocked by incubation for 2 hours in 5% (w/v) nonfat milk. The following primary

antibodies were used overnight at 4°C: Rabbit anti-human FECH antibody(; 1: 1000); Rabbit anti-human ALAs1 antibody(1: 500); Rabbit anti-human BCRP antibody(; 1: 500). Bound antibodies were detected using horseradish peroxidase-conjugated secondary antibodies (Beijing Biosynthesis Biotechnology Co. LTD). Finally, the membranes were visualized by chemiluminescence.

RNA Isolation and Real time Polymerase Chain Reaction for ALAs1 and FECH

Cell Culture

L-02 cells, a human fetal hepatocyte line, purchased from Cell Bank of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, purchased from Shanghai, China, were cultured according to the manufacturer’s instructions 15 at 37°C in 5% CO2. Cell culture materials were procured from Corning()

Discussion

Nevertheless, the ability of chemicals to activate PXR is species dependent. RFP is a human PXR specific activator that weakly affect on mouse (Lehmann, et al. 1998).

INH hepatotoxicity is thought to be dependent on metabolic activation by arylamine N-acetyltransferase and CYP2E1, but Li found AcHZ and hydrazine do not cause INH-related hepatotoxicity.

Hepatic heme synthesis leading to protoporphyria and possible impact with other metabolic systems (Davies, et al. 2005).

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