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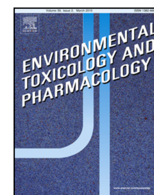

Zidovudine and isoniazid induced liver toxicity and oxidative stress: Evaluation of mitigating properties of silibinin

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ABSTRACT

HIV/AIDS patients are more prone for opportunistic TB infections and they are administered the combined regimen of anti-retroviral drug zidovudine (AZT) and isoniazid (INH) for therapy. However, AZT + INH treatment has been documented to induce injury and remedial measures to prevent this adversity are not clearly defined. Silibinin (SBN) is a natural hepatoprotective principle isolated from medicinal plant *Silybum marianum* and is currently used for therapy of various liver diseases. This study investigate the hepatotoxic potentials of AZT alone, INH alone and AZT + INH treatments and the mitigating potentials of SBN against these drugs induced toxic insults of liver in rats. Separate groups of rats (n = 6 in each group) were administered AZT alone (50 mg/kg b.w.), INH alone (25 mg/kg, b.w.), AZT + INH (50 mg/kg, b.w. and 25 mg/kg, b.w.), SBN alone (100 mg/kg, b.w.) and SBN + AZT + INH daily for sub-chronic period of 45 days orally. The control rats received saline/propylene glycol. INH alone and AZT + INH-induced parenchymal cell injury and cholestasis of liver was evidenced by highly significant increase in the activities of marker enzymes (aspartate and alanine transaminase, alkaline phosphatase, argino succinic acid lyase), bilirubin, protein, oxidative stress parameters (lipid peroxidation, superoxide dismutase, catalase, reduced glutathione, vitamins C and E) and membrane bound ATPases were evaluated in serum/liver tissue homogenates. Histopathological studies show ballooning degradation, inflammatory lesions, lipid deposition and hydropic changes in the liver tissue. All the above biochemical and pathological changes induced by AZT + INH treatments were mitigated in rats receiving SBN simultaneously with these hepatotoxins, indicating its hepatoprotective and antioxidant potentials against AZT + INH-induced hepatotoxicity. The moderate hepatoprotective and oxidant potentials of SBN could be due to its low bioavailability and this deficiency could be prevented by supplementation of phosphatidylcholines and studies are warranted on these lines to improve the therapeutic efficiency of SBN.

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1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) patients and Human Immunodeficiency Virus (HIV) infected individuals are vulnerable for tuberculosis (TB) co-infection, due to suppressed immunity and they are commonly treated with regiments containing antiretroviral drug zidovudine (Azidothymidine – AZT) and antitubercular drug isoniazid (INH). The World Health Organization (WHO) has reported that nearly 2.6 million HIV patients encounter co-infection with TB and it accounts for 1.8 million deaths worldwide. Though efficacious, combined administrations of AZT and INH for therapy in HIV/AIDS with TB co-infected patients are reported to stumble up on developments of 4.5% to 11.5% fulminent and subhepatic failure as adverse effects in Indian and Western populations (World Health Organization, 2011). Development of hepatotoxicity on conjoined treatment with AZT and INH in humans is a challenging issue and precise remedial measures for prevention against these drugs-induced toxic insults to the liver are poorly defined.

AZT (a pro-drug) a reverse transcriptase inhibitor drug, is reported to induce several adverse effects like myofilamentous abnormalities, lipid droplets accumulation in muscles, hepatomegaly, hepatic steatosis and liver failure when given alone or in combination with nucleoside analogues or with other reverse and non-reverse transcriptase inhibitor drugs. INH is the "first-line drug" used for chemotherapy of TB and its administration is well documented to induce hepatocellular failure, hepatic steatosis and necrosis in about 10% to 30% of its recipients in human when given alone or with other antitubercular drugs (Awodele et al., 2011;

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Mitchell et al., 1976) and experimental animals as well (Sarich et al., 1997; Karthikeyan, 2005).

It is proposed that oxidative stress, generation of peroxides and alterations in oxidative phosphorylation in hepatic mitochondria is the cause for AZT-induced hepatotoxicity (Tikoo et al., 2008; Majid et al., 1991) and liberation of highly reactive radicals during metabolism in liver is responsible for INH-induced hepatic necrosis and steatosis (Sarich et al., 1997; Whitehouse et al., 1983). Since imbalance in oxidative homeostasis is considered as an essential factor towards onset of both AZT and INH-induced hepatocellular damage, suppression in generation of reactive oxygen species (ROS) and thereby restoration of oxidant-antioxidant imbalance is considered as valuable therapeutic strategy towards mitigation of these drugs-induced toxic insults of the liver.

Silibinin (SBN), a flavonolignane is a diastreoisomer (containing silibin A, B and isosilibin A, B), extracted from the seeds of the medicinal plant *Silybum marianum*, commonly called “milk thistle”, has been shown to offer excellent hepatoprotective, antioxidant and hepatocellular membrane stabilizing properties and hence is used in medical practice for therapy of gall bladder problems, hepato biliary disorders and alcohol-induced hepatocellular necrosis, steatosis and fibrosis (Loguercio and Festi, 2011; Lee et al., 2007). Previous studies conducted in our laboratories have shown the hepatoprotective, antioxidant and membrane stabilizing potentials of SBN treatments against AZT alone (Raghu et al., 2015), diethyl and dimethyl nitrosamines-induced hepatic necrosis, cirrhosis and fibrosis (Ezhilarasan et al., 2012; Harrison et al., 2010). In the current study, we examined the hepatoprotective potentials and oxidative stress mitigating properties of SBN on its simultaneous administration against sub-chronic AZT and INH-induced toxic insults of the liver in rats.

2. Materials and methods

2.1. Drugs and chemicals

Agrinosuccinic acid disodium salt, 2,4-dichloro-1-napthol, bilirubin, 1,1,3,3 tetra methoxy propane (malondialdehude, MDA), 5-5′-dithio-bis 2-nitrobenzoic acid, α-tocopherol, 2-thiobarbituric (TBA), silibinin (SBN) and isoniconiticacid hydrazine (isoniazid, INH) were purchased from M/s Sigma-Aldrich Chemicals (St. Louis, Missouri, USA). Zidovudine (AZT) was obtained as gift from the Director, Central Drug Testing Laboratory, Chennai. Other chemicals used for various assay were analytical grade and procured locally.

2.2. Animals

Wistar albino rats of either sex, weighing (150 ± 20 g) aged 3–4 months procured from Institutional Animal House facility were used in this study. They were maintained at controlled environmental conditions (temperature – 24 ± 2 °C; relative humidity – 50–60%; 12 h dark–light cycle) in polypropylene cages over husk beddings and were provided standard pellet diet and water ad libitum. Institutional Animal Ethical Committee permission was obtained prior to experimentation (IAEC No: 02/010/2011).

2.3. Experimental protocol

Thirty six rats were allocated at random into six groups, each comprising of equal sex ratio and number (n = 6). Group I rats were treated saline and propylene glycol (vehicle) mixture and they served as control. Groups II and III were treated AZT alone (50 mg/kg b.w.) and INH alone (25 mg/kg b.w.) respectively. Group IV rats received AZT (50 mg/kg b.w.) and INH (25 mg/kg b.w.) as successive treatments. Group V rats received SBN (100 mg/kg b.w.) as simultaneous treatment after AZT (50 mg/kg b.w.) and INH (25 mg/kg b.w.) administration. Group VI rats were treated SBN alone (100 mg/kg b.w.). All the above treatments were given orally for sub-chronic period of 45 days. Since we observed maximum hepatotoxicity (as determined by status of marker enzymes of hepatotoxicity and bilirubin in serum) in AZT + INH treated rats on day 45 as compared to day 60 and 90 (data not shown), in this study, we restricted our data presentation till this day. The sustained decline of hepatotoxicity seen upon continued AZT + INH treatments on the above latter days could possibly be due to the physiological adaptations of the liver or due to its inherent regenerating potential. The dosage for AZT was selected from our previous studies (Raghu et al., 2015) and from the reports of other investigators (Harlass, 1996). Dose for INH was fixed based on our previous works (Victorrajmohan et al., 2005). For SBN the dose was selected based on its hepatoprotective and antioxidant properties against AZT, dimethyl nitrosamine and diethylnitrosamine induced hepatotoxicity (Raghu et al., 2015; Ezhilarasan et al., 2012; Harrison et al., 2010) in our laboratories in rats. AZT and INH were dissolved in saline and SBN was suspended in propylene glycol (grade 2000) and they were prepared just before use and the volume of all administrations were maintained at 0.3–0.5 ml/100 gm b.w. of rats.

2.4. Sample collection and preparation

Blood (2–2.5 ml) was withdrawn from retro-orbital plexus of ether anesthetized rats into clean tubes and they were allowed to clot. Serum was separated (1–1.5 ml) by centrifugation (3000 rpm; 15 min), aspirated and stored at cold (−20 °C) until further analysis. Rats were sacrificed by decapitation and livers were quickly excised. It was washed in saline to remove blood clot and cell debris. Liver tissue homogenates were prepared (1% or 10%) in tris-HCl buffer and were centrifuged (5000 rpm at 4 °C; 15 min). The clear supernatants were stored (at −20 °C) until further analysis. All biochemical assays were performed within 48 h after sample collection. For histopathological studies, a piece of liver tissue (approximately 5 mm^3) was sliced and fixed in phosphate-buffered-formal saline.

2.5. Determination of aspartate transaminase (AST) and alanine transaminase (ALT) activity

The activity of AST and ALT in serum and tissue homogenates were estimated as detailed by Reitman and Frankel (1957). This assay quantifies the pyruvate liberated by this enzyme on treatment with 2,4-dinitrophenyl hydrazine and sodium hydroxide at 520 nm using spectrophotometer. Its value are expressed as IU/L in serum and IU/g wet tissue for liver tissue

2.6. Determination of alkaline phosphatase (ALP) activity

Phenol liberated by ALP in presence of the substrate, disodium phenylphosphate and sodium carbonate-sodium bicarbonate buffer (pH 10) on treatment with Folin’s phenol reagent and sodium carbonate yield a blue colored complex, whose intensity was quantified at 640 nm as detailed by King (1965) in serum and liver tissue. Its values are expressed as IU/L and IU/gm tissue in serum and liver respectively.

2.7. Determination of argino succinic acid lyase (ASAL) activity

Serum ASAL activity was quantified as described by Campanini et al. (1970). Briefly, the arginine released from the substrate sodium argino succinate was allowed to react with sodium hypochloride buffer to form pink color, whose optical density was

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