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Tannic acid prevents azidothymidine (AZT) induced hepatotoxicity and genotoxicity along with change in expression of PARG and histone H3 acetylation

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Abstract

Azidothymidine (AZT) is known to decrease HIV virus replication and is one of the most frequently prescribed antiretroviral drugs used for AIDS treatment. Dose-limiting toxicities are the major curse associated with AZT therapy. Recently, we have reported that tannic acid; a PARG inhibitor prevents cisplatin induced nephrotoxicity. The present work was conceived to study the effect of tannic acid on AZT induced hepatotoxicity and genotoxicity. AZT induces increase in plasma levels of ALT, AST and alkaline phosphatase along with increase in micronucleus (MN) count in peripheral blood. Suggesting, AZT is hepatotoxic and genotoxic to mice. Treatment of tannic acid protects AZT induced hepatotoxicity by decreasing the ALT, AST and alkaline phosphatase levels. It also significantly reduces the oxidative damage by preventing reduction in glutathione and decreasing the level of malondialdehyde in liver of AZT treated mice. In addition, tannic acid decreases the PARG expression, PARP cleavage and histone H3 acetylation in liver of AZT treated mice. Moreover, treatment of tannic acid also decreases MN count in peripheral blood, suggesting its anti-mutagenic effect. In light of these findings we suggest the potential role of tannic acid treatment in preventing AZT induced toxicity.
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Keywords: AZT; Tannic acid; Oxidative stress; Hepatotoxicity; Micronucleus and genotoxicity

1. Background

Azidothymidine (AZT) is a potent inhibitor of HIV replication and the first clinically approved drug for AIDS. Mechanism involved in therapeutic action of this nucleoside analogue includes the incorporation of the AZT triphosphate into newly synthesized DNA template (Wurtzer et al., 2005), which results in chain termination of DNA synthesis and inhibition of viral reverse transcriptase (Cossarizza and Moyle, 2004). The major limitations of AZT chemotherapy are clinical toxicities that include dose-related bone marrow suppression manifested as severe anaemia and leucopenia, hepatic abnormalities, myopathy, limited brain uptake and short half-life in plasma and the rapid development of resistance against the virus. Pharmacokinetic studies in humans have shown that the AZT plasma half-life is approximately 1 h. Repeated and higher doses of AZT are administered for maintaining therapeutic levels in plasma, thus leading to bone marrow toxicity (Skoblov et al., 2004).

These drugs are also known to produce genotoxic manifestations that include mutagenesis, chromosomal aberrations and telomere shortening which eventually lead to micronucleus (MN) formation in erythrocyte (Sussman et al., 1999; Olivero et al., 2005). Metabolic pathways that result in the phosphorylation of AZT play a crucial role in AZT-DNA incorporation (Olivero et al., 1999) and may be altered after its prolonged treatment. Recently, Olivero et al. reported that thymidine kinase

Abbreviations: AZT, azidothymidine3′-azido-3′-deoxythymidine; ROS, reactive oxygen species; PAR, poly (ADP-ribose); PARG, poly (ADP-ribose) (PAR) glycohydrolase; PARP, poly (ADP-ribose) polymerase; AST, asparatate aminotransferase; ALT, alanine aminotransferase; MN, micronucleus; NCEs, normochromatic erythrocytes; NRTIs, nucleoside reverse transcriptase inhibitor; ERK, extracellular signal regulated kinase.

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1, the enzyme responsible for AZT mono-phosphorylation, is down-regulated during long-term exposure and thus appears to be associated with AZT induced inhibition of replication and accumulation of cells in S-phase (Olivero, 2007).

In addition, activation of poly-ADP-ribose polymerase and accelerated NAD+ catabolism have been observed in case of AZT treated animals. ROS mediated oxidative damages, activated ADP-ribosylation reactions and accelerated NAD+ catabolism play important role in the development of cardiomyopathy in animal model and liver toxicity in AZT treated AIDS patients (Szabados et al., 1999; Virag, 2005). The metabolism of poly (ADP-ribose) is mediated by PARG, key enzyme regulating PARP activation. Tannic acid, a PARG inhibitor has been shown to reduce cell death which is mediated by oxidative stress (Ying et al., 2001; Uchiumi et al., 2004).

Tannic acid (Gallotannin) is reported to prevent many ROS mediated drug toxicities. It is one of the important as well as functionally active antioxidant among polyphenols, possessing antioxidant, anticancer and antimutagenic properties (Zhao et al., 2005). The mechanisms underlying the protective effect of tannins include the scavenging of radicals and inhibition of superoxide radicals. Tannic acid treatment resulted in significant recovery of hepatic glutathione levels and phase-II metabolizing enzymes. It also, significantly decreases lipid peroxidation, xanthine oxidase, hydrogen peroxide generation and liver damage (Sehrawat et al., 2006). Polyphenols have been reported to quench lipid peroxidation, prevent DNA oxidative damage, and scavenge hydroxyl radical (Lin et al., 2001). The ability of several polyphenols to chelate iron or copper ions has been ascribed to their antioxidant activity (Andrade et al., 2005). Recently, we have reported the differential effects of tannic acid on cisplatin induced nephrotoxicity (Tikoo et al., 2007a). Hence, present work was under taken to study the effect of tannic acid on AZT induced toxicity.

2. Materials and methods

2.1. Chemicals

AZT was generously provided as gift from Ranbaxy, India. Tannic acid was purchased from Merck. All the chemicals were purchased from Sigma (St. Louis, MO, USA), unless otherwise mentioned.

2.2. Animal and drug treatment

All the experiments were approved by the Institutional Animal Ethics Committee (IAEC) and complied with the NIH guidelines on handling of experimental animals. Experiments were performed on male Swiss albino mice (20 ± 2 g). Mice were randomly divided into four groups containing six animals in each, namely, control, tannic acid control, AZT treated and AZT along with tannic acid.

Different doses of AZT (400, 800 and 1200 mg kg^{-1}, *p.o.*) was studied for optimization of toxicity. At 800 mg kg^{-1} dose of AZT moderate liver toxicity and genotoxicity was observed in mice. Hence, further study with tannic acid was carried out with this dose. AZT was administered (800 mg kg^{-1}, *p.o.*) for 28 days. Treatment of tannic acid (5 mg kg^{-1}, *i.p.*) was done after 30 min of AZT treatment for 28 days. Similarly, in tannic acid control group tannic acid (5 mg kg^{-1}, *i.p.*) was administered for 28 days.

2.3. Measurement of biochemical parameters

Blood samples were collected from the retro orbital plexus of mice under light ether anesthesia in heparinized centrifuge tubes and immediately centrifuged at 2300 × *g* for the separation of plasma. Plasma was stored at −80 °C until assayed. The plasma was used for the estimation of ALT, AST and alkaline phosphatase. Estimations were carried out as per manufacturer's instruction provided with commercially available kits (Accurex Ltd., Mumbai, India).

2.4. Assessment of oxidative stress markers

Oxidative stress markers were determined as described previously (Tikoo et al., 2007b). Briefly, after sacrificing mice by cervical dislocation, the livers were excised and rinsed with normal saline and weighed. After weighing, liver tissue was minced properly and the homogenate was prepared in cold phosphate-buffered saline (pH 7.4) and centrifuged at 700 × *g*. Supernatant was collected and used for estimation of thiobarbituric acid reacting substances (TBARS). The lipid peroxide level in animal tissues was measured according to method described by Ohkawa et al. (1979). For reduced glutathione, liver tissues were homogenized in 10 ml ice-cold homogenizing buffer combined with sulphosalicylic acid with two 10 s burst of tissue disintegrator. This tissue homogenate was used for measuring GSH content. The GSH content was estimated according to Ellmans' method (Ellman, 1959).

2.5. Histopathology of liver

Histopathology was performed as described previously (Tikoo et al., 2007a,b). Briefly, mice were anesthetized under light ether anesthesia, after surgery circulating blood was removed by cardiac perfusion with 0.1 M PBS (pH 7.4; 20–50 ml). After clearance of circulating blood, 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) was perfused for another 5 min (100–200 ml of fixative) to fix the tissues. Liver was removed from the animal, sliced transversely, and paraffin-embedded for light microscopic evaluation. Histopathological changes in liver structure were assessed in at least 25 randomly selected tissue sections from each group studied. Sections were stained with Mayer's hematoxylin and eosin to examine cell structure.

2.6. Micronucleus count

Peripheral blood slides for determining the frequency of micronucleated erythrocytes were prepared on the day of sacrifice. Slides were fixed in absolute methanol, and stained with Giemsa. For each, 2000 uniformly stained normochromatic erythrocytes (NCEs) were scored to determine the frequency of micronucleated cells, reflecting genetic damage and reflect events that occurred approximately 2–30 days previously. MN count is expressed as MN-NCEs per 1000 NCEs (% NCEs).

2.7. Protein isolation and Western blotting

Nuclei isolation and western blotting were performed as described previously (Tikoo et al., 2007a,b). Briefly, total proteins were isolated from tissue homogenates by sonication and nuclei were isolated. Nuclei were suspended in low salt buffer [1% NP-40, 10 mM Tris, 10 mM NaCl, 10 mM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mM PMSF (phenyl methyl sulfonyl fluoride)] prior to sonication and its protein concentration was determined by Lowry method. Immunoblot analysis was performed by using Anti PARP (rabbit 1:1000, cell signaling) and anti-actin (rabbit 1:2500, Sigma, St. Louis, MO, USA) and HRP-conjugated secondary antibodies (anti-rabbit) from Santa Cruz, CA. Proteins were detected with the enhanced chemiluminescence (ECL) system and ECL Hyperfilm (Amersham Pharmacia Biotech, UK Ltd., Little Chalfont, Buckinghamshire, England).

2.8. Immunohistochemistry

Liver was taken out as described earlier and processed on an automatic tissue processor (Leica). Sections (4 μm) were cut and mounted on slides coated

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