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Toxic Effects of Isoniazid and Rifampicin on Rat Brain Tissue: The Preventive Role of Caffeic Acid Phenethyl Ester

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Abstract: To the best of present knowledge, the possible protective effects of Caffeic Acid Phenethyl Ester (CAPE), on possible Isoniazid (INH) and Rifampicin (RIF) induced neurotoxic effects in brain tissue have not been investigated yet. As such, the purpose of this experimental study was to investigate the effects of CAPE, on INH and RIF induced neurotoxicity in rat brain tissue. We measured Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD) activities, Malondialdehyde (MDA) and nitric oxide (NO) levels in the brain tissue of rats. Male Sprague-Dawley rats were divided into eight experimental groups, with ten animals in each group. These groups are consist of control group, INH-treated group, RIF-treated group, INH+RIF treated group, INH+CAPE treated group, RIF+CAPE treated group, INH+RIF+CAPE treated group and CAPE treated group. MDA and SOD levels in brain tissue were significantly higher and TAC levels were lower in the INH, RIF and INH+RIF treated groups ($p<0.05$) and TAC levels were lower in the INH, RIF and INH+RIF groups than in the control group ($p<0.05$). CAPE plus INH and/or RIF treatment caused a significant decrease in MDA levels in brain tissue ($p<0.05$). In addition, CAPE plus INH and/or RIF treatment caused a significant increase in TAC levels ($p<0.05$). In conclusion, we have shown that administration of INH and RIF is accompanied by increased lipid peroxidation and oxidants in rat brain tissue. CAPE may protect against INH and RIF-induced neurotoxicity. Therefore, CAPE supplementation may be used as a potential neuroprotective drug for antituberculosis therapy with INH and/or RIF.

Key words: Isoniazid, rifampicin, oxidative stress, caffeic acid phenethyl ester, neurotoxicity

INTRODUCTION

Tuberculosis is the world's second most common cause of death from infectious disease, after HIV/AIDS (Frieden *et al*., 2003). Isoniazid (INH) and rifampicin (RIF), the most important first line antitubercular drugs have been used for the treatment of tuberculosis (Yossepowitch and Dan, 2007; Pal *et al*., 2008a). INH has been the mainstay of treatment of tuberculosis infection for 50 years (Lobue and Menzies, 2010). However, it is associated with adverse events, including peripheral neuropathies and seizures. Other presumed toxic reactions are autonomic neuropathy, encephalopathy, coma (Goldman and Braman, 1972; Cicek *et al*., 2005; Arbex *et al*., 2010). The concentration of RIF in the central nervous system is only 10-20% of the serum concentration of the drug. However, it is enough for the drug to be clinically effective. Nevertheless, RIF is associated with adverse events such as dizziness, headache and ataxia (Cicek *et al*., 2005). The most effective antituberculosis therapy is a combination of INH, RIF and pyrazinamide for eight weeks, followed by INH and RIF for a further four to seven months (Bass *et al*., 1994). INH+RIF are effective for the treatment of tuberculosis infection (Jasmer *et al*., 2000; Spyridis *et al*., 2007). INH and RIF continue to be effective drugs in treatment of tuberculosis (Pal *et al*., 2008b). RIF is a potent agent against actively dividing intracellular and extracellular organisms and has activity against semidormant bacilli that work primarily by inhibiting DNA dependent RNA polymerase, blocking RNA transcription. It is given as a daily oral dose of 10 mg kg^{-1} (Hershfield, 1999). Recent data indicate that INH and RIF, appear in measurable quantities in the cerebrospinal fluid and pass to some degree through non-inflamed meninges (Holdiness, 1987). Although there are rare reports of a neurologic side effects with RIF, it is not known whether RIF increases INH-induced neurotoxicity (Arbex *et al*., 2010). Which is mediated by induction of apoptosis (Bhadauria *et al*., 2007). INH metabolites have been identified, including hydrazine (HZ), ammonia and oxidizing free radicals. INH formation and elimination are

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genetically controlled. The mechanism underlying INH neurotoxicity has yet to be fully defined. However, several hypotheses have been suggested the metabolite(s) involved in this phenomenon remain obscure although a major role is clearly played by HZ and possibly also by the ammonia it releases (Preziosi, 2007). MDA and NO increase markers of oxidative stress and decrease TAC (Preziosi, 2007; Ergul *et al*., 2010; Uzar *et al*., 2012). CAPE is a structural relative of flavonoids, has powerful antioxidant properties (Ilhan *et al*., 2004). It is a neuroprotective and antioxidant molecule, an active component of propolis from honeybees which can induce expression of heme oxygenase-1 (HO-1) and Brain-derived Neurotrophic Factor (BDNF). Because HO-1 and BDNF induction has been suggested to protect neurons (Kurauchi *et al*., 2012). Combining INH and RIF with an antioxidant substance, such as CAPE, may reduce brain toxicity induced by INH and RIF. A previous study showed that CAPE, caused a neuroprotective effect on biochemical status in drug-induced oxidative damage in rat brain tissue (Uzar *et al*., 2010). The aim of the present study was to examine the protective effects of CAPE in INH and RIF induced brain neurotoxicity in a rat model of INH and RIF induced neurotoxicity.

MATERIALS AND METHODS

This study was approved by Dicle the University Animal Ethics Committee and it was performed in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, prepared by Dicle University, Animal Ethics Committee. Male Sprague-Dawley rats (aged 8-12 weeks) weighing 220±30 g (Mean+SD) were used in this experiment. The rats were placed in a temperature (22±2°C) and humidity (50+5%) controlled room in which 12 h light/dark cycles were maintained for one week before the start of the experiment. A standard diet and tap water were provided ad libitum. The rats were divided into eight experimental groups, with ten animals in each group: Control, INH-treated group, RIF treated group, INH+RIF treated group, INH+CAPE treated group, RIF+CAPE treated group, INH+RIF+CAPE treated group and CAPE treated group. The RIF doses and INH doses of treated groups were 50 mg kg^{-1} per day for both RIF/INH and these doses were given orally with tap water for 30 days (Attri *et al*., 2000; Gokalp *et al*., 2006). The control group was given plain tap water. CAPE was administered to the INH+CAPE, RIF+CAPE, INH+RIF+CAPE and CAPE groups at a dose of 10 μmol kg^{-1} i.p. as described previously for 30 days (Gokalp *et al*., 2006). Isotonic saline solution (an equal volume of CAPE) was given i.p. for 30 days to the control group. After all the rats received the above treatments, they were fed *ad libitum* until midnight, then they were anesthetized with ether and brain tissue samples were obtained. Half of these tissues were stored at -50°C until biochemical analysis.

Biochemical analyses: The excised brain sample were weighed and immediately stored at -50°C. Assays were performed on the supernatant of the homogenate which was prepared at 14, 000 rpm for 30 min at +4°C. The protein concentration of the tissues was measured by the Lowry method (Lowry *et al*., 1951). Superoxide dismutase (SOD) activity was measured according to the method described by Fridovich (1974). Lipid peroxidation level in the cerebrum was expressed as MDA and measured according to the procedure proposed by Ohkawa *et al*. (1979). NO levels were determined by the Griess method (Cortas and Wakid, 1990). The TAC of supernatant fractions was evaluated using a novel automated and colorimetric measurement method developed by Erel (2004). The TAC results are expressed as nmol Trolox equivalent/mg protein. The assay was calibrated with hydrogen peroxide and the results are expressed in terms of nmol H_2O_2 equivalent/mg protein (Hu *et al*., 1993; Aycicek *et al*., 2005). The unit of cerebrum tissue TAC was μmole H_2O_2 equivalent/gram protein and mmole H_2O_2 equivalent/gram protein, respectively.

Statistical analyses: Data are expressed as Mean±SD. The normality of the distribution for all variables was assessed by the Kolmogorov-Smirnov test. The Mann-Whitney U-test was used for variables that do not meet the normality assumption. A one-way Analysis of Variance (ANOVA) and post-hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine differences among groups. A p-value of $p<0.05$ was considered statistically significant.

RESULTS

Biochemical results of the rat brain tissue are shown in Table 1. MDA, NO and SOD levels were higher in the INH group than in the control group ($p = 0.036$, $p = 0.001$ and $p = 0.001$ respectively), while TAC levels were significantly lower in the INH group than in the control group ($p = 0.008$). CAPE+INH treatment caused a significant decrease in the MDA and NO levels compared to INH alone (for each parameter, $p = 0.001$). In addition, CAPE+INH treatment caused a significant increase in TAC levels compared to INH alone ($p = 0.001$). MDA and NO levels were significantly higher in the RIF group than those of the control group ($p = 0.006$ and $p = 0.001$, respectively). CAPE+RIF treatment caused a significant decrease in MDA and NO levels compared to RIF alone

Table 1: Biochemical parameters in the brain tissue of rats

Groups	TAC (mmol trolox meq. g^{-1} protein)	MDA (nmol g^{-1} wet tissue)	SOD (IU mg^{-1} protein)	NO (μmol g^{-1} protein)
1: Control	0.53±0.7	213.0±44.7	244.3±18.8	27.5±5.4
2: CAPE	0.60±0.35	193.5±32.6	244.6±27.6	31.5±6.8
3: INH	0.34±0.2	254.4±28.9	396.2±77.6	61.3±12.5
4: RIF	0.28±0.4	267.7±43.4	408.9±47.8	58.3±22.3
5: RIF+INH	0.28±0.2	255.2±42.6	409.9±29.6	38.3±10.2
6: INH+CAPE	0.71±0.1	169.3±21.9	240.1±19.1	36.9±3.9
7: RIF+CAPE	0.51±0.8	188.1±49.3	239.2±42.6	34.2±4.7
8: RIF+INH+CAPE	0.55±0.6	211.6±37.1	263.6±35.8	38.5±9.1
p-values				
1-3	0.008	0.036	0.001	0.001
1-4	0.001	0.006	0.001	0.001
1-5	0.001	0.033	0.001	0.055
3-6	0.001	0.001	0.001	0.001
4-7	0.002	0.001	0.001	0.001
5-8	0.001	0.028	0.001	0.971
3-5	0.380	0.970	0.510	0.001
4-5	0.980	0.520	0.960	0.001
1-2	0.330	0.320	0.990	0.473

NS: Not significant, MDA: Malondialdehyde, SOD: Superoxide dismutase, NO: Nitric oxide

(for each parameter, p = 0.001). TAC level activities were lower in the RIF group than in the control group (p = 0.001) and SOD activities were higher in the RIF group than in the control group (p = 0.001). CAPE+RIF treatment caused a significant decrease in MDA, SOD, NO activity in brain tissue compared to RIF alone (for each parameter p = 0.001). Moreover, CAPE+RIF treatment caused a significant increase in TAC levels compared to RIF alone (p = 0.002). MDA and NO levels in the INH+RIF group were higher than those of the control group (p = 0.033 and p = 0.055, respectively). CAPE+INH+RIF treatment caused a decrease in MDA generation in brain tissue compared to the INH+RIF group (p = 0.028). TAC levels were lower in the INH+RIF group than in the control group (p = 0.001). CAPE+INH+RIF treatment caused a significant increase in TAC levels and a significant decrease in SOD activity compared to the INH+RIF group (both parameters, p = 0.001).

DISCUSSION

Free radicals are characteristically toxic. They able to damage molecules (nucleic acids, lipids, proteins). Fortunately, cells possess appropriate defense mechanisms in the form of free radical scavengers and enzymes which metabolize free radicals or their precursors INH and/or RIF which can cause oxidative damage in tissues (Cicek *et al*., 2005; Bhadauria *et al*., 2007; Warlow *et al*., 2008; Uduman *et al*., 2011). MDA is the breakdown product of the major chain reactions that lead oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress. It is known that increased levels of MDA, are the marker of extent of lipid peroxidation, in the brain (Shivarajashankara *et al*., 2003; Erel, 2004; Cicek *et al*., 2005). It also has been revealed that increased Lipid Peroxidation (LPO) correlates with the degree of oxidative effects of INH in rat hippocampus (Cicek *et al*., 2005). INH has been shown to cause a significant increase in MDA levels of rat erythrocyte and co-administration of CAPE with INH decreased the MDA levels (Gokalp *et al*., 2006; Ergul *et al*., 2010; Kerman *et al*., 2012). There are rare reports of a neurologic side effect with RIF (Arbex *et al*., 2010). However, it is not known if RIF increases INH-induced neurotoxicity. Chen *et al*. (2011) reported that increased lipid peroxidation by INH and RIF induced hepatic injury in rats (Chen *et al*., 2011). Combination treatments (INH plus RIF) increased lipid peroxidation products (Saad *et al*., 2010). We found that both INH and RIF caused a significant increase in MDA levels in rat brain tissues. Our study has three main findings. First, we found that INH and/or RIF administration at a dose of 50 mg kg^{-1} per day resulted in a significant increase in NO and MDA levels and SOD activity in rat brain tissue, on the other hand they caused significant decreases in TAC levels. Second CAPE significantly decreased MDA and NO levels and SOD activity and significantly increased TAC levels when applied to the rats subjected to INH and/or RIF toxicity. Our third main finding is that there was no significant difference in neurotoxicity in rat brain tissue between INH+RIF treatment and INH treatment alone. RIF plus INH compared to INH alone does not lead to any significant increase in neurotoxicity in the rat brain. INH exposure causes increased Reactive Oxygen Species (ROS) generation along with alteration in levels of enzymatic antioxidants such as SOD (Bhadauria *et al*., 2007). RIF is considered a powerful inducer of mixed-function oxidase that increases the hepatotoxicity of isoniazid by enhancing the production of toxic metabolites from acetylhydrazine (Sarma *et al*., 1986).

Nevertheless, it is not known whether RIF increases INH-induced neurotoxicity in rat brains. Anti-tubercular drug mediated oxidative damage is generally attributed to the formation of highly ROS, which act as stimulators of lipid peroxidation and a source of destruction and damage to the cell membrane (Pal *et al*., 2008a, b) Furthermore, alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components have been reported to be involved in INH and RIF-induced hepatotoxicity (Attri *et al*., 2000; Saad *et al*., 2010). We found that both INH and RIF led to significant increases in NO and MDA levels in rat brain tissue; the increases may be due to overproduction or decreased discharge of oxidant substances. Increased lipid peroxidation and oxidant generation in the brain tissue of rats receiving both INH and RIF suggests that the neurotoxic effect was caused by oxidative insult. Present results suggest that INH and RIF augments oxidative stress either by modulating the production of free radicals, ROS. In this study, CAPE, decreased lipid peroxidation and oxidants in a significant manner. MDA and NO levels were reduced by CAPE and TAC levels were increased. These results indicate that CAPE might be a novel agent to protect the brain tissue from oxidative stress due to INH and RIF neurotoxicity. In addition, the fact that CAPE normalizes both MDA and TAC levels in brain tissue, which are respectively increased and decreased due to antituberculosis medication, tissue may indicate that CAPE is effective in brain tissue. NO is a free radicals that becomes elevated with cerebral neuronal damage. It reacts with, particularly the fatty acid component of membrane phospholipids (Lizasoain *et al*., 2006). Increasing evidence has suggested that NO has an important role in modulating oxidant stress and tissue damage (Koc *et al*., 2005). The primary product of the interaction between NO and superoxide anion is peroxynitrite. Which is a potent cellular oxidant (Habib and Ali, 2011). Peroxynitrite, as a short-lived ROS, is quickly protonated and then decays generating the highly toxic hydroxyl radical, which explains the cytotoxicity associated with the raised level of NO (Kim *et al*., 2005). The present study first indicated the marked elevation in NO level in brain tissue of INH and/or RIF-treated rats and CAPE significantly attenuated this increment. SOD catalyzes the conversion of superoxide radicals to H_2O_2. It protects cells against the toxic effects of superoxide radicals (Gokalp *et al*., 2006). In the brains of the treated INH+RIF group, there was a significant increase in SOD activity, which may be another sign of increased oxidative stress in brain tissue. CAPE might be a scavenger of free oxygen radicals and it serves to prevent oxidative stress in the INH+RIF treated rat brain. Therefore, it prevents the elevation of SOD enzyme activities in INH+RIF rat brain. We show that a combine therapy of INH and RIF increased MDA and NO levels and decreased TAC levels in rat brain tissue. CAPE efficiently decreased MDA and NO levels and increased TAC levels. When the combined therapy of INH and RIF was applied. However, no differences were seen in the biochemical results between the rats that received INH or RIF therapy alone and those that received the combination INH+RIF therapy. In conclusion our findings suggest that CAPE supplementation may be used as a potential neuroprotective drug for antituberculosis therapy with INH and/or RIF. This protective effect may be due to the antioxidant properties of CAPE, which scavenges the free radicals that can cause brain cell damage.

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