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In vitro toxic action potential of anti tuberculosis drugs and their combinations

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ABSTRACT

Tuberculosis (TB) is one of the leading infectious causes of death due to single infectious agent after HIV/AIDS. Rifampicin (RIF), Isoniazid (INH), Ethambutol (EMB), Pyrazinamide (PZA) and/or their combinations are extensively prescribed to treat TB. Despite several therapeutic implications, these drugs also produce several toxic effects at cellular level. MTT assay and Ames test were adopted in this study for the determination of cytotoxic and mutagenic potential of these anti-TB drugs. Among all tested drugs, cytotoxic potential of RIF was strongest with highly significant decline ($p < 0.001$) in cell numbers at the concentration of 250 μg/ml with LC_{50} at 325 μg/ml, while significant decline ($p < 0.01$) in cell count was observed in INH treated group at the concentration 500 μg/ml with LC_{50} at 1000 μg/ml. Moreover, combination RIPE demonstrated significant reduction ($p < 0.01$) in cell number at the concentration of 25–500–500–500 μg/ml with LC_{50} at 60–1200–1200–1200 μg/ml. It is apparent from the data that almost all drugs represented identical mutagenic pattern i.e., more significant results were achieved in TA100 with metabolic activation (+S9). RIF proved to be highly mutagenic of all tested drugs with significant mutagenicity ($p < 0.01$) at 0.0525 μg/plate against TA98 strain with S9. The combination RIPE exhibited highly significant mutagenic activity ($p < 0.01$) at concentration 0.125–3–3–3 μg/plate without S9, while addition of S9 resulted in similar activity at lower doses, i.e., 0.0525–1–1–1 μg/plate. It was concluded from the data that all anti-TB drugs possess significant cytotoxic and mutagenic potential, especially in combination, making TB patient more vulnerable to cytotoxic and mutagenic effects of anti-TB drugs, which could produce further health complications in TB patients.

1. Introduction

Globally, tuberculosis (TB) is one of the leading infectious causes of death due to single infectious agent after HIV/AIDS. In 2010, there were 8.8 million incidents and 1.4 million deaths from TB. Over 95% of TB linked deaths occurred in developing countries and were among the top three causes of women deaths aged between 15 and 44 years. Additionally, about half a million children suffered from TB out of which 64,000 children

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Table 1 – Description of different combination of drugs with respective codes.

No.	Drugs combination	Combination abbreviation	Drug concentration (μg/ml)[a]
1	Rifampicin (R), Isoniazid (I), Pyrazinamide (P), Ethambutol (E)	RIPE	R: 0.05, I: 1, P: 1, E: 1[a]
2	Rifampicin, Isoniazid, Pyrazinamide	RIP	R: 0.05, I: 1, P: 1
3	Rifampicin, Isoniazid, Ethambutol	RIE	R: 0.05, I: 1, E: 1
4	Rifampicin, Isoniazid	RI	R: 0.05, I: 1
5	Pyrazinamide, Ethambutol	EP	E: 1, P: 1

[a] *Note*: two fold dilutions were made of all these combinations up to eight concentrations.

reported dead (World Heath Organization 2010). TB is causing socio-economic problems especially in the developing Asian countries with over 60% incident rate, representing the largest TB burden among all continents. Annually, 0.3–0.48 million people in Pakistan suffered from TB and children contributed 75% of the total cases. Pakistan, with more than 48,000 deaths each year, is ranked sixth amongst the largest twenty two TB burden countries (World Heath Organization 2010).

According to WHO "daily observed therapy (DOT) program", treatment of TB involves administration of combination of Rifampicin (RIF), Isoniazid (INH), Ethambutol (EMB) and Pyrazinamide (PZA) for the first 2 months (initial phase), followed by RIF and INH for the next 4 months (continuation phase). RIF is a focal anti-TB therapy, which belongs to complex macrocyclic antibiotics. It inhibits DNA dependent RNA polymerase in *Mycobacterium tuberculosis* and inhibits transcription of messenger RNA (mRNA) by ensuing translation to different proteins (Corcoran and Hahn, 1975). Similarly, INH is also considered a corner stone of anti-TB cocktail (Youatt, 1969; Zhang et al., 1996) and is activated by mycobacterial enzyme (Zhang et al., 1992). EMB is a routinely used first line anti-TB drug along with other drugs and inhibits bacterial enzyme *arabinosyl transferase* involved in the cell wall biosynthesis (Takayama and Kilburn, 1989). Lastly, PZA is a synthetic pyrazine analogue of nicotinamide and is considered key component of anti-TB therapy, which help in shortening the duration of TB therapy from 9 to 12 months to 6 months (Zhang et al., 2003). PZA is converted by pyrazinamidase to pyrazinoic acid that inhibits mycobacterial fatty acid synthase I gene, which is involved in mycolic acid biosynthesis of *M. tuberculosis* (Zimhony et al., 2000).

Despite several therapeutical implications, these drugs also produce several toxic effects at cellular level. RIF was found to be clastogenic and induce dose dependent increase in chromosomal aberrations in mouse bone marrow after treatment with therapeutic and repeated doses (Aly and Donya, 2002). Moreover, drug induced chromosomal abnormalities in mouse spermatocytes also increased "sister chromatid exchanges (SCEs)" in mouse bone marrow (Aboul-Ela, 1995). INH and its metabolite are found strongly associated with hepatotoxic potential (Girling, 1977) because it is converted into reactive hydrazine, which is genotoxic. Moreover, increased INH linked malignancies has been reported (Stott et al., 1976). EMB-induced optic neuropathy is a medical condition associated with EMB therapy, which may cause permanent vision loss (Tsai et al., 2008). EMB is also documented to induce clastogenic effect in the bone marrow cells of rats. Anitha et al., have reported PZA as weak genotoxicant, which produced chromosomal aberrations and sperm shape abnormalities in mouse (Anitha et al., 1994). On the contrary, data is also available from different scientists who advocate non-clastogenic characteristics of anti-TB drugs (Ahuja et al., 1981; Speit et al., 1980). Additionally, some scientists claim that it is not the drug itself but the combination of drugs which produce synergistic effects to produce mutagenic aberrations (Gopal Rao et al., 1991).

Due to the non-availability of specific data about cytotoxicity and mutagenicity of these anti-TB drugs, it is inevitable to determine cytotoxic and mutagenic potential for these drugs for health and safety appraisal of TB patients. Hence, MTT assay and Ames test were adopted for the determination of cytotoxic and mutagenic potential of these anti-TB drugs (individually and in combination) (Table 1).

2. Materials and methods

2.1. Chemicals

Tested chemicals i.e., RIF, INH, EMB, PZA were provided by Pacific Laboratories, Lahore, Pakistan. Dimethylsulfoxide (DMSO) was purchased from Merck, Germany. Metabolic activation cellular fraction (S9) was obtained from Environmental Biodetection Products Inc. (EBPI, Canada) in lyophilized form along with its co-factors. All other chemicals and media used were of analytical grade. MTT 3-(4,5 dimethylthiazolyl-2-yl)2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma chemical company, USA.

2.2. Cytotoxicity assay

Cytotoxicity was evaluated by adopting MTT assay using baby hamster kidney cells (BHK-21), as described by Freshney (1998). Briefly, BHK-21 cell line was obtained from Quality Operation Laboratory (QOL), University of Veterinary and Animal Sciences (UVAS), Lahore. BHK-21 cells were maintained in M-199 medium and incubated at 37 °C to obtain 80–90% confluent monolayer. BHK-21 cells were seeded in 96 well cell culture plates at the cell density of 1–2 × 10^5 cells per ml and incubated at 37 °C for 48–72 h. Cells were then treated with different drug concentrations in triplicate (Tables 2 and 3). After overnight incubation, cells were treated with MTT dye, incubated for 4 h and after the addition of 10% DMSO again incubated for 2 h. Cell culture media, 10% DMSO and BHK-21 cells were used as negative control, while cell culture media and BHK-21 cell line was used as positive control. Cell survival percentage (CSP) was spectrophotometrically measured at 570 nm using ELISA reader in terms of optical density value (OD). Cell survival %