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Preliminary report

Effect of antituberculous drugs on human polymorphonuclear leukocyte functions in vitro

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Abstract

The aim of the study was to investigate antituberculous drugs effects on polymorphonuclear leukocyte (PMN) functions (phagocytic activity and intracellular killing activity) in vitro. PMNs obtained from healthy volunteers were incubated with antituberculous drugs (isoniazid [INH], rifampin [RIF], pyrazinamide [PZA], ethambutol [EMB], streptomycin [S], amikacin [A], ofloxacin [OFLX], prothionamide [PTH] and cycloserine [CyC]) and different combinations at therapeutic serum concentrations. Phagocytic activity of PMNs was significantly increased when compared with controls by PTH ($p<0.001$), A ($p<0.001$), OFLX ($p<0.001$), INH+RIF+S combination ($p<0.01$), A+OFLX combination ($p<0.05$), A+OFLX+CyC combination ($p<0.01$) and A+OFLX+CyC+PTH+EMB combination ($p<0.01$). Intracellular killing activity of PMNs was significantly increased by OFLX when compared with the control ($p<0.05$). No significant difference was observed in functions of PMN for other drugs when compared with control ($p>0.05$). Functions of PMN were significantly increased by OFLX when compared with A+OFLX combination ($p<0.05$). Phagocytic activity of PMNs was significantly increased by A+OFLX+CyC combination and A+OFLX+CyC+PTH+EMB combination when compared with A+OFLX+CyC+PTH combination and A+OFLX+CyC+PTH+PZA combination ($p<0.05$). No significant difference was found in functions of PMN between the other groups ($p>0.05$). In conclusion, some antituberculous drugs alone or in combination enhanced PMN functions, although in combination no additive or synergistic effects were detected. Moreover, none of the antituberculous drugs alone or in combination significantly decreased PMN functions. The drugs having adverse effects on immune functions would better be replaced with equally effective drugs or drug combinations having positive effects on PMN functions.

Keywords: Polymorphonuclear leukocyte; Antituberculous drugs; Phagocytosis; Intracellular killing

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1. Introduction

Tuberculosis became serious and major public health problem worldwide with the emergence of multidrug resistant tuberculosis (MDR-TB) and increased incidence of tuberculosis case by the concomitant human immunodeficiency virus (HIV) infection. It is estimated that one in every three patients in the world is infected with *Mycobacterium tuberculosis*. Approximately 3 million people worldwide die from tuberculosis annually [1–3].

Various studies have shown that there is an interaction between antibiotics and the function of host phagocytes distinct from their direct antimicrobial activity [4–8]. The investigation of the possible immunomodulatory influence of well-known antibiotics could be a new approach to the treatment of infections; instead of developing the newer generations of antibiotics for achieving higher efficacy, widening the spectrum and overcoming increased bacterial resistance [9]. A variety of methods of immunomodulation and immunotherapy were used to improve the efficacy of treatment in tuberculosis [3].

The aim of the present study was to investigate the effects of both first and second line antituberculous drugs (alone and in combination) on the functions of polymorphonuclear leukocytes (PMNs) in vitro.

2. Materials and methods

2.1. Antituberculous drugs

Isoniazid [INH], rifampin [RIF], pyrazinamide [PZA], ethambutol [EMB], prothionamide [PTH] and cycloserine [CyC] were kindly provided by Koçak Pharmaceutical Inc. (Istanbul, Turkey), streptomycin [S] by I.E. Ulugay Pharmaceutical Inc. (Istanbul, Turkey), amikacin [A] by Eczacibasi Pharmaceutical Inc. (Istanbul, Turkey), ofloxacin [OFLX] by Aventis Farma Pharmaceutical Inc. (Istanbul, Turkey). INH (5 μg/mL), RIF (7 μg/mL), PZA (40 μg/mL), EMB (7 μg/mL), S (25 μg/mL), A (24 μg/mL) and CyC (10 μg/mL) [1,10] were prepared as stock solutions at the therapeutic serum concentrations in sterile distilled water. OFLX (2.9 μg/mL) [10] was prepared as a stock solutions in Hank's balanced salt solution (HBSS, Sigma) and PTH (1.6 μg/mL) [11] was prepared as a stock solutions in methanol. Stock solutions were diluted as necessary in HBSS.

2.2. Preparation of PMNs

PMNs were prepared by modification of the method of Alexander et al. [12]. A 10 mL blood sample was collected from healthy volunteers by vein puncture in tubes containing ethylenediaminetetraacetic acid (EDTA, Sigma). PMNs were isolated from EDTA-whole blood by Ficoll (Sigma) gradient centrifugation as described by Boyum [13]. PMNs were washed three times with 3 mL of ice-cold PBS (0.1 M phosphate-buffered saline, pH: 7.2) and then they were resuspended in HBSS and adjusted to 1×10^7 PMN/mL [14]. The PMNs were found to be 98% viable by trypan blue exclusion.

2.3. Strain and inoculum

Candida albicans used in all experiments was a clinical isolate (*C. albicans* 4826) obtained from the Clinical Microbiology Laboratory in Marmara University Hospital, Istanbul, Turkey.

The inoculum was prepared as described by Boyum [13]. The test organism was maintained on Sabouraud dextrose agar. After yeast cells were incubated one night in Sabouraud dextrose broth, the yeast cells were washed twice in sterile saline, and then suspended in HBSS at pH 7.5 and adjusted to 10^7 CFU/mL. Before use, viability was assessed by means of the methylene blue dye (0.01% w/v in water) [15]. Only cultures with >95% viability were used.

2.4. Phagocytosis and candidacidal effect

A suspension of *C. albicans* in HBSS was opsonized in 10% pooled fresh human serum at a proportion of 4:1 in a separate tube at 37 °C 30 min. Before adding the opsonized yeast, PMNs were added to each sterile tube contained antituberculous drugs INH (5 μg/mL), RIF (7 μg/mL), PZA (40 μ/mL), EMB (7 μg/mL), S (25 μg/mL), A (24 μ/mL), OFLX (2.9 μ/mL), PTH (1.6 μ/mL) and CyC (10 μg/mL) alone and their different combinations at