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Original article

ASSESSMENT OF LEVOFLOXACIN EFFECT ON HUMAN NEUTROPHIL FUNCTIONS IN VITRO

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Abstract

The aim of the study was to determine the effects of levofloxacin on the phagocytic and intracellular killing functions of human neutrophils at different therapeutic concentrations and to investigate the possible differences between pure powder and pharmaceutical form of levofloxacin on the neutrophil functions. Neutrophils obtained from healthy volunteers were incubated with both pure powder and pharmaceutical form of levofloxacin at different concentrations (1.4 µg/mL, 2.8 µg/mL, 5.7 µg/mL, 11.4 µg/mL). At concentrations of 2.8 µg/mL solutions, prepared from both pure powder and pharmaceutical form of levofloxacin, were observed to decrease phagocytosis and intracellular killing comparing to control samples, but only the decrease for the pharmaceutical form's effect on phagocytic function was statistically significant ($p<0.05$). At concentration of 2.8 µg/mL solution, prepared from pharmaceutical form of levofloxacin, was observed to statistically significant decrease phagocytosis comparing to 2.8 µg/mL solution prepared from pure powder of levofloxacin ($p<0.05$). At concentration of 5.7 µg/mL solution, prepared from pharmaceutical form of levofloxacin, was observed to statistically significant decrease phagocytosis and intracellular killing comparing to 5.7 µg/mL solution prepared from pure powder of levofloxacin ($p<0.05$). Phagocytic activity of neutrophils was significantly decreased by levofloxacin solution prepared from pure powder at concentrations of 11.4 µg/mL when compared with control ($p<0.05$). As conclusion, the solutions prepared with pharmaceutical form have significantly inhibition on the phagocytic and intracellular killing functions of human neutrophils in vitro when comparing with the solutions prepared with pure powder.

Key words: *Phagocytosis, Intracellular killing, Levofloxacin, Neutrophil.*

Levofloksasin'in İnsan Nötrofil Fonksiyonlari Üzerine Etkisinin İn vitro Değerlendirilmesi

Çalışmamızın amacı, farklı terapötik konsantrasyonlardaki levofloksasinin; insan nötrofillernin fagositoz ve hücre içi öldürme fonksiyonları üzerine etkilerini belirlemek ve nötrofil fonksiyonları üzerine levofloksasin'in tablet ve hammadde formları arasındaki olası farkları araştırmaktır. Sağlıklı gönüllülerden elde edilen nötrofiller, farklı konsantrasyonlardaki (1.4 µg/mL, 2.8 µg/mL, 5.7 µg/mL, 11.4 µg/mL) hem hammadde hem de tablet formundaki levofloksasin çözeltileri ile inkübe edilmiştir. 2.8 µg/mL'lik hammadde ve tablet dozaj formunda hazırlanan çözeltiler ile nötrofillerin fagositoz ve hücre içi öldürme fonksiyonlarında kontrole göre azalma izlenmiştir; ancak sadece 2.8 µg/mL'lik tablet çözeltisi ile nötrofillerin fagositoz fonksiyonundaki azalma kontrole göre istatistiksel olarak anlamlı bulunmuştur ($p<0.5$). Levofloksasin'in 2.8 µg/mL'lik tablet formundan hazırlanan çözeltisinin, 2.8 µg/mL hammaddeden hazırlanan çözeltisiyle karşılaştırıldığında fagositozu istatistiksel olarak anlamlı azalttığı bulunmuştur ($p<0.05$). 5.7 µg/mL'lik tablet çözeltisinin 5.7 µg/mL'lik hammadde çözeltisine oranla nötrofillerin fagositik ve hücre içi öldürme etkilerini istatistiksel olarak anlamlı derecede azalttığı bulunmuştur ($p<0.05$). 11.4 µg/mL'lik hammadde çözeltisi ile nötrofillerin fagositoz fonksiyonu kontrole göre istatistiksel olarak anlamlı düzeyde azalmıştır ($p<0.05$). Sonuç olarak, tablet dozaj formundan hazırlanan çözeltiler ile inkübe edilen nötrofillerde, hammadde çözeltileri ile inkübe edilen nötrofillere oranla fagositoz ve hücre içi öldürme fonksiyonları üzerine anlamlı inhibisyon saptanmıştır.

Anahtar kelimeler: *Fagositoz, Hücre içi öldürme, Levofloksasin, Nötrofil.*

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INTRODUCTION

Various studies have shown that there is an interaction between antibiotics and the function of host phagocytes distinct from their direct antimicrobial activity (1-3). 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 (4,5).

The possible factors that affect the immune response of antibiotics were not well defined previously (1-2). This study was designed to meet two main objectives: the effects of concentration and adjuvant materials on immune response of levofloxacin. First objective was to investigate the effects of levofloxacin on the phagocytic and intracellular killing functions of human neutrophils at different therapeutic concentrations. The second objective was to examine the possible differences between pure powder and pharmaceutical form of levofloxacin on the neutrophil functions.

EXPERIMENTAL

Antibacterial agents

Levofloxacin were kindly provided by Aventis Farma Pharmaceutical Inc. (Istanbul, Turkey) in powder form and pharmaceutical form. Both pure powder (1.4 μg/mL, 2.8 μg/mL, 5.7 μg/mL, 11.4 μg/mL) and pharmaceutical forms (2.8 μg/mL, 5.7 μg/mL) of levofloxacin were prepared as a stock solutions at the different therapeutic concentrations in Hank's balanced salt solution (HBSS, Sigma) (6-8). The stock solutions were prepared with pharmaceutical form that contained at 2.8 μg/mL and 5.7 μg/mL concentrations of levofloxacin.

Preparation of neutrophils

A 10 mL blood sample was collected from healthy volunteers by vein puncture in tubes containing heparin (Mustafa Nevzat Pharmaceutical Inc, Turkey). Neutrophils were isolated from heparinized whole blood by Ficoll (Sigma) gradient centrifugation as described by Boyum et al. (6) Contaminating erythrocytes were lysed by suspension of the PMN pellet in distilled water for 30 s, followed by the addition of hypertonic saline to correct the tonicity of the solution. Neutrophils 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 x 10^7 neutrophils / mL (7). The neutrophils were found to be 99% viable by trypan blue exclusion.

Neutrophil phagocytosis and intracellular killing function

A suspension of *Candida albicans* in HBSS was opsonized in 10% pooled fresh human serum at a proportion of 4:1 in a separate tube at 37^{0}C 30 minutes. Before adding the opsonized yeast, neutrophils were added to each sterile tube contained levofloxacin at different therapeutic serum concentrations and the mixture of neutrophils and drugs were incubated at 37^{0}C for 30 minutes in a shaking incubator. At the end of the preincubation, opsonized yeast cells were added to the mixture of neutrophils and drugs. This mixture was incubated for 30 minutes at 37^{0}C and five minutes before incubation was complete, 1 mL of methylene blue (0.01%) was added to each tube at concentration 1:2 (v/v) in order to stain the dead yeast cells. Then neutrophil functions were measured on a slide under the microscope. Phagocytic activity of neutrophils were calculated as the percentage of neutrophils that include yeast cells and intracellular killing activity of neutrophils were calculated as a percentage of neutrophils that include dead yeast cells (3, 8) All assays were performed in duplicate.

Data analyses

Results are all expressed as means ± SD and the statistical significance was determined by the one-way analysis of variance (ANOVA). P values less than or equal to 0.05 were considered to be statistically significant.

RESULTS

At concentrations of 2.8 µg/mL solutions, prepared from both pure powder and pharmaceutical form of levofloxacin, were observed to decrease phagocytosis and intracellular killing comparing to control samples, but this was only statistically significant for the pharmaceutical form's effect on phagocytic function (Figure 1).

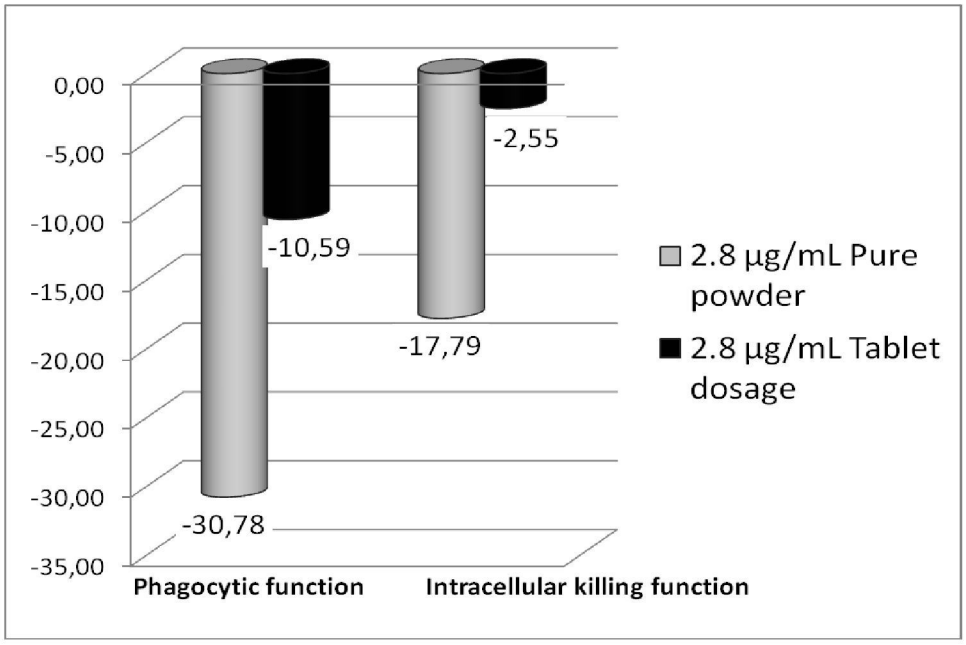

Figure 1. The percent difference of 2.8 µg/mL levofloxacin pharmaceutical form and pure powder effects on neutrophil functions compared to control group.

At concentration of 2.8 µg/mL solution, prepared from pharmaceutical form of levofloxacin, was observed to decrease phagocytosis and intracellular killing comparing to 2.8 µg/mL solution prepared from pure powder of levofloxacin, but this was only statistically significant for the effect on phagocytic function ($p<0.05$).

At concentration of 5.7 µg/mL solution, prepared from pure levofloxacin, was observed to stimulate small increases in both phagocytic and intracellular killing comparing to control. By contrast, the solution of pharmaceutical form produced a slight decrease in both phagocytic and intracellular killing functions. Comparing to control the results at this concentration were not however statistically significant ($p>0.05$). The percent difference of 5.7 µg/mL levofloxacin pharmaceutical form and pure powder effects on neutrophil functions compared to control group was shown in Figure 2. At concentration of 5.7 µg/mL solution, prepared from pharmaceutical form of levofloxacin, was observed to decrease phagocytosis and intracellular killing comparing to 5.7 µg/mL solution prepared from pure powder of levofloxacin, these decreases were statistically significant ($p<0.05$).

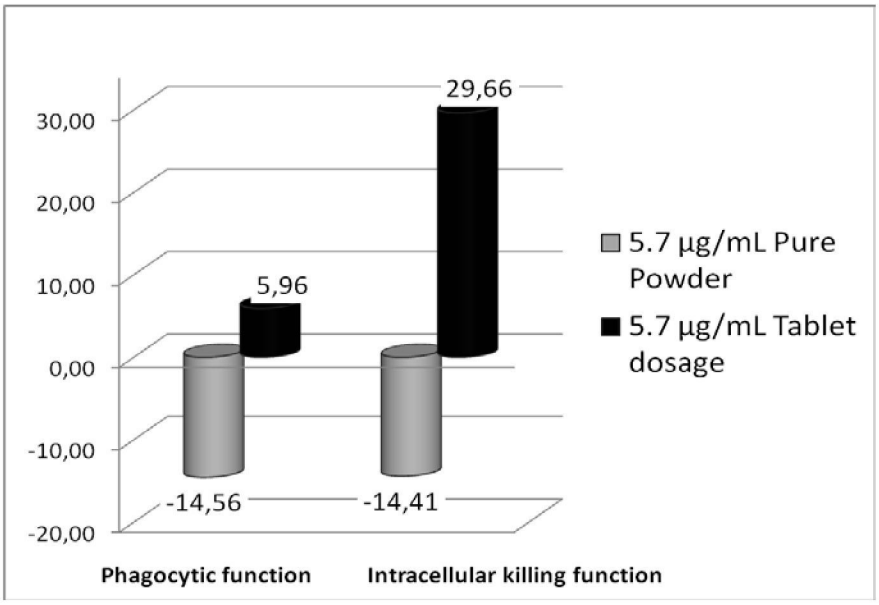

Figure 2. The percent difference of 5.7 µg/mL levofloxacin pharmaceutical form and pure powder effects on neutrophil functions compared to control group.

At concentrations of 1.4 µg/mL (subtherapeutic) and 11.4 µg/mL (above twice the therapeutic level) solutions prepared from pure levofloxacin produced decreases both in phagocytic and intracellular killing functions comparing to control, nevertheless, this was only significant for the phagocytic effect at concentration of 11.4 µg/mL solution prepared from pure powder levofloxacin. All the results are shown in Table 1.

Table 1. Effects of levofloxacin on neutrophil functions.

Levofloxacin Therapeutic Concentration and Form (µg/mL)	n	Phagocytic Activity (Mean±SEM [%])	Intracellular Killing Activity (Mean± SEM [%])
Control	7	43.14±8.40	16.86±6.49
11.4 pure powder	3	29.67±3.21	9.33±1.15
1.4 pure powder	3	36.67±4.16	12.67±3.21
5.7 pure powder	7	45.71±9.64	21.86±6.01
5.7 pharmaceutical form	7	36.86±4.56	14.43±6.27
2.8 pure powder	7	38.57±7.00	16.43±7.57
2.8 pharmaceutical form	7	29.86±9.10	13.86±6.74

n: number of experiments

DISCUSSION AND CONCLUSION

In the present study, all the solutions of levofloxacin (both prepared with pure powder or pharmaceutical form) decreased neutrophil functions except for 5.7 µg/mL pure powder solution when compared with control group. 2.8 µg/mL pharmaceutical form solution and 11.4 µg/mL pure powder dosage form solution were significantly decreased neutrophil phagocytic

function when compared with control group ($p<0.05$). Nevertheless, the decreases on intracellular killing function by the levofloxacin solutions were not statistically significant when compared with control group ($p>0.05$).

Although, there are several studies conducted for evaluation of levofloxacin effect on neutrophil functions, the data is conflicting due to non-standardized methods and use of different neutrophil functions by using various assays (2, 4).

Azuma et al. (9) demonstrated that ofloxacin (pure; 2.8 μg/mL) markedly potentiated the phagocytosis of *Escherichia coli* in PMNs. In the other study, both phagocytic activity and intracellular killing activity of PMNs were significantly increased by ofloxacin when compared with control (5).

Braga et al (10) found that gatifloxacin (pure; 1/16 and 1/32 of the minimum inhibitory concentration [MIC] for 10^6 bacteria/mL) only increased the neutrophil intracellular killing function and also showed that gatifloxacin had generally no effects on the other neutrophil functions.

The ciprofloxacin (pure; 0.5 mg/L;), lomefloxacin (pure; 1 mg/L;), fleroxacin (pure; 1 mg/L;) and ofloxacin (pure; 1 mg/L) were increased the intracellular killing of *Staphylococcus aureus* in human neutrophil granulocytes in a concentration- and time-dependent manner in the other study (11).

When comparing the effects of pure powder and pharmaceutical pharmaceutical forms of levofloxacin on neutrophil functions, both 2.8 μg/mL and 5.7 μg/mL pharmaceutical form solution had inhibitory effects on neutrophil phagocytic function when compared with pure powder solution at the same concentrations ($p<0.05$). 5.7 μg/mL pharmaceutical form solution was decreased the neutrophil intracellular killing function when compared with pure powder solution at the same concentrations ($p<0.05$).

There is only one study that investigated the possible difference between pharmaceutical form and pure powder clarithromycin solutions on the neutrophil functions in the literature. In this study, clarithromycin solution prepared with pharmaceutical form was decreased the neutrophil functions, which was similar with the present study (12). The solutions prepared with pharmaceutical form have significantly inhibition on the phagocytic and intracellular killing functions of human neutrophils in vitro when comparing with the solutions prepared with pure powder. Adjuvant materials in pharmaceutical form would be effect the response of levofloxacin on neutrophil function, so these materials will also be considered when evaluating the immunomodulator effects of antimicrobial agents.

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