Effect of ciprofloxacin on mitogen-stirrsulated human peripheral blood mononuclear cell proliferation

Cemalettin AYBAY, Kayhan ÇAĞLAR, Turgut iMİR

Department of Miciobiolopy *$t&* Kmiinolony and ImmChOfoglc Research and Application Centefj Medical School of Gazi University, Ankara, TURKEY

Ciprofloxacin, a DNA gytase inhibitor, was tested for its inhibitory or stimulatory effect on phytohemagglutinin (PHA) stimulated proliferation (measured using MTt 0-(4,5<iimethyithiazoi-2^yil)-^ nromide]) co-lor/mat ic assay) of human peripheral blood mononuclear cells. Ciprofloxacin did nut diminish or enhance mononucleai coll proliferation at the concentrations achievable in serum in its clinical applications. But proliferation of PHA-stimulated mononuclear calls was inhibited by ciprofloxacin when present in amounts of more than 12.5 \\g/ml. [Turk J Med* Res *1994; 12(1): 11-14}*

Key Words: Ciprofloxacin, Phytohemagglutinin

Ciprofloxacin is a member of the quinolone family, highly active bactericidal agent, effective ngaints a broad spectrum of gram positive ard gram- negative bacteria. The minimum inhibitory concentrations generally range between 0.01 and mg/ml (1). Ciprofloxacin inhibits DMA gyrase, a bacterial type II tcpoisometase that negatively supercolis DNA (1,2). Quinolone antibiotics affoct eukaryotic cells as well. Eukaryotic cells do not contain DNA gyrase, however, they do contain a conceptually and mechanistically similar type-II DMA topoisomerase that removes positive supercolis from eukoryotic DNA to prevent its tangling during replication (3). The prokaryotic topoisomerase II is approximately 100-fold more sensitive to inhibition by quinolooes than its eukaryotic counter-part (1). Quinoione antibiotics also inhibit eukcryotic DNA polymerase a, p. and terminal deoxynucleodityl transferase (4).

In this study, we investigated the effect of ciuiof'oxacin on mitogen stimulated and non stimulated human peripheral blood mononuclear cells using MIT colorimetric assay. This procedure employs the pale yellow tetrazolium salt (MTT [â-(4,5-dimëthyltliiàzol-2-yl)-2.r>- diphenylietrazolium bromide]) which is cleaved by active mitochondria to torn a dark blue formazan product that can be completely solubilized in acidic isopropanol and detected by a microtiter plate reader. This assay provides a simple way to detect living and growing cells without using radioactivity (5). Mosmann (6) originally reported that this assay can be utilized to measure proliferating cells as well as in cytotoxicity assays.

HpceivecJ: Nov. 22. 1993 Accepted: One. 2R, 1993

Correspondence: Cemalettin AYBAY
Dept. of Microbiology and
Immunology, Gazi UTF.
Besevler-Ankara, TfiiRKEY

ivIATKRiALS AND METHODS

Mononuclear ceils (MNCs) were isolated from fresh heparinized peripheral blood obtained from twelve healthy laboratory personnel by layering over Histopnque 1077 (Sigma) and centh'"ged 30 min at 1500 rpm (400xg), 18°-20°C. Using a sterile pipet, the upper layer containing the plasm* and most of the phtelors was removed and the MNC layerr was iransfcred to a centrifuge tube. The cells were washed three times by adding r-iPMi-1640 medium (Sigma) (3 times the volume of the mononuclear cell layer) and cenirifuged 10 min at 1300 rpm 18°-20°C. After the final wash, the supernatant was discarded anci the cell pellet was resüsperYded in RPMI-1640 medium (with L-glutamine, pH 7.4) sunpiemented with %t0 autologous serum. The cells were counted and adjusted to 3x10° celt/ml

Phytohemagglutinin (PHA) (Seromed, Germany Cat. No. M 5030) was used as the mitogenic agent at a final concentration of 5 pg/ml in wells.

Ciprofloxacin (Ciproxine 200, Bayer. 100 ml IV infusion solution contained 0.254 g ciprofloxacin lactate

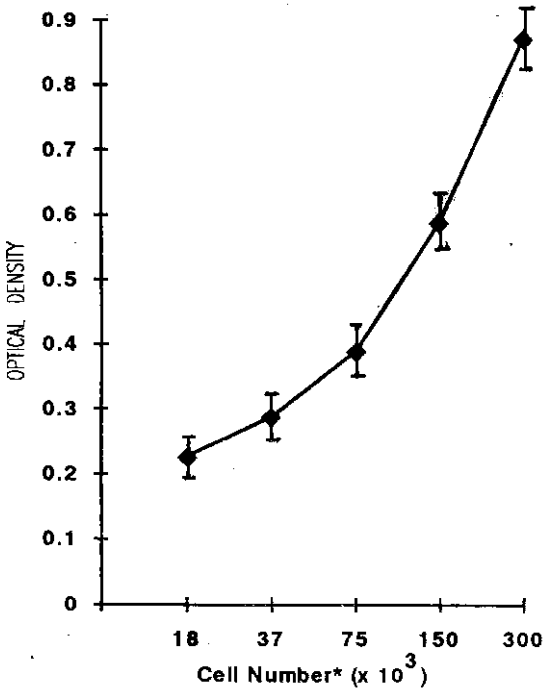

Figure 1. Relationship between cell number, and absorbance (OD) of MTT-formazan generated by living PHA-stimulated MNCs. MTT working solution was added to PHA-stimulated MNCs. MTT-formazan was dissolved in 100 jil propanol (0.04 N HCl) after 3 h incubation at 37°C with MTT. (OD: meanistandard error of the mean, n=6)

* Cell number represents the amount inoculated into wells on day 0.

as 0.2 g ciprofloxacin base) was prepared at the final concentrations of 0.8 to 200 ug/ml in wells.

MTT (Sigma, No. M-2128) was dissolved in Phosphate Buffered Saline to make a 5 mg/ml stock solution. After filter-sterilization of stock solution, MTT working solution was prepared as follows: 1 ml of MTT stock solution and 9 ml of RPMI-1640 supplemented with %5 fetal calf serum (SEBAK) were mixed.

In order to solubilize the formazan crystals, acidic-isopropanol (0.04 N HCl in isopropanol) was used.

The MNCs were cultured in U-bottomed sterile 96 well-microtitre plate at a density ox 3x10^5/200 ul per well and the groups, studied in triplicate, were organized as follows:

Group A: MNCs only (Control A).

Group B: MNCs+PHA (Control B).

Group C1 - C5: MNCs+four-fold dilutions of ciprofloxacin (0.8 to 200 pg/ml)

Group D1 - D5: MNCs+PHA+four-fold dilutions of ciprofloxacin (0.8 to 200 pg/ml)

The cells were incubated for 3 days in a 37°C, 5% C02 humidified incubator. After incubation, the plate was centrifuged 7 min at 1100 rpm (225xg) and without disturbing the cell pellet 170 pi of supernatant was discarned from every well. The plate was left on the rotator for 5 min at 100 rpm. After adding 50 pi of MTT working solution to each well, the plate was incubated for 3 hours in a 37°C, in a 5% CO2 humidified incubator. After incubation, 100 pi of acidic isopropanol was added to each well and pipetted up and down vigorously 5-6 times to dissolve the dark blue formazan crystals. The plate was protected from light by covering, with aliminium foil and kept for 30 min at 4°C for completely dissolving the crystals. After confirming microscopically that formazan crystals were completely solubilized, the plate was read in a microtiter plate reader (Autoreader ll. Ortho Diagnostic Systems Inc., NJ, USA) using a 550-nm filter.

Student's t-test was used to analyze the results.

RESULTS

The results in Figure 1. show that the absorbance (OD) is directly propportional to the number of living cells which were stimulated with PHA (5pg/ml) for 3 days at 37°C. After 3 days of incubation period, the OD of the cells in control B (PHA-Stimulated MNCs) was nearly two times of control A (Non-stimulated MNCs) (Table 1). Table 1. demonstrated that MNCs responded to mitogenic agent. Viability, assessed With trypan blue dye exlusion test, of MNCs before and after incubation (for all groups) was not below 94%±3.2 and 85%±5.3, respectively, variation of OD value among triplicates in each group did not exceedt 15%.

Ciprofloxacin, at the concentrations of 0.8 to 12.5 pg/ml, was found to be ineffective on human PHA-stimulated MNCs proliferation. But at the concentrations of more than 12.5 mg/ml, ciprofloxacin significantly decreased ($p<0.001$) PHA-stimulated human MNC proliferation (Figure 2).

Ciprofloxacin itself does not have a proliferative or a toxic effect on nonstimulated human MNCs at the concentrations of 0.8 to 50 mg/ml. But at the concentration of 200 mg/ml, ciprofloxacin significantly

Table 1. The OD values of control B and control A

B*	0.87	0.94	0.88	0.96	0.87	0.79	0.95	0.88	0.87	0.80	0.91	0.88
A	0.49	0.47	0.53	0.47	0.49	0.36	0.55	0.58'	0.41	0.43	0.38	0.45

* The OD was measured after 3 days of incubation period using a 550-nm filter

B: PHA-stimulated MNCs, n=12, A: Non-stimulated MNCs, n=12

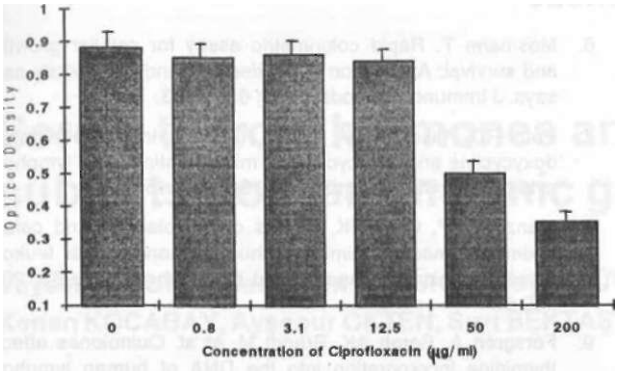

Figure 2. Proliferative response of MNCs to PHA in group B and group D1-D5. Optical density (MTT reaction) of PHA-stimulated human MNCs without ciprofloxacin (0) and in the presence of ciprolloxacin at different concentrations (0.8 to 200 pg/ml) after incubation at different concentrations higher than 12.5 pg/ml. The results (M±S.E.M.) represent experiments with MNCs from twelve volunteers.

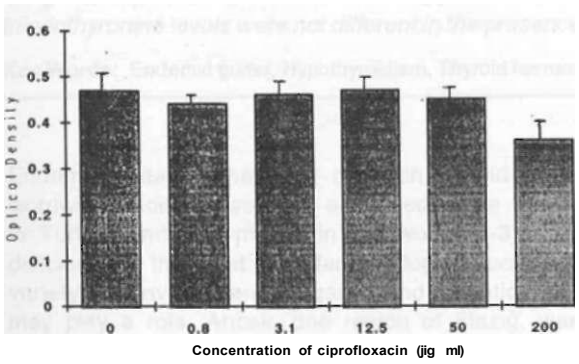

Figure 3. MTT reaction of MNCs in group A and group C1-C5. Optical density (MTT reaction) of non-stimulated humann MNCs without ciprofloxacin (0), and in the presence of ciprofloxacin at different concentrations (0.8 to 200 pg/ml) after incubation for 3 days demonstrated that ciprofloxacin decreased formazan production by MNCs only at highest (200 pg/ml) concentration. The results (M±S.E.M.) represent experiments with MNCs from twelve volunteers.

(p<0.001) decreased the formazan production by non stimulated MNCs (Figure 3).

DISCUSSION

The effect of several classes of antibiotics on MN proliferation has been studied by several investigators (7.8).

Hussy et al (1) reported that ciprofloxacin at the concentrations of 1-10 mg/ml had no effect on eukaryotic cell proliferation; however inhibited cell growth completely at the concentration of 100 mg/ml and led to cell death at 1000 mg/ml.

According to the report by Forsgren et al (9), ciprofloxacin (at the concentrations of 0.8-12.5 pg/ml) increased the incorporation of [^{3}H] thymidine into DNA of phytohemagglutinin-stimulated human lymphocytes, while decreased the [^{3}H] thymidine incorporation into MNCs at the concentrations of 50 pg/ml and 200 pg/ml.

Gollapudi et al (10) reported that ciprofloxacin did not diminish or enhance mononuclear cell proliferation *in vitro* at the concentrations of 5 to 125 pg/ml for ConA-stimmulated murine splenocytes. Gollapudi et al allso performed the assay with human MNCs stimulated with PHA. Although ciprofloxacin at the concentration of 25 pg/ml depressed the incorporation of [^{3}H] thymidine Into MNCs (45,604±5,600) in comparision with the control cells (51,089±1,704) (while not at 5 mg/ml (54,568±2,623), the statistical significance was not reported.

MTT colorimetric assay provides a simple and quantitative measurement for mammalian cell proliferation without the need of radioactive isotopes. For this reason MTT colorimetric assay was choosen to assess cell proliferation. As shown in Figure 1 the assay demonstrated that it was well corraleted with the number of viable cells and the OD. In agreement with data from the literature, we also found that ciprofloxacin had no inhibitory effect on PHA-stimulated human MNCs proliferation at the concentrations up to 12.5 pg/ml. Although ciprofloxacin depressed the PHA-stimulated MNC proliferation at 50 pg/ml, the OD of PHA-stimulated MNCs at this concentration was almost the same with that of non-stimulated MNCs (compare Figure 2 with Figure 3). At higher concentrations (200 mg/ml) ciprofloxacin decreased the formazan production by PHA-stimulated as well as by non-stimulated MNCs.

After ingestion of 400-600 mg., peak serum levels were found to be 1-3 mg/ml for ciprofloxacin (11). In this regard, we concluded that ciprofloxacin did not affect PHA stimulated human MNC proliferation at the concentrations achievable in serum in its clinical applications.

Ciprofloxacinin mitojen ile stimüle insan periferik kan mononükleer hücre proliferasyonu üzerine etkisi

DNA giraz inhibitörü olan ciprofloxacinin MTT ([3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide]) kolorimetrik test yöntemi kullanılarak phytohemaglutinin (PHA) ile uyarılmış insan periferik kan mononükleer hücreleri üzerine inhibitör veya stimülatör bir etkisinin olup olmadığı araştırıldı. Ciprofloxacinin klinik uygulamalarda ulaşılan serum konsantrasyonlarında mononükleer hücre proliferasyonu üzerine etkisi gözlenmedi. Fakat, PHA ile uyarılmış mononükleer hücre proliferasyonu 12.5 pg/ml denn daha yüksek konsantrasyonlarda inhibe ettiği gözlendi. [Türk J Med Res 1994; 12(1): 11-14]

REFERENCES

1. Hussy P, Maass G, Tummler B, et al. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase complex, topoisomerases I and Il, and growtn of mammalian lymphoblasts. Antimicrobial Agents and Chemotherapy. 1986; 29: 1073-8.
2. Shen LL, Pernet AG. Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: The target of the durgs in DNA, Proc. Natl. Acad. Sci. USA, 1985; 82: 307-11.
3. Mandell GL, Sande MA. Antimicrobial agents, the pharmacological basis of therapeutics (Ed. Alfred Goodman Gilman), New York USA: Pergamon Press. 1990: 1058.
4. Rusquet R, Bonhommet M, David JC. Quinolone antibiotics inhibit eucaryotic DNA polymerase *a.* and p terminal deoxynucleotidyl transferase but not DNA ligase. Biochemical and Biophysical Res Com. 1984; 121: 762-9.
5. Current protocols in Immunology, (Ed. John E. Coligan), Published by Greene Publishing Associates and Wiley-interscience. New York; 1992; 1: 636.
6. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65: 55-63.
7. Potts RC, Hassan HAA, Brown RA, et al. In vitro effects of doxycycline and tetracycline on mitogen stimulated lymphocyte growth. Clin Exp Immunol. 1983; 53' 458-64.
8. Manzella JP, Clark JK. Effects of moxolactam and cefuroxime on mitogen-stimulated human mononuclear leukocytes. Antimicrobial Agents and Chemotherapy. 1983; 23: 360-3.
9. Forsgren A, Bergh AK, Brandt M, et al. Quinolones affect thymidine incorporation into the DNA of human lymphocytes. Antimicrobial Agents and Chemotherapy. 1986;29: 506-8.
10. Gollapudi SVS, Prabhala RH, Thadepalli H. Effect of ciprofloxacin on mitogen-stimulated lymphocyte proliferation. Antimicrobial Agents and Chemotherapy. 1986; 29:337-8.