# EFFECT OF SOME ANTIMICROBIAL AGENTS ON MACROPHAGE FUNCTIONS

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## ABSTRACT

*six antimicrobials (azlocillin, ceftriaxone, clindamycin, cefodizim, norfloxacin and ofloxacin) were tested in vitro in concentrations equal to their therapeutic serum levels on three aspects of macrophage function, in order to evaluate phagocytosis of heat-killed Candida allicans, NBT reduction and macrophage production of migration inhibition factor. Clindamycin, cefodizim and ceftriaxone, in that order, exerted a significant stimulatory effect on macrophage phagocytosis. Clindamycin, ceftriaxone, cefodizim and ofloxacin were stimulatory to macrophage NBT reduction. Again, clindaymycin was the antimicrobial with maximal stimulatory effect on MIF production by peritoneal mice macrophages, followed by cefodizim and ceftriaxone.*

## INTRRODUCTION

From clinical experience, it is well known that, a functional host defense system is required or antimicrobial chemotherapy to be effective. Antimicrobials may have a significant bactericidal effect in vitro, but the effect in vivo may sometimes be disappointing. The is especially true in immunocompromised hosts.

Among specific host mechanisms of defense against infections, phagocytic cells provide a remarkable tool against a wide range of microorganisme. Polymorphonuclear leucocytes play a major role in the body's defense against acute infectious processes, while mononuclear phagocytes are concerned principally with the control of microorganisms which are able to survive intracellular residence and against which neutrophils are ineffective (Drutz and Mills, 1978). Antimicrobials may have a direct effect upon host cells including phagocytes, and it is important to understand the potential beneficial and deleterious effects of antimicrobials on the host's immune response.

## AIM OF THE WORK

The aim of this work is to study the effects of six antimicrobial agents on macrophage functions by assessing the following tests:

1. Phagocytosis of heat-killed Candida.
2. Nitro-blue tettra zolium reduction test.
3. Migration inhibition test.

## MATERIAL AND METHODS

Six antimicrobial agents, namely Azlocillin (Bayer), Norfloxacin (MSD), Ofloxacin (Hoechst), Ceftriaxone (Hoffmann La Roche), Cefodizim (Hoechst) and Clindamycin phosphat (Upjohn) were studied. The concentrations of these antimicrobials were adjusted to be the same

as their recommended therapeutic serum levels as follows:

| | |
|---|---|
| Azlocillin | 12.5 µg/ml. |
| Norfloxacin | 2 µg/ml. |
| Ofloxacin | 2 µg/ml. |
| gceftriaxone | 125 µg/ml. |
| Cefodizim | 5 µg/ml. |
| Clindamycin | 15 µg/ml. |

Macrophages were prepared from the peritoneal exudate of mice, according to the method desribed by Hamblin (1981).

## 1. PHAGOCYTOSIS OF HEAT-KILLED CANDIDA ALBICANS: (warren et al., 1967)

**Materials:**

1. Hank's solution pH 7.2.
2. Candida allican's suspension $5x10^6$ cells/ml.
3. Peritoneal macropage of mice in Hank's solution $2x10^6$ cells/ml.
4. Antimicrobial solutions in their therapeutic serum levels.
5. Pooled normal sera.

**Method:**

1. To a duplicate set tubes we added:
a-0.25 ml of macrophage suspension ($2x10^6$ cells/ml).
b- 0.25 ml of pooled normal human serum.
c- 0.25 ml of Hank's balanced salt solution.
d- 0.25 ml of heat-killed Candida suspension ($5x10^6$ cells/ml).
2. 0.25 ml of the tested antimicrobial agent at its therapeutic serm level was added to one set of tubes.
3.The tubes were then incubated at 37°C FOR 30 minutes and then entrifuged at 200 g for 5 minutey. The supernatant was removed, leaving a drop into which the sediment was resuspended.
4. Smears were prepared from the deposit, left to dry in air, and stained with May Grunwald-Giemsa.
5. 200 macrophages were examined, the number of ingested Candida associated with each cell was counted, and the mean particle number associated with each cell was calculated.

## II.NITRO BLUE TETRAZOLIUM TEST: (wilkinson, 1981)

**Materials:**

1. Nitro-blue tetrazolium (British Drug Houses).
2. Macrophage suspension ($5x10^6$/ml) in PBS.
3. The tested antimicrobial agents in their therapeutic serum levels.

**Method: (Unstimulated NBT)**

1. To a duplicate set of tubes, we added:
a- 0.5 ml of macrophage suspension in PBS ($5x10^6$/ml).
b- 0.2 ml of the freshly-made-up 0.15% NBT solution.
2. To one set of tubes we added 0.2 ml of the tested antimicrobial agents at their therapeutic serum level.
3. The tubes were then incbated at 37°C for 20 minutes, spinned gently at 400 g for 3-4 min. and the supernatant was removed. A drop of PBS was added to resuspend the cells.
4. A film was made, fixed by gentle heating and counterstained with diltute carbol-fuchsin.
5. Using a 100x oil-immersion objective, 200 macrophages were counted, and the percentage of NBT-positive cells containing blue deposits was determined.

## III.MIGRATION INHIBITION TEST: (Hamblin, 1981)

**Materials:**

1. Disposable migration plates with 12 chambers (sterilin).
2. Microcapillaries (Drummond«Micro-Caps» 20 µl).
3. P.H.A. Containing culture supernatants.

4. Culture medium containing serum (fetal calf serum).
5. Peritoneal macrophage of mice ($3x10^7$ cells /ml).
6. The tested antimicroboial agents at their therapeutic serum levels.
7. Culture supernatant containing M.I.F.

**Method:**

1. Microcapillaries were filled with a cell suspension (peritoneal macrophages in medium plus serum at suitable final cell concentration ($3x10^7$ cells /ml.).
2. The tested antimicrobial agent at its therapeutic serum level was added to the peritoneal cells suspension, and another sets of microcapillaries were filled with this suspension.

3. The micocapillaries were centrifuged at 300 g for 5 minutes at room temperature, and then the capillaries were scored at the cell-fluid interface using a diamond pen. The capillaries were then broken at the scoreline with forceps.
4. One capillary tube was then mounted in a migration chamber and the culture supernatant containing the M.I.F.was added to the well.

5. The migration chamber was covered with cover-slip ensuring an air-tight seal without bubbles.
6. The plate was then incubated at 37°C overnight (15- 18 h).
7. The areas of migrating cells were prsjected onto plain paper, and the outer margin of the migrating fan was drawn.
8. The area of migration was then determined by planimetry. The results were expressed as the mean migration area, or as the migration index (MI) as follows:

$$MI = \frac{\text{Mean area of migration in presence of antimicrobial agent}}{\text{Mean area of migration in control chambers}}$$

or as % of migration = M.I. X 100.

## RESULTS

1. TESTS FOR MACROPHAGE PHAGOCYTOSIS OF HEAT–UILLED CANDIDA (Table 1)

a. Azlocillin (12.5 µg/ml) when added to macrophage suspension resulted in no significant effect on macrophage phagocytosis of Candida compared to the control test.
The phagocytic index (mean number of killed Candida phagocytosed by 200 macrophages) in the control test was 3.545 ± 0.23 Candida/cell, while in the azlocillin test it was 2.84 ± 0.43 Candida/cell. Applying the student t test to compare the results in the 2 groups, it was found that $t = 0.27$ $p > 0.05$ (insignificant) .
B. Ceftriaxone. Two concentrations were tested:
1- At 125 µg /ml concentration, there was no increase in macrophage phagocytosis ($p > 0.05$).
2- At 250 µg/ml concentration, there was a highly significant enhancement of macrophage phagocytosis ($p < 0.01$).
c. Clindamycin phosphate 15 µg/ml showed a very highly significant enhancement ($P < 0.001$).
d. Cefodizim 5 µg/ml showed no significant enhancement on macrophage phagocytosis ($P < 0.05$).
e. Norfloxacin 2 µg/ml showed no significant enhancement on macrophage phagocytois ($P > 0.05$).
f. Ofloxacin 2 µg/ml showed also no significant enhancement ($P > 0.05$).

**Table (1): Effect of antimicrobials on macrophage phagocytosis of heat-killed Candida.**

| Anti-Microbial | Concentration µg/ml | Mean No. of killed Candida/macrophage «Phagocytic Index» | Student t test (fiona. 1984) | P |
|---|---|---|---|---|
| Control | - | 3.545 | - | — |
| Azlocillin | 12.5 | 2.84 | 0.27 | > 0.05 |
| Control | — | 2.82 | — | — |
| Ceftriaxone | 125 | 3.05 | 10.05 | > 0.05 |
| Ceftriaxone | 250 | 3.57 | 4.05 | < 0.01 |
| Control | — | 2.594 | | |
| Clindamycin | 15 | 3.445 | 8.1 | < 0.001 |
| Control | — | 2.574 | — | — |
| Cefodizim | 5 | 3.245 | 4.6 | < 0.01 |
| Control | — | 2.614 | — | — |
| Norfloxacin | 2 | 2.615 | 0.9 | > 0.05 |
| Control | — | 2.614 | — | — |
| Ofloxacin | 2 | 2.845 | 1.83 | > 0.05 |

Table (2): Effect of antimicrobials on macrophage NBT reduction.

| Anti-Microbial | oconcentration µg/ml | Percentage No. of cells containing formazan granules | Z test (Fiona, 1984) | P |
|---|---|---|---|---|
| Control | - | 20.9 | - | - |
| Azlocillin | 12.5 | 18 | 0.75 | > 0.05 |
| Control | - | 20.9 | - | - |
| Ceftriaxone | 125 | 57 | 7.6 | < 0.01 |
| Control | - | 24 | - | - |
| Clindamycin | 15 | 73 | 10.6 | < 0.001 |
| Control | - | 24 | - | - |
| Cefodizim | 5 | 50 | 5.3 | <0.001 |
| Control | - | 24 | - | - |
| Norfloxacin | 2 | 19 | 1.2 | > 0.05 |
| Control | - | 24 | - | - |
| Ofloxacin | 2 | 50 | 5.3 | <0.001 |

Table (3): Effect of antimicrobials on macrophage production of migration inhibition factor.

| Anti-Microbial | Concentration µg/ml | Mean % of migration | Z test** | Significance |
|---|---|---|---|---|
| **Azlocillin** | 12.5 | 137 | - 1.87 | Insignificant inhibition |
| **Ceftriaxone** | 125 | 33.7 | 2.36 | significant production |
| **Clindamycun** | 15 | 13.7 | 2.36 | Highly significant production |
| **Cefodizim** | 5 | 22.2 | 2.36 | Highly significant production |
| **Norfloxacin** | 2 | 136 | - 1.74 | Insignificant inhibition |
| **Ofloxacin** | 2 | 107.5 | V- 0.498 | Insignificant inhibition |

*Mean % of migration =
Mean area of migration in antimimicrobial plate

Table (4): summary of the effects of the six antimicrobials on the three tests performed.

| Antimicrobial | Phagocytosis of heat-killed Candida | NBT test | Migration inhibition test |
|---|---|---|---|
| Clindamycin | ++++ | ++++ | ++++ |
| Cefodizim | +++ | ++ | +++ |
| Ceftriaxone 125 µg/ml | ++ | +++ | +++ |
| Ceftriaxone 250 µg/ml | ---- | N.D. | N.D. |
| Azlocillin | -- | ---- | ---- |
| Norfloxacin | — | -- | -- |
| Ofloxacin | | + | - |

+ = Significant stimulatory effect.
- = Inhibitory effect.
N.D.= Not done.

## II. TEST FOR MACROPHAGE NITRO – BLUE TETRAZOLIUM REDUCTION (NBT TEST ) : ( Table 2 )

Azlocillin and norfloxacin showed an an insignificant stimulatory effect in the NBT test ( P> 0. 05 ), all other chemobiotics tested showed a very highly signicant stimulation of macrophage NBT reduction ( P < 0. 001 ) in this order : Clindamycin showed the most signifiant stimulatory effect, followrd by ceftriaxone, then cefodizim, then ofloxacin

## III. TEST FOR MACROPHAGE MIGRATION INHIBITION : ( Table 3 )

Azlocillin, norfloxacin and ofloxacin showed an inhibitory effect on the production of migration inhibition factor ( MIF ) by peritoneal mice macrophages in the following order : Ceftriaxone ( 125 ug / ml ) had a significant stimulatory effect on the production of MIF, cefodizim showed a highly sigificant stimulatory effect, while clindamycin phosphate showed the most statistically significant effect on MIF production .

Table (4 ) summarizes all the effects of the six antimicrobials stdied on the three facets of macrophage function tested .

## DISCUSSION

Traditionally, selection of an antimicrobial agent has been based on one criterion : the direct antimicrobial activity of the chemobiotic against the pathogen ( expressed as MIC ). Recent in vitre studies suggest that some antimicrobials may work to enhance the activity of certain components of the host's defense system as well as to weaken the pathogen's resistance to them. Antibiotic selection, therefore, should be based not only on the direct effect of the antibiotic on the bacteria, but also on the relationship bwtween the antibiotic and the host's defense mechanism .

Previous studis of the effects of antimicrobial agents and macrophage functions showed the following results :

1. Clindamycin phosphate : Preincubation of organisms with clindamycin phosphate led to an increase in phagocytosis and early intracellular illing by alveolar macrophages ( Hand et al., 1984 ) .
2. Cefodizim : Cefodizim interacts with NMRI mice peritoneal macrophages in vivo by increasing their secretory activity of acid hydrolases. Marked changes in the levels and distribution of the activities of the lysosomal enzyme were induced. There was an increase of secreted enzyme activity after intravenous and subcutaneous administration ( Limbert et al., 1984 ; .
3. Pefloxacin : Both low and high concentrations of pefloxacin significantly increased the phagocytic capacity and activity of alveolar macrophages ( Desnottes et al., 1986 ) .
4. Ceftrazidime : A subinhibitory concentration of ceftrazidime resulted in enhanced phagcytosis of bacteria by peritoneal mouse macrophages ( Cuffini et al., 1982 ) .
5. Cephalothin : After an initial 2 h incubation with alveolar macrophages, the growth of surviving intracellular staphylococcus aureus aureus was examined in the presence of subinhibitory concentrations of cephalothin. No inhibition of growth was seen ( Elliott et al ., 1982 ) .
6. Aminoglycosides : Gentamycin and netilmicin were found to inhibit macrophage phagcytosis in different ways in high concentrations, while sisomicin did not affect the same function. Intracellular killing of Candida albicans by peptone - elicited macrophages was not affected by incubation with different aminoglyco-

sides ( Lannello et al., 1982 ).
7. Penicillin : No inhibition of growth was Seen in surviving intracellular Staphylococcus aureus examined in the presence of subinhibitory conctrations of penicillin ( Elliott et al., 1982 ).
8. Nafcillin : Warran and Gray ( 1967 ) repoted that the addition of nafcillin to the incubation medium enhanced Stahylococcal phagocytosis by mouse macrophages .

To date, there are no available studies concerning the effects of azlocillin, norfloxacin, ofloxacin and cefrtiaxone on macrophage functions. Clindamycin phosphate and cefodizim were tested for their effects on other immune parameters. The results showed that, clindamycin potentiates opsonization of Streptococcus pyogeres ( M - type 6 ) by human complement and subsequent phagocytosis by freshly isolated human PMN in vitro. It was found that the potentiation of phagocytic uptake was dependent on the length of time that the bacteria were in contact with the antibiotic ( Gemmell et al., 1981 ). In another study, incubation of strains of group A Streptococcus or S. pneumoniae in subinhibitory concentrations of clindamycin reduced formation of the M and C antigens in the organisms. Treated organisms were phagocytosed and killed more readily (Gemmell et al., 1980 ) .

Forsgren et al. ( 1980 ), reported suppressed lymphocyte transformation to PHA with clindaycin, but only at a very high concentration ( 50 ug / ml ) of the drug. As regards cefodizim, it was found that, lymphocytes from Bulb / C mice treated with 3 and 30 mg / g / day of cefodizim display increased responsiveness to B - cell mitogen and specific antigens The amount of antigen - specific antibody producing plaque - Formimg cells was increased and was accompanied by a rise in the specific IgG heamagglutination titer ( Limbert et al., 1984 ) .

In this study, clindamycin showed the most statistically significant enhancement effect on the three performed tests. Cefodizim showed a ststistically significant enhancement of both phagocytosis of killed Candida and macrophage migration inhibtor tests, but less stmulatory effect on NBT reduction .

Ceftriaxone showed a statistically significant enhancement effect on the NBT reduction test and to a less extent on both phagocytosis of killed Candida and macrophage migration inhibition tests . Its effect on the phagocytosis of killed Candida was dose - dependent i. e., a significant stimulatory effect was observed at a dose of 250 ug / ml, but not at 125 ug / ml .

On the other hand, azlocillin showed inhibitory effects on the three tests performed followed by norfloxacin .

Ofloxacin showed a slightly significant stimulatory effect on NBT reduction, and and a slightly inhibitory effect on phagocytosis of killed Candida and macrophage migration inhibition tests .

Antimicrobials which stimulate phagocytosis may have a direct effect on phagocytic cells, or they may act by affecting the cell wall antigena of the organism, making them easily opsonized and phagocytosed by phagocytic cells .

## REFERENCES

*Cuffini, A. M., carlone, N. A. and xerri, l. (1982) : effect of ceftazidime on the marophafe phagocytisis of pseudomonas aeruginosa. institute of microbiology, University of torino, italy.*

*Desilnottes, J. F., jacotot, F., bruel, J. B., bassoullet, M. T. and niel, G. (1986) : effects of perfloxacin on phagocytosis function of rat macrophages and polymorphnuclear leucocytes. J. antimicrob. chemoth., 17 (suppl. B) : 52-57*

*Drutz, D. T. and Mills, J. ( 1978 ) : Immunity and infection in basic and clinical immunology. In Fundenberg ,H. H. (Editor ), Los Alto , California, P. 217 .*

*Eliott, G. R., Peterson, P. ., Verbrugh, H. A., Frieber , M. R., Hoidal, J. R. and Quie, P. G. ( 1982 ): Influence of subinhibitory concentrations of penicillin, cephalothin and clindamycin on staphyococcus aureus growth in human phagocytic cells. Antimicrob. Agent & Chemotherap., 23 : 781 - 784.*

*Fiona,B. P. ( Editor ) (1984 ): Medical statistics made easy. Churchill, Livingstone, Edinburgh, London, Melbourne and New York.*

*Forsgren.A., Bank, G., Bechman, H. and Bellahsene, A ( 1980 ): Antibiotic host - defense interaction in vitro and in vivo. Scand. J. Infect. Dis., 24 ( Suppl .) : 195 - 203*

*Gemmell .C. C. and Abdul - Amir, M. K. ( 1980 ) : Antibiotic induced changes in Streptococci with respect to their interaction with human PMNs. In : Current, J. D. and Grassi, C. American Society for Microbiology, Washington D. C., P. 810 - 811 .*

*Gemmell ,C. G., Peterson , R. ., Schmeling, D. and Quie, P. G. (1981 ) : The potentiation of phagocytosis of streptococcus pyogenes by treatment with various antibiotics .J. Clin. Invest., 67 : 1249 - 56 .*

*Hamblin ,A. S. ( 1981 ) : The clinical measurement of lymphokines. In : Techniques in clinical immunology. Thompson R. A. ( Editor ). Blackwell Scientific Publications, P. 254 .*

*Hand ,W. L., ing - Thompson, W. L. and Johnson, J. D. ( 1984 ) : Influence of bacterial antibiotic interactions on subsequent antimicrobial activity of alveolar macrophages. J. Infect. Dis., 149 : 271 - 276 .*

*Lennello, D., Toscano, M. A., Bonina , L., Nicoletti, G., Delfino, D. and Masttroeni, P. ( 1982 ) : In vitro studies of netilmicin a,d other aminoglycosides on human neutrophil. Microbiology Institute, University of Messina, Itaty .*

*Limbert, M., Bartlett, R. R., Dickneite, G., klesel, N., Schorlemmer, H. V., Seibert, G., Winkler I. and Schrinner, E. ( 1984 ) : Cefodizime an aminothiazolyl cephalosporin. IV: Influence on the immune system . J. Antibiotics, 37 : 1719 - 26 .*

*Warren,G. H. and Gray, J. ( 1967 ) : Influence of nafcillin on enzymatic lysis of staphylococcus aureus. Cand. J. M.croboil., 19 : 321 - 28 .*

*Wilkinson ,P.C. ( 1981 ) : Neutrophil leucocyte function tests. In : Techniques in Clinical Immunology. Thompson, R. A. ( Editor ) Blackwell Sientific Publications, P. 273 - 292 .*

# تأثير بعض المضادات الحيوية على وظائف الخلايا الأكولة

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تم فى هذا البحث اختبار سته مضادات حيوية وهى الأزلوسيلين والسيفتيراكزون والكلندا ما يسين والسيفودكسيم والنورفلوكسين والأفلوكساسين بتركيزات تعادل مستواهم فى المصل فى الجرعات العلاجية على بعض وظائف الخلايا الأكلولة بغرض تقويم تأثير هذه المضادات الحيوية عليها . وقد وجد بالبحث ان كل من الكلندا ما يسين والسيفودكسيم والسيفتيراكزون والأفلوكساسين لهم تأثيرات ايجابية على الخلايا الأكلولة بمعنى ان فى وجود احدهم تزداد قدرة الخلايا الأكولة على التهام البكتريا . ووجد ايضا ان الكلندا ما يسين كان له اكبر الأثر فى زيادة انتاج المعامل المثبط لهجرة الخلايا الأكولة فى الفيران ويأتى بعده السيفود كسيم ثم السيفتيراكزون .