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Effects of antibiotics on human polymorphonuclear leukocyte chemotaxis *in vitro*

Y. Ueyama, M. Misaki, Y. Ishihara, T. Matsumura

Department of Oral and Maxillofacial Surgery II, Okayama University Dental School, Okayama 700, Japan

SUMMARY. **The effects produced *in vitro* by minocycline (MINO), aminobenzyl penicillin (ABPC) and cefotiam (CTM) on polymorphonuclear leukocytes (PMN) chemotaxis were investigated in this study using the Boyden chamber technique and polarization assay. The following results were obtained: CTM brought about a significant increase of PMN chemotaxis at the therapeutic dose (10, 100 μg/ml), MINO reduced PMN chemotaxis significantly at a concentration as low as 1 μg/ml and ABPC did not influence PMN chemotaxis at concentrations up to 100 μg/ml in both methods. Furthermore, using a checkerboard assay, it was shown that CTM worked as a chemokinetic factor.**

INTRODUCTION

A variety of antibiotics are used in the treatment of infectious diseases. In the past, antibiotics have been evaluated primarily for antibacterial action (minimum inhibitory concentration) and pharmacokinetics in the host (concentration of blood levels, transfer to lesions). More recently, the effects of antibiotics have been evaluated from the viewpoint of host defense mechanisms against infection.[1] Some of the most interesting studies have focused on the effects of antibiotics on the functions of polymorphonuclear leukocytes (PMN) which play an important role in non-specific defense mechanisms against infection.[2,3]

The PMN functions can be divided into migration, phagocytosis and bactericidal activity. Of these, migration is regarded as one of the major initial protective mechanisms, as demonstrated by patients with such leukocyte disorders, who are liable to suffer from infectious diseases.[4] In this study, the effects of different groups of antibiotics on PMN migration *in vitro* were studied by the polarization assay, to observe morphological changes on PMN migration, and by the Boyden method, to detect alterations in function.

MATERIALS AND METHODS

Preparation of antibiotics

Three kinds of antibiotics were tested by the polarization assay and the Boyden method: minocycline (Lederle Japan Ltd, MINO), aminobenzyl penicillin (Meiji Seika Kaisya, Ltd, ABPC) and cefotiam (Takeda Chemical Industry Ltd, CTM).

Preparation of polymorphonuclear leukocytes

PMN were obtained from heparinized venous human blood of healthy volunteers by means of centrifugation at $300 \times g$ for 30 min in a density gradient using Mono-Poly Resolving Medium (Flow Laboratories, Australia). The residual red cells were lysed with 0.83% ammonium chloride. The PMN were then washed twice in Hanks balanced salt solution (HBSS) and suspended to the desired concentration in HBSS. Cells suspensions prepared contained greater than 95% PMN and the viability of PMN was over 98% by trypan blue exclusion.

Polarization assay for polymorphonuclear leukocytes

PMN suspensions (0.45 ml) adjusted at 2.0×10^6 cells/ml and 0.45 ml of the antibiotics at desired concentrations were mixed and incubated in a water bath (37°C) for 15 min. After these mixtures were exposed to 0.1 ml of 10^{-7} M *N*-formyl-*L*-methionyl-*L*-leucyl-*L*-phenylalanine (FMLP, Sigma Chemical, St Louis, MO), they were incubated in a water bath (37°C) for 10 min, the reaction stopped with 2 ml of 10% formalin in HBSS (pH 7.4) and the mixture kept in ice water for 30 min. The number of polarized PMN were counted in a hemocytometer.

Assay for migration of polymorphonuclear leukocytes

Assays were performed by use of a multiwell chemotaxis assembly (Neuro Probe, Cabin John, Md, USA).[5,6] Each of the wells on the bottom plate was filled with 25 μl of test specimens dissolved in HBSS or 25 μl of HBSS for control. A polycarbonate filter sheet (Neuro Probe) of 3 μm pore size and about 10 μm thickness was then placed on the bottom plate. A gasket and a top plate were fixed in place. 50 μl of PMN suspension (10^5 cells) was then added to each well on the top plate. The whole assembly was incubated at 37°C for 60 min in humidified air containing 5% (Vol/Vol) CO_2. After incubation, the filter

was removed, fixed and stained with Diff-Quick (International Reagents Co, Kobe, Japan). Migration of PMN from the upper well toward the bottom one containing the test specimens or HBSS for control was estimated by counting the number of PMN which had completely passed through the filter; 20 microscopic fields on the filter surface adjacent to the bottom wells were randomly selected for counting at $\times 1000$ magnification (by combination of a $\times 100$ oil immersion objective lens and a $\times 10$ ocular lens equipped with a microgrid (5 by 5 mm; Olympus Optical Co, Tokyo, Japan).

RESULTS

Effect of antibiotics on PMN polarization

Figure 1 shows the effect of several antibiotics on PMN motility examined with the polarization assay.

Polarized cells induced by FMLP decreased in number in the presence of MINO. This reduction occurred in a dose-dependent manner 7.8% (1 μg/ml), 12.4% (10 μg/ml) or 37.2% (100 μg/ml) as compared to controls.

ABPC had no effect on PMN polarization stimulated by FMLP in concentrations of 1, 10 μg/ml, but had a significant suppression effect at 100 μg/ml.

CTM increased polarized cells significantly, even at a concentration of 1 μg/ml, and accelerated the PMN migration significantly in a dose dependent fashion; the polarization rose 6.8% (1 μg/ml), 14.3% (10 μg/ml) or 22.9% (100 μg/ml) of control levels in the clinical dose range.

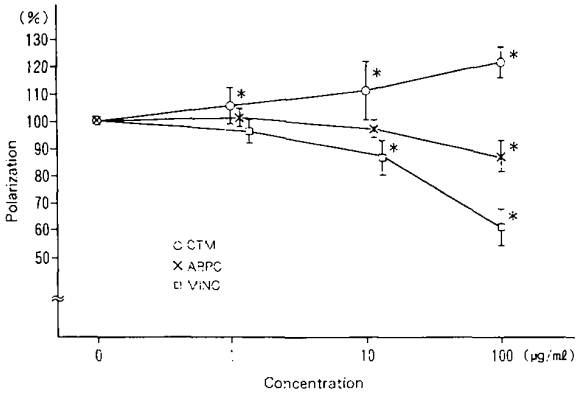

Fig. 1 – Effect of antibiotics on PMN polarization. Each value represents the mean ± 1 standard deviation of six analyses, where every analysis was made in triplicate. The difference between antibiotics and control was significant at a level of 5% (*) by Wilcoxon test.

Effect of antibiotics on PMN migration

Figure 2 shows the result of the PMN migration in response to antibiotics.

MINO significantly inhibited PMN migration at a therapeutically attainable concentration (1 μg/ml). At higher concentrations (10, 100 μg/ml), significant inhibition of PMN migration was effected in a dose-dependent manner (Fig. 2A).

ABPC did not significantly affect PMN migration within the clinical dose range of 1 to 100 μg/ml (Fig. 2B).

CTM slightly increased PMN migration at a concentration of 1 μg/ml, but not significantly. Concentrations of 10 and 100 μg/ml of CTM, within the clinical dose range, caused significant enhancement of PMN migration (Fig. 2C).

Checkerboard assay with CTM

To determine whether the increase of PMN migration caused by CTM was merely because of increased random migration of PMN(chemokinesis), or directed migration in response to a concentration gradient of CTM(chemotaxis), various concentration of CTM were added to either the upper well, the lower well, or both well in a checkerboard pattern (Table 1), and migration in these wells were compared. When there was no difference in CTM concentration between the upper and lower compartments,

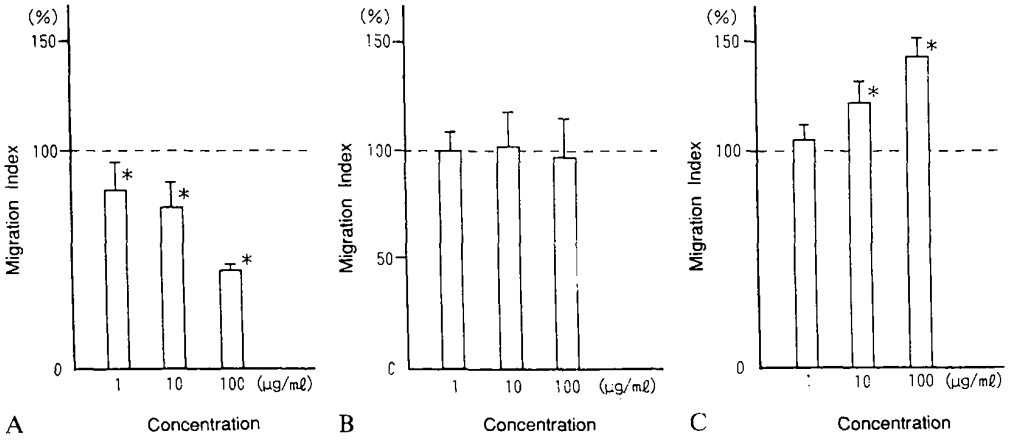

Fig. 2 – Effect of antibiotics on PMN migration. A) MINO B) ABPC C) CTM. Each value represents the mean ± 1 standard deviation of six analyses, where every analysis was made in triplicate. The difference between antibiotics and control was significant at a level of 5% (*) by Wilcoxon test.

Table 1 – Checkerboard assay of the effect of CTM on PMN migration. The number of PMN on the lower surface of a polycarbonate filter was counted in triplicate 20 microscopic fields to determine ± 1 standard deviation

Conc. (μg/ml) of cefotiam in lower well	Number of cells (mean ± SD) with increasing conc. (μg/ml) of cefotiam in upper well			
	0	1	10	100
0	48.9 ± 7.9	57.7 ± 4.3	71.7 ± 5.6	82.3 ± 4.4
1	55.9 ± 3.3	67.3 ± 8.0	68.9 ± 5.0	79.1 ± 3.7
10	59.1 ± 3.8	67.6 ± 4.8	74.6 ± 1.3	82.0 ± 4.9
100	63.3 ± 2.2	71.6 ± 4.7	82.8 ± 8.2	95.0 ± 4.8

PMN migration was enhanced in dose-dependent fashion. The enhanced migration also occurred when the concentration of CTM in the upper compartment exceeded that in the lower compartment. The assay result strongly suggested that the increased PMN migration caused by CTM was due to chemokinesis.

DISCUSSION

The Boyden method measures the number of migrating PMN and their migration distances. In contrast, the polarization assay records morphological changes related to function, and therefore, the results may represent chemokinesis of PMN or its initial migrating reaction.[7]

The Boyden method calculates the number of PMN which, in accordance with the concentration gradient of migration factors, pass through a millipore filter and migrate to the lower surface of filter. Various methods have been used to investigate the effects of antibiotics on PMN migration.[8–10] In our study we placed an antibiotic in the lower chamber and a PMN suspension in the upper in order to eliminate any effects by factors other than the antibiotic and also to investigate if the antibiotic itself acts as a chemotactic factor.

In 1974, Martin et al[11] reported that, by the Boyden method, tetracycline(TC) at 0.1–10 mg/ml inhibited PMN migration by 40–50%. Since then, various investigators have reported the effects of various antibiotics on PMN function. With respect to protein synthesis inhibitors, if selective toxicity of an inhibitor is quantitative, it may reduce the migrating function of leukocytes or inhibit protective function when administered in large doses. The reports on tetracycline-based antibiotics and protein synthesis inhibitors showed similar inhibition of the PMN migrating function *in vitro*. However, since most studies used antibiotics at levels much higher than those commonly found in serum during their clinical use, the results obtained could not be applied to the *in vivo* situation. In our study, both the Boyden method and the polarization assay revealed that PMN migration was inhibited by MINO at concentrations as low as 1 μg/ml, which supports the possibility of inhibition *in vivo*.

It has been reported that β-lactam antibiotics exert no effect on migration,[12] although penicillin-based antibiotics belonging to the β-lactam group are reported to inhibit migration at high dose.[9] In this study, ABPC had no apparent effect on migration at 1–100 μg/ml (close to blood levels common in clinical use).

Cephem antibiotics belonging to the β-lactam group are reported at high concentration (100–1000 μg/ml) to inhibit migration.[13] The present results reveal a dose-dependent enhancement of migration by CTM from doses as low as 1 μg/ml to 100 μg/ml, which are serum levels common in clinical use. Thus, in contrast to the report by Milatovic (1983),[10] these data reveale that some cephem antibiotics may stimulate PMN migration.

The Boyden method, like the other migration assays, does not indicate whether chemotaxis or chemokinesis is involved in the enhanced migration. The checkerboard method, which observes the effects of a concentration gradient on PMN migration, can distinguish one from the other. CTM enhanced the PMN migration even in a negative concentration gradient, indicating that CTM acted as a chemokinetic factor.

Antibiotics can influence the migration of PMN. And since the collection of PMN in infectious lesions during an acute phase response is due to the migrating function of the cells, those agents having a negative influence on migration may inhibit the body's protective mechanisms by impairing PMN functions. To administer these agents to patients with PMN dysfunction may further compromise their ability to cope with injection. We have presented evidence that agents which accelerate the PMN function exist, such as CTM, and this activity should be considered as an additional beneficial effect to the antibacterial activity when choosing antibiotics for treatment of infections.

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The Authors

Y. Ueyama DDS, PhD
Assistant Professor

M. Misaki DDS
Instructor
Y. Ishihara DDS, PhD
Associate Professor
T. Matsumura DDS, PhD
Professor
Department of Oral and Maxillofacial Surgery II
Okayama University Dental School
2-5-1 Shikata-cho
Okayama 700
Japan

Correspondence and requests for offprints to Y. Ueyama

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