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Comparative study of the effect of Teicoplanin and Vancomycin upon the phagocytic process of peritoneal macrophages

C. Barriga, I. Pedrera and A. B. Rodríguez

Departamento de Fisiología, Facultad de Ciencias,
Universidad de Extremadura, 06071 Badajoz (Spain)

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The effect of Teicoplanin and Vancomycin upon the phagocytic process was compared by evaluating the different activities of the peritoneal macrophage phagocytic function from mice treated with these antibiotics. The results indicated that teicoplanin and vancomycin increased both the substrate adherence and chemotaxis of peritoneal macrophages and that neither antibiotic, at the concentrations of 10, 25, 50, 75 and 100 mg/l, had any chemoattractant capacity for peritoneal macrophages. There was an increase in the attachment of *Candida albicans* only in the macrophages from mice treated with vancomycin. The phagocytosis of *C. albicans*, *Staphylococcus aureus*, *Escherichia coli* and inert particles as well as the nitroblue tetrazolium reduction capacity (microbicide capacity) increased in the presence of both antibiotics. The *C. albicans* digestion capacity increased only in the peritoneal macrophages from mice treated with teicoplanin.

Key words: Teicoplanin, Vancomycin, Macrophages, Phagocytic process.

Phagocyte cells, such as macrophages, possess a variety of processes that are involved in killing bacterial and fungal pathogens during infections. These processes are mediated by a range of destructive granule enzymes and a series of reactive oxidants that are generated during the respiratory burst via activation of NADPH oxidase complexes (10). Indeed, functional defects of this system are generally associated with severe recurrent infections. Antimicrobial chemotherapy aims equally at eradicating pathogens from the organism. Therefore, a knowledge of the interaction between bacteria,

Correspondence to C. Barriga (Tel: 24-289388, Fax 24-271304).

antibiotics and host defence mechanisms is highly important for a comprehension of the infection progress and its treatment (12).

Teicoplanin is a glycopeptide antibiotic with a model of action and spectrum of activity similar to that of vancomycin (19, 23). It is two to eight times more active than vancomycin against staphylococci and streptococci, especially *Streptococcus faecalis* and other enterococci (3, 7). It offers the potential advantages of a longer half-life, less-frequent dosing, and lower nephrotoxicity than vancomycin (19, 23).

An effective antibiotic should have a stimulating effect on phagocytosis. For this reason, the possibility that teicoplanin and vancomycin might modify certain of the stages of the phagocytic process in macrophages, as well as recognition of preinflammatory stimuli, cell activation by attachment and ingestion of pathogenic agents, and delivery of cytotoxic molecules to the phagocytosed pathogen has been analyzed.

Materials and Methods

Animals.– Laboratory animals used in this study were male Swiss mice (*Mus musculus*), aged 12 ± 4 weeks, maintained at a constant temperature (22 ± 2 °C) on a 12-hour light/dark cycle and given Sander Mus food and water *ad libitum*.

Antibiotics.– Teicoplanin purified to 100 % (Marion Merrell Dow S.A., Madrid) and vancomycin of 96.8 % purity (Lab. Lilly S.A., Barcelona) were dissolved freshly every day in phosphate buffered saline solution (PBS). Dosages seven times greater than that given to humans were used for the mice (1, 2, 14, 18). Mice receiving teicoplanin were injected with a single intramuscular dose of 42 mg/kg the first day and 21 mg/kg the following six days. The vancomycin group were injected with two doses per day of 100 mg/kg for seven days. There were controls for both groups consisting of animals that were injected with saline solution every 12 hours (vancomycin control group) or every 24 hours (teicoplanin control group). In addition, there was a basal control group of mice housed in identical conditions but which were not subjected to any type of treatment. One day after the end of the treatment, the animals were sacrificed by cervical dislocation and the macrophages isolated from the peritoneum. For the studies of the chemoattractant activity of teicoplanin and vancomycin on the peritoneal macrophages, concentrations of 10, 25, 50, 75 and 100 mg/l were utilized (18).

Bacteria.– *Escherichia coli* (ATCC 35218) and *Staphylococcus aureus* (ATCC 9144) from a stored collection were used. When required, overnight broth cultures were washed twice in saline solution and adjusted spetrophotometrically at 675 nm to a content of 1×10^6 Colony Forming Units (CFU)/ml.

Collection of peritoneal macrophages.– Peritoneal macrophages were obtained from each animal immediately after sacrifice. The abdomen was cleansed with 70 % ethanol, the abdominal skin carefully dissected without opening the peritoneum, and 4 ml of Hank´s solution (Sigma) adjusted to pH 7.4 injected intraperitoneally. The abdomen was massaged and the macrophages removed, allowing recovery of 90-95% of the injected volume. The cells (macrophages and lymphocytes) were counted and adjusted to a final concentration of 1×10^6 macrophages ml^{-1} in Hank´s medium. Cell viability was 98 ± 1 % as measured by the trypan-blue exclusion method (8). All samples were processed in duplicate.

Serum used for the attachment, phagocytosis and microbicide capacity tests was obtained from the basal mice.

Samples were incubated at 37 °C in a humidified atmosphere of 5 % CO_2. All samples were processed in duplicate.

Adherence.– The substrate adherence capacity was carried out using a method described by DE LA FUENTE *et al.* (8). Adherence index (AI) was calculated according to the equation:

$$AI = 100 - \frac{\text{(macrophages/ml supernatant)}}{\text{(macrophages/ml original sample)}}$$

Chemotaxis capacity.– Cell migration was evaluated according to a modification by RODRÍGUEZ *et al.* (21) of the original technique described by BOYDEN (4). N-formyl-l-methionyl-l-leucyl-phenylalanine (FMLP peptide) (5 ng/ml; Sigma) was put into the lower compartment to induce chemotaxis. The Chemotaxis index was calculated by counting at random the total number of macrophages in 16 fields on the lower face of the filters. Results were compared with the values obtained with Hank's medium (no chemoattraction, control values).

The chemoattractant activity of teicoplanin and vancomycin on peritoneal macrophages was also evaluated. 300 µl aliquots of macrophage suspension were each deposited in the upper compartment of the chamber and teicoplanin and vancomycin (at 10, 25, 50, 75 and 100 mg/l) were put into the lower compartment to induce chemotaxis.

Attachment, ingestion and digestion capacity for Candida albicans.– The attachment of *Candida albicans* was carried out following a method previously described by ORTEGA *et al.* (16). The attachment index (AI) represent the number of *C. albicans* attached per 100 macrophages counted.

The same procedure was used to determine the ingestion of *C. albicans*, but counting the number of *C. albicans* ingested per 100 macrophages (phagocytosis index, PI) after 15 and 60 min of incubation. To determine the capacity of digestion of *C. albicans*, 1.5 ml of methylene blue at a concentration of 0.01 % was added at 50 min of incubation, and after 10 min at 37 °C, the number of *C. albicans* which were not only phagocytosed but also destroyed per 100 macrophages was calculated (digestion index, DI). Results were also expressed as percentage of phagocytosis (percentage of macrophages with phagocytic capacity) and phagocytic efficiency (phagocytosis index/percentage of phagocytosis number of candidae phagocytosed by each active macrophage).

Ingestion of latex beads.– After formation of the adherent monolayer (16), 20 µl of latex beads (Sigma, 1.09 µm, diluted 1 % in PBS) and 200 µl of PBS were added. After 30 min of incubation, the plates were washed with PBS at 37 °C, fixed and stained. In order to quantify the phagocytosis, the number of particles ingested by 100 phagocytic cells was counted, giving the latex bead phagocytosis index. The results were also expressed as percentage of phagocytosis and phagocytic efficiency.

Phagocytosis of Staphylococcus aureus *and* Escherichia coli.– To evaluate the phagocytosis, a modification of the method described by BURLATELA *et al.* (5) was used as follows (17): Aliquots of 500 µl of macrophages were incubated for 15 minutes at 37 °C with 100 µl of serum and 500 µl of bacteria (*E. coli* or *S. aureus*) in a bath with shaking. Then the mixtures were washed three times with 5 ml of ice-cold PBS, using differential centrifugation to remove extracellular bacteria, and the

phagocytes were lysed in distilled water (160 x g, 5 min). Finally, 20 µl aliquots of the samples were seeded onto Mueller-Hinton agar plates and the CFU counted after one day of incubation at 27 °C.

Microbicide capacity.– The oxygen-dependent microbicide capacity of the macrophages was evaluated by means of superoxide anion (O_2^-) production according to DE LA FUENTE (8) with modifications. Aliquots of 250 µl of macrophages were mixed with 250 µl of NBT (Sigma, 1 mg/ml in PBS solution). Then, 25 µl of latex bead suspension were added to obtain the stimulated samples, as well as 25 µl of PBS to the unstimulated samples (samples without latex beads). After 30 minutes of incubation, the reaction was stopped, the samples centrifuged, the supernatants discarded, and the reduced NBT extracted with dioxane (Sigma). The absorbances of the supernatants at 525 nm were determined using dioxane as blank. The percentage stimulation was calculated by giving the value 100 to the absorbance of the unstimulated samples.

Statistical analysis.– All data are expressed as mean ± standard deviation of the number of experiments stated in the corresponding figures. In the statistical study, the results were analyzed using non-parametric tests: for the comparison of two groups of treatments, the Wilcoxon test (paired samples); for the multiple comparisons, the Anova two-way test by addition of Friedman ranges (paired samples) and the one-way Anova by addition of Kruskal-Wallis ranges (unpaired samples).

Results

The results for the substrate adherence of peritoneal macrophages from mice treated with teicoplanin and vancomycin are given in table I. In general, both antibiotics increase this property throughout the times of incubation, except for vancomycin at 60 min of incubation. Fig. 1 shows that teicoplanin and vancomycin augment macrophage mobility towards the chemoattractant (FMLP). The chemoattractant activities of teicoplanin and vancomycin (at doses of 10, 25, 50, 75 and 100 mg/l) on peritoneal macrophages were also evaluated, and the values obtained show no differences between the chemotaxis index from the controls (only PBS) and the different doses of either teicoplanin or vancomycin. The FMLP

Table I. *Substrate adherence index of peritoneal macrophages from Swiss mice treated with teicoplanin and vancomycin.*
Each value is the mean and SD of 8 experiments performed in duplicate.

Treatment	Time of incubation (minutes)			
	15	30	45	60
Basal	24 ± 14	42 ± 10	58 ± 12	74 ± 16
Teicoplanin control	17 ± 5	51 ± 15	62 ± 8	69 ± 8
Teicoplanin	55 ± 13[ab]	64 ± 7[ab]	77 ± 17[ab]	83 ± 7[b]
Vancomycin control	18 ± 4	52 ± 14	60 ± 9	70 ± 6
Vancomycin	44 ± 12[ab]	69 ± 13[ab]	77 ± 14[ab]	77 ± 19

(a) $p < 0.05$ with respect to basal values at the same time of incubation.
(b) $p < 0.05$ with respect to control values at the same time of incubation.

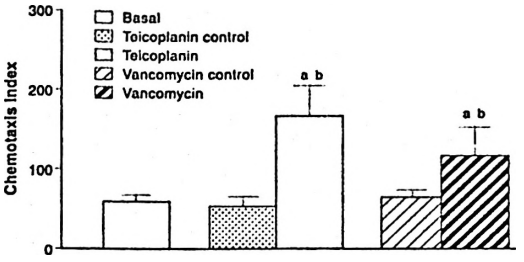


Fig. 1. *Chemotaxis index of peritoneal macrophages from mice treated with teicoplanin and vancomycin.* Basal values, without treatment. Teicoplanin control and vancomycin control, mice injected with saline solution every 24 h and 12 h, respectively. Each value is the mean ± SD of 8 experiments performed in duplicate. $p < 0.05$, (a) versus basal values, and (b) versus control values.

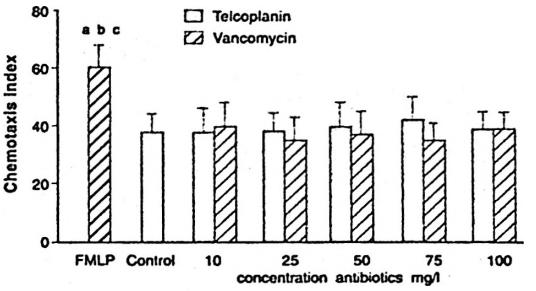


Fig. 2. *Chemoattractant capacity (Chemotaxis index) of teicoplanin and vancomycin for peritoneal macrophages.*
Each value is the mean ± SD of 8 experiments performed in duplicate. $p < 0.05$, (a) with respect to control (PBS); (b) and (c) versus all teicoplanin and vancomycin concentrations, respectively.

Table II. *Phagocytic activity of peritoneal macrophages from Swiss mice treated with teicoplanin and vancomycin.*
Each value is the mean and SD of 8 experiments performed in duplicate.

		Teicoplanin		Vancomycin	
	Basal	Control	Treated	Control	Treated
Candida albicans					
Attachment index	66 ± 20	60 ± 19	95 ± 25	63 ± 4	125 ± 20[a b]
Phagocytosis index	49 ± 11	41 ± 5	115 ± 19[a b]	45 ± 7	97 ± 9[a b]
% Phagocytosis	27 ± 8	27 ± 6	54 ± 19[a b]	25 ± 6	58 ± 9[a b]
Phagocytic efficiency	1.76 ± 0.25	1.38 ± 0.36	2.25 ± 0.19[a]	1.30 ± 0.32	1.47 ± 0.59
Digestion Index	22 ± 15	20 ± 14	49 ± 13 [a b]	21 ± 12	30 ± 25
Inert particles					
Phagocytosis index	220 ± 80	220 ± 70	430 ± 50[a b]	200 ± 50	330 ± 70[a b]
% Phagocytosis	57 ± 7	54 ± 10	80 ± 12[a b]	52 ± 7	81 ± 11[a b]
Phagocytic efficiency	3.3 ± 1	3.5 ± 0.7	7.5 ± 2.5[a b]	3.2 ± 0.4	4 ± 0.5

(a) $p < 0.05$ with respect to basal values.
(b) $p < 0.05$ with respect to its control values.

values were significantly higher than the controls, with both vancomycin and teicoplanin (fig. 2).

Table II lists the results on the attachment, phagocytosis and digestion of *Candida albicans* by the peritoneal macrophages after treatment with teicoplanin and vancomycin. The macrophages from mice treated with vancomycin present an increased attachment of *C. albicans*. The indices of phagocytosis of *C. albicans* by macrophages from mice treated with both teicoplanin and vancomycin were greater than the values of the controls and the

basal group. The digestion index was significantly augmented in the macrophages from mice treated with teicoplanin.

With respect to the phagocytosis of bacteria (fig. 3), the results indicated that both antibiotics increased the ability of macrophages from treated animals to phagocitose *S. aureus* and *E. coli*, although the increase was greater for *S. aureus* phagocytosis in both cases. Similarly, the two antibiotics augmented the inert particle (latex bead) phagocytosis (table II). Also the table II lists the results for the percentage of phagocytosis and phagocytic efficiency for *C. albicans* and inert particles. Whereas teicoplanin increased both the percentage of phagocytosis and the phagocytic efficiency, vancomycin only increased the percentage of phagocytosis.

Figure 4 shows the results corresponding to the microbicide capacity of macrophages (superoxide anion production). The macrophages from animals treated with antibiotics present higher values than those in the control and basal groups. There are no differences between the two antibiotics.

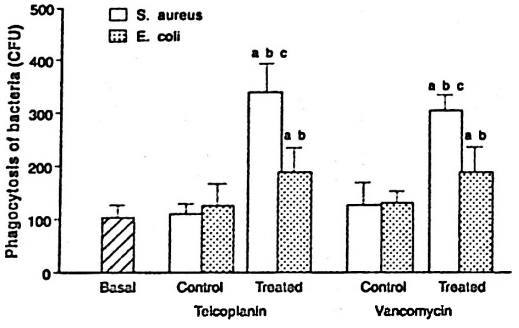
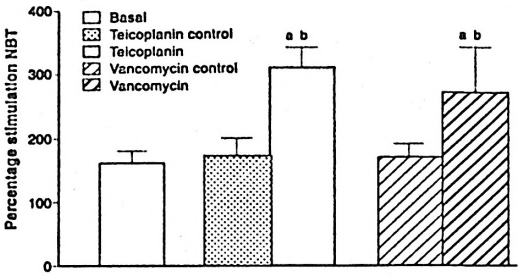


Fig. 3. *Phagocytosis of Staphylococcus aureus and Escherichia coli by peritoneal macrophages from mice treated with teicoplanin and vancomycin.*
Legend as in figure 1. $p < 0.05$: (a) versus basal values, (b) versus respective control ones, and (c) versus values of *E. coli.*



Fig, 4. *Percentage stimulation NBT by peritoneal macrophages from mice treated with teicoplanin and vancomycin.*
Legend as in fig. 1. $p < 0.05$: (a) versus basal values and (b) versus respective control ones.

Discussion

Many antibiotics have a demonstrated ability to influence the host defences in both the specific and non-specific immune response (11, 12, 15, 24). This immunomodulation by antibiotics is an interesting aspect of therapy in patients with a compromised immune system (11), who are far more susceptible to bacterial (*S. aureus* and *E. coli*) and fungal (*C. albicans*) infections, generally presenting neutropenia. The use in immunodepressed patients of an antibiotic which, apart from its specific action, could enhance phagocytosis, would be interesting.

In the present study, the effects of teicoplanin and vancomycin administration on different activities of the phagocytic function has been investigated, by using the commonest pathogens in infections of immunodepressed patients as material to be ingested, and as phagocyte the macrophages from mice which had been treated with these antibiotics for a week.

Adherence of phagocytes to the endothelium is the first event in the inflammatory response. This capacity of macrophages is usually evaluated by

means of the adherence of phagocytes to a plastic surface, which has been described as comparable to tissue (13). Our results indicated that teicoplanin and vancomycin increased the adherence capacity of murine peritoneal macrophages. Adherence precedes the migration (i. e. chemotaxis) of phagocytes towards the focus of infection, where the remaining stages of phagocytosis are completed (9). Thus, chemotaxis is a good index of the macrophages ability to carry out their non-specific function (9). Teicoplanin and vancomycin induced an increase in this property of macrophages. To determine whether teicoplanin or vancomycin might have a chemoattractant power, the study was performed by depositing the respective antibiotic in the lower compartment at the concentrations of 10, 25, 50, 75 and 100 mg/l, doses within the range of therapeutic plasma levels (18). Our results indicated that neither antibiotic is a chemoattractant for macrophages, since the values obtained were similar to those observed with Hank´s medium (a non-chemoattractant) and less than those obtained with FPML.

Before ingesting antigens, phagocytes attach themselves to them by specific receptors (22). To evaluate the effect of the antibiotics on attachment and subsequent ingestion, a microorganism (*C. albicans*) that is unaffected by the action of these drugs and whose size would allow us to differentiate by optical microscopy between attachment, ingestion, and destruction of the yeast was chosen. Macrophages from vancomycin treated mice had an augmented *C. albicans* attachment. The phagocytosis of opsonized antigens is another important stage in the phagocytic process. In the present study both antibiotics were found to stimulate peritoneal macrophages to ingest opsonized *C. albicans* and inert particles, which in the case of teicoplanin was due both to an increase in the number of macrophages with phagocytic capacity (percentage of phagocytosis) and to their phagocytic efficiency. The macrophages from mice treated with teicoplanin presented an augmented digestion of ingested material, which could be due to teicoplanin penetrating into the phagocytes (20). Both antibiotics stimulated the ingestion of *S. aureus* and *E. coli*, but more effectively in the case of from teicoplanin treated mice against *S. aureus*, similar to the finding of CARLONE *et al.* (6). To analyze whether the increased destruction capacity of the macrophages was due to activation of the oxidative metabolism, the production of superoxide anion was evaluated, where both teicoplanin and vancomycin were found to enhance this process.

In conclusion, our data showed that teicoplanin and vancomycin generally act on peritoneal macrophages from mice to stimulate the phagocytic process.

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C. BARRIGA, I. PEDRERA y A. B. RODRÍGUEZ. *Estudio comparativo de teicoplanina y vancomicina sobre el proceso fagocítico de macrófagos peritoneales.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (4), 215-222, 1996.

Se evalúa, de forma comparada, el efecto de la teicoplanina y de la vancomicina sobre el proceso fagocítico mediante la valoración de las diferentes actividades de la función fagocítica de macrófagos peritoneales procedente de ratones tratados con ambos antibióticos. Los resultados indican que ambos antibióticos incrementan la adherencia a substrato y la quimiotaxis y que a concentraciones de 10, 25, 50, 75 y 100 mg/l, carecen de capacidad quimioatrayente para los macrófagos peritoneales. Se

aprecia un incremento en la unión de *Candida albicans* por los macrófagos procedentes de ratones tratados con vancomicina. La fagocitosis de *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* y partículas inertes, asi como la reducción del nitroazul de tetrazolium (capacidad microbicida) aumentan en presencia de ambos antibióticos. La capacidad de digerir (destruir) *C. albicans* se incrementa en los macrófagos procedentes de ratones tratados con teicoplanina.

Palabras clave: Teicoplanina, Vancomicina, Macrófages, Proceso fagocítico.

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