REVISTA ESPAÑOLA DE FISIOLOGIA, 49 (4), 231-234, 1993

Stimulation of Phagocytosis against *Staphylococcus aureus* by Teicoplanin and Vancomycin

M. I Pedrera*, F. Pérez, A. B. Rodríguez and C. Barriga

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

(Received on May 28, 1993)

M. I. PEDRERA, F. PÉREZ, A. B. RODRÍGUEZ and C. BARRIGA. *Stimulation of Phagocytosis against* Staphylococcus aureus *by Teicoplanin and Vancomycin.* Rev. esp. Fisiol., 49 (4), 231-234, 1993.

The constructive interference of teicoplanin with phagocytosis of *Staphylococcus aureus* by murine macrophages and human neutrophils, studied *in vivo* and *in vitro* respectively, was compared with that of vancomycin. Both teicoplanin and vancomycin increased the phagocytosis by macrophages. All the concentrations of teicoplanin increased the phagocytosis by neutrophils, but only the highest concentrations of vancomycin (50 mg/l and 100 mg/l) had this effect, greater values being found with teicoplanin in all cases.

Key words: Teicoplanin, Vancomycin, Phagocytosis.

Phagocytes play an important role in the host defence against microbial infections (8). In fact functional defects of this system are generally associated with severe recurrent infections. Antimicrobial chemotherapy is directed at the same target: to eradicate the pathogens from the organism. Cooperation with the natural host defence system is of paramount importance in achieving a cure of the infection. Therefore, it is of interest to investigate the possible interference of chemotherapeutic agents in the activity of phagocytic cells. They can stimulate cellular functions or co-operate synergistically with them, or, on the contrary, depress or antagonize them, with a consequent inhibition of their activity (5).

Teicoplanin is a glycopeptide antibiotic derived from *Actinoplanes teicomyceticus* and is chemically related to vancomycin. It has potent *in vitro* activity against Gram-positive organisms and has slightly greater activity against *Staphylococcus aureus* than

* To whom all correspondence should be addressed (Phone: 34-24-274800/313-312; Fax: 34-24-271304).

vancomycin. Teicoplanin has a longer half-life than vancomycin, can be administered both intravenously and intramuscularly, and may be less toxic than vancomycin (6).

Studies were undertaken to detect how the possible interference of teicoplanin *in vivo* and *in vitro* with the phagocytosis of *S. aureus* by murine macrophages and human neutrophils compares with vancomycin.

Materials and Methods

Isolation of phagocytic cells. — Macrophages were obtained from mices sacrificed by cervical dislocation. The abdomen was cleansed with 70 % ethanol and the abdominal skin, carefully resected without opening the peritoneum, and 4 ml of PBS (phosphate buffered saline solution) were injected intraperitoneally. The abdomen was massaged and the peritoneal exudate cells (macrophages and lymphocytes) removed, with 90-95 % recovery of the injected volume. The macrophages were counted and then adjusted by dilution with PBS to 1×10^6 macrophages ml^{-1} of PBS. The cell viability was 98 ± 1 % with trypan-blue exclusion.

Neutrophils were obtained from heparinized venous blood of healthy volunteers by means of centrifugation at 300 × *g* for 30 min in a density gradient using Mono-Poly-Resolving-Medium (Flow, Virginia, U.S.A.). Neutrophils were then washed in PBS and adjusted to 1×10^6 cells ml^{-1} of PBS. The cell viability was 95 ± 5 % with trypan-blue exclusion.

Bacteria and serum. — *Staphylococcus aureus* (ATCC 9144) was obtained from hospitals through the Microbiology Department, Faculty of Medicine, Badajoz (Spain). When required, overnight broth cultures were washed twice in saline solution and adjusted spectrophotometrically to contain 1×10^6 Colony Forming Units (CFU)/ml.

The serum was obtained and pooled from venous blood from ten healthy volunteers.

Treatment with antibiotics. — Teicoplanin (Merrell–Dow) and vancomycin (Lilly) were dissolved freshly every day in PBS.

In vivo studies: Male Swiss mice of 15 ± 2 weeks of age were used. Dosages seven times greater than human dosages were used for the mice, for both antibiotics (1). The teicoplanin group 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). One day after the end of the treatment, the animals were sacrificed to obtain the macrophages. In addition, there was a basal group of mice in identical conditions that were not subjected to any type of treatment.

In vitro studies: The human neutrophils were incubated with 10, 25, 50, 75 and 100 mg/l of teicoplanin or vancomycin for 15 min at 37 °C, accompanied by antibiotic-free control samples.

Phagocytosis of S. aureus. — To evaluate the phagocytosis of *S. aureus* a modification of the method described by BURGALETA *et al.* (2), was used as follows.

In vivo studies: Aliquots of 0.5 ml of macrophages from the different groups described above for the *in vivo* treatment were incubated for 15 minutes at 37 °C with 100 µl of serum and 0.5 ml of *S. aureus* in a bath with shanking. Subsequent to incubation, 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 × 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 37 °C. All samples were processed in duplicate.

In vitro studies: The neutrophils were treated with teicoplanin and vancomycin and, to evaluate the phagocytosis, 0.5 ml of *S. aureus* grown in Mueller-Hinton-Agar in the presence of 100 µl of serum was added to each sample. Then the schedule described above for the *in vivo* study was followed.

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) was used; for 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) were used.

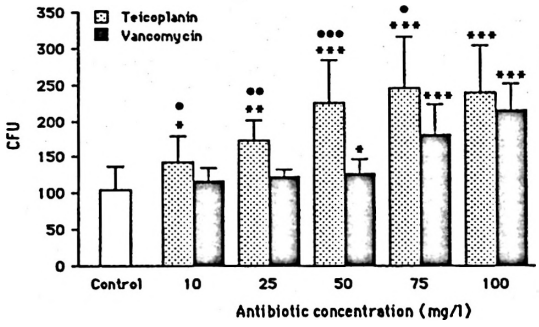

Fig. 1. In vivo *effect of teicoplanin and vancomycin on phagocytosis of* Staphylococcus aureus *by murine peritoneal macrophages.*
Each column represents the mean ± S. D. of 10 determinations performed in duplicate.
*** ($p<0.001$) with respect to the control. *** ($p<0.001$) with respect to values obtained in the basal group.

Results and Discussion

Teicoplanin has excellent antistaphylococcal activity (10) and is a possible alternative candidate to vancomycin. Since FIETTA *et al.* (5) reported that teicoplanin, but not vancomycin, was able to enhance killing of staphylococci ingested by neutrophils, this paper examined the effect of teicoplanin and vancomycin in the phagocytosis of *Staphylococcus aureus* by macrophages and neutrophils.

The phagocytosis of *S. aureus* by macrophages isolated from mice treated with teicoplanin and vancomycin for seven days, studied *in vivo*, was significantly greater ($p<0.001$) than that observed in the control and basal animals (fig. 1).

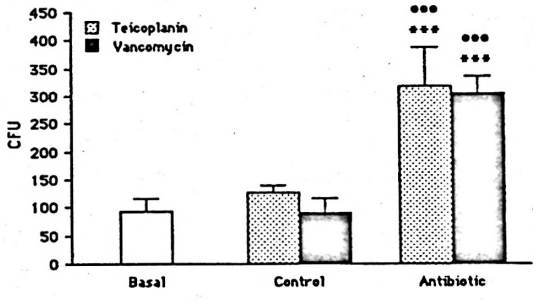

Fig. 2. In vitro *effect of teicoplanin and vancomycin on phagocytosis of* Staphylococcus aureus *by human neutrophils.*
Each column represents the mean ± S. D. of 10 determinations performed in duplicate.
*** ($p<0.001$) with respect to the control. * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) with respect to the same concentrations of vancomycin.

The results for the phagocytosis of *S. aureus* by neutrophils incubated for 15 minutes with 10, 25, 50, 75 and 100 mg/l of teicoplanin and vancomycin, studied *in vitro*, are shown in figure 2. For all the concentrations of teicoplanin and for 75 and 100 mg/l of vancomycin, the results were significantly superior to the controls. When the results for the two antibiotics are compared at each of the concentrations,

the teicoplanin values were always superior to those obtained with vancomycin. Similár findings have been reported by MORÁN *et al.* (7) using *Candida albicans* as the ingestion material, observing that indices of phagocytosis were more favourable when teicoplanin was used.

This is probably due to higher intracellular accumulation of teicoplanin (9) on account of its molecular weight being 20 % greater than vancomycin's (7), its having a longer half-life (4), or because teicoplanin transfers rapidly across the phagocyte membranes by simple diffusion as has been previously reported in macrophage membranes by CARLONE *et al.* (3). Therefore, teicoplanin is a good alternative to vancomycin when there appears to be a decline in the bacterial phagocytosis capacity.

Acknowledgements

This study was supported by a grant from Marion Merrell Dow (Madrid, Spain).

Resumen

Se estudia la interferencia de la teicoplanina en la fagocitosis de *Staphylococcus aureus* por macrófagos murinos *in vivo* por neutrófilos humanos *in vitro*, en comparación con la vancomicina. Tanto la teicoplanina como la vancomicina incrementaban la fagocitosis de *S. aureus* por macrófagos. A todas las concentraciones de teicoplanina se incrementa la fagocitosis por neutrófilos, teniendo la vancomicina este efecto sólo a las mayores concentraciones (50 and 100 mg/l) y siendo en todos los casos mayor la estimulación de la fagocitosis en presencia de teicoplanina.

Palabras clave: Teicoplanina, Vancomicina, Fagocitosis.

References

1. Barriga, C., Muriel, E., Benitez, P. and De la Fuente, M.: *Comp. Immunol. Microbiol. Infect. Dis.*, 14, 297-302, 1991.
2. Burgaleta, C., Moreno, T. and Loza, E.: *J. Antimicrob. Chemother.*, 23, 460-462, 1989.
3. Carlone, N. A., Cuffini, A. M., Ferrero, M., Tullio, V. and Avetta, G.: *J. Antimicrob. Chemother.*, 23, 849-859, 1989.
4. Carper, H. T., Sullivan, G. W. and Mandell, G. L.: *J. Antimicrob. Chemother.*, 19, 659-662, 1987.
5. Fietta, A., Bersani, C., De Rose, V., Grassi, F. M., and Gialdroni Grassi, G.: *J. Hosp. Infect.*, 7, 57-63, 1986.
6. Glupczynski, Y., Lagast, H. and Van der Auwera, P.: *Antimicrob. Agents Chemother.*, 29, 52-57, 1986.
7. Morán, F. J., Puente, L. F., Pérez-Giraldo, C., Blanco, M. T., Hurtado, C. and Gómez-García, A. C.: *J. Antimicrob. Chemother.*, 28, 415-418, 1991.
8. Peterson, P. K.: *Am. J. Med.*, 76, 2-10, 1984.
9. Van der Auwera, P., Matsumoto, T. and Husson, M.: *J. Antimicrob. Chemother.*, 22, 185-192, 1988.
10. Williams, A. H. and Gruneberg, R. N.: *J. Antimicrob. Chemother.*, 14, 441-445, 1984.