

Evaluation of silicon oil on bacterial growth

Avaliação dos efeitos do óleo de silicone no crescimento bacteriano

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ABSTRACT

Purpose: To analyze the antimicrobial properties of silicon oil (Óleo de Silicone®, Ophthalmos, Brazil) on *in vitro* bacterial growth of different microorganisms related to endophthalmitis.

Methods: The following microorganisms were analyzed: (1) *Pseudomonas aeruginosa* (ATCC 27583); (2) *Escherichia coli* (ATCC 25922); (3) *Staphylococcus aureus* (ATCC 25923); (4) *Staphylococcus epidermidis* (ATCC 12228); (5) *Candida albicans* (ATCC 10231); (6) *Klebsiella pneumoniae* (ATCC 13883); and (7) *Streptococcus pneumoniae* (ATCC 49619). The plates were incubated at 35 ± 2°C and its growth examined after 24 hours. An empty disk was placed in the center of each plate as a control.

Results: No inhibition halos were verified in any of the plates containing the four different concentrations of the bacterial inocula.

Conclusions: The silicon oil 1000 cps does not have any effect on bacterial growth of any of the studied microorganisms.

Keywords: Endophthalmitis; Silicon oils/analysis; Anti-infective agents; Bactericides; Antibacterial agents

RESUMO

Objetivo: Analisar as propriedades antimicrobianas do óleo de silicone (Óleo de Silicone®, Ophthalmos, Brazil) no crescimento *in vitro* de diferentes microorganismos relacionados à endoftalmite.

Métodos: Os seguintes microrganismos foram analisados: (1) *Pseudomonas aeruginosa* (ATCC 27583); (2) *Escherichia coli* (ATCC 25922); (3) *Staphylococcus aureus* (ATCC 25923); (4) *Staphylococcus epidermidis* (ATCC 12228); (5) *Candida albicans* (ATCC 10231); (6) *Klebsiella pneumoniae* (ATCC 13883); and (7) *Streptococcus pneumoniae* (ATCC 49619). As placas foram incubadas à temperatura de 35 ± 2°C e o seu crescimento examinado após 24 horas. Um disco de papel filtro neutro, sem óleo de silicone, foi posicionado no centro de cada placa como controle.

Resultados: Não foram encontrados halos de inibição em nenhuma das placas contendo as diferentes concentrações de inóculo bacteriano estudadas.

Conclusões: O Óleo de Silicone® 1000 cps não apresenta efeito no crescimento bacteriano de nenhum dos microrganismos estudados.

Descritores: Endoftalmite; Óleos de silicone/análise; Antimicrobianos; Bactericidas; Antibacterianos

INTRODUCTION

Silicon oil is a term designated to denominate a group of clear, inert and hydrophobic polymers, chemically derived from siloxane⁽¹⁻⁴⁾. The first reference of its use in ophthalmology dates back to 1958 with Stone, as a substitute of the vitreous humor in experimental intraocular surgery. But it was only after the 1970's, with modern microsurgery, that this technique was widely spread around the world, Jean Haute (France), Relja Zivojnovic (Holand) and Peter Lever (England) combined successfully intraocular injection of the silicon oil and *pars plana* vitrectomy. Currently, the main indications of its use are complicated cases, when long term tampon effects are required, and infectious endophthalmitis⁽¹⁻⁶⁾.

One of the most important indications and reason of this study is the infectious endophthalmitis, the most feared complication of the ophthalmological surgeons. According to previous studies, exogenous endophthalmitis incidence is higher in cases of ocular trauma (2.4 - 8%), trabeculectomy (0.2 - 9.6%) and cataract surgery (0.04 - 0.7%). In the event of an ocular trauma with intraocular foreign body, the incidence increases up to 30%, according to some authors^(3,4,7-24).

The most frequently responsible microorganisms of acute exogenous endophthalmitis are *Staphylococcus aureus*, *Staphylococcus*

epidermidis, *Enterococcus faecalis* and *Streptococcus pneumoniae*. As for the chronic, the most isolated microorganism in cultures is the *Propionibacterium acnes*^(18,22).

Some prospective and experimental studies, both *in vitro* and *in vivo*, suggested that the silicon oil has bactericidal activity against many microorganisms, besides increasing the intraocular concentrations of antibiotics, if present⁽²⁵⁻²⁹⁾.

The purpose of this study was to verify the effect of the silicon oil on *in vitro* bacterial growth of selected microorganisms.

METHODS

An experimental study was developed in the Laboratory of Microbiology of the Irmandade da Santa Casa de Misericórdia de São Paulo of the Department of Pathology Sciences of the Faculdade de Ciências Médicas da Santa Casa de Misericórdia de São Paulo (Brazil).

The silicon oil 1000 cps (Óleo de Silicone®, Ophthalmos, Brazil) was studied.

The following bacterial cultures from the American Type Culture Collection were studied: *Pseudomonas aeruginosa* (ATCC 27583), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Sta-*

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phylococcus epidermidis (ATCC 12228), *Candida albicans* (ATCC 10231), *Klebsiella pneumoniae* (ATCC 13883) and *Streptococcus pneumoniae* (ATCC 49619).

The bacteria were primarily incubated for 18-24 h in a nutrient broth at $35 \pm 2^\circ\text{C}$ until reaching 0.5 on the McFarland scale, which corresponds to 1.5×10^8 CFU/cc (Colony Forming Units) in Tryptic Soy Broth. For each microorganism, three more dilutions were used, corresponding to 1.5×10^6 CFU/cc, 1.5×10^4 CFU /cc and 1.5×10^2 CFU/cc. The four dilutions were submitted to the test.

The four dilutions of the inocula were then seeded on plates containing Muller-Hinton medium for all bacteria with exception of *S. pneumoniae*, which was seeded on blood agar, according to the modified Kirby-Bauer technique, standardized by the Clinical and Laboratory Standards Institute (C.L.S.I.)⁽³⁰⁾.

Following the preparation of the plates, $15 \mu\text{m}^3$ of the silicon oil (Óleo de Silicone® 1000 cps, Ophthalmos, Brazil) were evenly distributed in standard 6 mm paper filter discs using micropipettes (Eppendorf®, Hamburg, Germany).

The discs containing silicon oil were then positioned in the respective seeded plates, with ten discs for each microorganism, in each of the four different concentrations of bacterial inocula⁽³¹⁾.

The plates were once again incubated for 48 hours at $35 \pm 2^\circ\text{C}$. The analyses were made in 24 and 48 hours. Bactericidal and bacteriostatic activity was verified by sampling inhibition zones, when present, and seeding other plate with substrates from within the inhibition halos⁽³¹⁾.

In all plates, a blank paper filter disk was placed without silicon oil, to compare the bacterial growth, since it does not have an inhibition halo.

RESULTS

No inhibition halos were verified in any of the plates containing the four different concentrations of the bacterial inocula as shown in figure 1.

DISCUSSION

In our study, in disagreement with other authors, the silicon oil did not have any effect on growth of any of the three main families of ethiological agents of the infectious endophthalmitis: Gram + bacteria, Gram - bacteria and yeast^(26,27). Such a radical difference can be explained by the choice of distinct methods.

Mackiewicz et al. and Ozdamar et al., compared, using similar methods, the growth of different bacteria inoculated directly on silicon oil, saline solution and a nutrition solution. After the adequate

management of the material and incubation, checking daily the colony forming units, it was verified in both studies that after 5 - 7 days, the number of C.F.U. inoculated on silicon oil diminished through time and were extinct after approximately 20 days. On the nutrition solution this outcome was according to the natural growth pattern and, on the saline solution, it was stable throughout the length of the study^(26,27).

Nonetheless, these results do not seem to be reproduced *in vivo*. Bali et al., in 2003, demonstrated that eyes affected with severe acute infectious endophthalmitis treated with pars plana vitrectomy, antibiotics and injection of intraocular silicon oil had better anatomical and functional results when compared with eyes treated only with regular vitrectomy and antibiotics. These results suggest that this substance is useful in the surgical treatment of the infectious endophthalmitis⁽²⁵⁾.

If *in vitro* there is no confirmed antimicrobial activity of the silicon oil, the only possible explanation for the above results *in vivo* would be the nutritional deprivation that the substitution of the vitreous with silicon oil could cause to the intraocular pathogens, leading them to death by starvation and consequently the control of the infection process.

The most probable explanation for the different results of this study relies on the method. The use of filter paper discs on the evaluation of antimicrobial activity is proven to be a very easy method, with high sensitivity and specificity, since it analyzes solely the effect of the studied substance in direct and homogeneous contact with the microorganisms in growth. Other authors have chosen this method to assess the antimicrobial activity of different substances used daily in clinical and surgical practice, such as trypan blue, fluorescein and cyanoacrylate, obtaining reliable results⁽³²⁻³⁴⁾.

Through this method, we did not observe the presence of inhibition halos around the paper filter discs soaked with silicon oil in any of the plates containing the different concentrations of bacterial inocula, suggesting that the silicon oil at 1000 cps does not have any effect on *in vitro* bacterial growth.

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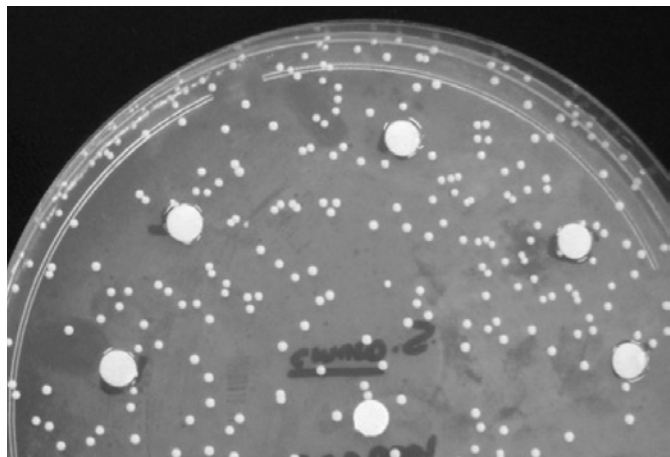


Figure 1. Müeller-Hinton plate inoculated with *Staphylococcus aureus* showing, in detail, the absence of inhibition halos around the impregnated discs. Laboratory of the Irmandade da Santa Casa de Misericórdia de São Paulo.

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