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SKIN DISC ANTIBIOGRAM: A NEW IN-VITRO MODEL TO TEST PHARMACODYNAMIC EQUIVALENCE OF ANTI-BACTERIAL TOPIC FORMULATIONS

Alizé Fargier (1), Manuel Sacha (1), Cornelia Keck (2), Eleonore Haltner (1)

(1): Across Barriers GmbH, Science Park 1, Saarbrücken, Germany

(2): Angewandte Pharmazie, Hochschule Kaiserslautern, Campus Pirmasens, Germany

INTRODUCTION

Generics represent an important part of new products on market. However there is a necessity to prove the equivalence between originator and generic products to obtain marketing authorization. The World Health Organization defines two products as therapeutically equivalent if they are pharmaceutically equivalent at the same dose and demonstrate similar safety and efficacy based on bioequivalence, pharmacodynamics, clinical or in-vitro studies; therapeutic equivalence is then assumed without requiring further supportive evidence (1).

Concerning antimicrobial products which target microbes rather than humans, supplementary appropriated tests should be used to establish efficiency against microorganisms (2).

The aim of this study was to adapt a well-known and described in-vitro assay “antibiogram disc diffusion” to prove and compare the efficiency of originator and generic antibiotic ointments. The investigated topical formulations are used for treatment of localized skin infections.

The Kirby-Bauer method (3) also called “disc diffusion antibiotic sensitivity testing” is primarily used by the clinicians to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy, and in monitoring resistance trends over.

In this method, small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in where bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible. The diameter of the inhibition zone is proportional to the sensitivity of the microorganism and the efficacy of the antibiotic.

In the described method, human dermatomed skin discs containing the antibacterial formulation on the stratum corneum were used instead of antibiotic impregnated discs, to evaluate the equivalence of products (generic and originator).

MATERIALS AND METHODS

In a pre-experiment the skin permeation behavior of antibiotic from the two formulations was



evaluated. The experiment was performed in sixfold with one skin donor (max. 300 µm mean thickness).

The permeated amount of the active ingredients was quantified over a time period of 48 hours by withdrawing 8 samples from the acceptor compartment of Franz diffusion cells.

The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values were determined for both products on *Staphylococcus aureus* using broth microdilution method (4). Samples over MIC point were plated on Muller Hinton Agar, incubated 24h at 37°C and colonies counted to determine MBC values.

In order to evaluate if a brief skin disinfection using ethanol at 70 % or the skin itself can have any influence on the bacterial growth, previously sterilized dermatomized (max. 300 µm mean thickness) human skin discs were prepared in 13 mm diameter and incubated on agar plates previously inoculated with the reference pathogen, *Staphylococcus aureus*. The survival of the microorganism was evaluated on different incubation times (18, 24 and 48 h).

After development of the method, bacteriostatic activity of generic and originator ointments were investigated and compared. The experiment was performed in sixfold.

RESULTS AND DISCUSSION

Results for both originator and generic formulations tested on *Staphylococcus aureus* are respectively MIC= 0.051 and 0.050 µg·mL⁻¹, MBC= 3.256 and 3.212 µg·mL⁻¹. The MIC and MBC values for both products are similar.

When comparing the ointments bacteriostatic activity using the “Skin disc antibiogram” no difference was observed regarding the inhibition zones between the originator and the generic (2.0 ± 0.2 cm versus 1.9 ± 0.2 cm). The experiment was performed in sixfold and confirmed the reproducibility of the results. Furthermore, no inhibition was observed after incubation in the control plates containing only skin without formulation. Even if the skin was only briefly and gently disinfected with 70 % ethanol, this procedure was sufficient to avoid a cross contamination of tested microorganisms.

Results are in accordance with permeation measurements: originator presents a Papp value of 8.39E-10 ± 7.43E-11 cm·s⁻¹ and a cumulative transport of 1.74 ± 0.44 µg·cm⁻² while the generic Papp value is 1.11E-09 ± 2.68E-10 cm·s⁻¹ and the total permeated amount of API after 48 h 2.66 ± 0.48 µg·cm⁻². The transport and the absorbed amount of API are similar for both formulations.

From this assay the generic and originator can be considered as equivalent.

CONCLUSION

The described method is the basis of a new tool as ex vivo pharmacodynamics equivalence test, using the principle of agar plate diffusion antibiogram. The application fields may be “in-vitro bioequivalence” testing of generic products, but also the galenic development of innovative antibacterial formulations.



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