LACK OF TRANSCUTANEOUS PASSAGE OF GENTAMICIN AND MICONAZOLE THROUGH THE SKIN OF THE CANINE EAR PINNA

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STUDY OBJECTIVE

EASOTIC\textsuperscript{®} is an ear suspension for the treatment in dogs of otitis externa of both fungal and bacterial origin. It contains miconazole, gentamicin and hydrocortisone aceponate. The aim of this study was to evaluate the transcutaneous passage of both gentamicin and miconazole after application of EASOTIC\textsuperscript{®} to the epidermal part of skin of the canine ear pinna, using an in vitro Franz-type diffusion cell method (static method – scheme of the apparatus below). This approach allows the evaluation of the cutaneous resorption of miconazole and gentamicin, and therefore the evaluation of the systemic safety of EASOTIC\textsuperscript{®}.

MATERIALS AND METHODS

Biological material and treatments

Skin (internal part of ear) was obtained from Beagle dogs (6 adult females). EASOTIC\textsuperscript{®} was applied (176 µL per 0.79 cm\textsuperscript{2} skin disc, i.e. 2.987 mg miconazole nitrate and 458 µg gentamicin sulfate) onto the epidermal face of the skin (non occlusive conditions). Liquid receptor (2.2 mL saline with 5% w/v BSA) was maintained at 32 ± 1°C in compliance with OECD guideline \textsuperscript{(1)}, and stirred with a magnetic stirring bar. The test product was applied for up to 48 hours, and three skin discs originated from three different dogs were used per incubation time. Liquid receptor was collected 1, 8, 24 and 48 hours after application, frozen at ca -80°C, and further analysed for gentamicin and miconazole contents.

Integrity of the skin was assessed with the TEWL (transepidermal water loss) measurement.

Quantification of miconazole and gentamicin

Miconazole was analysed in liquid receptor using a validated GLP HPLC-UV method (230 nm) following liquid/solid extraction with Oasis\textsuperscript{®} HLB 3 cc 60 mg cartridges. The LLOQ = 0.1 µg.mL\textsuperscript{-1}. GLP qualitative HPLC-fluorimetric method (λ<sub>ex</sub> = 340 nm, λ<sub>em</sub> = 440 nm) after liquid/solid extraction using Phenomena strata XC 33 µm cartridges Sorbent 200 mg/3 mL was validated for gentamicin at the concentration of 5 µg.mL\textsuperscript{-1}.

RESULTS

Integrity of the stratum corneum and therefore of the skin discs was demonstrated by low and similar TEWL values:

<table>
<thead>
<tr>
<th>Cell no</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
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</thead>
<tbody>
<tr>
<td>Dog</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Time (h)</td>
<td>1</td>
<td>8</td>
<td>24</td>
<td>48</td>
<td>C</td>
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<tr>
<td>TEWL (g.m&lt;sup&gt;-1&lt;/sup&gt;-h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>4.2 ± 0.5</td>
<td>4.7 ± 0.5</td>
<td>4.6 ± 1.4</td>
<td>4.4 ± 0.5</td>
<td>5.1</td>
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C: control (0 & 48 hours)

The use of previously validated quantitative (miconazole) and qualitative (gentamicin) HPLC analytical method with either UV or fluorimetric detection, showed that neither miconazole nor gentamicin was detected in the liquid receptor, whatever the sampling time and the replicate.

CONCLUSIONS

The use of this in vitro cell diffusion model provides information on absorption of test substance applied to excised skin \textsuperscript{(1)}.

When EASOTIC\textsuperscript{®} is applied onto excised skin without stratum corneum alteration (as shown by the measure of the TEWL) and originated from the internal part of the ear of dogs (target species for EASOTIC\textsuperscript{®}), neither miconazole, nor gentamicin crosses the stratum corneum and reaches the liquid receptor, even after 48 hours application.

Both miconazole and gentamicin are known for almost nil transcutaneous passage \textsuperscript{(2, 3)} and this study confirms thus this lack of percutaneous resorption even in case of really thick stratum corneum \textsuperscript{(4)} and when incorporated into an oily formulation.

This will allow any systemic adverse effects associated with either miconazole or gentamicin, for both the target species during the course of the treatment, and for the user, in case of skin contamination.

\textsuperscript{(1)}: OECD guideline 428
\textsuperscript{(2)}: Kosuzume et al., Iyakuhin Kenkyu, 1976
\textsuperscript{(3)}: GENTALLIN\textsuperscript{®} Injectable: RCP of this product
\textsuperscript{(4)}: Lansdown, Vet Record 1985

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