In vitro modelling of in vivo discoloration of the dapivirine-levonorgestrel vaginal ring using a range of simulated vaginal and menstrual fluids


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IN VITRO MODELLING OF IN VIVO DISCOLORATION OF THE DAPIVIRINE-LEVONORGESTREL RING USING A RANGE OF SIMULATED VAGINAL AND MENSTRUAL FLUIDS

Clare F. McCoy1, Diarmuid J. Murphy1, Yahya H. Dalal Bashi1, Peter Boyd1, Pat Spence2, Brid Devlin2, Kyle Kleinbeck2, Bindi Dangi2, Tiffany Derrick2, R. Karl Malcolm1

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Background

Surface discoloration has been observed in clinical trials of various silicone elastomer vaginal rings (VRs) including IPM’s 25 mg dapivirine and 90-day dapivirine + levonorgestrel (DPV+LNG) ring formulations (Fig. 1).

To investigate the potential causes of this discoloration, four different silicone VR formulations were exposed to various simulated vaginal fluids (SVF) and simulated menstrual fluids (SMF) for up to 60 days in vitro. A range of dyes (Table 1) representing personal care and household cleaning products, as well as hydrogen peroxide and copper intratuterine devices (IUDs) were included in the test compositions.

Objectives

1. Expose four silicone vaginal ring formulations (placebo; 25 mg dapivirine; 200 mg dapivirine; 200/320 mg DPV+LNG) to a range of simulated vaginal fluid and simulated menstrual fluid solutions for up to 60 days.

2. Assess the extent of surface and internal discoloration

Methods

SVF media containing either 20 µM H2O2, 20 µM H2O2 + copper IUD, methyl red (MR), toluidine blue (TB), or crystal violet (CV) were prepared, with SVF-only and ultrapure water as experimental controls.

SMF-based media (SMF-only, SMF + 20 µM H2O2 or SMF + 20 µM H2O2 + copper IUD) were prepared using a 1:1 mixture of horse blood and SVF containing 0.5% w/v xanthan gum.

Single rings from each formulation were placed in 100 mL of each media. Flasks were incubated (37°C/60 rpm) for 30 and 60 days with media replenished weekly. At scheduled timepoints, rings were removed, rinsed, dried and photographed alongside controls. Cross-sections of VRs were examined for interior staining using a Keyence digital microscope.

After 30 and 60 days, rings soaked in SVF-only, SVF + H2O2, SVF + H2O2 + IUD media showed no surface discoloration. Rings soaked in SVF + dye media showed uniform surface staining (Fig. 2).

Rings exposed to methyl red and toluidine blue showed significant colour ingestion throughout the ring. Rings exposed to crystal violet showed minimal colour ingestion (Fig. 3).

Staining patterns were similar to those observed with dapivirine rings (Fig. 1A–C) exposed to highly coloured personal care and household cleaning products during clinical trial use.

After 60 days, the surfaces of SMF-treated rings appeared yellow compared to controls (Fig. 4), with colour intensity correlating with duration of exposure. After 14 days, rings exposed to IUDs showed black/dark surface markings consistent with direct IUD contact (Fig. 5). Non-uniform red-brown staining – later demonstrated to be blood debris – was also observed on the surface of rings exposed to SMF-only and SMF + H2O2 (Fig. 6). No significant staining was observed beneath the surface of the ring.

Results & Discussion

Table 1. Physicochemical properties of APIs and synthetic dyes used in in vitro discoloration studies.

<table>
<thead>
<tr>
<th>API or Dye</th>
<th>Chemical Structure</th>
<th>Molecular weight</th>
<th>Log P (clogP)</th>
<th>Density to Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapivirine</td>
<td><img src="image" alt="Dapivirine Structure" /></td>
<td>319.4</td>
<td>2.3 ± 0.1</td>
<td>1.19 ± 0.01</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td><img src="image" alt="Levonorgestrel Structure" /></td>
<td>540.8</td>
<td>1.0 ± 0.1</td>
<td>1.23 ± 0.02</td>
</tr>
<tr>
<td>Methyl Red</td>
<td><img src="image" alt="Methyl Red Structure" /></td>
<td>225.2</td>
<td>3.0 ± 0.2</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>Toluidine Blue</td>
<td><img src="image" alt="Toluidine Blue Structure" /></td>
<td>351.7</td>
<td>3.0 ± 0.1</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td><img src="image" alt="Crystal Violet Structure" /></td>
<td>588.6</td>
<td>2.5 ± 0.1</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>Vaginal fluid</td>
<td><img src="image" alt="Vaginal Fluid Structure" /></td>
<td>1.113 ± 0.005</td>
<td>18 mg/mL at 30°C, 30.0 ± 0.1 mg/mL at 40°C</td>
<td></td>
</tr>
<tr>
<td>Dye media</td>
<td><img src="image" alt="Dye Media Structure" /></td>
<td>0.68 ± 0.1</td>
<td>NA</td>
<td>0.5 ± 0.01</td>
</tr>
</tbody>
</table>

Figure 1. Examples of post-use discoloration of silicone elastomer vaginal rings.

Figure 2. Representative images of DPV+LNG rings: A–untreated control; B–exposed to SVF + MR; C–exposed to SVF + TB; D–Exposed to SVF + CV media for 30 days.

Figure 3. Representative images of DPV+LNG ring cross-sections: A–untreated control; B–exposed to SVF + MR; C–exposed to SVF + TB; D–Exposed to SVF + CV media for 30 days. Images recorded at ×30 magnification.

Figure 4. Representative images of vaginal rings: A–placebo; B–200 mg DPV; C–200/320 mg DPV+LNG; D–25 mg DPV exposed to SMF + 20 µM H2O2 treatment media for 60 days.

Figure 5. A–25 mg DPV ring and B–placebo ring overlaid with a copper IUD. Both images display dark oval marks that correspond with the contact points between the ring surface and the copper component of the IUD.

Conclusions

Exposure of rings to SMF caused yellow surface discoloration and dark markings. Staining was identified as blood debris from the SMF and was consistent with the appearance observed in post clinical use VRs (Fig. 1D–F). Discolorations were not associated with any specific safety risks for the user but may impact acceptability.

Donor Acknowledgement:
IPM receives generous support from the Danish Ministry of Foreign Affairs, the German Federal Ministry of Education and Research (BMBF) through the KIW Development Bank, Irish Aid; the Ministry of Foreign Affairs of the Netherlands, UK aid from the UK Government’s Foreign, Commonwealth & Development Office, the American people through the US President’s Emergency Plan for AIDS Relief (PEPFAR) in partnership with the United States Agency for International Development (USAID), and the Bill & Melinda Gates Foundation.

1School of Pharmacy, Queen’s University Belfast, Belfast BT1 7EL, UK E: kmalcolm@qub.ac.uk
2International Partnership for Microbicides, Silver Spring, Maryland 20910 E: bdevlin@ipmglobal.org