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SHIV Infection Risk after Rectal Application of a Highly Osmolar Personal Lubricant

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Abstract

Background: Personal lubricant use is common for anal sex. Some water-based products with high osmolality and low pH damage rectal tissues. Additionally, the preservative polyquaternium 15 (PQ15) can enhance HIV replication *in vitro*. This has raised concerns that lubricants with such properties increase STD/HIV infection risk, although *in-vivo* evidence is scarce. In 2012, the WHO issued an interim advisory note to limit use of rectal lubricant formulations >1,200 mOsm/kg, with pH < 5.5, and containing PQ15. Here, we use an animal model to test the hypothesis that a highly osmolar (>8,000 mOsm/kg), water-based lubricant with pH of 4.4, and containing PQ15 increases risk for HIV infection .

Methodology: To determine susceptibility to infection, we compared virus doses needed for infection in 21 lubricant- or control-treated cynomolgus macaques. The macaques received six gentle rectal infusions of lubricant or buffer over 3 weeks, followed by rectal virus exposures at varying doses (1.25-25,000 tissue culture infectious doses [TCID-50] of $SHIV_{SF162P3}$), 30 minutes after the last infusion. We had previously observed rectal pro-inflammatory cytokines and epithelial tissue peeling after such treatment. Uninfected macaques were rested for at least 6 weeks, and then re-exposed to higher doses until 51 exposures and 15 infections (controls=7, lubricant=8) had occurred. We calculated and compared animal infectious doses (AID-50) by modeling HIV infection as a function of the $\log_{10}$ TCID-50 using logistic regression.

Results: The estimated AID-50 and 95% confidence interval (CI) for TCID-50 was 196,336 (1.0, 4.5x10) in lubricant-treated macaques and 1,808 (728, 4495) in controls. The estimated AID-50 ratio of lubricant treated macaques to controls of was not statistically significant different (144; p = 0.4452). Hence, there is not enough evidence that lubricant-treated macaques succumb to virus exposures at lower doses than control macaques.

Conclusions: The tested lubricant did not increase susceptibility to infection. This indicates that neither the type/extent of inflammation induced by this lubricant, nor the presence of PQ15 impacted infection risk in this model. Additional approaches could further clarify lubricant use and HIV risk by evaluating other products, vaginal and penile use, and by analyzing beneficial lubricant effects stemming from reduced condom breakage and tissue trauma during sex.

Background

- Personal lubricant use is common during anal sex.
- Some water-based products with high osmolality and low pH damage rectal tissues[1].
- The preservative polyquaternium 15 (PQ15) can enhance HIV replication *in vitro*[2].
- There are concerns that lubricants with such properties increase STD/HIV infection risk, although *in vivo* evidence is scarce.
- The WHO issued an interim advisory note[3] (2012) to limit use of rectal lubricant formulations >1,200 mOsm/kg, with pH < 5.5, and containing PQ15.
- Our animal model tests the hypothesis that a highly osmolar (>8,000 mOsm/kg)[2], water-based lubricant with pH of 4.4[2], and containing PQ15 increases risk for HIV infection.

Design and Rationale

- To determine susceptibility to infection, we compared virus doses needed for infection in 21 lubricant- or buffer-treated cynomolgus macaques.
- We had observed rectal tissue cytotoxicity in such an animal model (Fig. 1-3; Table 1; cytotoxicity phase of study).
- In the challenge studies, the macaques received six rectal infusions of lubricant/buffer during 3 weeks (Fig. 4), followed by rectal virus exposures at varying doses (1.25-25,000 tissue culture infectious doses [$TCID_{50}$] of $SHIV_{SF162P3}$), 30 minutes after the last application.
- Uninfected macaques were rested for at least 6 weeks, and then re-exposed to higher doses until 51 exposures and 15 infections (controls=7, lubricant=8) had occurred.
- We compared animal infectious doses (AID_{50}) by modeling HIV infection as a function of the log10 $TCID_{50}$ using logistic regression.

Study design (cytotoxicity)

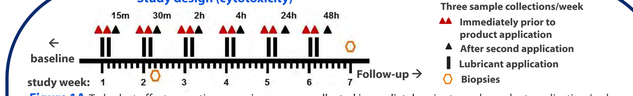
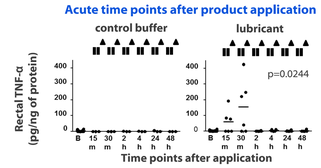


Figure 1A. To look at effects over time, specimens were collected immediately prior to each product application (red triangles), while more acute effects were analyzed with specimens taken 15 minutes to 48 hours after product application (black triangles). Rectal biopsies were taken at 30 minutes post-product application and after study conclusion.

Acute time points after product application



All time points during product application

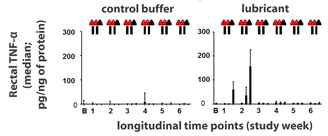


Figure 1B. Induction of pro-inflammatory cytokine TNF-α at acute time points [top panel; 15 minutes (m), 30m, 2hours (h) 4h, 24h, or 48h post-product application]; Induction of pro-inflammatory cytokine TNF-α at all time points (lower panel) during 6 weeks of product application; medians and ranges are graphed.

Cytokine	GM for controls at acute time points[1]	GM for lubricant-treated animals at acute time points[1]	p-value[2] (control arm versus lubricant-treated arm)
IL-1β	0.397	3.355	0.0460
TNF-α	0.091	3.245	0.0244
IFN-γ	0.090	0.611	0.0938
IL-12	0.522	8.032	0.0035
IL-15	1.229	5.785	0.0017
G-CSF	0.068	1.624	0.0134
IL-1Ra	14.896	38.164	0.0500
IL-8	8.406	45.936	0.2959
IL-1Ra	32.274	153.122	0.0500
MCP-1	1.575	10.020	0.0545
TGFα	9.211	25.170	0.0303
VEGF	20.898	76.576	0.1047
sCD40	1.560	13.273	0.0197
IL-4	1.016	5.813	0.0097
MIP-1α	1.443	3.688	0.0143

Table 1. Cytokine expression at acute time points in the two study arms. [1]To compare the cytokine expression at baseline and acute time points, we combined the measurements at 15m and 30m post-application, except for cytokines TNFα, IFN-γ, G-CSF, MIP-1α, and IL-4 where we combined readings at all four acute time points. The p-values shown are not corrected for multiple comparisons; PBS= phosphate buffered saline; GM=geometric mean; [2]=derived from the ratio of the GM ratios in the PBS and lubricant arms (unpaired t-test).

Epithelial sloughing and H&E stain of tissues in rectal lavage

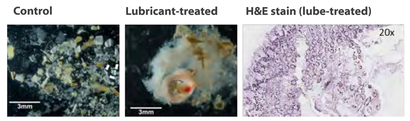


Figure 2A. Lubricant induces rectal shedding of epithelial cells. Examples of epithelial sloughing in a control (leftmost panel) and lubricant-treated animal (middle panel). The right panel shows a representative H&E stain of peeled epithelial cells (20x).

Acute and longitudinal time points

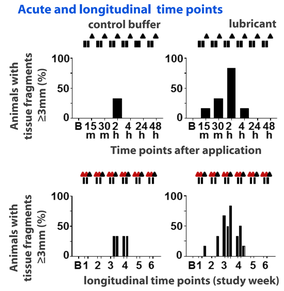


Figure 2B. Epithelial sloughing measured at acute time points collected after the 2nd weekly lubricant application (top panel), and those measured over the entire study (lower panel);

Tissue-associated blood

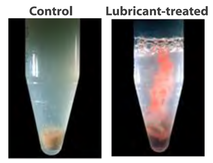


Figure 2C. Blood associated with rectal washes; photographs of microfuges containing rectal lavages.

Rectal biopsies

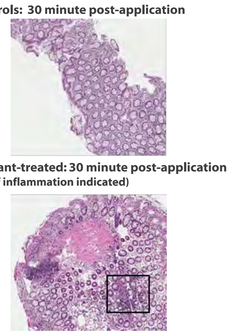


Figure 3. Hematoxylin and eosin stain (20x) of rectal biopsies before lubrication application (top panel) and 30 minutes after lubricant-application (lower panel) ; the lower panel shows focal infiltrates of inflammatory cells (square box), predominately mononuclear, seen in the lamina propria; there is no disruption of architecture.

Study design (challenge studies)

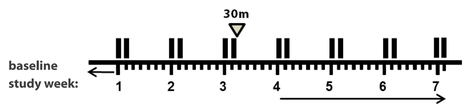


Figure 4. Study design (virus challenge phase). Lubricant was applied non-traumatically for three weeks, two consecutive days per week. The animal was challenged with SHIV162p3 30 minutes after the sixth lubricant application (open triangle). To check for plasma viremia and virus shedding, samples were collected for at least 2 weeks post-peak viremia. Relevant assays were performed on baseline blood draws before the first lubricant application to ensure that the animal is not infected.

Doses and infections

Virus dose (TCID50)	PBS-treated	Lube-treated
25000	+	-
5,000	+	+
	+	-
2,500	+	+
	+	-
	+	-
1,250	-	+
	-	-
	-	-
500	-	+
	-	+
	-	-
250	+	+
	-	+
	-	-
	-	-
	-	-
	-	-
	-	-
	-	-
50	+	-
	-	-
	-	-
	-	-
12.5	-	-
	-	-
1.25	-	-
	-	-

Table 2. SHIV162p3 challenge doses and infection. '+'=animal infected at the indicated dose,'-'=animal not infected; PBS=phosphate buffered saline; TCID50=tissue culture 50% infective dose; the historical controls are indicated in the area shaded in grey.

Risk of infection

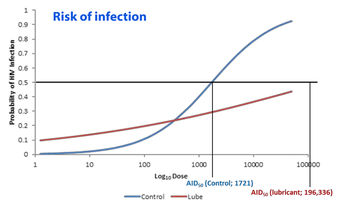
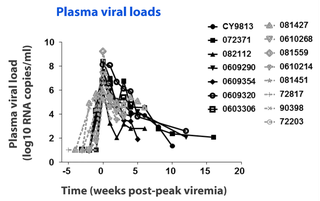


Figure 5. Risk of infection. The estimated AID-50 ratio between the study arms was not statistically significant different (144; p = 0.4467).

Plasma viral loads



Rectal shedding of virus

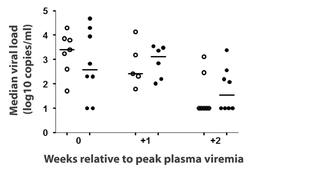


Figure 6. Plasma SHIV RNA levels (top panel) measured in PBS-treated (black symbols; solid lines) and lubricant-treated (grey symbols; broken lines) animals; SHIV RNA levels measured in rectal secretions (lower panel), in PBS-treated (open symbols) and lubricant-treated (closed symbols) animals; 0=time of peak plasma viremia.

Results

- Rectal cytokines, including pro-inflammatory ones, peaked at acute time points post-lubricant application (Fig. 1B; Table 1).
- We observed epithelial peeling and associated blood (Fig. 2ABC); rectal pH and microflora remained unchanged (data not shown).
- Rectal biopsies (30 minutes post-lubricant application) showed mild inflammation (Fig. 3).
- The estimated AID_{50} and 95% CI for $TCID_{50}$ were 196,336 (1.0, 4.5x10) in lubricant-treated macaques; and 1,721 (414, 7165) in controls (Fig. 5, Table2); the AID_{50} ratio was not significantly different (p= 0.4467).
- Also not significant in the two study arms were the differences in plasma viremia and rectal virus shedding (Fig 6).

Conclusions

- Although we observed acute cytotoxicity in rectal tissues, the test lubricant did not increase susceptibility to infection.
- Neither the type/extent of inflammation induced by this lubricant, nor the presence of PQ15 impacted infection risk in this model.
- This study constitutes an important first step in the *in vivo* evaluation of lubricants with regards to HIV transmission.

Study limitations

- Only one product was evaluated.
- Applications were non-traumatic.
- Since sex was not modeled, beneficial effects of lubricant not evaluated.
- This macaque model might not be sensitive enough to assess increased risk of HIV infection.

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