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ORIGINAL PAPER

Rectal Douching Practices Associated with Anal Intercourse: Implications for the Development of a Behaviorally Congruent HIV-Prevention Rectal Microbicide Douche

Alex Carballo-Dieguez[1] · Rebecca Giguere[1] · Cody Lentz[1] · Curtis Dolezal[1] · Edward J. Fuchs[2] · Craig W. Hendrix[2]

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Abstract

Tenofovir administration via rectal douching results in higher rectal-mucosa drug concentration than oral administration. Many who engage in receptive anal intercourse (RAI) use cleansing rectal douches. To inform development of a behaviorally-congruent tenofovir douche, 4751 individuals ≥ 18 years-old, born male, from all US states/territories, who engaged in anal intercourse responded to an online survey. Of those who reported RAI in the prior 3 months, 80% douched beforehand, 82% within 1 h, mean 2.9 consecutive applications; 27% douched afterwards, 83% within 1 h, mean 1.7 consecutive applications. Among multidose users, 78% applied doses within 2 min, and 76% retained liquid < 1 min. Most used tap water (89%) in an enema bottle (50%) or rubber bulb (43%), and douched for cleanliness (97%), to avoid smelling bad (65%), and to enhance pleasure (24%). 98% reported high likelihood of using an HIV-prevention douche. An ideal product will protect within a user's typical number of applications, within 1 h, and be dissolvable in tap water.

Keywords Rectal douching · Enemas · HIV-prevention · Rectal microbicides

Resumen

La administración de tenofovir por medio de una ducha rectal resulta en una concentración de droga en la mucosa rectal más alta que por vía oral. Muchos de los que tienen sexo anal receptivo (SAR) usan duchas rectales para limpiarse. Para informar el desarrollo de una ducha rectal congruente con los comportamientos habituales de los usuarios, 4751 individuos mayores de 18 años, nacidos varones, de todos los estados y territorios de los EEUU, que tenían sexo anal respondieron a una encuesta por Internet. De los que reportaron SAR en los últimos 3 meses, 80% usaron una ducha previamente, 82% dentro de una hora, con un promedio de 2.9 aplicaciones consecutivas; 27% usaron una ducha posteriormente, 83% dentro de una hora, con un promedio de 1.7 aplicaciones consecutivas. De los que usaron múltiples dosis, 78% aplicaron las dosis dentro de 2 minutos, y 76% retuvieron el líquido < 1 minuto. La mayoría usó agua corriente (89%) en una botella de enema (50%) o un bulbo de goma (43%), y se ducharon para estar limpios (97%), para evitar feos olores (65%), y para aumentar el placer (24%). 98% reportó alta probabilidad de usar una ducha que prevenga el VIH. El producto ideal debería proteger dentro del número típico de aplicaciones del usuario, dentro de una hora, y ser soluble en agua corriente.

Introduction

Pre-exposure prophylaxis (PrEP) effectively prevents HIV infection. This was demonstrated initially among individuals who took oral PrEP daily [1], and subsequently among those who used PrEP intermittently provided a minimum number of doses were taken [2]. Studies are currently underway to develop long-acting PrEP delivery systems (injections and subdermal implants) that could potentially facilitate adherence [3, 4].

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✉ Rebecca Giguere
rebecca.giguere@nyspi.columbia.edu

[1] HIV Center for Clinical and Behavioral Studies at New York State Psychiatric Institute and Columbia University, 1051 Riverside Drive, Unit 15, New York, NY 10032, USA

[2] Division of Clinical Pharmacology, Johns Hopkins University, Baltimore, MD, USA

Yet, both oral and long-acting PrEP are systemic, i.e., drugs that distribute throughout the body. Some individuals who are at risk of HIV infection do not want to be exposed to systemic drugs, mainly out of concern about long-term side effects [5]. For example, during MTN 017, a study in which participants had the chance to compare daily oral PrEP and a topical PrEP rectal gel candidate, 28% of participants reported that daily oral PrEP was their least favorite prevention strategy and that they were more likely to use a topical microbicide gel in the future if it were effective against HIV [6]. Another study in Peru also found that, among transgender women and men who have sex with men, 57% of participants would prefer a topical PrEP rectal gel compared to 29% who preferred oral PrEP [7].

Furthermore, although efforts are underway in the HIV prevention research field to develop a vaccine, as with oral PrEP, uptake among those at risk for HIV will likely depend on individual preferences and concerns about factors including vaccine efficacy, duration of protection, cost, and safety [8, 9]. Therefore, even in the event of a successful vaccine and in the current environment in which oral PrEP has proven efficacy, behaviorally-congruent prevention methods in the form of topical products are essential components of the HIV prevention toolbox.

One of the challenges of achieving HIV prevention with a topical product is ensuring that the product is present at each sexual risk occasion. Intra-vaginal rings could potentially offer topical prevention if women leave them in place for as long as recommended [10]. In the case of individuals who engage in anal intercourse (AI), rings are not feasible and other strategies are needed. Rectal gels applied prior to AI could be one such strategy, yet the need to use an applicator to deliver the gel intra-rectally is problematic [11–15].

Rectal douches present a tantalizing possibility for a topical rectal microbicide. Many individuals who engage in AI use cleansing douches regularly before and even after AI [16–23]. Studies have shown that a rectal douche could be a good vehicle to deliver an HIV-protective drug [24, 25], and that topical administration of a protective drug can result in higher drug concentrations in the colon tissue target cells than oral (systemic) administration [26]. Furthermore, many individuals who do not currently use rectal douches in association with AI indicate they would be likely to use them if they protected against HIV [18]. Consequently, efforts are underway to develop a "behaviorally-congruent" rectal microbicide douche, i.e., a product whose characteristics, mode of use, and adaptability to users' needs come as close as possible to the real-world experiences and ongoing practices of potential users (U19 AI113127, PI: Hendrix) [26].

Our group recently published a scientific literature review supplemented by a review of rectal douching videos posted on YouTube that highlighted the popularity of rectal douches [18]. Yet, some specific information (e.g., number of doses used, retention time) important for the development of a microbicide douche was missing. The goal of the present study was to obtain additional data on behavioral aspects of rectal douching associated with AI that could contribute to the development of a behaviorally congruent rectal microbicide douche.

Methods

Between October 27 and November 15, 2017, we conducted an online survey through Grindr, a social media app popular among men who have sex with men. Initially, an inbox message with the subject line "Clean, safe and ready!" was sent to all active Grindr users in the US and its territories. Users who opened the message were shown the study advertisement (Fig. 1).

A total of 1,245,426 Grindr users received the above inbox message. This message was opened 145,621 times (roughly 12%), which resulted in 9752 users clicking through to the study survey (6.70% of those who opened the message). After checking the geographical and racial/ethnic distribution of the initial respondents recruited using the inbox message, we published a text-based pop-up advertisement

Fig. 1 Survey advertisement sent to inbox of all Grindr users in US and its territories

(known on Grindr as a broadcast) to further reach racial/ethnic minorities in New York, NY; Atlanta, GA; Jackson, MS; and San Juan, PR. As a result, 1348 additional users clicked on the broadcast advertisement to proceed to the online survey. Of the total 11,100 respondents who proceeded to the landing page of the online survey, 7481 (67%) individuals clicked on an initial arrow to actually initiate the survey, of whom, 4772 (63%) answered at least one survey question. Overall, 4751 (99.6%) of respondents fit our eligibility criteria, reporting having been assigned "male" on their original birth certificate, being 18 years of age or older, and having engaged in AI.

The full survey, including pictures of a variety of known rectal douching devices, appears in the "Online Appendix". Purposely, the survey was kept short to ensure that it remained user-friendly, especially given that no monetary incentive was offered for completion.

Descriptive statistics were used to summarize demographics and survey responses. Some variables provided an "other, please specify" option, and these write-in responses were recoded into existing response categories whenever possible. Given that certain questions did not apply to some participants, and that we were ethically obliged to allow participants to refuse to answer questions, missing data were common. The results reported below show the N with non-missing data for each variable. Several outcomes were selected for further statistical analyses. Outcomes of interest with continuous variables were (1) frequency of RAI, (2) frequency of douching before RAI, and (3) frequency of douching after RAI; these variables were log-transformed prior to analyses due to skewed distributions. Outcomes with dichotomous variables were (4) if used enema bottle, (5) if used rubber bulb, (6) if used hose attached to faucet, (7) If used tap water, and (8) If used commercial douche. We tested whether these outcomes were associated with age, race (if White, non-Latino, Yes/No), HIV status (HIV+ vs. HIV−), and residence (urban, suburban, rural). Statistics included correlations, t-tests, Chi square tests, and ANOVAs, as appropriate for the variables involved.

Results

The 4751 individuals who agreed to take the survey and fit eligibility criteria came from all 50 states, the District of Columbia, Puerto Rico, U.S. Virgin Islands, and military post offices. Their demographic characteristics appear in Table 1. Respondents' average age was 36.63, ranging from 18 to 98. Slightly over half of the respondents were white, with the racial and ethnic distribution approximating that of the general population in the US. [27] The overwhelming majority identified as men, although 35 individuals identified as women or transgender and 38 as other (e.g., gender fluid, non-binary). Most of the respondents lived in urban or suburban areas, and 10% were HIV-positive.

Figure 2 shows the subsamples of participants responding to different survey questions.

Of the participants who replied to the question on frequency of receptive anal intercourse (RAI) in the prior 3 months, 22% indicated no RAI in the prior 3 months and 78% indicated they had had RAI. Of those who had engaged in RAI, 3349 responded to a question about douching before RAI and 80% reported doing so in the past 3 months. Similarly, 3262 responded to a question about douching after RAI and 27% reported doing so. There were 3245 participants who answered both questions and, of those, 25% said the douched both before and after, while 18% douched neither before nor after. Individuals who did not report RAI (including those with missing data) in the 3 months prior to the survey were asked if they ever had insertive anal intercourse (IAI) and 82% said yes.

Table 2 shows the sexual behavior reported by the respondents and their attitudes concerning rectal douches. Overall, respondents indicated that they had had a mean of 8.01 occasions of RAI in the prior 3 months, had douched before RAI on an average of 7.25 occasions and after RAI on an average of 1.61 occasions. When asked to check "all that apply" for reasons to douche before or after RAI, participants mainly chose to be clean (97%) and to avoid smelling badly (65%), although about a quarter of respondents also found that douching enhanced sexual pleasure (24%). Some believed that douching could prevent infections (10%) and a small number douched following a suggestion from a sexual partner (6%). The devices most commonly used were an enema bottle (50%) or a rubber bulb (43%); yet, about a third of the participants also indicated they used a hose attached to a faucet (32%). Tap water was by far the most frequently used liquid (89%), with about a quarter of respondents reporting also using commercial douches (26%).

Most individuals who douched before RAI did so between 10 and 60 min before sexual activity (77%). The mean number of douche applications before RAI was 2.9, with similar proportions of respondents reporting using on average 1 (21%), 2 (22%), 3 (26%), or more than 5 applications (20%). Douching after RAI tended to occur close to the time of sexual activity, with the majority of individuals douching within 30 min (60%) and using just one (55%) or two doses (28%), with a mean of 1.7 applications.

The largest proportion of respondents who used more than one douche application administered them within 30–60 s of each other (34%), and the largest proportion of respondents retained the liquid between 15 and 30 s following each application (32%).

Of the respondents who reported rectal douching within the prior 3 months, 98% expressed likelihood to use a rectal douche that could protect against HIV. Of the respondents

Table 1 Demographic characteristics of DREAM Internet Study respondents (N = 4751)

	Mean (SD) N (range)
Age	36.63 (12.88) 4038 (18–98)
	N (%)
Ethnicity/race	4119 (100%)
White, non-Latino	2182 (53%)
Latino	813 (20%)
African-American	607 (15%)
Asian/Pacific Islander	261 (6%)
Native American	36 (1%)
Other	220 (5%)
Gender identity	4125 (100%)
Man	4052 (98%)
Woman	8 (< 1%)
Transgender	27 (1%)
Other	38 (1%)
Current residence[a]	4101 (100%)
Urban	2159 (53%)
Suburban	1492 (36%)
Rural	450 (11%)
HIV status	4009 (100%)
Negative	3263 (81%)
Positive	404 (10%)
Don't know	258 (6%)
Prefer not to answer	84 (2%)

4751 were eligible, agreed to continue, and provided some data. Missing data were common, therefore the total N with non-missing data for each variable is noted in the table

[a]Respondents came from all 50 states, the District of Columbia, Puerto Rico, U.S. Virgin Islands, and military POs

who reported IAI, 95% expressed support for a partner to use rectal douches to prevent HIV; furthermore, two-thirds of them reported having ever asked a sexual partner to use a cleansing rectal douche before sexual intercourse. Interestingly, most individuals who reported not douching before or after RAI said that the main reasons were that they never thought about it (56%) or that they did not feel the need to do it (29%); a small proportion was concerned about harmful side effects, including cramps (6%). Yet, even among individuals who did not douche, 95% reported being likely to use a douche that could protect against HIV.

The exploratory analyses of outcomes of interest resulted in several significant findings: More frequent RAI was associated with White race ($p = 0.017$), HIV positive status ($p < 0.001$), and urban residence (lowest = Rural) ($p < 0.001$); more douching before RAI was associated with older age ($p = 0.003$), HIV positive status ($p < 0.001$), and urban residence (lowest = Rural) ($p < 0.001$); more douching after RAI was associated with non-white race ($p < 0.001$) and HIV positive status ($p < 0.001$); using an enema bottle was associated with older age ($p = 0.009$) and non-white race ($p < 0.001$); using a rubber bulb to douche was associated with younger age ($p = 0.003$), white race ($p = 0.031$) and HIV negative status ($p < 0.001$); using a hose/faucet was associated with older age ($p < 0.001$), white race ($p < 0.001$) and HIV positive status ($p < 0.001$); using tap water was associated with younger age ($p = 0.002$) and HIV positive status ($p = 0.039$); and finally, using a commercial douche was associated with older age ($p < 0.001$).

The qualitative data collected through an open-ended question asking respondents to explain reasons for wanting or not wanting to use and HIV-prevention douche helped gain further insight into participants' choices. Primary reasons given for wanting to use an HIV prevention rectal douche included to prevent HIV, preferring the convenience of a douche to that of an oral tablet, and viewing the douche as "dual purpose" for both cleaning and prevention. Exemplars of such responses appear in Table 3.

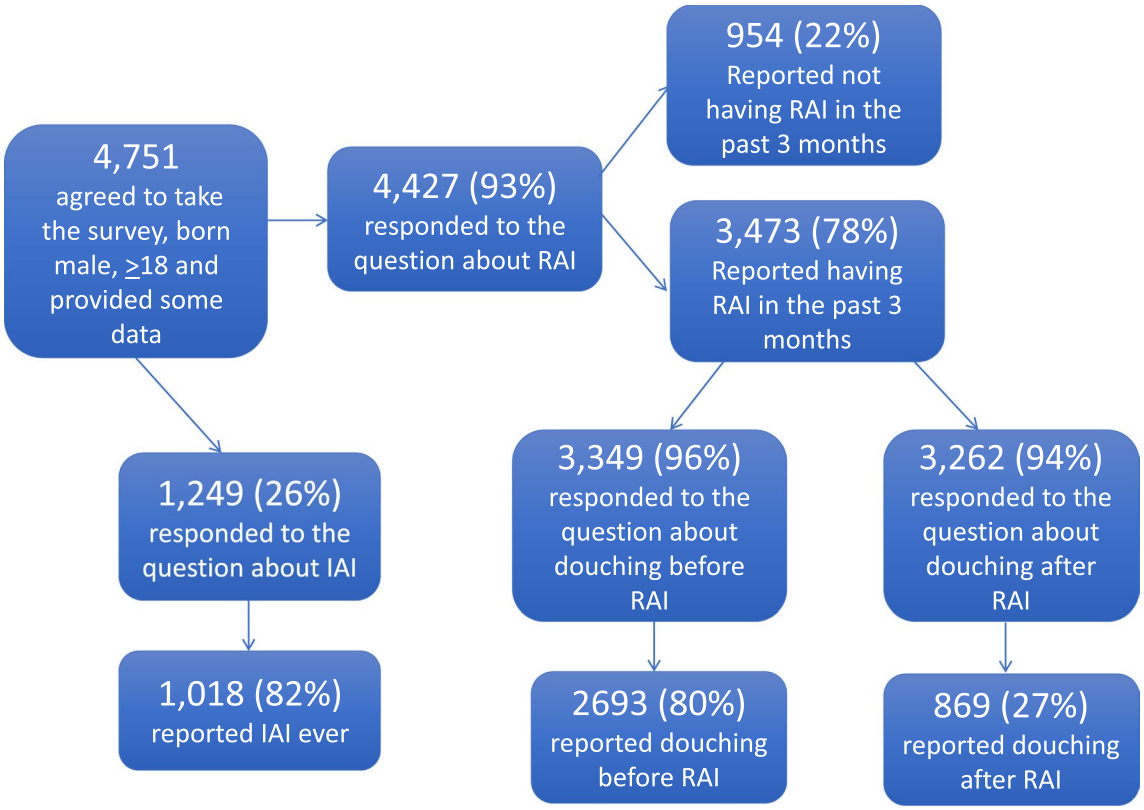

Fig. 2 Subsamples of participants answering different survey questions

Discussion

The goal of the present study was to provide quantitative detail on specific behavioral aspects of rectal douching associated with AI that could contribute to the development of a behaviorally-congruent HIV-prevention rectal microbicide douche. Several of our findings show that microbicide douches could become an important HIV-prevention tool.

First, our results show that 82% of respondents who had RAI in the 3 months prior to our survey had douched before, after, or both before and after RAI. This confirms the popularity of douching reported in prior studies. [18] Furthermore, of the individuals who douched, 98% indicated that they would definitely (70%) or probably (28%) use a douche with anti-HIV properties. It could be argued that self-reports on likelihood to use an HIV-prevention product have correlated poorly with actual use in microbicide studies. [28] The main difference in the case of the present study is that respondents report already using rectal douches, and provided that the microbicide douche does not significantly differ from regular rectal douches in its characteristics or mode of administration, using a microbicide douche could come down to choosing one douche over another. We could draw a parallel with individuals choosing to use fluoride toothpaste instead of a non-fluoride one once they learn that the former not only delivers the hygiene expected but also prevents disease. History shows that once a toothpaste with cosmetic and therapeutic benefits was developed, it quickly dominated the market [29].

Second, of the 18% of our respondents who had RAI but had not used rectal douches in the prior 3 months, the majority said the reason was not having thought about it or not feeling the need for a douche, something modifiable through education and availability of a microbicide douche. The fact that 94% of non-douchers indicated they were likely to use a microbicide douche may be encouraging.

Third, the conceptual models proposed for microbicide acceptability and adherence underscores the importance of partner support [25]. In our survey, 95% of those who engage in IAI stated they would support a partner using an HIV-prevention douche, and two-thirds of these respondents had a history of requesting that their partners use rectal cleansing douches.

Furthermore, while HIV infection may occur during the use of PrEP, the douche will likely create minimal risk for selection of resistant HIV for several reasons: oral and vaginal dosing are associated with very low risk for tenofovir resistance to date, rectal tenofovir dosing results in very low systemic exposure compared to oral dosing, and rectal tenofovir use is likely to be only episodic with

Table 2 Behavior and attitudes of DREAM Internet Study respondents (N = 4751)

Subsample	Behavior/attitude	Mean (SD) N[a] (Range)
All respondents	Receptive anal intercourse (RAI) occasions, past 3 months	8.01 (33.25) 4427 (0–1000[b])
Respondents who report RAI	Douche before RAI	7.25 (27.15) 3349 (0–1000[b])
	Douche after RAI	1.61 (5.70) 3262 (0–100)
		N[a] (%)
Respondents who douche before or after RAI	**Reasons for douching**[c]	2675 (100%)
	I wanted to be clean	2602 (97%)
	I didn't want to smell	1731 (65%)
	Douching enhances sexual pleasure	648 (24%)
	Douching might prevent infections	274 (10%)
	My sex partner suggested it	170 (6%)
	Device used[c]	2664 (100%)
	Enema bottle	1331 (50%)
	Rubber bulb	1145 (43%)
	Hose attached to faucet	853 (32%)
	Liquid used[c]	2652 (100%)
	Tap water	2349 (89%)
	Commercial douche	678 (26%)
Respondents who douche before RAI	**Timing of douche before sex**	2588 (100%)
	Less than 10 min	128 (5%)
	10–30 min	872 (34%)
	30–60 min	1117 (43%)
	More than 1 h	471 (18%)
	Number of douche applications before sex	2587 (100%)
	1	532 (21%)
	2	565 (22%)
	3	661 (26%)
	4	316 (12%)
	5 or more	513 (20%)
Respondents who douche after RAI	**Timing of douche after sex**	816 (100%)
	Less than 10 min	214 (26%)
	10–30 min	279 (34%)
	30–60 min	184 (23%)
	More than 1 h	139 (17%)
	Number of douche applications after sex	809 (100%)
	1	448 (55%)
	2	225 (28%)
	3	83 (10%)
	4	30 (4%)
	5 or more	23 (3%)
Respondents who use more than 1 douche application	**Time between first and second applications**	2051 (100%)
	Less than 30 s	305 (15%)
	30–60 s	697 (34%)
	1–2 min	586 (29%)
	2–5 min	339 (17%)
	More than 5 min	124 (6%)

Table 2 (continued)

		N[a] (%)
Respondents who douche before or after RAI	**Time until liquid is expelled**	2581 (100%)
	Less than 15 s	517 (20%)
	15–30 s	830 (32%)
	30–60 s	630 (24%)
	1–2 min	385 (15%)
	More than 2 min	219 (9%)
	Would use rectal douche to prevent HIV transmission	2580 (100%)
	Definitely not	17 (1%)
	Probably not	34 (1%)
	Probably yes	734 (28%)
	Definitely yes	1795 (70%)
Respondents who do not report RAI[d]	**Ever had insertive anal intercourse (IAI)**	1249 (100%)
	Yes	1018 (82%)
	No	231 (19%)
Respondents who report IAI	**Ever asked partner to use douche before IAI**	1006 (100%)
	Yes	637 (63%)
	No	369 (37%)
	Reaction if partner used rectal douche to prevent HIV transmission	841 (100%)
	Supportive	800 (95%)
	Opposed	6 (1%)
	Neither	35 (4%)
Respondents who did not douche before or after RAI	**Reasons for not douching**[c]	586 (100%)
	Never thought about it	329 (56%)
	Don't feel any need	171 (29%)
	Might be harmful to health	85 (15%)
	Cramps/side effects	37 (6%)
	Would use rectal douche to prevent HIV transmission	575 (100%)
	Definitely not	10 (2%)
	Probably not	21 (4%)
	Probably yes	208 (36%)
	Definitely yes	336 (58%)

4751 were eligible, agreed to continue, and provided relevant data

[a]Total Ns are those among the subsample with non-missing data

[b]Three participants reported 1000 occasions of RAI (next highest value = 537) and 1 participant reported 1000 occasions of douching before RAI (next highest value = 800)

[c]Multiple responses allowed

[d]Includes 313 who did not answer the question regarding frequency of RAI

brief exposures rather than sustained or fluctuating levels associated with oral dosing.

By definition, an on-demand, behaviorally-congruent HIV prevention douche should be similar in its formulation and its mode of use to what is most popular among its potential users. Thus, a desirable product profile for a rectal douche should consider the following behaviors:

- Given that most people use enema bottles or a rubber bulb filled with tap water, a liquid or powder that could be added to and dissolved in tap water would be ideal.
- Given that the number of applications used varies and that 21% of respondents on average use only one application before RAI and 33% of respondents reported using 4 or more cleansing douches, for an ideal HIV-prevention

Table 3 Qualitative results for DREAM Internet Survey (N = 4751)

Respondent's behavior	Reason to use an HIV prevention rectal douche	Examples
Has RAI	To prevent HIV	Would make me feel like I'm doing everything I can to prevent HIV
		The condom can break so I think using the HIV prevention douche is a plus
		Would be crazy not to use it if available
	Better than PrEP/convenience	I don't want to have to take PrEP daily and I'd rather have another option other than condoms
		I feel an anal douche would be more effective in that you can use it as you need rather than ingesting a pill, like PrEP
		I love bareback but I can't take PrEP… DREAM is a dream no doubt
		On prep now and having side effects. Knee cramps and weight gain fat deposits
	Two for one	I would use it because it's a twofer. It keeps the area clean and prevents HIV
		Having both cleanliness and prevention would be ideal
		Best of both worlds
		I always worry about being clean down there before anal sex, so if there was a douche that could clean and prevent HIV then that would be awesome
		I think it is a brilliant concept. It would emphasize the point to remain clean and sanitary while also maintaining a clean bill of health
		Sounds simple and kills two birds with one stone… or douche
		It's dual purpose. I'd totally start douching if it meant I could also help prevent HIV
Has IAI	Two for one	An enema that can keep him clean and prevent either of us from contracting something nasty is definitely going to receive my support. This is the definition of good, clean fun!
		This would take care of 2 of my main worries with anal sex
		Why not? It cleans in there and protects at the same time? Genius
	Convenience/better than PrEP	As long as it had been shown to be very effective it would be great. Then they wouldn't have to take a daily pill if they weren't at a daily risk of infection
		Sounds better than taking daily pills
	HIV prevention—for both	Because I'm positive but undetectable but would want them to feel secure
		Because it keeps both of us safe
		That would be a game changer—put my mind at ease

douche to provide sufficient protection within a user's typical number of douching applications, the product dose will need to be easily adjusted to douche number in order to avoid a complex sequence of medicated and non-medicated douches.

- Should douche number not be adjustable, given that one-third of respondents reported using 4 or more cleansing douches before intercourse, a strategy needs to be devised to ensure that too many microbicide douche applications do not result in toxicity and, conversely, that tap water douches applied after the microbicide douche (or the use of a faucet-attached douching device) do not remove the drug from the mucosa. To deal with this challenge, ideally, individuals should be allowed to follow their regular cleansing routines and to use the microbicide douche afterwards. Those who use devices attached to a faucet would also use a microbicide douche dispensed in an enema bottle afterwards.
- Should the product not be scalable with regard to dose, then the effect on drug delivery resulting from non-medicated douches preceding or following a medicated douche should be well understood to advise users regarding product sequencing with typical douching products.
- Faucet-attached douches, important to some respondents in our survey, will require additional device development to deliver the product in a fully behaviorally-congruent way.
- Given that expulsion of the douche liquid happens quickly, under 30 s for 52% of respondents, penetration of the drug into the mucosa needs to happen within this time frame.
- Given that 82% of participants reported douching within 1 h before intercourse, the microbicide douche would need to deliver protective levels of drug to the mucosa in this time frame.
- Given that some people douche after RAI, it would be ideal if a rectal douche could act as post-exposure prophylaxis. This could be especially useful in situations in which an individual did not anticipate RAI and may not have prepared for it.

- Given that individuals who have RAI but do not douche reported that they never thought about it or did not feel the need to do it, yet most of them still said they would be likely to use a microbicide douche, there seems to be an openness among individuals who do not douche to modify their behavior.
- Given that selection of douche product is a highly personal decision with wide options, a rectal microbicide for use as douche should be compatible with a wide variety of commercial douche products and demonstrate as much as part of clinical evaluation.

Although this list of recommendations stems from the behaviors reported by douche users, it may not be possible to develop a douche fully matching this ideal profile. It may be necessary to explore the feasibility of introducing and supporting behavior modifications that may lead to the successful use of a microbicide douche (e.g., whether individuals who cleanse using faucet-attached hoses would be willing to use a bottle-applied microbicide douche).

An important limitation to this study is that it was limited to the U.S. and participants were recruited using a single social media app (Grindr) whose subscribers may be different from individuals that could be recruited through different means. Also, they self-selected to participate in the survey. However, given the large sample size of MSM who responded to our survey, their geographic spread covering all 50 US states and some of its territories, and the similarity in the proportion of ethnic/racial minorities among our respondents to that of the latest US census, there is an increased likelihood that our findings may be generalizable. Furthermore, the sample size is large enough to offer parameters of ongoing rectal douching practices on which to build a behaviorally-congruent microbicide douche. More research is needed to understand douching behaviors globally among individuals whose AI practices may expose them to HIV.

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Compliance with Ethical Standards

Conflicts of interest None to declare.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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