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The vaginal environment: studying parameters which influence microbicide cytotoxicity. B.J. Catalone[a], F.C. Krebs[a], S.R. Miller[a], D. Malamud[b], M.K. Howett[a], and B. Wigdahl[a]. [a]Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, PA, USA and [b]Department of Biochemistry, University of Pennsylvania School of Dental Medicine, and Biosyn, Inc., Philadelphia, PA, USA

Continued increases in heterosexual transmission of human immunodeficiency virus type 1 (HIV-1) have fueled efforts to develop a female-controlled topical vaginal microbicide which is effective against HIV-1 and other sexually transmitted pathogens. In evaluating potential vaginal microbicides, parameters of the vaginal environment, such as protein, pH, osmolarity, and cervical mucous must be considered as they may alter the efficacy of the microbicide. In these studies, the effect of protein on nonoxynol-9 (N-9), C31G, and sodium dodecyl sulfate (SDS) cytotoxicity was examined using two human epithelial cell lines of cervical origin (HeLa-CD4-LTR-β-gal and ME-180). In experiments using ME-180 cells, the impact of protein on microbicide cytotoxicity was examined using serum-free media containing bovine serum albumin (BSA) at concentrations up to 10 mg/ml (equivalent to the protein content of 10% serum). At N-9, C31G, or SDS concentrations that resulted in 50% cell viability after exposure for 8 h in serum-free media, all three microbicides were equally cytotoxic after 8 h in serum-free media containing BSA at 0 to 10 mg/ml. The addition of BSA up to 5 mg/ml resulted in decreases in microbicide cytotoxicity compared to cells maintained in the absence of BSA. Above 5 mg/ml BSA, no further decreases in microbicide cytotoxicity were observed. These data demonstrate that under these conditions (pH ~7, 37°C), protein in the media at concentrations up to 5 mg/ml appears to lessen the cytotoxicity of N-9, C31G, and SDS. However, protein does not differentially impact microbicide cytotoxicity, suggesting that the effect of protein is independent of the agent or its mechanism of action. Future experiments will examine effect of other parameters including pH, osmolarity and cervical mucous.

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Sensitivity of HIV-1-susceptible immune cell targets to topica microbicides nonoxynol-9 (N-9), C31G, and sodium dodecy sulfate (SDS). S.R. Miller[a], F.C. Krebs[a], B.J. Catalone[a], D Malamud[b], M.K. Howett[a], and B. Wigdahl[a]. [a]Department o Microbiology and Immunology, Penn State College of Medicine Hershey, PA, USA, and [b]Department of Biochemistry, University of Pennsylvania School of Dental Medicine, and Biosyn, Inc. Philadelphia, PA, USA

HIV-1 transmission during heterosexual vaginal intercourse ha become an important aspect of the AIDS epidemic. Efforts t reverse this disturbing trend include the development of nontoxi broad-spectrum female-controlled microbicides for use durin intercourse. We have shown that microbicides nonoxynol-9 (N-9) C31G, and sodium dodecyl sulfate (SDS) are effective in vitr against HIV-1. Furthermore, exposure of these agents to vagina and cervical epithelial cells results in concentration-dependen cytotoxicity. Topical microbicides must also be evaluated for thei effect on immune cells that may serve as hosts for HIV-1 infectio within the genital tract as well as viral transport vehicles durin intercourse. We assessed the cytotoxicity of each agent after cell of monocytic and lymphocytic origin were exposed for 10 min, h, 8 h, or 48 h. We have shown that monocytic (U937 and THP-1 and lymphocytic cell lines (SupT1 and Jurkat) were very similar i their sensitivity to each agent. Experiments using primar monocytes and primary mixed CD4- and CD8-positive T cell demonstrated comparable sensitivity to N-9, C31G, or SDS. SD was consistently the least cytotoxic in all experiments. We als examined the hypothesis that HIV-1 infection might affe microbicide cytotoxicity. By comparing the viability of HIV- (strain IIIB)-infected SupT1 cells to uninfected SupT1 cells, w demonstrated that HIV-1 infection increased the sensitivity c SupT1 cells to N-9, C31G, or SDS exposure. These results sugge that exposure to N-9, C31G, or SDS may result in preferenti killing of HIV-1-infected cells, providing an additional margin c protection against transmission of HIV-1 during sexu intercourse.

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Sodium dodecyl sulfate (SDS) and C31G as effective microbicidal alternatives to nonoxynol-9 (N-9): Comparative cytotoxicity in primary human vaginal keratinocytes. F.C. Krebs[a], S.R. Miller[a], B.J. Catalone[a], P.A. Welsh[a], D. Malamud[b], M.K. Howett[a], and B. Wigdahl[a]. [a]Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, PA, USA, and [b]Department of Biochemistry, University of Pennsylvania School of Dental Medicine, and Biosyn, Inc., Philadelphia, PA, USA

An ideal topical vaginal microbicide must be minimally toxic to cells within the vaginal epithelium, including vaginal keratinocytes, as well as highly active against a variety of sexually transmitted disease pathogens. We assessed the sensitivity of primary human vaginal keratinocytes and the human cervical epithelial ME-180 cell line to potential topical vaginal microbicides nonoxynol-9 (N-9), C31G, and sodium dodecyl sulfate (SDS). Expression of epithelial cell-specific keratin proteins on both cell types was verified by immunofluorescence and FACS analyses. Experiments that compared agent cytotoxicity during a continuous, 48 h exposure demonstrated that primary vaginal keratinocytes were almost 10-fold more sensitive to N-9 compared to either C31G or SDS. Under identical conditions, ME-180 cells were less sensitive to N-9, C31G, or SDS exposure than primary keratinocytes. To evaluate the effect of multiple microbicide exposures on cell viability, primary vaginal keratinocytes were exposed to N-9, C31G, or SDS three times during a 78 h period. Although each of the three microbicide applications contributed equally to the total reduction in cell survival, C31G was considerably more cytotoxic at lower concentrations than N-9 or SDS within the range tested. When cytotoxicity over a 48 h period was examined, exposure to C31G for 18 h resulted in levels of cytotoxicity not achieved by either N-9 or SDS until 24 to 48 h. These results reveal interesting differences in time- and concentration-dependent cytotoxicity of N-9, C31G, and SDS, and suggest that primary cell types may be more effective indicators of in vitro microbicide cytotoxicity than continuous cell lines.

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Novasomes and sodium dodecyl sulfate (SDS) enhance the microbicidal efficacy of nonoxynol-9. F.C. Krebs[a], B.J. Catalone[a], S.R. Miller[a], D.C. Wright[b], M.K. Howett[a], and B. Wigdahl[a]. [a]Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, PA, USA and [b]Novavax Biologics and Research Division, Novavax, Inc., Rockville, MD USA

Development of topical vaginal microbicide products containing nonoxynol-9 (N-9), a widely used spermicidal agent, may reduce the risk of male-to-female transmission, an increasingly important component of the global AIDS epidemic. However, N-9 as well as other microbicides are associated with vaginal irritation, inflammation, and limited efficacy against some sexually transmitted disease (STD) pathogens. In this regard, our in vitro experiments have demonstrated N-9 cytotoxicity at concentrations well below those required for HIV-1 inactivation. Experiments which compared the sensitivity of human cervical HeLa cells to N-9 or sodium dodecyl sulfate (SDS) showed that N-9 was up to 450-fold more cytotoxic than SDS. In explorations of more efficacious N-9-based microbicides, we demonstrated that incorporation of N-9 into Novasomes (paucilamellar, non-phospholipid liposomes) resulted in a mixed microbicide (containing 4% N-9) that was less cytotoxic than N-9 alone. Novasome mixtures containing twice as much N-9 (8% N-9) were even less cytotoxic than either N-9 or the mixture containing 4% N-9. N-9-containing Novasome preparations were also measurably more capable of HIV-1 inactivation than equivalent concentrations of N-9. Addition of SDS, which may provide activity against non-enveloped viruses such as human papillomaviruses, did not adversely affect the cytotoxicity of N-9-containing Novasome preparations or the ability of N-9 to inactivate HIV-1. These investigations suggest that microbicides characterized by greater effectiveness against HIV-1 (and other STD pathogens) and less cytotoxicity than N-9 may be designed by combining N-9, Novasomes, and SDS. Future investigations will examine the relationship between microbicidal efficacy and variations of N-9, SDS, and Novasome content.