ORIGINAL RESEARCH ARTICLE

Safety Studies of a Recently Developed Microbicidal Contraceptive Gel (UniPron) in Female Baboons (*Papio anubis*)

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ABSTRACT

To identify any toxicity on the vaginal epithelium, liver and kidney following UniPron administration, ten healthy female olive baboons (*Papio anubis*) of reproductive age and of proven fertility were used. Five baboons were each treated with 15g of UniPron intravaginally twice a week for 20-weeks and venous blood collected before and after each treatment. Venous blood was collected from five control animals as in the experimental females, but these control animals were not given any treatment. The endpoints that were evaluated included clinical chemistry profiles on kidney and liver functions and vaginal histopathology. Female baboons treated with 15g of UniPron intravaginally showed no detectable adverse effects on clinical chemistry profiles investigated and vaginal histopathology. Repeated intravaginal exposure of female baboons to UniPron did not induce detectable vaginal irritation and there were no detectable histological changes. We conclude that administration of UniPron into baboon vagina did not cause any detectable toxicity *(Afr J Reprod Health 2009; 13[4]:95-104).*

RÉSUMÉ

Etudes pour déterminer la sécurité d'un gel contraceptif microbicide (UniPron) chez les babouins (Papio Anubis) qui viennent d'être mis au point. Pour identifier la présence de la toxicité sur l'épithélium vaginal, la foie et le rein suite d'une administration d'UniPron, nous avons utilisé dix babouins (Papio Anubis) olivâtres en bonne santé qui étaient en âge de procréer et d'une fécondité confirmée. Cinq babouins ont été soignés chacune au 15g d'UniPron par voie intravaginale deux fois par semaine pendant 20 semaines et le sang veineux a été collecté avant et après chaque traitement. Le sang veineux a été collecté de cinq animaux témoin comme dans le cas des femelles d'expérience, mais ces animaux témoin n'ont pas été traités. Les résultats qui ont été analysés ont compris les profils chimiques clinques sur les fonctions de la foie et du rein et l'histopathologie vaginale. Les babouins femelles qui ont été soignées au 15g d'UniPron par voie intravaginale n'ont pas eu d'effets négatifs détectables sur les profils chimiques étudiés et sur l'histopathologie. L'exposition intravaginale répétée des babouins femelles a UniPron n'a pas déclenché l'irritation vaginale détectable et il n'y avait pas de modifications histologiques détectables. Nous avons conclu que l'administration d'UniPron dans le vagin du babouin n'a causé aucune toxicité détestable *(Afr J Reprod Health 2009; 13[4]:95-104).*

KEYWORDS: HIV/AIDS, UniPron, Microbicide, Safety, Toxicity

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Introduction

With the number of unwanted/unplanned pregnancies on the rise, there is a need for safe, effective and reliable contraceptives now more than ever before. Overpopulation, particularly in developing countries, is complicated by the pandemic of sexually transmitted infections (STIs) including human immunodeficiency virus (HIV) [1]. As the HIV/AIDS epidemic continues to grow, people must find a way to protect themselves against HIV and other STIs. The high incidence of these infections is owing to heterosexual intercourse, and the infections spread more readily from men to women than from women to men[2-3]. Male or female condoms used correctly and consistently is the only available method shown to be effective in preventing both unwanted pregnancies and STI including HIV[4-5]. A dramatic increase in the use of condoms, however, reflects concerns about STIs including human immunodeficiency virus[6]. Women often have little power to negotiate the use of condoms with their partners and are unable to protect themselves from nonconsensual coercive sex[7-8]. Hence, a desperate need exists to provide people with new, easy to use, safe and affordable methods of protection that will allow women to take the necessary measures without having to negotiate with their partners.

A number of contraceptive options are available for women of reproductive age, including hormonal contraceptives, vaginal spermicides, intrauterine devices, barrier methods and tubal ligation[6]. Due to an increased incidence of HIV transmission, contraception research is beginning to focus on the development of spermicidal agents with antiviral activity [9]. Vaginal contraceptive products have been available for many years and some contain the membrane surfactant (e.g. nonoxynol-9 (N-9)) as one of the main ingredients [10]. However, the major drawback of using N-9 or other surfactants is their detergent-type cytotoxic effect on vaginal cells [11]. In addition to its poor efficacy, the use of N-9 has been associated with the development of lesions in the vaginal and cervical epithelia [9]. A compound with a combination of anti-HIV and sperm-immobilizing activity devoid of toxicity to vaginal mucosa and systemic toxicity would prove to be an ideal vaginal microbicidal contraceptive agent. Since these compounds would probably be used repeatedly over decades, an ideal spermicidal microbiocide should have an established safety record without genital epithelial toxicity [10]. Vaccines against HIV and immunocontraceptive vaccines have proved elusive for a long time of research. Topical microbicidal spermicides are now widely considered, as they would ideally provide a convenient, readily available method of self-protection against STIs in addition to preventing pregnancy. However, a major challenge has been to design mechanism-based microbicides that are highly effective against pregnancy and STIs/HIV infections while lacking detergent-type effects on epithelial cells and normal vaginal flora [7]. Both contraceptive and non-contraceptive

microbicides are currently under investigation [12-13].

A dual protection vaginal microbicidal contraceptive that is inexpensive and capable of preventing STIs including HIV as well as being a new option for family planning is therefore desirable.

In studies to assess the suitability of various anti–human antibodies directed against immunocompetent cells to identify components involved in cellular and humoral immune responses in the immune organs of a female baboon, most anti–human antibodies have been found to cross react with the baboon antigens [14]. The same reagents have been used to evaluate the immunobiology of its reproductive tract. The distribution of immune cells in the reproductive tract of the female baboon was found to be comparable to that in the human [14]. These studies demonstrate the potential for this primate to be used as a model for study of human reproductive biology.

Our study was designed to determine whether UniPron administration into the baboon vagina induces any toxicity on the vaginal epithelium, liver and kidney.

Materials and Methods

UniPron

UniPron is a clear fluffy, acidic and non-detergent lubricating gel. The product is heavily buffered with a pH of 3.4 and contains a number of raw materials and compounds. The buffering agent in UniPron is carbomer (carboxyvinyl polymers of high molecular weights). UniPron therapeutic classification is antifertility and/or microbicide agent. The product is very stable at both room (approximately 22 ^{o}C) and body temperature and has a shelf life of at least 24 months. UniPron was formulated and developed in partnership with a Pharmaceutical company, Universal Pharmaceutical Corporation Limited and is patented, Patent KE 218. Our recent studies have demonstrated that UniPron is a fully effective non-hormonal reversible contraceptive in baboon [15]. UniPron mechanism of action is lowering vaginal pH from 5.0 to 3.4 immediately after administration and maintaining the acidity for about 3 hours after which the pH returns to the normal range without causing any detectable irritation on vaginal epithelium[15]. UniPron has the ability to preserve an acidic vaginal microenvironment due to its highly buffered pH of 3.4. Like the BufferGel, a vaginal defense enhancer [16], UniPron is also a lubricant. In this study, 15g (≈15ml) of UniPron was determined to be the appropriate dose based on the baboon vaginal surface area.

Animals

10 healthy sexually mature cycling female olive baboons (*Papio anubis*) from the Institute of Primate Research, Karen were used in this project. The study protocol was approved by the Institutional "Animal Care and Use Committee" and "The Scientific and Ethical Review Committee" of the Institute of Primate Research. These committees are guided by the

Institutional Guidelines as well as the International Regulations including those of the National Institute of Health (NIH), Primate Vaccine Evaluation Network (PVEN) and Helsinki Convention. Feeding regimens and clinical evaluation on a daily basis were adhered to as per the standard protocols at the institute. The animals were housed in individual cages facility available at the institute.

Toxicity Study

10ml of blood was collected from five female baboons, which had not been given any treatment. The blood, collected from the femoral vein, was kept at room temperature for 2-3 hours and then centrifuged (JOUAN C422, USA) at 1610 revolutions per minute (rpm) for 10 minutes to obtain sera. The sera from these animals were used as control samples.

Five female baboons were used for experimental studies. UniPron was administered into the vagina of each animal twice a week for twenty weeks. The animals were immobilized by intramuscular injection with a mixture of Ketamine hydrochloride before sampling (KetasetR, Fort Dodge Laboratories Inc., Fort Dodge, Iowa) and xylazine (Chanazine, Chanelle Pharmaceuticals Manufacturing Ltd., Galway, Ireland), after which a sterile speculum was used to open up the vagina and 15g of UniPron administered using a syringe. This was done twice a week for twenty weeks. There was no leakage and there was complete gel retention since the volume administered was based on the baboon's vaginal surface area. 10ml of blood was collected from each animal twice a week for twenty weeks during the period of UniPron administration. Blood was also collected at the end of the final week of UniPron administration. The blood was kept at room temperature for 2-3 hours then centrifuged to obtain sera. All the sera were aliquoted into 2ml vials and kept at -20^0C until use.

Clinical Chemistry Profiles

All the tests were performed using reagents and methods provided by the manufacturer in a kit (Max-Planck-ring 21 – D 65205 Wiesbaden – Germany). Biochemical analysis was performed using CE6600 Multimode Computing UV Spectrophotometer, (Cambridge England). The aliquoted sera were used for the analysis of total protein (TP-g/L), albumin (ALB-g/L), blood urea nitrogen (BUN-mmol/L), Creatinine (CRE-µmol/L), aspartate aminotransferase (ASTµ/L), alanine aminotransferase (ALT-µ/L), alkaline phosphatase (ALP-µ/L), and total bilirubin (TBIL-µmol/L).

Histopathology

Vaginal biopsies for histopathological studies were taken from all animals in the UniPron treated and control groups after 20 weeks of the experiment. The biopsies were taken from either the right or left lateral sides of the vagina. Histological sections of the biopsy tissues were processed using paraffin wax method, stained with haematoxylin and eosin, and examined under light microscopy as

reported elsewhere [15]. The pathologist was blind to the origin of the individual biopsies examined.

Clinical Observation

During every UniPron application, a gynaecologist opened up the vagina of the baboon using a sterile speculum and observed the vaginal wall up to the cervix for any visually detectable changes.

Statistical Analysis

Statistics was done using InStat version 3.06. Differences were considered statistically significant at $p<0.05$.

Results

Blood Chemistry Findings

Analysis of blood chemistry parameters for female baboons revealed no significant differences between UniPron treated and the control group with respect to TP, ALB, CRE, AST, ALT, ALP and TBIL. The kidney function (BUN and CRE) and liver function (TBIL, AST, ALT, ALP, TP and ALB) were not adversely affected by the intravaginal administration of UniPron (Tables 1 and 2).

Histopathology

Histopathological results of biopsies obtained from UniPron treated and control baboon vaginal tissues clearly showed that UniPron is non-toxic and did not induce any detectable vaginal irritation. There were no detectable histological changes in any of the ten biopsies examined. All the animals had normal histology comprising of the non-keratinized stratified squamous epithelia, well-vascularized lamina propria, muscularis mucosae and adventitia for each group of baboons. For each animal, two to four independent fields per section were examined in 40 random tissue sections from ten different animals. The representative images obtained at x100 magnification are shown in Figure 1 and Figure 2.

Clinical Manifestations

The clinical manifestations were normal during the different menstrual cycle stages. Mild monilial infection and mild cervical erosion was observed in some animals in both the control and experimental groups. The monilial infection was self-curing and could not be attributed to UniPron application.

Discussion

There is an urgent need to develop a product that can be administered vaginally to offer dual protection before sexual intercourse to kill HIV and at the same time disable or kill sperm. Research efforts to develop topical microbicidal contraceptive for intravaginal use for prevention of pregnancy and sexually transmitted infections have been ongoing for nearly a decade [17]. Women need a product that they can control and even

Table 1: Blood Chemistry profiles for *P.anubis* given 15g of UniPron intravaginally

Test Component	Sample Points (Weeks)					
	0 (Before Application)	2	4	6	8	10
Total Protein	6.8±1.2	6.5±2.2	5.7±1.6	8.2±2.1	7.9±3.1	7.7±1.5
Albumin	5.4±0.7	4.2±1.7	4.2±1.2	4.7±1.3	4.8±0.6	4.2±0.6
Urea	54.7±5.7	53.4±12.7	54.8±21.8	63.3±7.2	61.3±21.1	55.0±13.7
Bilirubin	0.4±0.5	0.3±0.4	0.3±0.4	0.6±1.1	0.1±0.2	0.3±0.4
Creatinine	1.0±0.7	1.5±0.7	0.6±0.9	1.1±0.5	1.8±0.9	0.9±1.5
Alkaline Phosphatase	208.3±71.8	187.7±54.0	197.7±125.6	205.8±99.1	235.1±86.1	239.6±63.9
Alanine Aminotransferase	51.5±16.9	43.6±14.3	26.1±32.2	34.07±19.0	24.4±37.5	32.8±8.5
Aspartate Aminotransferase	33.1±5.6	39.6±2.7	36.3±12.5	37.7±8.3	37.27±7.1	33.8±6.8

Mean values of parameters evaluated from experimental animals for liver (TBIL, AST, ALT, ALP, TP and ALB) and kidney (BUN and CRE) function tests for ten weeks. The values are presented as mean±SD. Analysis was performed using One-way ANOVA with post-test. Column 0 was used as the control sample point, p >0.05, was considered not significant.

Table 2: Blood Chemistry Profiles for the Experimental and Control Animals (n=5)

Test Component (Units mentioned in Materials and Methods)	Experimental Animals Mean±SD	Control Animals Mean±SD
Total Protein	6.5±2.0	6.4±1.5
Albumin	4.3±1.0	4.8±0.4
Urea	56.8±13.9	55.2±15.0
Bilirubin	0.8±1.2	0.2±0.2
Creatinine	2.0±2.3	2.2±1.1
Alkaline Phosphatase	221.2±87.4	244.9±99.2
Alanine Aminotransferase	27.9±2.5	40.2±7.5
Aspartate Aminotransferase	43.3±8.0	62.1±41.1

Samples were taken every week for 20 weeks of UniPron administration from both groups and the mean±SD for each group determined. p >0.05 at 95% confidence interval was considered not statistically significant. The values fall within the normal range of baboon clinical parameters [29] indicating that UniPron did not cause any significant changes in the baboon model.

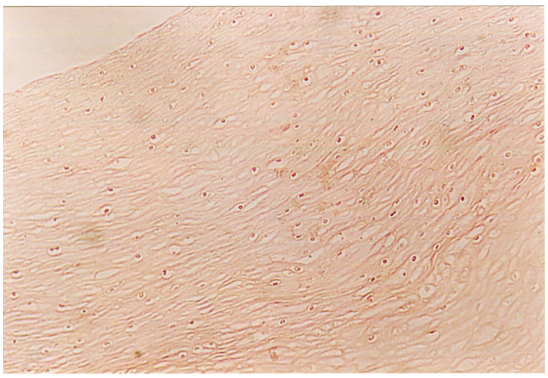

Figure 1: Vaginal biopsy tissue obtained from control animal during early follicular stage
Normal vaginal tissue from the female Olive Baboon (×100). Female Olive Baboons were not treated with UniPron for the 20 week experimental period. After 20 weeks, biopsies were taken from the vagina of the control animals. The biopsies were transported to the lab and fixed in 10% formaldehyde. Tissues were then embedded in paraffin and sections were subjected to routine hematoxylin and eosin staining as reported in other studies [15].

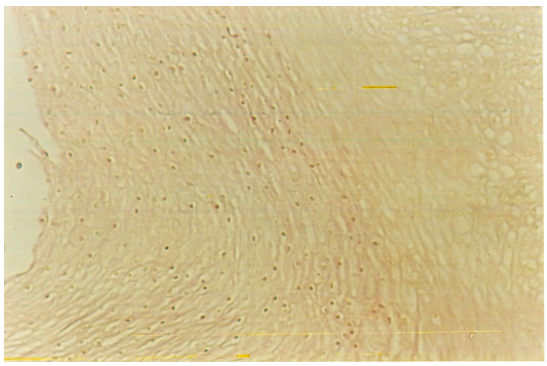

Figure 2: Vaginal biopsy tissue obtained from an experimental animal during early follicular stage
Effect of UniPron on vaginal epithelium (×100). Vaginal tissue from control (see Figure 1) and UniPron treated female baboons (see Figure 2) were processed using paraffin wax method, stained with haematoxylin and eosin, and examined under light microscopy. Representative tissue sections from different animals within each treatment group are shown. There were no detectable histological changes and no indication of vaginal irritation following UniPron treatment for 20 weeks.

use without their partner's consent as some women lack the power to ensure consistent and correct condom use [18]. The safety of any microbicidal contraceptive is an important parameter to consider when developing such products due to their use on a regular basis. Since insufficient vaginal lubrication during sex is a common problem [19], exogenous lubrication may improve sexual intercourse by increasing sexual pleasure of both partners [20]. In this study, we have evaluated the olive baboon as a possible model to study the potential effects and

toxicity of UniPron on human reproduction. Intravaginal administration of UniPron, a potent, dual-function anti-HIV and spermicidal agent, given to female baboons twice a week for five months caused no detectable adverse effects on clinical chemistry profiles and histopathology. The kidney and liver functions as well as nutrition status were not affected adversely by UniPron exposure and there were also no detectable histological changes. At present, probably all commercially available spermicides have detergent ingredients that disrupt cell membranes. The spermicidal activities of these surfactants are associated with their structural affinity to the membrane lipids [21-22]. Therefore, the major draw back of using N-9 or other surfactants is their detergent-type effect on epithelial cells and normal vaginal flora. Frequent use of N-9 as a vaginal contraceptive has been associated with an increased risk of vaginal or cervical infection, irritation, or ulceration [23-26]. Other products with acid buffering properties that are being tested for microbicidal and contraceptive potential include ACIDFORM [27-28]. For any of these products to be of real benefit as microbicides and contraceptives, they need to be not only effective with no side effects but also affordable.

Our previous studies showed that UniPron is a fully effective non-hormonal reversible contraceptive that does not interfere with normal vaginal microflora including Lactobacilli in baboon [15]. While microbicides may not completely address power imbalances, they could give women another option to reduce their vulnerability not only against STIs including HIV/AIDS, but also against pregnancy. In this study, the use of UniPron gel was found to be safe in the baboon model. UniPron could be a potential candidate for use as a microbicidal contraceptive and it should be tested as a microbicide in the prevention of HIV infection.

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