Development of a Nanoporous Elastomere Intra-Vaginal Ring (IVR) for the Sustained Release of Non-Hormonal Contraceptives

Saxena Brij B1, Koldras Kristen E1, Singh Mukul1, Nguyen Nancy1, Rathnam Premila1, Ledger William J1 and Lerner Sidney2

Abstract

The goal of this investigation is the development a new biocompatible intra-vaginal ring (IVR) composed of nanoporous poly (diol citrate) elastomers for a sustained release of non-hormonal contraceptives, namely ferrous gluconate to cause spermiosistasis, ascorbic acid to increase cervical mucus viscosity, and a mixture of ampholines and/or poly L-glutamic acid (PGLA) buffer to sustain the pH at 4 to cause spermiosistasis within 30 seconds, for the prevention of undesired pregnancy. The IVR of 5.5 cm in diameter and a rim of 0.5 cm is cast in a mold. The daily eluates of the IVR are tested for pH, spermiosistatic and/or spermicidal activity and effect on the viscosity of cervical mucus. Results of this study are efficacious in vitro; the IVR has a shelf life of one year and provides the basis for the newly developed IVR containing non-hormonal contraceptives for in vivo use in women to prevent undesired pregnancy as well as pelvic and HIV infection simultaneously or HIV infection alone.

Keywords: Intra-Vaginal Ring (IVR); Non-hormonal contraceptives; Nanoporous elastomeric polymer hydrogel

Significance: Development of a biocompatible, intra-vaginal ring (IVR), composed of nanoporous elastomere hydrogel for the delivery of non-hormonal contraceptives and/or anti-HIV agents. The IVR will be an effective multipurpose prevention technology (MPT) for protection against both undesired pregnancy and sexually transmitted HIV infection. The novel IVR is a reliable and easy-to-use device that can prevent the side effects of long-term hormonal contraceptive use. It can improve women’s lives by providing a better quality of maternal and fetal health, especially in regions where unsafe sex is the largest cause of disease in females. It is an innovative approach for family planning and protection against sexually transmitted infections that women can control themselves. The drugs and IVR are made of compounds that are already FDA approved for human use.

Introduction

Contraception is the term used to describe methods used for preventing undesired pregnancy. Hormonal methods include combined oral contraceptives (COC), mini pills, injections, implants, hormonal intrauterine devices (Mirena), and the hormonal vaginal ring (NuvaRing®) once a month [1]. Hormonal contraceptives, on the other hand, are the most convenient, but long-term use of these products can lead to an altered hormonal state, disrupt endocrine homeostasis, and are associated with increased incidence of cervical cancer [2]. Non-hormonal methods are natural family planning methods, the withdrawal technique (coitus interruptus) [3], male condoms, cervical caps, female condoms and spermicide gels [4]. There remains a significant failure rate mainly due to improper use of these methods. Female condoms are also available but not very popular. Surgical procedures, namely male and female sterilization, are invasive, costly, and difficult to reverse. Notwithstanding the development in the methods of family planning, the paradox exists that the rates of undesired pregnancy, pregnancy termination, and pelvic diseases, including HIV, are increasing globally. To overcome the above and to provide a safe and efficacious means of protection the intra-vaginal ring (IVR) containing non-hormonal agents described here can be an effective non-hormonal method that can provide protection from month to month to coincide with the menstrual cycle when women may change to a new ring. This new IVR, as compared to the earlier HEMA and acacia intra-vaginal rings [5,6], provide a better cost effective, reliant mode of family planning that benefits maternal-fetal health and overall quality of life and may also be used to deliver microbicides [7].

Materials and Methods

Preparation and properties of nanoporous poly (diol citrate) elastomeric hydrogel IVR

The nanoporous elastomere IVR (Figure 1) retained its flexibility for one year (Figures 2 and 3) indicating shelf life for ease of use: The IVR rings are made by using nanoporous elastomeres of biodegradable, hydrophilic, and biocompatible polymer hydrogel using poly (diol citrates). The biodegradable nano-pores act to control the release rates of the drugs [8,9], 1,8-Octanediol, citric acid and ascorbic acid were used in a 10:10:1 molar ratio. First, monomers

Figure 1: Nanoporous elastomere Intra-Vaginal Ring (IVR) of 5.5 cm in diameter with a rim of 0.5 cm, with no contraceptives.
were mixed and heated at 165°C for 10 minutes, followed by 140°C for an additional 60 minutes for prepolymerization. The formed poly-1,8-octanediol-co-citric acid-co-ascorbate (POCA) prepolymer was dissolved in 100% ethanol and purified by precipitation in a minimum 5:1 water:prepolymer solution ratio, followed by lyophilization for 3 days. The POCA prepolymer was dissolved at a 30% w/w solution in 100% ethanol and ferrous gluconate (0.8 g per ring), ascorbic acid (0.7 g per ring), and pharmalyte (0.5 cc per ring) was mixed in by simple dissolution of the compound in the prepolymer solution. This mixture was then left overnight for ethanol evaporation and the resulting, viscous polymer poured into a metal mold for a ring with inner diameter of 55 mm and cross-sectional diameter of 5 mm. The ring was post-polymerized for 4 days at 80°C, after which the ring was gently removed from the mold and washed with ethanol (3x).

In vitro and in vivo evaluation of poly-diol citrates have shown cell and tissue compatibility [10]. Ferrous gluconate and ascorbic acid was purchased from Sigma-Aldrich Co., and the mixture of polyamino-polycarboxylic acid (Ampholines) was purchased from Amersham Biosciences (Uppsala, Sweden) and Poly L-Glutamic Acid (PLGA):sodium bicarbonate buffer was supplied by the Guillin Peptide Technology Limited, China, for use as a pH stabilizer.

Collection of daily eluates of the IVR and analysis of release rates [5,6]: The IVR is placed in a petri dish containing 10 ml of phosphate buffered saline (PBS) or vaginal fluid stimulant [11]. The petri dish is closed airtight and placed on a shaker at low speed. Eluates are collected daily and the eluant is replenished over a 30 day period. The eluates are ultra-filtered and stored at 20°C until analyzed. The IVR is placed in a petri dish containing 10 ml of phosphate buffered saline or vaginal fluid stimulant [11] in vitro showed little difference in the release rates of non-hormonal contraceptives of the intra-vaginal ring (IVR) is also efficacious in vitro after a year of shelf life. The daily eluates of the IVR in either phosphate buffer saline (PBS) or vaginal fluid stimulant [11] in vitro showed little difference in the concentrations. The daily release of ferrous gluconate and ascorbic acid of the IVR in 2010 and 2011 for 30 days is shown in Figures 4A, 4B and 5A, 5B, respectively, along with the cumulative release of both ferrous gluconate (Figures 6A and 6B) and ascorbic acid (Figures 7A and 7B). Daily concentration of ferrous gluconate in 2010 and 2011 showed little change and sustained at an average daily release concentrate.

Results

Comparison of daily eluates of IVR initially and after one year of storage

The IVR after one year of shelf life retained its flexibility and physical and mechanical properties (Figures 2 and 3). The daily release rate of non-hormonal contraceptives of the intra-vaginal rings (IVR) is also efficacious in vitro after a year of storage. The daily eluates of the IVR in either phosphate buffer saline (PBS) or vaginal fluid stimulant [11] in vitro showed little difference in the concentrations. The daily release of ferrous gluconate and ascorbic acid of the IVR in 2010 and 2011 for 30 days is shown in Figures 4A, 4B and 5A, 5B, respectively, along with the cumulative release of both ferrous gluconate (Figures 6A and 6B) and ascorbic acid (Figures 7A and 7B). Daily concentration of ferrous gluconate in 2010 and 2011 showed little change and sustained at an average daily release concentrate.
rate of 1.396 mg/mL over 34 days and 1.455 mg/mL over 30 days, respectively. Cumulative concentration of ferrous gluconate was sustained with a total amount of 40.522 mg/mL in 2010 and 47.482 mg/mL in 2011 over 34 days. Daily concentration of ascorbic acid for 2010 and 2011 decreased only slightly from a daily average release rate of 0.197 mg/mL to 0.164 mg/mL over 34 days. Cumulative concentration of ascorbic acid showed little change after storage for one year with a total amount of 6.698 mg/mL in 2010 to 5.600 mg/mL in 2011 over 34 days. Hypothetical daily and cumulative release rates show a slight decrease, however, it is noteworthy to point out that the pooled eluates from the ring in 2010 and 2011 are still equally effective. Daily pH of eluates from 2010 and 2011 showed little change with a daily average of 4.4 to 4.3 over 34 days shown in Figures 8A and 8B, respectively. The pooled eluates of the IVR showed complete spermiostasis within 25 seconds up to 30 days (Figures 9A and 9B). It may be mentioned that the IVR eluates did not inhibit the growth of Lactobacilli in the vaginal flora obtained from healthy women, which is required for a healthy vaginal environment.

**Discussion**

With the world’s population at 7 billion and increasing, it is imperative to find a global balance between people and available resources. Lack of family planning is one of the pivotal causes of socio-economic issues that adversely affect maternal-fetal health, quality of life, and exposure to infectious diseases. Similarly, sexually active women not using contraception continue to be at risk for both unintended pregnancy and sexually transmitted diseases (STDs), including HIV. The intra-vaginal ring (IVR) is incorporated with non-hormonal contraceptives, namely ferrous gluconate, L-ascorbic acid, and mixtures of polyamino-polycarboxylic acid (Ampholines) or Poly L-Glutamic Acid (PLGA):sodium bicarbonate buffer to sustain a pH of 4.0. The compounds utilized are non-hormonal and are FDA approved for human use [5,6]. The contraceptives are delivered locally and bypass the systemic route to be effective, thus avoid adverse effects of long term use of hormones. In addition, anti-HIV agents, such as tenofovir (PAMPA) and Boc-lysinated betulonic acid (Boc-LBA) which are soluble in aqueous medium [15], may also be incorporated into the IVR. These microbicides act at different sites of viral life cycle, have low systemic absorption, and are nontoxic to the vaginal mucosa, thus they have the highest potential in preventing sexual transmission of HIV when used in combination at low doses to minimize the drug resistance [7]. The IVR may allow a compliant, safe, and effective method that can empower women for protection against undesired pregnancy, HIV, and/or pelvic infections.

During the development of the IVR, three objectives were identified as being necessary to inhibit sperm from fertilizing the ovum. The basis for localized non-hormonal contraception by ferrous gluconate, ascorbic acid, and a pH modifier, is to cause complete spermiostasis in less than a minute. Human spermatozoa are enriched with unsaturated fatty acids susceptible to lipid peroxidation, which is a type of cellular damage involving the formation of free oxygen radicals. When the bilayer of sperm tails are exposed to ferrous ion,
the propagation of lipid peroxidation leads to continuous formation and decomposition of lipid peroxides and complete spermiostasis [16-20]. Any systemic absorption of ferrous gluconate may compensate for the loss of blood during menstruation in undernourished or malnourished women. Ascorbic acid is a vitamin and reduces the disulfide bonds in mucopolysaccharides of the cervical mucus to increase its viscosity to change it from an open cellular structure, found at midcycle of the menstrual period, to the closed structure in order to form an impenetrable barrier for sperm motility. A mixture of polyamino-polycarboxylic acid (ampholines) and/or Poly L-Glutamic Acid (PLGA) sodium bicarbonate buffer sustain the vaginal pH at 4, which is healthy for vaginal flora, but lethal to sperm [21]. The IVR allows for coital independence without affecting normal gonadal functions. The contraceptive agents are released by the IVR over a 30 day period, act locally, and prevent undesired pregnancy. To inhibit the heterosexual transmission of HIV infection, the IVR can simultaneously deliver microbicides along with non-hormonal contraceptives to protect women from both undesired pregnancy and/or HIV infection.

The efficacy of these non-hormonal contraceptives has been amply demonstrated in vitro on human sperm and cervical mucus, as well as in vivo in rabbits [5]. In another study, women who used the formulation for 4 weeks showed complete spermiostasis with no adverse effects, irritation, or inflammation of the vaginal mucosa or phallic skin [22]. Safe and reliable use of the IVR will require the cooperation of both sexual partners. The IVR will also be cost effective for developing countries where inability of family planning often leads to insufficient nutrition and susceptibility to infectious diseases due to an overall decrease in quality of life.

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References


Author Affiliations

1Well Cornell Medical College, Department of Obstetrics and Gynecology, 515 East 71st Street, S-412, NY 10021, USA
2BioRings, LLC, 215 Lexington Avenue Room 1001, NY 10016, USA