An Intravaginal Ring for the Sustained Delivery of Antibodies

MANJULA GUNAWARDANA,[1,2] MARC M. BAUM,[1] THOMAS J. SMITH,[1,2] JOHN A. MOSS[1]

[1]Department of Chemistry, Oak Crest Institute of Science, Pasadena, California
[2]Auritec Pharmaceuticals, Inc, Pasadena, California

Received 14 March 2013; revised 23 July 2014; accepted 6 August 2014

Published online 17 September 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24154

ABSTRACT: Human monoclonal antibodies (mAbs) based on IgG and IgA have shown promise as topical microbicide candidates to protect women from HIV infection. Application of mAbs has been limited, however, by the inability of vaginal gels and conventional intravaginal ring (IVR) designs, the predominant vaginal product formulations, to effectively deliver biomolecules in a coitally independent fashion with retention of bioactivity. We have developed a novel pod-IVR platform that delivers ovine IgG (ov-IgG) as a model for IgG and IgA human mAbs. *In vitro* release of ov-IgG from the pod-IVRs was sustained for 14 days. Facile control of release rate was achieved by changing the size of delivery channels in the ring structure, and the feasibility of ov-IgG delivery in the range 0.5–30 mg day^{-1} from a 10-pod IVR was demonstrated. The activity of ov-IgG in pod-IVR formulations was maintained as confirmed by ELISA binding assay. Pod-IVRs delivering ov-IgG show promise for the effective sustained topical delivery of antibody-based microbicides. This significantly broadens the range of microbicides that can be delivered in a sustained fashion from IVRs and enables a new arsenal of topical biologic microbicide candidates beyond small molecule antiretrovirals. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3611–3620, 2014

Keywords: drug delivery systems; HIV/AIDS; mucosal drug delivery; controlled delivery; macromolecular drug delivery; lyophilization; poly(lactic/glycolic) acid (PLGA or PLA)

INTRODUCTION

The estimated global HIV incidence is more than 34 million, and over 2.5 million HIV-1 infections are still acquired annually despite significant efforts in the development of broad-spectrum microbicides and an effective vaccine.[1] Both tenofovir (TFV) gel[2,3] and oral tenofovir disoproxil fumarate (TDF) and emtricitabine combination[4] microbicides have shown promise in preventing sexual HIV transmission in clinical trials, but trial failures of other microbicide candidates[5–9] indicate that new effetive and safe microbicide candidates are needed urgently. The first candidates studied for topical HIV prevention were broadly acting, nonspecific microbicides such as nonoxynol-9,[5,6] λ- and κ-carrageenan (Carraguard),[8] or naphthalene sulfonate polymer (PRO 2000 gel).[7] More recently, microbicide efforts have focused on antiretroviral drugs such as TFV,[2–4] dapivirine,[10] or MIV-150.[11] Antiretrovirals target-specific stages of the virus lifecycle such as viral entry (CCR5 agonists), viral DNA replication (reverse transcriptase inhibitors), or viral genome insertion (integrase inhibitors). The high concentrations of these compounds in gel formulations have the potential for adverse safety effects by damaging the highly sensitive cervico-vaginal mucosal tissues, and CCR5 agonists are not active against X4 and dual tropic viruses.[12]

As an alternative, antibody-based microbicides applied topically to the vagina may play an important role in protecting women from HIV infection, from both efficacy and safety perspectives.[12] The broadly neutralizing human monoclonal antibodies (bNAbs) b12,[13,14] 2G12,[15] 2F5,[16] and 4E10[16] have demonstrated efficacy against SHIV infection in macaque models. These bNAbs neutralize a diverse range of primary HIV-1 isolates,[12,17] and more potent bNabs against a wider range of HIV-1 isolates have subsequently been identified including PG9, PG16, VRC01, and multiple PTG bNAbs.[18–21] The bNAb VRC01 protected against HIV-1 vaginal transmission in a mouse model and is the first *in vivo* demonstration of bNAb efficacy in human target cells.[12] The target of a bNAb microbicide is not limited to HIV: passive immunization against herpes simplex virus-2 (HSV-2) by FcRN-transported IgG delivered to the female genital tract was obtained in a mouse model.[22]

The practical application of bNABs as a topical microbicide has thus far been limited by the inability of gels and conventional intravaginal ring (IVR) designs,[23] the predominant topical vaginal product formulations, to effectively deliver biomolecules in a coitally independent fashion with retention of antibody bioactivity. Morrow et al.[24] developed an insert vaginal ring for delivery of hydrophilic and macromolecular drugs and demonstrated release of the antibody 2F5, but the delivery was only sustained over a maximum of 5 days with limited control of release rate. The pod-IVR,[25] a novel modular ring design consisting of polymer coated solid drug cores (pods) incorporated into a silicone IVR, was specifically designed for simultaneous delivery of multiple drugs, and in particular, relatively hydrophilic antiviral agents that are difficult to release from traditional matrix and reservoir IVRs. In pod-IVRs, the release rate for each drug pod is controlled independently, determined by the size of one or more delivery channels that are mechanically formed in the elastomer backbone during fabrication, as well as the pod's biocompatible polymer coating and the total number of pods per IVR. The pod-IVR platform has been applied to delivery of TFV,[26,27] TDF,[27] and acyclovir (ACV)[28] individually and in combination.[29] Pod-IVRs have the capability for simultaneous delivery of drug combinations spanning a wide-range of physicochemical properties. A five-drug multipurpose protection pod-IVR that simultaneously delivers, with independently controlled release rates, three

Correspondence to: John A. Moss (Telephone: +626-817-0883; Fax: +626-817-0884; E-mail: j.moss@oak-crest.org)

Journal of Pharmaceutical Sciences, Vol. 103, 3611–3620 (2014)

antiretroviral drugs against HIV (TFV, nevirapine, and saquinavir) and a progestin–estrogen contraceptive (etonogestrel and estradiol) have been developed and its pharmacokinetics investigated in a sheep model.[30] Described here is the application of the pod-IVR platform to delivery of mAbs and other highly water-soluble biological molecules using the ovine IgG (ov-IgG) antibody as a model for IgG and IgA human mAbs.

MATERIALS AND METHODS

Preparation of IgG Antibody Solid Formulation

Protein G purified sheep IgG (ov-IgG, >95% pure) was obtained from Innovative Research (Novi, Michigan) as a 4.7 mg mL^{-1} solution in pH 7.4 phosphate buffer (PBS, 0.02 M sodium phosphate, 0.15 M NaCl). A 12 mL aliquot of ov-IgG solution was freeze dried to obtain ~200 mg of a white, voluminous powder containing approximately 56 mg ov-IgG (29%), 105 mg NaCl (54%), and 21 mg Na_2HPO_4 (11%) and 11 mg Na_2HPO_4 (6%). The freeze-drying cycle consisted of initial flash freezing of the solution by immersing the lyophilizer flask in liquid nitrogen followed by drying on a Virtis Freezemobile 12SL (SP Industries, Warminster, Pennsylvania) at 0.1 Torr for 16 h.

Manufacture of Silicone Pod-IVRs

Intravaginal rings of the pod-IVR design containing ov-IgG were prepared using methods previously reported.[25] Briefly, cylindrical cores of 40 mg dry ov-IgG solid formulation (3.2 mm diam. × 2 mm ht.) were formed using compaction with a pellet press (Globe Pharma MTCM-I, North Brunswick, New Jersey). The compressed ov-IgG cores were coated with two layers of poly(D,L-lactide) (PLA, 10–18 kDa, ester-terminated) (Resomer R 202 S; Evonik Industries AG, Essen, Germany) from a 5% PLA solution in 2:1 dichloromethane:ethyl acetate to form ov-IgG pods. A 6 μL aliquot of PLA solution was dropped on one flat end of the cylindrical core using an automatic pipette and allowed to dry. The core was inverted and a second 6 μL aliquot applied to the opposite flat end. After drying at room temperature for ~4 h, a second PLA layer was applied using the same technique. The PLA-coated ov-IgG pods were embedded in silicone ring segments with one delivery channel per pod (channel diameter 0.75–2.0 mm) as described previously.[25] Delivery channel diameters reported here are the punch size used to form the channel. The measured channel diameter has been shown previously to agree closely with the punch diameter.[25]

In Vitro Studies

Studies to measure the *in vitro* release of ov-IgG into a simplified vaginal fluid simulant (VFS) were carried out on IVRs containing one ov-IgG pod. The VFS was adapted from Owen and Katz[31] and consisted of 25 mM acetate buffer (pH 4.2) with NaCl added to yield a 200 mOs solution. For all *in vitro* release studies, the IVRs were placed in glass vials containing 10 mL VFS at 25 ± 2°C and with shaking at 60 rpm on an orbital shaker. Aliquots of the release medium were removed at specified time intervals and analyzed by UV absorption spectroscopy at 280 nm (OD_{280}) using a Spectramax Plus384 96-well plate reader (Molecular Devices, Sunnyvale, California). The concentration of ov-IgG in the release solution was calculated using the Beer-Lambert law and the absorption coefficient 1.36 mg^{-1} cm^{-1} mL provided by the manufacturer on the product datasheet. Data fitting was carried out by nonlinear least squares minimization using Excel (Microsoft, Redmond, Washington) software.

Fluorescence Imaging of PLA Polymer Coatings

The thickness and uniformity of the PLA coating was evaluated by adding a Rhodamine 6G fluorescent dye to the coating solution and imaging sections of pods using fluorescence microscopy. Rhodamine 6G perchlorate (Acros, Geel, Belgium) was dissolved in dichloromethane and an aliquot added to the 5% PLA solution to obtain a dye concentration of 20 mM. Pods were coated with the fluorescent PLA solution as described above. The cylindrical pods were embedded in silicone and cut on two different cross-sections parallel and perpendicular to the round cylindrical pod faces for imaging. Low magnification (6×) light images were acquired using a 6X-50X stereo zoom microscope (Edmund Optics, Barrington, New Jersey) with a Nikon Coolpix 995 digital camera. Fluorescence images were acquired with an EVOS fl fluorescence microscope (AMG, Mill Creek, Washington) using a filter set that overlaps the Rhodamine 6G absorption ($\lambda_{max} = 530$ nm) and emission ($\lambda_{max} = 552$ nm) bands [EVOS RFP light cube: $\lambda_{ex} = 530$ nm, 40 nm bandwidth; $\lambda_{em} = 593$ nm, 40 nm bandwidth]. Thickness of the coating was measured using the ImageJ software package (National Institutes of Health, Bethesda, Maryland).[32,33] The number of pixels per micrometer for each image was determined using the Set Scale function of ImageJ and the calibrated scale bar provided by the EVOS fl software. The thickness of the fluorescent PLA film in each image was determined using the Measure function in ImageJ at 10 points equally spaced across the pod surface shown in each image. Thickness data is reported as mean ± SD for the set of ten measurements.

ELISA Measurements

The binding activity of ov-IgG was analyzed by ELISA. Rabbit $F(ab')^2$ Anti-sheep IgG (coating antigen) and Rabbit Anti-sheep IgG(H+L)-HRP (detection antibody) were obtained from SouthernBiotech (Birmingham, Alabama). MaxiSorp 96-well flat-bottom immune plates, wash buffer (1× PBS), Tween 20, TMB substrate solution, and SEA BLOCK blocking buffer were purchased from Thermo Scientific (Rockford, Illinois). ELISA plates were prepared by sequentially incubating overnight at 4°C with 100 μL per well of coating antigen (2 ng μL^{-1} in 1× PBS), 1 h at room temperature with 200 μL per well of SEA BLOCK blocking buffer, 1 h at room temperature with 100 μL of standard or sample, and finally 100 μL of detection antibody diluted 1:5000 in wash buffer. Plates were washed three times with wash buffer between each incubation step. Plates were developed by adding 100 μL TMB substrate solution to each well, followed by 50 μL 4N H_2SO_4 once color development was observed in low concentration standards. The absorption at 450 nm (OD_{450}) was measured using a SpectraMAX Plus384 micro plate reader. All ov-IgG samples were adjusted to a concentration of 0.41 mg mL^{-1} as determined by OD_{280} then further diluted 1:200 prior to ELISA measurement. For each ELISA experiment, a single plate was used for all ov-IgG standards and samples. Samples and standards were measured in triplicate. Standards at eight concentrations from 0 to 1000 ng/mL (as determined by OD_{280}) were prepared from a new batch of ov-IgG purchased within 1 week of the ELISA measurement. Plots of standard OD_{450} from ELISA versus concentration from