Journal of Controlled Release 235 (2016) 147–154

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Evaluation of microparticulate ovarian cancer vaccine via transdermal route of delivery

Suprita A. Tawde [a,*,1], Lipika Chablani [b], Archana Akalkotkar [c], Martin J. D'Souza [d]

[a] Akorn Pharmaceuticals, Research and Development, 50 Lakeview Parkway, Suite 112, Vernon Hills, IL 60060, USA
[b] Department of Pharmaceutical Sciences, St. John Fisher College, 3690 East Ave, Rochester, NY 14618, USA
[c] Wil Research Laboratories LLC, 1407 George Rd, Ashland, OH 44805, USA
[d] Vaccine Nanotechnology Laboratory, Department of Pharmaceutical Sciences, Mercer University College of Pharmacy and Health Sciences, 3001 Mercer University Drive, Atlanta, GA 30341, USA

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 25 April 2016
Accepted 25 May 2016
Available online 26 May 2016

Keywords:
Microparticles
Ovarian cancer
Vaccine
Transdermal
Spray drying

ABSTRACT

Ovarian cancer is the fifth most commonly occurring malignancy in women, with the highest mortality rate among all the gynecological tumors. Microparticulate vaccine can serve as an immunotherapeutic approach with a promising antigenic delivery system without a need for conventional adjuvants. In this study, a microparticulate vaccine using whole cell lysate of a murine ovarian cancer cell line, ID8 was prepared by spray drying. Further, the effect of interleukins (ILs) such as IL-2 and IL-12 was evaluated in a separate study group by administering them with vaccine particles to enhance the immune response. The vaccine microparticles were administered to C57BL/6 female mice via transdermal alone and in combination with the oral route. The transdermal vaccine was delivered using a metallic microneedle device, AdminPen™. Orally administered microparticles also included an M-cell targeting ligand, *Aleuria aurantia* lectin, to enhance the targeted uptake from microfold cells (M-cells) in Peyer's patches of small intestine. In case of combination of routes, mice were given 5 transdermal doses and 5 oral doses administered alternatively, beginning with transdermal dose. At the end of vaccination, mice were challenged with live tumor cells. Vaccine alone resulted in around 1.5 times tumor suppression in case of transdermal and combination of routes at the end of 15th week when compared to controls. Inclusion of interleukins resulted in 3 times tumor suppression when administered with transdermal vaccine and around 9 times tumor suppression for the combination route of delivery in comparison to controls. These results were further potentiated by serum IgG, IgG1 and IgG2a titers. Moreover, CD8 + T-cell, CD4 + T-cell and NK (natural killer) cell populations in splenocytes were elevated in case of vaccinated mice. Thus, vaccine microparticles could trigger humoral as well as cellular immune response when administered transdermally and via combination of route of delivery. However overall, vaccine administered with interleukins, via combination of route, was found to be the most efficacious to suppress the tumor growth and lead to a protective immune response.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Ovarian cancer is the most lethal gynecological cancer and the fifth most leading cause of cancer related deaths in women in the US [1,2]. The National Cancer Institute (NCI) has estimated 21,290 new cases and 14,180 deaths due to ovarian cancer in the US in 2015. When cancer incidences are compared worldwide, the mortality rate associated with ovarian cancer was found to be relatively high in the US and Europe [3]. Since it is very difficult to detect an ovarian cancer, especially in the early stages, it is referred to as a 'silent killer'. Only about 10% of ovarian cancers are usually found in the early stages. Patients with epithelial tumors, which account for approximately 90% of ovarian cancer, generally have poor overall survival and the 5-year survival for stages III–IV of these tumors is about 29.1% [4]. The first-line treatment for advanced ovarian cancer involves surgery to remove the tumor, followed by chemotherapy. However, the cancer relapses within relatively short periods of time even after treatment. It has been reported that up to 75% of patients responding well to the initial treatments face tumor relapse within 18–28 months [5]. Moreover, chemotherapeutic treatments for cancer are toxic and/or of minimal therapeutic value. Therefore, alternative approaches such as immunotherapy are being investigated to prevent relapse of cancer. Several vaccines are underway in clinical trials and most of them have not progressed beyond phase I/II studies [6,7].

* Corresponding author at: 50 Lakeview Parkway, Vernon Hills, IL 60060, USA.
E-mail address: suprita25@gmail.com (S.A. Tawde).
[1] Permanent Address: Department of Pharmaceutical Sciences, Mercer University College of Pharmacy and Health Sciences, 3001 Mercer University Drive, Atlanta, GA 30341, USA.

http://dx.doi.org/10.1016/j.jconrel.2016.05.058
0168-3659/© 2016 Elsevier B.V. All rights reserved.

Various proteins and peptides have been approved or are being evaluated in clinical trials for treatment of cancer. Due to limited oral bioavailability of such antigens, injectable routes of administration are currently being used. Scientists have been exploring the potential of delivering vaccine antigens orally or transdermally as these delivery routes have ease of administration, are non-invasive and patient compliant. Transdermal delivery is considered as the best route for vaccine administration because of the skin-associated lymphoid tissue which comprises of Langerhans cells, dermal dendritic cells, lymph nodes and subsets of T-lymphocytes. Microneedles have been used to pierce the upper layer-stratum corneum of the skin to enhance transdermal delivery by promoting the transport of macromolecules that cannot be delivered across the skin by passive diffusion alone [8,9]. Microneedles are micron-sized needles, which upon insertion into the skin result in formation of aqueous conduits forming a passage for the vaccine antigens towards the immune-competent skin layers. Due to their short needle length, they avoid contact with the nerve endings in the dermis thus remain to be a painless mode of immunization [10–12].

In addition, the microparticulate delivery system has several advantages over the usage of the antigens alone. Particulate antigens have been proven to be more immunogenic than soluble antigens [13,14]. Improved uptake of the particles compared to the solution results in higher cytotoxic T-lymphocytes (CTLs) response against the cancer cells. The antigen presenting cells (APCs) in the body easily phagocytose these microparticles recognizing them as an antigen and generate an immune response [15]. Further, they are drained into the nearby lymph nodes where they activate various other immune cells. Thus, the particulate delivery systems may mimic pathogens that are commonly recognized, phagocytosed and processed by professional antigen- presenting cells (APC) [16,17]. When administered transdermally, the microparticles are taken up by the immune cells in the skin, which trigger mucosal as well as systemic immune response [10]. Langerhans cells are dendritic cells that activate T cells and induce a strong immune response and occupy around 20% of the skin's area. On the other hand, M-cells are the microfold cells, which act as sampling ports for any foreign entities encountered in the small intestine upon oral administration [18–23]. These M cells house various dendritic cells and immune cells in them. Once the oral vaccine particle is sampled by M-cells, it is processed by a dendritic cell/antigen presenting cell (APC) and presented on MHC (major histocompatibility complex) Class I or MHC Class II molecules [24,25]. The antigens are further recognized by the immune cells in the vicinity leading to the cascade of an immune response. The immune response also includes humoral response by plasma B-cells, which leads to production of antibodies and their class switching. The role of B-cells has been debatable in past but a recent study by Mahmoud SM et al. shows that the humoral immunity is important in addition to cell-mediated immunity in prognosis of breast cancer [26]. Thus, we aim to trigger both humoral and cell-mediated immune response through this prophylactic cancer vaccine, which can impart resistance against tumor challenge. Moreover, the microparticulate drug delivery system can be used to assimilate various antigens in one delivery system that can reduce the number of doses as well as reduce the different vaccination regimen [13,14].

In this study, we have investigated whether vaccination with microparticles containing the ovarian cancer antigens can prevent/retard ovarian cancer growth. A murine ovarian cancer cell line, ID8 was used as a source of antigens for vaccine preparation. The cell line correlates closely to human ovarian cancer cell lines in signaling pathways and results in development of tumor in mice models similar to human ovarian cancer. Thus, ID8 cell line provides a unique model to study the immune response developed by the vaccine against the initiation and progression of ovarian cancer in mice with an intact immune system [2]. Therefore, we proceeded with a whole cell lysate of ID8 cells to prepare the vaccine for this study. Despite of advancement in recombinant technology and gene expression, the whole cell lysate vaccine still remains a very promising approach. Whole cell lysate provides a pool of tumor-associated antigens (TAAs) which can induce both CD8 + and CD4 + T cells [27].

In our previous study, microparticulate vaccine was found to be efficacious when administered orally [23]. Therefore, we aimed to evaluate the microparticulate vaccine via transdermal route alone and in combination with oral route. By combination route of administration, aim was to achieve merits of both oral and transdermal immunization [28]. The vaccine particles were administered for this purpose using a microneedle device called as AdminPen™. For this purpose, microparticles were prepared by spray drying technique using methacrylic copolymer Eudragit® FS 30 D and hydroxyl propyl methyl cellulose acetate succinate (HPMCAS) as described elsewhere [20,23]. These polymers have been reported their applications for transdermal delivery in form of patches as well as particulates [29,30]. Several others have mentioned their usage for oral sustained or controlled release delivery [31,32]. To target the vaccine formulation to M-cells in the Peyer's patches of the intestine upon oral delivery, M-cell targeting agent, *Aleuria aurantia* lectin (AAL) was used in the formulation [15,20,21]. In addition, immunostimulatory molecules such as IL-2 and IL-12 were added in order to enhance the overall potency of the formulated vaccines. Oral delivery was performed by using an oral gavage. Transdermal delivery was achieved using an AdminPen™ device comprised of an array of 43 metallic microneedles of 1100 nm length in 1 cm sq area of circular microneedle array made of SS316 stainless steel (as shown in Fig. 1). In the present study, we demonstrate and compare the efficacy of the vaccine formulation which was administered via two different approaches based on route of administration: (1) transdermal and (2) combination of transdermal and oral route in vivo in mouse tumor model.

2. Materials and methods

2.1. Materials

ID8 cell line was kindly provided by Dr. Katherine Roby, Kansas University Medical Center, Kansas City, KS. Six to eight week-old C57BL/6 female mice were purchased from Charles River Laboratories, Wilmington, MA. HPMCAS was purchased from AQOAT, FMC Biopolymers, Philadelphia, PA. Eudragit® FS 30 D was generously gifted by Evonik industries, Parsippany, NJ. Mouse plasma was obtained from Biochemed, Winchester, VA. AAL was obtained from Vector Labs, Inc., Burlingame, CA. Recombinant murine interleukins, IL-2 (5×10^6 units/mg) and IL-12 (1×10^7 units/mg) were purchased from Peprotech Inc., Rocky Hill, NJ. Flow cytometry cell markers were purchased from eBioscience, San Diego, CA. Goat anti-mouse HRP-IgG and anti-IgG subtypes were purchased from Bethyl Laboratories, Montgomery, TX and Sigma, St. Louis, MO respectively. AdminPen™ device was purchased from nanoBioSciences LLC. All other materials used were of analytical grade.

2.2. Preparation and characterization of whole cell lysate of ID8 ovarian cancer cell line

The whole cell lysate of the murine ovarian cancer ID8 cells was prepared using hypotonic buffer and freeze-thaw cycles as described elsewhere [23,33,34]. The lysate obtained was stored at —80 °C until used. The whole cell lysate of ID8 cell line was characterized for total protein content using Bio-Rad DC™ protein assay. The lysate was also screened for presence of the only known marker, by western blot analysis as described elsewhere [23,35].

2.3. Preparation and characterization of vaccine microparticles

The vaccine formulation was prepared by using spray drying technique as described elsewhere [20]. Briefly, hydroxyl propyl methyl cellulose acetate succinate (HPMCAS) and Eudragit® FS 30D were

Download English Version:

https://daneshyari.com/en/article/7861589

Download Persian Version:

https://daneshyari.com/article/7861589

Daneshyari.com