UDC 615

ISSN 0428-0296

PHARMACIA

3/2013

PUBLISHED BY BULGARIAN SCIENTIFIC PHARMACEUTICAL ASSOCIATION

Pharmacia *Vol. 60* *No. 3* *pp. 1-52* *Sofia, November 2013*

PHARMACIA

Volume 60 **2013** **Number 3**

JOURNAL OF THE BULGARIAN PHARMACEUTICAL SCIENTIFIC SOCIETY

Indexed in: MEDLINE, CAplus[SM]/Chemical Abstracts, TOXCENTER, EMBASE/Excerpta Medica, PASCAL, BIOTECHNOBASE, ExtraMED[TM], SCOPUS

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Volume 60 **2013** **Number 3**

CONTENTS

DEVELOPMENT OF CANCER VACCINE FOR TREATMENT OF BREAST CANCER: TARGETING CANCER ANTIGENS TO ELICIT ANTIGEN-DIRECTED IMMUNE RESPONSE

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Abstract. Many MUC1/CA 15-3 peptide-based cancer vaccines have been designed and tested in animal models and in clinical trials. It is proposed that MUC1/CA 15-3 vaccines of the future should include both peptides and glycopeptides to stimulate multiple forms of T cells recognizing cancer. 10 adult female mice weighing 20-30g were immunized with an antigen that was prepared by emulsifying the antigen in equal volume of adjuvant aluminum hydroxide and saline. Seven mice were used as experimental animals and three as control. 100 µl of serum of breast cancer patient was mixed with 100 µl of saline and 10 µl of alum. Mixture was allowed to stand for 30 minutes at room temperature and injected intraperitonially to mice separately. Blood sample containing serum polyclonal antibodies was obtained from mice by the procedure of cardiac puncture. SDS-PAGE analysis showed that proteins present in normal human serum were in the range of 374.07-20.29 kDa molecular weight with a raw volume ranges from 775.90-30032.75. While in the serum of breast cancer patients, the proteins were in the range of 380.46-19.86 kDa with raw volume ranging 3582.65 – 24311.38. Raw volume of MUC1/CA 15-3 having MW of 280 kDa was markedly increased in serum of breast cancer patients as compared to raw volume of 280 kDa protein in serum of normal subjects. Raw volume of cancerous protein MUC1/CA 15-3 was decreased when incubated with monoclonal antibodies i.e. from 6178.58 to 1131.44. Besides, the raw volumes of other two proteins having MW 109.07 and 28.13 kDa also decreased on treatment with antibodies. The *in vivo* and *in vitro* experiment of MUC1/CA 15-3 vaccine showed that treatment of aggressive cancer with antiMUC1 antibodies may increase survival rate in breast cancer, however, hurdles must be overcome to elicit the proficient and defensive immune responses and eradicate cancers.

Key Words: Mucinous CA 15-3, Vaccine, Breast cancer

Introduction

Breast cancer has been reported as one of the most common causes of death in women. The rates of incidence and fatality due the disease are on the rise globally, even in areas that previously had low rates [1]. The highest fatality rate due to the disease is reported for women of ages 40-55 [2]. Local studies in Pakistan found a seven percent incidence rate of breast carcinoma [3]. MUC1/CA 15-3 has a core protein mass of 120-225 kDa which increases to 250-500 kDa with glycosylation [4]. Normally MUC1/CA 15-3 is expressed on the apical surface of glandular epithelial cells of GIT, lung, pancreas, breast etc [5].

MUC1/CA 15-3 is concerned in many physiological mechanisms such as cell signaling, adhesion, development and differentiation [6,7]. The intracellular part of the MUC1/CA 15-3 protein may contribute in signal transduction pathway, through multiple interactions with intracellular proteins [8]. The intracellular tail of MUC1/CA 15-3 is phosphorylated and can interact with many signaling proteins and transcriptional factors [9]. It protects the body with infection i.e. the oligosaccharides in the extracellular domain of MUC1/CA 15-3 bind with pathogen and prevent it to reach the cell surface [10,11].

The major difference between normal MUC1/CA 15-3 and tumor MUC1/CA 15-3 in addition to their levels is that tumor MUC1/CA 15-3 carries tumor-specific shorter glycan chains with a much higher portion of negatively charged sialic acids and thus could give way more immunogenic epitopes [12]. A number of reports have identified anti-MUC1/CA 15-3 immune responses in cancer patients after active immunization with MUC1/CA 15-3 [13]. Three different forms of MUC1/CA 15-3 are observed i.e.

glycosylated protein, underglycosylated protein and unglycosylated synthetic peptide. These are able to elicit MUC1/CA 15-3 specific CTL response. The efficiency of processing and the resulting strength in CTL activity correlate inversely with the degree of glycosylation [14].

Vaccination with MUC1/CA 15-3-loaded dentritic cell (DC) is becoming a major immunotherapeutic strategy for the treatment of MUC1/CA 15-3/CA positive tumors due to its high immunogenicity. Glycosylated recombinant MUC1/CA 15-3 from a eukaryotic expression system might reduce the efficacy of based DCs immunotherapies [13, 15]. Use of recombinant proteins that are purified from bacteria, and thus are unglycosylated, may be a practical approach to enhance the immunogenic potential of DCs [16,17]. It is proposed that recombinant N-terminal part (2–147 amino acids) of MUC1/CA 15-3, may provide a diverse epitope repertoire, which could be utilized as an effective tumor antigen for DC-based cancer immunotherapy [18].

Many MUC1/CA 15-3 peptide-based cancer vaccines have been designed and tested in animal models and in clinical trials. It is proposed that MUC1/CA 15-3 vaccines of the future should include both peptides and glycopeptides to stimulate multiple forms of T cells recognizing cancer [12]. A distinctive confronts of developing cancer vaccines is to either prevent or treat cancer by selecting an appropriate antigen that invokes a specific anti-tumor immune response. This evoked immune response should carry minimal toxicity and must maintain a state of anti-tumor vigilance to be effective [19].

Present study was tried to synthesize vaccine *in-vitro* using the property of breast tumour to produce mucinous antigen. Most efforts to develop cancer vaccines have focused on the treatment of established cancers, targeting cancer antigens to elicit antigen-directed immune responses. Electrophoretic technique was used to confirm the production of antibody that may be effective against antigen of CA 15-3.

Materials and Methods

10 adult female mice weighing 20-30g were obtained from the PCSIR Laboratories, Lahore. Mice were immunized with an antigen that was prepared for injection by emulsifying antigen in equal volume of adjuvant aluminum hydroxide and saline. Aluminum hydroxide was prepared as described by Revoltella and Ovary [20]. 2N aluminumsulphate solution (Fisher, Reagent Grade) was mixed with equal volume of a 2N NaOH solution until gelification and washed three times with 0.15M NaCl. A stock solution of 60mg/ml of aluminum hydroxide was prepared and kept at room temperature until use.

MUC1 Vaccine preparation

Ten adult female mice weighing were included in the study. Seven were used as experimental animals and three as control. 100 µl of serum of breast cancer patient was mixed with 100 µl of saline and 10 µl of alum. Mixture was allowed to stand for 30 minutes at room temperature and injected intraperitonially to mice separately. 100µl saline was injected to control mouse. Procedure was repeated after every 48 h for a week. Blood sample containing polyclonal serum antibodies was obtained from mice by the procedure of cardiac puncture [21].

Blood sample was centrifuged at 3,000 rpm for 1-2 min. Serum containing antibodies was separated and incubated with serum of breast cancer patient at 37^{o}C for 30 min. Incubation mixture waselectrophoresed on 10% polyacrylamide gel to observe the inhibiting effect of monoclonal antibodies on cancer antigen. SDS-PAGE was performed according to the method described by Laemmli [22]. After staining with Coomassie brilliant blue R-250 dye (Sigma Chemical Co. USA) and destaining, the gels were photographed and their images were stored for protein quantification by Gene Genius Bio-imaging Gel Documentation System. This provided the data of molecular weight and percent raw volume for each of the fraction.

Results and Discussion

Electrophoretic results of MUC1 vaccine

It was observed that molecular weights and raw volumes of proteins present in normal human serum were in the range of 374.07-20.29 kDa and 775.90-30032.75, respectively (Fig. 1). While in breast cancer patients, the proteins were observed in the range of 380.46 to 19.86 with raw volume ranging 3582.65 to 24311.38.

Study showed that the raw volume of MUC1/CA 15-3 (280 kDa protein) markedly increased in serum of breast cancer patients as compared to raw volume of 280 kDa protein in serum of normal subjects i.e. from 1107.68 to 6178.58. It was observed that the raw volume of cancerous protein MUC1/CA 15-3 was noticeably decreased from 6178.58 to

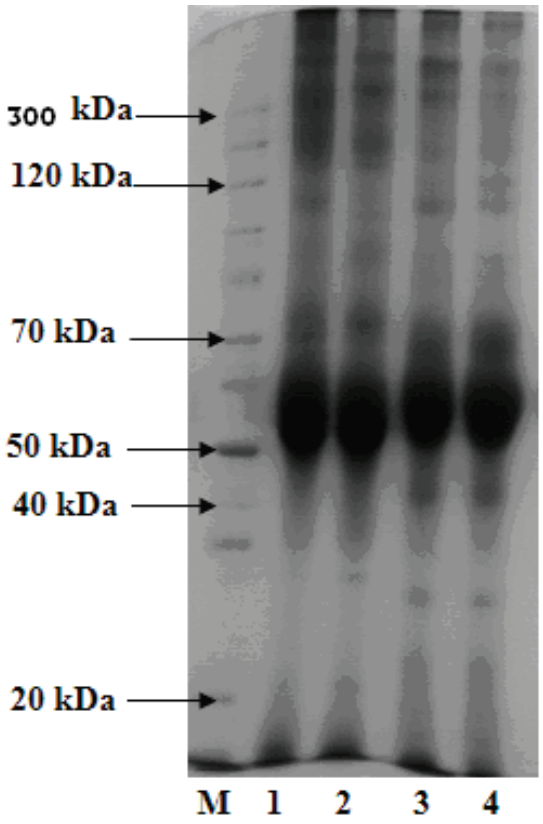

Fig.1 *SDS-PAGE profile of normal and cancerous serum samples. Lane M: Unstained protein ladder (Invitrogen); Lane 1: normal human serum; Lane 2: breast cancer serum; Lane 3 and 4: Incubation mixture of breast cancer serum and monoclonal antibodies.*

1131.44 when incubated with monoclonal antibodies. Besides, the raw volumes of other two proteins having MW of 109.07 kDa and 28.13 kDa were also decreased when treated with monoclonal antibodies.

Our experimental study confirmed that MUC1/CA 15-3 may be used as a novel therapeutic target for immunotherapy or anti-tumor vaccines for breast cancer patients. However, the decreased raw volumes of some proteinsindicated the toxic effect of vaccine which may be warranted. Monoclonal antibodies produced by *in vivo* methods can comprise various mouse proteins and other contaminants that might require purification.

Conclusion

The *in vivo* and *in vitro* experiments of MUC1/CA 15-3 vaccine showed that treatment of aggressive cancer with antiMUC1 antibodies may increase survival rate in breast cancer. Still there are many hurdles that must be overcome to elicit efficient and protective immune responses and eradicate cancers. Much work remains to be done to optimize immunization schedule and method for monitoring the success of immunological approach in cancer patients.

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