Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Some studies on Camel Nanobodies

A Thesis Presented By

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Abstract

Camel antibodies is unusual simplified non canonical antibodies (HCAb) which devoid of CH1 domain and light chain which gave it great chance for different application in diagnosis and treatment. In this study, immunization of Egyptian camel with tetanus toxoid for studying the structure and features of camel nanobodies, the serum were collected and purified and found that the main antibody in serum of camel is HCAb (75-80kDa) which appeared by SDS-PAGE. The prophylactic and therapeutic effects of nanobodies were tested in groups of Mice injected with tetanus toxin. The camel antibodies can enter the nerve cell and neutralize the toxin and relieve the signs of toxicity.

Dedication

To my lovely Wife

To my little son, Mohamed

To my spirit of my father

To all my family

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Yours,

Wafy Hamed

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1. Introduction

Antibodies or immunoglobulins (Igs) are soluble glycoproteins in blood and tissue fluids that play a central role in the humoral immune response of vertebrates. Antibodies are synthesized by B-lymphocytes in response to foreign biological and chemical substances (antigens) of great structural verity with a purpose to neutralize them. Owing to their high specificity and high affinity of antigen binding and to the possibility of antibody generation against practically limitless repertoire of antigens, antibodies and their derivatives are among the most important reagents for the basic, applied and medical research **(Honegger A, Pl¨uckthun A 2001).**

The conventional antibody molecule consists of 4 polypeptide chains; two are called heavy chains and the other two having low molecular weight are called light chains **(Utsumi and Karush 1964 &Burton,1985).** Although the antibodies have proven their worth protection against infections and as therapeutic drugs, they do suffer from a number of technical problems, including their slow clearance rate after injection into the body. Also the antibody molecular size (e.g., IgG) is over five times larger than the average therapeutic protein and if used against solid tumors, will not fully penetrate the cancerous tissue to destroy it. This has prompted the search for much smaller proteins that retain both the antigen-binding capability of the conventional antibodies and their ability to remove invading organisms.

In 1993, a group of Belgium scientists has published an important discovery(**Hamers*et al*., 1993)** indicating that in addition to classical

antibodies, there is a significant amount of unusual non-canonical antibodieswith simplified structure in blood of camelids (camel, lama, vicugna). These nanobodies consist of a dimer of only one shortened heavy chain, and the light chainsare absent (heavy chain antibodiesHCAb).

These nanobodies represent a new generation of antibodyproteins that retains the variable (V) region sequences within a much smaller protein framework. As an example is the single domain nanobodies developed by **Ablynx** Company, which has antigen-binding capability in a molecule of around 12,000 Da.

Nanobodies, derived from naturally occurring single-chain antibodies, are the smallest fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain.It is the smallest known protein with the ability to specifically recognize an antigen**(Fridy*et al* .,2014)**.

The nanobody technology was originally developed following the discovery that camelidae (camels and llamas) possess a unique repertoire of fully functional antibodies that lack light chains. Like conventional antibodies, nanobodies show high target specificity and low inherent toxicity; however, like small-molecule drugs, they can inhibit enzymes and can access receptor clefts. Their unique structure consists of a single variable domain (VHH), a hinge region, and two constant domains; CH2 and CH3**(Maggi and Scotti, 2017)**

The discovery of the single-domain antibody's potentials has stimulated their use in an increasing variety of fields. The rapid accumulation of articles describing new applications and further

developments of established approaches has made it. At present, the possibility to use nanobodies as enzyme inhibitors **(Martin *et al.*, 1997; Lauwe reys *et al.*,1998; Conrath *et al.*,2001; De Genst *et al.*, 2006; Koch-Nolte *et al.*, 2007)**, affinity ligands (**Kloostere*t al.*, 2007**) ; intrabodies (**Gueorguieva *et al.*, 2006; Rothbauer *et al.*, 2006; Verheesen*et al.*, 2006**), as probes in biosensors (**Pleschberger *et al.*, 2004; Huang *et al.*, 2005a; Saerens *et al.*, 2005**), as tools to trace antigens in live cells (**Rothbauer *et al.*, 2006**) and to study protein–protein interactions (**Huang *et al.*, 2005b**) has been demonstrated. Owing to their small size and reduced complexity of paratopes, Nanobodies are ideal candidates to design small peptide mimetic (**Marquardt *et al.*, 2006**). Nanobodies can also be used in consumer products. For example, Nanobodies prevent infection of lactic acid bacteria by phage, thereby accelerating fermentation in cheese production (**Ledeboer *et al.*, 2002; de Haard *et al.*, 2005**) or they can prevent dandruff when supplied in shampoo (**Dolk *et al.*, 2005**).

The aim of the present work was to elucidate the therapeutic potential of tetanus toxin-specific nanobodies in clinically tetanus toxin intoxicated mice. It is planned to achieve this aim by:

1. **Immunization of Egyptian camel with tetanus toxoid vaccine.**
2. **Separation of immunoglobulins from camel sera.**
3. **Study the nature of camel immunoglobulins by SDS-PAGE and colorimetric identification by Commassie blue.**
4. **Determination of the heat stability and pH stability of the prepared tetanus toxin- specific camel nanobodies.**
5. **Evaluation of the immune-prophylactic and immune-therapeutic potential of the prepared tetanus toxin-specific camel nanobodies.**

2. Review of Literature

2.1. Camel immunoglobulins:

Utsumi and Karush (1964) found in the conventional antibody, the variable region of the light chain (VL) and the variable region of the heavy chain (VH) are combined to make the antigen binding site, although, in some cases, the heavy chain alone can also bind antigen (Fig.1)

Burton (1985) recorded that papain protease can split antibody into two fragments: Fab (Fragment antigen binding) and Fc (Fragment crystallizable). Correspondingly, one part of the antibody molecule (Fab) defines its antigen specificity, and the other part (Fc) is responsible for effector functions, which lead to the antigen elimination. They recorded also that the hinge region located between CH1 and CH2 domains of H-chain is responsible for mobility of Fab-fragment. Papain cut immunoglobulin right in the hinge region above inter-chain disulfide bonds. CH2-domain is the place of carbohydrates attachment and of complement binding. CH3-domain interacts with the Fc-receptor at the surface of cells involved in immunological reactions. Right combination of heavy-chain variable region (VH) and light-chain variable region (VL) make the antigen binding site, and constant antibody domains do not participate directly in the antigen recognition. Single-chain protein construct (scFv) composed of both heavy chain and light chain variable domains that are connected by a linker sequence is the minimized derivative of the antigen-binding domain of conventional antibodies. The constant domains of antibodies however, are not involved in antigen recognition; the heavy chain constant regions CH2 and CH3 (Fc) are