



Review article

Single domain antibodies: a new approach in therapeutics**Mahesh Narkhede*, Rohan Narkhede, Mangesh Mapari**

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Single domain antibodies are rapidly comes into therapeutics in last two decades due to their therapeutic advantages. Most of drugs are derived from antibodies based proteins. Single domain antibodies having advantages over the monoclonal antibodies such as small size, heat resistant, stability, hydrophobicity, low immunogenicity, high solubility. These are small in size hence called nobodies. Their production is carried out using mammalian cells for therapeutic uses. These single domain antibodies are now employed in drug delivery system. They are also utilized in identification of toxin. Single domain antibodies are recently employed in treatment diseases like cancer, Alzheimer's disease, Parkinson's disease etc. They are also employed in virus detection. This is a cross sectional and correlational-descriptive study conducted among 197 nurses working in Golestan Hospital of Ahvaz. Moslach Burnout Inventory (MBIQ) and General Health Questionnaire (GHQ) were instruments for data collection. SPSS software was applied for analyzing the data.

Keywords: Single domain antibodies, Nano bodies, Polymer some, Complementarity, ADCC, Multimerization.**INTRODUCTION**

The field of recombinant antibody technology has rapidly progressed during the last two decades, mainly because of the interest in their human therapeutic use. The ability to select specific human antibodies by display technologies and to improve their affinity, stability, and expression level by molecular evolution has further boosted the field. Approximately 30% of drugs in development are biologics, most of which are Ab-based proteins used as treatment for inflammatory diseases, cancer, and allergies. The success of the anti-tumor necrosis factor (TNF) Abs has boosted the search for other Abs. In 2013, 22% of the sales of large pharmaceutical companies were biologics and this figure is expected to rise still further. Monoclonal Abs (mAbs) have become indispensable therapeutic and research tools. Given that they are difficult and expensive to produce, they impose a heavy burden on healthcare and research budgets. Moreover, they are not suitable for some applications. First, they are large molecules (150 kDa), which limits their tissue and/or tumor penetration and bio-distribution. Second, they can elicit immune

reactions that neutralize their activities, which sometimes limits the long-term use of chimeric and humanized Abs available on the market. Third, mAbs typically have a half-life of several days and this limits their use in molecular imaging because of the intense background signal. The disadvantages of Abs are related to their large size, efforts have been made to minimize them. This leads to the development of antigen-antibody fragments (Fab fragments), variable fragments (Fv fragments), and single-chain variable fragments (scFv fragments). The stability of these newer antibodies also enhanced.

During the early 1990s, Hamers, Casterman and her team discovered a new antibody in Camelidae members. Compared to older conventional antibody immunoglobulin G (IgG) Antibodies, camelid antibodies found to express antibodies devoid of light chains, called "heavy-chain-only antibodies (HcAbs). Although single-domain antibodies later were also identified in particular cartilaginous fish, most research on the biotechnological application of single-domain antibodies was done using camelids because of their ease of handling, including immunization.

Although they do not originate from humans, Nbs have a low immunogenicity because of a large sequence identity with the human VH gene family III, making them suitable for chronic indications. In nine clinical studies with incidence of antidrug antibodies was low (3%) and their presence mainly transient.

“A single domain antibody called Nano body is an antibody fragment costing of single monomeric variable antibody domain.”

PROPERTIES OF SINGLE DOMAIN ANTIBODIES:

These are much smaller (12-15 kDa) than common antibodies (150-160kDa) [9]. A single domain antibody is a peptide chain of about 120 amino acids long comprising of one variable heavy chain domain (VHH) of a heavy chain antibody or of a common IgG. Single domain antibodies have same affinity towards antigen as whole antibodies but, are more heat resistant and stable towards detergent and high concentrations of Urea. Nano bodies derived from camelid are less lipophilic and more water soluble because several of the hallmark hydrophilic amino acid residues of VHHs was more stable than the original VH fragment. In contrast to common antibodies, two out of six single-domain antibodies survived a temperature of 90 degree Celsius without losing their ability to bind antigens in 1999 study.

Stability towards gastric acid and protease. The comparatively low molecular mass leads to a better permeability in tissues. Unlike whole antibodies, they do not show complement system triggered cytotoxicity because they lack an Fc region. These are potentially weak immunogenic.

Structure of single-domain antibodies

Variable domains derived from the anti-body heavy (VH) and light (VL) chains are shaded dark gray and light gray, respectively, whereas constant domains (CH and CL) are not shaded. Note the absence of the light chain and CH1 domain in heavy-chain antibodies. Anti-body domains that pair by non-covalent interactions are indicated by overlaying them.

These contains two constant domains (CH2 and CH3), a hinge region, and an antigen-binding or variable heavy chain domain (VHH) called the Nb, which retains full antigen-binding capacity. The framework regions present surrounding to complementarity determining regions (CDR). It might participate in Ag identification and binding. about 61-80% of contact with Ag is through this region. Nbs are small (15 kDa) and can have a long protruding CDR3 loop. Their prolate shape exposes a convex paratope and both these features help them to access receptor clefts or binding pockets more easily that are inaccessible to Abs.

Also the CDRs of VHHs contain some characteristic features. The N-terminal part of CDR1 is more variable. Secondly, many dromedary VHHs have an extended CDR3 that is often stabilized by an additional disulfide bond with a cysteine in CDR1 or

resulting in the folding of the CDR3 loop across the former VL interface.

Advantages of single-domain antibodies

Fast clearance

Single-domain nature

No decrease in library size because of reshuffling of VL and vh domains

Efficient refolding due to increased hydrophobicity and single-domain nature increased hydrophobicity

Small size and extended flexible CDR3 small size

Efficient folding due to increased hydrophobicity and single-domain nature

High solubility

Increased functional size of immune libraries

Production of single domain antibodies

Most functional complete antibodies can be efficiently produced using mammalian cells, especially when their appropriate glycosylation is required for therapeutic applications. The antibody fragments having absence of Fc with its N-linked oligosaccharide are preferably produced in microbes. These have a shorter development time from gene to product and require simple well-established fermentation conditions that can be performed on large-scale resulting in low cost. The microbial production is mainly based on E. coli, yeasts, or filamentous fungi. In E. coli they can be produced by secretion into the oxidizing periplasmic space or expression in the reducing cytosol. In fungi the production can be done by cumbersome refolding of antibody fragments.

VHHs are mostly produced in E. coli. There is only one example of VHH production that can be produced in filamentous fungi, which results in limited proteolytic degradation of the secreted product due to high levels of proteases secreted by filamentous fungi. The yeast *Saccharomyces* are also capable of producing VHHs. The N-glycosylated VHHs can also be produced by yeast. This can affect antigen binding. Furthermore, it could complicate their therapeutic use because the addition of yeast specific high-mannose oligosaccharides results in a high immunogenicity and decreased serum half-life because of binding to specific mannose receptors on cells of the reticulo-endothelial system.

Several VHH sequence pattern can be associated with their production level. First his presence of a potential N-linked glycosylation site increases production levels in yeast. Second, the presence of unpaired C-terminal cysteines reduces expression levels. Third, replacement of hydrophobic residues of conventional VH domains normally interacting with CH1 increased scFv production in E. coli suggesting that the hydrophilic mutations that naturally occur at these positions in VHHs also contributes to their high expression level. There are many examples of VHHs that differ by only a few

amino acids and are produced at highly variable levels where the exact amino acid change responsible for the difference in production level is difficult to predict. Furthermore, without such knowledge, VHH production can be enhanced by random molecular evolution using deoxyribonucleic acid shuffling, as has often been done for conventional antibody fragments. In baker's yeast, the specific VHH production rate is correlated with growth rate, and can be up to fivefold increased by growing on carbon source like ethyl alcohol. The medium is provided with supplements like sorbitol, casamino acids, or ethylenediamine tetraacetic acid improves VHH production by *P. pastoris*.

VHs cannot recruit effector functions such as ADCC and CDC on their own. This limits their therapeutic application. Although such effector functions can be indirectly recruited using conventional antibody fragments binding to host immunoglobulin it may be more efficient to recruit these functions by fusing VHs to host Fc domains. Production of such functional antibodies requires the correct glycosylation of the CH2 domain, which until recently could only be accomplished using higher eukaryotic cells but not by microbial production. But, this may now be feasible using *P. pastoris* strains with an engineered glycosylation machinery that are capable to produce proteins with a specific human glycoform. Furthermore, transgenic mice containing hybrid llama/ human antibody loci that contain llama V regions and human D, J, and C regions have recently been used to generate human heavy-chain antibodies in mice.

In addition to monovalent VHs, several expression formats for the production of VHH multimers have been described. These include genetic fusions of two or three VHs that either recognize different antigens or the same repeating antigen to increase functional affinity. Although such VHH fusions are less efficiently produced than their monovalent versions, their production level exceeds that of their conventional-antibody-based fusion counterparts without aggregation or low solubility. However, antigen binding by the C-terminal VHH in such fusions can be compromised presumably because of steric hindrance by the N-terminal VHH [1-7].

Applications of single domain antibodies Nbs incorporated into drug delivery systems

Nbs can also be chemically attached to the surface of other drug delivery systems, such as nano-sized drug carriers or NPs, which can then be encapsulated with nonspecific drugs for active delivery to the site of interest. This is an attractive approach because it protects the body against systemic toxicity and allows solubilization of hydrophobic drug in hydrophilic structures, such as liposomes or micelles. Additionally, it permits administration of larger drug doses simultaneously, which could reduce the administration frequency and immunogenicity.

Important advances have been made with an Nb coupled to a drug delivery system.

A new interesting class of carrier systems is the polymer some, which architecturally resembles liposomes but is highly stable and can encapsulate larger amounts of hydrophilic drugs compared with micelles. This makes them particularly interesting for the delivery of cargo intracellular or for the controlled release of drugs. As an example, tumor vessel-targeting polymerases decorated with Nbs that target PlexinD1 a Tran's membrane protein overexpressed in tumor vasculature.

Toxin identification and detoxification

The use of Nbs could further enhance toxin neutralization. Given their good tissue distribution, Nbs can more easily reach and neutralize toxins. However, some toxins are small, no immunogenic polypeptides, so obtaining a proper immune response can be problematic. Nbs have been raised against different toxic venom fractions of the *Androctonus australis hector* scorpion venom (AahG50, AahII, and AahI'). Multiagency induced by polymerization of two Nbs against the two most toxic venom fractions improved their affinity. Also, proof-of-principle was obtained from experiments on rats, in which the specific Nbs had good pharmacodynamics properties and effectively protected against envenoming. *Hemiscorpius lepturus* is another scorpion and the most dangerous in Iraq; hemineneolysin (HNc) is the major hemolytic and dermo-necrotic venom fraction known from this species. Anti-HNc Nbs were raised and completely protected against HNc-induced envenoming. Neutralizing antivenom Nbs against a-cobratoxin were also generated and fused to a human Fc fragment, thus retaining their high binding affinity to the toxin via the Nb but exerting the immunological properties of conventional Abs.

Treatment of cancer

As monoclonal antibodies single domain antibodies (nanobodie) are distributed homogeneously in tumor tissue. As anti-cancer biological agents, Nbs can be used as antagonistic drugs, but due to the absence of an Fc-effector domain, their efficacy as a pure immunotherapeutic is inferior to that of mAbs. Nevertheless, the absence of the Fc-domain in Nbs can reduce the number of unwanted immune-mediated adverse effects that are elicited by this domain. However, more promising approaches were recently introduced, such as their use as targeting moieties linked to effector domains and radionuclides. Additionally, they can be decorated on nanoparticles (NPs) that can be filled with other (small-molecule) anticancer drugs for active targeting to the specified tumor cells. Two aspects need to be considered when such Nb – effector domain complexes are generated. First, the stability of the Nbs, which are reportedly very stable, might be attenuated and, second, a change in Nb-binding affinity has been reported.

Targeting bacteria and phages

Nbs to combat bacteria can be raised against bacterial surface proteins to block bacterial attachment to host cells. Based on this principle, Nbs against the lactin domain of F18 fimbrial adhesion of the enterotoxigenic *E. coli* and Shiga toxin-producing

E. coli prevented attachment in vitro. Pentameric Nbs enhance antigen agglutination, and pentavalency of Nbs can be conferred by exploiting the homo penta merization properties of nontoxic verotoxin B via linking the Nbs to that toxin. Subsequently, the high-avidity pentabodies that bound the flagella of *Campylobacter jejuni* and other specific protease-resistant anti-flagella Nbs demonstrated remarkable stability and potently inhibited the motility of *C. jejuni*. The pentabodies was also potent in vivo, reducing *C. jejuni* colonization in the ceca of infected chickens. By targeting the flagella, both bacterial motility and biofilm formation can be inhibited, as in the use of anti-flagella Nbs against *Pseudomonas aeruginosa*. Another protein that is important during biofilm formation is the biofilm-associated protein (Bap) and, consequently, anti-bap Nbs were developed as a strategy to combat *Acinetobacter baumannii*. Nbs that prevent bacterial secretion of toxins can also be designed, such as Nbs against the type VI secretion system of Gram-negative bacteria.

Single-domain antibodies against fungi and protozoans

The potentials of single-domain antibodies have been only scarcely applied to fungi and protozoans. It is the case of the antibodies raised against a cell wall protein of *Malassezia furfur*, a fungus implicated in dandruff. Since the selected VHH antibodies should be potentially included in a shampoo formulation, they had to resist to the harsh chemical conditions brought about by elevated concentrations of anionic and nonionic surfactants. Therefore, the panning-washing conditions were adapted to reflect the high detergent content of shampoos. This approach enabled the recovery of VHHs with specifically increased stability under denaturing. Nbs that target the paraflagellar rod protein of different trypanosomes have been described, but they are mainly useful as diagnostic markers of trypanosomiasis [8, 9].

Strategies for virus detection and neutralization

Nbs can interfere at different levels of the viral replication cycle, such as by preventing virus–cell attachment, viral entry, and viral uncoating. An Nb directed against hepatitis C virus (HCV) specifically prevents viral cell entry and cell–cell transmission. An intracellular expressed Nb that interferes with Rev Multimerization of HIV-1, a protein that is involved in nuclear trafficking of viral mRNAs, efficiently inhibited this crucial step in virus replication. Additionally, Nbs are useful in the study of the mechanisms of oligomer assembly of HIV. In other studies, an Nb expressed in the cytosol that targets the nucleoprotein of influenza virus potently inhibited nuclear translocation and another Nb expressed in the cytoplasm blocked the

replication of porcine reproductive and respiratory syndrome virus (PRRSV). Prophylactic Nbs can be generated too, for example by generating modified lactobacilli that produce VHH antibody fragments, called ‘lacto bodies’. Oral administration of lacto bodies expressing surface-anchored anti-rotavirus (RV) Nbs might be prophylactic against RV-induced diarrhoea.

Lactobacillus paracasei expression of bivalent Nbs or co-expression of two individual Nbs even led to protection against escape mutants and can also be used therapeutically.

Nbs in neurodegenerative and other amyloid disorders

There are currently only symptomatic treatments for neurodegenerative disorders; no disease-modifying or neuroprotective therapies that alter the natural disease course are available. Consequently, new and affordable therapies are needed.

Alzheimer’s disease

AD is the most common neurodegenerative disease. It is characterised by the cerebral deposition of aggregated amyloid- β (Ab) peptide plaques and the formation of neurofibrillary tangles, resulting in dementia and loss of cognitive function. Ab plaques are formed via proteolytic cleavage of a large precursor protein, amyloid precursor protein (APP), by enzymes such as Beta-site APP-cleavage enzyme (BACE-1). Nbs that are selective for different amyloid (precursor) peptides have been produced, and Nbs that can prevent the formation of mature Ab fibrils by stabilising Ab protofibrils have been identified. For diagnostic purposes, Ab-specific Nbs coupled to ^{99m}Tc enabled the in vivo detection of vascular and parenchymal Ab deposits because they could cross the disrupted BBB, making them promising tools for such applications.

Parkinson’s disease

Some attempts have been made to use Nbs to tackle the second most common neurodegenerative disease, Parkinson’s disease (PD). PD is characterized by the loss of dopaminergic neurons in the substantia nigra, and misfolding of α -synuclein (α -syn) into fibrillar aggregates seems to have a prominent role in the pathogenesis of this disease. Consequently, reduction of the intracellular levels of α -syn is a logical therapeutic approach, the aim being the prevention of misfolding, aggregation, and toxicity. NbSyn2 is an Nb directed against the C-terminal part of monomeric α -syn, but the Nb could not prevent aggregation because it bound both monomeric and aggregated protein. Nevertheless, those Nbs provide information about possible

conformational rearrangements during fibrillar maturation and, therefore, are valuable for gaining knowledge about the structure of α -syn [10].

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