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Review

Immunotherapies and Nano-vectorization: New Trends on Scorpion Envenomation Treatments

Immunothérapies et Nanovectorisation : Nouvelles Perspectives pour le Traitement Antiscorpionique

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ABSTRACT

Scorpion envenomation is a real medical emergency and life hazard in many countries of the world, especially in rural tropical areas of Africa, Asia and America. Children and elderly are the most affected persons. In this non exhaustive report, we present recent contributions in the scorpion envenomation treatments. From recent advances of antivenom immunotherapy, an immunopreventive treatment combined with immunotherapy could be for interest, since envenomation remains an important health problem. Venom neurotoxins (3-7 kDa) can easily diffuse in tissues and blood circulation and their rapid action on ions channels of excitable cells lead to the dangerous pathophysiology of scorpion envenomation.According to the small molecular weight, their vectorization with biocompatible nanoparticles to antigen presenting cells could induce a protective immunity against scorpion envenomation. The present review will report the latest development of clinical and experimental scorpion immunotherapies as well as approaches using nanoparticles as vectors, to improve the classical immunotherapy and vaccine protocols against venoms or toxins.

KEYWORDS: scorpion envenomation, treatments, nanoparticles, vector, biocompatibility.

RESUME

L'envenimation scorpionique est une urgence médicale dans de nombreuses régions tropicales and subtropicales, notamment en Afrique, Asie et Amérique latine. Les enfants ainsi que les personnes âgées sont les personnes les plus vulnérables. Dans cet article de synthèse, nous relaterons un certain nombre de contribution en rapport avec l'optimisation des traitements de l'envenimation scorpionique. Compte tenu des résultats prometteursportant sur l'amélioration de l'efficacitéde l'immunothérapie antivenimeuse, l'immunoprevention serait d'un grand apport aussi bien dans la prévention des personnes des régions à haut risque, ce traitement pourrait être aussi associé à l'immunothérapie. L'apport de la nanovectorisation utilisant des vecteurs biocompatibles spécifiques aux cellules immunitaires pourrait induire une immunoprotection efficace contre la physiopathologie induite par les venins de scorpions. La présente revue discutera des dernières avancées en matière d'immunothérapies antiscorpionique ainsi que des études récentes utilisant des nanoparticules comme vecteurs spécifiques, visant à améliorer l'immunothérapie classique mais aussi des protocoles vaccinaux contre les venins ou les toxines.

MOTS CLES : envenimation scorpionique, traitements, nanoparticules, vecteur, biocompatibilité

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1. Introduction

Nanotechnology is an expanding field, which includes the development of human-made materials in the 10–200 nanometer size range. This reduced scale results in significant changes of typical physical and chemical properties of materials, makes them very attractive for novel and innovative applications in various fields. Nanotechnology has been employed in medicine for therapeutic drug delivery and development of several treatments for various diseases and biological disorders. Indeed, nanomaterials have been used successfully in magnetic and fluorescent bioimaging, as carriers for drugs, and even as medicines themselves (e.g., antimicrobial agents).

Scorpion envenomation is a severe syndrome which lead to a complex pathogenesis causing important morbidity and mortality in Maghreb regions [1,2]. The toxicity of scorpion venoms is mainly due to their neurotoxins that can bind to the sodium voltage-gated channels thus causing multi-organs failure and death [3-5]. Specific immunotherapy associated with symptomatic treatments according to the degree of severity remains the currently used approach to treat stung patients. The efficiency of this immunotherapy against scorpion envenomation presents some limits due mainly to the delay taking between the sting and the antivenom administration to patients of at-risk regions. For this reason, many experimental immunotherapies using next generation of antibodies are in development to improve the existing therapy.

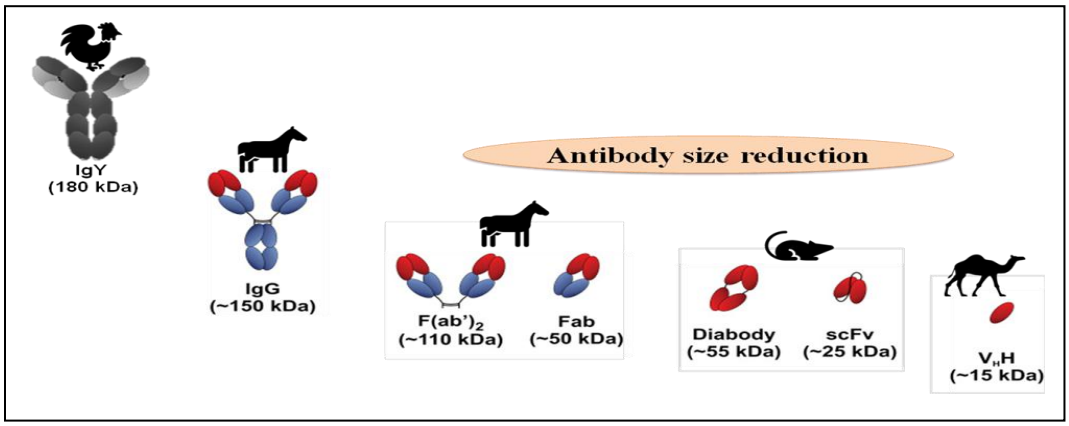

Figure 1: Antibody engineering (according to Laustsen et al., 2018)

IgY: chicken IgY antibody. IgG: whole IgG antibody. F(ab')2: pepsin-digested IgG antigen-specific region. Fab: papain-digested antigen-specific region. Diabody: non-covalent dimers of scFv fragments. scFv: single-chain variable fragments. VHH: single-domain antigen-

2. Specific antivenom immunotherapies

1.1. Classical immunotherapy

Passive immunotherapy (or classical immunotherapy) is right now the only specific treatment against scorpion stings. It is based on the acquisition of specific immunity induced in equine animal producers after repeated administration of the venom. The first produced scorpion antiserum was produced by Todd in 1909 in Cairo [6]. In Algeria, Dr Etienne Sergent made many efforts about classical immune therapy by removing animal proteins such as albumin in order to obtain a less immunogenic products and to increase human compatibility or tolerance [7]. The improvement of the classical immunotherapy efficacy could be based on the optimization of immunization protocols, considering the type of antigen (venom vs. toxins), adjuvant type, immunization dose and its frequency and animal choice (horse vs. camelid, sheeps or chicken) [8,9]. In 1998, Laraba-Djebari and 74Hammoudi used the toxic fraction FtoxG50 isolated from the whole venom of *Androctonus australis hector* as antigen and the neutralizing properties of antibodies appeared to be higher after animal immunization with multiple injections of

toxic fraction than those obtained with the whole venom [10]. High toxicity of the venom and its toxic fraction could also limit the efficiency of immune serum preparation. Animal producers may have chronic injuries due to the toxic administration during the immunization schedule having an impact on the quality and efficiency of the antivenoms [2].

For this purpose, attenuation of antigens to lower its toxicity and enhance its immunogenicity was tested. Immunization with attenuated *Aah* venom by gamma irradiation assayed in mice yielded immunoprotection against 10 lethal doses 50,6 months after the last immunization [11]. The choice of the used adjuvants in immunization protocols is also an important factor to be considered. The use of saponin as an alternative to Freund's complete adjuvant, lead to important specific neutralizing antibody titers in rabbits, with fewer local reactogenicity [8].

The choice of neutralizing molecule in scorpion envenomation treatment is also important to improve the classical immunotherapy. To better neutralize the small components of venoms such as neurotoxins (60-70 kDa) and to reduce reactogenicity, produced equine IgG (150 kDa) were hydrolyzed by pepsin to obtain F(ab')2 (110 kDa) or by papain to obtain Fab (50 kDa) (Figure 1). The effectiveness of antibodies is linked to their neutralizing capacity but also to their tolerance and their pharmacokinetic. Several factors such as the antibody form, the route of injection and the time delay before their administration can limit the clinical efficacy of this specific treatment [4]. The effects of different antibody forms Fab, F(ab')2 fragments or their synergistic action were also tested in order to enhance the efficacy of immunotherapy against *Aah* scorpion venom [12,13].

The efficiency of these preparations were tested against the venom of *Androctonus australis hector* (*Aah*), its whole toxic faction FToxG50 or its main toxin *Aah II*, Pathohistological analyses demonstrated that treatment with a mixture of Fab and F(ab´)2 was the more effective treatment, in terms of inhibiting myocardial and pulmonary damage (hemorrhage, interstitial and intra-alveolar edema, leukocyte infiltration). This study reports that immunotherapy was significantly improved when the mixture of the two antibody fragments was tested [13]. The development of next generation immunotherapies,more effectives than the classical one, is needed to improve patient care [14].

2.2. Recombinant antibodies, nanobodies and immunotherapy advances

Different experimental approaches undertaken in mice have been considered to improve the existing immunotherapy. The expression of isolated toxins from scorpion venom has been performed in different systems to improve and design a possible recombinant antibody (Ab). Several recombinant antibodies were generated in order to neutralize toxin *Aah II* of *Androctonus australis hector* [15]. Synthetic peptides mimicking *Aah* II toxin or *TsV* II toxin of scorpion venoms used to immunize animals, lead to neutralizing antibodies, but with very low titers [16,17].

Despite the neutralizing potential of the recombinant antibodies, their therapeutic importance was diminished by their murine origin. New forms of molecules (rFab, scFv) have also been developed for therapeutic use. The structures of small fragments scFv (monomeric, dimeric, trimeric, and tetrameric) are formed by two variable domains (VL and VH) of immunoglobulin, these molecules showed pharmacological and biological activities similar to the initial antibody both *in-vitro* and *in-vivo* [18]. The use of natural toxoid (*Amm* VIII *from Androctonus mauretanicussmauretanicus* venom) to induce neutralizing antibodies against most scorpion venom toxins, particularly *Aah* II toxin lead to an immunoprotective effect in mice up to 42 LD 50 against this toxin [19].

KAah 1, a natural peptide isolated from *Aah* venom is able to induce the production of specific antibodies having cross-reactivity with *Aah* II toxin and can neutralize up to 5 LD 50 of *Aah* venom toxic fraction FtoxG50 [20].

Experimental nanobodies (Nbs) produced in hyper immunized camels, have been also introduced to neutralize *Androctonus australis hector* venom. The bispecific Nb (NbF12–10) seemed to be more efficient against scorpion envenoming in preclinical studies than classic based therapy [21]. NbF12–10 was designed against *Aah* I/*Aah* II toxins. A subsequent intravenous injection of 85 µg of NbF12–10 protected all mice subcutaneously injected with a lethal dose of *Aah* venom (32.4 µg of crude venom/20 g of mice) . However, *in-vivo* monitoring of radiolabeled nanobodies and F(ab')2 fragments revealed that the nanobody-based molecules were cleared from blood faster than the F(ab')2 antivenom due to the lower molecular mass of nanobodies. Moreover, a major difference was observed in the

organ accumulation of antibodies. Monovalent nanobodies and the bispecific constructaccumulated mainly in the kidneys, whereas F(ab')2 fragments were predominantly retained in the liver [22,23].

2.3. Chicken immunoglobulin (IgY)

Three immunoglobulin classes, analogues to the mammalian immunoglobulin classes have been shown to exist in chicken, IgA, IgM and IgY (IgG). [24,25]. IgY is the major low molecular weight serum immunoglobulin in oviparous (egg laying) animals [26]. The overall structure of IgY is similar to the mammalian IgG, with two light (L) and two heavy (H) chains. The use of IgY as an alternative antivenom has been proposed since 1990 [27]. More recently, an efficient and purified IgY antivenom against *Aah* scorpion venom was produced in laying hens characterized by an inability to react with mammalian complement make them an attractive alternative to equine antivenoms [9]. The optimization and the improvement of a preventive treatment such as vaccine against the pathophysiology induced by scorpion envenomation remains a real challenge. Research and assessment of new adjuvants that can boost the immunogenicity of scorpion venom antigens is nowadays envisaged [8,28-30]

Table 1: Recent studies highlighting some nanovectors delivery against various antigens

Nanovector	Antigen agent	Antigen formulation	Experimental model	Reference
Calcium alginate	*Aah* venom	Encapsulation	Mice, Rabbits	Nait Mohamed and Laraba-Djebari, 2016
Poly (D, L-lactide)	*Aah, Bot* venom	Encapsulation	Mice	Ayari-Riyabi et al., 2016
Bacterial Ghosts	Tumor antigens	Encapsulation	Dendritic Cells	Dobrovolskienė et al., 2018
Bacterial Ghosts	*Acinetobacter baumannii* antigens	Encapsulation	Mice	Pulido et al., 2018
Silver Nps	*Bothrops jararacussu* venom	Adsorption	Prokaryotic cells	Oliveira et al., 2019
PEGylated GNP	NKCT1 toxin	Adsorption	Human Myelogenous Leukemic Cells	Bhowmik et al., 2013
PLGA-Dopamin	BSA	Conjugation	RAW 264.7 cells	Lee et al., 2017
Fe-amine-functionalized PEG	CTX	Conjugation	Human Tumors cells	Veiseh et al., 2009

3. Preclinical vaccine approach

One of the first experiments based on the use of scorpion *Centruroidesnoxius* venom detoxified bychemical modification (glutaraldehyde polymerization) was performed [31].

The use of detoxified venom of *C. noxius* lead to the protection of rabbits against 50 LD 50 of the toxic fraction of the crude venom of C. noxius (1 LD 50 : 0.26 mg/kg); however the time and the effectiveness of the protection were limited. Other studies report the attenuation of the venom toxicity by entrapping it in liposomes [32]. The detoxification of *A. australis* venom using γ-radiation mixed with Alum adjuvant was also performed and lead to a substantial reduction in the venom toxicity (LD 50 25 times higher than the LD 50 value obtained with untreated venom) with an important middle-term mmunoprotection against 4 LD 50 in mice [28]. The immunization with irradiated *Aah* venom associated with Alum induces a low inflammatory response without any major adverse effects.

This safe formulation presents also the same immunoprotective effect against scorpion envenomation [30]. An effective immune response activation is also observed when the oil-in-water adjuvant MF59, was associated with *Aah* venom, leading to a best specific immune response compared to Alum adjuvant [8,29]. To better targets specific immune cells like dendritic cells, nanovectors were recently used as specific antigen delivery systems. Designing a nanovector for antigen delivery presents both theoretical and practical challenges. Nanoparticles (Nps) must be nontoxic, biocompatible, vectorize a large amount of antigen

with a high affinity for the targeted tissues. They have to cross biological barriers and protect the antigens until their site of action [33]. Biodegradable particles have great potential for application as vectors of biologically active molecules. Encapsulated antigens have been shown to have good potential for system release and adjuvating (Table 1). These particles allow to the controlled release of the antigen that can reduce the number of immunization doses or develop single-dose vaccines [34]. Natural polymeric nanoparticles such as calcium alginate or chitosan, are able to: i) stabilize the antigens by protecting them from the biological environment; ii) increase their bioavailability; iii) improve the targeting of antigen presenting cells (APCs), mainly the dendritic cells [35,36]. Encapsulated scorpion

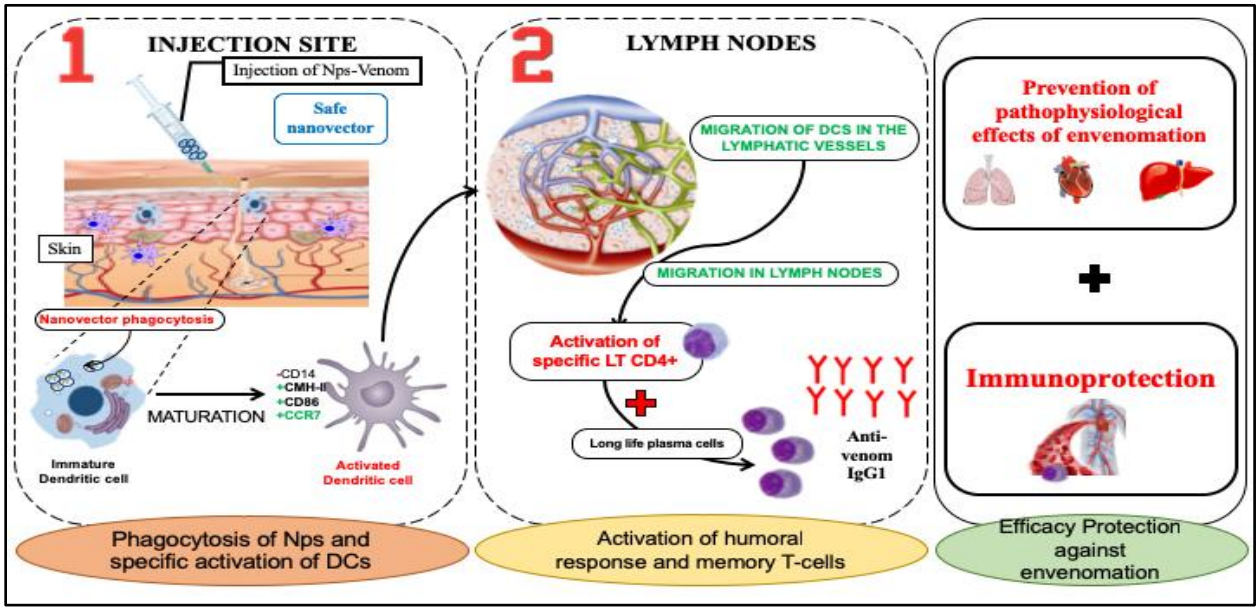

Figure 2: Induced immune response after nanovector phagocytosis for immunoprotection (Smith et al., 2013, Heath et al., 2013, Nait Mohamed and Laraba-Djebari. 2016)

Tityusserrulatus venom in sphingomyelin-cholesterol liposomes and their use in murine immunization yield to specific antibodies with high protection [37]. Immunization of mice with encapsulated *T. serrulatus* scorpion venom lead to a protective effect against toxic fraction *Tst*-G50 up to 3 LD50 [38]. The Animal immunization with encapsulated *Androctonus australis hector* venom in calcium-alginate or poly (D, L-lactide) polymer nanoparticles used vectors induced a strong and efficient antigen delivery [39-41]. Once injected subcutaneously, the synthesized nanoformulations encapsulating irradiated *Aah* venom were phagocytized by the dendritic cells and then activated and migrated into the lymph nodes. The activation of venom-specific LT CD4+ induces the formation of high-affinity memory and long-lived circulating plasma cells and specific synthesis of IgG1. This innovative vaccine protocol allowed to a strong effective immunoprotection up to 6 LD 50 of *Aah*venom, preventing pathophysiological effects caused in the heart, liver and lungs by the envenomation [41–43], Figure 2). *T. serrulatus* venom-loaded in chitosan nanoparticles seems to successfully mimicking the slow protein release identified for the BSA-loaded hitosan nanoparticles. The released *T. serrulatus* venom depended on the venom loading, displaying the flexibility and feasibility of these particles to modulate the antigen delivery. The nanoconjugation of scorpion toxins such as chlorotoxin (CTX) isolated from *Leiurus quinquestriatus* scorpion venom with an amine-functionalized polysilane and super magnetic iron oxide nanoparticles preferentially binds to glioma cells, as compared with non-neoplastic cells or normal brain cells [44]. Moreover, the CTX-conjugates deactivated membrane-bound MMP2 and caused an increase in the internalization of lipid rafts.

Conclusion

Immunotherapy as a specific treatment is recommended after scorpion stings in most at-risk regions. However, to be more efficient, this therapy needs to be improved, optimized, and standardized considering its limitations (delay, antibody format, soluble or freeze-dried, dose, route of injection). Therefore, nanovectorization could be a promising alternative not only to enhance classical immunotherapy but also as an active immunotherapy for developing a long lasting protection against scorpion envenomation in at risk regions.

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Conflicts of interest

Authors do not declare any conflict of interest.

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