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Significances of Antimicrobial Peptides (short communication)

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Abstract :

Antimicrobial peptides is very important because it is used as therapeutics, biomarkers and modified antimicrobial peptides but it has some limitation like high production cost, toxicity, susceptibility to proteolysis.

Keywords : Therapeutics, Biomarkers, Modified AMPs.

In insects, injury infection elicits the production of antimicrobial peptides in the fat body (the insect equivalent of the vertebrate liver) and within a few hours renders the insect hemolymph (blood) antimicrobial. Philipp Bulet et al (2005) reported antimicrobial peptides are the parts of insects have developed to fight off pathogens. Pawel Mak et al. (2001) reported antimicrobial peptide purification (AMP) & biochemical characterization of two inducible AMP from the hemolymph of Galleria mellonella.

AMPS AS THERAPEUTICS

AMPs are excellent candidates for development of novel therapeutic agents and compounds to conventional antibiotic therapy because they generally have a broad range of activity. Their effectiveness is mainly attributed to their ability to disrupt the cell membrane and to cause the cell lyses. Therefore the AMPs are able to kill a broad spectrum of bacteria even multidrug resistant as well as fungi, viruses, protozoa or tumor cells in a short period of time. The explanation of this miraculous effectiveness is very simple- the targets of AMPs, as already mentioned, are mostly the biological membranes. To become resistant to AMPs it would mean for the cells to redesign their membranes, i.e. to change the composition of the membrane by reorganization and usage of different kinds of its components. Moreover, the sequences of AMPs mostly do not contain any uncommon or unique areas to be marked as typical recognition sites for the concrete peptide in order to be destroyed. This is the main aspect which makes the AMPs so promising in vaccine development. Recently, the investigation of many naturally occurring peptides as well as their derivatives has been performed. They can be used for diverse therapies from anti-

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pathogenic through to anti- cancerogenic. Many of antimicrobial peptides have already entered clinical trials. Their status in the clinical trial can be searched and checked easily (http://clinicaltrials.gov/).

AMPS AS BIOMARKERS

Another possible application of AMPs in medicine is biomarkers (especially, human ?-defensins). Biomarkers have been detected in malignant cells and in body fluids of patients with tumor, e.g. in case of metastatic colorectal cancers (Albrethsen, J, et al; 2006), lung tumors (Bateman A, et al; 1992), renal cell carcinomas (Muller et al; 2002), and others. Moreover, their increased concentration (cca. 40 ng/ ml) in body fluids could be an indicator of an inflammatory syndrome, or when measured in milligrams, it could be a marker of sepsis (Panyutich et al; 1993).

MODIFIED AMPS

Some naturally occurring AMPs have been proved not to be suitable candidates as therapeutics and therefore not suitable for further pharmaceutical development. The reasons for this can be diverse: unfavorable pharmacokinetics, the loss of activity under physiological conditions, low microbiological efficiency, quick degradation etc. To overcome all these problems modified AMPs are used. AMPs are diverse and they possess various properties as well as structures. Despite this diversity, they do have some common features - all of them are charged and their active regions are generally amphipathic (Tossi et al; 1997). The modified AMPs share these common properties. Moreover, they have to fulfill several requirements: to have the smallest amino acid domain which has full antimicrobial activity, to maintain the same activity in different environment, e.g. biological fluids, to be specific to selected organism as well as to be resistant against degradation. There are many ways how to modify AMPs but the detailed description of this process is above the frames of this thesis. In short, AMPs can be modified according to following models:

A. AMPs hybrids- they consists of active regions of 2 or 3 natural peptides, this increases their activity and decreases their cytotoxicity for host cells, e.g. cecropin A-melittin (Fox et al; 2012 and Wade et al; 1992);

B. AMP conjugates- they are conjugated to some receptor ligand or specific antibody which is situated on the outer surface of a specific pathogen, therefore they can be used in lower concentration and their side effects are decreases, e.g. Fusarium spp-specific antibody (Peschen et al; 2004).

C. AMP congeners- these compounds are related to another in composition and they have similar/antagonistic effects. A number of disulfide bonds can be reduced in

congeners and it results in higher antimicrobial activity, abolishment of chemotactic activity, etc., e. g. SMAP29 (Kalfa et al; 2001) or CAP18 (Larrick et al; 1993).

D. AMP mimetics- these AMPs are synthetic but not peptide molecules, even though they have the same properties and activities. They have similar chemical and physical properties, e.g. peptoids (Palermo et al; 2010).

E. Cyclotides- they are isolated from plants and are characterized by their head-to-tail cyclic backbone. Thanks to their cyclic structure they are more stable in diverse environment and resistant to degradation by proteolytic enzymes and by heating. Moreover, they are more active. Their application as anti-HIV agents (Ireland et al; 2008), insecticidal, antibacterial etc. is broad (for example, a circularized peptide analogue of rabbit defensin NP-1 (Yu Q. et al; 2000).

F. Immobilised AMPs- AMPs are incorporated into broad spectrum of materials and surfaces through plastics up to films as they are able to kill the pathogens. This method is used in food industry for preservation (Cutter et al; 2001) as well as in medical research, for example, lysozyme, nisin or nisaplin.

LIMITATIONS OF AMPS

However the AMPs are very promising as new therapeutics there are also several aspects which should be taken into consideration. Thus, as everything, the application of AMPs is also limited in a certain way. The main limitations are:

1. High production cost

Antimicrobial peptides have relatively high molecular weights compared to most antibiotics. To keep prices down they have to be produced as recombinant proteins and in higher yields. The production of recombinant proteins has become common because it is effective and the costs are lowered when produced in a large-scale. However, there are some technical difficulties especially when large amounts are required. At the current production efficiency, for each 100 kg of recombinant peptide one million liters of fermentation mixture is necessary (Hof et al; 2001). Moreover, it is very important to choose suitable expression system, so- called, fusion partner that is crucial for protein stability, yield and purification. Another way is to produce peptides synthetically, but its cost is from 5 to 20 times higher as compared to modern antibiotics - the cost per gram can differ from $50 to $400 (Marr et al; 2006). The only way how to overcome this cost problem is to produce synthetic peptides massively to lower the cost of all reagents as it was done by Roche Company in case of T-20 peptide, the suppressor of HIV-1 replication, for example.

2. Toxicity

Several studies have shown that some antimicrobial peptides, especially some cationic ones, are toxic for mammalian cells, for example antimicrobial peptides from the venom reservoirs of wild bees (Slaninova et al; 2012). However, in spite of the fact that not many studies on AMPs toxicity have been published, it was shown that a lot of antimicrobial peptides have neglectable toxicity.

3. Susceptibility to proteolysis

Pharmacodynamic and pharmacokinetic issues. Another aspect to discuss is AMPs susceptibility to proteolysis in the body, peptide aggregation problems, the in vivo half-life and required dosage frequency. AMPs are relatively labile to proteolysis which affects their applicability. In this context, several protection strategies have been proposed. AMPs can be protected by incorporation of negatively charged or lipophilic proteins (Sorensen et al; 1999), or by other chemical modifications as insert of uncommon amino acids (i.e. isovaleric acid, hydroxyproline) to prevent binding to the active sites of proteolytic enzymes (Banerjee A et al; 1996) or to prevent cleavage by alkylation of nitrogen atoms (Ostresh et al; 1996). To increase the stability of peptides, amidation of N-terminus, non-natural amino acids or peptide cyclizations are performed. Except the already mentioned limitations, significant differences of AMPs effects were noticed under in vivo and in vitro conditions. Exploration of high antimicrobial activity in in vitro tests does not always mean the same results in in vitro experiments, as AMP activity can be lost under physiological conditions. Human ?-defensin is (Goldman et al; 1997) one of the best confirmation of this observation. The discovery of antimicrobial peptides itself as well as their presence in almost all living organisms and their importance in defending organism against pathogens have become a milestone of this millennium. AMPs are very important compounds which help to maintain organism alive as well as protected them against microbes without specific immune responses. Their discovery has shown new possible direction in designing new therapeutics.

As it was already mentioned, it has been proved that AMPs are effective against a broad range of microbes, viruses, in some tumor cases. Therefore many research teams pay attention to this topic and try to find solutions how to use the AMPs against the existing pathogens as well as in prevention of pathogen caused diseases. One of the directions in searching for new effective compounds is the research of AMPs in arthropods in general and in ticks, in particular.

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