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Listeriolysin from *Listeria* and Their Effecting on AHNP

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Abstract:

Conventional anti-cancer therapy involves the use of chemical chemotherapeutics and radiation and are often non-specific in action. A major shortcoming in current treatment has been the development of drug resistance and inability to penetrate tumor cells. Alternative antitumor medicines with higher specificity and efficacy have therefore been explored. There is Significant interest in exploring the use of microbes as potential anticancer agents. We will introduce bacterial anti-cancer therapy with a focus on the various mechanisms involved in tumor targeting and suppression in this review. The bacteriotherapy in the design of novel treatments, approaches in combination with conventional cancer treatment may be effective in cancer therapies. We're focusing on the progress that has been made in the treatment of bacterial cancer. Patients in cancer treatment are attracted to tumor targeting peptides. These peptides are widely studied, delivering anticancer agents to tumor sites. In this study, we produced a new form of recombinant listeriolysin O (LLO) with genetically fused Anti HER2/neu peptide (AHNP) sequence adding to its C terminal end. The aim of the study was to engineer this pore forming toxin to make it much more specific to tumor cells. The results show that the LLO C terminal should not be changed, and it appears for the purpose of engineering and adding peptide modules, the N terminal of the toxin should be preferred. development of the small molecule that these constrained peptides provide drugs, but also provide insight into the atomic features of protein-protein interactions.

Keywords: Listeria –Listeriolysin –recombination.

Litreture Review

A foodborne pathogen that causes serious infections in humans (1,2,3). Meningitis, meningoencephalitis and bacteremia are the most common symptoms in non-pregnant adults (4). In murine infection models, *Listeria.monocytogenes* is a facultative intracellular parasite and immunity is cell-mediated . The molecular and cellular basis of the intracellular life of this parasite has been largely elucidated *L monocytogenes* is known for its unique ability to cause severe systemic

infections (bacteremia), affect the central nervous system, and cause infections that are difficult to treat (5,6) Listeriosis primarily affects vulnerable groups such as immunocompromised people, pregnant women, Foodborne pathogen *L monocytogenes* is a facultative anaerobe that is gram-positive. Immunocompetent individuals who are infected may experience mild gastrointestinal complications, but pregnant women, the elderly, and immunocompromised populations may experience more severe symptoms. An estimated 1600 individuals are estimated to contract *L monocytogenes* each year and admitted to hospitals in the USA, where up to 5% of patients do not survive, resulting in an 19% of all foodborne pathogen-related deaths are caused by *L.monocytogenes* infections . Because of the elevated death rate, the food industry is closely monitoring *L.monocytogenes,* which is accountable for $28 billion in medical costs and recall costs every year .

LLO is a member of the large family of bacterial pore-forming toxins (PFTs) known as the cholesterol-dependent cytolysins (CDCs), which are primarily found in gram-positive bacteria (7,8). As the molecular mechanisms underlying the formation of transmembrane pores from the assembly of soluble monomeric proteins once they are bound at the surface of lipid membranes are well understood, CDCs may be the most studied family of PFTs. . According to a study , three distinct expression structures for the synthesis of the LLO toxin were compared in 2005. Purification by column was not possible in the first structure using the fusion system. 350 μg/L of protein was purified in the second structure by inserting His-Tag in the N-terminal of the toxin gene, and 250 μg/L of protein was purified in the third structure by inserting His-Tag in the C-terminal of the toxin gene. According to this study, adding a His-Tag to the toxin's Cterminal reduces the amount ofprotein expressed.

The role of Listeriolysin O (LLO) and other virulence factors in the pathogenesis of *L. monocytogenes*. The pathogenesis of *L. monocytogenes* depends on tissue type, numerous bacterial virulence factors (9) ,and depends on host protein recruitment., which ultimately affects cell signaling pathways and host gene expression (10) Although the main intracellular role of LLO is thought to be due to the collapse of intracellular vacuoles, it also plays important roles in the extracellular space before internalization and in the cytoplasmic environment after vacuole escape (11), The extracellular role of *L. monocytogenes* is that it is internalized by professional phagocytes (e.g.g, macrophages, neutrophils) or by non-cellular cells, including cells of the intestinal epithelium (enterocytes). It can be internalized by triggering internalization by cells that are considered phagocytic., hepatocytes and endothelial cells (12,13) The central virulence factors involved in internalization into nonphagocytic cells are the surface proteins InlA and InlB, which interact with host membrane receptors such as E-cadherin and Methionine (14,15)

Once exposed to the cytosol, LLO must be rapidly inactivated to prevent cytotoxicity, enable bacterial replication, and spread to other host cells. Therefore, LLO activity must be tightly controlled, and this can be achieved in a variety of ways. Initially, LLO transcription is controlled by bacteria and is regulated by temperature , reactive oxygen species , pH . sugar availability , and branched-chain amino acids Once LLO is secreted by bacteria, it is exposed to the environment and is primarily controlled by host cells. Intestinal cells are a common site of *L.monocytogenes* entry, particularly at sites of cell extrusion where E-cadherin is temporarily exposed (16,17) Furthermore, LLO can also mediate bacterial internalization through the formation of pores on the cell membrane, leading to endocytic entry into epithelial cells or hepatocytes (18,19) This type of entry is caused by changes in the influx of extracellular Ca2+ ions following binding of LLO and pore formation on the host plasma membrane (20,21)

At the same time, the influx of extracellular Ca2+ triggers another membrane resealing mechanism, allowing repair of the plasma membrane, during which cells can absorb specific molecules from the extracellular environment. (14, 18, 19) Furthermore, we showed that addition of LLO to cell

monolayers decreased trans epithelial electrical resistance and increased monolayer permeability. After addition of LLO, changing at tight junctions and associated cytoskeleton, indicating that LLO may affect the enterocyte barrier by affecting the apical membrane*L.monocytogenes* virulence factors play essential roles in bacterial cell invasion, intracellular survival, and cell-to-cell spread. LLO plays a central role in these processes, and represents the most important pathogen ,because LLO primarily acts at the level of lipid membranes. The helical bundles play an important role in the formation of trans membrane pores . the molecular properties of LLO and the mechanisms of membrane damage are important and will be discussed. The transition from the pre-pore to the pore state is such that monomers and small oligomers are not inserted into the membrane and larger aggregations are required to cooperatively overcome the energy barrier required for membrane permeation and insertion of the β-hair pin .It happens in a coordinated manner because the body requires it. Therefore, the inserted arc assembly is composed of approximately 20 monomer subunits of 1,2-Dioleoyl-sn-glycero-3-phosphocholine DOPC.These well-defined LLO assemblies require detailed structural characterization to elucidate the details of the various functional moieties at the atomic level. In general, the physicochemical properties of lipid membranes can significantly influence pore formation by pore-forming proteins . LLO binding does not disrupt the organization of the lipid bilayer, but increases the mobility of the phospholipid head group and disruption of the hydrophobic core of the more fluid cholesterol-rich membrane . Similarly, all-atom molecular dynamics simulations show increased lateral mobility of lipid molecules in membranes containing insertion complexes compared to cytosolic leaflets or bare bilayers . In contrast, the presence of sphingomyelin or saturated phospholipids in ordered cholesterol-rich membranes reduces both effects, as determined by solid-state NMR . Lateral mobility also decreases significantly near the D4 domain in region as cholesterol density increases .(20)

LLO initially forms a small pore in the phagosome membrane, allowing the passage of protons and Ca2+.his delays the fusion of phagosomes and lysosomes, allowing time for bacteria to escape from the phagosomes and leading to degradation of within the phagosomes . Initial pore formation occurs within the first 5 minutes after bacteria enter the phagosome , and 30 minutes after bacteria enter the phagosome they escape into the cytosol (13) The pores of the phagosomal membrane have been reported to be large enough to allow proteins to pass through after 15 min (12) One possibility is that LLO-secreting bacteria that tightly adhere to host cell membranes cause local membrane disruption sufficient for invasion in tissue culture.We also cannot exclude the possibility that other virulence factors specific to *L.monocytogenes* are involved, except for internalinB.Pore-forming toxins (PFTs) of the cholesterol-dependent cytolysin family, on the other hand, produce enormous persistent protein-lined pores in the plasma membrane. To fix this type of to repair the damage, the protein hole must be removed from the cellular membrane. As a result, PFT-induced plasma membrane damage exists. Believe that the pore is repaired either by shedding of pore-containing microvesicles or by endocytosis of the pore. (16)

LLO has very little effect on bacterial uptake by skilled phagocytes. Furthermore, it has recently been suggested that LLO-mediated plasma membrane perforation by cytosolic bacteria promotes cell-to- LLO's function in is crucial. Internalization of *L. monocytogenes* by hepatocytes. Additional research revealed that the creation of LLO pores on the plasma membrane triggers the extracellular Ca_2+ influx. Moreover, the C-terminal toxin's AHNP-targeting peptide prevents the toxin from attaching to the target cell's membrane. Some article investigated the effecting of LLO recombinant toxin and LLO-AHNP toxin on breast cancer cell lines MCF-7 and MDA-MB-231. The results showed that the cytotoxic activity of recombinant toxin against these cell lines is dependent on protein concentration. The findings indicate that LLO-AHNP has less cytotoxic effects than LLO recombinant toxin, which is likely because the AHNP peptide was inserted into the C-terminal toxin and interfered with the toxin's bonding domain.This suggests that the toxin's lethal properties are negatively influenced by the receptors present in

the MDA-MB-231 cells. On the other hand, when the engineered toxin was tested on the MCF-7 cell line, it showed a decrease in effect by about two times compared to the MDA-MB-231 cell line. This indicates that the AHNP receptor does not impact the C-terminal side of the LLO toxin. Comparing the activities of the natural and engineered toxins on the MDA-MB-231 cell line, which serves as the negative control, reveals that the engineered toxin (LLO-AHNP) experiences more than a 9-fold decrease in activity in cells lacking the AHNP receptor. In other words, the addition of the AHNP peptide resulted in a toxin activity loss of over 7 times in AHNP receptor-free cells.(21) Discovering new treatments for cancer is a priority for many research institutions.There are many treatment options for cancer, including chemotherapy and radiation therapy.(22,23)

Biological treatments based on monoclonal antibodies, enzymes, and toxins have opened new avenues to address the issue of specificity in cancer treatment. Several researchers are attempting to develop specific therapeutics and diagnostics using tumor-targeting peptides (24, 25).Toxins attach to various cells through their catalytic domains and kill them..To specifically target the catalytic domain of a toxin, several technical steps need to be performed on the toxic molecule. This ability of LLO to form pores in eukaryotic membranes is the basis of its cytolytic activity. However, they are still not real candidates for cancer therapy due to the lack of binding specificity to the target membrane. The strategy to use LLO molecules to attack tumor cells was to fuse antibody fragments against the tumor antigen Lewis Y with LLO molecules.toxin molecule. This study demonstrates a new approach to identifying LLO molecules in specific cancer cell types. Our goal was to determine whether more specific pore-forming toxins could be obtained using targeting peptides that more selectively target specific cell types. This was achieved by fusing a targeting peptide sequence to the C-terminus of the LLO molecule. Many studies have used targeting peptides to increase the specificity of biomacromolecules such as toxins and enzymes.(26) Targeted drug delivery is aimed at improving therapeutic specificity and reducing side effects.reveals its unique characteristics.[21] Because bacterial toxins can efficiently kill cells, many toxins have been investigated as potential anticancer agents.[22]

Since higher hemolytic activity was observed for LLO than for LLO AHNP, it is possible that these toxins have a binding site on the C-terminal side, as suggested by (27).There is presence of the AHNP targeting peptide in the C-terminal toxin disrupts the binding of the toxin to the target cell membrane.The effect of the engineered toxin on the MCF 7 cell line was approximately two times smaller than on MDA MB 231 cells. This indicates that the AHNP receptor does not affect the C-terminal side of the LLO toxin. Comparing the activity of natural and engineered toxins in MDA MB 231 cells as a negative control, the engineered toxin (LLO AHNP) was shown to reduce activity more than 9-fold in cells lacking AHNP receptors.In other words, addition of AHNP peptide reduced toxin activity more than 7-fold in negative AHNP-R cells. Previous studies have shown that cytolysin (CylA) is widely used as a bacterial toxin and antineoplastic agent. Cytolysins are pore-forming agents that form multimeric pores in eukaryotic membranes and induce apoptosis via a caspase-mediated pathway. Cytolysins are usually obtained from *Escherichia coli* or *Staphylococcus aureus* (28).

Studies have shown treating mice with *Salmonella typhimurium* or *E.coli. E.coli* strains expressing the ClyA toxin showed inhibition of tumor growth (29,30).Scientist developed a cytolysin-producing *Escherichia coli* K-12 strain and combined it with radiation therapy to slow tumor growth and prevent metastasis in mice bearing CT26 murine colon cancer tumors (31). Improving tumor targeting facultative anaerobic bacteria, such as *Salmonella enterica* and *Listeria monocytogenes*, can survive in oxygen-rich environments and are toxic to normal tissues. Therefore, improving tumor targeting of facultative anaerobes is of paramount importance. Long-term treatment with AHNPs resulted in inhibition of tumor xenograft formation (i.e., prevention of tumor

formation), (32) and intraperitoneal administration of AHNPs induced small palpable tumors derived from T6-17 fibroblasts. Tumor development was suppressed.AHNP inhibited the progression of tumorigenesis. These animals. These observations suggest that AHNPs can inhibit the growth progression of established tumors. Since AHNPs showed increased apoptosis of tumor cells when treated with chemotherapeutic agents in vitro, we investigated the same effect in vivo. The small size and high binding affinity of AHNPs to the extracellular domain make them suitable candidates as immunotherapeutic and diagnostic agents. However, the pharmacokinetic profile of AHNP is not optimal for therapeutic or diagnostic purposes.(33,34,35)

To improve the binding affinity and antitumor efficacy of p185her2/neu, we developed a fusion protein combining AHNP with a non-antitumor protein . It contains an immunoglobulin protein scaffold, streptavidin (SA).(36) Recombinant protein AHNP-SA (ASA) binds p185her2/neu with high affinity, inhibits the proliferation of cells overexpressing p185her2/neu, and inhibits tumor growth induced by p185her2/neu transformed cells. To do. Suppresses reduction.(37,38,39) Peptidomimetics utilize modifications to side chain groups or the peptide backbone to improve peptide stability and/or biological activity. Since most linear peptides are easily degraded by enzymatic proteolysis, modification of the peptide backbone can help reduce the rate of degradation. The highly charged side chain groups of peptidomimetics increase receptor binding affinity and selectivity for these peptidomimetics, thereby reducing undesirable side effects and improving therapeutic efficacy.(40,41,42). Science has clarified other types of treatment for cancer, especially breast cancer, as stated in research. The pathogenesis of breast cancer is unknown, but number of factors are associated with an increased risk which includes genetic factors which involved oncogenes and tumor suppressor genes.(43) The critical tools for studying cancer drug screens and cell biology have been made available by the 3D spheroids of cancer cells,because of the type of culture system it is possible to replicate a large number of aspects of in vivo cancer cell conditions. The spheroid culture conditions, including a decrease in extracellular mass from peripheral cells of spheroids to their core, can describe the physicochemical gradients within cancer cells. , and spheres availability.(44) On the other hand ,one of the emerging biotherapeutics that needs to be used is oncolytic virotherapy. a more efficient in vitro tumor model to overcome the two-dimensional (2D) monolayer tumor cell culture model's inability to maintain tissue-specific structure. This is to provide significant predictive preclinical evaluation findings. (45,46,47,48) The properties of peptidomimetics make them useful as bioactive agents and as agents with pharmacological activities such as protease inhibition, antibacterial, anticancer, analgesic, antiviral, and antimalarial effects.It's very interesting.This review focuses on the contribution of peptidomimetics in the synthesis, pharmacological activity, and further development of new drugs that can be effectively used in the treatment of various diseases.

Conclusion

Our findings indicated that the N-terminus of toxin should be favored for peptide module assembly and addition. The introduction of bacterial therapy use here in a new field in cancer treatment. The exceptional capacity of bacteria to attain target specificity was varied with therapeutic effects points to the effective of bacterially mediated cancer therapy.

Synthetic biology and genetic engineering tools have allowed microorganisms to be adapted to transport medicinal payloads more efficiently. A combination of bacterial therapy, chemotherapy, and radiotherapy can assist overcome tumor heterogeneity and result in excellent outcomes. However, safety and bacterial biodistribution issues remain a worry. More study and development in the field of bacterial therapy could bring a new dimension to cancer treatment to our knowledge. AHNP is the first tiny peptidomimetics designed specifically targeting receptors that bind to the extracellular domain of tumor proteins. We hope that this method will result in the discovery of small compounds that can be employed as innovative receptor-based cancer therapies in people.

Our findings show that biologically active synthetic exocyclic peptides can be designed to improve functional characteristics utilizing structured oligomerization and then recombinantly generated via bacterial expression.

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