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Acanthamoeba Keratitis With Emphasis On Need And Role Of Emerging NANO-Tech Based Treatments: A Review

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Abstract : **Acanthamoeba keratitis (AK) is a sight-threatening, sub-acute or chronic inflammation of the cornea, mostly associated with contact lens wearers that affect both healthy or immunocompromised individuals. The infected individuals usually have the history of trauma/ injury/abrasion to the cornea along with the exposure to Acanthamoeba sp. through contaminated water or soil. It usually produces severe ocular pain and congestion of the conjunctiva. Other features presented are scattered sub-epithelial infiltrates, anterior uveitis, stromal perforation and inflammation of the sclera. Glaucoma is commonly reported and occasionally posterior segment signs such as nerve edema, optic atrophy and retinal detachment may be observed. In untreated eyes, blindness may eventually result as the necrotic region spreads inwards. Diagnosis is often delayed, in patients of AK, leading to further delay of treatment and ineffectiveness of drugs, thus resulting in blindness ie; complete loss of sight. The conventional methods for AK treatments are frequently unsuccessful due to lack of sufficiently effective drugs and these treatment methods usually involve the use of multiple antibiotics and/or surgery. Therefore, realizing an acute need of new anti-Acanthamoeba drugs, mainly when resistance to the classical therapeutics is encountered, researchers have now thought of considering nanotechnology as a tool to combat such problematic diseases. Nanotechnology has emerged as a promising solution that has the potential to revolutionize the diagnosis, treatment and management of such diseases as they are more targeted and serve to offer improved drug absorption. This document serves to generalize the current need and role of nanotechnology in drug delivery mechanisms by addressing to some of the helpful novel ocular treatment possibilities that may be applied to AK for its possible cure.**

Keywords: ***Acanthamoeba*** **keratitis, ocular, nanotechnology, diagnosis, treatment, drug delivery methods.**

1. INTRODUCTION

Acanthamoeba species are ubiquitous and most prevalent free-living protozoa found in a wide range of environmental habitats [17]. These are small, aerobic, amphizoic amoebae, capable of living opportunistically inside human host and cause disease. The pathogenic species of *Acanthamoeba* is responsible to cause granulomatous amoebic encephalitis (GAE). It is the infection of the brain and may prove to be fatal for immunocompromised individuals. *Acanthamoeba polyphaga* is known to cause sub-acute or chronic inflammation of the cornea which is severely painful and potentially sight-threatening disease of the human eye. This disease is known as *Acanthamoeba* keratitis [16,7]. The infected individuals usually have the history of trauma/ injury/abrasion to the cornea along with the exposure to *Acanthamoeba sp*. through contaminated water or soil. Diagnosis is often delayed, in patients of AK, leading to further delay of treatment and ineffectiveness of drugs, thus resulting in blindness ie; complete loss of sight. AK treatments are frequently unsuccessful usually involving the use of multiple antibiotics and/or surgery and lack of sufficiently effective drugs [13]. Realizing the need of new anti-*Acanthamoeba* drugs, mainly when resistance to the classical therapeutics is encountered, researchers have now thought of considering nanotechnology as a tool to combat such problematic diseases. Nanotechnology has emerged as a promising solution that has the potential to revolutionize the diagnosis, treatment and management of such diseases as they are more targeted and serve to offer improved drug absorption. This document serves to summarize the current need and role of nanotechnology in drug delivery mechanisms by addressing to some of the helpful novel ocular treatment possibilities that may be applied to AK for its possible cure.

2. MAJOR RISK FACTORS, SYMPTOMS AND CLINICAL FEATURES OF THE DISEASE

A. polyphaga is naturally found in soil and water. It is found in two forms, the active trophozoite and the dormant cystic form. Trophozoite stages are known to be infective stages and are capable of causing disease in healthy as well as immunocompromised individuals. *Acanthamoeba* keratitis may be caused due to contact of cornea with contaminated water, especially while swimming, contact lens wear and its improper handling [23]. In India, besides, contact lens wearers, mainly rural people, farmers and rural vendors are the at-risk groups of catching the disease. They often utilize

outdoor contaminated water bodies, such as ponds, rivers, pools for swimming and bathing [24]. Not only this they often rub their eyes with dirty contaminated hands, wash eyes with contaminated water. Affected individuals may complain of disease symptoms that include eye redness, sensation of something in the eye or irritation, excessive pain, excessive tearing, photophobia, lid edema and blurred vision [4]. In rare circumstances, *Acanthamoeba* can spread to the posterior part of the eye as well [18] leading to further complications.

3. DIAGNOSIS

AK is often mistakenly diagnosed as herpes simplex keratitis or fungal keratitis or bacterial keratitis [23]. A diagnosis of AK should be considered following advanced techniques, such as PCR, real time PCR and/or confocal microscopy. These techniques are often used as a diagnostic and monitoring tool also. Similarily, Confocal microscopy also has been found to be a powerful tool for diagnois of AK, it helps to find the infection in vivo, hence, there is no need to wait for long and time taking culture and microbiological analysis. In such an in-vivo microscopy, the diagnosis is made at that very moment and there is no need for invasive biopsy anymore. In confocal, microscopy, one can easily observe cystic stages as round, hyperreflective lesions and the diagnosis is made. Then, there are multiplexed quantitative PCR methods also, which are able to discriminate individual *Acanthamoeba* sp. in mixed samples of bacteria and *Acanthamoeba* [5]. H1 nuclear magnetic resonance (NMR) has been also used for diagnosis of Acanthamoeba, in vitro by Hauber. et. al.[10]

4. TREATMENTS

Acanthamoeba keratitis may be treated well only if it is diagnosed at an early stage. Treatment may vary from person to person, depending upon the severity of the disease and the way, the patients are responding to the drugs. Treatment still relies on topical biguanides and diamidines. These biguanides is usually, poly hexamethylene biguanide (PHMB), 0.02% or chlorhexidine 0.02% in combination with a diamidine (propamidine is ethanoate 0.1% or hexamidine 0.1%)[5]. prolonged use is required for these drugs to act. The diamides are usually well tolerated, but prolonged use can lead to a (reversible) toxic keratopathy. Other drugs such as neomycin has been not in use as this drug may induce hypersensitivity in patients. This drug is also found to be resistant to acanthamoebae now [4].When the drugs do not work, then, at later stages, invasive approaches and surgery may be required which may cause discomfort as well as complications. Since there have new developments in our understanding of the disease, with new techniques for diagnosis and treatment Therefore, we are more compelled to switch over to more sophisticated/ reliable and promising techniques such as nanotechnologies.

5. ADVENT OF NANOTECHNOLOGY IN TREATMENT OF *ACANTHAMOEBA* KERATITIS

Nanotechnology has the potential to overcome ocular barriers and sustain drug delivery following topical administration, offering an efficient ocular drug delivery system, which can provide maximum precorneal residence time, leading to better results during the treatment of the disease. nanotechnology, could be defined as “the design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property.”[3]. In Nanomedicine, the medical application of nanotechnology, promises a neverending range of applications from biomedical imaging ;ie, diagnosis to drug delivery and therapeutics ie., treatments. Nanomedicine results from the manipulation of atoms and molecules that can range in size from 1 to 100 nm, where, a nanometer is one-billionth of a meter. At this size, particles begin to show entirely different physicochemical properties than that of the parent material. During the recent years, developments in targeted drug delivery have been facilitated for various specific tissues. Nanomedicine has emerged into a billion-dollar industry because of these compounds’ inherent ability to overcome solubility and stability issues, localize drug delivery, as well as to diagnose via in vivo imaging. Such upcoming developments and innovations in nano medicine may develop a capacity to generate multifunctional entities capable of simultaneously diagnosing, monitoring and even delivering therapeutic agents. Several such particle types and structures have been discovered. At present, many nanotechnology based ocular drug delivery systems, such as nanoparticles, niosomes, liposomes, dendrimers and micelles are preferred mode of delivery for various eye related diseases. We will be discussing about some of the nano-components, in brief, that may be applied to the effective treatment of *Acanthamoeba* keratitis:

Nanoparticles: Nanoparticles can be a potential carrier for targeted and controlled drug delivery to the posterior segment as well. It helps improving the residence time of nanoparticles in cul-de-sac. To avoid organic solvents and polymer toxicity , solid-lipid nanoparticles have also been attempted in ocular drug delivery. Due to extremely small dimensions, solid, lipid nanoparticles (SLN) are presumably entrapped and retained in the mucin layer covering the corneal epithelium.

Nanosuspensions: This is a colloidal suspension of nano sized drug particles, stabilized by surfactants. such formulation helps in easy eye drop preparations, enhanced solubility, lower dosage and improved physical stability and improved bioadhesion that helps in rapid corneal penetration and reduced ocular irritation.

Liposomes: are small lipid vesicles (.08µm-10µm) containing an aqueous core enclosed by phospholipid bilayers. They help in prolonged drug retention, improved drug absorption and protection of active molecules from metabolic enzymes at the corneal epithelium interface. Properties of liposomes vary substantially with lipid composition, size, surface charge and methods of preparation.

Niosomes: This is formed by the self assembly of non-ionic amphipathic molecules in aqueous media and have a bilayered structure physically similar to liposomes. These vesicles have been used to encapsulate both hydrophilic and lipophilic drugs, either in aqueous layer or in a lipid membrane. It has greater stability and ease of storage, low toxicity, biodegradability and biocompatibility.

Discosomes: represent modified forms of niosomes, which have large disc shaped structure. These vesicles are derived from the niosomes. Larger size of discosomes prevents drainage into systemic pool and disc shape provides a better fit in ocular cul-de-sac, discosomes can be selected as the carrier for controlled ocular administration of water soluble drugs.

Micelles: Micelles are nanosized, amphiphilic, spherical colloidal particles with a hydrophobic interior (core) and a hydrophilic exterior (shell). Their main utility is in the preparation of pharmaceutical formulations. Their individual particle size is less than 50 nm in diameter, which provides benefits over liposomes.

Dendrimers: Dendrimers are a highly branched, globular macromolecules. It has got a repeated branching around the central core that results in a nearly-perfect three dimensional geometrical pattern. They have numerous cavities within their branches to hold therapeutic and diagnostic agents. Dendrimers may be toxic because of their ability to disrupt cell membranes. Because dendrimer synthesis is controlled, they can be "made to order" to fit target binding sites of specific viruses [22].

6. CONCLUSION

Acanthamoeba has been found to colonise the nasal mucosa in up to 24% of environmentally exposed populations [15], although its pathogenic activity is much more rare, as indicated by medical and health professionals. *Acanthamoeba* keratitis has a large variation in reported rates between countries, which is thought to be largely due to differences in diagnostic criteria, rather than differences in populations. In developing countries like India, where, an individual poses a higher risk/threat of the disease due to unhygienic, unsanitary conditions, poor public water supply quality, more risk of exposure to contaminated soil and water, in various ways and various life activities. The disease has been tagged as rare. There are no methods of dissemination of knowledge about this disease. Even the medical and health professionals are not well aware of the disease as well as its incidence rate in India. Due to poverty, lack of awareness and ignorance, such diseases are not been reported in India. But, this does not mean that their incidence rate is also low, especially in a country that has a high exposure rate of the causal organism and suitable conditions for disease propagation. Schaumberg et al. found a US incidence of 2 cases per million contact lens wearers in the late 1980s [23] as compared to over 21 cases per million contact lens wearers in the UK in 1998 [21]. Considering this data, we need to accept that *Acanthamoeba* keratitis is becoming an increasingly significant problem in days to come. Contact lens wear is not always the main risk factor for infection. In a recent epidemiological study from India [14], only 0.9% of reported cases of AK were thought to be associated with contact lens wear. The major risk factors were associations with eye trauma and poor water supply. The first case of *Acanthamoeba* keratitis (AK) was reported in 1973, of an American patient in Dallas, while, the first published reports emerged in the UK in 1974 [19]. In those days, diagnosis was made through light microscopy, where, with the help of retrieved tissues from both cases, it was found that cystic stages of *Acanthamoeba* sp. were present. Both these cases were in need of surgical procedures where, one required grafting and the other, enucleation respectively. Since, then, there has been significant increase in the rise of *Acanthamoeba* keratitis patients and even in the disease severity [1]. This actual rise has been shown to be related to increased rates of contact lens wear [2], in particular soft lenses and the use of one-step cleaning solutions, which may prohibit to kill all the amoebic infection present in the contact lens, hence causing the disease [12]. Extensive studies on this topic have been conducted by Illingworth and Cook in 1998 [11], Hammersmith in 2006 [9], and Dart et al. in 2009 [6] *Acanthamoeba* keratitis is a potentially sight threatening, devastating disease that are tagged as rare by medical and health professionals of India. The disease constantly presents difficulties in diagnosis and treatment. Since 1973, we have expanded our knowledge of the clinical manifestations of this disease and have come to recognize them with the advancements of science and technologies. Recent advances of PCR and confocal microscopy have not only started to improve our diagnostic ability but, they have also become more recognized and available. It is hoped that they will serve us more in clinical practice in near future. Although mainstream, treatment still relies on topical biguanides and diamidines for straightforward cases, we are still learning about novel and advanced technologies about treating the disease. We have also learnt that steroids may be used safely in cases with a significant inflammation. We have learnt that deep lamellar keratoplasty (DLK) may provide good outcomes whilst maintaining the host endothelium [5]. It has also been observed that Laser photokeratectomy may become more important as we continue to explore its indications. But, we are aware of the fact that surgical and invasive treatment as well as prolonged use of topical drugs do have side effects and complications. In addition, corneal and conjuctival epithilial barriers, lacrimal drainage, prevent therapeutics from penetrating. Even though nanoparticles are a promising tool for enhancing ocular bioavailability, various safety issues still

need to be considered. New advancements in nanotechnology promise desired therapeutic effects while ameliorating side effects associated with many traditional medications.

Nanomedicines is meant to solve problems associated with the solubility, bioavailability and immunocompatibility, of these traditional medications [8]. There have been interesting developments in nanomedicine and nanopharmaceuticals that has generated a number of advancements throughout recent years with a focus on engineering of novel applications. Additionally, diseases management could be done using multifunctional agents encompassing both imaging and therapeutic capabilities, thus allowing simultaneous monitoring and treatment. Nanotechnology also offers the ability to detect diseases at much earlier stages. Such diagnostic applications and practices may build upon conventional procedures using nanoparticles, nanosuspensions, micelles, liposomes, discosomes and many such useful novel structures. A detailed evaluation of these formulations are essential to expand and upgrade our current knowledge on nanotechnology and nanomedicine applications. Despite the progress in the field, influence of various properties of nanomicelles such as size, shape, surface charge, rigidity of structure on ocular disposition need to be studied in further details to develop an efficient nanocarrier system as well as safety and long-term effects of nanoformulations must also be evaluated. This review will provide a brief discussion of the some of the major nanotech based updates with reference to *Acanthamoeba* keratitis. The aim of this paper is to bring the reader up to date with current and emerging practices.

REFERENCES

[1] Ashton N. and Stamm, W., "Amoebic infection of the eye: a pathological report," *Transactions of the Ophthalmological Societies of the United Kingdom*, vol. 95, no. 2, pp. 1975, 214–220.

[2] Badenoch, P. R. "The pathogenesis of Acanthamoeba keratitis," *Australian and New Zealand Journal of Ophthalmology*, vol. 19, no. 1, 1991. pp. 9–20.

[3] Bawa R. Patents and nanomedicine. Nanomedicine;2: 2007, 351-74.

[4] Claerhout, I. and Kestelyn, P. "Acanthamoeba Keratitis: A Review". *Bull. Soc. belge Ophtalmol.*, , 274, 1999, 71-82.

[5] Clarke, B., Sinha, A., Dipak, Parmar, N. and Sykakis, E. *Review Article* Advances in the Diagnosis and Treatment of Acanthamoeba Keratitis. Volume 2012, Article ID 484892, 2012, 1-6.

[6] Dart, J. K. G., Saw, V. P. J. and S. Kilvington, "Acanthamoeba Keratitis: Diagnosis and Treatment Update 2009," *American Journal of Ophthalmology*, vol. 148, no. 4, 2009, pp. 487.e2–499

[7] De Jonckheere, J.F., "Ecology of Acanthamoeba". *Rev Infect Dis.* Mar-Apr; 13 Suppl 5, 1991 :S385-7.

[8] Goldberg M, Langer R, Jia X. Nanostructured materials for applications in drug delivery and tissue engineering. J Biomater Sci Polym Ed 2007;18:241-68.

[9] Hammersmith, K. M. "Diagnosis and management of Acanthamoeba keratitis," *Current Opinion in Ophthalmology*, vol. 17, no. 4, 2006.,pp. 327–331,

[10] Hauber, S., Parkes, H., Siddiqui, R. and Khan, N. A."The use of high-resolution 1H nuclear magnetic resonance (NMR) spectroscopy in the clinical diagnosis of Acanthamoeba," *Parasitology Research*, vol. 109, no. 6, 2011., pp. 1661–1669.

[11] Illingworth C. D. and Cook, S. D. "*Acanthamoeba* keratitis," *Survey of Ophthalmology*, vol. 42, no. 6, 1998., pp. 493–508.

[12] Jasim, H., N. Knox-Cartwright, S. Cook, and D. Tole, "Increase in Acanthamoeba keratitis may be associated with use of multipurpose contact lens solution," *British Medical Journal*, vol. 344, Article ID e1246, 2012.

[13] Khan, N. A., The immunological aspects of Acanthamoeba infections *American Journal of Immunology* 1 (1): 2005.24-30,

[14] Lalitha, P.,. Lin, C. C,. Srinivasan M., et al., "Acanthamoeba keratitis in South India: a longitudinal analysis of epidemics, *Ophthalmic Epidemiology*, vol. 19, no. 2, 2012. pp. 111–115.

[15] Lawande, R. V., Abraham, S. N., John, I. and Egler, L. J. "Recovery of soil amebas from the nasal passages of children during the dusty harmattan period in Zaria," *American Journal of Clinical Pathology*, vol. 71, no. 2, 1979. pp. 201–203.

[16] Marciano-Cabral, F. and Cabral, G., "*Acanthamoeba* spp. as Agents of Disease in Humans". *Clin Microbiol Rev*. Apr 2003 Apr;16(2):273-307.

[17] Mergeryan, H., "The prevalence of Acanthamoeba in the human environment". *Rev Infect Dis.* Mar-Apr;13 Suppl 5:, 1991, S390-1.

[18] Moshari, A., McLean, I. W., Dodds, M. T., Damiano, R. E. and McEvoy P. L. *Chorioretinitis after keratitis caused by Acanthamoeba: case report and review of the literature.* Ophthalmology 108**:** 2001, 2232-*2236*

[19] Naginton, J., Watson, P. G., Playfair, T. J., McGill, J., Jones, B. R. and Steele, A. D. "Amoebic infection of the eye," *The Lancet*, vol. 2, no. 7896. 1974, pp. 1537–1540.

[20] Niederkorn, J.Y., Alizadeh, H., Leher, H., McCulley, J. P. The pathogenesis of *Acanthamoeba* keratitis;Review. *MicrobesInfect* 1999; 1 :437 -42.

[21] Radford, C. F, Minassian, D. C. and Dart, J. K. G. "Acanthamoeba keratitis in England andWales: incidence, outcome, and risk factors," *British Journal of Ophthalmology*, vol. 86, no. 5, 2002, pp. 536–542.

[22] Rosa Borges, A., Schengrund, C. L. Dendrimers and antivirals: a review. Curr Drug Targets Infect Disord 2005;5:247-54.

[23] Schaumberg, D. A., Snow, K. K. and Dana, M. R. The epidemic of *Acanthamoeba* keratitis: where do we stand? Cornea 17**:,** 1998., 3-10.

[24] Shukla, K., *Protozoa and food: amphizoic amoebae and other protozoa in relation to edible biological products.* 2011. VDM Verlag Dr. Müller, Germany.