Photodynamic therapy for ocular diseases: The futuristic approach.

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Received: *31-Dec-2023, Manuscript No. AACOVS-24-124182;* ***Editor assigned:*** *02-Jan-2024, AACOVS-24-124182 (PQ);* ***Reviewed:*** *16-Jan-2024, QC No. AACOVS-24-124182;* ***Revised:*** *08-Jan-2025, Manuscript No. AACOVS-24-124182 (R);* ***Published:*** *15-Jan-2025, DOI: 10.35841/AACOVS.9.1.497-500*

Abstract

Photodynamic therapy has recently gaining more importance in the treatment of micro vascular diseases due to its tunable/precise delivery of payload. As ocular drug delivery is facing challenge due to various barriers and peculiar anatomy of eye the delivery of drugs using assistance based therapy is currently warranted. In this regimen this mini review will examine the basis of photodynamic therapy and its components. The various photosensitizer loaded nano formulations for multifactorial applications has been discussed with special emphasis has been provided towards the utilization of photodynamic therapy for ocular drug delivery.

Keywords: Photodynamic therapy, Low density lipoprotein, Photofrin, Hyperproliferating, Chlorin e6.

Introduction

Ocular drug delivery

The unique anatomy and physiology and complex nature of the eye create a major challenge for ocular drug delivery. The conventional formulations are inefficient, whereas systemic administration ends up with significant toxicity. There is a pressing need to develop novel drug delivery increasing ocular bioavailability especially at the posterior pole of eye. Recently, the emergence of lipid-based nanocarrier has provided a viable means of enhancing the bioavailability of ophthalmic formulations. A number of these formulations have been found to be clinically active.

The static barriers, dynamic barriers, and efflux pumps create a significant challenge for delivery of a drug to the posterior segment of eye. Transporter-targeted delivery, stimuli responsive delivery, etc. are gaining importance. Nanoparticles, nanomicelles, nanodispersions, solid lipid nanoparticles, nanoemulsions, liposomes, and microemulsions have been widely explored to overcome the challenges associated with ocular drug delivery. Designing noninvasive drug delivery systems exploring the feasibility to reach the posterior segment of eye *viz.* the light activated drug delivery may drastically improve drug delivery in the years to come.

Photodynamic therapy: Photodynamic Therapy (PDT) is a minimally invasive theranostic treatment modality for ocular diseases/cancer/skin diseases/microbial infections in which the diseased tissues were destroyed by its dual approach of light and specific photosensitizers (e.g. porphyrins, chlorines, pthaallocyanines, porphycenes etc.). The singlet oxygen ($^1O_2$) is produced on choroidal neovascular capillaries [1] upon light exposure of photosensitizer's results in apoptosis. Even if the apoptotic pathway is blocked, the damaged cells may still die by autophagic or necrotic pathways [2]. By itself, the photosensitizers are harmless and non-damaging. However, upon light irradiation at appropriate wavelength, the photosensitizer becomes excited and may transfers its energy to the surrounding molecular oxygen and generate the cytotoxic reactive oxygen species. The produced $^1O_2$ will oxidize critical cellular macromolecules, like lipids, nucleic acids, and amino acids, which alters the cellular permeability with the consequence of cell death.

Laser light: The electromagnetic radiation (light) which covers the visible region of 400 to 700 nm is relevant to photodynamic therapy. The absorption of light by the molecule causes energy of absorbed photons to get excited *via* transfer its energy to ground state will yields reactive oxygen species. Lasers were the standard light source to activate photosensitizers, which provide the advantages of ease of control, produce high intense monochromatic light and ease of focus to small spots. The current localization difficulties include fluence non-optimization, size and flexibility of light source, timing of illumination, energy requirment and cost.

Singlet oxygen: The singlet oxygen generation of photosensitizer was triggered by its light absorption of the photosensitizer which gets transformed from its ground state (S_0) to the excited state (S_1). The generation of fluorescence

occurs from the excited state itself, whereas the intersystem crossing to the triplet state (T1) at electronic level will produce the type II photodynamic mediated $^1O_2$ (short life span) which may induce photooxidative damage to biological targets. The half-life of $^1O_2$ is very short (≤ 0.04 μs) in biological systems [3].

Photosensitizers: Photosensitizers are photoactive compounds which induces short lived cytotoxic $^1O_2$ species and produces intrinsic fluorescence over a broad range of excitation/emission wavelengths upon non-thermal light irradiation, this photophysical and photobiological properties elicits its application in photomedicine [4]. The conceptual applications of photosensitizer in medical field appeared by the exposure of *Paramecium caudatum* (by a German medical student) to acridine orange and *in vitro* light which leads to the death of microorganism [5]. The clinical usage of photosensitizers has been decreased substantially due to its low tumor targeting efficacy and poor water solubility characteristics [6]. Recent reports suggest that nano formulations containing photosensitizer may improve the targeting (chemotherapeutic drug targeting) and therapeutic potentialities (synergistic effect) for micro vascular diseases treatment [7]. Preferentially the photosensitizers are taken up by normal and hyperproliferating retinal cellss, but they were rapidly cleared off from normal cells, retained for longer time in diseased cells due to overexpression of Low Density Lipoprotein (LDL) receptors in those cells [8,9]. The ideal characteristics of photosensitizers are:

Photosensitizer based formulations: Depending on the properties, photosensitizers were classified in to three classes, the first generation, second generation and third generation photosensitizers. The first generation photosensitizers (photofrin) slowly cleared from healthy cells and have shorter wavelength absorption. The second generation photosensitizers (porphyrin, metallated derivatives) exhibits intense longer wavelength absorption. The third generation photosensitizers (hypericin) exhibits good clearance from the healthy cells, improved localization and targeting ability to the mitochondria of the cells. Several photosensitizer based formulations have been developed by many researchers; initially the liposomal formulation of verteporfin (1st generation photosensitizer) using dimyristoyl phosphatidylcholine and egg yolk phosphatidyl glycerol was developed by Ichikawa, et al. in order to check the enhanced anti-cancer action *via* active targeting. In order to improve the efficiency of photosensitizers and to overcome the drawbacks of photosensitizers, scientists utilized a nano sized drug carrier or quenching/dequenching systems [10].

Literature Review

The liposomal formulation showed remarkable phototoxicity in ECV304 endothelial cell lines and anti angiogenic effect in meth-A-sarcoma bearing mice when this formulation was exposed to laser irradiation for 15 minutes at an activation wavelength of 690 nm after intravenous injection. Biodistribution of developed liposomal formulation after iv administration in BALB/c mice indicated that 2nd/3rd of the active drug molecule are able to accumulate at the tumour site. In an attempt to target αvβ3 integrin receptors Frochot, et al. developed RGD peptide conjugated tetraphenylchlorin photosensitizer formulations. The results of their study indicates that the $^1O_2$ production yield for the conjugated photosensitizer was still lower than un conjugated photosensitizer, they studied the phototoxicity and cellular uptake in HUVEC cells and found the higher cellular uptake and phototoxic effect in the conjugated form, due to the targeting capabilities of both linear and cyclic RGD motif [11].

Protoporphyrin IX conjugated glycol chitosan nanoparticles for the treatment of cancers using PDT was developed by Lee, et al. with an average size of 350 ± 15 nm. Conjugated nanoparticles showed enhanced phototoxicity when compared to free protoporphyrin IX molecules in SCC-7 cells which is due to higher cellular uptake of conjugated nanoparticles. The double combination of chemotherapy (doxorubicin) and PDT (methylene blue) based nanoparticles for its potential benefits in diseases management was reported by Khdair, et al. [12]. Drug loaded nanoparticle with a size of 62 nm was prepared by multiple emulsification cross linking method. Around 23% release of doxorubicin and 12% release of methylene blue for a period of 24 h in RPMI cell culture medium was observed. Increase in doxorubicin cellular uptake even in the nucleus of NCI/ADR-RES cells and ROS production than free drugs have been documented. Light activated combination therapy induced more ROS generation in comparison with light activated methylene blue treatment. The double combination therapy exhibited superior cytotoxic effect with significant apoptosis and necrosis induction.

The poly (ethylene glycol) block poly D, L-lactic acid loaded doxorubicin and photosensitizer hematoporphyrin mono methy ether nanovesicles was developed by Xiang, et al. They formulated four nanovesicles of blank, individual and dual drug loaded nanovesicles. The apoptotic rate of dual drug (doxorubicin and hematoporphyrin mono methy ether) loaded nanovesicles was higher when compared to individual drug loaded nanovesicles in HepG2 cells. Multifunctional targeted nanovesicular drug delivery system may improve the solubility of photosensitizer and targeting ability of doxorubicin, moreover it reduce the side effect of doxorubicin and provides a synergistic effect on HepG2 cells.

The third generation hypericin loaded poly lactide co-glycolide nanoparticles was developed by Labouebe, et al. using solvent evaporation technique with a drug loading of 0.03 to 0.15% and size range of 200-300 nm. Hypericin loaded nanoparticles (0.025 mg/l) exhibited a concentration dependent higher photoactivity than pure drug in Nu Tu-19 ovarian cancer cells, similarly by increasing the light dose and incubation time the photosensitizer loaded nanoparticles exhibited 4-folds higher phototoxicity which may be due to the cellular penetrating properties of nanoparticles [13].

In a view to enhance the production of $^1O_2$ for an efficient photodynamic therapy, the photosensitizer chlorin e6 conjugated human serum albumin nanoparticles was developed by Jeong, et al. with an average size of 88 nm. The amount of

$^1O_2$ produced by the human serum albumin conjugated photosensitizer was similar to pure drug chlorin e6 and human serum albumin nanoparticles do not yield any $^1O_2$. The cellular uptake of chlorin e6 conjugated nanoparticles and pure chlorin e6 by the HeLa cells were similar and it was takenup by the cytoplasm rather than the nucleus. The developed nanoparticles exhibited a 70% of tumour reduction in comparison to pure drug and saline control in HT-29 tumor bearing mice by laser irradiation. Chlorin e6 loaded magnetic nanoparticles was developed by Huang, et al. with a mean particle size of 24.3 ± 1.9 nm, and it showed a 7 fold increase in $^1O_2$ production after conjugation of chlorin e6 with magnetic nanoparticles. The occurrence of intense red fluorescence around the nucleus of MGC803 cells indicated the intracellular uptake of photosensitizer loaded magnetic nanoparticles. Cells treated with developed nanoparticles showed the cytotoxic effect which is inversely proportional to the drug and light dosing. The swelling/inflammation of mitochondria of cells occurs due to $^1O_2$ productivity of the developed nanoparticles. The targeting and imaging ability of the developed photosensitizer loaded magnetic nanoparticles was proved in nude mice [14]. Similarly, photosensitizer (HB) incorporated lipid coated gold nanocages was developed by Gao, et al. for its higher efficiency by photothermal effect [15].

The drug delivering nanoparticles can be programmed to be light sensitive by incorporating light sensitive polymers, functional dyes and metallic nanoparticles. The surface plasmon resonance effect of silver and gold nanoparticles influences the encapsulated photosensitizer to be released destructively/non-destructively by the heat (hyperthermia effect) generated by such nanoparticles [16]. It has been reported that upon illumination the nanoparticles may produce small quantity of heat sufficient to exceed the glass transition of the polymer complex, thereby decreasing the shell permeability till the laser light source illumination being stopped, thus the increased permeability allows the encapsulated photosensitizer to be released in a controllable manner [17].

Discussion

Photosensitizer nano formulations for ocular diseases

Augustin, et al. adopted a triple combination therapy involving photodynamic therapy (verteporfin), steroids and anti-angiogenic drugs for robust and lasting treatment of choroidal neovascularization in AMD [18].

With an objective to achieve enhanced production of singlet oxygen ($^1O_2$) for the management of age related macular degeneration a nanoparticulate photodynamic approach has been reported by us. Here in we incorporate nano silver to induce the production of $^1O_2$ which offer a surface plasmon mediated catalyzing effect. Hypocrellin B and nano silver loaded nanoparticles have been formulated with poly lactide-co-glycolide (HBS-NPs) and PLGA-α-tocopherol polyethylene glycol 1000 succinate (HBS-CP-NPs). The HBS-NPs/HBS-CP-NPs showed the spherical shaped particles in the range of 89.6-828.2 nm with negative zeta potential and narrow polydispersity index. The DSC thermograms show that the physical status of HB inside the HBS-NPs/HBS-CP-NPs may be of amorphous form. The average encapsulation of HB in HBS-NPs was 92.9 ± 1.79% and in HBS-CP-NPs was 84.06 ± 11.43%. HB release from the HBS-NPs/HBS-CP-NPs was found to be biphasic with an initial burst relase of 1.41%-3.50% in the first 8 h followed by sustained pattern of 47.82%-48.91% for 3 days. Photodynamic on/off mechanism of the developed naonparticles was checked by exposing the nanoparticulate formulation in harsh conditions (1-5% SDS) mimicking the acidic cellular environment. Increase in the fluorescent intensity and particle size indicated the production of $^1O_2$ inside the cellular environment and thereby eliciting its photodynamic on/off switch mechanism.

The attachment of HB over the nano silver was confirmed by the observed increase in spectral intensity of raman spectrum. The ROS generation level of HBS-NPs/HBS-CP-NPs was significantly higher than that of same nano formulations without nano silver (HB-NPs/HB-CP-NPs). The $^1O_2$ generating efficiency of HBS-NPs/HBS-CP-NPs was significantly higher than HB-NPs/HB-CP-NPs. The HBS-NPs/HBS-CP-NPs exhibited concentration and time dependent phototoxicity on A549 cell lines. The CAM treated with HBS-NPs/HBS-CP-NPs showed significant anti angiogenic effect compared to blank formulation. *In vivo* bodistribution studies reveal that intravenous administration of HBS-NPs/HBS-CP-NPs has exposure to the posterior segment tissues such as retina and vitreous humor. Enhancement in $^1O_2$ production on light exposure at cellular level using nano silver coupled photosensitizer (HB) based nanoformulations will create a new avenue in the future photodynamic treatment of micro vascular diseases like AMD [19,20].

Conclusion

As the importance of PDT is getting enhanced in the recent years, even the very specific self-lighting PDT also creating a major avenue due to its deep penetration to the diseased tissues. Choosing photosensitizers with the right activation wavelength indicate the success of PDT. Researchers should focus to bring effective photosensitizer loaded nanoformulations for the treatment of posterior segment ocular diseases with an approach to induce the precise production of $^1O_2$.

References

1. Allison RR, Mota HC, Bagnato VS, et al. Bio-nanotechnology and photodynamic therapy—State of the art review. Photodiagnosis Photodyn Ther. 2008;5(1): 19-28.
2. Chowdhary RK, Shariff I, Dolphin D. Drug release characteristics of lipid based benzoporphyrin derivative. J Pharm Pharm Sci. 2003;6(1):13-9.
3. Ash DV, Brown SB. New drugs and future developments in photodynamic therapy. Eur J Cancer. 1993;29(12):1781-3.

4. Vaz GM, Paszko E, Davies AM, et al. High content screening as high quality assay for biological evaluation of photosensitizers *in vitro*. PLoS One. 2013;8(7):e70653.
5. Kessel D. Photodynamic therapy: From the beginning. Photodiagnosis Photodyn Ther. 2004;1(1):3-7.
6. Jeong H, Huh M, Lee SJ, et al. Photosensitizer-conjugated human serum albumin nanoparticles for effective photodynamic therapy. Theranostics. 2011;1:230-9.
7. Xiang GH, Hong GB, Wang Y, et al. Effect of PEG-PDLLA polymeric nanovesicles loaded with doxorubicin and hematoporphyrin monomethyl ether on human hepatocellular carcinoma HepG2 cells *in vitro*. Int J Nanomedicine. 2013;8(1):4613-22.
8. Ichikawa K, Takeuchi Y, Yonezawa S, et al. Antiangiogenic Photodynamic Therapy (PDT) using Visudyne causes effective suppression of tumor growth. Cancer Lett. 2004;205(1):39-48.
9. Hasan T, Ortel B, Solban N, et al. Photodynamic therapy of cancer. Cancer Med. 2003;7:537-48.
10. Lee SJ, Koo H, Lee DE, et al. Tumor-homing photosensitizer-conjugated glycol chitosan nanoparticles for synchronous photodynamic imaging and therapy based on cellular on/off system. Biomaterials. 2011;32(16): 4021-9.
11. Frochot C, di Stasio B, Vanderesse R, et al. Interest of RGD-containing linear or cyclic peptide targeted tetraphenylchlorin as novel photosensitizers for selective photodynamic activity. Bioorg Chem. 2007;35(3):205-20.
12. Khdair A, Handa H, Mao G, et al. Nanoparticle-mediated combination chemotherapy and photodynamic therapy overcomes tumor drug resistance *in vitro*. Eur J Pharm Biopharm. 2009;71(2):214-22.
13. Zeisser-Labouèbe M, Lange N, Gurny R, et al. Hypericin-loaded nanoparticles for the photodynamic treatment of ovarian cancer. Int J Pharm. 2006;326(1-2):174-81.
14. Huang P, Li Z, Lin J, et al. Photosensitizer-conjugated magnetic nanoparticles for *in vivo* simultaneous magnetofluorescent imaging and targeting therapy . Biomaterials. 2011;32(13):3447-58.
15. Gao L, Fei J, Zhao J, et al. Hypocrellin-loaded gold nanocages with high two-photon efficiency for photothermal/photodynamic cancer therapy *in vitro*. ACS Nano. 2012;6(9):8030-40.
16. Alanazi FK, Radwan AA, Alsarra IA. Biopharmaceutical applications of nanogold. Saudi Pharm J. 2010;18(4): 179-93.
17. Bédard MF, de Geest BG, Skirtach AG, et al. Polymeric microcapsules with light responsive properties for encapsulation and release. Adv Colloid Interface Sci. 2010;158(1-2):2-14.
18. Augustin AJ, Puls S, Offermann I. Triple therapy for choroidal neovascularization due to age-related macular degeneration: Verteporfin PDT, bevacizumab, and dexamethasone. Retina. 2007;27(2):133-40.
19. Krishnaswami V, Ponnusamy C, Sankareswaran S, et al. Development of copolymeric nanoparticles of hypocrellin B: Enhanced phototoxic effect and ocular distribution. Eur J Pharm Sci. 2018;116:26-36.
20. Natesan S, Krishnaswami V, Ponnusamy C, et al. Hypocrellin B and nano silver loaded polymeric nanoparticles: Enhanced generation of singlet oxygen for improved photodynamic therapy. Mater Sci Eng C. 2017;77:935-46.

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