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PHOTODYNAMIC THERAPY- A NOVEL APPROACH TO TREAT CANCER AND INFECTIOUS DISEASES

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ABSTRACT

In the medical arena, the treatment of skin diseases with the aid of light has been performed since 1400 BC [1]. Since then it was applied in several areas of medicine. Compared to traditional chemotherapy and radiotherapy, PDT (Photodynamic therapy) based cancer treatment significantly reduces side effects and improves target specificity [2, 3]. The use of PDT can be extended to other areas of medicine to treat resistant pathogens, because irrational use of antibiotics and the failure of some patients to complete their treatment regimen also exacerbate the problem. Methicillin-resistant *Staphylococcus aureus* and vancomycin - resistant enterococci are two resistant species that are causing much concern at present [4].The outgrowth of this serious threat made the scientists around the globe to lead major research work to find alternative antibacterial therapeutics to which, bacteria will not be easily able to develop resistance. At present, one of the best alternative and novel therapy available is PDT and numerous studies, that have examined photodynamic inactivation (PDI) of antibiotic-resistant pathogens, cancer cells was found to be promising. This review focus on insights of photodynamic therapy and their future perspective.

Key Words: photodynamic therapy, chemotherapy, antimicrobial peptides

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INTRODUCTION

Photodynamic therapy (PDT) employs a non-toxic dye, termed a photosensitizer (PS), and low intensity visible light which, in the presence of oxygen, combine to produce cytotoxic species. PDT has the advantage of dual selectivity, in that the PS can be targeted to its destination cell or tissue and, in addition, the illumination can be spatially directed to the lesion [5].

Photodynamic therapy (PDT) for cancer and other diseases has received regulatory approval for several indications in many countries [3]. PDT for infectious diseases involves local delivery of the PS into the infected area by methods such as topical application, instillation, interstitial injection or aerosol delivery and irradiating the PS, that leads to formation of antimicrobial cytotoxic agents such as ROIs. In contrast, PDT for cancer, where the PS is usually injected into the bloodstream and accumulates in the tumour, followed by activation of PS that leads to destruction of tumour cells [5].

History of PDT

The concept of photodynamic therapy (PDT) originated during 1900s. A medical student Oscar Raab, from Germany [6] made an unexpected discovery that microorganisms, which had been incubated with certain dyes, could be killed when exposed to light, but not in the absence of light. Also later it was discovered that oxygen was also necessary for this antimicrobial effect to occur, since then the term 'photodynamic action' was used. After these discoveries several efforts were made to use this principle for cancer therapy, by applying dyes on to superficial skin tumours and then exposing them to light.

The modern era of PDT started in the 1970s, due to the efforts of Dr Thomas Dougherty working at Roswell Park Cancer Institute in Buffalo, NewYork. Thomas Dougherty along with his co-workers introduced the first photosensitizer, 'haematoporphyrin derivative' (HpD), and a more purified preparation later became known as Photofrin [6]. Photofrin has many disadvantages including skin photosensitivity and a relatively small absorbance peak at 630 nm making it somewhat inefficient to treat large tumours where light penetration is problematic [7]. Since then chemists have attempted to synthesize and discover molecules that could act as better PSs, and today several compounds have been proposed as potent PSs for treating cancer and other infections.

Need for PDT

PDT is essential in the present medical era for the following two reasons:

First, with the continuous increase of antibiotic resistance among bacterial population due to irrational use of antibiotic, spontaneous mutation and non-compliance of the patient to the dosage regimen, it is hard to deliver effective medical care for the human well-being. It is obvious that awareness about antibiotic usage among people may bring about considerable change in effective treatment, but still it will be good to find a way to combat antibiotic resistant bacterial pathogens through a novel method to which the pathogens could not develop resistance. Secondly, the limitations and disadvantages of conventional therapy (radiation therapy, chemotherapy and surgery) available for treating cancer includes damage to healthy tissues by radiation, systemic toxicities due to chemotherapeutic agents, affecting the quality of life after the removal of an organ through surgery.

Advantages of PDT

Most of the PDT procedure cause minor effects upon the normal tissue,since only non-toxic dyes and their derivatives are used. PDT procedure can be easily performed in a physician's office or outpatient setting, which favours the application of this therapy in several environments, since PDT does not need great structural pre-requisites [8]. Also microbes may not have the ability to develop resistance against the cytotoxic reactive oxygen intermediates (ROIs) developed during irradiation process.

PRINCIPLE OF PDT

A successful PDT requires only three components, i.e., a potent photosensitizer, a suitable light source, and oxygen. The therapeutic effect is achieved by activation of a photosensitizing agent, by suitable light of appropriate wave length and in the presence of oxygen, toxic reactive oxygen intermediates are formed which irreversibly oxidize essential cellular components, causing apoptosis and necrosis [9].

Mechanism of PDT using PS has been elucidated in several studies [2, 10, 11]. Briefly, PS in the ground state is activated to an excited singlet state upon irradiation with suitable light. The excited singlet state is very unstable, loses its energy and can relax back to ground state through emission of light (fluorescence) or convert into the stable triplet state via inter-system crossing caused by a change in the spin of electrons. The triplet state is more stable than singlet and long lifetime of microseconds allows them to interact with surrounding molecules and thus produces ROS through Type I and Type II reactions. Type I reaction involves generation of free radicals by the transfer of either hydrogen atom or an electron between the excited PS and the substrates. And in turn, these radicals then react with oxygen, resulting in the production of ROS such as superoxide and hydroxyl radicals. The Type II reaction involves the formation of highly reactive state of oxygen known as singlet oxygen ($^1O_2$) through the energy transfer process between the excited PS and the molecular oxygen in the ground state ($^3O_2$). The resulting ROS

((superoxide & hydroxyl radicals, singlet oxygen) cause irreversible damage to tissues and cells.

Mechanism of Photo damage

Photo toxicity of a PS strongly depend on various factors, the most prominent one is localization of PS [12]. Depending on its characteristics, a PS will generally localize towards organelles such as the plasma membrane, lysosomes, mitochondria, Golgi apparatus or endoplasmic reticulum (ER)[10]. The main mechanisms of photo damage induced cell death that have been described includes: apoptosis, necrosis and autophagy. The ability of PDT to activate multiple cell death pathways circumvents the major obstacle (apoptosis resistant cells in tumours) of conventional cancer therapeutics [13].

Apoptosis

Apoptosis is a highly regulated process that leads to controlled mechanism of cell-death that can be initiated by PDT-induced damage of several organelles. PSs that localize to mitochondria are the most likely to induce apoptosis [10]. Photo damage to mitochondria induces a change in the permeability of its membranes, which results in leakage of cytochrome c into the cytosol [14]. This, in turn, will initiate the apoptotic pathway.

Necrosis

Necrosis is initiated with higher PDT-dosage depending on other factors such as concentration of PS and lightused; increasing cell damage is observed [15]. The site of action and localization of PS also plays a critical role in bringing out necrotic cell death. Necrosis is more often observed when the PS site of action is the plasma membrane. For instance, apoptotic cell death is induced when Photofrin® get localized and activated in the cytoplasm,. On the other hand, when it is activated in the plasma membrane by modifying the incubation procedure, it induces more necrotic cell death [16].

Autophagy

Autophagy is the process by which a cell recycles damaged organelles and cytoplasmic components. Generally autophagy is a cytoprotective mechanism, but it also have been observed as a cell death mechanism in response to photodynamic action [17]. When apoptosis is impaired, autophagy seems to be the main cause for cell death [18]. Also it appears to be dose dependend;autophagy cell-death can be initiatedwith higher PDT doses [19].

Understanding the effect of PDT at a cellular level is complex. But from experimental studies with photosensitizers indicate that severity of cytotoxicity increases with the dose used. High doses of PDT lead to necrosis; the predominant form of cell death, mild dose initiates apoptosis, whereas low dose leads to autophagy [11].

Photodynamic Inactivation of Microbes

Two basic mechanisms have been proposed that account for the lethal antimicrobial activity by PDT: (i) DNA damage and (ii) damage to the cytoplasmic membrane, allowing leakage of cellular contents or inactivation of membrane transport systems and enzymes. Experimental results of several studies by researchers have indicated that treatment of bacteria with various PS and light leads to DNA damage [20]. The photodynamic action is not only limited to DNA damage but also it brings out other lethal effects such as alteration of cytoplasmic membrane proteins [21], impairment of cell wall synthesis, loss of potassium ions from the cell [22].

COMPONENTS OF PDT

Photosensitizer

Photosensitizers are molecules that can be activated by light in to order to generate reactive oxygen species that can damage cell structures including diseased mammalian cells and microorganisms.

Properties of ideal PSs

An ideal PS is often described based on the following favourable characteristics [10, 23, 24]

- The PS should be a single pure compound and can be easily produced under GMP conditions with quality control and low cost of production
- Better stability on storage
- They should have low dark toxicity without allergic reactions at administration.
- Method of administration should be easy and feasible via different routes without any pain.
- It should have a high absorption band, preferably in the near infrared (NIR), for optimal tissue penetration, yet with enough energy to generate singlet oxygen.
- It should have a high yield of ROS during illumination.

- High tumour selectivity and rapid clearance from the body will thereby minimizing the side effects of photo toxicity
- One of the focus points is water solubility to improve PS circulation and efficacy in aqueous surroundings.

Classes of photosensitizers

Hematoporphyrin (Hp) was the first porphyrin used as PS [25]. Later on, chemical modification of hematoporphyrin led to the discovery of a hematoporphyrin derivative (HpD), which was advantageous in terms of selectivity for tumour tissues, low level skin photosensitization in comparison to Hp. Subsequently, several significant studies were carried out around the globe to design and discover a potent photosensitizer molecule to rule out the disadvantages associated with previous compounds that lead to the production of several new non-porphyrinoid PS molecules. Some of PSs developed over the decades, includes metalloporphyrins (Lutrin® and Lutex®), porphycenes, pheophorbides (Tookad®), purpurins (Purlytin®), phthalocyanines, chlorins (Foscan®), protoporphyrin IX precursors (Hexvix®, Metvix® and Levulan®), phenothiazines (methylene blue, and toluidine blue), cyanines (merocyanine 540), dipyrromethenes, hypericin, and xanthenes (Rose Bengal) (31)[12]. This section emphasize on overview of classes of several PSs

TETRAPYRROLE STRUCTURES

Tetrapyrrole structures are one of the largest groups of PSs that have been employed for anticancer applications. Tetrapyrrole based compounds occur naturally in several important biomolecules such as haem, chlorophyll etc. Many tetrapyrrole PSs, except bacteriochlorins predominantly produce Type II singlet oxygen [6]. The number of tetrapyrrole compounds used as PSs in PDT is too numerous to list them all, so we have tried to give the overview of the most important compounds.

Porphyrins

HpD and Photofrin were the first PSs to receive regulatory approval [23]. ALA induced protoporphyrin IX is also a porphyrin and is widely used around the world, mainly by dermatologists [26]. Several attempts have been made over two decades to prepare new porphyrin compounds and those works were well documented such as a novel porphyrin-based PS (5,10,15,20-tetrakis[(5-diethylamino)pentyl] porphyrin, (TDPP) with four diethylami- nopentyl side-chains was reported by Li *et al.* That showed ahigh $^1O_2$ yield with the ability to kill human esophageal cancer cell lines (Eca-109) and significantly reduce the growth of Eca-109 xenograft tumors in BABL/c nude mice [27]. Red light is most often used to activate porphyrins *in vivo*.

Chlorins

Chlorin-type PSs is more advantageous than porphyrins because of their intense absorption in relatively harmless NIR region, which helps in deep penetration into biological tissues. However poor water solubility character of chlorin type PSs limits them to function as potent PS [28]. Therefore, chlorin-type PSs have been modified to improve the solubility, by conjugation with amino acids, peptides, and sugars for PDT studies. For example, chlorin P6-based watersoluble amino acid conjugate prepared by Meng et al. [29] has shown promising results *viz* strong absorption in phototherapeutic window, relatively high $^1O_2$ quantum yield and significant antitumour efficiency.

Chlorins include several most important clinically useful PSs, namely m tetra hydroxyl phenyl chlorin (Temoporfin or Foscan) (30), benzoporphyrin derivative (Verteporfin) (31) and Bremachlorin. Chlorin (e6) (32) is derived from naturally occurring chlorophyll and formulated either as the trisodium salt known as photodithazine [33] or dissolved in polyvinylpyrrolidone [34]. Red light between 650 and 700 nm is used to activate chlorins depending on the exact structure.

Bacteriochlorins

The bacteriochlorin group of compounds are also used as PSs for photodynamic therapy. Among several bacteriochlorin compounds, the most potent bacteriophaeophorbide derivative known as TOOKAD [35] and its water soluble derivative have been tested in clinical trials for prostate cancer [36]. Another popular bacteriochlorin compound that hasbeen under study is LUZ11 [37].Near-infrared light between 700 and 800 nm is used to activate bacteriochlorins [6].

Phthalocyanines

Phthalocyanines are synthetic dyes with considerable importance in the PDT field during 1980s and 1990s. A mixture of sulfonated chloroaluminium phthalocyanines known as CASPs gained significant attention in the PDT field as photosensitiser [38]. Other compounds such as zinc-phthalocyanine [39], silicon – substituted phthalocyanine PC4 [40] have been studied extensively in establishing PDT. Cationic phthalocyanines have been studied for antimicrobial applications, in vivo [41] Phthalocyanines are activated by far-red light in the 670 nm range.

SYNTHETIC DYES

Phenothiazinium salts

The most popular phenothiazinium dyes Methylene Blue and Toluidine Blue have been widely studied for their antimicrobial applications and to an little extent for anticancer therapy [42, 43, 44, 45].

Rose Bengal

Rose Bengal is a fluorescent dye belongs to xanthene class. Rose Bengal has been modified by the introduction of heavy atoms into the rings, such as the halogen atoms bromine and iodine to increase the triplet yield of the molecule by facilitating intersystem crossing [6]. Rose Bengal,as a photosensitizer has been explored for antimicrobial applications [46] and anti-cancer applications [47] in several studies.

Phenalenones

The compound phenalenone (PN) or perinaphthenone was often used as a universal reference standard for production of singlet oxygen due to the fact that a yield of singlet oxygen is high [48]. Experimental studies by Spath A *et al* have recently shown that a quaternized cationic derivative of phenalenone can function as a potent antimicrobial PS [49].

Squaraines

Squaraines are organic dyes that have been renewed recently due to their photosensitizing ability- intense absorption in between 600 - 850 nm. Squaraine dye can be produced by dicondensation reactions between electron rich substrates and squaric acid. In similar to xanthenes, the introduction of halogen substituents into the ring increases the triplet yield by the heavy atom effect [6, 50].

BODIPY dyes

The boron-dipyrromethene (BODIPY) dyes are under extensive study for past decade. These dyes usually constitute a popular class of fluorophores [51]. Recently Xian-Fu Zhang and Nan Feng studied about naphthalene substituted BODIPY dye and their results indicate that there is 5-fold increase in the formation efficiency of excited triplet state and singlet oxygen in polar solvents, which shows BODIPY dyes could be used as a potent Photosensitizer [52]. Addition of heavy halogen atoms in the pyrrole rings of BODIPY structure increases the triplet yield and allows the molecules to function as excellent photosensitizer [53].

NATURAL PRODUCT

There are several natural constituents that have been isolated and extensively tested in animal models as PSs. Some of them are described in following section

Riboflavin

Riboflavin (vitamin B2) has been explored as an antimicrobial PS [54]. Riboflavin has two peaks in the UVA (360 nm) and blue (440 nm) regions. Maisch *et al*. [55] have synthesized a cationic version of riboflavin and tested for its antimicrobial potential.

Hypericin

Hypericin is a phenanthroperylenequinone naturally occuring in St John's wort, (Genus: Hypericum) a well-known medicinal plant [56]. Hypericin is a hydrophobic molecule that requires formulation in a drug-delivery vehicle (nanoparticles, micelles, liposomes) [57]. Hypericin can be activated by orange light in the range of 600 nm.

Hypocrellin

Hypocrellinsbelongs to the perylenequinonoid pigments, which includes hypocrellin A (HA), B (HB and deacetylatedhypocrellin A (DAHA). Hypocrellins have been isolated from *Hypocrellabambuasesacc.,* a parasitic fungus. These compounds have been used in PDT studies after identifying the cause for hypericism, (a state of skin sensitivity to visible light) in animals after oral ingestion of large quantities of Hypericum plants [58].

Curcuminoids

Curcumin, a chemical constituent isolated from *Curcuma longa L*, a century old spice and medicinal compound [59]. In recent days it has been under study for photosensitising property. Curcumin is a hydrophobic molecule and required to be formulated suitably to allow it to be used as a PS [60]. Curcumin

is activated by blue light [61]. Curcumin has found most applications as an antimicrobial PS in dentistry to eradicate oral pathogens [62].

FACTORS AFFECTING PDT

Improved and increased understanding of the mechanisms underlying photodynamic action at cellular and molecular levels has helped scientists in developing strategies for further improvement of PDT. A significant success have been observed over a period of ten years in synthesizing pure PSs with absorption range between 650-800 nm, which ease the penetration deep in to the tissues. And also priority was given to the targeted delivery of the PS to particular cellular organelles, increasing PS uptake by targeted cells and accelerating PS clearance from the organism [63, 64].

Light

Illumination plays a critical role in a successful PDT. The clinical efficacy of photodynamic therapy dependent on dose, exposure time and properties of the light source used, because it has to penetrate the barriers before reaching the target cells and be able to activate the PS in situ. Nevertheless, the optical properties of the tissues also play a significant role in determining the efficacy of the process. Several endogenous chromophores within the tissues influence the scattering and absorption of light. For instance, at about less than 650 nm, efficacy can be limited due to the absorption by endogenous chromophores such as haemoglobin, whereas at longer wavelengths of greater than 1300 nm, water molecules can absorb light. This limits the use of light in the specific wavelength range to optimally penetrate the tissue. However, light with a longer wavelength that is, greater than 850 nm doesn't activate the PS to its triplet state and to generate singlet oxygen. So, the "therapeutic window" for the most PDT applications lies in the spectrum range of 620 and 850 nm for achieving optimal tissue penetration and PS activation [10, 65]

Extensive studies have been carried out on different light sources and in designing the equipments to deliver light for the optimal performance. The range of light sources includes broad-spectrum continuous-wave light sources (blue, red, green light), coherent monochromatic sources (intense pulsed light, potassium titanyl phosphate lasers, pulsed dye lasers, infrared lasers), incoherent polychromatic sources (gas discharge lamps, light-emitting diodes), photo pneumatic technology, and sunlight have all been studied [66].

Uptake of PS

Localization of the PS molecule is thekey factor in determining the extent of photodamage,Since the lifespan of photo-generated reactive specie such as ROIs in the biological environment is too short . This in turn will have a major impact on the PDT outcome.Three properties of the PS molecule – charge, lipophilicity and three-dimensional structure determines the PS uptake and distribution.

Charge

Investigations on the effect of electrical charges on PS uptake have revealed that positively charged PSs are electrostatically attracted by the negatively charged components of the plasma and other membranous structures of the cell, that they are efficiently taken up by cells and accumulate intracellularly at higher concentrations than in the environment. On the other hand negative charges on a PS molecule are unfavourable for transport across the membranes. A critical force driving such positively charged molecules inside the cells is the transmembrane potential [67]. Anionic PSs are taken up via endocytosis, which leads to localization towards lysosomes [68]. Low negative charges on the PSs can be compensated by its sufficient lipophilicity character. [10]

Lipophilicity

Studies show that altering the lipophilicity of a PS affects its uptake and localization. The more hydrophilic photosensitizers mostly bind albumin, whereas the amphiphilic PS bind high-density lipoproteins, and the hydrophobic ones, that are administered with a solubilisation vehicle, mostly localize in the inner lipid core of low-density lipoproteins [10]. Study by Ezzeddine R *et al* about the effect of molecular characteristics on uptake of Zn(II) N-alkylpyridylporphyrins by cancerous cell, showed that the uptake of the PS by cancer cells is strongly influenced by the length of the side chain [69]. Uptake of PS molecule by the cells increases by increasing the length of alkyl chain from 1 to 8 carbons [70].Also it was found that an increase in the

length of the alkyl chains above certain limits leads to suppression of cellular uptake [71].

Shape, Size and Three-Dimensional Structure of the Molecule

Cellular uptake of PS is also influenced by the three dimensional shape of the molecule. Engelmann et al. investigated how the lipophilicity of asymmetric porphyrin affects their incorporation in membranes and found that lipophilicity alone was not responsible of membrane binding. Results of this study indicate that the spatial structure of the molecule is also a fundamental factor in determining penetration on of PS molecule into the bilayer lipid membranes [72].

Oxygen

Apparently, the efficacy of PDT outcome directly related to the yield of $^1O_2$which in turn depends on the concentration of ground state oxygen in the target tissue [73, 74]. Hypoxic cells are very resistant to photosensitization and hypoxia is usually induced by impaired tumor vasculature which proves to be a major obstacle in the treatment of solid tumors [75].

Applications of PDT

In the current scenario, applications of photodynamic therapy is to too narrow, that it can be used to treat superficial microbial infections and certain type of localized tumours. Its use can be extended to numerous area of medical care, if their limitations can be overcome. Wide variety of chemical compounds have been evaluated for Photodynamic therapeutic potential, but few of them were approved for use in humans to treat tumours, microbial skin infections and as well as in dentistry. Table-1 focus on the list of clinically approved photosensitizers that have gained considerable importance in the field of medicine and health care [76].

Table-1 An overview of clinically approved photosensitizers and their applications in the field of PDT

Category	Generic name	Manufacturer	Application
First generation	Photofrin®	Axcan Pharma, Canada	Esophageal cancer, Lung adenocarcinoma, Endobronchial cancer
Second generation	Ameluz	DUSA, USA	Mild to moderate actinic Keratosis
	Metvix	Galderma, UK	Non-hyperkeratotic actinic keratosis and basal cell carcinoma
	Foscan	Biolitec,Germany	Advanced Head and neck cancer
	Laserphyrin	Meiji Seika, Japan	Early centrally located lung cancer
	Visudyne	Novartis, Switzerland	Age related macular degeneration
	Red aporfin	Luzitin, Portugal	Biliary tract cancer
Dyes	Methylene blue	German and Swiss red cross	Sterlization of freshly frozen plasma

Apart from the molecules mentioned in the above table, here is a list of photosensitizers which are presently in the clinical trials- Fotolon (Apocarepharma , Germany), Radachlorin (Rada-Pharma, Russia), Photochlor (Rosewell park), TOOKAD (Negma-Lerads), Antrin (Pharmacyclics),Photrex (Miravant, USA), Talaporfin (Meiji Seika, Japan). In near future the number of clinically approved photosensitizers may increase in

large numbers which is good sign for the betterment of human well-being.

CONCLUSION AND FUTURE PERSPECTIVES

PDT has a history of more than five decades started since its accidental discovery that microorganisms when incubated with certain dyes could be killed when exposed to light in the presence of oxygen. Since then a vast amount of effort were made by biologist, chemist and physicist to evaluate the chemical compounds including those exist in nature for the so called photo-sensitizing ability, to use in the medical field for the improvement of human well-being. For the past several years, numerous compounds have been under examination, but very few of them got approval for use in clinical practice around the globe.

Despite amazing progress in the field of PDT, a number of challenges still remain toward clinical applications viz, developing PS drugs with strong absorbance at long wavelengths with high chemical and photo-stability. Also toxicity and targeted delivery of PS drugs, delivery of light used to activate the PS molecule are the still significant components of PDT to be addressed. Nanotechnology has a profound impact on PDT in several ways; use of bio-compatible nanomaterial can offer solutions to address crucial limitations of conventional PS drugs. Vast amount of experimental data indicated that harnessed nanomaterial combined with PS drugs can bring out abundant progress such as enhanced solubility of hydrophobic PS drugs, improved target-specific delivery of PS drugs.

The perspective of PDT has seen an unimaginable transformation in recent years after the merge with nanotechnology which had widen up the endless possibilities in extending the PSs applications in diagnosis. However we are still far from achieving optimal objectives since PS based imaging is often impaired by certain limitations such as penetration of light and specific localization of PS leading to poor imaging contrasts between tumour and healthy tissues. The discovery of novel PS molecules with desired pharmaceutical properties and the application of PS in conjunction with nanomaterial are challenging tasks in therapeutic and diagnostic field. In near future, with the significant successes, it is expected that PDT combined with nanotechnology will soon be accessible for every individual as robust and well established treatment protocol rather than as experimental studies.

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