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Review Article

RESISTANCE IN MECHANISMS OF MYCO-BACTERIUM TUBERCULOSIS: INTRODUCING NEW CLASS OF THERAPEUTICS

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Background:

Tuberculosis (TB) is a serious public health problem worldwide Tuberculosis (TB) is reported as one of the most prevailing life-threatening health problems, affecting almost one third of the population globally. It is one of a major reason of death with an imposing amplified socio-economic impact. Tuberculosis patients have infrequent endocrine and metabolic derangements, but they are important when they occur. Multiple drug regimen, poor patient compliance, and stiff administration schedule are factors that are answerable for the development of and extensive drug resistance (XDR) and multi drug resistance (MDR) instances in TB along with poor drug targeting effects. The emerging resistance strains and high transmittance rate of the disease have

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prompted the need for studies in advanced drug delivery, particularly nanotechnology for the management of TB. A better knowledge of the mechanisms of drug resistance of M. tuberculosis and the relevant molecular mechanisms involved will improve the available techniques for rapid drug resistance detection and will help to explore new targets for drug activity and development. This review article discusses the mechanisms of action of anti-tuberculosis drugs and the molecular basis of drug resistance in M. tuberculosis.

Keywords: drug resistance; molecular mechanisms; Mycobacterium tuberculosis

Introduction

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide. TB is caused by the bacillus *Mycobacterium tuberculosis* (*Mtb*), which is spread *via* airborne droplets. Approximately one in four people worldwide demonstrate an immunological response to *Mtb* infection, which can remain dormant or progress into active disease forms [1]. Patients infected with TB who have no active signs or symptoms of disease were previously deemed to have latent TB, more recently changed to TB infection [2].

Patients with TB infection have a 5–10% lifetime risk of developing TB disease, which increases in varying states of immunodeficiency up to a 16% annual risk of activation of TB infection into TB disease in HIV patients [3]. In 2019, there were an estimated 10 million new incident cases of active TB disease worldwide [1]. Approximately two-thirds of all cases arise in eight countries alone, the vast majority of which have overwhelmed health services with limited resources. This significant global burden of disease has been recognized by the World Health Organization (WHO) who launched the End TB initiative in 2016. Their aim is to reduce incidence, morbidity and mortality of this disease by improving diagnostic and therapeutic practices, as well as developing preventative strategies, through innovative research and education. By 2035, the goal is to reduce TB mortality by 95% and reduce overall incidence of TB by 90% worldwide [4]. Owing to the work of our predecessors, it has been estimated that 60 million lives have been saved globally in the 21st century so far [5].
Effective TB treatment is dependent on:

- Prompt diagnosis of TB and recognition of drug resistance;
- Promoting and ensuring patient adherence to regimens;
- Robust contact tracing and prophylactic treatment of contacts; and
- Screening for TB infection in high-risk groups.

There is ongoing extensive research into developing accurate, timely methods of detecting drug resistance, even in resource poor settings. Many effective, less toxic medications are under development. Furthermore, methods of promoting and ensuring drug adherence are being

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reviewed. In addition, there is vital research ongoing in proactive areas of TB prevention, such as screening for, and treatment of, TB infection and developing efficacious vaccines to halt the spread of this killer disease. The aim of this article is to: review current practice in the diagnosis and treatment of TB; outline new diagnostic techniques under development; discuss new drug therapies and treatment regimens under review; and review the evidence for vaccination. The following sections will review the mode of action and resistance mechanisms of the main anti-TB drugs as well as new drugs recently described with anti-TB activity.

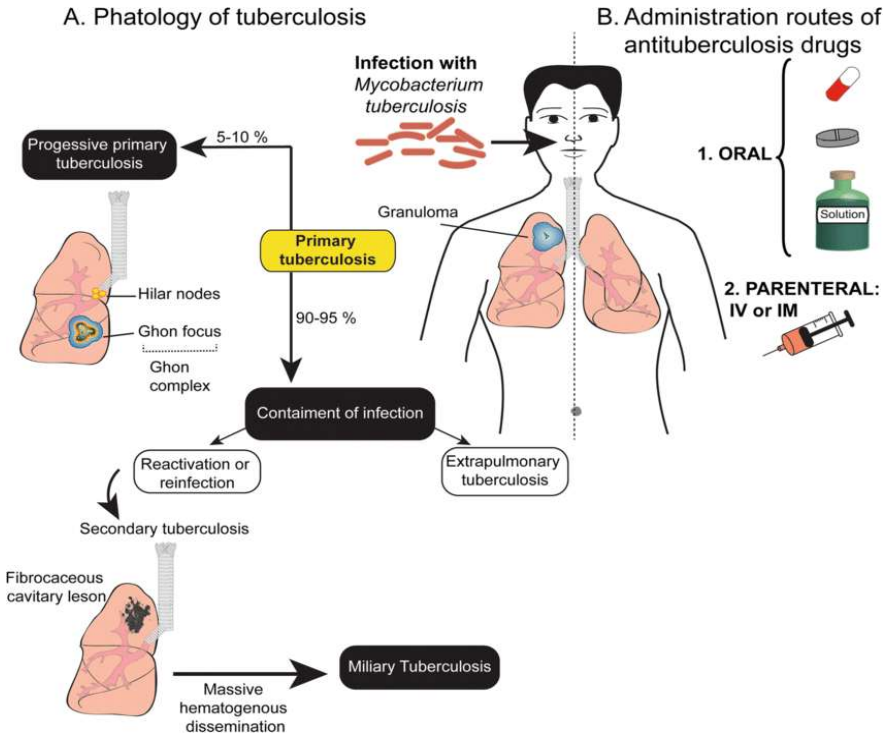

Fig.1 Pathophysiology of T.B.

Conventional Therapeutics involve in Tuberculosis

First-Line Anti-TB Drugs

A. Rifampicin

Rifampicin is a rifamycin derivative introduced in 1972 as an anti-tuberculosis agent. It is one of the most effective anti-TB antibiotics and together with isoniazid constitutes the basis of the multidrug treatment regimen for TB. Rifampicin is active against growing and non-growing (slow metabolizing) bacilli [6]. The mode of action of rifampicin in M. tuberculosis is by binding to the β-subunit of the RNA polymerase, inhibiting the elongation of messenger RNA [7]. The majority of rifampicin-resistant clinical isolates of M. tuberculosis harbor mutations in the rpoB

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gene that codes for the β-subunit of the RNA polymerase. As a result of this, conformational changes occur that decrease the affinity for the drug and results in the development of resistance [8]. In about 96% of M. tuberculosis isolate resistant to rifampicin, there are mutations in the so-called ―hot-spot region‖ of 81-bp spanning codons 507–533 of the rpoB gene. This region is also known as the rifampicin resistance-determining region [9].

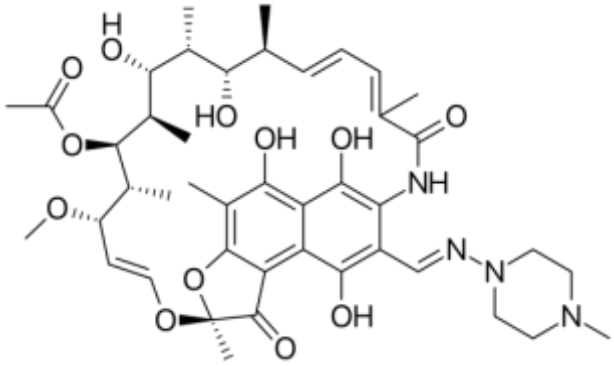

Fig.2 Rifampicin scaffold for tuberculosis treatment.

Mutations in codons 516, 526 and 531 are the most commonly associated mutations with rifampicin resistance in the majority of studies [10,11]. Although less frequent, some reports have also noted the occurrence of mutations outside of the hot-spot region of rpoB [12,13]. Cross-resistance with other rifamycin's can occur. Mutations in some codons (e.g., 518 or 529) have been associated with low-level resistance to rifampicin but still susceptible to other rifamycin's, such as rifabutin or rifalazil [14,15]. This is important for TB patients that need to receive antiretroviral therapy since rifabutin is a less effective inducer of the cytochrome P450 CYP3A oxidative enzyme [16]. On the other hand, monoresistance to rifampicin is quite rare and almost all rifampicin-resistant strains are also resistant to other drugs, especially to isoniazid. This is the reason why rifampicin resistance is considered as a surrogate marker for MDR-TB [17].

B. Ethambutol

Ethambutol was first introduced in the treatment of TB in 1966 and is part of the current first-line regimen to treat the disease. Ethambutol is bacteriostatic against multiplying bacilli interfering with the biosynthesis of arabinogalactan in the cell wall [18]. In M. tuberculosis, the genes embCAB, organized as an operon, code for arabinosyl transferase, which is involved in the synthesis of arabinogalactan, producing the accumulation of the intermediate D-arabinofuranosyl-P-decaprenol [19].

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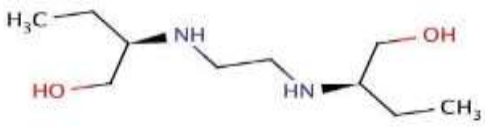

Fig.3 Ethambutol scaffold

The recognized mechanism of resistance to ethambutol has been linked to mutations in the gene embB with mutations at position embB306 as the most prevalent in most of the studies performed [20, 21]. Some studies, however, have also found mutations in embB306 in ethambutol susceptible isolates [22].

Moreover, a study with a large number of M. tuberculosis isolates found that mutations in embB306 were not necessarily associated with resistance to ethambutol but with a predisposition to develop resistance to increasing number of drugs and to be transmitted [23]. In fact, allelic exchange studies have shown that individual mutations causing certain amino acid substitutions produced ethambutol resistance, while other amino acid substitutions had little or no effect on ethambutol resistance [24]. The same authors have more recently reported that mutations in the decaprenyl phosphoryl-B-D-arabinose (DPA) biosynthetic and utilization pathway genes, Rv3806c and Rv3792, together with mutations in embB and embC accumulate, giving rise to a range of MICs of ethambutol depending on mutation type and number [25]. These findings could have influence on the correct detection of ethambutol resistance by current molecular methods. Mutations in embB306 then, cause variable degrees of ethambutol resistance and are required but are not enough to cause high-level resistance to ethambutol. There remain about 30% ethambutol resistant strains that do not present any mutation in embB stressing the need to identify other possible mechanisms of drug resistance to this drug.

C. Pyrazinamide

Pyrazinamide was introduced into TB treatment in the early 1950s and constitutes now part of the standard first-line regimen to treat the disease. Pyrazinamide is an analog of nicotinamide and its introduction allowed reducing the length of treatment to six months. It has the characteristic of inhibiting semi-dormant bacilli residing in acidic environments such as found in

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the TB lesions [26]. Pyrazinamide is also a pro-drug that needs to be converted to its active form, pyrazinoic acid, by the enzyme pyrazinamidase /nicotinamidase coded by the pncA gene [27, 28]. The proposed mechanism of action of pyrazinamide involves conversion of pyrazinamide to pyrazinoic acid, which disrupts the bacterial membrane energetics inhibiting membrane transport. Pyrazinamide would enter the bacterial cell by passive diffusion and after conversion to pyrazinoic acid it is excreted by a weak efflux pump. Under acid conditions, the protonated pyrazinoic acid would be reabsorbed into the cell and accumulated inside, due to an inefficient efflux pump, resulting in cellular damage [29].

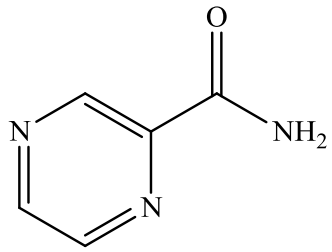

Fig.4 Pyrazinamide.

One study has also found that pyrazinoic acid and its n-propyl ester can inhibit the fatty acid synthase type I in replicating M. tuberculosis bacilli [57,58]. A recent study, however, has challenged the previous model by proposing that pyrazinoic acid inhibits trans-translation, a process of ribosome-sparing in M. tuberculosis [30]. The study was performed in pyrazinamide-resistant strains lacking mutations in pncA but that had mutations in rpsA identifying the ribosomal protein 1 (RpsA) as the proposed target. Overexpression of RpsA conferred increased resistance to pyrazinamide and pyrazinoic acid was confirmed to be bound to RpsA [31]. While a very intriguing hypothesis as a target for pyrazinamide, the failure to perform allelic transfers in this study makes it difficult to conclude that in fact mutations in rpsA are the target of pyrazinamide. Mutations in the gene pncA remain as the most common finding in pyrazinamide resistant strains. These mutations, however, are scattered throughout the gene but most occur in a 561-bp region in the open reading frame or in an 82-bp region of its putative promoter [32,33]. Some few studies have reported the occurrence of pyrazinamide resistant strains without any mutation in pncA stating that the resistance could be due to mutations in another not yet identified regulatory gene [33]. Based on the current evidence, the contribution of mutations in rpsA to pyrazinamide resistance remains limited [34–36].

D. Streptomycin

This Originally isolated from the soil microorganism Streptomyces griseus, streptomycin was the first antibiotic to be successfully used against TB. Unfortunately, as soon as it was prescribed, resistance to it emerged, a result of being administered as monotherapy [37]. Streptomycin is an

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aminocyclitol glycoside active against actively growing bacilli and its mode of action is by inhibiting the initiation of the translation in the protein synthesis [38]. More specifically, streptomycin acts at the level of the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA coded by the genes rpsL and rrs, respectively [39]. Consequently, mutations in rpsL and rrs are the major mechanisms of resistance to streptomycin but account for 60%–70% of the resistance found [40]. Among the mutations reported in rpsL, a substitution in codon 43 from lysine to arginine has been the most commonly reported. This mutation produces high-level resistance to streptomycin.

In rrs the most common mutations occur around nucleotides 530 and 915. There remain an important percentage of strains resistant to streptomycin that lack mutations in either of these two genes, suggesting additional mechanisms of resistance. In the last years, it has also been reported that mutations in gidB, a gene encoding a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA, confers low-level resistance to streptomycin [41, 42].

2.0 Second-Line Anti-TB Drugs

2 (a). Fluoroquinolones

Fluoroquinolones are currently in use as second-line drugs in the treatment of MDR-TB. Both ciprofloxacin and ofloxacin are synthetic derivatives of the parent compound nalidixic acid, discovered as a by-product of the antimalarial chloroquine [42]. Newer-generation quinolones such as moxifloxacin and gatifloxacin are being evaluated in clinical trials and proposed as first-line antibiotics with the purpose of shortening the length of treatment in TB [43,44]. The mode of action of fluoroquinolones is by inhibiting the topoisomerase II (DNA gyrase) and topoisomerase IV, two critical enzymes for bacterial viability. These proteins are encoded by the genes gyrA, gyrB, parC and parE, respectively [45]. In M. tuberculosis, only type II topoisomerase (DNA gyrase) is present and, thus, is the only target of fluoroquinolone activity [46].

2 (b). Kanamycin, Capreomycin, Amikacin, Viomycin

These four antibiotics have the same mechanism of action by inhibiting the protein synthesis but, while kanamycin and amikacin are aminoglycosides, capreomycin and viomycin are cyclic peptide antibiotics. All four are second-line drugs used in the management of MDR-TB. Kanamycin and amikacin inhibit protein synthesis by alteration at the level of 16S rRNA. The most common mutations found in kanamycin-resistant strains are at position 1400 and 1401 of the rrs gene, conferring high-level resistance to kanamycin and amikacin. However, mutations at position 1483 have also been reported [47, 48].

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Full cross-resistance between kanamycin and amikacin is not complete, as previously thought. Some studies have shown variable levels and patterns of resistance suggesting that other mechanisms of resistance might be possible [49].

In concordance with this, a low-level resistance to kanamycin has been associated with mutations in the promoter region of the eis gene, encoding an aminoglycoside acetyltransferase [50]. Mutations at position −10 and −35 of this is promoter led to an overexpression of the protein and low-level resistance to kanamycin but not to amikacin. These mutations were found in up to 80% of clinical isolates showing low-level resistance to kanamycin [51,52]. Capreomycin and viomycin, on the other hand, have a similar structure and bind at the same site in the ribosome, at the interface of the small and large subunits [52]. They show full cross-resistance as reported in previous studies [53]. Mutations in the tlyA gene have also been associated with resistance to capreomycin and viomycin. TlyA is an rRNA methyltransferase specific for 2'-O-methylation of ribose in rRNA. Mutations in tlyA determine the absence of methylation activity [54]. Although some studies did not find this association, a recent meta-analysis, evaluating the association of genetic mutations and resistance to second-line drugs, has confirmed the presence of tlyA mutations in addition to mutations in RRS [55].

3. New Anti-TB Drugs

Notwithstanding the alleged lack of interest of the pharmaceutical industry for the development of new antibiotics, there are several anti-tuberculosis drugs in the pipeline and some of them are already being evaluated in clinical trials and in new combinations with the purpose of reducing the length of TB treatment.

3 (a). Delamanid: Delamanid, previously known as OPC-67683, is a derivative of nitro-dihydro-imidazooxazole with activity against M. tuberculosis that acts by inhibiting the synthesis of mycolic acid and is undergoing clinical evaluation in a phase III trial []. The structure of delamanid is shown in Figure 2. Delamanid was previously shown to have a very good in vitro and in vivo activity against drug-susceptible and drug-resistant M. tuberculosis [56], as well as good early bactericidal activity comparable to that of rifampicin [57]. Delamanid has more recently shown its safety and efficacy in a clinical evaluation for MDR-TB [58].

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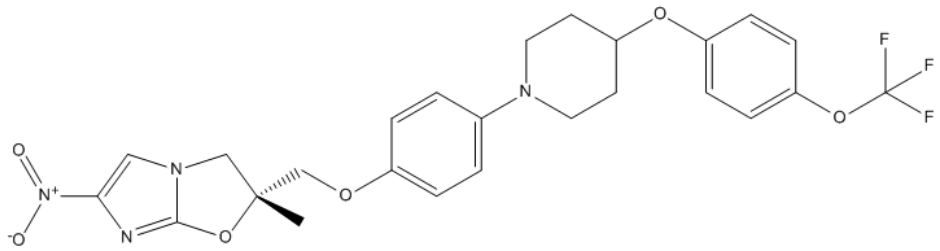

Delamanid (OPC-67683)

Fig.5

The specific mode of action of delamanid is by inhibition of the mycolic acid synthesis but it differs from isoniazid in that, it only inhibits methoxy- and keto-mycolic acid while isoniazid also inhibits α-mycolic acid [57]. Delamanid also requires reductive activation by M. tuberculosis to exert its activity. In experimentally generated delamanid-resistant mycobacteria, a mutation was found in the Rv3547 gene, suggesting its role in the activation of the drug [57].

3. BPA-824

PA-824 is a bicyclic derivative of nitroimidazole that showed specific activity against M. tuberculosis [59]. The structure of PA-824 is shown in Figure 3. This small-molecule compound showed a very good in vitro and in vivo activity in animal models [60] and it also showed to be safe and well tolerated [61]. PA-824 is currently undergoing further clinical evaluations.

PA-824 needs to be activated by a nitroreductase to exert its activity and it inhibits the synthesis of protein and cell wall lipids. The mechanism of resistance to PA-824 has been shown to be most commonly associated with loss of a specific glucose-6-phosphate dehydrogenase (FGD1) or the dezaflavin cofactor F420. More recently, a nitroimidazo-oxazine-specific protein causing minor structural changes in the drug has also been identified [62].

3 (c). Benzothiazinones

A new class of drug with anti-mycobacterial activity, 1,3-benzothiazin-4-one or Benzothiazinones (BTZ), was recently described. The lead compound, 2-[2-S-methyl-1,4-dioxa-8-azaspiro[4.5]dec8-yl]-8-nitro-6-(trifluoromethyl)-4H-1,3-benzothiazin-4-one (BTZ043) was found to have in vitro, ex vivo and in vivo activity against M. tuberculosis. It was also found to be active against drug-susceptible and MDR clinical isolates of M. tuberculosis [62s]. Structure of BTZ043 is shown below:

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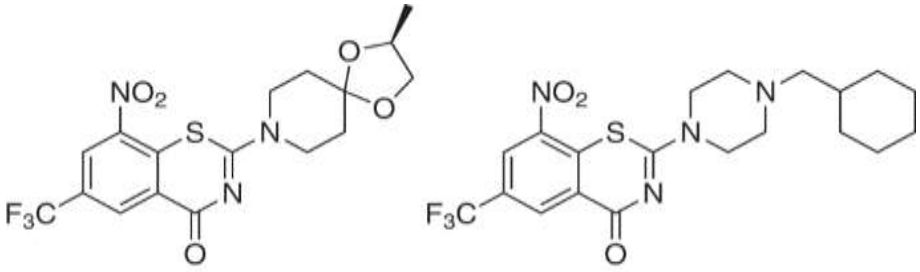

4.0 Conclusion

Drug resistance in TB remains a man-made phenomenon. It emerges as a result of spontaneous gene mutations in M. tuberculosis that render the bacteria resistant to the most commonly used anti-TB drugs. Among the reasons for this, the non-compliance with the treatment regimens is signaled as the first cause. The standard treatment of TB calls for a six-month regimen of four drugs that in the case of MDR-TB is extended to 18–24 months involving second-line drugs. This makes compliance with the treatment regimens very challenging and the rates of non-adherence could be high, resulting in poor outcomes and further dissemination of MDR strains. Notwithstanding the fact that mutations in a number of genes are clearly associated with drug resistance in M. tuberculosis, there are still many cases where resistant strains do not harbor any known mutation. For example, a recent study using whole-genome sequencing identified new genes and intergenic regions that were associated with drug resistance and its evolution, showing that TB drug resistance is a phenomenon more complex than previously assumed [64]. More clarification is needed on the role of specific gene mutations and the development of MDR- or XDR-TB, or the relation between drug resistance and fitness of the bacteria. A better knowledge is also required on the role of efflux pump mechanisms and the development of clinical drug resistance, or the role of porins, if any, on the intrinsic resistance to certain antibiotics.

5.0 Future Prospective

Tuberculosis (TB) remains a significant global health threat, with millions of cases reported each year. One of the biggest challenges in controlling TB is the emergence of drug-resistant strains of Mycobacterium tuberculosis (MTB), the bacteria that causes TB. New classes of therapeutics are urgently needed to combat the growing problem of drug-resistant TB. Researchers are exploring a number of different approaches, including, targeting novel mechanisms of action involves identifying new ways to kill MTB that are not affected by existing drugs. Development of new drugs this includes both new antibiotics and other types of drugs, such as those that can shorten treatment times or improve the effectiveness of existing drugs. Repurposing existing drugs this involves finding new uses for existing drugs that may be effective against MTB. The future of TB treatment is likely to involve a combination of these approaches. New classes of therapeutics

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are essential to overcome the challenge of drug resistance. In addition, new diagnostic tools are needed to rapidly identify drug-resistant strains of MTB so that patients can be treated with the most effective drugs. Personalized medicine with a better understanding of the mechanisms of drug resistance, it may be possible to tailor treatment regimens to individual patients based on the specific strain of MTB they are infected with. Combination therapy new drugs are likely to be used in combination with existing drugs to improve efficacy and reduce the risk of resistance emerging. Shorter treatment regimens current TB treatment regimens can be long and difficult to adhere to. New drugs that can shorten treatment times would improve patient outcomes and help to prevent the spread of drug-resistant TB. The development of new TB therapeutics is a complex and challenging process, but it is essential to address the growing problem of drug resistance. With continued research and investment, new classes of therapeutics can be developed to help control TB and save lives.

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