DEVELOPMENT OF NANODELIVERY SYSTEMS FOR ANTI-TUBERCULOSIS DRUGS USING METAL HYDROXIDES NANOLAYERED HOSTS

SAIFULLAH

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By

SAIFULLAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

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DEDICATION

I dedicate my thesis to my beloved father, my paternal grandmother Mai Bhaun Bhain (Mai Amna), my beloved father Ahmed Ali Bullo, my mother, my siblings and to rest of my family.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

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Chair: Prof. Mohd Zobir Bin Hussein, PhD
Faculty: Institute of Advanced Technology

Development of biocompatible and biodegradable nanodelivery systems with sustained release properties is the key research area of nanomedicine technology. Inorganic nanolayers are the promising material due to fascinating properties like ease of preparation, ability to intercalate different type of anions (inorganic, organic, biomolecules and even genes, etc.), high thermal stability, delivery of the intercalated anions in the sustained manner, high biocompatibility and easy biodegradation, etc. In this research, anti-tuberculosis (anti-TB) nanodelivery formulations were designed by intercalating anti-TB drugs into inorganic nanolayered hydroxide to be used as nanodelivery systems. *Tuberculosis* (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis* (MTB) and has been lethal to humans since centuries. Chemotherapy of TB involves multi-drug therapy, frequent dosing, and side effects of anti-TB drugs and long term treatment duration of 3-24 months. These complications are responsible for patients' noncompliance to the treatment. Drug development is very lengthy, high budget consuming and multistage process and there has been no new anti-TB drug in the market since about the last 5 decades. Research on the development of drug delivery systems is of prime importance to overcome the above scenario. Drug delivery systems (DDS) offer many advantages over the conventional chemotherapy, such as targeted delivery and protection of drugs from physico-chemical degradation, which ultimately will reduce the side effects associated with drugs. The DDS releases the drugs in a sustained manner for a longer period of time that would result in the reduction in the dosing frequency. The nano-sized DDS have the tendency to easily penetrate the bacteria and cancer cells. All of these advantages would ultimately improve the patients' compliance to the treatment.

In this research, several anti-TB nanodelivery formulations were developed by intercalating anti-TB drugs namely para amino salicylic acid (PAS) and isoniazid (INH)

into metal hydroxide nanolayers namely zinc layered (ZLH), zinc-aluminium layered double hydroxides (Zn/Al-LDHs) and magnesium-aluminium layered double hydroxides (Mg/Al-LDHs). A total of 8 nanocomposites based on the aforementioned anti-TB drugs and metal nanolayers hydroxides were prepared. The developed nanocomposites of anti-TB drugs with metal hydroxides nanolayers (inorganic nanolayers) showed higher biocompatibility with normal human lung cell lines (the most common residing place of mycobacterium tuberculosis) and with 3T3 mouse fibroblast cells (the most sensitive cells to toxicity). The release of the intercalated anti-TB drugs was sustained in human body simulated phosphate buffer (PBS) solutions of pH 7.4 and 4.8. Therapeutic efficacy of the anti-TB nanocomposites against *mycobacteria* tuberculosis was found to be much better compared to para amino salicylic acid and isoniazid in their free form. The *in vitro* study results are extremely encouraging to conduct the further study on animal models (in vivo study) and these biocompatible anti-TB nanocomposite formulations could be very useful in coping with tuberculosis effectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBANGUNAN SISTEM PENGHANTARAN NANO BAGI UBAT ANTI-TUBERKULOSIS MENGGUNAKAN PERUMAH HYDROKSIDA LOGAM BERLAPIS NANO

By

SAIFULLAH

Oktober 2014

Chairman Prof. Mohd Zobir Bin Hussein, PhD
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Pembangunan sistem penghantaran nano yang bio-serasi dan bio-terurai dengan ciri-ciri pelepasan yang berterusan adalah merupakan tumpuan utama penyelidikan dalam teknologi perubatan-nano. Lapisan nano tak-organik adalah bahan yang menjanjikan harapan tinggi kerana sifat menariknya seperti penyediaannya yang mudah, keupayaannya untuk disisipkan dengan pelbagai jenis anion (tak-organik, organik, biomolekul dan juga gen, dll.), kestabilan haba yang tinggi, penghantaran anion tersisip secara mampan, bio-serasi yang tinggi dan mudah bio-terurai dan sifat-sifat lain lagi. Dalam kajian ini, rumusan anti-batuk kering (anti-TB) penghantar nano telah direkabentuk dengan menyisipkan ubat anti-TB ke dalam bahan tak-organik lapisan-nano hidroksida untuk digunakan sebagai sistem penghantaran nano. *Tuberculosis* (TB) adalah penyakit berjangkit bawaan udara yang disebabkan oleh *Mycobacterium tuberculosis* (MTB) yang telah membawa maut kepada manusia sejak berabad-abad lamanya. Kemoterapi TB melibatkan terapi pelbagai dadah, dos yang kerap dan kesan sampingan dari ubat-ubatan anti-TB, dan tempoh rawatan yang panjang, 3-24 bulan. Komplikasi ini bertanggungjawab terhadap ketidakpatuhan pesakit terhadap rawatan. Pembangunan ubat-ubatan baru memakan masa yang sangat panjang, bajet yang tinggi dan proses pelbagai peringkat, dan tiada ubat anti-TB baru di pasaran semenjak kira-kira 5 dekad yang lalu diperakukan. Penyelidikan terhadap pembangunan sistem penyampaian ubat adalah sangat penting untuk mengatasi senario di atas. Sistem penyampaian ubat (DDS) menawarkan banyak kelebihan berbanding dengan kemoterapi konvensional, seperti penghantaran yang dapat disasarkan dan perlindungan dadah daripada degradasi fiziko-kimia, yang akhirnya akan mengurangkan kesan sampingan yang dikaitkan dengan dadah. DDS yang mengeluarkan dadah secara berterusan untuk tempoh yang lebih lama akan menyebabkan pengurangan dalam kekerapan dos. DDS yang bersaiz nano mempunyai kecenderungan untuk lebih mudah menembusi bakteria dan sel-sel kanser. Semua kelebihan ini akhirnya akan meningkatkan pematuhan pesakit

terhadap rawatan. Dalam kajian ini, beberapa rumusan penghantar nano anti-TB dihasilkan dengan menyisipkan ubat anti-TB iaitu para-amino asid salisilik (PAS) dan isoniazid (INH) ke dalam lapisan nano hidroksida logam iaitu zink berlapis (ZLH), zink-aluminium hidroksida berlapis ganda (Zn/Al-LDH) dan magnesium-aluminium hidroksida berlapis ganda (Mg/Al-LDH). Sebanyak 8 nanokomposit berdasarkan ubat anti-TB dan lapisan nano logam hidroksida yang dinyatakan di atas telah disediakan. Nanokomposit yang dibangunkan dengan menggunakan ubat anti-TB dengan hidroksida logam berlapis nano tak-organik menunjukkan sifat serasi-bio yang lebih tinggi terhadap sel paru-paru manusia biasa (tempat yang paling biasa bagi *Mycobacterium tuberculosis*) dan dengan sel-sel 3T3 fibroblast tikus (sel-sel yang paling sensitif terhadap keracunan). Pembebasan ubat anti-TB tersisip ke dalam larutan simulasi badan manusia, fosfat penampan (PBS) pada pH 7.4 dan 4.8 menunjukkan sifat lepasan terkawal. Keberkesanan terapeutik bagi nanokomposit anti-TB terhadap batuk kering *mycobacteria* didapati jauh lebih baik berbanding dengan para-amino asid salisilik dan isoniazid dalam bentuk bebas mereka. Keputusan kajian vitro ini amat menggalakkan, dan seterusnya kajian lanjut menggunakan model haiwan (kajian in vivo) bagi rumusan nanokomposit anti-TB yang serasi-bio ini sangat berguna untuk digunakan sebagai perawatan batuk kering yang lebih berkesan.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Inorganic nanolayers are the two dimensional nanomaterial whose structure is made up of infinite layers and the thickness of their layers in nanoscale range. Layered double hydroxides (LDHs) and layered hydroxide salts (LHS) are the types of inorganic nanolayered material having thickness in nanoscaled range (1-100nm)[1] **.** Drug delivery, gene delivery, biosensing science and bioimaging technology are the key tools of the modern-day biomedical sciences. Different materials are used for the application of the above mentioned biomedical tools but there are certain issues related to different materials such as cytotoxicity, lack of biodegradation and different types of material is used for individual application [2]. Inorganic nanolayers have many advantages for biomedical applications. It has been proven to be very high in vitro and in vivo biocompatibility, biodegradable, tendency to release the intercalated drugs in a sustained manner and is single material can be applied as drug delivery, gene delivery, biosensing and in bioimaging sciences [3-6]. Inorganic metal nanolayered hydroxides have been widely applied for the delivery of various pharmaceutical drugs and have found to be promising in improving the bioavailability of drugs, better biocompatility and therapeutic efficacy [6-8].

1.2 Problem statement

Tuberculosis (TB) is an airborne infectious disease caused by the bacterium *Mycobacterium tuberculosis (MTB).* Tuberculosis can be classified in two types based of the infections, (1) Pulmonary tuberculosis, when MTB infect the lungs and (2) Extra pulmonary tuberculosis when MTB infect other organs of humans except lungs such as liver, spleen, kidneys, tonsils, intestine, brain and bones etc [9]. Tuberculosis has been lethal to humans for centuries till today and according the most recent global TB report (2013) by world health organization (WHO) there are about 8.6 million people were new cases of TB and approximately 1.3 million human died from the disease in the year 2012 [10]. Chemotherapy of TB is complicated by number of factors such as multidrug prescription, frequent dosage, adverse side effects anti-TB drugs and long treatment duration (6-24months). All of these factors result in the patients' noncompliance to the chemotherapy of tuberculosis and patients' noncompliance is the most common cause for the treatment failure. And unfortunately no new anti-TB drug has been introduced in the market in last 5 decades as the drug development is very lengthy and budget consuming process [10]. In this scenario when there has been no new anti-TB drug introduced in the market. We need to make better utilization of currently available anti-TB drugs. We can do this by reducing the adverse effect of the drugs, improving the

bioavalibity of drugs and by reducing the concentration of dosages and by decreasing the dosing frequency of drugs. We can achieve all these important properties with biocompatible and biodegradable drug delivery systems having sustained release properties. Inorganic nanolayered metal hydroxides have all of these characteristics to be applied as drug delivery systems for anti-TB drugs.

1.3 Objectives

Following are the objective of the study

1. To prepare anti-TB nanodelivery formulation based anti-TB drugs Isoniazid and Para aminosalicylic acid using metal layered hydroxides host (ZLH, Zn/Al-LDHs and Mg/Al-LDHs). To characterize the designed anti-TB nanodelivery systems using different analytical techniques (XRD, FT-IR, HPLC, CHNS, UV/Vis and FESEM)
2. To determine the *in vitro* release of these anti-TB drugs in human body simulated PBS solution from the nanolayered host.
3. To determine their *in vitro* cytotoxicity on human normal lung cell MRC-5 and 3T3 fibroblast cells.
4. To determine their therapeutic effect against mycobacteria tuberculosis and other Gram positive and Gram negative bacteria.

1.4 Significance of study

This study was carried out to design sustained release formulations of anti-TB drugs with biocompatible capabilities for the anti-tuberculosis drugs namely para aminosalicylic acid and isoniazid with inorganic nanolayers. The sustained release nanocomposites formulations have the potential to enhance the bioavailability of drug for longer period of time by sustained release of the drugs, thereby would reduce the dosing frequency of the drugs, protect the drug from physico-chemical degradations inside the body, and reduce the adverse effects to the drugs. All of these advantages would improve the patients' compliance to the treatment of tuberculosis and would make the chemotherapy of tuberculosis patients' friendly.

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CHAPTER 9

CONCLUSION AND RECOMMENDATIONS

In this research different anti-tuberculosis (anti-TB) nanodelivery formulations were designed and synthesized by intercalation of anti-tuberculosis drugs namely para-aminosalicylic acid (second line anti-TB drug) and isoniazid (first line anti-TB drug) using the metal layered hydroxides (inorganic nanolayers) as hosts. Zinc layered hydroxides, zinc aluminium layered double hydroxides, and magnesium aluminium layered hydroxides were used as metal layered hydroxides host.

Para amino salicylic was successfully intercalated into zinc layered hydroxides using zinc oxide and zinc nitrate hexahydrate as precursors. Para amino salicylic acid was also intercalated into zinc aluminium layered double hydroxides by using coprecipitation and ion exchange methods.

Isoniazid was successfully intercalated into zinc aluminium layered double hydroxides applying coprecipitation and ion exchange methods. Isoniazid was also successfully intercalated into magnesium aluminium layered double hydroxides using ion exchange and coprecipitation methods.

The anti-TB drugs para-aminosalicylic acid and isoniazid from the metal layered hydroxides hosts (inorganic nanolayers) were found to be release in two stages manner initially fast followed by more stable slow release. However, the overall release of these drug was found to be sustained. The in vitro sustained release of the designed anti-TB nanodelivery formulations would enhance the bioavalibity of these drugs in body for longer period of time.

Both of these drug tare thermally stabilized by the metal layered hydroxides (inorganic nanolayers) due to the electrostatic interactions between the negatively charged drug and positively charged nanolayers. In addition the metal layered hydroxides would protect the drug from physico-chemical degradations inside the body and this would also result in the reduction in the adverse affects associated with these drugs.

The anti-mycobacterial and antimicrobial studies were conducted for the therapeutic comparative studies. The minimum inhibitory concentration (MIC) of free para amino salicylic acid (PAS) against mycobacteria tuberculosis was found to be 5.0 µg/mL. The effective MICs for the PAS-Zn/Al-LDHs (prepared by co precipitation method), PAS-Zn/Al-LDHs (prepared by ion exchange method), PAS-Zinc layered hydroxides (using ZnO precursor) and PAS-Zinc layered hydroxides (using zinc nitrated hexahydrated) were found to be 1.97 µg/mL, 1.98 µg/mL, 0.83 µg/mL, and 1.40 µg/mL respectively against bacteria mycobacterium tuberculosis. The efficacy of PAS was improved 2.5 times by PAS-Zn/Al-LDHs (prepared by co precipitation method), PAS-Zn/Al-LDHs (prepared by ion exchange method). The efficacy of PAS was also found to be enhanced 5 times by PAS-Zinc layered hydroxides (using ZnO precursor) and it was increased 4 times by the PAS-Zinc layered hydroxides (using zinc nitrated hexahydrated).

Furthermore PAS based nanocomposites were also found active against Gram positive and Gram negative bacteria.

The MICs of the free drug, isoniazid against mycobacteria tuberculosis was found to be 2.3 µg/mL and in comparison to this, effective MICs of isoniazid-Mg/Al-LDHs prepared by ion exchange and coprecipitation methods were found to be 0.37 µg and 0.44 µg,

respectively. The therapeutic efficacy of isoniazid was enhanced about 5 times by its nanodelivery formulations with Mg/Al-LDHs compared to the free isoniazid.

The effective MICs of isoniazid zinc aluminium layered double hydroxides (INH-Zn/Al-LDHs) prepared by coprecipitation and ion exchange methods were found to be 0.91 µg and 0.62 µg against the mycobacteria tuberculosis. Comparison of MIC of free drug isoniazid with effective MICs of nanocomposites revealed that the therapeutic efficacy were enhanced by about 2.5 times and 3.7 time by isoniazid-Zn/Al-LDHs prepared by coprecipitation and ion exchange methods, respectively.

The enhanced efficacies of para amino salicylic acid and isoniazid in metal layered hydroxides intercalated forms can be attributed to the nanoscaled size, which helps the better internalization inside the mycobacteria tuberculosis and sustained release of these drugs for longer period of time.

The all of above mentioned nanocomposites-based para aminosalicylic acid and isoniazid showed approximately four fold higher biocompatibility with human normal lung fibroblast cells MRC-5 and 3T3 fibroblast cells for up to 72 hours longer incubation period at the highest concentration of 50 µg/mL. Only exception to this was PAS-zinc layered hydroxides (prepared using ZnO precursor), which was found to show cytotoxicity in time concentration dependent manner with 3T3 fibroblast cells. However PAS-zinc layered hydroxides (prepared using ZnO precursor) was found to be highly biocompatible to human normal lung cells.

It can be concluded that metal layered hydroxides (inorganic nanolayers) fulfilled all the criteria of ideal drug delivery systems for anti-TB drugs and also for the other drugs due their high biocompatibility which would reduce the side effects, tendency to release the drugs in sustained manner, can protect the drugs from physico-chemical degradation, and enhances the therapeutic efficacy of the drugs.

The current research opens the horizon for the patient friendly tuberculosis treatment with high efficacy, low adverse affects and better therapeutic efficacy which would results in enhancement of patients; compliance to the chemotherapy of tuberculosis.

Table 9.1 Summary of the Research

Compounds	(%) Drug Loading	Sustained Release Period	(%) Viability of Human normal lung cells After 72 hours of incubation	MIC nanocomposites against bacteria M. tuberculosis	Effective MIC M.tuberculosis	Reference
Isoniazid (INH)	-	1-2 hours only	20	2.3 µg/mL	2.3 µg/mL	
INH-Mg/Al-LDHs (by coppt)	10.34%	3-5 days	86	3.60 µg/mL	0.37 µg/ mL	Saifullah et al. 2014 (International Journal of nanomedicine
INH-Mg/Al-LDHs (I.Exchane)	12.35	3 – 5 days	86	3.60 µg/mL	0.44µg/mL	
INH-Zn/Al-LDHs (Co-ppt)	21.00	4-6 days	80	7.40µg/mL	0.91 µg/mL	
INH-Zn/Al-LDHs (I. Exchange)	31.00	3-5 days	80	7.40µg/mL	0.62 µg/mL	Saifullah et al. 2014 J.Nanobiotechnology (UR)
P. Salicylic acid (PAS)	---	1.5-2 hours	80	-	5.00 µg/mL	Saifullah et. 2013 Chemistry central. Journal
PAS-ZnLH LDHs (using ZnO)	17.00	1 - 5 days	80	5.50 µg/mL	0.83 µg/mL	Saifullah et al. 2014 DDDT
PAS-ZnLH LDHs (using $ZnZNO_3$)	22.40	0.5 - 5 days	80	6.10 µg/mL	1.40 µg/mL	Saifullah et al. 2014 Scientific World J.
PAS-Zn/Al-LDHs (Coptt)	22 .00	3 – 5 days	80	7.90 µg/mL	1.73 µg/mL	Saifullah et al. 2013DDT
PAS-Zn/Al-LDHs (I.E)	16.6 0	3-5 days	80	12.40 µg/mL	2.00 µg/mL	Saifullah et al. 2014. DDDT

9.1 Selection of best nanodelivery system from the designed carriers

Almost all of the designed showed good drug delivery characteristics such as sustained release of the intercalated drugs, better therapeutic efficacy against the pathogenic bacteria mycobacteria tuberculosis and high biocompatibility with human normal lung cells. At this stage we cannot select best nanocomposite as all of them are found excellent nanodrug delivery systems in this *in vitro* research. Further *in vivo* studies for therapeutic efficacy on TB infected animal models and cytotoxicity studies on both healthy and infected animal are required for the selection the best delivery system among these designed drug delivery systems.

9.2 Recommendations

Further works should be carried out

a) In designing the nanodelivery formulation-based on metal layered hydroxides (inorganic nanolayers) namely layered double hydroxides or layered hydroxy salts for different drugs, biomolecules, and genes etc., metal hydroxides such as magnesium hydroxides, zinc hydroxides and aluminium hydroxides can be used instead of using salts of these metal which contain competing anions.
b) Use of metal hydroxides as precursors offer easy intercalation of neutral drugs molecules, even anionic drugs would easily be intercalated and further more final product would be free from contaminant, especially competing unwanted anions like nitrates, chlorides and acetate anions etc.
c) *In vivo* studies of these developed anti-TB nanocomposites based on para amino salicylic acids, isoniazid and metal layered hydroxides should carried out for TB infected animals.
d) *In vivo* anti-inflammatory studies using these nanocomposites should be carried out to investigate the affect on the animal tissues and organs.
e) Pharmacokinetic studies can also be conducted using the developed anti-TB nanocomposites to determine extent of bioavailability of the drugs and to study the removal of degraded metal ions from the body.
f) Nanocomposites prepared by using isoniazid as active agent should be tested against different cancer cell lines and isoniazid has been reported to possess anticancer activity.

LIST OF PUBLICATIONS

9.4.1 Research Publications

1. **Bullo Saifullah and** Mohd Zobir B Hussein . Inorganic nanolayers structure, preparation and biomedical application (Review article). International Journal of Nanomedicine-2014 (Accepted).

2. **Bullo Saifullah**, Palanisamy Arulselvan, Mohamed Ezzat El Zowalaty, Sharida Fakurazi, Thomas J Webster, Benjamin M Geilich, Mohd Zobir B Hussein. Development of biocompatible nanodelivery system for tuberculosis based on Isoniazid-Mg/Al Layered Double Hydroxide and its comparison with Isoniazid-Zn/Al- Layered Double Hydroxide. International Journal of Nanomedicine. 2014:9

3. **Bullo Saifullah**, Mohamed E.El Zowalaty, Palanisamy Arulselvan, Sharida Fakurazi, Thomas J Webster, Benjamin M Geilich, Mohd Zobir Hussein. Antimycobacterial, antimicrobial activities and biocompatibility of nanocomposites of Para aminosalicyclic acid with ZnLH and Zn/Al LDHs. (Drug Design Development Therapy. Drug Design, Development and Therapy 2014:8 1029–1036.

4. **Bullo Saifullah,** Palanisamy Arulselvan, Mohamed Ezzat El Zowalaty, Sharida Fakurazi, Thomas J Webster, Benjamin M Geilich, Mohd Zobir B Hussein. Development of a highly biocompatible anti-tuberculosis nanodelivery formulation based on para-amino salicylic acid – zinc layered hydroxide nanocompositese Scientific World Journal Volume 2014, Article ID 401460, 12 pageshttp://dx.doi.org/10.1155/2014/401460.

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14. Mohd Zobir Hussein, **Bullo Saifullah,** Palanisamy Arulselvan, Mohamed Ezzat El Zowalaty, Sharida Fakurazi, Thomas J Webster, Benjamin M Geilich. Nanodelivery system for anti-tuberculosis drug based on Mg/Al and Zn/Al layered double hydroxides-isoniazid nanocomposites. The International Conference for Academic Disciplines 16-19 June 2014 at Casa Convalescencia, Sant Antoni Maria Claret 171, Barcelona, Spain.

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ORIGINAL RESEARCH

Antimycobacterial, antimicrobial, and biocompatibility properties of para-aminosalicylic acid with zinc layered hydroxide and Zn/Al layered double hydroxide nanocomposites

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Abstract: The treatment of tuberculosis by chemotherapy is complicated due to multiple drug prescriptions, long treatment duration, and adverse side effects. We report here for the first time an in vitro therapeutic effect of nanocomposites based on para-aminosalicylic acid with zinc layered hydroxide (PAS-ZLH) and zinc-aluminum layered double hydroxides (PAS-Zn/Al LDH), against mycobacteria, Gram-positive bacteria, and Gram-negative bacteria. The nanocomposites demonstrated good antimycobacterial activity and were found to be effective in killing Gram-positive and Gram-negative bacteria. A biocompatibility study revealed good biocompatibility of the PAS-ZLH nanocomposites against normal human MRC-5 lung cells. The para-aminosalicylic acid loading was quantified with high-performance liquid chromatography analysis. In summary, the present preliminary in vitro studies are highly encouraging for further in vivo studies of PAS-ZLH and PAS-Zn/Al LDH nanocomposites to treat tuberculosis.

Keywords: Zn/Al-layered double hydroxides, zinc layered hydroxides, tuberculosis, para-aminosalicylic acid (PAS), antimicrobial agents

Introduction

Tuberculosis (TB) has been lethal to humans for centuries and despite significant technological advances, it still claims millions of precious human lives. According to a recent global TB report by the World Health Organization, about 8.6 million new cases of TB were reported with about 1.3 million deaths in 2013.[1]

TB is a bacterial infectious disease caused by *Mycobacterium tuberculosis*, which generally targets the lungs (pulmonary TB) but can also infect other body organs like the liver, spleen, kidneys, tonsils, brains, intestine, etc, and is called extrapulmonary TB.[2] The aforementioned classification is based on the target site of the infection. However, TB can also be classified according to the treatment prescription, namely: 1) drug susceptible TB (DSTB) (the most common form of TB, which can be cured by the four first-line anti-TB drugs: isoniazid, rifampicin, pyrazinamide, and ethambutol), 2) multidrug-resistant TB (MDR-TB) (the form of TB when bacteria become resistant to multiple anti-TB drugs, especially against isoniazid and rifampicin; MDR-TB is treated with second-line anti-TB drugs, namely para-aminosalicylic acid [PAS], cycloserine, aminoglycosides, fluoroquinolones, thioamides, and cyclopeptides),[3] and 3) extensively resistant TB, where the bacteria become resistant to first-line anti-TB

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ORIGINAL RESEARCH

Development of a biocompatible nanodelivery system for tuberculosis drugs based on isoniazid-Mg/Al layered double hydroxide

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Abstract: The primary challenge in finding a treatment for tuberculosis (TB) is patient non-compliance to treatment due to long treatment duration, high dosing frequency, and adverse effects of anti-TB drugs. This study reports on the development of a nanodelivery system that intercalates the anti-TB drug isoniazid into Mg/Al layered double hydroxides (LDHs). Isoniazid was found to be released in a sustained manner from the novel nanodelivery system in humans in simulated phosphate buffer solutions at pH 4.8 and pH 7.4. The nanodelivery formulation was highly biocompatible compared to free isoniazid against human normal lung and 3T3 mouse fibroblast cells. The formulation was active against *Mycobacterium tuberculosis* and gram-positive bacteria and gram-negative bacteria. Thus results show significant promise for the further study of these nanocomposites for the treatment of TB.

Keywords: tuberculosis, isoniazid, Mg/Al LDH, nanodelivery system

Introduction

Mycobacterium tuberculosis (MTB) causes pulmonary tuberculosis (TB) when the bacterium infects the lungs, and extrapulmonary TB where it infects other organs such as the kidneys, liver, spleen, intestine, tonsils, bones, and brain.[1] Patient non-compliance is the most common challenge in the treatment of TB; this is due to long treatment duration, adverse effects of anti-TB drugs, multidrug prescriptions, and frequent dosing.[2] TB has been a threat to human beings for centuries; the latest global TB report states that there were ~8.6 million people infected with TB and ~1.3 million died from the disease in 2012.[3]

Isoniazid (INH) is one of the most powerful anti-TB drugs among four first-line anti-TB drugs; namely rifampin, isoniazid, pyrazinamide, and ethambutol.[4] Patients are required to take a 300 mg dose of isoniazid daily for 6 months in combination with other anti-TB agents.[3] However, there are many undesirable side effects associated with isoniazid such as hepatotoxicity, jaundice, hyperacute liver failure, and hydralazine hypotension.[4–7] Due to these adverse affects, the isoniazid dosage concentration is limited, and when the disease is treated with subtherapeutic doses, it can possibly develop resistance to that particular drug.[8] Unfortunately, there have been no new anti-TB drugs introduced to the market over the past 5 decades.

Biocompatible drug delivery systems have been the focus of numerous studies since they can improve the efficacy of existing drugs.[4] For example, drug delivery

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Research Article

Development of a Highly Biocompatible Antituberculosis Nanodelivery Formulation Based on Para-Aminosalicylic Acid—Zinc Layered Hydroxide Nanocomposites

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Tuberculosis is a lethal epidemic, difficult to control disease, claiming thousands of lives every year. We have developed a nanodelivery formulation based on para-aminosalicylic acid (PAS) and zinc layered hydroxide using zinc nitrate salt as a precursor. The developed formulation has a fourfold higher efficacy of PAS against mycobacterium tuberculosis with a minimum inhibitory concentration (MIC) found to be at 1.40 μg/mL compared to the free drug PAS with a MIC of 5.0 μg/mL. The newly developed formulation was also found active against Gram-positive bacteria, Gram-negative bacteria, and *Candida albicans*. The formulation was also found to be biocompatible with human normal lung cells MRC-5 and mouse fibroblast cells-3T3. The *in vitro* release of PAS from the formulation was found to be sustained in a human body simulated phosphate buffer saline (PBS) solution at pH values of 7.4 and 4.8. Most importantly the nanocomposite prepared using zinc nitrate salt was advantageous in terms of yield and free from toxic zinc oxide contamination and had higher biocompatibility compared to one prepared using a zinc oxide precursor. In summary, these promising *in vitro* results are highly encouraging for the continued investigation of para-aminosalicylic acid and zinc layered hydroxide nanocomposites *in vivo* and eventual preclinical studies.

1. Introduction

Tuberculosis (TB) has remained lethal to humans for centuries and is of great public health concern. There were about 1.4 million human deaths from TB and about 8.7 million people infected in 2012 [1, 2]. TB is also the second greatest killer of humans in the world by a single infectious agent after HIV/AIDS [1]. The situation has become even more dire by the reemergence of multidrug resistant TB (MDR-TB) and in 2012, approximately 450,000 people developed MDR-TB and there was about 37% deaths of MDR-TB [1].

Chemotherapy of TB has been complicated by multidrug prescriptions, dosing frequency, longer treatment duration, and adverse side effects associated with anti-TB drugs [3, 4]. Since the drug development is lengthy, costly, and time consuming, it should not be surprising that no new anti-TB drug has reached the market in over 5 decades with the last anti-TB drug approved (rifampicin) in 1963 [3–5]. To cope

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REVIEW

Controlled-release approaches towards the chemotherapy of tuberculosis

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Abstract: Tuberculosis (TB), caused by the bacteria *Mycobacterium tuberculosis*, is notorious for its lethality to humans. Despite technological advances, the tubercle bacillus continues to threaten humans. According to the World Health Organization's 2011 global report on TB, 8.8 million cases of TB were reported in 2010, with a loss of 1.7 million human lives. As drug-susceptible TB requires long-term treatment of between 6 and 9 months, patient noncompliance remains the most important reason for treatment failure. For multidrug-resistant TB, patients must take second-line anti-TB drugs for 18–24 months and many adverse effects are associated with these drugs. Drug-delivery systems (DDSs) seem to be the most promising option for advancement in the treatment of TB. DDSs reduce the adverse effects of drugs and their dosing frequency as well as shorten the treatment period, and hence improve patient compliance. Further advantages of these systems are that they target the disease area, release the drugs in a sustained manner, and are biocompatible. In addition, targeted delivery systems may be useful in dealing with extensively drug-resistant TB because many side effects are associated with the drugs used to cure the disease. In this paper, we discuss the DDSs developed for the targeted and slow delivery of anti-TB drugs and their possible advantages and disadvantages.

Keywords: *Mycobacterium tuberculosis*, drug-delivery system, targeted delivery, anti-TB drug, TB, patient compliance

Introduction

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, remains a most lethal disease in humans. The tubercle bacillus has tremendous ability to cope with the human immune system and has developed the ability to survive and do well within macrophage phagosomes. Despite technological advances, TB continues to threaten humans. According to the World Health Organization's 2011 global report on TB 2011 World Health Organization (WHO) report,[1] 8.8 million TB cases were reported in this year, approximately 1.35 million of which were fatal with an additional 0.35 million fatalities in individuals with HIV. TB remains among the three major causes of death among females aged 15–44 years old and approximately 320,000 women died due to TB in 2010. The identification and suitable treatment of multidrug-resistant TB (MDR-TB) remain the most important aspects to be addressed. Furthermore, TB's resistance to contemporary TB antibiotics is due to its dormant form in the host cells. It is known that rifampin, isoniazid, and ethambutol (but not pyrazinamide) require bacteria to reproduce in order to perform their function. It is thought that the bacteria's ability to remain dormant allows them to be phenotypically resistant to prescribed antibiotics.[2,3]

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RESEARCH ARTICLE Open Access

Sustained release formulation of an anti-tuberculosis drug based on para-amino salicylic acid-zinc layered hydroxide nanocomposite

Bullo Saifullah[1], Mohd Zobir Hussein[1*], Samer Hasan Hussein-Al-Ali[2], Palanisamy Arulselvan[3] and Sharida Fakurazi[3,4]

Abstract

Background: Tuberculosis (TB), is caused by the bacteria, *Mycobacterium tuberculosis* and its a threat to humans since centuries. Depending on the type of TB, its treatment can last for 6–24 months which is a major cause for patients non compliance and treatment failure. Many adverse effects are associated with the currently available TB medicines, and there has been no new anti tuberculosis drug on the market for more than 50 year, as the drug development is very lengthy and budget consuming process.
Development of the biocompatible nano drug delivery systems with the ability to minimize the side effects of the drugs, protection of the drug from enzymatic degradation. And most importantly the drug delivery systems which can deliver the drug at target site would increase the therapeutic efficacy. Nanovehicles with their tendency to release the drug in a sustained manner would result in the bioavalibilty of the drugs in the body for a longer period of time and this would reduce the dosing frequency in drug administration. The biocompatible nanovehicles with the properties like sustained release of drug of the target site, protection of the drug from physio chemical degradation, reduction in dosing frequency, and prolong bioavailability of drug in the body would result in the shortening of the treatment duration. All of these factors would improve the patient compliance with chemotherapy of TB.

Result: An anti tuberculosis drug, 4 amino salicylic acid (4 ASA) was successfully intercalated into the interlamellae of zinc layered hydroxide (ZLH) via direct reaction with zinc oxide suspension. The X ray diffraction patterns and FTIR analyses indicate that the molecule was successfully intercalated into the ZLH interlayer space with an average basal spacing of 24 Å. Furthermore, TGA and DTG results show that the drug 4 ASA is stabilized in the interlayers by electrostatic interaction. The release of 4 ASA from the nanocomposite was found to be in a sustained manner. The nanocomposite treated with normal 3T3 cells shows it reduces cell viability in a dose and time dependent manner.

Conclusions: Sustained release formulation of the nanocomposite, 4 ASA intercalated into zinc layered hydroxides, with its ease of preparation, sustained release of the active and less toxic to the cell is a step forward for a more patient friendly chemotherapy of Tuberculosis.

Keywords: Para Amino salicylic acid (PAS/4 ASA), MDR TB, Zinc layered hydroxide, Nanocomposite, 3T3 cell lines

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ORIGINAL RESEARCH

Antituberculosis nanodelivery system with controlled-release properties based on para-amino salicylate–zinc aluminum-layered double-hydroxide nanocomposites

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Abstract: We report the intercalation and characterization of para-amino salicylic acid (PASA) into zinc/aluminum-layered double hydroxides (ZLDHs) by two methods, direct and indirect, to form nanocomposites: PASA nanocomposite prepared by a direct method (PASA-D) and PASA nanocomposite prepared by an indirect method (PASA-I). Powder X-ray diffraction, Fourier-transform infrared spectroscopy, and thermogravimetric analysis revealed that the PASA drugs were accommodated within the ZLDH interlayers. The anions of the drug were accommodated as an alternate monolayer (along the long-axis orientation) between ZLDH interlayers. Drug loading was estimated to be 22.8% and 16.6% for PASA-D and PASA-I, respectively. The in vitro release properties of the drug were investigated in physiological simulated phosphate-buffered saline solution of pH 7.4 and 4.8. The release followed the pseudo-second-order model for both nanocomposites. Cell viability (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] assays) was assessed against normal human lung fibroblast MRC-5 and 3T3 mouse fibroblast cells at 24, 48, and 72 hours. The results showed that the nanocomposite formulations did not possess any cytotoxicity, at least up to 72 hours.

Keywords: drug-delivery system, slow-release nanocarrier, tuberculosis, biocompatible nanocomposites

Introduction

Layered double hydroxides (LDHs) have a hydrotalcite-like structure in which some of the divalent cations are replaced by trivalent cations, which results in positively charged brucite-like sheets stacked on top of one another, resulting in a layered structure. The positive charge of these brucite-like nanosheets is neutralized by the anions and water molecules.[1] A variety of anions can be accommodated between the layers to counter balance the positive charge and this tendency makes them versatile to be used for different applications. The anions can be inorganic, like halides, nitrates, and sulfates, and organic, like drugs, amino acids, dyes, polymers, and DNA etc.[1,2] LDHs are represented by the general formula $(M^{II}_{1-x}M^{III}_{x}[OH]_2)(A_{x/n}{}^{n-}) \cdot yH_2O$, in which M^{II} is a divalent metal ion, M^{III} is a trivalent metal ion, and anions are used to neutralize the positive charge of the sheets. The LDHs have many applications, such as their use for extraction of toxic waste from water,[3] in catalysis,[4] and as a flame retardant,[5] and they have also been used in chiral separation.[6] Recently, the most important application of LDHs has been investigated, particularly slow-release drug-delivery systems. Different drugs have been intercalated recently into nanosheets of LDHs, such as anticancer drugs, antihistamines, antidiabetics, and antimicrobials.[7,8]

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9.4.2 Conferences /Workshops/ Posters/ Proceedings

1. **Bullo Saifullah,** Mohd Zobir Hussein . Drug delivery application of graphene oxide using para-aminosalicylic acid as model drug. Workshop on Advanced Materials and Nanotechnology 2014 (WAMN 2014), UPM, Malaysia, August 25-26, 2014.

2. **Mohd Zobir Hussein*, Bullo Saifullah et al.** Graphene as a platform for an anti-tuberculosis nanodelivery system based on isoniazid. Advanced Material Conference 2014. Langkawi, Malaysia. 25-26 November 2014.

3. **Mohd Zobir Hussein, Bullo Saifullah et al.** Nanodelivery system for anti-tuberculosis drug based on Mg/Al and Zn/Al layered double hydroxides-isoniazid nanocomposites The International Conference for Academic Disciplines 16-19 June 2014 at Casa Convalescencia, Sant Antoni Maria Claret 171, Barcelona, Spain.

4. **The 6th Nanotechnology cncer Asia-pacific (NCAP)** Network Meeting (Healthcare Session I of APAN 37th Video Conferencing meeting) IDEC Alpha University Putra Malaysia 23 January 2014.

5. **Workshop on Advanced Materials and Nanotechnology 2013 (WAMN),** Organized by Institute of Advanced Technology (ITMA), University Putra Malaysia (UPM), 10-11 September 2013

6. **Nanomaterials and Nanodelivery Workshop 2013.**
   Organized by Laboratory of Molecular Medicine, Institute of Bioscience, University Putra Malaysia 25th April

7. **43rd Union World Conference on Lung Health 2012.**
   Held in Kuala Lumpur from 13-17 November 2012, organized by the International Union Against Tuberculosis and Lung Disease (The Union).

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