Frontiers in Clinical Drug Research

(Anti Infectives)

Editor:
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Bentham Books

Frontiers in Clinical Drug Research-Anti-Infectives

(Volume 6)

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Frontiers in Clinical Drug Research-Anti-Infectives

Volume # 6

Editor: Atta-ur-Rahman, *FRS*

ISSN (Online): 2452-3208

ISSN (Print): 2352-3212

ISBN (Online): 978-981-14-2574-5

ISBN (Print): 978-981-14-2573-8

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PREFACE

The 6th volume of **Frontiers in Clinical Drug Research – Anti Infectives** comprises five chapters that cover a variety of topics including prolonging antibiotic life, antimicrobial materials and devices, treatment of various infectious diseases.

In chapter 1, Sencanski and Glisic present an overview on Direct-Acting antiviral drugs for treatment of Hepatitis C Virus (HCV) that covers the latest therapeutic advances that can potentially convert chronic HCV into a routinely treatable disease. The introduction of direct-acting antivirals (DAAs) has improved efficacy and tolerance of treatments with high cure rates. A literature review was conducted to identify published clinical trial results regarding DAA combination therapy with third generation NS3/4a protease inhibitors. Detailed attention is given to the chemistry of the approved NS3/4a drugs and candidate therapeutics in advanced stages of development. In this regard, a review of key drug design and organic synthesis stages is presented for anti-NS3/4A DAAs.

In chapter 2, Soares *et al* explain how lattice plants can be used as a source of anti-infective compounds, Bucio *et al* in chapter 3 present recent developments on current antimicrobial materials as well as strategies for obtaining antimicrobial surfaces and coatings and their properties. In addition, the safety assessment of biomedical applications and international standards are also discussed in this chapter.

Chapter 4 by Mostafa El-Sayed gives an update on the current progress in the development of new drugs for the treatment of toxoplasmosis. In the last chapter of the book by Irena *et al.* the authors provide an insight into the current knowledge of the usage of antibody preparations, efficacy and mechanisms of action, with respect to specific diseases, including the treatment of infectious diseases and future prospects.

I would like to thank all the authors for their excellent contributions that will be of great interest. Also, I would like to thank the editorial staff of Bentham Science Publishers, particularly Mr. Mahmood Alam (Director Publications), Mr. Obaid Sadiq (In-charge Books Department) and Miss Asma Ahmed (Manager Publications) for their support.

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CHAPTER 1

Direct-Acting Antiviral Drugs for Treatment of Hepatitis C Virus Infection-Clinical Trials Data and Chemistry of NS3/4a Protease Inhibitors

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Abstract: The hepatitis C virus (HCV) infection is a major and rising global health problem, affecting more than 71 million people worldwide. HCV is connected with several hepatic and extrahepatic disorders, containing several malignancies. Improved HCV detection with combined simple, well-tolerated treatments could reduce the need for liver transplantation and HCV related mortality. The latest therapeutic advances might convert chronic HCV into a routinely treatable disease. The introduction of direct-acting antivirals (DAAs) has improved efficacy and tolerance of treatments with high cure rates. DAAs target specific nonstructural proteins of the HCV with consequential interference with viral replication and consequently infection. The majority of the FDA approved drugs for HCV and those pending approval are small molecule drugs, especially those that utilize the viral inhibitor mechanisms of action and favor the HCV nonstructural proteins as their targets. Therefore, DAAs represent the most promising anti-HCV drugs that carry the least risk of drug failure during clinical trials. NS3/4a protease inhibitors have become the basis for HCV treatment as most new therapies contain an inhibitor from this class. It is reported that the approach for combating chronic viral infections is best achieved by a combination of several strategies, by means of inhibiting several targets. Moreover, the best promising strategy for fighting HCV is most similar to the anti-HIV therapy. A literature review was conducted to identify published clinical trial results regarding DAA combination therapy with third generation NS3/4a protease inhibitors. Detailed attention is given to the chemistry of the approved NS3/4a drugs and candidate therapeutics in the advanced stages of development. In this regard, a review of key drug design and organic synthesis stages is presented for anti-NS3/4A DAAs.

Keywords: Chemical Synthesis, Clinical Trials Data, Drug Design, Direct-Acting Antivirals, Hepatitis C Virus, NS3/4A protease, Protease Inhibitors.

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INTRODUCTION

Chronic HCV infection with an approximate worldwide prevalence of 1% is a worldwide health problem, affecting 71 million people with 1.75 million persons newly infected each year [1, 2]. HCV has been reported as the principal cause of chronic liver disease, cirrhosis, and liver cancer [3].

A sustained virological response (SVR) to antiviral therapy remarkably alters the course of liver disease related to the HCV infection by lowering the frequency of hepatic decompensation, liver cancer, liver-related mortality, all-cause mortality, and liver transplantation [4, 5]. The previous standard-of-care treatment for chronic HCV, before 2011, was a PEGylated interferon (PEG-IFN) and ribavirin (RBV) combination (PEG-IFN/RBV), a dual therapy that has been used for more than 15 years [6]. This long and costly therapy was associated with serious adverse effects [6, 7]. The introduction of therapy with direct-acting antivirals (DAAs), anti-HCV drugs that directly target HCV proteins, is considered as a key advancement in HCV therapy offering higher cure rates and the least adverse events. NS3/4A protease inhibitors - telaprevir and boceprevir in 2011 became the first FDA-approved DAA drugs. With the treatment with one of the first generation NS3/4A protease inhibitors in combination with PEG-IFN more than 75% patients infected with the HCV genotype 1 achieved an SVR, but this therapy was associated with serious side effects, increased daily pill burden and drug resistance [8, 9]. As a result of drawbacks of the first-generation HCV protease inhibitors better therapeutics of the second-generation were developed. Reduced demand for the first generation drugs due to the availability of newer HCV drugs with higher efficacy and fewer side effects, along with the fact that they were no longer recommended by the WHO, has stopped their production [10]. In an astonishing revolution in the treatment of chronic HCV the second phase commenced in 2015 with a regimen of DAAs in combination with 2 or 3 second-generation DAAs that target HCV viral proteins (NS3/4A protease inhibitors, NS5B nucleos(t)idic and non-nucleos(t)idic polymerase inhibitors, NS5A replication complex inhibitors) without IFN and RBV for 8 to 16 weeks based on baseline factors such as the stage of fibrosis, viral genotype and subtype, baseline viral load, former treatment history (naive or experienced) and resistance-associated variants. The majority of the new HCV treatment combinations have an immense antiviral impact (virological cure or a SVR > 95%), fair tolerance and a lower pill burden [11]. The third phase in the HCV treatment revolution has recently emerged with the introduction of the pangenotypic DAAs, suitable for all HCV genotypes. Current, up-to-date, oral antiviral DAA combination therapy shows supreme treatment efficacy, safety and tolerability. The third-generation pangenotypic NS3/4A protease inhibitors (mainly glecaprevir (GLE) and voxilaprevir (VOX)) possess both high antiviral

activity and a genetic resistance barrier with cure rates of over 95% regardless of the presence of baseline resistance associated variants [12]. In addition these regimens are well tolerated with low incidence of side effects, even in the difficult-to-treat population (*e.g.* compensated cirrhosis, end-stage renal disease and patients who failed previous DAA treatment) [12, 13]. Similarly, in another difficult-to-treat subgroup of HCV patients with genotype 3, which constitutes 30% of the global HCV population, pangenotypic protease inhibitor, glecaprevir–GLE coformulated with pibrentasvir, an NS5A inhibitor, show potential with high SVR rates [14].

Despite improved HCV detection, still fewer than 20% of people living with HCV are aware of their infection, so attention should be directed to engage, screen, and diagnose everyone in need of therapy while improving access to quick, simple, and affordable HCV diagnostics at the point of care. The above mentioned issues are crucial to attain global HCV elimination [15, 16]. According to current international guidelines genotype testing is still recommended before HCV treatment commencement [17, 18], although the new pangenotypic DAA regimens no longer depend on quantitative HCV RNA or genotype data to stratify the duration of treatment. Also many current international clinical trials collect evidence of the efficacy of simplified approaches for diagnosis and treatment monitoring [16, 19]. Current new DAA combinations with a pangenotypic third generation NS3/4A protease inhibitor represent an opportunity in low and middle-income countries with limited resources to treat HCV infected patients without prior costly genotype testing. These issues regarding diagnostic simplification and cost-reduction are crucial for carrying out HCV screening and treatment in resource limited countries [20].

HCV NS3/4a protease inhibitors inhibit the enzymatic activity of NS3/4A and have a crucial role in the development of contemporary therapies for HCV. Of note is that this class of drugs has become a mainstay of HCV treatment as most new therapies contain an inhibitor from this class. HCV is a positive-stranded RNA virus whose genome encodes a polyprotein processed into at least 10 viral structural and nonstructural (NS) proteins: C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [21]. HCV NS proteins, originating from the proteolytic degradation of the polyprotein are part of the cellular replication complex which is essential for viral replication. The NS3 protein has an N-terminal serine protease domain and a C-terminal RNA-dependent ATPase domain. NS4A represents a co-factor for the activity of NS3 serine protease [21]. Since HCV NS3-4A protease is essential for viral replication it represents an attractive target for developing new anti-HCV therapies.

Surrogate endpoints are often used in clinical trials, as they allow for indirect

CHAPTER 2

Plant Latices As Anti-Infective Compounds

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Abstract: Infectious diseases are caused by pathogenic microorganisms, such as bacteria viruses,or fungi, and can be spread, directly or indirectly, from one person to another. Among these diseases, AIDS, tuberculosis, influenza, hepatitis and neglected tropical diseases, such as dengue and malaria, stand out. On the other hand, emerging infectious diseases are infections that have recently appeared in a population or those whose incidence or geographical range is rapidly increasing and/or threatens to increase in the near future. Since the 1970's, approximately 40 infectious diseases have been discovered, including SARS, MERS, Ebola, Chikungunya, avian and swine flu, and, most recently, Zika. The transmission modes for the different existing infectious diseases vary greatly, among them the most common are through water and food, vectors, zoonosis (diseases transmitted by animals), sexual transmission, blood, air and soil. Anti-infective drugs development and the remarkable eradication of smallpox in the 1980's, following a global immunization campaign led by the WHO (World Health Organization), generated the hope that infectious diseases could be controlled and even eliminated. However, the current perception that infectious diseases continue to emerge and re-emerge (including the possibility of bioterrorism), highlights the future challenges in infectious diseases research. The modern medicine needs new anti-infective drugs in order to treat specific drug-resistant infections at the same time as the drug development pipeline is almost dry. Thus, the development of new drugs and anti-infective compounds is needed, such as the use of plant-derived latex, which is well studied and present great potential. Therefore, the objective of the present chapter is to give an overview regarding the use of plant-derived latices as anti-infective compounds, as well as to point out new perspectives on this matter.

Keywords: Anti-infective, Enzymes, Plant latex, Secondary metabolites, Traditional knowledge

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INTRODUCTION

Nature has been a source of medicinal products for millennia, with many useful drugs developed from plant sources [1]. In addition, medicinal plants and fruits have been used by indigenous peoples for centuries as sources of extracts used in the treatment of a variety of problems. Among the parts of plants used, latex has broader ethno-pharmacological applications as it is used by local tribal communities for wound healing, burns, joint pain, and for controlling worm infections. Moreover, plant latex is used in industry to make all sort of products, *e.g*. paintings, elastics, swim caps, condoms, catheters, medical gloves, balloons and chewing gum. It is also mixed in cement as an additive, providing quick physical strength for solidification [2].

About 20,000 species from 40 families are estimated to have laticifers, although so far only 12,500 plant species of 22 families have been identified [3]. Latex is found in dicotyledonous and monocotyledonous plants and, actually, nearly 10% of families and species produce latex [4], which are found in unrelated plant orders, suggesting polyphyletic origins [5].

On the other hand, latex is more frequently observed in tropical plants, with high prevalence percentages of laticiferous families and species (respectively, 12.2% for families and 14.0% for species) [6]. Some notorious laticiferous plants are: *Hevea brasiliensis*, *Carica papaya* and *Ficus* spp.

Latex is an aqueous suspension or emulsion, derived from living cells. It is a complex mixture of peptides, enzymes, triterpenoids, diterpenoids, amino acids, alkaloids, starches, sugars, oil, tannins, and other compounds [7]. In most plants, latex is white, but some have yellow, orange, or scarlet latex [4]. Moreover, latex tends to be more phytochemically diverse than resins, mucilages and gums [8]. Resin is distinct from latex because it is kept in canalicular inter-cellular spaces called resin ducts and is not kept inside cells [9]. Resin is a plant exudate common in conifers and some angiosperms (*e.g*., Anacardiaceae), rich in terpenoids and phenolics, and stored in intercellular spaces. Mucilage is another plant exudate, composed of sticky polysaccharides that is often clear and exude from the phloem. Gum is distinct from the above mentioned plant exudates once it is composed of water-soluble polysaccharides that are exude from cellular cavities or bark [10]. On the other hand, latex is produced and then stored, rarely, in parenchyma cells or more frequently in the tube structures known as laticifers [11].

Laticifers are tissues composed of extremely elongated cells that extend throughout the plant that exude the latex, whose main origin is from vacuoles. In general, these cells are distributed in various tissues of the plant body, *e.g*. roots,

stems, petioles and leaves [9]. Based on their development and structural characteristics, laticifers can be classified as: articulated and nonarticulated laticifers [11 - 13].

Nonarticulating laticifers are formed from single cells that often branch, but do not loop or reconnect. Since the laticifers branch, but never merge, the formed laticifers have tree-like shapes without loop structures. The cells undergo karyokinesis without cytokinesis, resulting in coenocytic cells [14 - 16]. On the other hand, articulated laticifers have a compound origin as they develop from multiple cells. Articulating laticifers form loops and are often connected by perforations in the cell walls of neighboring laticifers [10, 14, 15].

Thus, if a plant with laticifers are injured, latex will be immediately secreted. Not unlike the blood of animals, when it occurs, latex oozes out, becomes sticky when exposed to air, and quickly coagulates. The coagulation process is vital for plant defence against possible pathogens attack [4, 10]. Unlignifed organs (soft) are food for insects, while lignifed organs (hard) are attacked by microbes at wound sites, so the diversity in latex structure and composition is most likely an organ-specific adaptation to different potential pests [17].

Plants are continuously challenged by bacteria, fungi, oomycetes, nematodes, insects and other pathogens and herbivores. Various secondary metabolites and proteins provide resistance to herbivores *via* toxicity, antinutritive and “mire” effects. In general, specialized latex metabolites are biochemical end products that do not reenter primary metabolism. There is no strict association between compound class and laticifer type [2, 5, 10, 18]. Latex metabolites vary between species and families of plants, for example, we can find terpenoids in *Hevea brasiliensis*, cardiac glycosides in the Apocynales, alkaloids (morphine, codeine, and papaverine) in *Papaver somniferum*, phenolic glucosides in *Cannabis sativa*, sugar in Asteraceae and tannins in Musa, Aroideae [16]. Konno [9] reviewed in details the chemicals (mostly secondary metabolites but some primary metabolites) and proteins from plant latex and other exudates (Fig. **1**).

Latex protein constituents vary among organs in a single species, for example, as shown for young petioles and immature fruit of *Ficus carica* [17]. Different classes of enzymes and proteins have been found from plant latex, for example: papain-like cysteine proteases (PLCPs), chitinases, lectins, protease inhibitors and oxidases [9]. Let's take a good look at these proteins.

According to the Commission on Enzymes of the International Union of Biochemistry, proteases or peptidases, enzymes that catalyze the hydrolysis of the peptide bond between amino acid residues of a protein, are classified as EC 3.4. Therefore, proteases belong to group 3 (hydrolases), and subgroup 4 (which

CHAPTER 3

Antimicrobial Materials and Devices for Biomedical Applications

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Abstract: Bioaccumulation in sanitary devices, caused by opportunistic pathogens, intervenes negatively in the recovery of a patient since these are able to provoke a mild or life-threatening infection. Thus, surfaces of certain materials such as gauzes, catheters, sutures, *etc.*, which are adjacent or directly exposed to a healing zone, are prone to become sites for the growth, proliferation, and spread of pathogenic microorganisms. Although in surgical or healing processes, sterile materials are usually applied, the time of contact with biological interfaces is long enough to make the sterilization but not enough to control and prevent an infection since pathogens abound in the surroundings. Air, water, and soil can be potential vectors, without considering those factors related to iatrogenesis that also play a role in the opportunities for the patient's recovery. Within this context, engineered materials are currently being developed and explored towards devices and biomaterials with improved design, performance, duration, biocompatibility aiming to be safer for the user. The surface functionalization of materials with antimicrobial agents is a highlighted alternative to overcome this issue. This chapter addresses current antimicrobial materials, as well as strategies for obtaining antimicrobial surfaces and coating as well as their properties. In addition, the safety assessment of biomedical applications and international standards are discussed.

Keywords: Antimicrobial agents, Antimicrobial surfaces, Advanced materials, Biomedical devices, Biological evaluation, Bioactive molecules, Coatings, International standards, Safety assessment, Surface functionalization.

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OVERVIEW OF ANTIMICROBIAL MATERIALS AND SURFACES

Since ancient times, the history of humanity reveals a constant struggle to fight bacteria and other pathogenic microorganisms. Infections are responsible for a vast number of deaths around the world, and before the outbreak of antibiotics discovery, for instance, several materials with anti-microbial properties were applied. Therefore, an adequate anti-infective strategy is crucial in hospitals and demands great efforts against the outbreaks of infections, which are triggered by a wide variety of microorganisms.

Some of the most common pathogens infections are caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Candida aspergillus*, *etc.*, which are responsible for several illnesses that tend to be developed from mild to life-threatening if undesirable proliferation takes place in the human body. These include skin and wound infections, eczema, abscesses, endocarditis (biocontamination of heart valves), pneumonia, onychomycosis, candidiasis or thrush (both vaginal and oral), aspergillosis, *etc.*

Nevertheless, the pathogens are not restricted to the colonization of surfaces but also they invade interstitial extracellular cavities, and are capable of internalizing in the cells of the connective tissue [1, 2]. Thus, the pathogens can survive to chemotherapy treatments, because these are well protected by the intracellular reservoir, ready to start the infection again, once antimicrobial concentrations have lost their efficacy.

Consequently, the use of antibiotics able to reach intracellularly hidden bacteria is crucial. One of the challenges is that the antibiotics penetrate the membranes of eukaryotic cells, since antibiotics as gentamicin are incapable of reaching the intracellular compartment, and even the gentamicin-laden biomaterials, occasionally turn into ineffective or even a counterproductive option in cases where strains capable of cell internalization are involved in the infection, such as *S. aureus*, which is a typical pathogen [3].

In principle, the use of microbial agents would be a perfect fit if bacterial resistance, among other problems, would not arise from the use, misuse or overuse of antimicrobial agents. Within this context, technological advancements have been directed to control pathogenic growth or population on surfaces, for example, as fighting infections locally seems a lot more effective and rationale than fighting it at a systemic level, as it would require fewer amounts of antibacterial agents and control the infection on site.

The concept of controlling and designing antibacterial surfaces has a direct and deep impact on reducing healthcare-associated infections and mortality. Overall,

this promising technology holds a relevant contribution economically, industrially and from a healthcare perspective. In addition, such materials, also named self-cleaning devices, compose a more sustainable system as depending on the technology applied, none or almost no antimicrobials are released to the ecosystem, thus also being entitled as environmentally friendly.

The topic is, focused on the ability to control the pathogenic population in distinct environments, specially, bound to catheter materials or devices, when in direct or indirect contact with the body over time. At first glance, sterilization of medical devices assures acceptable levels of decontamination concerning biomedical applicators. However, it is limited and variably effective in controlling the pathogenic microorganisms that will persist grow, and induce biofilm formation on the material or devices over time.

In such conditions, strategies to control bioburden abound and not limited to selective killing towards a specific pathogen, or using antimicrobial or static agents is paramount. This measure in addition, is driven towards controlling or modifying surfaces to block or modulate bacterial adhesion or growth.

METHODS TO ACHIEVE ANTIMICROBIAL SURFACES

One way to classify antimicrobial materials is based on physical, chemical, and biological synthesis methods. In this section, the chemical methods are referred to those, which are compounds involved in the interaction *via* covalent bonds, to immobilize or functionalize materials, regardless of the origin of the antimicrobial agent. On the other hand, the physical methods are limited to surface coating or blending where there is no chemical transformation of the matrix or biomedical devices and both retain their whole functional identity. The third approach comprises the modifications or functionalization with antimicrobial biomolecules. This type of coating and/or immobilization is frequently studied separately as the antimicrobial properties are featured by the biomolecule or its derivative that provides effectiveness. These methods usually are less harsh as they require milder reaction conditions, medium, pH, or temperature.

In fact, some materials require the use of a combination of these methods to provide effective antimicrobial measures. The main criterion in choosing an adequate method and type is in accordance with the nature of the matrix and the reactants. Indeed, most of the current antimicrobial materials involve both chemical and physical types of modifications, these can be considered mixed methods [4].

Despite the significant number of methods listed in literature for obtaining

CHAPTER 4

Recent Advances in the Treatment of Toxoplasmosis

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Abstract: Toxoplasmosis is caused by an obligate intracellular parasite, *Toxoplasma gondii* (*T. gondii*) which infects about thirty percent of the world human population. Infection occurs by the consumption of *Toxoplasma* tissue cysts in raw or undercooked infected meat, intake of the contaminated food or water with *Toxoplasma* oocysts shed in the feces of an infected cat, blood transfusion, placental transmission or organ transplantation. Toxoplasmosis is the most common opportunistic infection in the pregnant women and the immunocompromised individuals resulting in severe complications as encephalitis, pneumonitis and myocarditis with high mortalities. Unfortunately, few effective drug therapies for this disease are available, their aim being the decrease of the parasite replication rate to prevent more pathological changes in organs involved as well as to avoid the serious complications. The recommended therapy for treatment or prophylaxis of toxoplasmosis is the combination of pyrimethamine and sulfadiazine, in spite of this combination is so effective against acute toxoplasmosis but being unsuccessful in the treatment of toxoplasmic chorioretinitis, encephalitis, and congenital toxoplasmosis. Anti-Toxoplasmic drugs need to be effective against the all stages and strains of *Toxoplasma* parasite with a higher penetration into the cerebral, ocular and placental tissues and have no side effects as fetal toxicity and teratogenic effects. Up till now, there is no available drug has all these advantages. The occurrence of side effects, the development of resistant strains and the lack of effectiveness against *Toxoplasma* tissue cyst are the main disadvantages of the current drug therapies. The discovery of drug therapies with a lower toxicity and able to prevent and treat toxoplasmosis would represent a novel era in the treatment of this infectious disease, especially in immunosuppressed patients. Therefore, the researches technology with regard to the parasite's proteomics and functional genomics are needed for the development of new and safer drug agents. Currently, an increase orientation of the pharmacological action of natural agents and medicinal plants are usually considered to be safer than synthetic drugs. The value of these plants as sources of natural product bioactive molecules to medicine related to their chemotherapeutic effect and considered as template molecules for the manufacture of new drug agents. Recently, most of the researches in the drug development for toxoplasmosis focus on the use of nanotechnology for improving the

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pharmacokinetic profile of drugs. The combination of nanoparticles and plant extracts to give rise broad spectrum of drugs may be a greater chance for defeating *Toxoplasma* infection. This chapter aimed at giving an update on the current progress in the development of new drugs for the treatment of toxoplasmosis.

Keywords: Current therapeutics, Medicinal plants, Nanotechnology, *Toxoplasma gondii*, Treatment.

INTRODUCTION

Toxoplasmosis is an infectious disease caused by the intracellular parasite, *Toxoplasma gondii*. Humans acquire the infection by consumption of undercooked or raw meat from infected animals or consumption of food or water contaminated with oocysts excreted infected cats. Other different routes of transmission include transplacental, organ transplantation and blood transfusion. The laboratory workers who handle cultures and animal models with this parasite and also butchers and slaughterhouse workers are in danger [1 - 3].

Toxoplasmosis affects about 30% of the world's population. This wide geographic distribution is expounded to many factors, as food habits contact with infected cat excreta and variations in climate. In immunocompetent individuals, *T. gondii* infection is mostly asymptomatic or may produce flu-like symptoms, and sometimes associated with lymphadenopathy. It is usually followed by a lifelong latent infection [4]. Toxoplasmosis occurred in a fetus and immunocompromised hosts might lead to a severe illness or maybe fatal damage [5]. The outcome of the infection by *Toxoplasma* depends on the interaction of the many factors, including the status of the host immune system and parasite factors, like parasite inoculum, infective stage, and genotype of isolate.

T. gondii organism has different stages: tachyzoites (active stage), tissue cyst stage containing bradyzoites (dormant stage), and sporozoites (in oocysts). The biology of these stages is considered a vital area in research that will be important for the discovery of anti-toxoplasmic agents. In the acute phase of toxoplasmosis, metabolically active tachyzoites destroy tissues and disseminate throughout the body, resulting in clinical manifestations and most drugs used to treat toxoplasmosis significantly affect the tachyzoites. When the chronic infection establish, the tachyzoites turn into dormant tissue cysts (the resistant form) that persist with the host's life. If the host's immunity suppressed, the tissue cysts might rupture, and the bradyzoites will turn back to active tachyzoites, resulting in severe complications [6]. Most anti-*Toxoplasma* agents do not have an effect on the tissue cysts as they encircled by a resistant cyst wall. Moreover, *Toxoplasma* tissue cysts generated within the brain are protected by the blood-brain barrier that results in difficultly in the treatment [7].

During the infection process, *Toxoplasma* bradyzoites or sporozoites invade the intestinal epithelium and spread to the mesenteric lymph nodes and then enter the bloodstream, and come across the biological sites as the blood-brain-barrier, the blood-retina barrier and also the placenta [8]. *Toxoplasma* invades various organs and infects a wide variety of cells [9]. The primary step of the cell penetration by *T. gondii* is that the recognition of an attachment point by its special organelles, rhoptries and micronemes, each discharging proteins throughout the invasion methods [10]. After the cellular invasion, the organism allays in a vacuole, derived from the host cell's plasma membrane [11] and asexually multiplies causing cellular disruption and death. Leading necrosis attracts inflammatory host cells, as lymphocytes and monocytes. This response causes the major pathology in infected individuals. As host resistance develops, usually around 3 weeks post-infection, tissue cysts may form in several organs, primarily within the brain and muscles. These dormant cysts enable *T. gondii* to evade the adaptive host immunity. As tissue cysts periodically rupture, the free bradyzoites are killed by the immune system of the host. If the immune system becomes compromised due to chemotherapy or AIDS, these bradyzoites transform to tachyzoites, causing active infection [9]. Immunocompromised patients need therapy to control progressive disease, and the therapy should be continued for the period of cell-mediated immunological disorder to prevent recrudescence [12].

Toxoplasma gondii has 3 main genotypes which differ in the virulence and epidemiological manner. Type I strain is a high virulence for mice [13] and has been recorded in the patients with ocular [14] and congenital toxoplasmosis and additionally was related to offspring cerebral toxoplasmosis [15]. Type II is non-virulent for mice but causes latent infection with the formation of tissue cysts. This strain is also most commonly related to human infections in Europe and North America [13]. Type II strain has been registered in patients with congenital disease and AIDS patients [16, 17]. Type III is non-virulent for mice and less occurring than type II in Europe and North America. This Type is the most recurrent strain in the animals [13].

Toxoplasma gondii is able to infect the most internal organs and tissues of the mammalian host. In the host cells, *T. gondii* generates DNA damage and causes rapid cell death with rupture and liberation of the free organisms and soluble antigens that lead to several histopathological changes in the influenced organs and tissues ranging from mild congestion to severe degeneration [18, 19].

Because of the medical importance of toxoplasmosis, it's necessary to study the prevalence of this parasitic infection among different human populations from the different areas of the world. Epidemiological studies help in evaluating the morbidity level and the burden of human toxoplasmosis and should be useful for

CHAPTER 5

Antimicrobial Immunoglobulin Prophylaxis and Therapy

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Abstract: Immunoglobulins, either natural or induced, represent powerful means for eliminating microbial threats. For decades, vaccines have been used which function by inducing antibodies for the successful elimination of microbes, preventing disease and mortality.

Polyclonal immunoglobulin products from human plasma were used for the first time to treat immune deficiency during the 1950s. Nowadays, the administration of immunoglobulin, either intravenous or subcutaneous, is the cornerstone of the treatment of primary immunodeficiency affecting the humoral immune system. The therapy can help people with weakened or underdeveloped immune system fight off infections. This is also the treatment of choice for certain autoimmune and neurological diseases.

The polyclonal antibody preparation consists of both natural antibodies and antigen-induced antibodies and reflects the history of antigen experience of the donor population. Natural antibodies are germline-encoded, polyspecific, low-affinity antibodies that also have a regulatory role in maintaining the immune system homeostasis. Antibody preparations, enriched for certain desired specificities, or hyperimmune human globulins are used for prophylaxis and treatment of infectious diseases with a high mortality rate.

It was shown that lymphocytes from survivors of viral infections, without available vaccines, could be used for protective monoclonal antibodies production. This chapter provides insight into the current knowledge of the usage of antibody preparations, efficacy and mechanism of action, with respect to specific diseases, including the treatment of infectious diseases and future prospects.

Keywords: High mortality rate infectious diseases therapy, IVIG, Inflammation, Immunodeficiency, Induced antibodies, Monoclonal antibodies, Natural antibodies, Passive immunotherapy, SCIG, Sepsis.

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Atta-ur-Rahman (Ed.)

ANTIBODIES

Antibodies are one of the components of humoral immunity excreted by specialised cells -- B lymphocytes, also known as plasma cells. In terms of localisation, antibodies can be membranous, present at the surface of B cells, or soluble, present in the serum, or secretions. Membrane-attached antibodies are part of the B cell receptor complex, which when activated induces the release of antibodies in the soluble form. Several grams of antibodies per litre of plasma circulate throughout the body *via* the bloodstream. These antibodies have multiple specificities and can reach remote locations in the periphery within minutes. Antibodies are involved in a variety of biological responses in both health and disease. They can have both protective and pathological roles. Immunization-induced specific antibodies account for the therapeutic effects of the overwhelming majority of vaccines, whether prophylactic or therapeutic.

Each antibody molecule is composed of two structural units: the variable antigen binding domain, which is dual in each molecule and is designated Fab, and a constant region which is constant within an antibody class/subclass, termed the Fc region. Antibodies bind antigens through the Fab regions with a wide range of affinities and the majority of biological activities of antibodies are mediated by the Fc region which interacts with a wide range of molecules (receptors) leading to different effector functions.

Based on the kind of heavy chain Fc region, there are 5 different antibody classes or isotypes (IgD, IgM, IgA, IgG and IgE). Additionally, some antibody classes comprise structurally different groups of molecules and hence in humans, there are four IgG subclasses (IgG1, IgG2, IgG3 and IgG4), which were numbered according to their abundance in the serum such that IgG1 is the most abundant. Each antibody class/subclass has a specific role in the immune response; in directing the immune response based on the type of antigen that provoked the response. IgA is the main antibody in mucosal defence, IgE in defence against parasitic worms, whereas antibody-mediated response against other pathogens comprises highly specific IgG. In terms of abundance in the serum, antibody classes follow IgG>IgA>IgM>IgD>IgE.

Based on the mode of induction, antibodies can be divided into natural antibodies or infection/immunisation-induced antibodies.

Natural Antibodies

In the sera of healthy humans and animals, antibody molecules exist which are not induced by a foreign antigen, and which are called Natural antibodies (NAb). Apart from serum, NAbs can be found in other bodily fluids such as the

colostrum, saliva and cerebrospinal fluid [1 - 4]. NAb can be of the IgM, IgA and IgG class [5]. NAb in the newborn mostly belong to IgM antibody class (80%), whereas in adults IgG class predominates [6]. NAbs are not initially induced in a specific immune response [7]. In mice, NAbs are secreted by specialised B cells. Immediately after birth, the main and probably the only source of natural IgM antibodies is a small population of B lymphocytes designated as B1 cells [8]. B1 cells in mice differ from conventional B cells in terms of origin, activation requirements, and signal transduction pathway upon the engagement of the B cell receptor. The characteristic of these B1 cells is the CD5 surface antigen expression (which is also expressed on T cells) which, upon activation, suppresses the BCR-induced proliferation signal. The differentiation of B1 cells takes place during fetal development, after which these cells are mainly located in the pleural and peritoneal cavity throughout life. B1 cells are long-lived with the capacity to self renew.

The major characteristics of NAbs are that they are polyreactive, specific for either endogenous structures, part of self, or for foreign antigens [4]. Research on the topic revealed that NAbs actually mostly recognize self-antigens, therefore a more accurate description would be that NAbs repertoire is mostly composed of autoantibodies which have the capacity to bind pathogens as well. During maturation, NAb producing B1 cells go through a positive selection towards self antigens, without the development of autoimmunity, which is probably due to the low affinity of these antibodies.

The level of circulatory NAbs is stable during a lifetime, while the repertoire and reactivity profile of these antibodies is exceptionally stable within a species and even between species. The stability is caused by genetics. Namely, the genes for variable (V) regions of NAbs, for both the heavy (V_H) and light (V_L) chains., are generally not subjected to somatic hypermutation and display much reduced, or non-existent, N-region additions and therefore the offspring actually receives a conserved defence antibody pool. Antigen receptor diversity is increased during VDJ recombination when the enzyme terminal deoxynucleotidyl transferase is present, which adds N-additions to the V-D and D-J junctions. Such germline characteristics have been shown to be essential in NAbs' ability to protect against infection [9].

Although interesting from a mechanistic point of view, the vast differences between mice and humans are evident in the elusiveness of a well defined human B1 cell populations. It appears as if these B1 cells in humans could belong to the innate-like lymphoid cells.

A model has been proposed by Kruetzmann *et al.* [10] in which, in humans, IgM

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Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.