

Frontiers in Drug Design and Discovery



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Bentham Books

Frontiers in Drug Design & Discovery

(Volume 10)

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Frontiers in Drug Design and Discovery

Volume # 10

Editors: Atta-ur-Rahman & Muhammad Iqbal Choudhary

ISSN (Online): 2212-1064

ISSN (Print): 1574-0889

ISBN (Online): 978-981-14-2156-3

ISBN (Print): 978-981-14-2155-6

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PREFACE

Development of new therapeutics requires a strong science base in terms of understanding the diseases at the molecular levels, and then identifying new drug leads against novel targets. The world scientific literature is now inundated with a lot of work in this field, and it is often difficult for a researcher to find focused and comprehensive accounts of the topic of his interest. The book series, “*Frontiers in Drug Design and Discovery*” was an attempt to fill this important gap. Volume 10 of the series is a collection of four scholarly written reviews and a research article, contributed by leading experts in the field of drug discovery and development.

Ansari *et al.* have contributed an excellent review on the recent progress made in the research, manufacture, and quality assurance of various classes of industrial, therapeutic and diagnostic proteins. Collectively termed biopharmaceuticals, these proteins are used for the treatment of haemophilia, insulin-dependent diabetes, and various immune and cardiovascular diseases. Recombinant expression systems used in the production of biopharmaceuticals are the focus of this article. Another review by Hefferon *et al.* provides an excellent insight into the emerging field of virus like particles (VLP), various nanoparticles (VNP), and the safe and effective applications in drug delivery and transportation of biomedical agents. Badavath and Jayaparakash have contributed an article on the recent development of monoamine oxidase inhibitors (MOAs) as new and effective antidepressants. Among the MOAs, 4,5-dihydro--H-pyrazole derivatives have special merit due to their interesting molecular architecture, and nanomolar inhibitory potential against MOA enzymes from various sources. Korkotian *et al.* have focused on the problems associated with the treatment of alcohol intoxication. They propose to employ polyphenolic substances of plant origin, collectively called flavonoids, for this purpose. Flavonoids are known to possess a wide range of biological activities. The authors have presented the effects of flavonoids from plants of Scrophulariaceae family on functional properties of rat hippocampal neural cultures in the presence of ethanol. The last review of Barradas *et al.* is an account of the development of novel drug delivery systems, based on smart polymeric scaffolds, for topical applications. Their physicochemical properties, safety, and stimuli-response characteristics as effective drug nanocarriers are presented.

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We are extremely grateful to authors for their excellent scholarly contributions, and for the timely submission of their reviews. The 10th volume of the ebook series is the result of the efficient coordination and excellent management Ms. Mariam Mehdi (Assistant Manager Publications), and team leader Mr. Mahmood Alam (Director Publications). We are confident that this volume will receive wide appreciation from students, young researchers, and established scientists.

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CHAPTER 1**Recombinant Protein Production: from Bench to Biopharming****Rais A. Ansari^{1,*}, Shakil A. Saghir^{2,3}, Rebecca Torisky² and Kazim Husain⁴**¹ Department of Pharmaceutical Sciences, College of Pharmacy, Health Professions Division, Nova Southeastern University, 3200 S University Drive, Fort Lauderdale, FL 33328, USA² Scotts Miracle-Gro, 14111 Scottslawn Road, Marysville, OH 43041, USA³ Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan⁴ Department of Gastrointestinal Oncology, Moffitt Cancer Center and Research Institute, 12902 USF Magnolia Drive, Tampa, FL 33612, USA

Abstract: The needs for purified proteins in modern medicine, research and industrial application are immense and production of proteins using recombinant technology offers solutions; proteins are used in simple laboratory experiments like protein-protein and protein-DNA interactions and in diagnostic, therapeutic and industrial applications. Some examples of the application of purified recombinant proteins for the treatment of diseases include clotting factors (Factor VIII and IX) for the treatment of hemophilia, insulin-dependent diabetes, and adenosine deaminase for severely compromised immune disease. Recently, human monoclonal antibodies, like anti-tumor necrosis factor- α (Adalimumab) for the treatment of rheumatoid arthritis and Repatha (proprotein convertase subtilisin kexin type 9 or PCSK9) inhibitor antibody for the treatment of and reduction in the risk of myocardial infarction, stroke and revascularization of coronary artery diseases, are produced using protein overexpression methodology described in this chapter. Use of recombinant protein technologies has enabled industries to produce proteins of human significance at a tremendous pace. Production of therapeutic proteins at large scale for millions of individuals to treat diseases is one of the essential needs of mankind. From simple proteins like albumin, growth factors, cytokines, viral vaccines and human monoclonal antibodies, all are being produced utilizing the recombinant protein expression technology and purification processes, whether in a laboratory or biopharming scale in microorganisms, animals and/or plants. This chapter summarizes various recombinant expression systems and their pharmaceutical applications.

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Keywords: Adenovirus Expression System, Baculovirus-Mediated Expression System, Biopharming, CHO Expression System, Eukaryotic Expression System, Gene of Interest, History of Biopharming, Mammalian Expression System, Possible Contaminants in Expression Systems, Prokaryotic Expression System, Protein Expression System, Recombinant Proteins, Shuttle Vector, Vaccinia Virus Expression System, Yeast Expression System.

INTRODUCTION

Human beings use proteins or smaller peptides in different ways, which could be enzymes added to soap or use of growth hormone for the treatment of pituitary-driven dwarfism. Such proteins can be obtained from various sources. However, yields were previously low and the cost of purifying them was quite high, limiting their production and use. Advancements in the area of recombinant protein production has changed the trend making the yields much higher and the cost much lower, allowing the production of such proteins on industrial scale, opening the door for the treatment of multiple diseases and disorders discussed in this chapter. For example, bovine and porcine insulins had been used for the treatment of insulin-dependent diabetes, and they have now been replaced by human insulin produced in *Escherichia coli* using the recombinant technology. This technology has also enabled us to avoid potential contaminations from pathogens of animal origin, like viruses. By using recombinant protein technology, we can overexpress desired proteins and biopharm them using microorganisms, animals, and/or plants.

BASIC EXPRESSION CONCEPT

In almost all systems for expression of recombinant proteins, either plasmids carry or are used to create the expression viruses with a gene of interest (GOI) which is driven by a promoter from another gene (a heterologous system) which is active in the organism wherein the protein is being expressed. Isolation of proteins and their purity remains the issue; therefore, an affinity tag is added to either the amino-terminal (*N*-terminal) or carboxyl-terminal (*C*-terminal) of the proteins. The tags serve for isolation and purification of the protein and read in frame of the GOI. In order to add the tag at either *N*- or *C*-terminal, 4-6 uncharged amino acids are used between tag and protein (Fig. 1). Usually, an endopeptidase site is present between the GOI and the tag so that tag can be removed enzymatically. For a majority of tags (*e.g.*, glutathione-S-transferase [GST], maltose binding protein [MBP], chitin, strep-tag, polyarginine [p-Arg], and 6xhistidines [6xHis]) affinity resins are used, while for other tags (*e.g.*, small ubiquitin-related modifier [SUMO], FLAG-tag, c-myc peptide, and 1D4 epitope) (for the list of affinity tags and acronyms, see (Table 1) antibody-resin affinity columns are employed for purification [1, 2]. A single tag, either at the *N*- or *C*-terminal, is not efficient for

obtaining sufficiently quality proteins, therefore, dual tags (one at the N- and the other at the C-terminal or in tandem) are routinely used to further enhance the purity of the proteins. A single step 6xHis tag GOI purification using nickel-nitrilotriacetic acid (Ni-NTA) or other metal-containing resins does not produce a satisfactory purified protein. With dual fusion, one additional affinity purification, following 6xHis-affinity purification, removes the contaminating proteins. To increase the purification further, the dual affinity purified sample is subjected to high pressure liquid chromatography (HPLC) or specific protocols developed for the purification of that GOI.

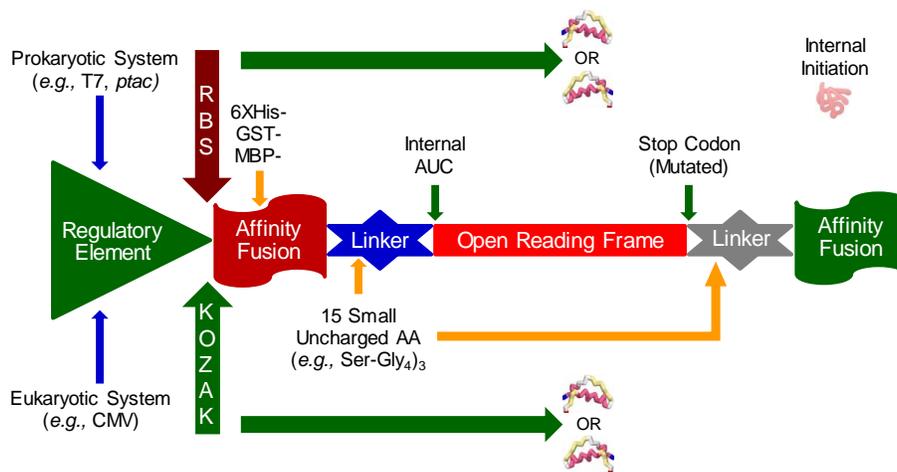


Fig. (1). The basic concept of recombinant proteins and its expression system.

Table 1. List of the affinity/tag.

Polyhistidine (3-10 histidines, usually 6 histines)
Polyarginine (usually 4-5 arginine)
FLAG (N-DYKDDDDK-C)
Metal Affinity Tag (MAT= N-HNHRHKH-C)
Strep-tag (N-WRHPQFGG)
c-MYC peptide (N-EQKLISEEDL-C)
Dual tags with MAT/FLAG and MAT/c-MYC
Hemagglutinin antigen tag (N-YPYDVYA-C)
Calmodulin-binding peptide
Cellulose-binding domain
Acyl carrier protein (8kd)
Small ubiquitin related modifier (SUMO) with 6xHistidines

Plant Virus Nanoparticles and Virus like Particles (VLPs): Applications in Medicine

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Abstract: Both virus like particles (VLPs) and virus nanoparticles (VNPs) are viable platforms for the transportation of drugs, imaging agents, immunogenic ligands and other materials. They can be loaded with genetic material and/or drugs for therapeutic purposes. VLPs possess multivalent molecular settings, which help stimulate various molecular interactions for a potent immune response. VNPs are biodegradable and biocompatible nanoparticles that occur in nature and can be modified with genetic and chemical protocols for therapeutic purposes. There has been considerable research on the use of different VLPs and VNPs as safe and viable platforms for vaccine development, tumor therapy and other medical applications. The following chapter provides insight into applications of plant VLPs and VNPs in medicine.

Keywords: Chimeric VLP, Vaccines, Virus like particles (VLPs), Virus nanoparticles (VNPs), Plant-derived vaccine.

INTRODUCTION

Virus nanoparticles (VNPs) can originate as novel biomaterials from a variety of sources including the structural proteins of viruses infecting plants, animals and bacteria [1]. These biomaterials can self-assemble through noncovalent bonds, resulting in systematic-structured nanoparticles which vary in shape and size [2]. Since the morphology of these complex structures is controlled by genetic variation, it is facile to alter them using synthetic biology [2]. Plant virus nanoparticles ranging from 10-100 nm are a subcategory of these bio-nanoparticles and are expressed in various host systems. The wide variety of plant VNPs provide for a diversity of applications.

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Virus-like particles (VLPs) are particles that resemble viruses but lack nucleic acid [3]. Changes in ionic strength and pH can be used to disassemble viruses into genetic material and proteins. The resulting coat proteins can self-assemble to form virus-like particles (VLPs) [4] with either single or multiple structural proteins organized in numerous layers [3].

In the following chapter, we describe different types of VLPs, methods for their production and various ranges of VLP applications in medicine. The focus is on VLPs derived from plant viruses; however, to deliver a better overview, non-plant VLPs are also discussed.

VIRUS LIKE PARTICLES (VLPS)

VLPs are complexes of structural proteins that can be expressed in recombinant systems by spontaneous assembly. These structures resemble naturally occurring viruses with respect to conformation and organization but differ as they lack a viral genome [5].

Types of VLPs

VLPs of Structurally Simple Viruses

VLPs of structurally simple viruses consist of non-enveloped viruses that have a nucleocapsid encoded by a single virus encoded protein. Thus, it is easy to generate VLPs of such viruses since the assembly process is dependent on the expression of a single protein. The first single-protein, simple VLPs produced in plants were Norwalk virus (NV) 34 [6]. The Alfalfa mosaic virus (AIMV) coat protein forms VLPs of various shapes and sizes [7]. A modified form of AIMV coat protein bearing HIV-1 and rabies virus epitopes was allowed to express in tobacco plant using a TMV vector. The infected leaf tissue displayed ellipsoid particles of the modified subunits of AIMV [8].

VLPs with Lipid Envelope

Many pathogenic viruses are encapsulated with an envelope derived from the cell membrane of the host. This envelope comprises lipids and proteins of the host cell membrane and viral glycoproteins. The generation of neutralizing antibodies that can target these envelope proteins are essential in vaccine research. Tobacco mosaic virus produces VLPs that are rod-shaped. The first vaccine to have been

produced using enveloped VLPs consisted of 17 to 25 nm diameter spherical particles of Hepatitis B surface antigen enveloped with a host cell membrane [9].

VLPs with Multiple-Protein Layers

Numerous non-enveloped viruses consist of several copies of polypeptides within their capsids. These diverse polypeptides are either produced by the processing of polyprotein precursors or by translation from various open reading frames [7]. VLPs of multiple capsid proteins that must interact with one another are more difficult to produce in comparison to those generated by one or two core capsid proteins. One challenge lies in the fact that proteins encoded by numerous discrete mRNAs are liable to be localized differently within the cell, thereby influencing the efficiency of the assembly process [10]. Reoviruses are double-stranded non-enveloped RNA viruses consisting of three spheres bearing four diverse VPs produced by discrete genome segments [7]. VLPs of Cowpea mosaic virus (CPMV) were shown to exhibit antitumor activity against B16F10 lung melanoma [11].

Methods of Production of VLPs

Non-infectious VLPs lacking the viral genome can be produced through the expression of viral proteins in plant hosts. The initial step for VLP production in plants involves the formation of a suitable plasmid for expressing proteins essential for VLP assembly. These proteins include both the capsid/shell and structural proteins of the VLPs [7]. The desired sequence is injected into a suitable vector for steady genetic transformation and utilized for either plasmid or nuclear transformation through standard techniques [12]. The plants at this stage are allowed to regenerate, self-fertilize and generate true-breeds. Plastid transformation is advantageous over nuclear transformation because transgenes encoded by plastids have a low risk of contaminating the environment as they are maternally inherited and not transferred through pollen [7].

The VLPs are expressed transiently through two methods: utilization of replicating plant virus vectors or through a non-replicating binary vector; both can be delivered to the plant by agroinfiltration. A range of plant viruses serve as expression vectors [13] including Potato virus X (PVX) [14], Cowpea mosaic virus (CPMV) [15], and Tobacco mosaic virus (TMV) [16], *etc.*

VLP purification from the plant consists of homogenization of the tissue sample, cell debris removal and plant extract enrichment to obtain the expressed VLPs. The rigid cellulose in the cell wall of the plant cell is removed by either enzymatic

CHAPTER 3

MAO Inhibitory Activity of 4, 5-Dihydro-1H-Pyrazole Derivatives: A Platform To Design Novel Antidepressants**Vishnu Nayak Badavath*** and Venkatesan Jayaprakash*Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology-Mesra, Ranchi-835215, Jharkhand, India*

Abstract: Emergence of treatment-resistant depression is the new challenge before us. As antidepressants currently existing in the market are of little or no use, clinicians are looking for newer and effective antidepressants to handle situations. Inhibition of Monoamine oxidase, an effective strategy discontinued a few decades before due to selectivity related issues. Technological advancement in chemistry and biology interface is now availing hopes of achieving the design and synthesis of novel, isoform-selective and tissue-specific inhibitors. This has renewed the interest in re-exploring the MAO inhibitors in the past decade. Under this background, the chapter reviews MAO inhibitory activity and antidepressant activity of 4, 5-dihydro-1H-pyrazole derivatives reported to date. Since different sources of enzymes (rat, bovine, human, *etc.*) were used by different groups to evaluate the newly synthesized compounds, any discussion on structure-activity-relationship may not be justified. Hence, the authors made an attempt to summarize the literature based on the chemical architecture of the compounds that may help the medicinal chemists to further explore the unexplored chemical space. Further, efforts by the scientific community to report the effect of chirality of compounds on activity and selectivity, experimentally or through computational simulations are also documented.

Keywords: 4, 5-Dihydro-1H-Pyrazole Derivatives, Antidepressant Activity, Chiral Separation and Computational Studies, MAO Inhibitory Activity.

INTRODUCTION

According to the WHO, depression is a common mental disorder, characterized by loss of interest or pleasure, sadness, low self-worth or feelings of guilt, appetite or disturbed sleep, poor concentration and feelings of tiredness.

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Depression can be long-lasting or recurrent, substantially impairing an individual's ability to function at work or school or cope with daily life. At its most severe, depression can lead to suicide. When mild, people can be treated without medicines but when depression is moderate or severe, they may need medication and professional counseling. Depression can be easily diagnosed and treated by non-specialists as a part of primary health care. Only a small proportion of individuals who do not respond to first-line treatment approaches specialists for treatment of complicated depression.

As per the WHO report, globally there are more than 350 million people of all age groups suffering from depression. It is a leading cause of disability worldwide which affects women more than men. There are nearly one million deaths every year globally due to depression leading to suicides. In India, around 36% of the population is suffering from Depression (Source: WHO factsheet, date of citation 15/04/2019). Geo-political and socio-economic conditions play a major role in the onset and progression of depressive illness. Counseling and appropriate treatment at the right time may effectively prevent the progression of depression. Treatment generally aimed at restoring the level of Noradrenaline and Serotonin in the brain. One of the successful approaches is to inhibit the Monoamine oxidase-A (MAO-A), the enzyme responsible for the degradation of Noradrenaline and Serotonin [1].

Monoamine oxidases (MAO) are responsible for maintaining the level of neurotransmitters (Noradrenaline and Serotonin) in the central nervous system (CNS). Increased activity of MAO-A is responsible for depression, while the increased activity of MAO-B causes neurodegenerative disorders such as Parkinson's and Alzheimer's disease [2]. Therefore, Monoamine oxidases are valid drug targets for designing drugs for the treatment of depression, Parkinson's and Alzheimer's disease. MAO inhibitors, introduced into clinical practice during the 1960s, were abandoned due to adverse effects, such as hepatotoxicity and the so-called "cheese reaction", which was characterized by hypertensive crisis [3]. Further, it was understood that most of the adverse effects were due to non-selective inhibition of MAO-isoforms [4]. This led to an intensive search for novel MAO inhibitors (MAOIs), selective towards isoforms and this effort has increased considerably in recent years. Selective MAO-A inhibitors such as Clorgyline (irreversible) and Moclobemide (reversible, efficacy moderate) are effective in the treatment of depression [5]. Similarly, selective and irreversible MAO-B inhibitors such as Selegiline and Rasagiline are useful in the treatment of Parkinson's and Alzheimer's diseases [6, 7]. Most of the inhibitors in the clinical practice are either selective and irreversible or non-selective reversible. The reported literature stated that, selective and reversible MAOIs can reduce the adverse effects, (such, as hepatotoxicity and the so-called "cheese reactio", which

is characterized by hypertensive crisis) caused by non-selective and irreversible MAO inhibitors [4].

Monoamine Oxidases Enzyme and their Mechanism of Action

Monoamine oxidases are FAD containing enzymes bound to the outer mitochondrial membrane and are responsible for the oxidative deamination of neurotransmitters and dietary amines [8] to produce the corresponding aldehyde, ammonia and hydrogen peroxide using oxygen as an electron acceptor [9]. This led to the rapid degradation of these molecules and ensures the proper functioning of synaptic neurotransmission, regulation of emotional behavior and other brain functions.



Increased activity of MAO enzymes due to their over expression may cause low level of neurotransmitter and higher oxygen consumption (local hypoxia). Decreased level of neurotransmitter led to the behavioral disturbances (Depression). On the other hand the byproduct of MAO-mediated reactions encompasses several chemical species (H_2O_2 generated Reactive Oxygen Species (ROS)) with potential neurotoxic property leading to the onset and progression of neurodegenerative disorders (Fig. 1) [2].

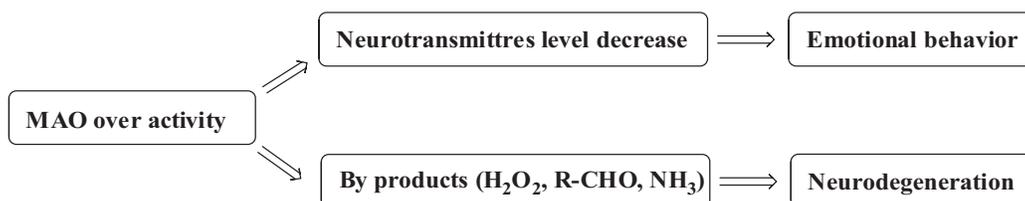


Fig. (1). Schematic diagram on pathological outcome due to overexpression of MAO.

Mary Bernheim discovered MAO enzyme for the first time in the liver [10]. They exist as two different isoforms, hMAO-A and hMAO-B, which differ by their sequence (70% sequence identity as deduced from their cDNA clones) [11], specificity towards their substrate and selective inhibitors [8, 12]. Both hMAO-A and hMAO-B are found in astroglia and neurons, although the brain exhibits high concentration of MAO-A and MAO-B, their regional and cell specific localization are quite different. MAO-A is predominantly found in catecholaminergic neurons, mammillary complex and coeruleus hypothalamus, while MAO-B is found serotonergic neurons, astrocytes and histaminergic cells. MAO-A is also found outside the central nervous system (lung, liver, small intestine, and placenta),

Flavonoids Antagonize Effects of Alcohol in Cultured Hippocampal Neurons: A Drug Discovery Study

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Abstract: Alcohol dependence is one of the top priority public health problems on a global scale. The costs of medical treatments of patients with alcohol dependence, a decrease in labor productivity, an increased risk of developing somatic and mental disorders, and early mortality are all consequences of acute and chronic alcohol abuse. The brain is one of the main targets of alcohol intoxication. Extensive neurobiological studies have revealed a number of synaptic and extra-synaptic mechanisms, affected by alcohol. A primary target of it is GABAergic transmission. Nevertheless, the exciting and disinhibiting actions of alcohol at the system and cellular levels have not been satisfactorily elucidated. It remains unclear whether effects of ethanol are highly complex, manifested only at the level of entire brain or concerns also individual cells, their subcellular structures, organelles, ion channels and receptors. With this approach, small, cultured neural networks that are isolated from the rest of the brain are of particular interest. A serious problem of modern pharmaceuticals is the lack of drugs that have a therapeutic effect on alcohol toxicity of the brain and nervous system, despite the abundance of so-called “traditional medicines”. Substances obtained from some herbs containing a mixture of biologically active substances that exhibit a wide range of properties are of particular interest. Among them - flavonoids, which are polyphenols of plant origin and often reveal a sign of sedative, neuroprotective, antidepressant properties, and may improve cognitive function. The aims of our study is to reveal the mechanisms of various concentrations of ethanol, as well as its chronic effects on the functional properties of neurons in small neural networks such as the primary neuronal culture of the rat hippocampus. We have also performed a complex neuropharmacology screening and the study of flavonoids, extracted from *Scrophulariaceae* plant family, which is known in the traditional medicine for its anti-alcohol properties.

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Keywords: Calcium Imaging, Electrophysiology, Ethanol, Flavonoids, Hippocampal Culture, Inhibition, SK-channels.

INTRODUCTION

Alcohol dependence is a top priority public health hazard on a global scale. According to WHO [1], excessive alcohol consumption is among the leading causes of morbidity and premature death in many countries where the purchase of alcohol is not regulated legislatively, having a serious impact on the quality and duration of human life. The costs of medical treatment of patients with alcohol dependence, a decrease in labor productivity, an increased risk of developing somatic and mental disorders, and early mortality are all consequences of acute and chronic alcohol abuse.

The brain is a major target of alcohol intoxication. The potential costs associated with brain damage produced by alcohol are enormous. In 7-10% of the population of developed countries, alcohol dependence is diagnosed; of which 9% have clinical brain damage. It is shown that the brain retains the dysfunctions accumulated in the past, even if alcohol toxicity is discontinued [2]. It is shown that along with chronic alcohol consumption, the spatial memory, which is stored in the hippocampus and is responsible for forming memories of the location in space, combined with information about related events, deteriorates [3, 4]. Perhaps therefore the strong alcohol intoxication is accompanied by a poor memorization of the events, including those related to movement in space. Thus, studies related to hippocampus, can make a valuable contribution to understanding the mechanisms of ethanol (EtOH).

It should be noted that the concentration of alcohol in the blood, after its consumption in any form of alcoholic beverages, reaches values of 0.5-1 ppm (0.05-0.1%) in a case of light and easy form of drunkenness, 2-3 ppm (0.2-0.3%) in a case of average levels of drunkenness and about 4-5 ppm (0.4-0.5%) with a very strong intoxication. A further increase in the volume of alcohol in the blood is considered with a life hazard [5].

According to the modern view, the main cause of death in acute alcohol intoxication is its depressive effect on cellular activity in the vital breathing center of the medulla oblongata, which leads to an arrest of breathing and subsequent coma. However, the specific level of achievement of this condition depends on a whole range of factors, such as the dynamics of alcohol intake and the individual tolerance of the organism to its accumulation and effects. The limiting dose of alcohol in the blood in different patients can fluctuate in the range from 0.5 to 0.8% and even higher [6, 7].

At the same time, the correlation between the level of alcohol in the blood and in the brain is not that unambiguous. In particular, it was found that the concentrations of EtOH in the brain during the first 5-15 minutes after its intake exceed those of the blood by 1.5 times, and in a case of rapid intake - by 3 times [8]. Thus, physiological, life-compatible concentrations of EtOH in the brain can reach 1.5-2%, but hardly exceed 2.5%, and the concentration of EtOH above 3%, used in some *in vitro* experiments, when a complete inhibition of neuronal activity is observed, cannot be recognized as physiologically relevant [9, 10]. Thus, despite the arbitrary nature of the study of alcohol intoxication *in vitro*, there is a very good correlation between *in vitro* alcohol levels, at which a strong decline in neuronal activity begins and the attainment of concentrations incompatible with life in the brain *in vivo*.

Extensive neurobiological studies have revealed a number of synaptic and extra-synaptic mechanisms, affected by alcohol. It interacts with lipids and thereby influences the viscosity of cell membranes [11]. The molecular targets of acute (short-term) effects of alcohol in the brain have been suggested [12 - 15], including potassium channels [16, 17], glutamate and GABA [18, 19] receptors as well as synaptic scaffold proteins [20, 21].

The primary target of ethanol is likely to be GABAergic transmission: either directly, by affecting synaptic and extra-synaptic GABA receptors, or by the involvement of neurosteroids [22 - 29]. However, the exciting or disinhibiting effects of alcohol at the structural and cellular levels have not been satisfactorily elucidated. Similarly, the stimulating and disinhibiting effect of alcohol on the psyche have not yet received a clear mechanistic explanation [30, 31].

Particularly, it remains unclear whether effects of EtOH in humans are manifested only at the level of specific brain structures or the entire nervous system, or concerns also individual cells, their subcellular structures, organelles, ion channels and receptors. With this approach, small, cultured neural networks that are isolated from the rest of the brain are of particular interest. Such a testing model system can demonstrate the effect of different pharmacological substances on the local activity, without the involvement of concomitant effects of incoming afferents from external structures or blood supply of the tested region of interest.

Besides all the above, the mechanism of chronic (long-term) effect of alcohol on neurons remains unclear as well. Using a dissociated culture of central neurons, a diversity of conflicting morphological and chemical consequences of chronic exposure to EtOH was found. On one hand, they include neuronal death [32], a decrease in the density of dendritic spines and their degree of maturity [20], but on the other hand - an increase in the size of dendritic spines associated with an

Hybrid Smart Materials for Topical Drug Delivery: Application of Scaffolds

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Abstract: The recent advances in materials science have enabled great achievements in the development of polymer scaffolds, which can constitute innovative platforms for the development of novel topical drug delivery systems (TDDS) associated with site-specific or prolonged drug release. The application of polymer scaffolds as drug delivery systems often relies on their combination with many types of nanocarriers, such as liposomes, solid nanoparticles, micelles, nanogels and metallic nanoparticles. The combination of polymer scaffolds and drug nanocarriers and the association of controlled drug release properties provide novel materials, considered hybrid as they gather two therapeutic effects: scaffolding and drug delivery. Such hybrid scaffolds have been shown to be suitable for delivering drugs at controlled rates and site distribution. Many drug carriers are often associated with stability issues, drug leaking or considerable interaction with undesirable cells, hindering their clinical function. Hence, for topical application, drug nanocarriers are often introduced in conventional secondary vehicles such as creams and lotions in order to provide the viscosity, extended residence time and adhesiveness, properties necessary for the administration route. In addition, smart stimuli-responsive polymers can be used in the formulation of both scaffolds and nanocarriers, being promising approaches in the topical treatment of various diseases. In this context, hybrid smart polymer-based scaffolds are versatile platforms for the development of novel TDDS. Such smart materials, in addition to being able to combine the benefits of different structural components, can also respond to external stimuli such as temperature, pH, and redox status, which can increase the effectiveness of therapeutic agents and decrease harmful effects on the surrounding

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tissue. In this chapter, the different polymer-based scaffolds, most nanocarriers and stimuli-responsive polymers are described as well as their most varied applications in the field of technological development of topical delivery systems for ideal drugs, which is still a challenge for formulation scientists.

Keywords: Hybrid systems, Nanoparticles, Polymer-based scaffolds, Polymers, Smart materials, Topical drug delivery.

INTRODUCTION

Lately, the main challenge of modern drug therapy is not finding more potent drugs but rather providing improved approaches to deliver those drugs to a specific place or target at the rate required inside the body [1]. To achieve this, great efforts have been made to develop novel drug delivery systems that can optimize drug absorption, distribution, half life time, release rate and site distribution. The research progress in nanotechnology and novel drug delivery systems allowed the development of novel pharmaceutical products, which bring several therapeutic advantages such as patient compliance and improvements in drug pharmacokinetics and pharmacodynamics.

Topical drug administration produces local effects, reducing the systemic drug circulation [2 - 4]. There are at least two relevant reasons for choosing topical drug delivery: (i) when systemic administration causes toxic side effects due to drug interaction with other biological compartments; (ii) when the affected tissue is difficult to be reached at sufficient drug concentration, or perfusion rate is reduced [5, 6].

Many drug carriers are used for topical and controlled drug delivery. Most of them are nano-sized carriers such as nanoparticles, micelles, liposomes and other lipid-based nanoparticles. Drug encapsulation into nanocarriers enables the production of tailor-made drug release rate, depending on the physicochemical properties of both drug and polymer matrix composition, whether polymeric or lipid [6].

Polymer-based scaffolds have been optimized over the last decade, in order to improve biocompatibility, cell proliferation capacity and incorporate more functionalities to local treatment. They are mostly constituted by biopolymers that arise as interesting materials due to their biocompatibility and biodegradability [7, 8].

Some examples of biocompatible polymers applied as scaffolds for drug delivery are: collagen, chitosan, alginate, poly (lactic acid) (PLA), poly (glycolic acid)

(PGA), poly (co-glycolic lactic acid) (PLGA), polystyrene (PS), poly (l-lactic acid) (PLLA), polydioxinone (PDO), poly (ϵ -caprolactone-co-lactic) (PCLA) and poly (ϵ -caprolactone) (PCL) [9, 10]. Polymers are widely used for scaffolds production by the techniques as phase separation, self-assembly, electrospinning *etc.* Among those techniques, electrospinning is one of the most studied and applied in recent years [8, 11].

The selection of the proper fabrication method can provide the development of porous materials with high drug diffusivity, being favorable to originate novel drug delivery systems. The therapeutic potential of scaffolds can be enhanced when combined with drug delivery vehicles as nanocarriers. Such combination can overcome some of the main drawbacks in TDDS and enables novel applications for scaffolding materials, opening a new window of opportunities for these hybrid materials. Moreover, the application of stimuli-responsive smart polymers that can change their macrostructure of self-assemble with external stimuli such as polarity, pH, temperature and redox potential or yet, sensible to enzymatic/hydrolytic degradation can provide novel materials with different and interesting drug release properties [12 - 14].

Hybrid smart systems that therefore combine drug nanocarriers with polymeric stimuli-responsive scaffolds can represent a new concept of rational and versatile therapy by providing multifunctionality and the development of more complex drug delivery systems capable of treating different conditions of human health more effectively. In a general aspect, these materials can be classified as nanocomposites, being formed by two phases: one dispersed composed by the nanocarriers and the other, continuous, composed by the polymeric scaffolds matrix. The development of nanostructured polymeric hybrid smart scaffolds can be seen as a multidisciplinary area, due to its enormous potential in various branches of science and technology. In this chapter, various nanocarriers associated to polymeric scaffolds and stimuli-responsive polymers are described and their applications are presented. The numerous possibilities for developing innovative and advanced materials in the field of pharmaceutical technology for new TDDS will be addressed. In this way, Fig. (1) shows the summary chart containing the types of polymer-based scaffold that can be applied into some tissues such as bone, topical (skin, eyes, gastrointestinal tract and vagina), neuronal and vascular. Also, examples of biomolecules and nanoparticles that can be incorporated into the scaffolds are mentioned in Fig. (1).

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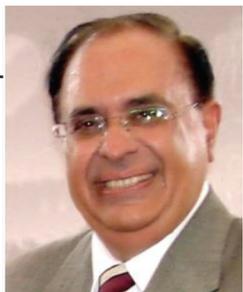
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