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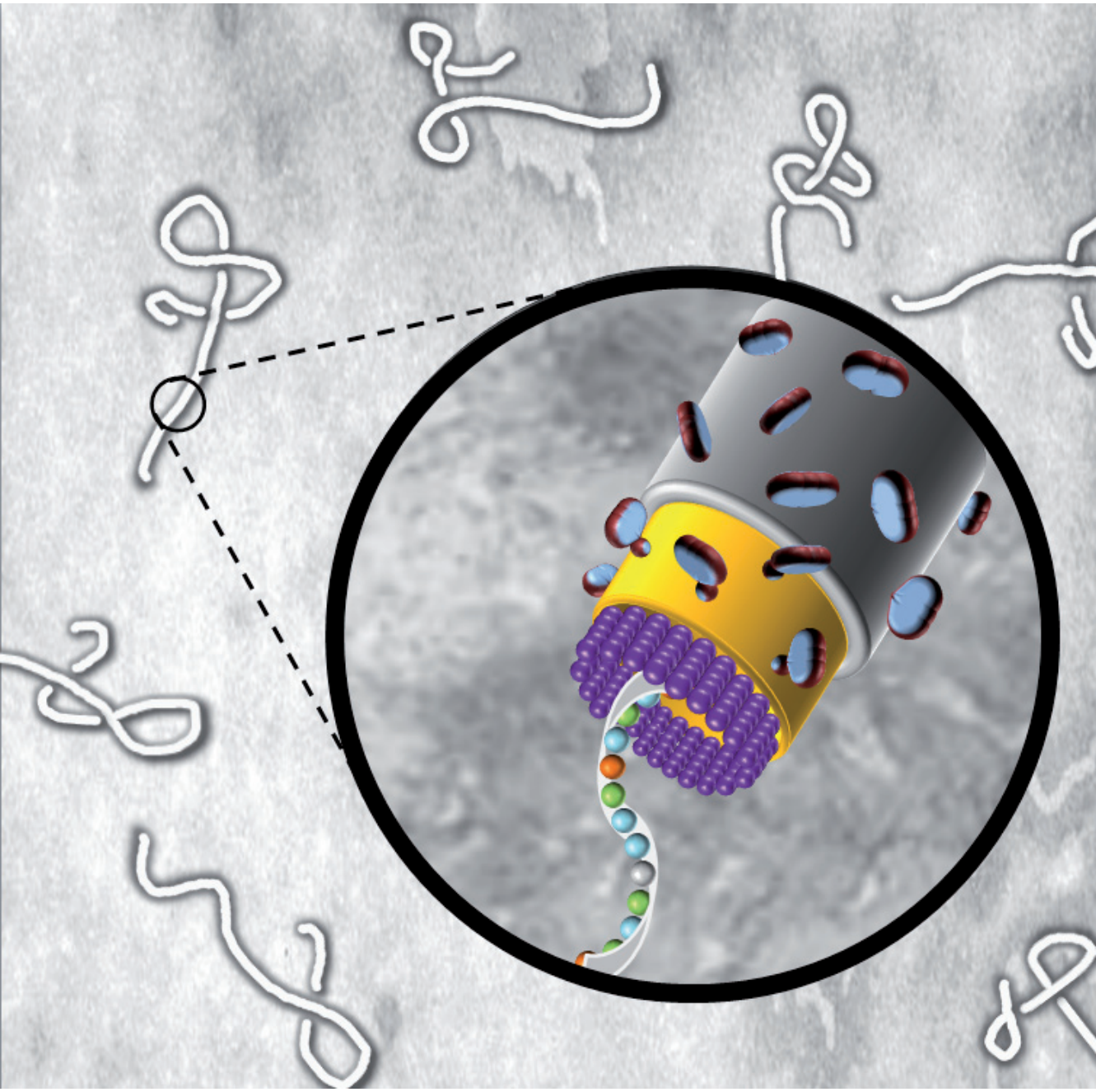

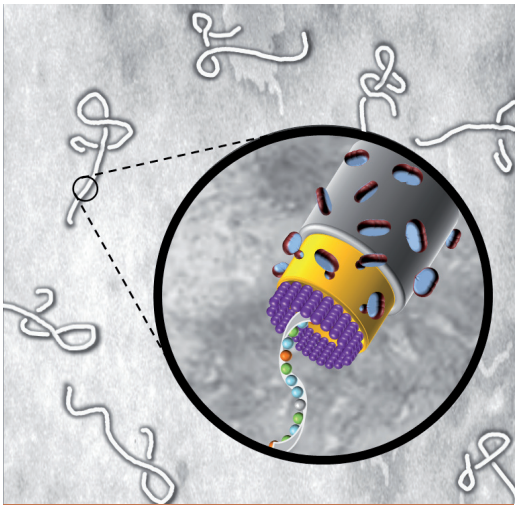

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Cover:
Ebola virus carries a negative-sense RNA genome in virions that are usually tubular-shaped, and contain viral envelope (derived by budding from domains of host cell membrane), matrix, and nucleocapsid components. Virions are approximately 80 nm in diameter, and have a virally encoded glycoprotein projecting as 7-10 nm long spikes from its lipid bilayer surface. The length of the whole virion is typically 800 nm, but sometimes up to 1000 nm long.

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Dear readers,

The first number of the „Journal of Metallomics and Nanotechnologies“ in 2015 is dedicated to research of viruses. Our international editorial board has been enlarged by Assoc. Prof. Kledi Xhaxhiu, University of Tirana, Albania. And we are still trying to update the form and usefulness of website of JMN. Now, it is easy to find the instructions for authors including templates and citation library for our journal. Also the workflow scheme has been proposed to clearly inform the authors how the process in such journal works (Fig. 1). All these improvements are necessary to push our journal to higher level for achievement of the indexing in scientific databases which is still being a long distance run.

This number contains nine reviews and two original articles. The both sections of reviews and articles are focused on the area of research of human/mammalian threating viruses such as Ebola, HIV, HPV or SARS. Six scientific abstracts focused on metallomics study in area of electrochemistry are closing this issue.

The second number of the issue will be dedicated to cancer research and novel findings in area of metal biochemistry, biomedicine and modern nanotechnologies. The contributions focused to metallomics of cancer, analysis of real samples and novel results, concerning a cancer treatment or detection will be warmly welcomed.

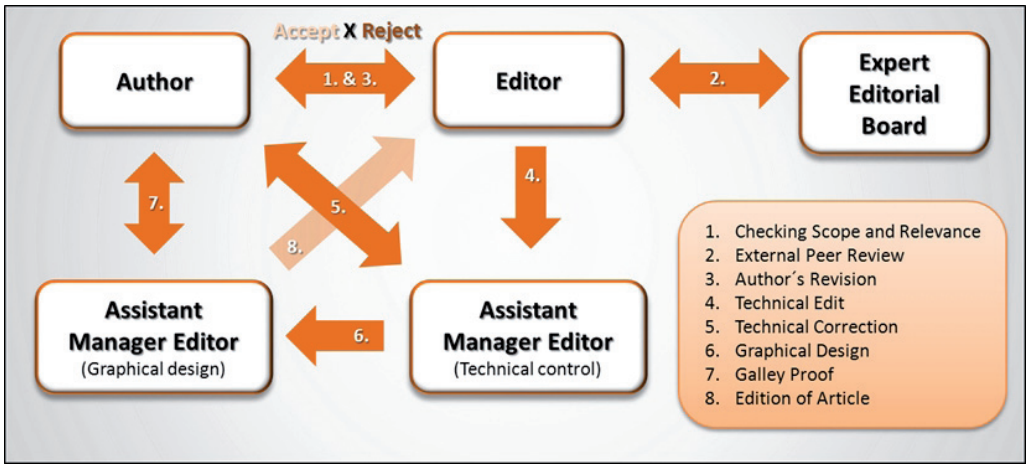

Figure 1: The workflow scheme of paper processing in JMN

Ondrej Zitka
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The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study.

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Review

Article

Scientific abstract

REVIEW

West Africa Ebola Outbreak 2014

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Ebola is caused by Ebola viruses (EBOV), members of the group of haemorrhagic fevers and it is one of the most dangerous infection diseases with mortality rates up to 90%. Ebola was firstly described in 1976 and since then occurred sporadically in Central Africa. Till 2014, more than twenty outbreaks were described, but number of deaths not exceeding 300 per outbreak. In 2014 came West Africa Ebola outbreak and the changeover appeared in Ebola epidemiology. During 2014 Ebola virus disease crossed the borders, affected five countries and number of cases and victims increased thousand times. The World is at attention and some countries have declared special rules for travellers from affected areas. This review aims to provide an overview of history of Ebola virus disease outbreaks, with the emphasis on 2014 West Africa Ebola outbreak.

Keywords: Ebola viruses; 2014; West Africa Ebola outbreak

1. Introduction

Ebola virus disease (EVD) is a sporadically occurring and high mortality disease caused by Ebola viruses (EBOV). There are five closely related strains of Ebola viruses. Four of them commonly cause disease in humans: Ebola virus (ZEBOV, formally known as Zaire); Sudan virus (SUDV); Tai Forest or Côte d'Ivoire virus (TAFV); and Bundibugyo virus (BDBV). The fifth, Reston virus (RESTV), has been caused disease only in nonhuman primates (monkeys, gorillas, and chimpanzees) [1, 2].

EBOV together with Marburg and Lloviu viruses make up the filoviruses family. All of the members of Filoviridae have similarities in the structure of virion, genome formation and most of them cause fatal haemorrhagic fevers in humans [3]. The illness included symptoms such as fever, headache, and malaise at onset, with profuse vomiting and diarrhea occurring 2-4 days later, haemorrhagic symptoms occur in severe cases. Ebola is one of the most lethal diseases. Mortality rate ranges from 53-88%. Ebola viruses are endemic to Africa and to the Philippines. Due to fact that Ebola is highly pathogenic disease with such as high mortality, must be conducted in a Biosafety Level 4 laboratory (together with Marburg virus, Lassa virus, Crimean-Congo haemorrhagic fever and various other haemorrhagic diseases).

EVD was firstly described in 1976 during two concurrent outbreaks [4, 5]. It was given the name 'Ebola' after the small river near the catholic mission of Yambuku, the epicentre of the 1976 Ebola haemorrhagic fever outbreak [13]. Between 1976 and 2014 twenty-four epidemics of EVD were verified, mostly caused by ZEBOV in Equatorial Africa [2]. These were typically occurs in Sub-Saharan Africa on limited area with number of cases from tenth to hundreds and number of victims not exceeding three hundred. West Africa Ebola outbreak 2014 (WAEO)

is currently the largest one in human history [6, 7]. There are several differences between WAEO 2014 and previously occurred outbreaks.

The differences are in size of geographically spread, number of cases and duration. Previously occurred outbreaks are mostly limited on villages closely related with rainforests and do not widespread to the large area, but WAEO exceeded not only countries borders (four countries of Equatorial Africa: Guinea, Liberia, Sierra Leone and Nigeria), but also the continent borders (Spain and America) [6]. Number of laboratory confirmed human cases were lower (maximum 425, Sudan 2000-2001) and duration of previously described outbreaks were shorter than one year. WAEO 2014 crossed also these established rules. The number of reported cases is almost 20,000, and it is assumed that outbreak takes more than one year, because number of cases and deaths still increase.

2. Virology

EBOV belongs to the genus Ebolavirus and family Filoviridae, in the Mononegavirales [8]. EBOVs are linear, single-stranded RNA viruses which genome has approximately 19 kb with a coding capacity for seven structural proteins [9]. The noncoding sequences located at the 3' and 5' ends of the viral genomes are of significantly different lengths [10, 11]. The ribonucleoprotein complex of virion consists of the genomic RNA encapsulated by the nucleoprotein, which is associated with VP30, VP35, and RNA-dependent RNA polymerase to the functional transcriptase-replicase complex [12]. VP35 serves also in an interferon antagonism [13] and protein VP40 is plugged as the matrix protein and also mediates formation of virions [14]. Structural protein (VP24) is associated with the virus membrane, interferes with interferon signalling [9]. The glycoprotein is the only transmembrane surface protein of the virus and forms trimeric spikes consisting of glycoprotein 1 and 2 [10]. The virion is pleomorphic, producing 'U'- shaped or '6'-shaped structure, but the predominant forms of the virion most frequently seen by electron microscope are long and filamentous, which gave the Filovirus family its name [1, 8, 9]. The ZEBOV is responsible for the current outbreak, which have been introduced in West Africa from Central Africa in the last few decades [15]. Ebola virus, infections with the ZEBOV have the highest case-fatality rates (60–90%) followed by those for the SUDV (40–60%). Case fatality of BDBV was estimated to 25%, based on one outbreak. Only one patient with Tai Forest Ebola virus was identified and survived [16].

The incubation period of Ebola is usually 1 - 2 weeks. Initial clinical symptoms are nonspecific (fever alternating chills, myalgia, and nausea). This stage is followed by influenza-like symptoms (rhinitis, cough, and breath difficulties and body aches); gastrointestinal symptoms (diarrhea, nausea, vomiting), and finally haemorrhagic symptoms, multi organ failure and deaths, which are connected with severe cases [1, 2].

Diagnosis of Ebola can be difficult initially, because the symptoms can be confused with those of diseases that are more common in Equatorial Africa such as malaria or Lassa fever [2]. Infection occurs through close contact with body fluids (skin or mucosal surfaces of infected patients). Most of cases occur at persons, who care about patients (family members or medical personal). Amplified transmission occurs in hospitals or health care centres, approximately one quarter of cases occurring among health care workers [2].

3. Epidemiology of Ebola viruses

The first outbreak of Ebola in Sudan (SUDV, 284 cases, mortality rate of 53%), occurred in Nzara, Maridi and the surrounding area and was spread mainly through close personal contact within hospitals and many medical care personnel were infected [17]. The second Ebola virus emerged from Yambuku, Zaire (ZEBOV, 318 cases, mortality 88%), occurred in Yambuku, and was spread by close personal contact and by use of contaminated needles [18]. The third strain of Ebola, RESTV was identified in 1989 when infected monkeys were imported into Reston (USA) from the Philippines. Only few people were infected with RESTV (seroconverted), but Ebola haemorrhagic fever never

developed in them. The last known strain of Ebola, TAFV was discovered in 1994 when a female ethologist performing a necropsy on a dead chimpanzee from the Tai Forest, Cote d'Ivoire, accidentally infected herself during the necropsy.

According to Feldman et al. Ebola haemorrhagic fever is thought to be a classic zoonosis [1]. Fruit bats are believed to be the natural reservoir [19]. On the other hand Gire et al. sustained human-to-human transmission during 2014 WAEO, with no evidence of additional zoonotic sources [7]. By Hayden et al. the high lethality of filovirus infection in monkeys, humans and other apes suggested that primates were not the natural hosts: if a virus kills too many of its hosts, then it cannot propagate and dies out [3]. It is obvious that despite the tremendous effort of researchers, Ebola's natural reservoir was never identified.

4. 2014 WAEO and previous Ebola epidemics

EVD was firstly identified in 1976 [5] during two outbreaks, which were observed concurrently in Southern Sudan, and in Northern Zaire [16, 20]. One year later was noticed one fatal case of Ebola in Zaire [21], and even later small outbreak in 1979 [22]. Based on serological tests, strains from Zaire and Sudan were different, and studies in mice and monkeys also confirmed differences in pathogenicity [23]. After epidemics in period 1976 - 1979 there is no evidence of EVD outbreaks between 1979 and 1994. But after 1994 the number of outbreaks increased, even that two new Ebola virus species were discovered, namely TAFV in 1994 in the Ivory Coast and BDBV in 2007 in Uganda [8].

Ebola haemorrhagic fever remains a plague for the population of equatorial Africa, with an increase in the numbers of outbreaks and cases since 2000 [1]. The current outbreak is thought to have begun in southeastern Guinea in December 2013, when a two-year-old boy died of a mysterious illness that quickly spread to family members and health-care workers [3, 15]. However, onset of the new outbreak was not recognized until March 2014 [15], which facilitated the spread to Sierra Leone, Liberia and Nigeria [24]. The June 18, 2014 the total EVD case count reported for all three countries was 528, including 364 laboratory-confirmed, 99 probable, and 65 suspected cases, with 337 deaths (case-fatality rate = 64%). Most of cases were reported in Guinea (398 cases), Sierra Leone (97 cases) and Liberia (33 cases) [25]. The August 8, 2014 the World Health Organization (WHO) declared the Ebola virus disease (EVD) outbreak in West Africa as a Public Health Emergency of International Concern (PHEIC) [25, 26] and highlighted the need for international cooperation to control the outbreak. The August 29, 2014 WHO noticed 3052 cases and 1546 deaths [25]. By September 18, 2014 the WHO reported of 5335 cases (confirmed, suspected and probable) with 2622 deaths, resulting in a case fatality rate of around 50% [27]. On the September 18, 2014 a total of 5335 cases with 2622 reported deaths have been notified, in Guinea, Liberia, and Sierra Leone [25]. According to WHO, the October 31, 2014, 13 540 people have been diagnosed with Ebola virus disease in eight countries, including 4951 deaths [25]. Dedicated doctors, nurses, and other health-care workers have made great efforts to contain the epidemic. WHO reports that 450 of these health-care workers have developed the disease and more than 230 died [25]. As of December 18, 2014 this outbreak has 19078 reported cases resulting in 7413 deaths [20, 28]. One month later, 7th January, number of cases was 20747 and 8235 deaths [25].

5. Transmission

It is often mentioned that Ebola virus is introduced into human populations by handling of infected animal (bush meat and carcasses) [1, 8, 19]. If the first source of transmission is an animal found dead or hunted in the forest, followed by person-to-person transmission from index case to family members or medical-care staff [8, 17, 18]. Animal-to-human transmission occurs when people come into contact with tissues or body fluids of infected animals, especially nonhuman primates [30]. Animal-to-human transmission has been reported in Côte d'Ivoire, an etiologist was infected through handling an infected, dead chimpanzee [16].

Year	Country, village	EboV subtype	Number of human cases	Number of deaths	Mortality	source and spread of infection
1976	Zaire, Yambuku	Ebola virus	318	280	88%	contaminated needles and syringes in hospitals - spread by close contact
1976	Sudan, Nzara and Maridi	Sudan virus	284	151	53%	spread by close contact within hospitals, many hospital staff were infected
1976	England	Sudan virus	1	0		Laboratory infection, accident - stick of contaminated needle
1977	Zaire, Tandala	Ebola virus	1	1	100%	Noted retrospectively
1979	Sudan, Nzara and Maridi	Sudan virus	34	22	65%	Recurrent outbreak at the same site as the 1976
1989	USA, Viriginia and Pennsylvania	Reston virus	0	0		EboV was introduced into quarantine facilities by monkeys from the Philippines
1990	USA, Viriginia	Reston virus	4	0		Reintroducuing into quarantine facilities by monkeys from the Philippines
1989-1990	Philippines	Reston virus	3	0		Source: macaques from USA. Three workers (animal facility) developed antibodies, did not get sick.
1992	Italy	Reston virus	0	0		Source: monkeys imported from the Philippines, that was also involved in the United States episodes.
1994	Gabon	Ebola virus	52	31	60%	Initially thought to be yellow fever, identified as Ebola in 1995
1994	Côte d'Ivoire	Taï Forest virus	1	0		Scientist became ill after autopsy on a wild chimpanzee (Tai Forest).
1995	Democratic Republic of Congo (Zaire)	Ebola virus	315	250	81%	Case-patient worked in the forest, spread through families and hospitals.
1996	Gabon	Ebola virus	37	21	57%	Source: chimpanzee found dead in the forest was eaten by hunters, spread in family members
1996-1997	Gabon	Ebola virus	60	45	74%	Case-patient was a hunter from forest camp. Disease was spread by close contact.
1996	South Africa	Ebola virus	2	1	50%	Infected medical professional traveled from Gabon to Johannesburg, nurse who took care became ill and died.
1996	USA, Texas	Reston virus	0	0		Source: monkeys imported from the Philippines.
1996	Philippines	Reston virus	0	0		Source: monkey in export facility in the Philippines
1996	Russia	Ebola virus	1	1	100%	Laboratory contamination
2000-2001	Uganda	Sudan virus	425	224	53%	Providing medical care to Ebola case-patients without using adequate personal protective measures
2001-2002	Gabon	Ebola virus	65	53	82%	Outbreak occurred over the border of Gabon and the Republic of the Congo.
2001-2002	Republic of the Congo	Ebola virus	57	43	75%	Outbreak occurred over the border of Gabon and the Rep. of the Congo.
2002-2003	Republic of the Congo	Ebola virus	143	128	89%	Outbreak in the districts of Mbomo and Kéllé in Cuvette Ouest Département.
2003	Republic of the Congo	Ebola virus	35	29	83%	Outbreak in villages located in Mbomo distric, Cuvette Ouest Départemen.
2004	Sudan, Yambio	Sudan virus	17	7	41%	Outbreak concurrent with an outbreak of measles, and several cases were later reclassified as measles.
2004	Russia	Ebola virus	1	1	100%	Laboratory infection.
2007	Democratic republic of the Congo	Ebola virus	264	187	71%	The outbreak was declared over November 20. Last death on October 10.
2007-2008	Uganda	Bundibugyo virus	149	37	25%	First reported occurrence of a new strain
2008	Philippines	Reston virus	6	0		Six workers (pig farm) developed antibodies, did not become ill.
2008-2009	Democratic republic of the Congo	Ebola virus	32	15	47%	
2011	Uganda	Sudan virus	1	1	100%	The Uganda Ministry of Health informed the public a patient with suspected Ebola Hemorrhagic fever died on May 6, 2011 in the Luwero district, Uganda.
2012	Uganda, Kibaale	Sudan virus	11	4	36%	Laboratory tests of blood samples were conducted by the UVRI and the CDC.
2012	Democratic republic of the Congo	Bundibugyo virus	36	13	36%	This outbreak had no link to the contemporaneous Ebola outbreak in the Kibaale, Uganda.
2012-2013	Uganda	Sudan virus	6	3	50	CDC assisted the Ministry of Health in the epidemiology and diagnosis of the outbreak.
2014	Democratic republic of the Congo	Zaire virus	66	49	74%	The outbreak was unrelated to the outbreak of Ebola in West Africa.

Table 1: Ebola outbreaks between 1976 and 2014, adapted from WHO [29]

It was laboratory confirmed that the deaths of chimpanzees were indeed by the Ebola virus. In Gabon and the Republic of the Congo, outbreaks in humans were associated with extensive deaths of chimpanzees and gorillas [31]. After analysis of the risk factors for transmission of EBOV from anthropological point of view, it is noticeable that the increase in Ebola outbreak since 1994 is frequently associated with drastic changes in forest ecosystems in tropical Africa [8]. Transmission of Ebola could be connected with human activities like hunting, farming, ministration of funeral rites, processing of bush meat from bats and exceptionally because of scientific activities [8, 32]. Sexual transmission has been suggested in humans since filoviruses can be found in semen [33]. Aerosol infection is questioned since people sharing the same space with infected persons do not contract the infection. Even though aerosol infection of nonhuman primates (NHP) has been demonstrated in the laboratory [34]. The highest risks of transmission of WAEO are people visiting and taking care of infected patients in their homes or in hospitals.

6. Therapy and prevention

Specific treatment or vaccine against the EBOV is not commercially available. Treatment strategies of EVD are pointed on the early start of supportive care which provably improved the survival of infected patients. They include rehydration therapy or giving intravenous fluids as well as treating symptoms.

WHO approved the use of convalescent serum and whole blood products to treat affected patients [35].

Regarding passive immunotherapy, the most promising drug seems to be ZMapp that combines three humanized monoclonal antibodies [36]. Specific antiviral EVD treatment strategies are still in the experimental phase [27]. There are three most discussing experimental antiviral drugs (TKM-Ebola, Favipiravir and BCX4430). TKM-Ebola interfere RNA molecule to silence expression of two Ebola genes which virus needs to replication. This drug prevented infection in all the laboratory animals given a lethal dose of Ebola virus [37]. Favipiravir (T-705), a viral RNA polymerase inhibitor, has shown efficiency against EV in mice [38]. BCX4430 (nucleoside analog that blocks viral RNA synthesis) has shown promising results in rodents and monkeys, but pre-clinical toxicology and phase 1 data for this drug are lacking [39]. The prevention of EVD requires improving our understanding of the epidemiology of the disease, especially the role of wildlife, including bats, in the transmission of Ebola virus to humans [8].

7. Conclusions

West Africa Ebola outbreak 2014 is occurring on a scale not seen before in Equatorial Africa. Such events perform as reminders of the potential of viruses to cause pandemic and endanger the health of the world's population. Rapid and wide spread is facilitated by insufficient medical facilities, poor sanitation of medical material, travel, air plane transport and unsafe burial practices. Because of the initial nonspecific symptoms of EVD (fever, nausea and flu-like symptoms) is difficult to distinguish EVD from other Africa endemic diseases and set up preventive and treatment strategy. Specific antiviral drug against EVD are still in the experimental phase. And there is no available and effective vaccine. It is available only supportive treatment strategies, due to this facts medical stuff have nearly empty hands. There is need to understand transmission on example of the current Ebola virus outbreak and development of animal model for testing of novel vaccines and antiviral drugs.

Acknowledgments

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements" for biomedical papers.

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Structure of influenza viruses, connected with influenza life cycle

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Influenza represents one of the biggest threats to the global population [1] and is considered as the one of the potential most dangerous pandemic agents. Not surprisingly, the World Health Organization (WHO) initiated the Global Influenza Program (GIP), which provides to member states strategic guidance, technical support and coordination of activities necessary for improving the preparedness to combat with effects of seasonal (or pandemic) influenza, which may represent danger to the health and lives of global population [2, 3]. According to WHO is seasonal influenza responsible for several million cases and almost half a million deaths annually [4]. Aim of this article is provide an overview of the structure of influenza virus and linking of the individual structures in the life cycle of influenza virion.

Keywords: Influenza virus; highly pathogenic avian influenza; pandemic; virion structure

1. Introduction

Social, economic and environmental impacts of annual seasonal epidemics are considerable, it is almost impossible to estimate what would happen in the case of a pandemic. Economic losses associated with Highly pathogenic avian influenza (HPAI) in the US were estimated on tens to hundreds millions of dollars [5]. In Europe the situation wasn´t better. In the Netherlands HPAI outbreak lasted two months, affecting 255 poultry farms and more than 30 million domestic fowl had to be killed [6].

Impact of influenza pandemics are known from the history of the 20th century, when three big influenza pandemics were described: Spanish Flu (1918), the Asian flu (1957) and Hong Kong-Flu (1968) [7]. The Spanish flu is regarded as the biggest in history, the number of victims was estimated on 50 million [8]. In the 21st century, two epidemics with pandemic potential (avian and swine flu) were recorded [9, 10]. Recently new subtypes such as H7N7 and H7N2 [11], H9N2 [12], H7N9 [13] are able to cause human infection. Many researches dealing that there is significant emergence of another pandemic. The question is when and where it will come and how much serious it will be [14-16].

Currently we are better prepared for the fight against epidemics representing by improved prevention, diagnosis and therapy [17-19]. On the other hand risk of extremely rapid spread of the pandemic increase because of the globalization, airplane transport and growth of population [20]. For this reason it is necessary to search new possibilities in the fields of prevention, treatment and diagnosis.

2. Structure of influenza virion

Influenza virions (Fig. 1) are enveloped, the capsid of the virus may be spherical or filamentous. The genome of influenza viruses is a linear, segmented and formed by (-) ssRNA. Influenza genom was encoded in 1976 [21, 22]. Till 2001, eight genome segments (Influenza A and B), and ten proteins, encoded by them, were described: nucleoprotein (NP), haemagglutinin (HA), neuraminidase (NA), proteins of polymerase

complex (PB1 PB2 and PA), matrix proteins (M1 and M2) and non-structural proteins (NS1 and NS2) [23]. In 2001, mitochondrial protein PB1-F2 [24] was described and up till 2012 six other proteins were found: PB1-N40 [25], PA-X [26] and NS3 [27], M42 [28], PA-N155 [29] and PA-N182 [29].

Total size of the Influenza A genome is 13.5 kbp, the size of different genome segments varies between 890 and 2341 bp [30]. Most of influenza A and B proteins are enclosed by lipid bilayer membrane, only three of them constitute an exceptions – two of them has antigenetic characters: trimer HA and tetramer NA. The third one, called M2, is integrated into the membrane and serves as a ion channel [31].

All of the segments of vRNA are associated with polymerase complex and nucleoprotein (NP), and they form ribonucleoproteins (RNP), which are responsible for transcription and replication of influenza [32, 33]. Structure of the native RNP was described by Arranz et al. [34].

Influenza proteins, coded by relevant segments:

- PA, PB1, PB2, PB1-F2 transcripts are on 1st, 2nd, and 3rd segments. PA, PB1, PB2 forms polymerase complex, PB1-F2 is a product of an alternative open reading frame.
- HA (Haemagglutinin) transcript is on the 4th segment.
- NP (Nucleoprotein) transcript is on the 5th segment.
- NA (Neuraminidase) transcript is on the 6th segment.
- M1 (Matrix) transcript is on the 7th segment.
- M2 (M2 ion channel) transcript is on the 7th segment, splicing of M1 transcript.
- NS1 (Non-structural protein) transcript is on the 8th segment.
- NS2 (NEP Nuclear export protein) transcript is on the 8th segment, and is formed by splicing of NS1 transcript.

2.1 Viral polymerase complex (PB1, PB2 and PA)

All of segments of influenza genome are assembled into complexes, containing RNA, polymerase complex and nucleoprotein. These complexes are characterised as ribonucleoproteins (RNPs) and represent minimal transcription and replication equipment of influenza virus.

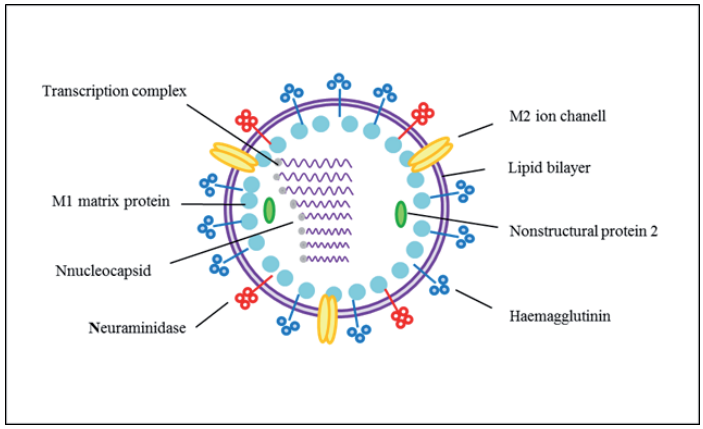

Figure 1. Scheme of influenza life cycle

Viral polymerase formed complementary RNA (cRNA) during replication. cRNA is used for synthesis of new vRNA copies [35, 36]. Molecular mechanisms of transcription and replication is not fully understood, but recent studies suggest that transcription can be implemented by cis-acting RNA polymerase, while replication by trans-acting RNA polymerase [37]. Viral polymerase contains two alkaline polymerase proteins (PB1, PB2) and one acidic protein PA, assembled into structure of trimer, where C end of PA binds N end of PB1 and C end of PB1 binds N end of PB2 [34, 35]. PB1 represents active side for binding of 5´and 3´ terminal ends of vRNA and cRNA [38]. PA and PB2 play the key roles in initiation of transcription process, binding and cleavage of the host pre-mRNA [37]. Many of influenza strains expresses the PB1-F2 protein, which is transcribed from an alternative open reading frame (+1 ORF) PB1. PB1-F2 protein is involved in the induction of apoptosis of host cells, reacts with PB1, influences activity of the polymerase complex, and participates to the viral pathogenesis of some influenza virus strains [39].

2.2 Haemagglutinin

Haemagglutinin (HA) is trans-membrane glycoprotein (size 13.5 nm, molecular weight 76 kDa). HA is target molecule for neutralising antibodies. Primary function of HA is initiation of infection, and interaction with sialic acid receptor of host cell [33, 40]. Interaction of HA with sialic acid receptor leads to virus entry into the host cell, release of viral RNA and viral replication. Currently, the most widespread HA subtypes of influenza A in the human population are H1 and H3. Exceptionally avian HA subtypes can cause disease in humans: H5 [41, 42]; H7 [43] and H9 [44].

HA monomers are synthesized separately as precursor HA0, which are proteolytically cleaved into two sub-unites (HA1 and HA2) [45, 46]. After cleavage of HA0, three cleaved HA structures form the mushroom-shaped trimer [47]. Top of the mushroom consists of antiparallel β-sheet region HA1 subunits and lower part of mushroom consists of three spirally twisted α-helices (HA2) [48]. HA1 subunit (receptor binding site) allows binding of the virus to host cell receptors [47]. Human HA preferentially recognize α-2,6 glycosidic bond on receptors, whereas avian viruses prefer α-2,3 [33, 47]. Preference of human or avian type of receptor is given by number of aminoacids in HA.

2.3 Nucleoprotein

Nucleoprotein (NP) is part of transcription equipment, and is bond with viral envelope by M1 protein [37]. NP is RNA binding protein, forms NP-RNA complex, and poses the template for transcription and replication [49]. NP has the ability to polymerize in trimeric structure, formed by NP monomers connected together through loops and pockets of neighbouring NPs [32, 50]. Although NP is considered phylogenetically as conserved protein, influenza B NP (unlike type A) is tetramer [51]. Most important functions of the NP are: covering vRNA, facilitating NP´s folding into structures dsRNP [52]. NP also interacts with PB1 and PB2 subunits of viral polymerase [33, 37]. Structure of NP as a trimeric complex consisting of head, body and tail was described using surface plasmon resonance (SPR) with high-resolution [53].

2.4 Neuraminidase

Neuraminidase (NA) represents second surface antigen of influenza, and it is involved in releasing of newly formed virions out of host cell. Neuraminidase has to cleave sialic acid from the surface of host cells enzymatically, before releasing of new virions [40]. NA is an enzyme with hydrolytic activity, cleaving the glycosidic bond between the sialic acid (N-acetylneuraminic acid) and D-galactosamin or D-galactose, which represents HA receptor on host cell surface [54, 55]. NA is plugged during the penetration of the virus through the mucin layer of the mucosa, budding of the virus and releasing of the virus from the host cells [56]. In 2012 NA mutation D151G of A/Tanzania/205/2010 strain, which allows NA to assume the function of HA and mediate the binding of host cell receptor, was described [57]. In 2013 Hooper et al. designed mutation G147R, which resulted in take-over of all HA functions. This mutation was developed under laboratory condition but can occurs also by naturally way [55].

Antibodies against neuraminidase prevents the spread of infection between cells, but they do not have neutralizing activity [58]. NA, as well as HA, undergo to antigenic drift, which may resulted in occurrence of resistance to neuraminidase inhibitors (NAIs) [59]. Substitution of arginine (R) on the position 292 by lysine (K) R292K is manifested as resistance to Oseltamivir and Zanamivir [59, 60]. Another mutation, which brings resistance to Oseltamivir, is H274Y [61].

2.5 Matrix proteins (M1 and M2)

The seventh segment of vRNA encodes the matrix protein (M1) and the ion channel protein (M2). M1 protein forms structured layer under the viral membrane, and forms a bridge between the viral envelope and the core (vRNP). M2 is a multifunctional membrane protein forming a proton channel [62]. The process of viral entry into the host cell and release of the RNP requires coordinated action of M2 and M1 proteins [36, 62]. After entry of virus into the host cell and release of virion out of endosome, the activity of the M2 ion channel increases,

thereby increase the flow of positively charged moleucules, which results in acidification. Acidification of the internal environment of the virus, leads to the disruption of the bond between HA and M1 and uncoating of viral particles, followed by merging of HA with the endosomal membrane, RNP transport close to the nucleus, and beginning of viral RNA synthesis [30].

2.6 Nonstructural proteins (NS1 and NS2)

The eighth segment of type A influenza virus encodes two proteins, known as non-structural proteins (NS1 and NS2) [63]. These proteins are produced by alternative mRNA splicing [64]. Both proteins play key role in replication. Due to this fact they are considered as targets for development of new drugs. NS1 is a multifunctional protein, and is important for escaping out of the host immune system [65]. NS1 blocks synthesis of α/β interferons [66, 67]. NS1 is RNA binding protein, which is involved in regulation of many cell processes: inhibition of host mRNA polyadenylation, inhibition of export of polyadenylated host mRNA, inhibition splicing of mRNA and inhibition of interferon-mediated anti-viral response [68-70]. NS1 reduces both synthetic as well as pulmonary proinflammatory cytokines [71]. NS2 was firstly described as a part of purified viral particles and in the nucleus of infected eukaryotic cells [67, 72]. NS2 is also known as NEP and is involved in the transport of RNA and polymerase protein complex during replication, comparing to NS1, which is less described [73]. A number of studies showed NS2 participation in the regulation of viral RNA replication [74, 75].

3. Replication

Influenza viruses are replicated in the columnar epithelial cells of the respiratory tract [76] and are spread via respiratory secretions in small aerosol particles, generated during sneezing, coughing, and speaking [77]. The incubation period is 1-4 days. H1N1 and H3N2 subtypes (influenza A) and influenza B currently circulate in human population [4], the prevalence of these three flu strains may vary in time or geographically within countries, between countries or continents during one flu season [76].

The initial step of influenza infection is binding of virion to the host cell surface. Interactions between host and pathogen are mediated by HA antigen (on the side of virus) and sialic acid receptor (on the side host cell). After the successful attachment of the virus to the receptor, membrane fusion occurs and new envelope is formed around the virion [71]. New compartment (endosome) is formed in the next step, thereafter host cell begins to digest the endosome. Decrease of pH (< 6.0) leads to the activation of HA [78-80]. HA trimer becomes unstable and is partially unfolded [33, 81]. Inner content of virion is released into host cell cytoplasm after membrane fusion. Viral RNA (vRNA) polymerase complex and RNA-dependent RNA polymerase are transported into the cytoplasm of the host cell [55, 78]. Subsequently polymerase complex is transported into the host cell nucleus where the RNA dependent polymerase make positive complementary cRNA which is exported into the cytoplasm and translated, or

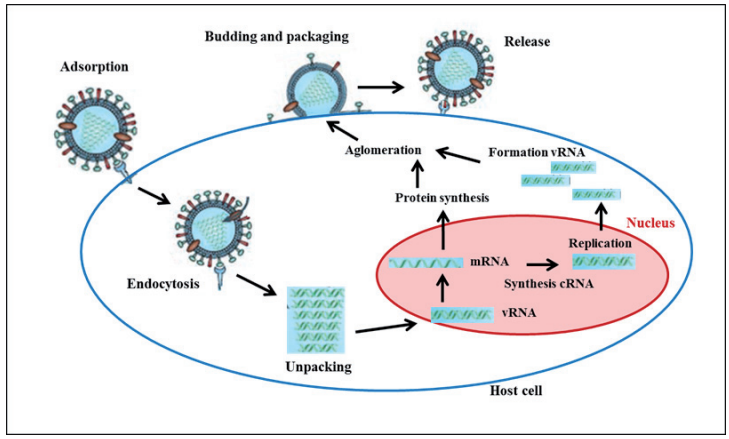

Figure 2. Scheme of influenza life cycle

remains in the nucleus. Influenza viruses are not capable to encode apparatus to produce 5'cap on its own mRNA. 5'cap is cleaved from the host mRNA, and thereafter bonded to viruses

mRNA [78]. Newly synthesized viral proteins are transported using the Golgi apparatus to the cell surface, or transported to the nucleus where they bind vRNA and contribute to assembling of new virions. Synthesized RNA contains a lot of uncorrected errors (one nucleotide for each 10kbp) which leads to the fact, that almost each virus contains mutation [36].

Other viral proteins have a wide range of functions, such as cleavage of cellular mRNA to obtain nucleotides for the synthesis of vRNA or inhibition of translation of host mRNA. vRNA and synthesized viral proteins are assembled into shape of new virions inside the host cell. Thereafter budding unit (in which RNP is inserted) is formed on the surface of the host cell, which is covered by HA and NA antigens on the surface [40]. In order to leave of newly formed virions out of the host cell, sialic acid receptors must be enzymatically cleaved (this receptor was used to bind the virus in early infection by HA) [24, 82]. The death of the host cell occurs after the release of newly replicated virions.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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REVIEW

Nanomedicine in the future of HIV treatment

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Although highly active antiretroviral therapy (HAART) has resulted in remarkable decline in the morbidity and mortality in AIDS patients, controlling HIV infections still remain a global health priority. In particular approaches, which offer site-specific, effective and safe drug delivery can be helpful in global elimination of AIDS progression. Currently, several nanoscaled vehicles such as polymeric nanoparticles, micelles, liposomes, dendrimers are intensively explored. Moreover, paramagnetic or magnetic nanoparticles or monocytes/macrophages based delivery can be employed to improve the delivery of drugs across the blood - brain barrier. The presented study describes the possibilities of nanomedicine in controlling HIV.

Keywords: AIDs; blood-brain barrier; dendrimers; liposomes; micelles; nanoparticles

1. Introduction

The human immunodeficiency virus (HIV) is a lentivirus, causing acquired immunodeficiency syndrome (AIDS) [1]. HIV is still responsible for more than 25 million deaths worldwide and estimated 34 million people are infected with HIV across the globe [2]. From development of nucleoside analog reverse-transcriptase inhibitor zidovudine (AZT) in 1987 [3] significant advances in HIV therapy have been achieved. Moreover, subsequent development of highly active antiretroviral therapy (HAART), using a cocktail of antiretroviral drugs significantly improved the life expectancy and quality of patients suffering from HIV [4]. Despite the striking successes in disease management, HAART is still associated with disadvantages such as inability to inhibit the drug-resistant viral strains, serious adverse effects, high costs and inability to eradicate HIV from its reservoirs [5]. As such, killing HIV in cellular and tissue reservoirs represents a major challenge for eradicating HIV infection. The infection of a relatively few cell types makes it desirable to direct drug therapy only to infected cells. Moreover latently infected cells do not show any signs of infection on their surface. Thus, active targeting of HIV drugs to HIV-infected cells has been difficult to achieve [6].

The recent advances in the development of nanomaterials have shown promises to revolutionize diagnosis, treatment and prevention of many diseases/pathogens, including HIV.

The present study discusses particularly the possibilities and advantages of nanoscaled materials and technologies in control of HIV.

2. Nanomedicine in HIV treatment

The use of nanomaterials in medicine raises high expectations for human health and nanotechnology is already contributing to the development of new drugs, biologics, and medical devices [7]. The improvement of existing therapeutics has the potential to give promising solutions to many illnesses, aiming for a better, more efficient and affordable healthcare [8]. The larger surface to volume ratio of nanoparticles allows much higher loading and dissolution rates, which significantly influences the biological half-life of employed drugs. Further, nano-based drugs exhibit advantages in sen-

se of drug release kinetics. Higher amount of drug in nanoparticle results in an initial burst release, followed by a constant slow release [6]. The increased blood circulation time prolongs the associated release of drug and hydrophilic coating can reduce the dose frequency of poor soluble drugs [9]. Finally, by selective targeting, the side effects can be minimized. Several nanoscaled systems such as liposomes, dendrimers, different nanoparticles, micelles and others have gained considerable interest for the treatment of AIDS, as is shown below.

2.1 Superparamagnetic iron oxide nanoparticles

Superparamagnetic iron oxide nanoparticles (SPIONs) comprise magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most commonly employed nanoparticles in biomedicine. Their superparamagnetic behavior makes them superior and offers possibilites as targeting using external magnetic field ("remote control" to move the drug-loaded on SPIONs to specific site) or observation of their distribution by magnetic resonance imaging (MRI) [10]. Moreover, synthesis of SPIONs is quite easy, low--cost and highly monodispersed suspension can be synthesized easily [11]. Combination with other nanoparticles, such as liposomes (forming magnetosomes) or quantum dots offers multifunctional particles, suitable for broad spectrum of applications [12]. It is general fact that numerous molecules such as drugs, enzymes or proteins can be immobilized on the SPIONs surface [13], nevertheless application in HIV/AIDS is limited. Saiyed et al. (2009) showed that AZT can be immobilized on SPIONs surface; however the inhibition effect on nanoformulation remains comparable with free drug [14]. The same group further formulated magnetosomes bearing AZT and observed their transport across BBB and it was demonstrated that magnetosomes-AZT permeability was enhanced by threefold in comparison with free AZT [15]. It can be stated that modified formulas of SPIONs can reduce drug clearance, metabolism and entrapment by reticuloendothelial system (RES).

2.2 Liposomes

Liposomes are usually small (80 - 100 nm), using variety of encapsulation methods [16]. Surface of liposomes can be easily modified to improve its properties [17], while incorporation of hydrophilic polyethylene glycol (PEG) can prevent interactions with plasma proteins, thus retarding recognition and removal by RES. Liposomes utilized for delivery of anti-HIV/AIDS drugs can be classified as ionic-stabilized, immune-stabilized or sterically-stabilized [18]. It was demonstrated that half-life of lipo-zalcitabine in the brain of Sprague-Dawley rats can be prolonged to 23 h as compared with 1.1 h for non-encapsulated drug [19]. Jin et al. revealed that encapsulation of AZT-myristate (prodrug of AZT) can lead to longer half-life and higher concentrations of AZT in brain and other organs [20]. In elimination of HIV-related infections, several fold increase in the concentration of amphotericin B was observed when BBB was acrossed by liposomes with amphotericin B tethered with RMP-7 (bradykinin B2 receptor agonist) [21]. Freeling and colleagues suggested lipid nanoparticles, containing triple-drug--cocktail of lopinavir (LPV), ritonavir (RTV) and tenofovir (PMPA) [22]. In primates their nanoparticles produced over 50-fold higher intracellular concentrations of LPV and RTV in lymph nodes, when compared to free drug and these levels were enhanced and sustained up to 7 days. Despite the demonstrations that liposomal drugs can be highly effective in increasing of successes of treatment of HIV/AIDS, few disadvantageous issues such as low drug entrapment ability for water-soluble drugs, still exist.

2.3 Dendrimers

Dendrimers are a class of monodisperse polymers distinguished by their repeated branching structure emanating from a central core [23]. They can be engineered in the size range of 10 - 100 nm and may contain many reactive functional end groups and internal void spaces, which make them potent for drug delivery systems [24]. The nature of dendrimers thus offers both possibilites - encapsulation and surface bioconjugation. In HIV research, dendrimers

are mostly studied for drug delivery to brain across the BBB. Dendrimers can be employed for delivery of anti-HIV siRNA as was demonstrated by Jimenez et al. [25]. Dendrimer/siRNA complex exhibited good permeability across the BBB and resulted in significant inhibition of the viral replication. Further, carbosilane dendrimers bearing sulfonate or carboxylate groups achieved 85 - 90% HIV inhibition without inducing inflammation or vaginal irritation in mice [26]. The limited application of dendrimers is associated with complicated production and their polycationic nature, which is toxic for negatively charged membranes.

2.4 Micelles

Micelles are aggregates of surfactant molecules dispersed in a liquid colloid, formed from hydrophilic head and hydrophobic single-tail regions in the micelle centre with diameter varying from 1-50 nm [27]. The inner cavity can be utilized as the encapsulation space. Currently there are three types molecules - micelles, block copolymers, surfactants and polymer-lipid conjugates used for preparation of micelles [28]. It was demonstrated that administration of P85 micelles with zidovudine, lamivudine and nelfinavir resulted in 78-92% down-regulation of p24 expressing monocyte-derived macrophages [29], whereas application without dendrimer resulted only in 62% inhibition during 2 weeks postinoculation of HIV. The largest disadvantages of all types of micelles is their significant instability, however they are still matter of concern, due to their exceptional ability to reduce premature drug release.

2.5 Bioconjugates

The drug-polymer bioconjugate-based nanocarriers can significantly influence HIV therapy by specific localization to viral reservoir sites. The most studied polymers for CNS delivery are currently acrylic, polyesters and poly(butyl cyanoacrylate) (PBCA) [30], however there are concerns about their degradation (toxic degradation products, as formaldehyde byproducts in case of PBCA) or ability to permeate the BBB. It was shown that PBCA nanoparticles are able to deliver an improved amount of drug, and the permeability of zidovudine and lamivudine can be increased by 8-20 fold or 10-18 fold, respectively [31]. Further, BBB permeability of acrylic polymers through receptor-mediated endocytosis can be enhanced by adsorption of apolipoproteins on surface of polymer [32]. The group of polymeric bioconjugates contains broad spectrum of applicable substances, however further experiments in vitro and in vivo are required to understand the phenomenon of metabolical degradation, transport across BBB and their undesired toxicity.

3. Conclusions

Insufficient concentration and short retention of anti-HIV drugs are the major causes, leading to failure of eradicating HIV from reservoirs and development of multi-drug resistance against anti-HIV drugs. Recently, some novel nanocarrier-based drug delivery systems shown remarkable properties to overcome many of the problems connected with HIV treatment. Possibility of targeting, elevation of drug residence and ability to across BBB makes nanoscaled carriers promising platforms for treatment HIV/AIDS; however further research to resolve other issues such as immunogenicity, unwanted drug leakage and carrier metabolism will be required.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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Regulation of human oncogenes' expression by human papillomavirus (HPV) 16 E6 protein

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Human papillomavirus (HPV) is a DNA virus from the Papillomaviridae family that is capable of infecting humans. Infection with HPV causes anogenital neoplastic lesions in men and in women. HPV is transmitted by sexual contact generally by asymptomatic carriers. These viruses establish productive infections only in keratinocytes of the skin or mucous membranes. Most HPV infections are subclinical and will cause no physical symptoms; however, in some peoples subclinical infections will become clinical and may cause benign papilloma or cancers, which mainly involve genital regions, and the head and neck areas. In this manuscript, we discuss HPV virology, the molecular mechanisms of carcinogenesis and the regulation of human oncogenes' expression by E6 HPV 16 protein.

Keywords: cancer; genome expression; human papillomavirus; protein p53, protein pRb,

1. Introduction

The human papillomaviruses (HPVs) are a diverse group of DNA viruses that establishes productive infections only in the laminated epithelium of the skin and in mucous membranes. More than 100 HPV types are known and the standard classification in types of high and low risk of these viruses was performed based on their cancer pathogenesis[1].

Most HPVs cause no symptoms, currently; the available studies show that the prevalence of asymptomatic genital HPV infections in the population is very high [2].Some types of HPVs can cause warts, while others can cause subclinical infections, which can lead to cervical cancer, in women or cancer of the anus and penis in men [3]. The most often transmission of HPVs occurs through skin to skin contact, so viral particles can penetrate first into mucosa and then, into epithelial cells [4]. Other HPV types affect non-anogenital localizations, such as the head and neck areas. The term head and neck cancer includes malignancy in an area that comprises the skin, oral cavity, salivary glands, lip, pharynx, larynx, nasal cavity, paranasal sinuses and soft tissues of the neck and ear [5,6].

The molecular mechanism of HPV carcinogenesis can be explained by the regulation and function of the two viral oncogenes E6 and E7. These oncogenes, increase the rate of cell division or inhibit programmed cell death, thereby they will increase the risk of malignant transformation [7,8].The great advances for fight against these viruses have been developed, such as prophylactic vaccination and high sensitivity-specificity methodologies of detection [9].

2. HPV Genome

HPVs have an icosahedral capsid and their genome is double-stranded circular DNA, with 8,000 nucleotide base pairs long. The HPV genome is divided into an early region (E), encoding

six genes (E1, E2, E4, E5, E6, and E7) that are expressed immediately after initial infection of a host cell, and a late region (L) encoding a major capsid protein L1 and also a minor capsid protein L2 and a non-coding regulatory region (NCR) of approximately 1 kb [6,10].

The L1 and L2 late proteins form capsomers of the virus that encapsidates the viral DNA. The L1 is the major capsid protein. L1 self-assembles into pentameric capsomers. L1 capsids assembled in vitro are the basis for prophylactic vaccines against several HPV types, because the amino acid sequences of L1 are well-conserved. The L2 protein is a minor component of the viral capsid. In addition to cooperation of L2 with L1 by the packaging of the viral DNA into the virion, L2 interacts with a number of cellular proteins during the infectious entry process. After the initial binding of the virion to the cell, L2 must be cleaved by the cellular protease [11].

The E1 encodes a protein that binds to the viral origin of replication. It has a helicase activity for separation of DNA strands. E2 is a transcriptional activator of virus gene expression. The E2 protein binds to E1 and stimulates viral DNA replication. E2 regulates a distribution of viral genomes to each daughter cell after cell division. The genetic changes, which inactivate E2 expression, tend to increase the expression of the E6 and E7 oncogenes, resulting in further genetic destabilization [9].

The E4 protein is expressed in a later phase of viral infection with a role in the assembly and release of the viral particle protein, facilitating virion release into the squamous epithelial tissue. Viral mutants incapable of expressing E4 do not support the replication of the viral DNA. E4 has also participated in arresting cells in the G2 phase of the cell cycle. The E5 are small and hydrophobic proteins that destabilize the function of many membrane proteins. E5 stimulates the transforming activity of the epidermal growth factor receptor resulting in the increased cell proliferation. These proteins associated with cancer activate the signal cascade initiated by epidermal growth factor and could prevent the infected cell from being eliminated by killer T cells [12].

The E6 and E7 HPV proteins are implied in tumorigenesis and are known to induce degradation of the tumor suppressor genes p53 and pRb, respectively (Fig. 1) [13]. They can suppress apoptosis and alter the function of factors involved in cell-cycle regulation, thereby facilitating prolongation of the proliferative stage of keratinocyte differentiation [14]. The major role of E6 is the degradation of p53, reducing the cell's ability to respond to DNA damage.

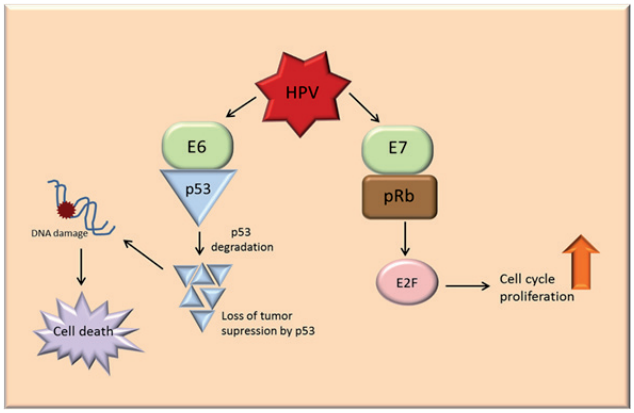

Figure 1: E6 and E7 oncoproteins of human papillomavirus (HPV) prevent tumor suppressor protein (p53) and retinoblastoma protein (pRb) from stopping the growth of damaged cells

The E6 protein also activates telomerase, an enzyme that maintains the telomeric DNA at the ends of linear chromosomes. Without telomerase, telomeres shorten upon each cell division, and causes cell senescence. Almost all human cancers and immortalized cell lines have highly active telomerase. Additionally, E6 can act as a transcriptional cofactor and also bind to signaling proteins and impede normal protein activity. The E6 protein is the target of therapeutic HPV vaccines designed to eradicate established cancer tumors.

The E7 protein is a small polypeptide of 100 amino acids. The carboxyl-terminus of E7 contains a similar zinc-binding domain as E6 [15].

The primary function of the E7 protein is to inactivate the members of the pRb family of tumor suppressor proteins. Together with E6, E7 serves to prevent apoptosis and promote cell cycle progression, for replication of the viral DNA. E7 also interacts with various other proteins, like important regulators of the cell growth. The E7 protein induces abnormal centrosome duplication, abnormal mitoses, aneuploidy and genomic instability [14-16]. E6-E7 oncogene expression is considered necessary for carcinogenesis and maintenance of the malignant phenotype of these cancers [6,17].

3. Regulation of the genome expression by E6 protein

HPV-16 was included into the high-risk HPV types, and is the most common one in all cases of cervical cancer; also raise the risk of developing oropharyngeal cancer [5,18]. HPV-16 genome is about 7900 bp long (Fig. 2). High--risk HPV types act as primary transforming viral proteins to inactivate the p53 and pRb pathways that result in cell proliferation and resistance to apoptosis [13,15]. This leads to the accumulation of DNA damage and mutations that give rise to cell transformation and carcinoma development [19].

Yu et al investigated the relation between high-risk HPV-16 infection and p53 mutation in lung carcinomas and its association with tumor behavior. The study indicated that mutation in the p53 and HPV-16 infection might coordinate the development of lung squamous cell carcinomas, and their coexistence was associated with a poor prognosis[20].

Similarly, Fujita et al investigated HPV infection and the expression of p53 in verrucous carcinoma (VC). The presence of HPV-16 DNA and the E6 protein was inversely associated with the expression of p53. Oral VC tumorigenesis may involve the inactivation of p53, which is associated with HPV infection [21].

The E6 protein of HPV-16 is a small polypeptide (150 amino acids) that contains two zinc-binding domains [22]. This protein has a transcription-modulatory activity for a wide variety of viral promoters, mainly adenovirus or herpes simplex virus [23].

The mechanism in the regulation of the E6 HPV-16 gene expression was induced by post--transcriptional RNA splicing. Kezhi Lin et al established a method to specifically amplify E6 HPV-16 associated transcripts. Six groups of E6 transcription patterns were identified from HPV- 16 positive cervical tumor tissue. The transcription pattern of the E6 HPV-16 gene was closely associated with the stages of cervical carcinogenesis, and may also be involved in the development of cervical cancer [24].

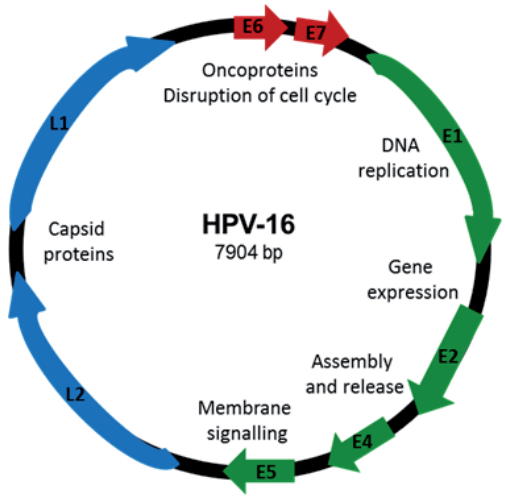

Figure 2: HPV-16 genome, early genes are designated E1 to E7 and late genes, L1, and L2 and description of their functions are shown in this figure. E6 and E7, oncoproteins, are indicated in red boxes, E1-E5 in green boxes and capsid proteins in blue boxes

Other studies about regulation of the E6 HPV-16 gene expression showed that mouse fibronectin (FN) gene as a putative cellular target whose expression is up-regulated by E6 oncoprotein. E6 HPV-16 transactivates the FN promoters in a p53-independent manner [25]. Notch1 gene is a determinant of keratinocyte differentiation and functions as a tumor suppressor in mammalian epidermis. This gene can be down-regulated by E6 through p53 degradation in normal human epithelial cells [26]. On the other hand, the involvement of HPV-16 infections in oral cavity of squamous cell carcinoma (HNSCC) and non–small cell lung cancer (NSCLC) remains elusive [27,28].

A protein known as, Foxhead box M1

(FOXM1), regulates cell cycle so as to promote tumor progression. Also this protein is upregulated by E2F1, which is released by pRb phosphorylation through p53 inactivation [29]. FOXM1 interacts with E7 HPV-16 to promote the transformation of primary embryo fibroblasts and FOXM1 also interacts with E6 HPV-16 to increase the expression of mediated NKX2-1 (also known as thyroid transcription factor 1). Consequently, FOXM1 induced by the E6/NKX2-1 axis is responsible for HPV-mediated tumor progression and metastasis [29,30].

4. Conclusions

Nowadays, there is a lot of analytical and clinical information about HPVs available that can be used for detection, development the vaccines against these viruses, and in the prognostic assessment of the patients who have developed cancer.

But further insights into the mechanistic actions of the E6 and E7 oncoproteins, on tumor progression at a level of regulation expression of these genes are needed for to support the fight against different types of cancer that are caused by HPVs.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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REVIEW

Application of nanotechnologies in the diagnostic of human papillomavirus

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The human papillomaviruses (HPV) are a diverse group of DNA virus belonging to the family of the Papillomaviridae and represents one of the most common infections of sexual transmission. Papillomaviruses cannot be cultivated on tissue culture, so the DNA diagnostics is the main and accurate method for the detection of clinical specimens. Commonly used methods in laboratories include the use of the polymerase chain reaction. Conventional methods often do not specify the types of HPV and only distinguish the presence of high-risk or low-risk type of HPV. In recent decades, the number of techniques is based on diagnostic of human papillomavirus using nanotechnologies, such as nanoparticles, their modification, magnetic separation or antisense therapy.

In the focus of this research is the preparation of a composite material, consisiting of graphene oxide and nanoparticles of zinc oxide (ZnO), silver phhosphate (Ag_3PO_4) and silver. Graphene oxide was prepared by Hummers method, involving the oxidation of graphite flake in sulfuric acid with permanganate with subsequent addition of the necessary components for the formation of metal nanoparticles. Physicochemical methods are considered for their characterisation. The size of nanoparticles ranges from 50 – 200 nm. Subsequently, the antibacterial effect of composites was tested by disk method on bacterial cultures of *S.aureus, E.coli*, methicilin-resistant *S. aureus* (MRSA). Selenium nanoparticles exhibit the highest antibacterial activity from selected nanoparticles containing graphene oxide composite with an inhibitory zone with the size 5 ± 1 mm. Silver nanoparticles display also a distinguishable antibacterial effect with inhibition zones of 2 ± 1 mm. Graphene oxide which is modified by zinc oxide nanoparticles shows no inhibitory effect. The obtained results show suitability of the prepared composite materials as candidates for alternative antimicrobialmaterials.

Keywords: Diagnostic; human papillomavirus; nanotechnologies

1. Human papillomavirus

Sexually transmitted human papillomavirus (HPV) infection has been identified as a cause of cervical cancer, and it is now widely recognized as being responsible for more than 95% of cervical cancer causes [1]. Since the discovery of HPV 16 and 18 DNA in cervical cancer tissue, more than 120 different types of the human papillomavirus have been isolated; N40 (E1-derived peptide) infect the epithelial lining of the anogenital tract and other mucosal areas. Epidemiological data from the U.S. National Health and Nutrition Examination Survey determined that the prevalence of HPV infection in a representative sample of women was highest in those aged 20–24 years (44.8%). Thus, cervical HPV infection is one of the most common sexually transmitted infections (STIs) in women.

Human papillomaviruses are small circular, double-stranded DNA viruses infecting epithelial tissues. HPV types can be classified both as high-risk and low-risk. Of the more than 120 different identified types of HPV, the majority are involved in infections of the genital tract, cancer or cervix, vulva, vagina and penis, and of non-anogenital localizations, such as the head and neck areas. From the point of view of the infection, human papillomaviruses have developed several molecular mechanisms to suppress apoptosis in infected cells [2].

The transmission of HPV infection occurs most often through the sexual contact. The newborn child infectin might be experienced through the contact with infected cells lining birth canal [3].

Viral particles penetrate by tiny cracks in mucosa or skin and infect the basal epithelial cells. Infected cells have characteristic cytological features. These include pycnosis of nuclei hyperchromasia and especially perinuclear vacuolation called coilocytosis. Such findings are typical indicators of viral infection [4].

The discovery of human papillomavirus DNA in cervical cancer by Harald zur Hausen sparked 30 years of research that established that persistent cervical infection by certain HPV genotypes causes cervical cancer. This research has led to revolutionary technical advances for the prevention of cervical cancer: prophylactic HPV vaccination and sensitive molecular HPV testing for screening. These promising technologies can be used to complement or enhance established cervical cancer prevention programs, and to provide robust solutions in low-resource settings without screening programs [5].

The available studies show that the prevalence of asymptomatic genital human papillomavirus infections in the population is very high. Bauer and his coworkers investigated university students by the method PCR (polymerase chain reaction) and at 46% have demonstrated HPV infection. A study of 1992 estimated incidence of HPV infections in the US for more than 30 million cases [6].

Human papillomavirus (HPV) is linked to anal cancer through high HPV DNA-detection rates. Here, in one of the largest international studies to date, HPV DNA was detected in more than 88% of anal cancers and more than 95% of anal intraepithelial neoplasias grades 2/3 [7].

2. General diagnotic of human papillomavirus

Papillomaviruses cannot be cultivated on tissue culture, so the DNA diagnostics is the main and accurate method for the detection of clinical specimens.

Commonly used methods in laboratories include the use of the polymerase chain reaction. Conventional methods often do not specify the types of HPV and only distinguish the presence of high-risk or low-risk type of HPV.

Diagnosis of HPV infection can be carried out more often visually or by different types of tests: i) vinegar (acetic acid) solution test; ii) Pap test or iii) DNA test.

Using of vinegar solution test turns HPV-infected genitals to white color. This may help to identifying of difficult-to-see flat lesions.

Pap tests can reveal abnormalities that may lead to cancer. These tests are performed from collection of cells from cervix or vagina. The principle of the Pap test is to wipe the cells from the outer opening of the cervix of the uterus and the endocervix. Swab is then microscopically examined to look for abnormalities in the sample [8]. The Pap test complements tissue biopsy;

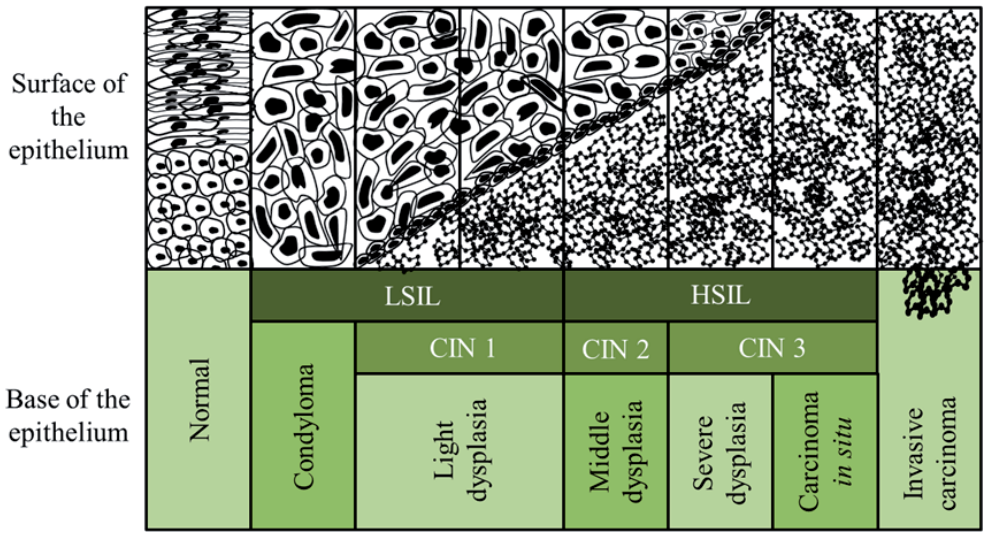

Figure 1: Continuum of squamous transformation

the tests do not compete. Each has some advantages and limitations. A Pap test is inexpensive, rapid, and very simple to obtain and process. It produces no injury to tissues. This allows frequent repetition of cellular sampling, which is especially important in the evaluation of the progressive or posttreatment regression of a disease. It contains samples of cells originating from a wider surface area than that obtained by a biopsy [9].

DNA test recognize the DNA of the high-varieties of HPV that have been linked to genital cancers. The test is conducted on a sample from cells taken from cervix. It is recommended for women older than 30 in addition to the Pap test.

Clinical forms of HPV infection is therefore defined as lesion visible to the naked eye. Subclinical form of infection can be detected by colposcopy, cytology and histology. Latent form of HPV infection causes no morphological changes in the squamous epithelium and for its detection are necessary to use methods of molecular biology. Cytology uses so-called Papanicalaou´s classification (PAP IV) and smears from the cervix.

Histology classifies the biopsy as a cervical intraepithelial neoplasia (CIN) grade I to III and invasive carcinoma (INCA). Newer classification system from 1988 value the finding of lesions as a low-grade (low squamous intraepithelial traditional treatment lesions (LSIL)), high-grade (high squamous intraepithelial lesions (HSIL)), invasive carcinoma and atypical cells (atypical squamous cells of unknown significance (ASCUS)/atypical glandular cells of unknown significance (AGUS)) [10]. Morphological methods, especially cytology are important screening methods. However, many studies points out some shortcomings of cytology. Above all, it has a poor reproducibility, false negativity (15 – 50%) and false positivity (10%) [11, 12]. The success of these screening programs depends on their good organization and capture in the widest possible population [13, 14].

The molecular-biological methods can help detect also the latent form of infection. Among them are two methods, whose sensitivity and reproducibility is sufficient that their use can be envisaged in routine diagnostic. One is polymerase chain reaction (PCR), when HPV DNA is amplified and thus high sensitivity is achieved. The second method is test based on a direct hybridization test with complementary DNA probes with chemiluminescent signal amplification (HC = hybrid capture), which is now commercially available (distributed by Abbott). Using PCR method was HPV DNA detected in up to 100% of biopsies INCA and CIN III. This method is fast and relatively simple. It must be strictly comply to some principle in

order to avoid possible contamination. Due to robustness of PCR method various materials can be investigated. The method of direct detection by hybridization is somewhat less sensitive than the PCR method. New improved HC system on microplates (HCM) brought compared to the original system carried out in test tubes (HCT) the improvement of sensitivity (HCT = 74 – 94%, HCM = 95%). Preliminary studies indicate that a combination of methods HC with PAP smears can achieve sensitivity up to 91 – 100% [15].

Use of HR HPV detection in gynecology is currently very serious issue around the world. Current data suggest that the introduction of HR HPV detection as a secondary diagnostic test for women with the cytology finding ASCUS/AGUS in women younger than 35 years would allow up to 100% capture of women with HSIL lesions. The introduction of testing on HR HPV together with cytology is also considered for a primary screening for women older than 35 years. In the long term so conceived screening program should bring in particular the reduction of incidence and mortality for cervical cancer, while this program should be economically recoverable.

3. Diagnostic of human papillomavirus using micro- and nanotechnologies

In recent decades, the number of techniques for detection of DNA, RNA or proteins, has been developed. For these purposes are used so--called biochips or microarrays. This technique became widely used for diagnosis of diseases, in the discovery of novel genes and drugs [16]. DNA microarray is a powerful tool for the parallel determination of nucleic acids and other biologically significant molecules [17]. DNA microarray technology can be efficiently used for the simultaneous detection and identification of many biological contaminants and health risky agents [18]. This technique was commonly used for diagnosis of HPV infection in patients using the Greiner Bio-OnePapilloCheck1DNA chip assay [19].

Among the most common used nanomaterials for the diagnostic of human papillomavirus belong nanoparticles. In order to overcome the shortcomings of the fluorescence-based detection technique, many researcher adopt nanoparticles to label biomolecules instead of fluorescence dyes [20-23]. Nanoparticles can be modified in various ways. For example, in the study of Piao et. al. [24] were used fluorescent nanomaterials, such as quantum dots, fluorophoredoped silica nanoparticles and metal nanoparticles for the diagnosis of human papillomavirus DNA.

To speed up the diagnostic technique can be used the method ultra-fast PCR based primers nanoparticles conjugates. It runs one thermal cycle in 8.5 s or one complete 40-cycle PCR protocol in 5 min and 40 s [25]. The polymerase chain reaction (PCR) technique is very important technique in the modern life science. Accurate and especially rapid diagnosis of the causative agents is built into the foreground of detection methods in terms of early treatment of patients [26]. PCR is often used for its speed, sensitivity and specificity in the detection of pathogens [27]. Currently, the technique of polymerase chain reaction with respect to the speed of determination dramatically reduced from three hours to few minutes. This area is requires improvement and further development for certain applications [28]. This technique was also used in many studies for diagnosis of viral particles [29, 30].

Other nanotechnology ways for papillomavirus diagnosis are magnetic microparticles. Magnetic microparticles have attracted vast attention because they have good biocompatibility and can be readily separated from reaction mixtures with the aid of an external magnetic field [31]. Magnetic microparticles have been extensively used for DNA hybridization, cell separation, immunoassay, protein and enzyme immobilization, and drug delivery [32], which can be further employed for fast and accurate HPV diagnostic in complex biological matrices. Using of magnetic microparticles for detection of presence of nucleic acids/protein from viruses was also patented [33].

For the diagnosis based on nanotechnologies can be also used the basic enzyme-linked immune sorbent assay (ELISA), which can be modified

by conjugated antibodies with quantum dots. Utilization of magnetic particles and antibodies can be also used together for diagnosis of HPV [34]. Recently, attention has been focused on the development of biolabels in the field of nanomaterials. For this purpose have been studied previously mentioned quantum dots [35]. Between the greatest advantages of quantum dots in nanotechnologies undoubtedly belong their unique optical properties, long-term imaging and "multiplexing" (simultaneous detection of multiple signals) [36]. Quantum dots with regard to nanotechnologies and microarrays have three disadvantages: fluctuations of photoluminescence and thereby reducing of quantum efficiency; toxicity of quantum dots containing heavy metals; their excitation maximum in the UV region [17].

Other way is antisense therapy. Antisense oligodeoxynucleotides (AS-ODNs) are designed to specifically downregulate target gene expression utilizing Watson-Crick pairing rules [37]. Antisense moieties have been widely used for gene silencing and therapeutic purposes. Márquez-Gutiérrez for example developed two AS-ODNs (419 and 434) directed to region that resulted in the destruction of HPV-16 E6/E7 mRNA in vitro and in vivo, inhibition of tumor cell proliferation, anchorage-independent growth, and tumor growth in an animal model [38].

4. Conclusions

There exist a number of analytical or molecular biological methods that can be used for the diagnosis of human papillomavirus. Given the need to accelerate the process of identifying the causative agent several available methods are described. They range from classical such as PCR and DNA/RNA hybridization to new identification methods based on nanotechnologies. Methods based on nanotechnologies discussed in this review represent a choice selection of available techniques for diagnostic applications related to human papillomaviruses.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products,services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform reguirements" for biomedical papers.

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SARS Coronavirus: Minireview

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The infection of SARS Coronavirus (CoV) was first observed in 2002. Since 2004, there have not been any new cases of this disease. However, new coronaviruses able to infect humans were found in bats and during the outbreak of Middle East Respiratory Syndrome Coronavirus. To effectively fight the coronavirus infections, the properties of SARS-CoV are still studied. In this review, we describe the genome, replication and transcription and important proteins of SARS--CoV. Also the ability of SARS-CoV to evade the innate immune system of host is described. The second part of this review discusses the methods for detection and treatment of SARS-CoV.

Keywords: coronavirus; neutralizing antibodies; severe acute respiratory syndrome

1. Introduction

In 2002, an outbreak of respiratory illness emerged from China, later named as a Severe Acute Respiratory Syndrome [1,2]. It was the first previously unknown pandemic transmissible disease of 21st century [3]. The disease spread quickly throughout the world (affecting 32 countries) with more than 8500 individuals infected and 900 dead (case fatality rate of 10%). It can be transmitted via respiratory droplets, direct contact or airborne processes [4].

The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is a virus belonging to the Betacoronavirus genus from family *Coronaviridae* [5]. Coronaviruses are closely related to influenza viruses and both SARS and MERS (Middle East Respiratory Syndrome) are more lethal than other human coronaviruses [2]. SARS-CoV nucleocapsid has a helical symmetry; it is enveloped with a positive sense, non--segmented, capped and poly-adenylated RNA genome the size of 30 kb [2].

SARS-CoV is a zoonotic virus, it was transmitted to humans from its natural reservoir horseshoe bats (of the Rhinolophus genus) after still undefined mutations, possibly through intermediate hosts such as palm civets (Paguna larvata) from live animal market [3]. Since 2004, there were no new cases of SARS-CoV in humans, although a SARS-like coronavirus strain able to infect humans can still be found in Chinese bats (named as a SL-CoV-W1V1) [6]. It uses the human angiotensin converting enzyme II receptor to enter the cells, thus it does not require any mutations or intermediate hosts to infect human cells [7]. Moreover, the recent emergence of MERS in Middle East shows that coronaviruses are still a threat to worldwide population.

2. Molecular biology of SARS Coronavirus

The genome of SARS Coronavirus contains 9 mRNA transcripts with 28 open reading frames (ORFs). Coronaviruses in general have a unique position in the RNA virus world because of their RNA-synthesizing machinery [8]. ORF1a and ORF1b form about 75% of the SARS-CoV genome and they are translated in a cap-dependent manner into large polyproteins [9], pp1a and pp1ab. Thiel et al. showed that pp1a and pp1b create 16 non-structural

proteins (nsp) via the proteolytic cleavage [10]. The replication and transcription of coronavirus RNA are mediated by these nsps. Snijder et al. found out that the nonstructural proteins encoded in ORF1b (nsp12-16) are expressed at significantly lower levels than those in ORF1a (nsp1-11) [11]. The nonstructural proteins associate into replicative/transcription complex after the maturation of polyproteins. This complex is responsible for the synthesis of new viral genomes and subgenomic mRNA encoding structural and accessory proteins [12]. The nsp12 encodes the RNA-dependent RNA polymerase, responsible for RNA replication and transcription of the viral RNA. SARS-CoV also possess helicase and capping enzymes, 3´-5´ exonuclease, involved in proofreading, as was described by Minskaia et al. [13]. Ivanov et al. have also found endoribonuclease in SARS Coronavirus [14].

SARS Coronavirus has four major structural proteins – spike (S), envelope (E), nucleocapsid (N) and membrane (M) - encoded by the remaining two thirds of the genome [9]. The spike, envelope and membrane proteins were found to be translated on the membrane-bound polysomes on endoplasmic reticulum [15].

The spike protein is glycosylated, situated on the surface of SARS-CoV particle and it is responsible for receptor binding and virus-cell fusion [16]. Li et al. observed that it initiates the entry of SARS Coronavirus into host cells by binding to receptors for angiotensin-converting enzyme 2 [17]. The minimal receptor-binding domain was found to be a 193-amino acid fragment in the S1 subunit of spike protein [18]. After the binding and some structural changes, the membranes fuse and the viral genome is delivered to the cytoplasm. Aydin et al. have determined that the membrane fusion is mediated by S2 subunit of spike protein [19]. Even minor changes in S protein can affect the tissue and species tropism and the virulence of coronaviruses, as was described by Li et al. [17].

The envelope protein is very small, containing only 76 amino acids [20]. It is not essential for genome replication or subgenomic mRNA synthesis; however, it was found, that it affects viral morphogenesis [21], budding [22], assembly, intracellular trafficking [23] and virulence. The alterations in E protein showed a high impact on SARS-CoV pathogenesis due to the induction of stress and unfolded protein responses and changes in the cellular ion concentrations [24]. Jimenez-Guardeno et al. suggested that the PDZ domain-binding motif of E protein targets PDZ domains present in cellular proteins [24].

The nucleocapsid protein is translated on free polysomes, it is highly basic and forms helical ribonucleoprotein complexes with a newly synthesized viral RNA [15], thus comprising the core structure of the SARS-CoV virion [9]. Chen et al. determined that these complexes are also involved in transcription, replication and virus packaging [25].

The membrane protein triggers virus budding after interaction with the envelope protein in ER-Golgi intermediate compartment [26]. Membrane protein then interacts with spike and nucleocapsid proteins and the formed virions accumulate in large vesicles. Theses vesicles are released from the host cell after the fusion with the cell membrane [27]. Hsieh et al. observed that the membrane protein together with envelope or nucleocapsid protein can form virus-like particles [28].

SARS Coronavirus also contains eight ORFs encoding accessory proteins unique for SARS-CoV and SL-CoV-W1V1 – p3a, p3b, p6, p7a, p7b, p8a, p8b and p9b [29]. These accessory proteins were found to be nonessential for SARS-CoV replication, but contribute to its pathogenesis [30], they are involved in cell proliferation, DNA synthesis, induction of caspase-dependent apoptosis, activation of stress response pathways and pro-inflammatory cytokine production [31].

The 3a protein, containing 274 amino acids [32], contributes to the increased virulence of SARS-CoV. It can form homo- and heterotetramers in infected cells [33] and ion channels selective to potassium ions [34], important in the virus life cycle and its pro-apoptotic function [35]. Minakshi et al. determined that calcium can serve as a ligand to the gated channel, causing conformational changes in the protein [36]. The 3a protein also assists in the internalization of viral spike protein from cell surface to

the intracellular sites [37] and Minakshi et al. confirmed a tyrosine-based sorting motif and a di-acidic motif present in this protein [38].

SARS Coronavirus has developed a mechanism to evade the antiviral activities of innate immune signaling pathway [39]. It contains a papain-like protease with deubiquitination activity, serving as an antagonist to the interferon [40] by the disruption of the signaling required for its induction [41]. Ratia el al. determined that the papain-like protease prefers the ubiquitin chains in comparison with mono--ubiquinated molecules [42]. It also blocks the induction of the interferon regulatory factor-3 after its phosphorylation [43], but does not inhibit its dimerization, nuclear localization or DNA binding [44].

3. Detection and treatment of SARS Coronavirus

The presence of SARS-CoV in patients can be detected using serological tests. However, Mahony et al. showed that seroconversion usually occurs 2 or 3 weeks after the infection [45], the development of methods for early detection of SARS-CoV is therefore necessary. Molecular methods can be employed for this early diagnosis. Peiris et al. used real-time RT-PCR method for the amplification of p1b gene [46]. The group of Hadjinicolaou et al. have described a real-time RT-PCR assay for the detection of spike, envelope, membrane and nucleocapsid genes, using molecular beacons with tolerance to mismatches. This method can be also used for the detection of generically non-identical strains that can emerge in the future [47].

However, traditional real-time RT-PCR suffers from varying levels of sensitivity to different RNA viruses [48]. Therefore, Keightley et al. used the real-time nucleic acid sequence-based amplification for the detection of polymerase and nucleocapsid genes and this method demonstrated equivalent sensitivity to SARS-CoV in comparison with traditional real-time RT--PCR [49]. Another molecular method employable for the early detection and epidemiological surveillance of SARS-CoV can be the single nucleotide polymorphism DNA microarray. The gene encoding spike protein contains 27 single nucleotide polymorphisms, closely related to the virus phylogenicity, virulence and pathogenesis, as was described by Kan et al. [50]. Guo et al. suggested a microarray able to detect and genotype samples with 100% accuracy [51].

Sunwoo et al. used antibody-based sandwich enzyme-linked immunoassay (ELISA) with monoclonal antibodies for the detection of spike protein. Using a bi-specific monoclonal antibody for the detection, they were able to increase significantly the sensitivity of their method [52]. The group of Roh et al. developed a method using RNA aptamer conjugated with quantum dots for the detection of SARS-CoV nucleocapsid protein, which can be easily used as a basis for a chip [53].

Nowadays, several vaccine strategies are being studied. Protective and neutralizing antibodies against coronavirus infection are often engineered to target spike proteins [54], especially its receptor binding domain on S1 subunit [55,56]. The possibility of post-infection passive immunization was studied by Yasui et al. with the increased elimination efficacy of homologous neutralizing antibodies in comparison with heterologous, caused by the cooperation of anti-SARS antibodies and phagocytic cells (monocyte-derived infiltrating macrophages and partially alveolar macrophages) [57].

However, vaccination against one type of coronavirus can significantly increase the probability of infection with other coronaviruses [58]. Moreover, Wang et al. determined that using antibodies against SARS-CoV spike protein might trigger the antibody dependent enhancement effect, thus undesirably increasing the virus yields [59]. This effect is caused by infection of large numbers of susceptible cells, mediated by immunoglobulin receptors such as Fc that facilitate the uptake of virus-antibody complexes in phagocytes and enhance the target cell infections [60].

The deletion or modification of E protein PDZ domain-binding motif and internal regions in the carboxy terminus of E protein, resulting in attenuated virus, can be another effective way of vaccine preparation [61]. Wong et al. also described peptidomimetic inhibitors for the main protease of SARS Coronavirus [62].

4. Conclusions

The recent emergence of MERS Coronavirus showed that there still are unknown coronavirus strains with high virulency in humans. The first dangerous strain observed was SARS Coronavirus in 2002 with 900 casualties. The molecular attributes of coronaviruses are very similar. SARS Coronavirus is probably the most studied. Due to the new insights into molecular biology of SARS, novel strategies for the detection and treatment of coronaviruses were proposed.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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Structural attributes and binding role of HIV-1 exterior envelope gp120

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Glycoprotein 120 (gp120) plays pivotal role in HIV-1 entry into the host cells, resulting from interaction of gp120 highly conservative CD4 binding site and CD4 receptor on the surface of CD^{4+} T cells. Therefore, gp120 became a major target in development of vaccines for HIV-1 management, based on competitive neutralization of gp120, disabling entry into the host cell. Present study describes the structure of HIV-1 virion and its pivotal protein - gp120, together with mechanism of viral entry through interaction between gp120 CD4 binding site and CD4 receptor. Finally, basic antibodies, suitable for HIV-1 vaccination are commented, with focus on RV144 or the Thai trial (ongoing clinical trial, phase III).

Keywords: CD4-binding site; glycoprotein; HIV envelope; virus entry; T-cells

1.Introduction

HIV/AIDS is a global deadly disease. Since its discovery in 1983, the Human Immunodeficiency Virus has sustained one of the major pandemics in the history of mankind [1]. Human immunodeficiency virus type 1 (HIV-1) is characterized by extensive genetic variability, as a consequence of high replication and mutation rates and frequent recombination [2,3]. The most up-to-date estimates that more than 30 million people are now living with HIV-1 infection, most of them in sub-Saharan Africa [4]. The virus depends on the physiological state of its target cells for efficient replication, and, in turn, viral infection perturbs the cellular state significantly.

A cure for HIV is still urgently needed and has become a global research priority. A unique cohort of HIV-infected individuals who spontaneously control HIV exists, and these are known as ‚elite controllers' [5]. These subjects represent a model with long term control of viral replication and HIV remission, based on natural $CD4^+$T cells depletion.

$CD4^+$ T cells play important role particularly in the adaptive immune system by releasing T cell cytokines, thus helping to suppress or regulate immune responses [6]. CD^{4+} T cells are a primary target of HIV viral entry, because of interaction between HIV exterior envelope glycoprotein 120 (gp120) and CD4 receptor found on the surface of $CD4^+$ T cells [7,8]. Thereof, perfect understanding of interaction between gp120 and CD4 receptors could lead to development of novel chemical substances with ability to block this interaction and inhibit the viral entry into the host cells.

The present paper focuses on description of role of gp120 in entry into the host cells through interaction with their CD4 receptors and further summarizes recent knowledge in development of HIV vaccines based on interaction with gp120.

2. Structure of HIV-1 virion

HIV is a member of the lentivirus genus, which includes retroviruses that possess complex genomes and exhibit cone-shaped capsid core particles around 120 nm in diameter. Like all

retroviruses, HIV's genome is encoded by RNA, which is reverse-transcribed to viral DNA by the viral reverse transcriptase (RT) upon entering a new host cell [9].

HIV is enveloped by a lipid bilayer, derived from the membrane of the host cell. Lipid bilayer possesses fundamental surface structures - surface gp120, which are anchored to a virus via interactions with the transmembrane gp41 [10,11] (schematic structure of HIV-1 virion is depicted in Fig. 1). Gp120 possesses a high level of glycosylation, which together with the presence of surface-exposed variable loops and conformational flexibility may reflect evolved viral defense against the host humoral immune response [12,13]. Elements of gp120, which are relatively conservative fold into a core, which has been crystallized in a complex with the two amino-terminal domains (D1D2) of CD4 and the antigen binding fragment (Fab) of the human neutralizing antibody, 17b [12]. The gp120 core is composed of inner and outer domains, which reflects the likely orientation of gp120 in the assembled trimer, and a bridging sheet. Components of both domains and the bridging sheet contribute to CD4 binding [14]. The lipid bilayer of virion further contains several cellular membrane proteins derived from the host cell, including major histocompatibility antigens, actin and ubiquitin [15]. Inner surface and a conical capsid core of the viral particle are covered by a matrix shell (p17, app. 2000 copies of the matrix protein) and the capsid protein (p 24, app. 2000 copies) [16]. Inside the conical capsid core, two copies of the unspliced viral genome are encapsulated in nucleocapsid (p7), stabilized as a ribonucleoprotein complex together with three fundamental virally encoded enzymes: protease (p11), reverse transcriptase (p66) and integrase (p31). Furthermore, virus particle contains additional accessory proteins Nef, Vif and Vpr [17].

3. Interaction providing host cell entry

Productive HIV-1 infection depends on host machinery, including a broad array of cellular proteins [19]. The current dogma is that the major HIV reservoir originates from activated CD4+ T cells that have been infected and survive while reverting to a resting state, thereby becoming a memory cell [20]. HIV-1 entry is based on the sequential interactions with the CD4 receptors on the CD4+ T cells and proteins from the chemokine receptor family [21]. The entry of HIV-1 into cell is mainly mediated by the viral envelope glycoproteins (gps) [13], which are synthesized as an ~850- amino acid precursors. After trimerization and posttranslational modification by carbohydrate, 160-kDa glycoprotein (gp160) is formed. Subsequent proteolysis of gp160, carried out in the Golgi apparatus, provides a transformation to gp120 exterior envelope glycoprotein containing relatively conserved CD4 binding site and gp41 trans-membrane envelope glycoprotein [10,11,22,23]. In the mature HIV-1 envelope glycoprotein trimer, the three gp120 subunits are non-covalently bound to three membrane-anchored gp41 subunits. For entry of HIV-1 into a host cell, the gp120 subunit associates with the CD4 receptor and the CCR5 coreceptor and this induces series of

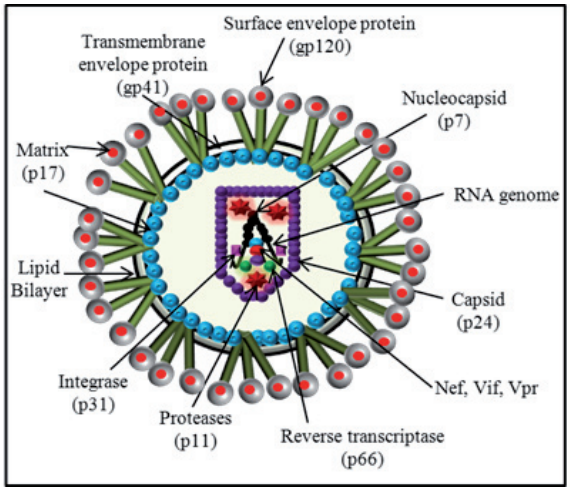

Figure 1: Schematic illustration of HIV virion showing its pivotal proteins and RNA genome, encapsulated in nucleocapsid. Adapted from [18] and redrawn

conformational changes culminating in virus and host cell membrane fusion. Most primary HIV-1 strains use the chemokine receptor CCR5 as coreceptor in conjunction with CD4 for virus entry. However, some strains have evolved to use related receptors [24]. Binding of gp120 to CD4 causes conformational changes observable in variable loop regions V1/V2 and V3, causing the V3 loop to evaginate, and thus becoming exposed to the coreceptors [25]. The precise mechanisms of interaction between V1/V2, V3 and chemokine receptors are not well understood [26]. The final step of viral entry, fusion of the viral components with target membrane, is achieved by gp41 [27,28]. After binding of gp120 to CD4 and coreceptors, conformational changes occur, leading to gp41 unfolding and the hydrophobic fusion peptide sequence extends towards the host cell membrane [29]. The insertion of the peptide leads to fold into a hairpin-like structure, believed to be responsible for the fusion of the HIV to the host cell [30].

4. Potential vaccines based on interaction antibody-CD4 binding site

Due to the nature of fusion, there are several possible targets for the development of drugs with synergistic effects on inhibition of viral entry steps, at which the interference can be attempted [18]. Generally, these targets may affect viral entry by the inhibition of CD4 binding due to a blocking of conservative CD4 binding site of gp120. Hence, glycoprotein cannot interact with receptors and coreceptors and process of conformational changes, whereas the triggering the fusion is stopped.

The first HIV CD4 binding site of gp120-specific human monoclonal antibody (mAb), b12, was identified in 1994 by Burton and colleagues [31]. It can bind to gp120 via CD4bs and successfully compete with soluble CD4 for binding to gp120. Nevertheless, the breadth of neutralization is limited either by variations in sequence of CD binding site or by distal mutations, affecting accessibility of b12 to its epitope [32]. Thus, it was shown that proper positioning of the gp120 plays crucial role for antibody for effective CD4 binding site recognition.

Currently, RV144, also known as the Thai trial (clinical trial, phase III) is the only HIV-1 vaccine demonstrating efficacy against HIV-1 acquisition. RV144 combines two vaccines (ALVAC/AIDSVAX) that failed, during vaccinating in Thailand in 2003 until 2006 [33]. The major components of RV144 are the antibodies (CH58 and CH59) against the V1V2 region of gp120 and it was revealed that these antibodies seem to play a predominant role in protection against HIV-1 acquisition [34]. CH58 and CH59 could bind to gp120 antigen and also to a HIV-1 envelope variable region 2 peptide [35], and thus work as the broadly neutralizing antibodies for HIV-1 management.

5. Conclusion

Exterior envelope glycoprotein gp120 is a unique protein structure, fundamental for HIV attachment to specific cell surface receptors and HIV entry into cells. Inhibition of such interaction is a „holy grail“ in development of vaccines for HIV management; however targeting gp120 has proven extremely difficult, particularly due to its shielding and partial variability. Further research of novel antibodies towards the most conservative sites of CD4 binding site of gp120 can thus led us to success as is currently shown in phase III by a mixture of monoclonal antibodies - RV144.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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REVIEW

Epidemiology and pathogenesis of Ebola viruses

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The family *Filoviridae* consists of three genera, the Ebolaviruses, Marburgviruses, and the recently described Cuevaviruses. The two first of them belong among the most virulent viruses. The Ebola viruses, respectively Zaire species of this genera is the causative agent of the biggest ebola outbreak, namely West Africa Ebola Outbreak, which started in 2014, and in which the case-mortality rate has been reported to be at about 50 percent, with the number of total cases exceeding twenty-four thousands[1]. In comparison with earlier Ebola outbreaks mortality have been higher (80 - 90 percent), but number of cases was a thousand times lower. In this study will be presented epidemiology and pathogenesis of Ebola virus disease including new findings resulting from the studies linked with 2014 Ebola Outbreak in West Africa. The EVD epidemiology, pathogenesis, and clinical characterization will be discussed in this study.

Keywords: Ebola virus, Ebola virus diseases , hemorrhagic fever, West Africa Ebola Outbreak

1. Basically about Ebola viruses

The Ebola viruses (EBOV) are enveloped, non-segmented, negative-sense, single-stranded RNA viruses, with size of genome approximately 19 kbp [2]. The virion is pleomorphic, producing 'U'- shaped, '6'-shaped, or circular forms [3]. The EBOV may cause a disease in humans as well as in non-human primates, called Ebola virus disease (EVD). EBOV are firstly described in 1976, since than five species of the EBOV were described since. Four of them: Sudan virus (SUDV), Ebola virus (EBOV, known as Zaire), Taï Forest virus (TAFV), and Bundibugyo virus (BDBV) cause acute and lethal disease in human population [4, 5]. The fifth, Reston virus (RESTV), differs from each other, it has been caused disease only in monkeys, chimpanzees, and gorillas and it is apparently maintained in an animal reservoir in the Philippines and has not been found in Africa [6, 7]. Since 1976, more than twenty outbreaks of EVD have been reported in Africa [8]. The current outbreak, West Africa Ebola Outbreak 2014 (WAEO), first reported by the World Health Organization (WHO) on 22 March 2014, was caused by ZEBOV and is responsible for more than ten thousands death [9]. In addition to the three most affected countries in sub-Saharan Africa (Guinea, Liberia ad Sierra Leone), EBOV cases have been reported in the Great Britain, the United States and Spain [1, 10]. The behavior of WAEO was so alarming, that some countries decided to implement special measures for air plane transport of passengers from affected countries [11].

2. Ebola virus disease

EBOV cause severe hemorrhagic fever with high percentage of fatal outcome in humans, and several species of non-human primates (NHPs) [12]. Human Ebola outbreaks usually occur abruptly from a vaguely defined source, with subsequent rapid spread from person to person [13]. In the past, EBOV were classified as „hemorrhagic fever viruses", based on the

clinical manifestations, which include coagulation defects, bleeding, and shock [14, 15]. But this term is no longer used because not all of Ebola patients developed significant hemorrhage symptoms, which usually occurs only in the terminal phase of fatal illness [16].

3. Epidemiology of EVD

Two main modes of transmission into human populations have been suggested: either direct contact to a reservoir or contact to other wildlife that also contracts EBOV from the reservoir [17]. Epidemiologic observations showed that chimpanzees were the source of one human case in two recently described outbreaks (Coˆte d'Ivoire in 1994 and Gabon in 1996) [13]. Transmission of EBOV in human population happens by direct contact with infected blood, or other bodily fluids (saliva, sweat, semen, milk), and tissues from dead or living infected persons [18]. Transmission via inanimate objects contaminated with infected bodily fluids is possible, the principal mode of transmission in human outbreaks is human-to-human transmission through direct contact with a symptomatic or dead EVD case or with contaminated surfaces and materials (bedding, clothing) [19, 20]. The risk for transmission is lower in the early stage of human disease (prodromal phase), but viral loads in blood and secretions rapidly increase during the course of illness, with the highest levels of virus shedding observed late in the course of illness of severely ill patients [21, 22]. Burial ceremonies and handling of dead bodies play an important role in transmission [20]. Amplified transmission occurs in hospitals (approximately one quarter of cases occurring among health care workers) [6].

Human, non-human primates and some other mammalian can be infected by EBOV and regarded as host, but reservoir of Ebola viruses was for a long period hidden. An intensive efforts were expend to identify the natural reservoir [18]. Previously some researchers suggested, rodents and bats could play role as reservoir animals of EBOV [23, 24]. First report about EBOV infected bats was verified by detection of antibodies and viral RNA in three bats species [25, 26]. What proved, that bats are involved in circulation of EBOV in nature [27]. Transmission of EBOV to humans in sub-Saharan Africa thought the contact with dead or living infected animals (non-human primates, antelopes and bats), although some studies traced cases back to the skinning and butchering of carcasses [28, 29]. Hunting and butchering of chimpanzee and fruit bats has been identified in previous outbreaks as a potential source of infection [3, 7]. Leroy et al. presented, the human outbreaks consisted of multiple simultaneous epidemics caused by different viral strains, and each epidemic resulted from the handling of a distinct gorilla, chimpanzee, or duiker carcass [30]. Due to this facts surveillance of wildlife health and monitoring of their mortality may help to predict and prevent next Ebola outbreaks.

4. Clinical characterization and pathogenesis of EVD

The incubation period of EVD ranges between seven to ten days [31], but can be shorter (two days) or longer (21 days) [7]. Virus presence in blood is usually detectable one day before clinical symptoms appear, using polymerase-chain-reaction-based methods, increasing amounts of virus in the bloodstream are accompanying events of the fatal illness [32]. Case fatality rates have varied from 44 to 90 percent, depending on the strain of EBOV, the level of medical care, and public awareness, etc. [33]. Typically clinical symptoms of EVD are sudden onset of fever, accompanied by flu-like symptoms, such as headache, malaise, myalgia, eventually vomiting, and diarrhea [20]. Only in 30–50 percent of EVD patients developed hemorrhagic symptoms [34]. Severe cases of disease are characterized by hepatic damage, renal failure together with multi-organ dysfunction, and central nervous system complication [7]. Mortality is caused by multi-organ failure and severe bleeding sites [7, 35]. During the terminal phase of illness, infected patients bleeding massively from the gastrointestinal tract affected by disseminated intravascular coagulation are relatively rare [7, 15]. Conversely, patients who survive infection show a decrease in amounts of circulating virus and clinical improvement

around day 7–10, what is usually linked with the occurrence of EBOV-specific antibodies [36]. Non-fatal or asymptomatic cases tend to be associated with a specific IgM and IgG responses and early and strong inflammatory response, including interleukin β, interleukin 6, and tumor necrosis factor α [7]. EBOV infected animals showed renal lesions with disseminated microvascular thrombosis evident in glomerular capillaries leading to acute kidney injury due to hypoperfusion [37]. Minimal or mild changes with no clinical significance were observed on lungs as a respiratory failure is not a common feature of EBOV [38]. Lesions on livers of infected macaques developed hepatic necrosis with mild to moderate inflammation, moreover elevation of hepatocellular and pancreatic enzymes are frequently presented [39, 40].

The pathogenesis of the disease is still not completely known. EBOV can enter the host body mostly via mucosal surfaces, or injuries in the skin [41]. Also infection through the intact skin cannot be excluded, although it is considered unlikely. Aerosol infection (RESTV) has been demonstrated in non-human primates under experimental conditions in dispersion chambers [42, 43]. EBOV infection is characterized by immune suppression and a systemic inflammatory response, which could cause damage of the vascular, and immune systems, that can lead to multiorgan failure and shock [7]. Geisbert et al. presented study in non-human primates and showed that EBOV replicates in monocytes, macrophages, and dendritic cells; however, in situ hybridization and electron microscopy have also shown the presence of virus in endothelial cells, fibroblasts, hepatocytes, and adrenal cells [44]. Del Rio et al. demonstrated, the EBOV disseminates to the lymph nodes, liver, and spleen [6].

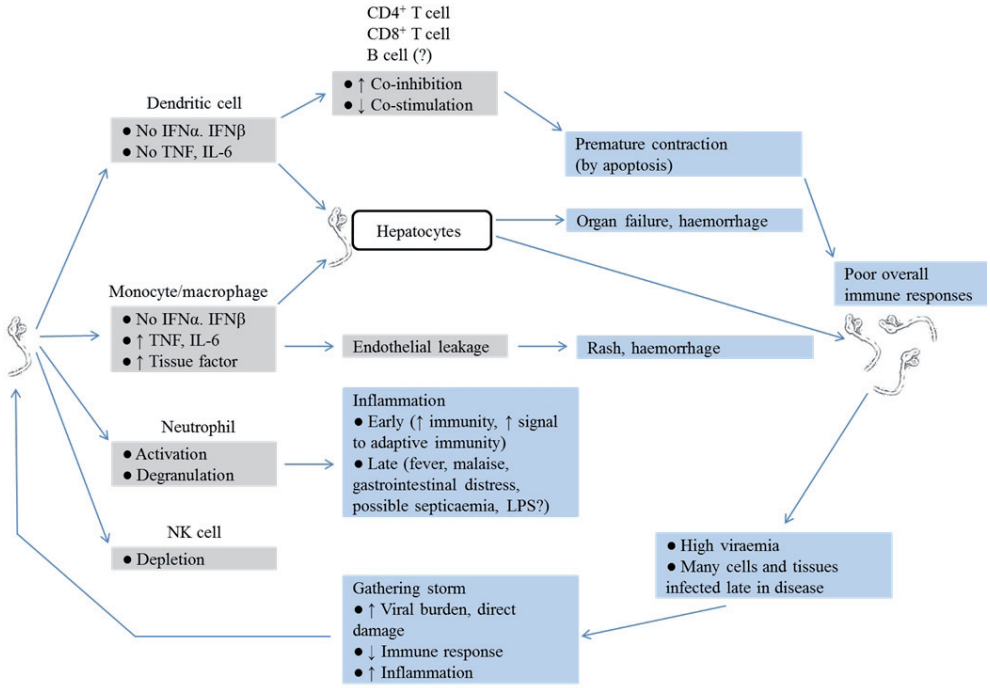

Figure 1: Overview of Ebola virus pathogenesis

4. Conclusions

The understanding of EBOV pathogenesis is crucial for the development of efficacious treatments and vaccines. Targeted therapy and vaccination are an immediate and highest priority. The danger, hidden in high virulent and infectious EBOV, breakdown of public health and preventive measures, and create significant challenges ahead. Resolving all of issues connected with EBOV will require long term and collaboration of scientific community with multinational organizations.

Acknowledgments

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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Hemagglutinin structure, membrane fusion and virus entry

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Hemagglutinin (HA) is an antigenic glycoprotein, which is placed on the surface of the influenza viruses. It is responsible for binding the virus to the host cell, that is being infected. The name „hemagglutinin" comes from the ability of protein to cause erythrocytes to agglutinate („clump together"). The process is like this: Hemagglutinin (HA) binds to the monosaccharide sialic acid which is present on the surface of its target host cells. The cell membrane then engulfs the virus through endocytosis and followed by formation of endosome. The cell then attempts to begin digesting the contents of the endosome by acidifying its interior and transforming it into a lysosome. When the pH decrease to 6.0, the HA molecule becomes partially unfold, and release a hydrophobic portion of peptide chain that was previously hidden. This so-called „fusion peptide" acts like a molecular grapple hook for lock on the endosomal membrane. The rest of the HA molecule refolds into a new structure and pulls the endosomal membrane right up next to the viral membrane, causing the two to fuse together. When it happened, the viral RNA genome enters into the cell's cytoplasm.

1. Introduction

Influenza A viruses belong to the order Orthomyxoviridae and are responsible for significant annual morbidity and mortality. They are classified serologically based on the antigenic properties of their surface glycoproteins: the hemagglutinin (HA) and the neuraminidase (NA) [1]. To date, 18 HA subtypes, caused by antigenic shift, have been determined [2, 3]. These subtypes can be divided into 6 clades and two groups and this variability makes it difficult to effectively aim any drug against this structure. Furthermore, also antigenic drift can strengthen the ability to escape the virus from effective blockage [4].

2. Structure and function of hemagglutinin

HA is a type I transmembrane glycoprotein with a signal sequence that is removed post-translationally, a membrane anchor domain near the C terminus, and a short cytoplasmic tail [5]. Its size si about 13.5 nm and a molecular weight of about 76 kDa [6]. HA is a target molecule for neutralizing antibodies, and therefore is considered as the major surface antigen [7]. Primary function of HA is the initiation of infection, HA also involved in the host cell recognition and binding of the virus to host cell receptor, which is composed of sialic acid [6, 8]. The virus-receptor binding is followed by virus entry into the host cell and release of viral RNA from the virion, which allows subsequent replication. Nowadays, H1 (H1N1) and H3 (H3N2) are the most widespread HA influenza subtypes in human population. Also other subtypes, which are typically occured in waterfowl, may cause human infection or deaths such as H5 [9, 10], H7 [11], and H9 [12].

HA monomers are synthesized are assembled noncovalently to trimer in the endoplasmic reticulum, where also glycosylation occurs, and transported through the Golgi complex to the cell surface of infected cells as an uncleaved,

fusionin-incompetent precursor HA0, which is proteolytically cleaved into two smaller subunits (HA1 and HA2) [13-16]. Disulphide bridges, which linkage HA1 and HA2 subunits, which were formed in viral replication in the HA0 folding proces, are cleaved [17, 18]. HA0 cleavage can take place either in the Golgi apparatus or extracellularly (using enzymes produced by cells of the respiratory system), the process HA0 cleavage is essential for infectivity of the virus particles [19, 20]. After cleavage of HA0 precursors, three HA structures form a trimer, which has a mushroom shape [18]. Mushroom consists of antiparallel beta-sheet region HA1 subunits and elongated membrane-proximal domain (stem region) dominated by intertwined and interconnecting α-helices (HA2) [21]. Large membrane-distal, globular HA1 subunit, or the receptor binding region enables the binding of the virus to glycan receptors on host cells, which are formed by the sialic acid bounded by galactose [18, 22, 23].

3. Membrane fusion and virus entry

Sialic acid, linked to complex glycans on either glycoproteins or glycolipids, is the receptor for influenza binding. No significant conformational change of HA appears during receptor binding and virion is just attached to the cell surface. Fusion-inducing conformational change is activated by binding of one or more of protons, as the pH in the endosome goes lower [24]. Enveloped viruses enter cells through fusion of their viral membrane with a host cell membrane. This fusion process is thermodynamically favorable but kinetically very slow [25].

Influenza infection is initiated by the viral HA binding to sialic acid receptors on the surface of the host cell. It is widely accepted that the human-adapted HA subtypes preferentially bind to the α(2,6)-sialic acid linkage, whereas the avian-adapted HA subtypes preferentially bind to the α(2,3)-sialic acid linkage [26, 27]. Membrane fusion between host cell and influenza virus is a thermodynamically favorable process, but a high kinetic barrier is crossed as the two bilayers approach each other [28]. When bound to the specific sialic acid receptor on the target cell, the influenza virion is endocytosed in coated pits and vesicles, and delivered to endosomes [17]. This process is cell-type dependent and influenza virus can enter the host cell using either clathrin-dependent and clathrin-independent endocytosis or by macropinocytosis [29-31]. Proton pumps in the membranes of endocytic vesicles induce an accumulation in protons and therefore lowering of the pH between 5 and 6, which is essential for HA cleavage and causes the HA1 'head' to separate from the HA2 'stem' and enables a set of HA2 conformational transformations [13, 32]. This change causes the exposure of the N terminus of HA2, known as the fusion peptide and is required to promote fusion between the viral envelope and the target membrane and therefore is essential for virus infection [33-35].

4. Conclusions

The infection process of HA has been well documented throughout the years. But, the understanding of the influenza viral attack mechanism is still crucial for designing of new antiviral therapeutics such as protease and fusion inhibitors and cross-neutralizing antibodies that interfere with the fusion process.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements" for biomedical papers.

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Paramagnetic particles for immobilization of bacteriophage λ

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This study presents synthesis of paramagnetic particles formed by nanomaghemite (γ-Fe_2O_3) core, whose surface was modified with $LaCl_3$ and entire composite is able to specifically bind the bacteriophage λ. In this study we described the structure of bacteriophage λ, its biochemical properties and role in the therapy, biotechnology and nanomedicine. In the next part we summarized accessible information about paramagnetic particles, their attributes and specificity of binding the bacteriophage λ. Paramagnetic particles show the ability to immobilize bacteriophage λ for subsequent analysis using ion-exchange chromatography with Vis detection. The presence of bacteriophage λ on the surface of paramagnetic beads was demonstrated by sodium dodecyl sulphate gel electrophoresis (SDS-PAGE). It can be stated that paramagnetic beads show significant binding ability towards bacteriophage λ. This phenomenon can be employed for magnetic separation of bacteriophages prior their possible modifications and subsequent application as a cytostatic nanotransporters or antimicrobial agents usable in hard-to-heal bacterial infections.

Keywords: Bacteriophage λ; gel electrophoresis; ion-exchange chromatography, superparamagnetic iron oxide nanoparticles

1. Introduction

Bacterial viruses, or bacteriophages, are viruses of bacteria, which are estimated to be the most widely distributed and diverse entities in the biosphere. From initial research defining the nature of viruses, trough deciphering the fundamental principles of life, to the development of the molecular biology science, phages have been suitable model organisms [1]. Thus, related to their nature, they can serve as the potential antibacterial agents. Recently, the idea of phage therapy which is the use of bacteriophages for both the prophylaxis and the treatment of bacterial infections, has gained special significance in view of a dramatic rise in the prevalence of highly antibiotic-resistant bacterial strains concurrently by the rescission of the pharmaceutical industry from research of new antibiotics. For the development of new antibiotics it is possible to use bacteriophage-encoded lysis-inducing proteins, either as recombinant proteins or as lead structures. Two additional potential medical applications of phages are the treatment of viral infections and their use as immunizing agents in diagnosis and monitoring of patients with immunodeficiences. Due to the immunomodulatory activity of bacteriophages, endogenous phages can have a possible role in maintaining the homeostasis of the immune system [2]. Phage research in more recent years has revealed not only their abundance and diversity of form, but also their influence on the evolution of microbial populations, and their possible applications [3]. Bacteriophages play a fundamental role in this new post-genomic era of phage biology, from

information emerging from genomics and metagenomics approaches through to applications in therapy [4], biotechnology and nanomedicine [5]. Bacteriophage λ is a coliphage (infects *Escherichia coli*) from *Siphoviridae* family, which belongs to tempered phages. The DNA molecule is packaged in the phage's head containing single stranded complementary ends (cos sites) that hybridize shortly after injection into the host. In the cell the phage λ can enter the lytic or lysogenic pathway [6]. During lysogenic cycle, 100 new virions are created from a single virion [6]. The viral capsid is a special type of often studied protein nanocarriers [7].

Bacteriophage λ could be used as such nanocarrier; the capsid has the icosahedral form with a diameter of 50-60 nm. Its genome contains approximately 50 genes in a linear double-stranded the DNA of 48 502 bp [8].

Superparamagnetic iron oxide nanoparticles (SPIONs) are novel material with exceptional attributes, which is currently more and more employed in various scientific areas, such as analytical chemistry to improve the analytical performance [9], biosensors development to enhance the specifity and sensitivity [10], and others. Due to possibility of surface modification, SPIONs can provide binding site towards broad spectrum of analytes, and thus serve for its immobilization, isolation of target delivery. Therefore, the main goal of this study is synthesis and characterization of modified SPIONs for immobilization of bacteriophage λ with aim to improve the operation possibilities for further applications in antimicrobial treatment or cytostatic encapsulation.

2. Results and Discussion

To demonstrate the binding ability of different paramagnetic particles (PMPs) prepared by us, we selected phage λ, a well-known bacteriophage from *Siphoviridae* family. In the first place, we compared a binding capacity of eleven paramagnetic particles synthesized by us. Before PMPs application it was necessary to include six washing steps with phosphate buffer saline (PBS), to remove the undesired impurities, contained in the solid PMPs after synthesis. Next, to achieve the highest yield of phage λ binding on surface of PMPs, the incubation was performed according to our previous study, engaged in the isolation of H7N7 virions [11]. After incubation, the solution containing unbound phage λ, PMPs were removed using the force of external magnetic field provided by the permanent magnet (Chemagen, Baesweiler, Germany). For characterization of phage λ binding attributes towards the paramagnetic particles the Tris-Glycine SDS-polyacrylamide gel electrophoresis was used. Tris-Glycine SDS--PAGE has been commonly used for protein analysis for more than forty years [12]. This method allows for the separation of proteins mixture according to their molecular weight [12]. Employed system allows effective separation of proteins in the range approximately from 300 kDa to 15 kDa. The intensity of the major constitutive protein - gpE on gel was measured and it was revealed that nearly all of particles were able to immobilize the tested organism. The highest yields of phage λ bound on surface of PMPs were observed in case of MAN81, modified by $LaCl_3$ (**Fig. 1A**). To further investigate the interaction between phage λ and modified SPIONs, IEC-Vis was employed, however prior to analyses remaining solid PMPs with bound phage λ had to be dissolved in
3 M HCl to disrupt complex protein structure of bacteriophages. After dissolution particles were evaporated by nitrogen blow-down evaporator. Evaporated analyte was finally resuspended with dilution buffer and analyzed. As it is shown in Fig. 1B, the highest sum of total amino acids (about 114 μM) was again observed in case of MAN81 (**Fig. 1B**). Strong charge-charge interaction may be utilized for maintenance of specificity of PMPs-based isolation of phage λ. Utilization of natural affinity phage λ based on different charges offers a tool for its isolation using different nanoparticles.

3. Experimental Section

3.1. Chemicals

Lanthanum(III) chloride, sodium tetrahydridoborate, sodium citrate and all buffer solutions were obtained from (Sigma-Aldrich, St. Louis, MO, USA) in ACS purity unless noted otherwise. Methyl cellosolve and tin chloride were purchased from Ingos (Prague, Czech Republic).

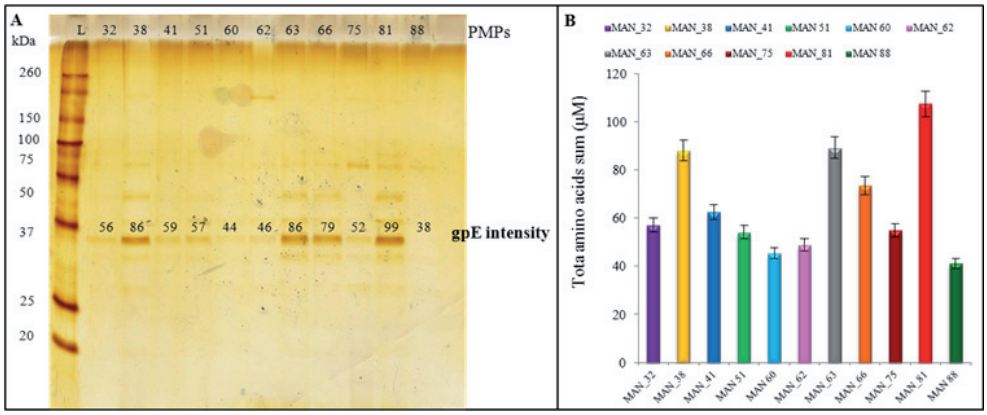

Figure 1: (**a**) Tris-Glycine SDS-PAGE of phage λ immobilized on a surface of variously modified SPIONs. Highlighted is band of major head protein - gpE (**b**) The IEC-Vis results showing total sum of amino acids determined to be bond on a surface of different types of modified SPIONs

3.2. Synthesis and modification of superparamagnetic iron oxide nanoparticles

Nanometric maghemite nanoparticles were prepared by dissolving of 7.48 g of iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \bullet 9H_2O$) in 400 mL of water. Reduction was carried out by addition of 1 g $NaBH_4$ in 50 mL of 3.5% NH_3 (w/v). Resulting mixture was warmed at 100°C for 2 hours and washed five times with water. Washed product was resuspended in the water (500 mL). For experiments, obtained superparamagnetic nanoparticles were further modified instead of MAN32 (pure nanomaghemite). Modifications were as follows: MAN38: 3-aminopropyl triethoxysilan (APTES) and triethoxysilane (TEOS), as is described in our previous study [11], MAN41: addition of 5 mL of $RuCl_3$, MAN51: addition of 1 g of amberlite, MAN60: 100 mg of GR-4 and 1 g of glucose, MAN62: 1 mM $HAuCl_4$ and 0.75 mL of 0.1 M trisodium citrate, MAN63: 100 mg of graphene oxide, MAN66: 3 g of polyvinylpyrrolidone (PVP), MAN75: 30 mL of i-PrOH and 4 mL of 28% APTES, MAN81: 5 mL of $LaCl_3 \bullet 7H_2O$ and MAN88: 75 mL of i-PrOH with 10 mL of ammonium and 1.66 mL of N1-(3-trimethoxysilylpropyl)diethylenetriamine (BAATMS). All products were stirred overnight, separated using an external magnetic force field and dried at 40°C.

3.3. Cultivation and purification of bacteriophage λ

Phage λ-producing strain of Escherichia coli was cultivated in Luria-Bertani broth (1% tryptone, 0.05% yeast extract and 1% sodium chloride) with 0.2% maltose for 24 h at 37°C and 600 rpm. After cultivation, the culture was mixed with chloroform at 6:1 ratio and incubated for 1 h at 25°C to kill the producing *E. coli*. The samples were centrifuged at 5200 g and 4°C for 10 min to remove *E. coli* and then at 10000 g and 4°C for 6 min to remove remaining contaminants. Next the supernatant containing phage was ultracentrifuged at 130000 g and 4°C for 3 h. The pellet containing phage was resuspended in PBS at protein concentration of 15 $\mu g.mL^{-1}$ and stored at 4°C.

3.4. Tris-Glycine SDS-PAGE

Tris-Glycine SDS-PAGE was performed according to Laemmli et al. [13]. For electrophoresis Mini Protean Tetra apparatus with gel dimension of 8.3 × 7.3 cm (Bio-Rad, USA) was used. First 12.5% (m/V) running, then 5% (m/V) stacking gel was poured. The gels were prepared from 30% (m/V) acrylamide stock solution with 1% (m/V) bisacrylamide. The polymerization of the running or stacking gels was carried out at room temperature for

45 min. The samples were loaded in 5 µL Sample Buffer (20 % glycerol, 0.1% bromophenol blue, 50 mM Tris, 2% SDS) in 2:1. For determination of the molecular mass, the protein ladder #7703 from New England Biolabs (Ipswich, MA, USA) was used. The electrophoresis was run at 200 V for 33 min at 20 °C (Power Basic, Bio--Rad USA) in Tris-Glycine running buffer (0.025 M Trizma-base, 0.19 mol.L^{-1} glycine and 3.5 mmol.L^{-1} SDS, pH = 8.3). The silver staining was performed according to Krizkova et al. [14]. The intensity of gpE proteins on gel was measured using the Carestream In vivo Xtreme software (Carestream Health, Inc., Rochester, NY, USA).

3.5. Preparation of sample PMPs for bacteriophage isolation

For isolation, we employed 50 µL of dispersion, comprising of 40 mg.mL^{-1} PMPs in 1mL PBS (pH 7) with 250 µL of phage λ. After isolation (37°C, 1250 rpm, 30 minutes) in thermomixer (Eppendorf thermomixer comfort, Germany), the sample was dissolved in 3 M hydrochloric acid (250 µL) and evaporated using nitrogen evaporator Ultravap RC (Porvair Sciences, Leatherhead, UK). Finally, the evaporated sample was resuspended with ACS H_2O (250 µL) and analysed using on ion-exchange chromatography AAA 400 (Ingos, Prague, Czech Republic).

3.6. Ion-exchange chromatography

For the identification of paramagnetic particles binding capacity the ion-exchange liquid chromatography AAA 400 with post column derivatization by ninhydrin was used and the absorbance detector in the VIS range set to 440 nm was employed. Glass column, tempered to 60°C with inner diameter of 3.7 mm and 350 mm length was filled manually with strong cation exchanger in sodium cycle LG ANB with approximately 12 µm particles and 8% porosity. Experimental conditions were applied according to our preliminary study [9].

4. Conclusion

In this study we characterized the binding attributes of paramagnetic particles towards bacteriophage λ. The highest binding yields of phage λ were achieved by using of SPIONs modified with $LaCl_3$. Since phage λ is able to efficiently kill dangerous pathogen - E. coli, it might play important role in management of these hard-to-heal bacterial infections. Our preliminary results show that bacteriophages could be effectively immobilized on a surface of nanomaterial, which can thus serve as a platform for manipulation with phages for further operations - driven application, drugs encapsulation of phages surface modifications.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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ARTICLE

Technical concept of 3D printed fluidic biosensor with polydimethylsiloxane chip based on fluorescence detection system

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There is a rapidly growing interest in low-cost, fast and sensitive biosensors. In particular, direct determination of important metabolites, serving as biomarkers of various pathological states can significantly enhance the treatment successes. In our study, we introduce a technical concept of a 3D printed biosensor, which employs polydimethylsiloxane chip with volume of 50 μL as an inert and optically clear reservoir for recognition element and fluorescence detection. By using a 3D printing technology, low production cost and high crafting reproducibility were achieved. Due to a presence of controlled electromagnet, the biosensor can be utilized for a broad spectrum of applications, based on paramagnetic nano- or microscaled materials.

Keywords: 3D printing; acrylonitrile butadiene styrene; biomarkers; fluorescence

1. Introduction

A biosensor is an analytical device, which can be easily employed for detection of broad spectra of analytes, including molecules, bacteria, viruses, etc. [1]. The development of biosensors provides an accurate, sensitive and specific detection, together with portability and the ability to furnish continuous real time signals of analytes levels in various matrixes. According to the definition, firmly established in the analytical field "biosensor" is a detection system that relies on a biomolecule for molecular recognition and a transducer to produce an observable output [2]. Molecular recognition element (e. g. antibodies, nucleic acids, receptor proteins, whole cells or bacteria) is fundamental component that interacts with the studied analyte [3]. Currently, very promising recognition elements are produced as a result of driven recombination in biological engineering. The transducers can work in different ways, such as physicochemical, optical, piezoelectric, electrochemical, etc.). Their role is the transformation of obtained signal, which goes from the analyte-recognition element interaction [4]. The transformation leads to more easily measured and quantified signals. Nowadays, few commonly used biosensors such as glucose biosensor are in every-day use all over the world. The development of novel biosensors for easy, fast and cheap detection of various types of biomarkers for cancer, oxidative stress or metabolical disorders, can significantly enhance the treatment successes and the survival rates. From these reasons, we suggested a technical concept of 3D printed biosensor with polydimethylsiloxane (PDMS) chip, utilizing the fluorescence detection, which is one of the most sensitive, to serve as a universal platform for detection of various types of biomarkers. Proposed biosensor was particularly designed to work in coupling with quantum dots-based labeling, which offers exceptional quantum yields. Various recognition biomolecules can be easily labeled by quantum dots, thus high specifity and boosted sensitivity can be achieved.

2. Results and Discussion

2.1. PDMS chip

PDMS belongs to a group of polymeric organosilicon compounds, commonly referred to as silicones. PDMS is optically clear, inert, non-toxic and non-inflammable and these attributes makes PDMS ideal material for large scale of applications, such as production of contact lenses and other medical devices, heat-resistant tiles and it is also used as antifoaming agent in food [5]. In our case, beneficial attributes of PDMS made us to decide to utilize this material for preparation of chip with reservoir where the interactions between analyte and recognition element are carried out. In particular, inertness and optical clearness are fundamental for fluorescence-based biosensors. PDMS chip was produced in 3D printed pattern (Fig. 1a, b), which was smoothed for creating of chips without surface imperfections (Fig. 1c, d). 3D printed patterns are cheap and their manufacturing is highly reproducible, thus their utilization offers few advantages, such as possibility of low-cost, large-scale production of chips with identical attributes.

Resulting volume of reservoir in chip was 50 µL and comprised an input connected with reservoir and an output for waste (Fig. 2). Both, the input and the output were tightly connected with syringe needles, to avoid an unwanted leakage of sample.

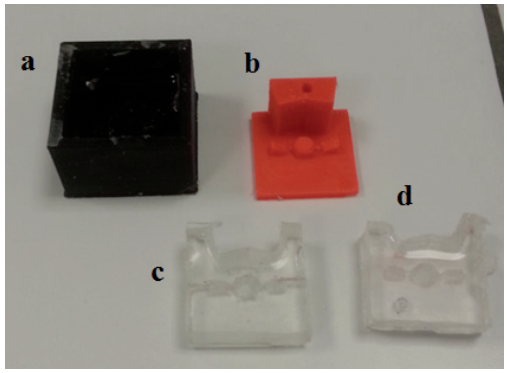

Figure 1: Photography of the patterns used for chip fabrication. The patterns (a, b) were manufactured by using 3-D printing, using acrylonitrile butadiene styrene (ABS) and (c, d) PDMS chips.

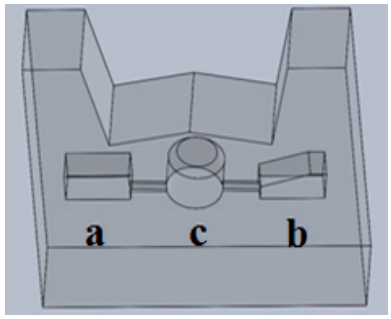

Figure 2: Schematic illustration of polydimethylsiloxane chip with (a) input, (b) output and (c) reservoir with volume of 50 µL.

2.2. Construction of fluidic device

As it is shown in a schematic drawing in Fig. 3A, the entire biosensor contained following 3D printed parts: holder for PDMS chip (3), emission (9) and excitation (10) filters, input (4) and output syringes (5) and part for pressing the PDMS in the holder (1). Other parts were purchased and were as follows: emission bandpass filter (λ = 550(55) nm) (8) and shortpass excitation filter (λ = 425 nm) (7), UV-Vis optical fibers with diameter of 3 mm (6) and electromagnet (11). In the case of excitation part, there is a slot for high-power LED (λ = 400 nm) as an excitation source. In emission part, the biosensor was connected with a control unit Arduino, which was linked to a LED driver and UV-Vis photodiode with photomultiplier. As system was designed to be flow-through, the leakage of analyzed liquid forms a large problem. Hence the PDMS chip with syringe needles was pressed between the holder and the pressing part by using four M3 screws. Such seal allowed for washing of the chip during the load tests without observable leakage. The electromagnet can be employed for both - immobilization of paramagnetic particles during magnetic separation or stirring of samples with paramagnetic materials, and is controllable through the control unit.

As is mentioned above, biosensor is designed particularly for manipulation with paramagnetic nano-, microscaled materials. Due to

communication between electromagnet and control unit, paramagnets can be easily immobilized or stirred, in dependence on application. Thus, the device can be employed for a broad spectrum of applications, such as particle-based immunoassays [6], magnetic separation of low--mass compounds [7], direct isolation of viral particles [8] or study of complex paramagnetic nanostructures, comprising fluorescence labels [9].

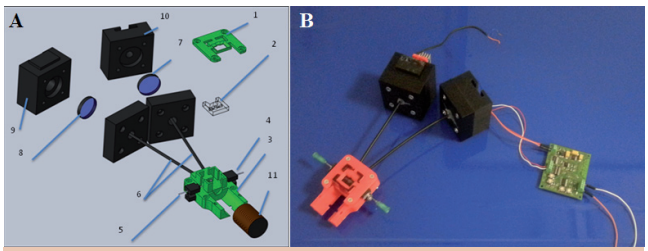

Figure 3: (A) The schematic drawing of individual parts employed for biosensor, where 1 stays for pressing part, 2 for PDMS chip, 3 for PDMS holder, 4 for input, 5 for output, 6 for optical fibers, 7 for shortpass excitation filter, 8 for bandpass emission filter, 9 for emission filter platform and 10 for excitation filter platform, (B) the assembled biosensor ready-to-use

3. Experimental Section

3.1. Fabrication of 3D-printed biosensor device

The platforms for PDMS chip, excitation, emission filters and optical fibres were manufactured by using 3D-printing technology (acrylonitrile butadiene styrene - ABS as the production material) by using 3D printer PROFI3DMAKER (Aroya, Straznice, Czech Republic).

3.2. Preparation PDMS chip

PDMS chip was fabricated in the pattern, made from ABS to produce the resulting structure with reservoir with the volume of 50 µL. Prior to use in biosensor, PDMS (Sylgard 184, Dow Corning, Midland, MI, USA) was evacuated to remove the unwanted air bubbles, which can interfere with optical light paths. For one chip 2 mL of PDMS and 100 uL of curing agent were mixed. Resulting mixture was further evacuated, filled into 3D printed pattern and left at 25 °C.

3.3. Detection system parts

As excitation source was employed power LED (λ = 400 nm, optical power 200 mW) (Roithner LaserTechnik, Vienna, Austria). Emission bandpass filter (λ = 550 ± 55 nm) and excitation shortpass filter (λ = 425 nm) were purchased from Semrock (Rochester, NY, USA). Optical fibers (UV-Vis transparent, core fiber diameter 1960 µm, numerical apparatus 0.5) were obtained from Edmund optics (Barrington, NJ, USA). The system was driven by the control unit Arduino linked to the LED driver. Evaluation of signal from UV-Vis photodiode S3991-01 (Hamamatsu Photonics, Hamamatsu, Japan) was done by multifunction two channels amplifier board (Digi board, Sglux, Berlin, Germany).

4. Conclusions

Biosensors can serve as promising tools for fast, cheap and sensitive analyses of broad spectrum of analytes. There exists many technical concepts and ways how to design and fabricate diverse platforms. 3D printing in combination with non-toxic, inert materials offers a lot of advantages, such as low production costs, biocompatibility, significant reproducibility and fast crafting time. Moreover, in conjunction with fluorescence detection, high sensitivity can be achieved.

Acknowledgments

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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SCIENTIFIC ABSTRACT

Zinc and its importance for organisms

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ABSTRACT

Zinc (Zn) is essential element, which plays important role in biosynthesis and stabilization of fundamental proteins and DNA. Further, it was revealed that Zn is connected with more than 300 enzymatic reactions. Insufficient amount of Zn in food leads to alterations of neuronal functions, and alterations in physical and psychical development. Into cell, Zn is transported mostly through ZiP1 transporter and intracellularly is further utilized into proteins structure. The control of Zn equilibrium is carried out by metallothionein proteins, which contain cystein residues that exhibit high affinity towards metals, and thus can maintain the redox state.

One of the most crucial utilization of Zn in organism is its incorporation into protein structure to form zinc finger motif. Zn finger motif (ZnF) plays substantial role in stabilization of molecular structure and proper functions of transcription factors. Zn finger is formed by simple amino acid sequence, using duo of histidines and cysteines for zinc stabilization (Fig. 1).

ZnF motifs are highly stable structures which rarely undergo conformational changes, which can be used in the design and preparation of artificial ZnF proteins with high affinity to a specific sequence for use in the treatment of a wide spectrum of diseases.

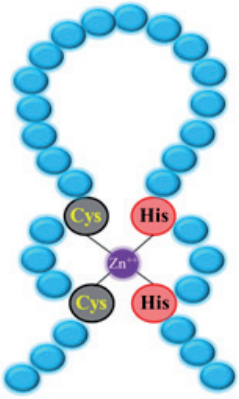

Figure 1.: Schematic drawing of secondary structural motif - ZnF

Keywords: Metals; Transcription factor; Signalling; Zinc finger;

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Electrochemical study of *Escherichia coli* bacterial culture with the cloned gene for metallothionein (*MT-3*) and effect of cadmium and lead ions

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ABSTRACT

The most common cause of environmental contamination by heavy metals is agriculture and industry. Because of this issue, it is necessary to look for new techniques to prevent this contamination particularly in terms of human health and the environment. For these purposes, the electrochemical methods for detection of interactions between human metallothionein genes cloned in Escherichia coli bacterial culture with heavy metal ions were used.

This study was focused on the application of electrochemical methods for study of bacterial strain Escherichia coli without and with cloned human metallothionein gene (MT-3) before and after the application of cadmium or lead ions in four concentrations (25, 50, 75 and 150 μM).

Study demonstrated changes in electrochemical metallothionein records through interaction with the heavy metal ions. This interaction caused the decrease in signal of Cat2 peak with increased interaction time due to the binding of metal ions to cysteine. Electrochemical determination also proved that the cadmium ions are more binding into the bacterial cells without the presence of plasmid with the MT-3 than lead ions. Then completely opposite situation was observed for the Escherichia coli strain with MT-3 gene. This was probably caused due to the higher affinity of MT-3 to lead ions than to cadmium ions.

Our results describe the analysis of Escherichia coli bacterial strains and effect of transformed gene presence.

Keywords: Cloned gene; E. coli; Electrochemistry; Heavy Metal Ions; Metallothionein;

Acknowledgments

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Properties and toxicity of extracellularly biosynthesized quantum dots formed by *Escherichia coli*

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ABSTRACT

Nanobiosynthesis belongs among the most recent methods for nanoparticles synthesis. This type of synthesis bears many advantages such as uniformity in particles shape- and size. Biosynthesis is also considered due its significant advantage regarding the properties of the particles obtained. In this study, we characterized the basic properties and composition of quantum dots (QDs), obtained by the extracellular biosynthesis of Escherichia coli. Furthermore, the toxicity of biosynthesized QDs and QDs prepared by microwave synthesis was compared. The obtained results revealed the presence of cyan CdTe QDs after removing substantial amounts of organic compounds, which stabilized the surface of the nanoparticles. QDs toxicity was evaluated using three cell lines (HFF, PC-3 and MCF-7) using the MTT assay. The test revealed toxicity differences between variants of QDs, varying about 10% in the HFF and 30% in the MCF-7 cell lines. Biosynthesized QDs were evaluated to be about 35% less toxic to the PC-3 cell lines than the QDs prepared by microwave synthesis.

Keywords: quantum dots, biosynthesis, Escherichia coli (E. coli), CdTe, toxicity

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Monitoring of metal ions in the plasma of children with tumour diseases

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ABSTRACT

Human exposure to metals is common due to wide presence in industry and long-term environmental persistence. Among the general population, exposure to a number of metals is widespread but generally at substantially lower levels than have been found in industry. Accumulation of metal ions in fatty tissues and circulatory system, negative effects on central nervous system and functioning of internal organs as well as acting as triggers of several serious diseases including tumour ones can be listed as adverse effects of metal ions on humans. Metals are toxic especially for children, because of their tolerance to poisons is lower.

In this study the changes of metal ions levels (Zn, Cd, Pb and Cu) were monitored in the blood plasma of child patients treated for various oncological diseases. Electrochemical method differential pulse voltammetry with fully automated system and atomic absorption spectrometry was used for determination of the metal ions.

It was found an increased amount of metal ions in the blood plasma of patients suffering from cancer disease in comparison with physiological values in healthy people. Highest levels of Zn(II) were detected in neuroblastoma and hepatoblastoma, Cd(II) in the non-Hodgkin lymphoma, Pb(II) for nephroblastoma and testicular germ cell tumour and Cu(II) in testicular germ cell tumour and hepatoblastoma.

Keywords: AAS; Electrochemical Analysis; Metals; Oncological Diseases; Plasma;

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SCIENTIFIC ABSTRACT

Interaction of heavy metals with carbon and iron based nanomaterials

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ABSTRACT

The environmental pollution, especially groundwater and waste water, by heavy metals is a global issue and it is solved by many developing and also developed countries. For this reason, many scientists are focused on the creation of new materials with specific properties which would allow the adsorption of heavy and transition metals on their surface.

This study was focused on the adsorption of different heavy or transition metals (Cd(II), Pb(II), Cu(II) and As(III)) on the surface of carbon based (reduced grapheme oxide, MWCNT, expanded carbon, graphene oxide, graphite oxide, partially reduced graphene oxide) and iron based materials (γ-Fe_2O_3 nanoparticles). There were used different times of interaction (1, 5, 10, 15, 30 minutes and 1, 3, 6, 12 and 24 hours) and the adsorption efficiency was determined using difference pulse voltammetry.

Electrochemical determination proved that the adsorption efficiency of Cd(II), Cu(II) and Pb(II) was 100% from applied concentration 100 μM and 24 hours interaction in case of reduced graphene oxide and iron nanoparticles. In case of expanded carbon and MWCNT the adsorption efficiency reached the maximum value of 85%. The adsorption capacity of reduced graphene oxide and iron nanoparticles was determined as 100 μM for all used heavy metals. For As(III) the graphene oxide, graphite oxide and partially reduced graphene oxide as adsorbents were used. In case of graphene oxide the adsorption efficiency reached 35% in highly acidic pH. These results were confirmed by atomic absorption spectrometry.

Keywords: Heavy metals, carbon based materials, iron nanoparticles, electrochemistry

Acknowledgments

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Interaction study of arsenic (III and V) ions with metallothionein gene (mt2a) fragment

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ABSTRACT

Arsenic is classified as a global pollutant and human carcinogen, to which humans are exposed, occurring in the environment coming from both natural and/or anthropogenic sources. The toxicity depends on the oxidation state or methylation level during the biotransformation in the organism, whereas oxidative stress induced by arsenic exposure is suggested as a potential mode of its carcinogenic action. Inorganic forms of arsenic appears in two biologically important oxidation states: As(V) (arsenate) and As(III) (arsenite), which are highly toxic. There are currently over 100 active clinical trials involving inorganic arsenic or organoarsenic compounds registered with the Food and Drug Administration (FDA) for the treatment of cancers. Arsenic trioxide is presently the most active single agent in the treatment of acute promyelocytic leukemia (APL).

In our work, we focused on studying of interactions of As(III) and/or As(V) with DNA. Interactions between arsenic ions and DNA were monitored by UV/Vis spectrophotometry by measuring absorption and fluorescence spectra, atomic absorption spectrometry, electrochemical measurements (square wave voltammetry) and agarose gel electrophoresis. Using these methods, we observed a stable structure of DNA with As(III) within the concentration range 0.4 – 6.25 $\mu g.mL^{-1}$. Higher As(III) concentration caused degradation of DNA. However, similar effects were not observed for As(V).

Keywords: Anticancer drug; Arsenic; DNA Interaction; Electrochemistry; Spectrometry;

Acknowledgments

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