Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 2, 202–207
www.aaem.pl

REVIEW ARTICLE

Bacillus anthracis infections – new possibilities of treatment

Dorota Żakowska[1], Michał Bartoszcze[1], Marcin Niemcewicz[1], Agata Bielawska-Drózd[1], Józef Knap[2], Piotr Cieślik[1], Krzysztof Chomiczewski[3], Janusz Kocik[4]

[1] *Biological Threats Identification and Countermeasure Centre, Military Institute of Hygiene and Epidemiology, Puławy, Poland*
[2] *Warsaw Medical University, Department of Epidemiology, Institute of Social Medicine, Warsaw, Poland*
[3] *Faculty of Chemistry and New Techonlogies, Military University of Technology, Warsaw, Poland*
[4] *Military Institute of Hygiene and Epidemiology, Warsaw, Poland*

Żakowska D, Bartoszcze M, Niemcewicz M, Bielawska-Drózd A, Knap J, Cieślik P, Chomiczewski K, Kocik J. *Bacillus anthracis* infections – new possibilities of treatment. Ann Agric Environ Med. 2015; 22(2): 202–207. doi: 10.5604/12321966.1152065

Abstract

Introduction and objective. *Bacillus anthracis* is one of biological agents which may be used in bioterrorism attacks. The aim of this study a review of the new treatment possibilities of anthrax, with particular emphasis on the treatment of pulmonary anthrax.

Abbreviated description of the state of knowledge. Pulmonary anthrax, as the most dangerous clinical form of the disease, is also extremely difficult to treat. Recently, considerable progress in finding new drugs and suitable therapy for anthrax has been achieved, for example, new antibiotics worth to mentioning, levofloxacin, daptomycin, gatifloxacin and dalbavancin. However, alternative therapeutic options should also be considered, among them the antimicrobial peptides, characterized by lack of inducible mechanisms of pathogen resistance. Very promising research considers bacteriophages lytic enzymes against selected bacteria species, including antibiotic-resistant strains.

Results. Interesting results were obtained using monoclonal antibodies: raxibacumab, cAb29 or cocktails of antibodies. The application of CpG oligodeoxynucleotides to boost the immune response elicited by Anthrax Vaccine Adsorbed and CMG2 protein complexes, also produced satisfying therapy results. Furthermore, the IFN-α and IFN-β, PA-dominant negative mutant, human inter-alpha inhibitor proteins and LF inhibitors in combination with ciprofloxacin, also showed very promising results.

Conclusions. Recently, progress has been achieved in inhalation anthrax treatment. The most promising new possibilities include: new antibiotics, peptides and bacteriophages enzymes, monoclonal antibodies, antigen PA mutants, and inter alpha inhibitors applications. In the case of the possibility of bioterrorist attacks, the examination of inhalation anthrax treatment should be intensively continued.

Key words

inhalational anthrax, treatment, antibiotics, antibodies, peptides

INTRODUCTION

Nowadays, *Bacillus anthracis* is regarded as one of the biological warfare agents, and there is a potential threat of its use during bioterrorism attacks. This is why intensive research is carried out worldwide, aimed at the development of effective methods which could effectively protect humans and animals against this biological agent. To-date, antibiotics, such as doxycycline, ciprofloxacin, penicillin, levofloxacin, and vancomycin, have been the basic drugs used for the treatment of *B. anthracis* infection [1]. However, more recent research indicates the risk of low effectiveness of antibiotics against *B. anthracis* strains that show antimicrobial resistance (*B. anthracis* ST-1, *B. anthracis* Sterne) [2, 3, 4]. Furthermore, these drugs can fail to protect the organism during the advanced phase of anthrax infection, when bacterial toxins are intensively produced. Thus, there is an increasing need for the development of alternative methods of anthrax treatment, especially in terms of the inhalational form of the disease which is the most difficult to treat [4, 5, 6, 7].

Address for correspondence: Dorota Żakowska, Biological Threats Identification and Countermeasure Centre, Military Institute of Hygiene and Epidemiology, 24-100 Puławy, Lubelska 2 Str., Poland
E-mail: dzakowska@wihe.pulawy.pl

Received: 30 July 2013; accepted: 05 March 2014

Description of the state of the knowledge – Pulmonary anthrax. Pulmonary anthrax develops after inhalation of *B. anthracis* spores into the pulmonary airway, which is why this form of infection is also termed inhalational anthrax. The inhalational form of infection can develop when *B. anthracis* spores reach the pulmonary alveoli, where they are phagocytized by macrophages. Part of the spores is disintegrated and destroyed, while the remainder are transmitted by the lymph vessels into the mediastinal lymph nodes, where favourable conditions occur for conversion into the vegetative form capable of toxin production [6, 7, 8, 9]. When the process of spores germination takes place the disease develops very quickly because of intensive synthesis of toxins causing haemorrhage, edema and necrosis.

The classic approach to inhalational anthrax treatment is based on the administration of antibiotics as quickly as possible, and balancing the disrupted electrolytic and acid-base homeostasis. The following sections of this article present the results of the most up-to-date studies on new therapies used in the treatment of inhalational anthrax in animals.

Antibiotics. In recent years, many studies have been published describing the *in vivo* activity of new antibiotics against *B. anthracis*, such as levofloxacin, which is absorbed very quickly in the organism, reaching its maximal concentration

in serum 60 min. after administration [10, 11]. Levofloxacin was intravenously administered to rabbits in humanized doses (treatment with half of the daily dose every 12 h), taking into consideration the metabolism of animals. After determination of antigenemia [10, 11] and confirmation of bacteremia, treatment with levofloxacin was conducted for 5 days at a total daily dose of 25 or 12.5 mg/kg, resulting in nearly 90% and 70% protection of the animals, respectively. This antibiotic eliminated anthrax infection already after the first day of treatment, proving that intravenous injection of levofloxacin may be used as an effective therapeutic against inhalational anthrax [10, 11].

Some new antimicrobial agents, examined as potential new therapeutics against anthrax, can target bacterial cytoskeletal proteins. This property was shown in the case of oligochlorophen analogs based on the monomer 4-chloro-2,6-dimethylphenol, which exert inhibitory actions against *B. anthracis*. Oligochlorophen analogs may target the cytoskeletal protein FtsZ and have a minimal inhibitory concentration (MIC) of 160–320 nM against *B. anthracis*. FtsZ is a GTPase that assembles into the Z ring at the site of cell division, thus being involved in the remodeling of bacterial cell walls to produce a daughter cell. Analogs, such as 3Z1, are small molecules acting as inhibitors of FtsZ. MIC of 3Z1 against *B. anthracis* was ~320 nM, which was comparable to MIC values for tetracycline, penicillin G and riphampicin *B. anthracis* developed resistance to oligochlorophens at a rate of 4.34×10^{-10} (95% confidence interval, 7.26, 2.03) per generation. This was 10-fold lower than the resistance developed against other antibiotics used for anthrax treatment in humans, i.e. riphampicin and penicillin G [12].

Another new antibiotic showing *in vivo* activity against *B. anthracis* is daptomycin, belonging to a group of peptide antibiotics, which MIC_{90} was 4μg/ml. Female BALB/c mice were infected with aerosolized *B. anthracis* Ames spores in a LD_{50} dose (3.4×10^4 CFU/whole body), and treated with antibiotic 24 h after challenge, at a dose of 50 mg/kg of body weight twice a day, for 14 or 21 consecutive days. The following results were obtained: on the 43rd day after challenge, 6 of 10 mice treated for 14 days, and 9 of 10 treated for 21 days survived. The survival rate was comparable with the results of treatment with ciprofloxacin. Furthermore, animal tissues removed at the termination of the experiment were negative for *B. anthracis*, indicating that daptomycin can be considered as a potential therapeutic agent against anthrax infections [13].

The next antibiotic investigated was gatifloxacin. A study was performed to determine the pharmacokinetic-pharmacodynamic (PK-PD) measure of efficacy of gatifloxacin in order to establish the survival rate in a murine *B. anthracis* inhalational infection model. Gatifloxacin belongs to the fourth generation fluoroquinolones. In this study, 6 and 8 weeks old non-neutropenic female BALB/c mice were challenged with aerosol of *B. anthracis* Ames strain, in a 50% lethal dose. Gatifloxacin was administered at 6 or 8 h intervals, starting 24 h after challenge, for the following 21 days, in doses mimicking the concentration profile for humans. During that time, the survival rate of mice was determined. The experiment revealed that for adults the daily dose of gatifloxacin should be 400 mg, whereas in the case of children, the dose should amount to 10 mg/kg of body weight, allowing to obtain a 100% probability of attaining the PK-PD target (ED_{99}) for *B. anthracis* [14].

A novel, semi-synthetic second generation lipoglycopeptide is dalbavancin, which can be administered in a once-weekly dose in humans. Dalbavancin was demonstrated to have potent *in vitro* activity against *B. anthracis* (MIC range, <0.03 to 0.5 mg/liter MIC_{50} and MIC_{90}, 0.06 and 0.25 mg/liter, respectively). Efficacy of this antibiotic was studied on murine inhalation anthrax model. It was shown that peak concentrations of dalbavancin in mouse plasma 2 h after administration of single intraperitoneal doses of 5 or 20 mg/kg of body weight, were 15 and 71 mg/kg, respectively. In this experiment, treatment with the antibiotic began 2 h after infection. In the case of the 20 mg/kg dose, the presence of dalbavancin could be detected even 6 days after injection, indicating that the treatment with this antimicrobial agent could be possible at long intervals between doses. When mice were challenged with a lethal dose of *B. anthracis* Ames, efficacy of the treatment with dalbavancin was 80–100%, as determined by the survival rate at 42 days. In this case, the treatment was initiated 24 h after experimental infection, administering the antibiotic every 36 h in a dose of 15–120 mg/kg, or every 72 h in higher doses: 30–240 mg/kg. Treatment with dalbavancin 36 or 48 h post-challenge in a dose of 60 mg/kg (in the case of 36 h intervals), or 120 mg/kg (in 72 h intervals), provided a 70–100% protection of animals against anthrax infection. The low MIC and long efficacy duration *in vivo* indicate that dalbavancin can be a promising alternative in the treatment of *B. anthracis* infections [15].

Studies on blocking protective antigen (PA). CMG2 is the major cell surface receptor showing high affinity for PA and mediating anthrax toxin lethality *in vivo*. Studies have shown that a fusion of the extracellular domain of human CMG2 and human IgG Fc can be used as an alternative to anti-PA antibodies in protection against anthrax. CMG2-Fc fusion protein was produced in plants. This purified fusion protein, administered to rabbits in a dose of 2 mg/kg body weight at the time of experimental infection with *B. anthracis* spores, gave full protection to the challenged animals. The animals not only survived the initial challenge with *B. anthracis*, but also a re-challenge performed after 30 days. Glycosylation is known to affect binding of Fc receptor; thus, further studies tested whether two different generated forms of CMG2-Fc, having Asn – glycosylated ($CMG2\text{-}Fc_G$) or Gln – aglycosyl form ($CMG2\text{-}Fc_A$) – at the normal site of N – glycosilation in Fc, have an impact on the potency of this fusion protein. Both forms, $CMG2\text{-}Fc_A$ and $CMG2\text{-}Fc_G$, had the same neutralizing effect *in vitro*, as shown by TNA (toxin neutralization assay), at EC_{50} effective concentration of 50 ng/ml, and both proteins protected animals against anthrax ($CMG2\text{-}Fc_A$ gave a 100% protection in a dose of 2 mg/kg). The study demonstrated that CMG2-Fc significantly neutralized lethal toxin *in vitro* [16].

This chimeric fusion protein was composed of the von Willebrand factor A (VWA) domain of human capillary morphogenesis 2 (CMG2) anthrax toxin receptor, and the Fc region of human immunoglobin G (IgG). The use of plants in the production of such recombinant proteins is an important step towards synthesizing a large biomass of this therapeutic. This fusion protein may be an effective therapeutic agent against *B. anthracis* infections [16, 17].

Human inter-alpha-inhibitor proteins (IαIp) are endogenous plasma proteins identified in humans, functioning as serine protease inhibitors. IαIp can block the systemic release of proteases in sepsis, and inhibit furins

preventing the assembly of PA. Administration of IαIp 1 h or 24 h after the spore challenge of mice with *B. anthracis* Sterne strain protected animals from death. It was demonstrated that a combination of antimicrobial agents plus IαIp resulted in a better effect of anthrax treatment in comparison to the use of single therapeutics [18].

Some dominant negative mutants (DN-PA) may oligomerize with the wild type of protective antigen preventing its translocation and inhibiting pores formation. Random mutations in the second domain of PA protein enabled three new DN-PA mutants to be obtained: V377E, T380S and I432C, which inhibited the activity of anthrax toxin. One of these mutants – V377E, was shown to be the most potent inhibitor, protecting mice previously exposed to anthrax toxin. It was demonstrated that the three dominant negative mutants affected the formation of PA oligomers inhibiting the conversion of prepores into pores. V377E protected the sensitive murine cells from toxin action, indicating that it could be a good inhibitor of anthrax toxin [19].

Some *in vivo* studies on the effectiveness of LF inhibitors used in combination with ciprofloxacin after post-challenge treatment of *B. anthracis* infection, showed that these inhibitors can protect from inhalational anthrax. In the experiment, mice were challenged with *B. anthracis* infection and 24 h later the treatment was initialized, using ciprofloxacin alone (50 mg/kg) or in combination with LF inhibitors: B1–11B1, B1–11B2 or B1–11B3 used in a dose of 5 mg/kg. Animals were monitored twice a day until the 14th day post-challenge. Survival rate of mice treated with B1–11B3 in combination with ciprofloxacin was 40%, whereas in the case of mice treated with ciprofloxacin alone, only 20% of animals survived. LF inhibitors protected macrophages from the cytotoxic activity of LF, and this effect was synergistic with the effect of the antibiotic used *in vivo*.

The results indicate that 60% activity of macrophages should be sufficient to prevent bacterial growth after infection [20].

Antibodies. In response to the bioterrorism attacks in USA in 2001, Human Genome Science (HGS) has developed an alternative to antibiotics therapeutic agent against anthrax: Raxibacumab, which is a monoclonal antibody which blocks the production of anthrax toxin. Raxibacumab was approved by Food and Drug Administration (FDA), based on the results of studies on monkeys and rabbits infected with *B. anthracis*. The drug then underwent clinical tests on 326 healthy adult volunteers [21, 22, 23]. Raxibacumab injected intravenously in a dose of 40 mg/kg body weight had a half life of 20–22 days [22]. This monoclonal antibody acts against bacterial toxin released into the blood and tissues, by blocking PA binding to its cell membrane receptor, thus protecting the cells from the entry of anthrax toxin into the cell. A single dose of Raxibacumab (also called Abthrax) [23, 24] increased survival rates of monkeys infected with inhalational anthrax even by 64%. This antibody is safe and well-tolerated by healthy humans after intramuscular or intravenous injection. This indicates that Raxibacumab can be used for prophylaxis and treatment of inhalational anthrax. In 2009, the HGS provided 20,000 doses of Raxibacumab to the Strategic National Stockpile [23, 24, 25, 26].

ETI-204 (Anthim) is another anti-PA monoclonal antibody showing effectiveness in anthrax treatment, although its use has not yet been approved by the FDA. Phase I tests showed that ETI-204 is safe and can be used in anthrax treatment, providing an efficient concentration of the drug in serum to protect against toxins produced by *B. anthracis* [27].

Research has also shown that sera from animals immunized with an unencapsulated live vaccine strain of *B. anthracis* producing toxin have anti-spore activities, and this property was associated with the antitoxin humoral response. The results demonstrated that the anthrax vaccine absorbed (AVA) and PA-immune sera from rabbits, enhanced the phagocytosis of spores by murine macrophages when virulent *B. anthracis* Ames strain was injected intraperitoneally, together with the Sterne vaccine strain. It seems that antitoxin antibodies act mainly by inhibiting germination of bacterial spores, whereas anti-sera containing anti-PA IgG stimulate phagocytosis of spores by macrophages [28].

Anthrax toxin can be also effectively neutralized by a chimeric monoclonal anti-PA antibody – cAb29, produced in genetically-modified CHO cells. cAb29 binds with monomeric or heptameric PA, preventing the formation of transmembrane pore by PA. Binding of cAb29 to prepore prevents its transition to the transmembranal pore inside acidic endosome, thus inhibiting the process of intoxification, i.e. the translocation of LF/EF into the cytoplasm of the host cell. When this antibody binds to the prepore it prevents the changes in pH, blocking the translocation of LF and EF into the cell. It was demonstrated that cAb29 binds the $2\alpha_1$ loop in domain 2 of PA, and in normal conditions this loop undergoes major conformational changes during pore formation. Rabbits experimentally challenged with *B. anthracis* and treated with cAb29 antibody 12 h after exposure showed 100% survival rate, proving that cAb29 has strong neutralizing activity associated with its ability to block the conversion of prepore into pore [29].

To maximize the protective efficacy of mAb treatment, an approach using a cocktail of antibodies recognizing different epitopes or different microorganism virulence markers have been tested. Animal models have been applied to produce antibodies used in modern anthrax therapy, i.e. anti-PA is produced in rats, mice, rabbits and monkeys; anti-LF in Balb/C and A/J mice and Fischer 344 rats; anti-EF in A/J mice and chimpanzees; and anti-capsule mAbs also in mice and chimpanzees. Cocktails of mAbs against different virulence factors may increase the efficacy of treatment mainly by the synergistic effect of the antibodies used. Passive immunization with murine anti-capsule monoclonal antibodies resulted in significant protection of mice against spores of the Ames strain; however, the administration of anti-capsule mAbs 11D and 4C produced in chimpanzees, provided even better protection than murine mAbs, protecting the animals not only after administration at the pre-exposure period, but also when administered with *B. anthracis* spores 20 h after challenge. These antibodies could be used in the treatment of infections caused by antibiotic-resistant strains. Anti-PA monoclonal antibodies may neutralize PA by different mechanism, such as: inhibition of receptor binding, interference with LF and EF binding to PA, blocking the enzymatic cleavage of PA into PA63, or disruption of formed PA heptamer through formation of a supercomplex [30].

Two human monoclonal antibodies against PA (anti-PA): p6CO1 and p6FO1 were shown to have strong neutralizing activities towards lethal toxin *in vitro*, even at low concentrations, i.e. IC_{50} p6CO1–0.12 μg/ml and p6FO1–0.45 μg/ml. Passive transfer of these antibodies to A/J mice before

challenge with the lethal toxin resulted in 80–90% protection of these animals. Most of the anti-PA mAbs produced after vaccination with AVA act by binding to domain 3 of PA and preventing its oligomerization. Studies with the use of such antibodies provide important information about human response to AVA vaccine and PA epitopes binding antibodies induced after vaccination, which are capable to neutralize anthrax toxin [31].

A different approach uses DNA vaccines in passive immunization. One such method is based on codon-optimized plasmid DNA encoding *B. anthracis* PA, which has been used to immunize rabbits to stimulate production of anti-anthrax antibodies utilized in passive immunotherapy. The obtained anti-sera were of high titer, and protected J774 macrophages by neutralization of the cytotoxic effect of the anthrax lethal toxin administered exogenously, as well as the toxin released by *B. anthracis* spores after infection. These anti-sera were also shown to protect mice against aerosol challenge with *B. anthracis* Sterne strain applied in a LD_{50} dose. Protection was noted when anti-serum was provided 1 h before or 1 h after infection. These studies indicate that the obtained high titer anti-sera may be used in immunization, which could supplement antibiotic therapy to improve the survival of patients infected with anthrax [32].

Synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG motifs also act as potent immune adjuvants and may be used to improve the immune response of the organism. Combining CpG ODN with AVA vaccine resulted in increased speed, magnitude and avidity of anti-anthrax response. Administration of CpG ODN in complex with AVA to mice previously challenged with *B. anthracis* stimulated the production of high levels of anthrax-neutralizing antibodies. Studies exploring the ability of CpG ODN to improve the immune response elicited by AVA have shown that macaques immunized subcutaneously with AVA in combination with CpG ODN showed stronger immune response after challenge by 10^5 of *B. anthracis* Sterne spores. The response was faster and more pronounced in comparison to animals immunized only with vaccine, and accelerated the protective immunity against anthrax. Simultaneous application of CpG ODN and AVA vaccine induces the production of a high titer of anthrax-neutralizing antibodies, whereas passive immunization with sera from macaques immunized with AVA plus CpG ODN protected nearly half of the recipient mice from challenge by 30 LD_{50} Sterne strain *B. anthracis* spores [33].

Another study examined the immunostimulatory abilities of CpG ODN adsorbed onto cationic polylactide-co-glycolide microparticles (CpG ODN-PLG). Co-administration of CpG ODN-PLG with AVA resulted in a stronger and faster immunoglobulin G response against anthrax PA than AVA vaccine alone. Mice immunized with the complex: CpG ODN-PLG plus AVA were protected from anthrax challenge already within 1 week of vaccination, and the level of protection correlated with serum immunoglobulin G titers of anti-PA antigen. Synthetic oligodeoxynucleotides (ODN) containing immunostimulatory CpG motifs can cause a 4–30-fold augmentation of the immune response when administered together with AVA. CpG ODN regulates the activity of B cells and plasmacytoid dendritic cells stimulating production of cytokines and chemokines. Biodegradable cationic polylactide-co-glycolide (PLG) improved the adsorption and processing of antigen by antigen-presenting cells.

The combination of CpG ODN adjuvant with AVA also boosted the protective immune response in specimen exposed to *B. anthracis*. The results of the study demonstrated that total IgG anti-PA titer was 97% accurate as a surrogate marker of survival following anthrax challenge, whereas toxin-neutralizing antibody showed 91% accuracy [33, 34].

Peptides. Anti-microbial peptides (AMPs) comprising a group of oligo- and poly-peptides have an ability to destroy microorganisms and inhibit their growth. Some AMPs, such as peptides from the family of β-defensins (human β-defensin HBD), have bactericidal properties against *B. anthracis* [35]. Proline-rich polypeptides (PRP) contain 10–15 amino acids and four proline residues. PRP-1, consisting of 15 amino acids, and G_x-NH_2 – a proline-rich polypeptide, are two examples of such compounds which have been tested *in vivo* on guinea pigs and mice for treatment against *Bacillus anthracis* and anthrax strain N55 vaccine. Both polypeptides show strong prophylaxis and therapeutical properties. In contrast to antibiotics, the peptides already act at low concentrations and need to be administered only in one or two doses. PRPs increase macrophages activity and viability, promoting IL-1 and TNF biosynthesis in macrophages, which prevents *B. anthracis* introduction into blood and eliminates the pathogen from the organism. Interestingly, after administration of cytokines, the formation of antibodies against *B. anthracis* became more intensive. Further studies are necessary to reveal the mechanism of cytokines transport into *B. anthracis* cells, and the direct effect of peptides on cytokines expression [36].

Other studies are aimed at the isolation of peptide ligands capable of binding to the native capsule of *B. anthracis* from a commercial phage display library using a synthetic form of the capsule consisting of 12 γ -D-glutamic acid residues [37].

There is also a group of novel synthetic peptides termed CAMELs, which show stronger antibacterial activity against *B. anthracis* than ciprofloxacin. It is thought that CAMEL peptides, having high activity against *B. anthracis*, may also show activity against *Bacillus subtilis* and *Bacillus megaterium* [38].

Cathelicidin hCAP-18 is a protein discovered in humans, which is cleaved to liberate an active LL-37 peptide having antibacterial activity. Synthesis of LL-37 was detected in bone marrow, keratinocytes of infected skin, as well as respiratory and oral epithelial cells [35]. Studies on the susceptibility of different *Bacillus* species to LL-37 peptide revealed that *B. subtilis* had a low level of resistance to antibacterial effect of LL-37 (50% growth-inhibitory concentration GI_{50}, 1μg/ml), *Bacillus cereus* and *Bacillus thuringiensis* showed intermediate resistance (GI_{50s}, 33μg/ml and 37μg/ml, respectively), whereas *B. anthracis* showed the highest level of resistance (GI_{50s}, 40 to 66 μg/ml). Degradation of LL-37 by *B. anthracis* culture supernatant was blocked by metalloprotease inhibitors, i.e. EDTA and 1,10-phenanthroline. It was additionally noted that the gene encoding the protease responsible for LL-37 degradation was not plasmid-borne, suggesting that aside from the classical plasmid-based virulence determinants, extracellular metalloproteases of *B. anthracis* may also play a role in the survival of the microorganism in the host [39].

Antimicrobial peptides are a component of the innate immune response and have a broad range of antimicrobial activities. Aside from their antibacterial activities, these peptides also play the role of mediators of inflammation

and stimulators of the immune system. In the case of LL-37 peptide, its direct antimicrobial activity is mediated by disruption of the lipid bilayer of target cells through formation of toroidal pore, which leads to osmotic lysis and cell death [39]. In *in vitro* cultures, *B. anthracis* secretes metalloproteases that provide resistance of the microorganism to the bactericidal effect of LL-37, and thus plays a role in *B. anthracis* virulence, especially in the case of infections caused through the inhalational rout. It has been revealed that *B. anthracis* possesses genes encoding extracellular proteases, responsible for degradation of the host antibacterial peptide LL-37, therefore contributing to virulence. After 16 hour culture of different *Bacillus* species in the presence of increasing concentrations of LL-37 (from 0–200 μg/ml), the 50% growth-inhibitory concentration (GI_{50}) of this peptide for *B. anthracis* Ames, *B. anthracis* Sterne and *B. anthracis* UM23-CI2 was 66, 68 and 63 μg/ml, respectively. It is possible that the resistance of *B. anthracis* to LL-37 is mediated by extracellular proteases, since in the presence of metalloprotease inhibitors, e.g. EDTA, the resistance to LL-37 could be eliminated when the concentrations of the inhibitor were greater than 125 μg/ml (decreasing bactericidal activity of LL-37 was observed). This indicates that metalloproteases play a key role in the virulence of *B. anthracis*. The advantages of LL-37 over conventional antibiotics include their broad spectrum of activity and no signs of resistance, which constitutes a major problem in the case of conventional antibiotics [39].

Interferons. Interferons (IFNs) play an important role in innate immunity during many viral, as well as bacterial infections. Studies have shown that recombinant murine IFN-β, as well as type I IFN inducer, Poly-ICLC, protects mice from infection with inhalational *B. anthracis* [40]. Poly-ICLC is a synthetic double-stranded RNA complex consisting of polyriboinosine-polyribocytidylic acid stabilized with poly-l-lysine and carboxymethylcellulose. This compound is a strong inducer of both type I IFN-α and –β. The best protective effect was obtained when Poly-ICLC was administered intranasally in a dose of 25 μg 24 h before an intranasal challenge with 3 LD_{50} Ames strain spores. Similar efficacy of protection was noted when Poly-ICLC was given at the time of challenge with *B. anthracis*. Delayed death of animals infected with 3 LD_{50} Ames strain spores was also observed after intramuscular administration of this IFN inducer; however, the effect was less significant. These results indicate that IFN defence can protect mice (up to 60%) against lethal inhalational anthrax, and therefore may have medical implications for the therapy of human anthrax [40].

Therapies based on inhibition of bacterial DNA synthesis and replication. *Bacillus anthracis* is innately resistant to trimethoprim (TMP), a compound which selectively inhibits several bacterial dihydrofolate reductases (DHFRs), but with no influence on human DHFR. Purified human recombinant DHFR (rDHFR) and *B. anthracis* rDHFR were also used to determine the 50% inhibitory concentrations (IC_{50}) of the derivatives. MIC for the investigated derivatives ranged from 12.8–128 μg/ml, and the IC_{50} values for *B. anthracis* rDHFR ranged from 46–600 nM, while in case of human rDHFR, the values IC_{50} were above 16,000 nM [41]. The selective inhibition of *B. anthracis* rDHFR by 2,4-diaminopyrimidine derivatives, and their activity against *B. anthracis* proved *in vitro,* demonstrate that this class of compounds can be potentially used in the development of new therapeutics for the treatment of infections caused by TMP-resistant bacteria, such as *B. anthracis* [41].

Another study investigated the activity of some novel lead series of inhibitors of dihydrofolate reductases showing 82-fold higher activity than trimethoprim. Studies were conducted on inhibitory activity of known antifolates against *B. anthracis* DHFR (BaDHFR), showing their substantial potency in inhibiting the bacterial enzyme, as well as growth of *B. anthracis* [42].

Lysins. Lysins are lytic enzymes produced by phages infecting bacteria. These enzymes cause lysis of bacterial cell wall enabling the release of bacteriophages. Structurally lysins are modulator proteins with an N-terminal domain that gives the enzymatic activity for a peptidoglycan bond and a C-terminal domain responsible for the binding specificity to carbohydrate epitopes in the bacterial cell wall. Lysins have an advantage over antibiotics in that they affect only targeted bacterial strains.

PlyPH lysins are enzymes which retain their lytic acivity in a broad pH range, hence their name. Due to their high specificity PlyPH may destroy chosen bacterial species, also those showing antibiotic resistance. Cloning technique was applied to characterize PlyPH lytic enzyme, which was shown to be specific for *B. anthracis* Sterne strain and *B. cereus* strain RSVF1. The obtained PlyPH lysin retained activity between pH 4–10.5, and a single dose of this enzyme protected a substantial percentage of mice intraperitoneally challenged with attenuated *B. anthracis* strain. After intraperitoneal injection of mice with 400 μl of purified PlyPH (prepared in a concentration of 3 mg/ml in 50 mM acetate buffer, pH 5.5), around 40% of the mice were protected against death resulting from anthrax infection, while 100% of *B. anthracis* challenged animals receiving only acetate buffer injections died within 38 h post-challenge. The results obtained demonstrate that PlyPH is a highly flexible enzyme, retaining its catalytic activity over a PlyPH can cause a selective lysis of RSVF1 bacteria in a mixture of various bacterial cells [43]. Thus, lysins, such as PlyPH and PlyG from γ phage, may be taken under consideration as a novel therapeutic against anthrax [43].

CONCLUSIONS

Bacillus anthracis spores are still the most dangerous weapon among pathogens which can be used in a terroristic attack. In this case, research for new anti-anthrax preparations is of primary importance for the protection of humans and animals. This overview of the most recent data shows the many new promising possibilities in finding effective anti-anthrax preparations. The most effective of them should be available in the national stockpile in the event of a biological crises.

REFERENCES

1. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis. 2001; 7(6): 933–944.

2. Brook I, Elliott TB, Pryor II HI, Sautter TE, Gnade BT, Thakar JH, et al. In vitro resistance of *Bacillus anthracis* Sterne to doxycycline, macrolides and quinolones. Int J Antimicrob Agents. 2001; 18: 559–562.
3. Athamna A, Athamna M, Abu-Rashed N, Medlej B, Bast DJ, Rubinstein E. Selection of *Bacillus anthracis* isolates resistant to antibiotics. J Antimicrob Chemother. 2004; 54: 424–428.
4. Brook I. The prophylaxis and treatment of anthrax. Int J Antimicrob Agents. 2002; 20: 320–325.
5. Żakowska D, Bartoszcze M, Niemcewicz M, Bielawska-Drózd A. Nowe możliwości hamowania infekcji *B. anthracis*. Medycyna Wet. 2011; 67(10): 665–668 (in Polish).
6. Żakowska D, Bartoszcze M, Niemcewicz M, Bielawska-Drózd A, Kocik J. New aspects of the infection mechanisms of *Bacillus anthracis*. Ann Agric Environ Med. 2012; 19(4): 613–618.
7. Hicks CW, Sweeney DA, Cui X, Li Y, Eichacker PQ. An overview of anthrax infection including the recently identified form of disease in injection drug users. Intensive Care Med. 2012; 38: 1092–1104.
8. Fowler RA, Shafazand S. Anthrax bioterrorism: prevention, diagnosis and management strategies. J Bioterr Biodef. 2011; 2: 107.
9. Wang JY, Roehrl MH. Anthrax vaccine design: strategies to achieve comprehensive protection against spore, bacillus, and toxin. Med Immunol. 2005; 4: 4.
10. Leffel EK, Bourdage JS, Williamson ED, Duchars M, Fuerst TR, Fusco PC. Recombinant protective antigen anthrax vaccine improves survival when administered as a postexposure prophylaxis countermeasure with antibiotic in the New Zealand white rabbit model of inhalation anthrax. Clin Vac Immunol. 2012; 19(8): 1158–1164.
11. Yee SB, Hatkin JM, Dyer DN, Orr SA, Pitt MLM. Aerosolized *Bacillus anthracis* infection in New Zealand white rabbits: natural history and intravenous levofloxacin treatment. Comp Med. 2010; 60(6): 461–468.
12. Foss MH, Weibel DB. Oligochlorophens are potent inhibitors of *Bacillus anthracis*. Antimicrob Agents Chemother. 2010; 54(9): 3988–3990.
13. Heine HS, Bassett J, Miller L, Purcell, BK, Byrne WR. Efficacy of daptomycin against *Bacillus anthracis* in a murine model of anthrax spore inhalation. Antimicrob Agents Chemother. 2010; 54(10):4471–4473.
14. Ambrose PG, Forrest A, Craig WA, Rubino ChM, Bhavnani SM, Drusano GL, et al. Pharmacokinetics-pharmacodynamics of gatifloxacin in lethal murine *Bacillus anthracis* inhalation infection model. Antimicrob Agents Chemother. 2007; 51(12): 4351–4355.
15. Heine HS, Purcell BK, Bassett J, Miller L, Goldstein BP. Activity of dalbavancin against *Bacillus anthracis* in vitro and in a mouse inhalation anthrax model. Antimicrob Agents Chemother. 2010; 54(3): 991–996.
16. Wycoff KL, Belle A, Deppe D, Schaefer L, Maclean JM, Haase S, et al. Recombinant anthrax toxin receptor – Fc fusion proteins produced in plants protect rabbits against inhalational anthrax. Antimicrob Agents Chemother. 2011; 55(1): 132–139.
17. Arzola L, Chen J, Rattanaporn K, Maclean JM, McDonald KA. Transient co-expression of post-transcriptional gene silencing suppressors for increased in *Planta* expression of a recombinant anthrax receptor fusion protein. Int J Mol Sci. 2011; 12: 4975–4990.
18. Opal SM, Lim Y-P, Cristofaro P, Artenstein AW, Kessimian N, DelSesto D, et al. Inter-alpha inhibitor proteins: a novel therapeutic strategy for experimental anthrax infection. Shock. 2011; 35(1): 42–44.
19. Wu G, Feng Ch, Cao S, Guo A, Liu Z. Identification of new dominant – negative mutants of anthrax protective antigen using directed evolution. Appl Biochem Biotechnol. 2012; 168(5): 1302–1310.
20. Forino M, Johnson S, Wong TY, Rozanov DV, Savinov AY, Li W, et al. Efficient synthetic inhibitors of anthrax lethal factor. PNAS. 2005; 102(27): 9499–9504.
21. Editor's Blog at WRAL Tech Wire. FDA approves GlaxoSmithKline drug for treatment of anthrax by Staff, telegram reports. GSK, Biotech&Life Science, 2012.
22. Migone T-S, Subramanian GM, Zhong J, Healey LM, Corey A, Devalaraja M, et al. Raxibacumab for the treatment of inhalational anthrax. N Engl J Med. 2009; 361(2): 135–144.
23. Mazumdar S. Raxibacumab. mAbs. 2009; 1(6): 531–538.
24. Schneemann A, Manchester M. Anti-toxin antibodies in prophylaxis and treatment of inhalation anthrax. Future Microbiol. 2009; 4: 35–43.
25. Support use of Raxibacumab (Abthrax) for the treatment of inhalation anthrax. www.drugs.com/clinical_trials/data-published-new-england-journal-medicine-support-raxibacumab-abthrax-inhalation-anthrax (acces: 2013.07.30).
26. Ramasamy S, Liu CQ, Tran H, Gubala A, Gauci P, McAllister J, et al. Principles of antidote pharmacology: an update on prophylaxis, post-exposure treatment recommendations and research initiatives for biological agents. Br J Pharmacol. 2010; 161: 721–748.
27. Monoclonal antibody for treatment of inhalation anthrax. A service of the U.S. National Institutes of Health. www.ClinicalTrials.gov (acces: 2013.07.30).
28. Welkos S, Little S, Friedlander A, Fritz D, Fellows P. The role of antibodies to *Bacillus anrhracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. Microbiology 2001; 147: 1677–1685.
29. Mechaly A, Levy H, Epstein E, Rosenfeld R, Marcus H, Ben-Arie E, et al. A novel mechanism for antibody – based anthrax toxin neutralization: inhibition of prepore – to – pore conversion. J Biol Chem. 2012; 287(39): 32665–32673.
30. Chen Z, Moayeri M, Purcell R. Monoclonal antibody therapies against anthrax. Toxins. 2011; 3: 1004–1019.
31. Smith K, Crowe SR, Garman L, Guthridge C, Muther JJ, McKee E, et al. Human monoclonal antibodies generated following vaccination with AVA provide neutralization by blocking furin cleavage but not by preventing oligomerization. Vaccine 2012; 30(28): 4276–4283.
32. Herrmann JE, Wang S, Zhang Ch, Panchal RG, Bavari S, Lyons CR, et al. Passive immunotherapy of *Bacillus anthracis* pulmonary infection in mice with antisera produced by DNA immunization. Vaccine 2006; 24: 5872–5880.
33. Klinman DM, Xie H, Little SF, Currie D, Ivins BE. CpG oligonucleotides improve the protective immune response induced by the anthrax vaccination of rhesus macaques. Vaccine 2004; 22: 2881–2886.
34. Xie H, Gursel I, Ivins BE, Singh M, O'Hagan DT, Ulmer JB, et al. CpG oligodeoxynucleotides adsorbed onto polylactide–co-glycolide microparticles improve the immunogenicity and protective activity of the licensed anthrax vaccine. Infect Immun. 2005; 73(2): 828–833.
35. Mirski T, Gryko R, Bartoszcze M, Bielawska-Drózd A, Tyszkiewicz W. Peptydy przeciwdrobnoustrojowe – nowe możliwości zwalczania infekcji u ludzi i zwierząt. Medycyna Wet. 2011; 67(8): 517–521 (in Polish).
36. Galoyan AA, Grigoryan SL, Badalyan KV. Treatment and prophylaxis of anthrax by new neurosecretory cytokines. Neurochem Res. 2006; 31: 795–803.
37. Beer M, Liu Ch-Q. Panning of a phage display library against a synthetic capsule for peptide ligands that bind to the native capsule of *Bacillus anthracis*. PLoS ONE. 2012; 7(9): e45472.
38. Wade D. CAMELs against anthrax. http://preprint.chemweb.com/medichem/0111002 (acces: 2013.07.30).
39. Thwaite JE, Hibbs S, Titball RW, Atkins TP. Proteolytic degradation of human antimicrobial peptide LL-37 by *Bacillus anthracis* may contribute to virulence. Antimicrob Agents Chemother. 2006; 50(7): 2316–2322.
40. Walberg K, Baron S, Poast J, Schwartz B, Izotova L, Pestka S, Peterson JW. Interferon protects mice against inhalation anthrax. J Interferon Cytokine Res. 2008; 28: 597–602.
41. Barrow EW, Dreier J, Reinelt S, Bourne PC, Barrow WW. In vitro efficacy of new antifolates against trimethoprim-resistant *Bacillus anthracis*. Antimicrob Agents Chemother. 2007; 51(12): 4447–4452.
42. Beierlein JM, Frey KM, Bolstad DB, Pelphrey PM, Joska TM, Smith AE, et al. Synthetic and crystallographic studies of a new inhibitor series targeting *Bacillus anthracis* dihydrofolate reductase. J Med Chem. 2008; 51(23): 7532–7540.
43. Yoong P, Schuch R, Nelson D, Fischetti VA. PlyPH, a bacteriolytic enzyme with a broad pH range of activity and lytic action against *Bacillus anthracis*. J Bacteriol. 2006; 188(7): 2711–2714.