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LOOKING FOR THE NEW PREPARATIONS FOR ANTIBACTERIAL THERAPY II. CLINICAL TRIALS; NEW β-LACTAM ANTIBIOTICS AND β-LACTAMASE INHIBITORS

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ABSTRACT

To obtain a status of a medicinal product, a compound possessing potential antimicrobial activity and displaying no cytotoxicity, must undergo three phases of clinical trials to prove its therapeutic efficacy, safety and quality. Properties of the compound should be based on the results of studies meeting specific criteria. Studies should be: randomized, double-blind, involving sufficient number of volunteers, concerning the infections localized in strictly defined area and caused by identified microorganisms. After the medicinal product is authorized to be on the market, clinical trials of the fourth phase are carried out to detect adverse effects, overdose symptoms, interactions of the new drug with other medicinal products and to establish characteristic of activity among groups such as children, elderly, women in pregnancy and patients suffering from other diseases, but only if the benefits of receiving treatment outweigh the risks.

This article is a second part of the series associated with searching for new antibacterial agents and it relates to performance of clinical trials and the new compounds belonging to the class of β-lactams. Among the 9 presented compounds, candidates to become medicinal products, two belong to the cephalosporins (CXA-101, S-649266), one to carbapenems (razupenem), three to monobactams (BAL30072, BAL30376, MC-1) and three to β-lactamase inhibitors (NXL-104, MK-7655, ME1071).

Key words: *clinical trials, novel antibiotics, β-lactams*

The previous issue of Przegląd Epidemiologiczny contains the article titled „New antibiotics and chemotherapeutics on the market" (1), first from the series „Looking for the new preparations for antibacterial therapy," discussing the new compounds introduced into the treatment of bacterial diseases in the twenty-first century. The process of the clinical trials required for the registration of a medicinal product, new β-lactam antibiotics and β-lactamase inhibitors, currently at the stage of clinical trials are presented in this paper.

CLINICAL TRIALS

Before the introduction a new medicinal product into the market, a number of tests, proving its therapeutic efficacy, safety and quality are required. Firstly, preclinical studies must to be carried out. Antibacterial activity using the standard and clinical strains, as well as toxicity on cell lines are evaluated as the *in vitro* studies. Relationship between the structure and activity (SAR – Structure-Activity Relationship) is determined for synthetic compounds, only. At the stage of *in vivo* studies performed on experimental animals, parameters such as: cytotoxicity, genotoxicity, mutagenicity, carcinogenicity, and pharmacokinetics are also defined. Speed of processes of absorption, distribution, biotransformation and excretion of the compound from the body (ADME - Absorption, Distribution, Metabolism, Excretion) are also assessed. ADME analysis provides basic information about the properties of the compound, eg. solubility, permeability (PAMPA - Parallel Artificial Membrane Permeability Assay), partition coefficient (logP), as well as stability of the compound in plasma and blood. Satisfactory results of preclinical studies allow for the introduction of the compound into the stage of clinical trials.

Clinical trials are carried on in Poland in accordance with the Regulation of the Minister of Health on the requirements for the conduct of basic documentation of a clinical trial (2). The Regulation sets out the requirements concerning the planning, monitoring, recording

and storage of basic clinical trial documentation, it also describes mode of action of sponsor, clinical researcher and researcher monitoring clinical trial in this area.

The clinical studies consist of three phases of evaluation of the proposed medicinal product, before its registration and marketing authorization. Phase I involves a preliminary determination of the safety, pharmacodynamics and pharmacokinetics and assessing of tolerance to increasing doses of the drug, including the determination of the maximum tolerated dose (MTD). In addition, the influence of food intake on the absorption of the drug has to be examined. Possible adverse effects have to be also monitored. The study involves a small group of healthy volunteers, 20-80.

Phase II is the continuation of the phase I of clinical studies. It involves larger number of healthy volunteers and consists of number of studies to evaluate whether the proposed drug is effective and safe. Therefore, the minimum dose with maximum efficiency, as well as pharmacokinetics of the compound according to gender and age, have to be defined. The efficacy of new drug is compared to placebo or more often, the drug traditionally used in the therapy using double-blind method. People involved in the study are randomly assigned to a treatment group or a control group. The group usually includes 50-200 people.

Phase III of clinical studies decisively confirms the therapeutic efficacy of the proposed drug in comparison to the traditionally used medicinal product. The trials are designed as randomized, double-blind, and multicenter. There are randomized trials, performed a double-blind, multicenter normally. In addition, the tests may include a risk assessment between safety of use and therapeutic efficacy in the short-term and long-term use. At this stage of the study, large groups of patients are examined, generally from 300 to more than 3000 people. This is the most difficult stage, the longest and most expensive. Indications for the application of the proposed drug can be extended, if the examination of the representative number of patients with the disease, proves the efficacy of the medicinal product.

Preparations passing three phases of clinical trials obtain an entry into the list of medicinal products registered in Poland by the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products (URPLWMiPB) (national procedure) or they maybe registered by the European Medicines Agency (EMA) in the case of a central registration procedure.

After obtaining the marketing authorisation, phase IV of clinical trials related to accurate assessment of the safety of the treatment in approved indications are conducted. The studies allow for the detection of: rare or resulting from long-term use adverse effects, overdose symptoms, new drug interaction with other active ingredients, specific activity among groups, such as children, elderly, pregnant women, and patients suffering from chronic diseases.

In Poland, adverse effects of medicinal products are monitored by URPLWMiPB.

Dynamically growing resistance of bacteria to applied drugs results in reducing the number of effective medicinal products. Furthermore, decrease in number of approved each year new antimicrobial compounds provides an additional risk to the safety related with the lack of effective therapeutic options. Search for new active compounds with antimicrobial activity against multidrug-resistant strains becomes an obligation.

β-LACTAMS ANTIBIOTICS

β-lactam antibiotics are the oldest and still widely used class of compounds with antibacterial activity due to the presence of β-lactam ring in the molecule. Penicillins, cephalosporins, carbapenems, monobactams, as well as β-lactamase inhibitors belongs to this group of substances. Similar mechanism of action and low toxicity are features common for all β-lactam antibiotics. Range of antimicrobial activity, pharmacokinetics, the rate of growing resistance, as well as the indications for use diversify those substances (3). Mechanism of action of β-lactams is based on combining with the penicillin binding proteins (PBP) – enzymes, which are involved in the final stage of the biosynthesis of peptidoglycan, element of bacterial cell wall, their inactivation and consequently cell lysis.

Microorganisms created four main mechanisms of resistance to β-lactam antibiotics: (i) production of β-lactamases – enzymes hydrolyzing the amide bond in the β-lactam ring, (ii) the synthesis of new or modified enzymes PBP with low affinity to β-lactam, but retaining biological function, (iii) in the case of Gram-negative bacteria, cell permeability modification by temporary closure of porin channels present in the outer membrane or reducing the number of porin channels in the next generations of microorganisms and (iv) active removal of the antibiotic - *efflux* mechanism (4).

The source of information on the various phases of clinical trials of described compounds, was primarily the official register and results database (5).

NEW COMPOUNDS FROM CEPHALOSPORINS GROUP

Among V generation of cephalosporin antibiotics, two compounds were introduced into the market: ceftobiprole medocaril (EMA - 2008) and ceftaroline fosamil (FDA - 2010, EMA - 2012). Two new compounds CXA-101 and S-649266 are at the stage of clinical trials.

Compound named CXA-101 (ceftolozane, FR264205) exhibits broad spectrum of antibacterial activity against *Enterobacteriaceae* and *Pseudomonas aeruginosa*. The lowest concentration of the antimicrobial agent inhibiting the visible growth of a 90% microbial strains (MIC_{90}, *Minimum Inhibitory Concentration*) is 8 μg/mL against *P. aeruginosa*, indicating at the compound CXA-101 as the most effective of all cephalosporins currently available on the market; 2-8 times more potent in comparison to the ceftazidime. The advantage of this compound is low tendency to induce resistance and increased stability to β-lactamase type AmpC. Unfortunately, the efficacy against Gram-positive and anaerobic bacteria is low, also the MRSA strains are resistant to CXA-101. Furthermore, CXA-101 is a compound susceptible to hydrolysis by the ESβL enzyme and carbapenemase, including KPC (*Klebsiella pneumoniae* carbapenemase), which significantly reduce its role as an antibacterial agent applied in monotherapy. The combination of CXA-101 with β-lactamase inhibitor – tazobactam in a concentration of 8 μg/mL restore the *in vitro* activity against *Enterobacteriaceae* producing ESβL. This combination is called CXA-201 and it shows efficacy against more than 90% of the *Enterobacteriaceae* producing extended spectrum β-lactamase type CTX-M (6). CXA-201 is an interesting therapeutic option in the treatment of infections caused by multidrug-resistant Gram-negative bacteria, including strains resistant to piperacillin with tazobactam.

In April 2010, Calixa Therapeutics, Inc. completed phase II of clinical trials on safety and efficacy of the CXA-101 in comparison to ceftazidime, among patients with complicated urinary tract infections (cUTI). Moreover, Cubist Pharmaceuticals conducts two research projects for the application of CXA-201, in which participants to phase III of clinical trials are being recruited. The first project relates to the treatment of complicated intra-abdominal infections (cIAI), and the second is for therapy of cUTI including pyelonephritis. Both projects concern parenteral administration.

Compound S-649266 is poorly understood cephalosporin possesing antimicrobial activity against strains producing New Delhi metallo-β-lactamase-1 (NDM-1) and is currently undergoing phase I of clinical trials conducted in Japan by Shionogi & Co. Ltd. in collaboration with GlaxoSmithKline (7).

NEW COMPOUNDS FROM CARBAPENEMS GROUP

During the last 10 years four carbapenems have been introduced to the treatment: ertapenem (FDA - 2001, EMA - 2002), biapenem (Japan - 2002), doripenem (Japan - 2005, FDA - 2007, EMA - 2008) and tebipenem pivoxil (Japan - 2009). Razupenem is currently undergoing clinical development.

Razupenem (PZ-601, SM-216601, SMP-601, PTZ-601) exhibits broad spectrum of activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *Enterococcus faecium* (VREF). MIC_{90} value is 0.015 mg/mL against methicillin-sensitive *S. aureus* (MSSA) and 0.03 μg/mL against *Staphylococcus epidermidis*. Unfortunately, the efficacy of razupenem against Gram-negative bacteria is lower in comparison to the registered carbapenems. Inferior activity was observed against *Enterobacteriaceae* and non-fermenting rods. MIC_{90} value is 8 μg/mL against *Serratia marcescens*, 32 μg/mL against *P. aeruginosa* and 4 μg/mL against *Enterobacteriaceae* producing ESβL. In February 2009, phase II of clinical trials evaluating the safety, efficacy and pharmacokinetics of the compound PZ-601 in the treatment of complicated skin and soft tissue infections (cSSSI) was completed (8).

It should be mentioned that in 2007, without giving any official reason Japanese company Daiichi Sankyo interrupted project to introduce tomopenem to the market. Tomopenem achieved phase II of clinical trials and it demonstrated a wide spectrum of antibacterial activity, especially against MRSA. However, in 2009, another Japanese company Meiji Seika Pharma Co., Ltd. announced the abandonment of research concerning the compound ME-1036 - new carbapenem, highly effective against MRSA, VISA and VRE, following by reports informing about skin reactions in patients receiving ME-1036 at the stage I of clinical trials (9).

NEW MONOBACTAM

High efficacy of the new generation of monobactams was based on the use of "Trojan horse" strategy through the incorporation of iron chelating groups into the structure of the molecule of a drug (10). Under conditions of iron deficiency, microorganisms use one of the fundamental mechanisms of its acquisition by extracellular secretion of low molecular weight compounds chelating iron - siderophores. Created complexes are being recognized by receptors of the outer membrane of the bacterial cell and transported to the periplasmic space. Compounds such as: BAL30072, BAL30376 and MC-1 possess in its structure moieties chelating iron, so that such a molecule can be introduced into cells as a carrier of iron ions - siderophore.

BAL30072 displays antibacterial activity against multidrug-resistant *Enterobacteriaceae* and *Acinetobacter* sp., *Burkholderia* sp., *P. aeruginosa* and *Stenotrophomonas maltophilia* strains. The lowest concen-

tration of an antimicrobial agent inhibiting the visible growth of a 50% microbial strains (MIC_{50}) is ≤ 2 μg/mL against multidrug-resistant *P. aeruginosa*, *Acinetobacter baumannii* non-susceptible against meropenem, and *Enterobacteriaceae* producing MBL (11). BAL30072 is effective also as an inhibitor of β-lactamases of classes A and C. Basilea Pharmaceutica Company conducts phase I of clinical trials assessing the pharmacokinetics, safety and tolerability of BAL30072 after multiple ascending doses of intravenous infusions. According to preliminary data, the product is well tolerated and shows no clinical adverse effects. Compound named MC-1, similar in structure to the BAL30072, possesses higher antibacterial activity against *Enterobacteriaceae* and *P. aeruginosa* in comparison to BAL30072 with MIC_{50} value of ≤ 0.5 μg/mL against the indicated strains. Compound MC-1 is still in the preclinical phase.

Compound named BAL30376 is unique in terms of design, but now still in the preclinical phase. Acronym BAL30376 describes combination of three compounds: BAL19764, BAL29880 and clavulanic acid, each fulfill individual function. BAL19764 (Syn2416, PTX2416) is an analogue of aztreonam and contains in its structure the iron chelating group, which allows the molecule for quick penetration into the bacterial cell. Unfortunately, the compound is susceptible to hydrolysis by cephalosporinases type AmpC and β-lactamases type ESβL. Other compounds are designed to inhibit these enzymes. BAL29880 is an inhibitor of β-lactamases class C, and clavulanic acid blocks the activity of other serine β-lactamases. MIC_{90} values expressed in the compound BAL19764 are in the range of ≤ 4 μg/mL against most strains of *Enterobacteriaceae*, including those producing MBL, 2 μg/mL against *S. maltophilia*, 8 μg/ml against multidrug-resistant *P. aeruginosa* and 16 μg/mL against multidrug-resistant strains of *Acinetobacter* sp. and *Burkholderia* sp., respectively. Presence of compound BAL29880 greatly increases the efficacy against *Citrobacter freundii* and *S. marcescens* (12).

NEW β-LACTAMASE INHIBITORS

The conception of achieving efficacy, while maintaining safety and quality without the simultaneous development of resistance, by creating a fixed dose combination of β-lactam antibiotics and β-lactamase inhibitor, which has been proved since a long time and shown by the clinical success of Augmentin (UK, 1985), consisting of amoxicillin and clavulanic acid. The inhibitor is also used in combination with ticarcillin (Timentin). Other β-lactamase inhibitors currently used in medicine are: sulbactam combined with ampicillin (Unasyn) or cefoperazone (Sulperazone) and tazobactam with piperacillin (Tazocin).

As a result of the appearance of new types of β-lactamases, such as MBL capable of hydrolyzing carbapenems, the need for effective inhibitors of these enzymes has increased significantly. Compounds with the following acronyms: NXL-104, MK-7655, and ME1071 are at the stage of clinical trials. None of them has in its structure classic β-lactam ring.

NXL-104 (avibactam, AVE1330A) is a representative of the new class of diazabicyclooctanes compounds with broad-spectrum of inhibition of serine β-lactamases class A, C and selected D, including ESβL. The efficacy of the inhibition of β-lactam ring hydrolysis is extremely high. To stop the activity of one molecule of β-lactamase, only 1-5 molecules of compound NXL-104 is needed, whereas for this purpose 55-214 molecules of clavulanic acid or tazobactam are required. In addition, the half-life of the enzyme-inhibitor complex for compound NXL-104 is longer than 7 days, while for the tazobactam, the half-life is approximately 5 hours (13). Two cephalosporin antibiotics: ceftazidime and ceftaroline were combined with compound NXL-104.

Ceftazidime (CAZ) in combination with an inhibitor NXL-104 is a product being developed by Novexel Inc. (now part of AstraZeneca). Addition of an inhibitor at a concentration of 4 μg/mL provides effective protection against hydrolysis by β-lactamase type ESβL produced by *Escherichia coli* and *K. pneumoniae*, and class C enzymes produced by *E. coli*, *Enterobacter cloacae* and *Enterobacter aerogenes*. This combination also displays activity against β-lactamase of *P. aeruginosa*. Compound NXL-104 is well tolerated at the dose of 2 g given parenterally and shows similar pharmacokinetic profile to ceftazidime. Due to the weak absorption, it cannot be administered orally. Like most of β-lactam antibiotics, the product is mainly excreted in the urine. AstraZeneca conducts three research projects with the use of ceftazidime combined with an NXL-104. In April 2011, phase I of clinical trials on safety, tolerability and pharmacokinetics of the substance NXL-104 used alone or in combination with ceftazidime was completed. In addition, in June 2010, phase II of comparative clinical trials on ceftazidime with NXL-104, compared to imipenem-cilastatin in cUTI therapy, was completed. Since the combination CAZ/NXL-104 has not shown activity against many strains of anaerobic species, the efficacy of the treatment cIAI was increased by the addition of metronidazole. In November 2009, phase II of clinical studies containing ceftazidime, NXL-104 and metronidazole compared to meropenem in the treatment of cIAI, was completed. No antagonist activity of the compounds used in this combination was observed (14).

Ceftaroline (CEF) is the only registered β-lactam antibiotic exhibiting activity against MRSA and other resistant strains of Gram-positive bacteria, but its efficacy against Gram-negative microorganisms is

limited. Product effective against *Enterobacteriaceae* producing β-lactamases class C and ESβL, but still without bactericidal activity against *P. aeruginosa* has been received by the combination of ceftaroline with NXL-104 at a concentration of 4 μg/mL. Now, combination of ceftaroline with NXL-104 inhibitor in being in the phase II of comparative clinical trials with regard to doripenem in the cUTI therapy. Cerexa Company, Inc. also completed phase I of clinical trials to estimate the effect of products CEF/NXL-104 and CAZ/NXL-104 on QT/QTc interval on ECG in comparison to placebo and moxifloxacin (Avelox), in healthy male volunteers.

The compound MK-7655 belongs to the group of diazabicyclooctanes, and its properties have been used in combination with imipenem. Addition of an inhibitor in a concentration of ≥ 4 μg/mL enhances activity against resistant strains of Gram-positive bacteria. Optimal antimicrobial activity against *P. aeruginosa* was obtained by combination of imipenem with MK-7655 in concentration of 8 μg/mL. After finishing in March 2012 phase I of clinical trial of the pharmacokinetics of MK-7655 compound in patients with impaired of renal function, Merck began recruiting participants for two parallel projects phase II of clinical trials. Both projects relate to the assessment of safety, tolerability and efficacy of MK-7655 substances in combination with imipenem and cilastatin in the treatment of cUTI and cIAI.

Compound ME1071, derivative of maleic acid exhibiting efficacy against *Enterobacteriaceae* producing MBL enzymes is still poorly understood. The use of an inhibitor at a concentration of 32 μg/mL with carbapenems (imipenem, doripenem and biapenem) and ceftazidime increases susceptibility of *P. aeruginosa* producing MBL from 8% to 27% (15). Meiji Seika Pharma Company Co., Ltd. conducts phase I of clinical trials on the use of ME1071 as parenteral administration.

CONCLUSIONS

The complicated process of clinical trials, often difficult to carry out, long-lasting and extremely expensive, is necessary to obtain the registration of medicinal products and the marketing authorization.

In the situation of the gradual increase in the number of multidrug-resistant strains of bacteria, there is a constant need to introduce new, more effective products into medicinal practice. The problem of resistance, particularly in the case of microorganisms producing β-lactamases with extended spectrum and carbapenemases should be an important issue in determining the appropriate antibiotic. The choice of an effective treatment should be carried out basing on the result of the antibiogram, reducing the spread of the resistance gene especially among clinical strains.

Compound CXA-101 displaying the best efficacy in comparison to commercially available cephalosporins, or razupenem exhibiting the highest antibacterial activity against multidrug-resistant strains of Gram-positive bacteria among the registered carbapenems may create an important alternative in the search for the appropriate treatment option. Promising are also compounds eg. combination of three compounds ceftazidime with β-lactamase inhibitor NXL-104 and metronidazole, or product BAL30376, that uses an unique penetration strategy into the bacterial cell – as a carrier of iron ions – siderophore.

There is a chance that the new β-lactam compounds currently undergoing clinical trials, may receive an approval of medicinal products registration authorities and will be available on the market as new antibacterial agents.

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