EXAMINATION OF ANTIMICROBIAL ACTIVITY OF SELECTED NON-ANTIBIOTIC PRODUCTS

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A variety of compounds which are involved in the management of diseases of non-infectious aetiology have shown some antimicrobial activity in vitro, against bacteria and other microorganisms (1–3). Such compounds are called “non-antibiotics”. By the end of the nineteenth century the dyes were known to possess antimicrobial activity, for instance Paul Ehrlich used methylene blue (one of phenothiazines compounds) as an antimicrobial agent (4). So far, a lot of attention has been focused on thioxanthenes, phenothiazines, and other agents with affinities to cellular transport systems which influence the structure of cellular membrane or ions transport etc. (5, 6).

Antimicrobial activity was found among some “non-antibiotics” present at Polish pharmaceutical market, during few years of controlling of the drugs in the National Medicines Institute. So far, about 900 drugs which were randomly chosen from different groups of pharmaceutical products have been examined. During the study (7–12), it was indicated that some of preparations inhibited growth of at least one of the four examined standard microbial strains. The drugs with the following active substances showed significant antimicrobial activity: amlodipine, acepromazine, butorphanole, cisapride, cisplatin, clomipramine, diltiazem, emadastine, fluvastatine, ketamine, levocabastine, matiprananol, methotrexate, nicergoline, perphenazine, proxymetacaine, sertraline, tegaserole, tetrahydrozoline, ticlolidine and tropicamide.

The aim of this study was to continue of the search and characterization of the antimicrobial activity of non-antibiotic drugs, obtained during a routine state control of pharmaceutical products from the Polish market performed by National Medicines Institute.

EXPERIMENTAL

Materials

The following microorganisms: Escherichia coli – ATCC 8739, Pseudomonas aeruginosa – ATCC 15442, Staphylococcus aureus ATCC – 6538P, Candida albicans – ATCC 10231 were used in the study.

The following pharmaceutical products available at the Polish market were randomly chosen for the analysis: Aescuven forte 30 mg tabl. (Hippocastani extr.), Agapurin SR 600 mg tabl. (pentoxifylline), Alpha-Vibolex 300 mg, 600 mg caps. (DL-α-lipoic acid), Amphochol 242 mg tabl. (Fumaria officinalis), Anesteloc 40 mg tabl. (pantoprazole), Atorvox 10 mg, 20 mg. tabl. (atorvastatin), Atorwastatyna 10 mg, 20 mg, (atorvastatin), Azathioprine 50 mg tabl. (azathioprine), Bioprazol

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10 mg, 20 mg tabl. (omepazol), Bisogamma 10 mg tabl. (bisopropol ole fumarate), Calcium pantothenicum 100 mg tabl. (calcium pantethenate), Captopril 25 mg tabl. (captoptril), Cardonit Prolongatum 40 mg caps. (isosorbide), Cerazette mg tabl. (verapamil), Sulfasalazin EN 500 mg tabl (sulfasalazine), Tabex 1.5 mg tabl. (citisine), Tamiflu 30 mg caps. (oseltamivir), Tegretol 400 mg (carbamazepine), Tertensif Kombi 4 mg + 1.25 mg tabl. (tert-buty lamine perindopril, indapamide), Toramide 10 mg tabl. (torasemide), Tramal 50 mg caps. (tramadol), Tulip 20 mg (atorvas tatin), Urosal 300 mg + 300 mg tabl. (salol + methenamine), Warfin 5 mg, tabl. (varfarin), Zokardis 30 mg tabl. (zofenopril), Zylocine 100 mg tabl. (allopurinol).

Initial screening of antimicrobial activity

The sterile blotting-paper disks were soaked with 10% (v/v or w/v) solutions of tested drugs in 0.08 M phosphate buffer pH 7 and placed onto Mueller-Hinton 2 Agar (BioMerieux). Plates were inoculated with standardized cells suspension 0.5 unit (Mcf Farland scale) of tested strains. The inhibition of bacterial growth was seen as a halo around the disk containing the tested compound. Size of inhibition zone was correlated with the antimicrobial activity of the drug.

Minimal inhibitory concentration (MIC) determination

Appropriate dilution of the drug in 0.08 M phosphate buffer pH 7.0 was mixed with 19 mL of a Mueller-Hinton 2 Agar, cooled to 45°C. The suspension of particularly strain of density 0.5 unit (McFarland scale) – 2 µL was applied on agar surface. The lowest concentration of active substance in the tested drug, which totally inhibited growth of examined strain, was evaluated as MIC value.

RESULTS AND DISCUSSION

It was shown that the drugs listed below inhibited growth of at least one of the examined strains: Nolvadox 20 mg tabl. (tamoxifen), Atorv / Tulip / Atorvastatyna 20 mg tabl. (atorvas tatin), Rilutec 50 mg tabl. (riluzole), Alpha Vibolex 600 mg caps. (α-lipoic acid), Deprim forte 425 mg caps. (Hyperici herbae extr. siccum), Zokardis 30 mg tabl. (zofenopril), Fevarin 50 mg tabl. (fluvoxamine), Pernazinum 100 mg tabl. (perazine), Rebetol 200 mg caps. (ribavirine) (Table 1).

Staphylococcus aureus was susceptible to over 70% of the drugs listed in Table 1. Fluvoxamine and perazine inhibited growth of S. aureus in concentration 2 mg/mL and E. coli in concentration 2 mg/mL and 4 mg/mL, respectively. Other chemical com-
Examination of antimicrobial activity of selected non-antibiotic products showed activity against this microorganism in concentrations between 4 and 36 mg/mL. C. albicans was susceptible to over 60% of the drugs listed in Table 1. The strongest senstivity C. albicans showed to antiepileptic substance riluzole 3 mg/mL. Additionally, fluvoxamine and perazine inhibited growth of C. albicans in concentration 4 mg/ml. Interestingly, natural product – Deprim forte – dry extract from Hyperici herbae in concentration 33 mg/mL. P. aeruginosa was susceptible to fluvoxamine and ribavirine (MIC – 10 mg/mL).

Kristiansen et al. (1, 5, 13) confirmed that non-antibiotic compounds enhance the in vitro activity of certain antibiotics against specific bacteria. For instance omeprazole and nizatidine enhance the inhibition effect of metronidazole on Helicobacter pylori growth (14) or phenothiazines could be supporting compounds in the treatment of multidrug-resistant Gram-negative rods (15). So, our further investigations will focus on this type of activity. Moreover, the antimicrobial activity of such non-antibiotic drugs emphasises a necessity of the neutralization of their activity during the microbial purity tests of pharmaceutical products (16, 17). This is the reason why any product should be validated towards its possible inhibition against microorganisms. In this study we examined mostly tablets and capsules in which addition of preservatives occur very randomly. From other examined products, in dermatological cream Emla any ingredients (including preservatives) do not influence the growth of tested microorganisms in testing concentration (1 g/10 ml), therefore test of microbial purity does not required any neutralization of this product.

REFERENCES


Table 1. Antimicrobial activity of selected non-antibiotic drugs

<table>
<thead>
<tr>
<th>Active substance (Pharmaceutical product)</th>
<th>Minimal inhibitory concentration (MIC) in mg/mL of active substance in drug</th>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
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<tr>
<td>Antagonist oestrogenorum* – tamoxifen (Nolvadox 20 mg tabl.)</td>
<td>6</td>
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<tr>
<td>Antiatheroscleroticum – atorvastatin (Atorvost/Atorvastyna/Tadip 20 mg tabl.)</td>
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<tr>
<td>Anticonvulsativum, Antiepilepticum – riluzole (Rilutec 50 mg tabl.)</td>
<td>5</td>
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<tr>
<td>Antidotm, Hepatoprotectivum – α-lipoic acid (Alpha Vibolex 600 mg caps.)</td>
<td>36</td>
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<tr>
<td>Cholereticum, Sedativum – Hyperici herbae extr. sicc. (Deprim forte 425 mg caps.)</td>
<td>33</td>
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<tr>
<td>Inhibitor ACE – zofenopiril (Zokandis 30 mg tabl.)</td>
<td>15</td>
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<tr>
<td>Thymolepticum – fluvoxamine (Fevarin 50 mg tabl.)</td>
<td>4</td>
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<tr>
<td>Neurolepticum – perazine (Pernazinum 100 mg tabl.)</td>
<td>2</td>
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<tr>
<td>Virustaticum – ribavirine (Rebetol 200 mg caps.)</td>
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* the names of pharmaceutical groups according to Podlewski et al. (18); ** lack of microbial growth inhibition