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Antimicrobial activity of ibuprofen: New perspectives on an "Old" non-antibiotic drug

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ABSTRACT

Pharmaceutical industry has been encountering antimicrobial activity of non-antibiotics during suitability tests carried out prior to routine pharmacopoeial microbiological purity analysis of finished dosage forms. These properties are usually ignored or perceived as a nuisance during pharmaceutical analysis.

The aim of this study was: (i) to compare the available data to our method suitability test results carried out on products containing ibuprofen, i.e. to demonstrate that method suitability can be a valuable tool in identifying new antimicrobials, (ii) to demonstrate the antimicrobial activity of ibuprofen and ibuprofen lysine.

Microbiological purity method suitability testing was carried out according to European Pharmacopoeia (EP), chapters 2.6.12. and 2.6.13. Antimicrobial activity of ibuprofen and ibuprofen lysine was demonstrated by a disk diffusion method, a modification of the European Committee for Antimicrobial Susceptibility Testing method (EUCAST), against test microorganisms recommended in the EP.

It was confirmed that ibuprofen may be responsible for the broad spectrum of antimicrobial activity of the tested products, and that method suitability tests according to the EP can indeed be exploited by the scientific community in setting guidelines towards future research of new antimicrobials. In the disk diffusion assay, inhibition zones were obtained with more than 62.5 μg and 250 μg for *Staphylococcus aureus,* 125 μg and 250 μg for *Bacillus subtilis*, 31.3 μg and 125 μg for *Candida albicans*, 31.3 μg and 62.5 μg for *Aspergillus brasiliensis*, of ibuprofen/disk, and ibuprofen lysine/disk, respectively. For *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* inhibition zones were not obtained.

Antimicrobial activity of ibuprofen is considered merely as a side effect, and it is not mentioned in the patient information leaflets of ibuprofen drugs. As such, for the patient, it could represent an advantage, but, it could also introduce additional risks during usage. Further microbiological, pharmacological and clinical trials are of great importance.

1. Introduction

Non-antibiotics are substances that aim at eukaryotic targets in the treatment of pathological conditions of non-infectious aetiology. They are mainly used as active pharmaceutical ingredients (APIs) in tablets, capsules, oral suspensions, creams, ointments, infusions and other pharmaceutical dosage forms. Antimicrobial properties are usually discovered afterwards and are considered to be a side effect.

The antimicrobial potential of non-antibiotics is not a recent discovery. More than 100 years ago, Paul Erlich reported antimicrobial activity of methylene blue and its ability to decrease the mobility of bacteria. The latter characteristic subsequently led to the development of the neuroleptic drug chlorpromazine, while the antimicrobial activity was no longer interesting after the discovery of penicillin. Antimicrobial properties of other

Abbreviations: API, active pharmaceutical ingredient; COX, cyclooxygenase enzymes; EP, European Pharmacopoeia; EUCAST, European Committee for Antimicrobial Susceptibility Testing; MHA, Mueller–Hinton agar; NSAID, non-steroidal anti-inflammatory drug; SDA, Sabouraud dextrose agar; TAMC, total aerobic microbial count; TSB, tryptic soy broth; TYMC, total yeast/mould count.

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non-antibiotics faced a similar fate, up until the end of the 20th century and the growing need for new antimicrobial substances (Martins et al., 2008; Mazumdar et al., 2010).

Some non-antibiotics have a direct antimicrobial activity through various effects on bacterial and/or fungal cells, for example, membrane effects, metabolic alterations, DNA intercalation, adhesion suppression, etc. Others have an indirect antimicrobial activity as helper compounds or as immune system modulators. Helper compounds are enabling further usage of conventional antibiotics by acting synergistically when co-administered or by reverting resistant microbial phenotypes, for example, by altering the permeability of the membranes. Some helper compounds may inhibit replication of the plasmids that can carry resistance genes and even eliminate them from the cells. Immune system modulators help the host in fighting the infection, for example, by stimulating cytokine secretion from the T-cells, or by potentiating the killing of the phagocytised microorganism inside of the macrophage (Martins et al., 2008; Mazumdar et al., 2010).

Pharmaceutical industry of today frequently encounters the antimicrobial activity of non-antibiotics during suitability tests carried out prior to routine pharmacopoeial microbiological purity analysis, one of the quality requirements upon release of the drug to the market, which assures microbiological safety for the patient. Suitability tests are conducted with a sole purpose of adjusting all method conditions to specific physical, chemical and biological characteristics of the sample in order to achieve the highest possible sensitivity and reliability. If the sample contains an antimicrobial substance, lower recovery rates and/or weaker colony growth of the test microorganisms are detected in its presence. Sadly, in most of the cases this information is perceived only as a nuisance, and great effort is made in order to neutralise such activity (European Pharmacopoeia, chapter 2.6.12., 2010; Kruszewska et al., 2012).

This work represents research inspired by several years of method suitability studies carried out at Belupo, Inc. pharmaceutical company, during which many antimicrobial samples were encountered, including products containing ibuprofen as an API. Ibuprofen is one of the favourite *over-the-counter* drugs, a non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory properties. It is a non-selective inhibitor of the cyclooxygenase (COX) enzymes, which are primary enzymes of the prostaglandin biosynthesis: constitutive COX-1 (responsible for physiological functions) and inducible COX-2 (involved in inflammation). COX convert arachidonic acid into prostaglandin PGH_2, which is converted into other prostaglandins (pain, inflammation and fever mediators) and thromboxane A_2 (which leads to blood clot formation) (Davies, 1998; Dollery, 1999; Milani and Iacobelli, 2012).

Antibacterial and antifungal activity of ibuprofen was mentioned for the first time by Hersh et al. in 1991 and by Sanyal et al. in 1993, respectively (Elvers and Wright, 1995). Therefore, antimicrobial activity of ibuprofen, both direct and indirect, has been known for more than 20 years. Surprisingly, only about 20 articles can be found on the subject, while some aspects of the antimicrobial activity of ibuprofen have not been explored at all, such as the mechanisms of the antibacterial action.

It is true that most of the antimicrobial effects of ibuprofen, as well as the effects of other non-antibiotics, happen at concentrations that are far above those that are normally achieved in the patient's blood upon administration of therapeutic doses. But, the potential lies in the topical application of ibuprofen (Elvers and Wright, 1995; Pina-Vaz et al., 2000) and in the treatment of other localised infections, for example, urinary tract infections, since it has been shown that ibuprofen reaches inhibitory doses in the patient's urine (Pina-Vaz et al., 2000). Additionally, it has been proven that low ibuprofen plasma concentrations that may not be high enough for a direct antifungal activity, are sufficient to induce efflux pump inhibition and the reversion of the azole resistant phenotypes in *Candida* species (Pina-Vaz et al., 2005). Ibuprofen could even be used as a coating of the prosthetic devices (Del Prado et al., 2010) or maybe as a *lead compound* for the synthesis of new antimicrobial agents with novel mechanisms of action (Mazumdar et al., 2010).

Unfortunately, antimicrobial activity of ibuprofen is still considered merely a side effect, which is not mentioned in the patient information leaflets of ibuprofen containing drugs. As such, for the patient, it may represent an advantage, a hidden added value of the products of an unknown extent. But, it may also introduce additional risks during usage, especially during simultaneous use of ibuprofen products with antibiotics (Finquelievich et al., 2002; Mandell and Coleman, 2002; Hynninen et al., 2006) or probiotics (Jiménez-Serna and Hernández-Sánchez, 2011), or, for example, if used rectally, during which it could cause imbalance in the gut microbiota.

The aim of this study was: (i) to compare the available data to our results and conclusions of the method suitability tests carried out on products containing ibuprofen as an API, i.e. to demonstrate that method suitability can be a valuable tool in identifying new antimicrobials and in setting guidelines towards their future research, even when it is carried out on finished dosage forms; and (ii) to demonstrate the antimicrobial activity of ibuprofen API as a pure substance and in the form of ibuprofen lysine, an ibuprofen salt, which is more soluble in water and which is used as an API in "fast-acting" ibuprofen formulations (Moore et al., 2014).

2. Materials and methods

2.1. Method suitability testing according to European Pharmacopoeia (EP)

Microbiological purity method suitability testing was carried out on the following dosage forms containing ibuprofen (manufactured by Belupo, Inc., Koprivnica, Croatia): non-aqueous oral forms (Neofen forte 400 mg tablets, Ibuprofen 600 mg film-coated tablets, Ibuprofen 800 mg film-coated tablets), aqueous oral forms (Neofen syrup, Ibuprofen 100 mg/5 ml oral suspension), preparations for cutaneous use (Neofen spray for the skin, Neofen plus gel) and a preparation for rectal use (Neofen 125 mg suppositories). Microbiological purity requirements for these products were set according to the EP chapter 5.1.4. Therefore, the following parameters were tested: TAMC (total aerobic microbial count), TYMC (total combined yeasts/moulds count), and the presence of *Escherichia coli, Staphylococcus aureus* and/or *Pseudomonas aeruginosa* in 1 g of the sample (EP, chapter 5.1.4., 2010).

Sample solutions of 1:10 (m/V), and their following decimal dilutions were prepared. TAMC and TYMC tests were carried out as described in the EP 2.6.12. (EP, chapter 2.6.12., 2010), with the pour-plate method being preferred, i.e. chosen as the starting method. The tests for the specified microorganisms were carried out as described in the EP 2.6.13. (EP, chapter 2.6.13., 2010), with the following specifics: 100 ml of tryptic soy broth (TSB) were used as the starting volume in the first step and 1 μl loops were used as the starting subculturing method.

As test microorganisms, commercially available ATCC test strains recommended in the EP were used. Bacterial and *Candida albicans* cultures were prepared fresh, by overnight incubation in TSB, at 30–35 °C, while *Aspergillus brasiliensis* was grown on Sabouraud dextrose agar (SDA) slant through 5–7 days, or until good sporulation was obtained, then it was washed with buffered sodium chloride peptone solution according to the EP, with 1 g/l of