SHORT COMMUNICATION
Polish Journal of Microbiology
2023, Vol. 72, No 4, 507–513
https://doi.org/10.33073/pjm-2023-038

Do NSAIDs and Other Pain Relief Drugs Can Inhibit the Growth of *Lactobacillaceae*?

HANNA KRUSZEWSKA*, ANNA ZAWISTOWSKA-ROJEK and STEFAN TYSKI

Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland

Submitted 4 July 2023, accepted 13 September 2023, published online 10 October 2023

Abstract



Non-steroidal anti-inflammatory drugs (NSAIDs) commonly used in clinical practice may cause gastrointestinal injuries and influence the gut microbiota. This study investigated the effects of various NSAIDs and some analgesics on the viability of *Lactobacillaceae* strains (including probiotic strains) *in vitro*. It was found that diclofenac, ibuprofen, ketoprofen, dexketoprofen, flurbiprofen, and acetylsalicylic acid inhibited the growth of lactobacilli at a concentration of 0.05–3.2 mg/ml. These MICs of NSAIDs are well above therapeutic plasma concentrations achieved in humans, indicating that the tested drugs should not inhibit the growth of lactobacilli in the human digestive tract.

K e y w o r d s: analgesics, NSAIDs, *Lactobacillaceae*, probiotic bacteria, non-antibiotics

Some drugs that do not belong to the group of antibiotics and antibacterial chemotherapeutics may have direct (Tyski 2003; Kruszewska et al. 2012; Laudy et al. 2017; Lagadinou et al. 2020; Kruszewska et al. 2021) or indirect antibacterial activities, e.g., affect the activity of membrane efflux pumps (Nair et al. 2004; Laudy et al. 2018) or the *quorum sensing* systems (Gajdács and Spengler 2019; Elshaer et al. 2022). These drugs are called non-antibiotics, and we determined whether frequently and sometimes chronically taken non-steroidal anti-inflammatory drugs (NSAIDs) and pain relief drugs could affect bacteria of the family *Lactobacillaceae* present in the microbiota, as well as those administered in probiotic preparations. A group of analgesics and NSAIDs, of which some presented antibacterial activity, was selected for the study. They are widely available (over-the-counter – OTC drugs) and are taken by patients with, among others, osteoarthritis, rheumatoid arthritis, acute gout attack, rheumatic fever, and juvenile chronic arthritis or for blood thinning (Phillips et al. 2004; Bacchi et al. 2012). The basis of the mechanism of action of NSAIDs in humans relies on the inhibition of the activity of cyclooxygenase (COX), an enzyme involved in the synthesis of prostaglandins. COX-1 is an enzyme physiologically active in various sites such as platelets, kidneys, blood vessels, and the stomach, whereas the activity of COX-2 increases rapidly in inflamed tissues (Yu et al. 2016). Due to the frequency and high doses of NSAIDs, gastrointestinal toxicity is likely to be a significant side effect, especially in predisposed patients (Bjarnason et al. 2018). The question is whether these ailments can be reduced using probiotic bacteria and whether NSAIDs will affect their growth.

Some studies have shown the effects of NSAIDs on the growth and virulence of symbiotic and pathogenic human bacterial strains. Antibacterial activity has been demonstrated for diclofenac, ibuprofen, acetylsalicylic acid (Laudy et al. 2016; Zimmermann and Curtis 2017; Lagadinou et al. 2020), flurbiprofen (Zimmermann and Curtis 2017; Lagadinou et al. 2020), piroxicam, indomethacin, and ketoprofen (Elshaer et al. 2022). Moreover, Laudy et al. (2016) showed that ibuprofen, diclofenac, acetylsalicylic acid, and mefenamic acid were substrates for efflux pumps in Gram-negative rods. Due to the differences in the susceptibility of bacteria to NSAIDs, it is interesting whether they can inhibit the growth of bacteria from the *Lactobacillacae* family and, in the case of a positive finding, at which concentrations. *Lactobacillaceae* strains are ubiquitous and can colonize the oral cavity, digestive tract, and vagina, constituting symbiotic protection. Besides, they can be present in soil, water, manure, sewage, and silage. Some

* Corresponding author: H. Kruszewska, Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland; **e-mail: h.kruszewska@nil.gov.pl**

© 2023 Hanna Kruszewska et al.
This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (https://creativecommons.org/licenses/by-nc-nd/4.0/).

bacteria from this family can be classified as probiotic microorganisms that may have local and systemic effects on the host organism. The main benefits resulting from the presence of probiotic microorganisms are stimulation of the immune system, production of antibacterial substances, regulation of the composition of the intestinal microbiota, antimutagenic and anticancer effects (Kerry et al. 2018; Klopper et al. 2018; Zawistowska-Rojek and Tyski 2022), and protection of the intestinal tract by the biosynthesis of cytoprotective short-chain fatty acids (Syer et al. 2015; Markowiak-Kopeć and Śliżewska 2020).

People often supplement antibiotic therapies with some probiotics or use them for digestive system ailments (Blaabjerg et al. 2017; Zawistowska-Rojek and Tyski 2022). However, the constant anti-inflammatory and analgesic use of NSAIDs (Phillips et al. 2004), also in the prevention and treatment of colorectal cancer (Kmieć et al. 2014), might threaten the intestinal microbiota; moreover, gastrointestinal side effects caused by NSAIDs could be associated with affecting the survivability of the probiotic bacteria.

Therefore, this study's main goal was to estimate whether commonly available anti-inflammatory and analgesic drugs affect the survivability of bacteria from the *Lactobacillaceae* family, which could impact the feasibility of using probiotic preparations.

We determined the effects of various active substances of NSAIDs, namely acetylsalicylic acid (Mikromol), diclofenac (Amoli Organics), indomethacin (Dr. Ehrenstorfer), ibuprofen (Mikromol), naproxen (USP), ketoprofen (LGC), dexketoprofen (Menarini), flurbiprofen (Sigma-Aldrich), piroxicam (USP), meloxicam (Ph Eur. CRS), celecoxib (Sigma-Aldrich), etoricoxib (Merck), nabumetone (Sigma-Aldrich) nimesulide (Sigma-Aldrich) and of analgesics, such as paracetamol (Sigma-Aldrich) and tramadol (LGC) on the viability of 49 *Lactobacillaceae* strains isolated from probiotic products (dietary supplements, foods for special medical purposes, yogurts) and strains isolated from healthy people (cervix or colon swabs). Clinical isolates were derived from the strain collection of the Department of Pharmaceutical Microbiology and Bioanalysis of the Medical University of Warsaw, Poland. The following strains were used: *Lactobacillus acidophilus* (n = 19), *Lacticaseibacillus rhamnosus* (10), *Lacticaseibacillus casei* (6), *Lacticaseibacillus paracasei* (3), *Lactiplantibacillus plantarum*, and *Limosilactobacillus fermentum* (1). Three reference strains *L. acidophilus* ATCC® 4356™, *L. rhamnosus* ATCC® 53103™, and *L. plantarum* ATCC® 14917™ were also included.

The assessment of the antibacterial activity of the selected agents was carried out in two stages. For the initial screening, 14 NSAIDs and two analgesics were investigated. The tested substances were dissolved in DMSO (POCH S.A., Poland) and diluted in 0.9% NaCl (bioMérieux, France) to 1 ml, to achieve a final concentration of 3.2 mg/ml in the MRS agar (De Man, Rogosa and Sharpe Agar; Merck Millipore, Germany). As a control, DMSO without active substances was used. The strains were harvested from the MRS agar to prepare the cell suspensions at a density of 0.5 on the McFarland scale. Subsequently, 2-µl aliquots of the suspensions of the tested strains were dripped onto the agar surface. The plates were incubated at 37°C for 72 h in an atmosphere with 5% CO_2. The compounds for which no growth was observed for at least one of the tested strains were selected for further investigation. In the second stage of antibacterial activity testing, the minimal inhibitory concentrations of diclofenac, ibuprofen, ketoprofen, dexketoprofen, and flurbiprofen in the MRS liquid medium (MRS broth; Merck Millipore, Germany), were determined in triplicate according to the CLSI guidelines (CLSI 2015).

The following NSAIDs: nabumetone, celecoxib, nimesulide, meloxicam, etoricoxib (all belonging to COX-2 inhibitors), naproxen, piroxicam and indomethacin, and the analgesics tramadol and paracetamol at a concentration of 3.2 mg/ml, did not inhibit the growth of all strains of the family *Lactobacillaceae in vitro*.

For compounds showing activity against both COX-1 and COX-2, the degree of growth inhibition depended on the used NSAID.

Based on the MIC determination, diclofenac, ibuprofen, ketoprofen, dexketoprofen, flurbiprofen, and acetylsalicylic acid inhibited bacterial growth at concentrations of 0.05–3.2 mg/ml, depending on the tested strain (Table I). The highest activity against most of the tested strains was found for diclofenac (MIC = 0.05 mg/ml for two strains and MIC = 0.1 mg/ml for 30 strains). The second most active compounds were ibuprofen (MIC = 0.4 mg/ml for 19 strains) and flurbiprofen (MIC = 0.4 mg/ml for 9 strains). The MIC of ketoprofen and dexketoprofen was 0.8 mg/ml for four strains. The lowest activity against all strains was obtained for acetylsalicylic acid (MIC = 3.2 mg/ml) (Table I). This value agreed with the previous data obtained by Kruszewska et al. (2021) for the probiotic strains *L. plantarum* and *L. acidophilus.* Also, the results obtained in this study regarding the activity of nimesulide against lactobacilli strains were consistent with previous research (Kruszewska et al. 2021). In contrast, ibuprofen in our study was more active than dexibuprofen (its *S*-enantiomer) in all tested strains (Kruszewska et al. 2021). In the study of Laudy et al. (2016), the highest activity showed diclofenac (MICs: 0.2–1.6 mg/ml), followed by ibuprofen (MICs: 1.6–3.2 mg/ml) and acetylsalicylic acid (MICs: 1.6–3.2 mg/ml), whereas naproxen and nimesulide showed no activity against most Gram-negative bacteria. Analyzing Gram-positive cocci,

Table I
Minimal inhibitory concentration (MIC) of selected NSAIDs against 49 *Lactobacillaceae* strains.

Species	Source/(number of strains)	MIC (mg/ml)																	Acetylsalicylic acid
		Flurbiprofen				Ketoprofen			Dexketoprofen			Ibuprofen			Diclofenac				
		0.4	0.8	1.6	3.2	0.8	1.6	3.2	0.8	1.6	3.2	0.4	0.8	1.6	0.05	0.1	0.2	0.8	3.2
L. acidophilus	ATCC® 4356™	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1
	Probiotic products (n = 10)	4	2	4	0	0	9	1	0	9	1	2	5	3	0	5	2	3	10
	Clinical isolates (n = 9)	0	4	5	0	0	6	3	0	4	5	1	8	0	0	4	5	0	9
L. rhamnosus	ATCC® 53103™	0	0	1	0	0	0	1	0	0	1	0	1	0	0	0	1	0	1
	Probiotic products (n = 4)	1	3	0	0	0	0	4	0	0	4	0	4	0	0	1	3	0	4
	Clinical isolates (n = 6)	0	0	5	1	0	0	6	0	1	5	0	6	0	0	4	2	0	6
L. casei	Probiotic products (n = 4)	2	2	0	0	0	3	1	0	4	0	4	0	0	1	3	0	0	4
	Clinical isolates (n = 2)	0	2	0	0	0	2	0	1	1	0	2	0	0	1	1	0	0	2
L. paracasei	Probiotic products (n = 3)	2	1	0	0	2	1	0	2	1	0	3	0	0	0	3	0	0	3
L. plantarum	ATCC® 14917™	0	1	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1
	Probiotic products (n = 3)	0	3	1	0	1	2	0	0	3	0	3	0	0	0	3	0	0	3
	Clinical isolates (n = 4)	0	2	1	0	0	4	0	0	4	0	1	3	0	0	3	1	0	4
L. fermentum	Probiotic products (n = 1)	0	1	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1
TOTAL	49	9	22	17	1	4	29	16	4	29	16	19	27	3	2	30	14	3	49

Leão et al. (2020) showed that for *Staphylococcus aureus* strains, naproxen and piroxicam were inactive, whereas the MIC of diclofenac and acetylsalicylic acid was 2 mg/ml. According to a review by Zimmermann and Curtis (2017), the MICs of ibuprofen for *S. aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* ranged from 0.05–1.25 mg/ml, and that of diclofenac for *S. aureus* was 0.05 mg/ml.

In studies on the effect of indomethacin (10 mg/kg) on the intestinal microbiota in mice, it was found that indomethacin does not affect the viability of *Lactobacillus* (Nadatani et al. 2012; Liang et al. 2015). In our study, indomethacin also did not inhibit the growth of lactobacilli. Moreover, two weeks of intragastrical treatment of rats with aspirin changed the oral microbial composition and increased the numbers of *Lactobacillaceae* (Cheng et al. 2018). However, Montrose et al. (2016) reported that chronic treatment of mice with celecoxib (1,000 ppm) caused a decrease in *Lactobacillaceae* abundance in the ileal content and feces.

Regarding the present study, it is important to relate the MIC values of the tested compounds obtained *in vitro* to their concentrations in the body fluids of people taking these drugs. The maximum plasma blood concentration of NSAIDs depends on the pharmaceutical form, and the most popular forms are tablets, capsules, suppositories, drops, syrups, and injections, which are less common. Oral doses of diclofenac are usually 100–150 mg daily in adults (Sarzi-Puttini et al. 2013). According to Willis et al. (1979), the maximum plasma concentration after a single oral dose (100 mg) and rapid intravenous diclofenac sodium injection ranges from 1.4 to 3.0 μg/ml. After administration of suppositories (50 mg) the maximum concentration (C_{max}) of the plasma was 1.95 ± 0.8 μg/ml (Novartis Pharmaceuticals UK Ltd. 2023). In the case of diclofenac, where the MIC against lactobacilli was 0.05 mg/ml (Table II), which is much higher than the maximum plasma concentration in the human body (0.003 mg/ml), it cannot influence *Lactobacillaceae* strains.

Ibuprofen is generally dosed from 200 to 600 mg, with a daily dose of up to 2,400 mg (Sarzi-Puttini et al. 2013). According to Janssen and Venema (1985), the maximum plasma concentration after administration of 600 mg tablets of ibuprofen twice, three times, and four times daily ranged from 39.4–66.4 μg/ml. After administration of suppositories (125 mg) C_{max} was an average of 23.3 μg/ml (EMA 2003). In this case, where the MIC against lactobacilli was 0.4 mg/ml (Table II), which is much higher than the maximum plasma concentration in the human body (approximately 0.06 mg/ml), ibuprofen cannot influence *Lactobacillaceae* strains.

For ketoprofen, the average dosage is approximately 150 mg per day, with a maximum of 200 mg (Sarzi-Puttini et al. 2013). According to Roda et al. (2002), after the administration of 200 mg of ketoprofen in the form of oral tablets, C_{max} was 5.91 ± 0.66 μg/ml. After rectal administration (100 mg) the mean maximum concentration of the plasma was 6.56 μg/ml (Caillé et al. 1980). The MIC against lactobacilli was 0.8 mg/ml (Table II), which is much higher than the maximum plasma concentration in the human body, and 0.006 mg/ml of ketoprofen cannot impair the viability of *Lactobacillaceae* strains.

Dexketoprofen is the (*S*)-enantiomer of ketoprofen, and the total daily dose should not exceed 75 mg, with a maximum single dose of 25 mg (Aronson 2016). According to González-Canudas et al. (2019), after administration of 25 mg in an oral solution, the blood C_{max} of dexketoprofen was 4.3 ± 0.9 μg/ml. The MIC against lactobacilli was 0.8 mg/ml (Table II), which is much higher than the maximum blood plasma concentration in the human body, and 0.004 mg/ml of dexketoprofen cannot influence the viability of *Lactobacillaceae* strains.

The daily dose of flurbiprofen should not exceed 400 mg (Valentovic et al. 2007). According to Yilmaz and Erdem (2015), after oral administration of a 100 mg formulation tablet, the C_{max} of flurbiprofen was 42.1 μg/ml, and after rectal administration (75 mg), the maximum concentration of the plasma was 72.4–77.3 μg/ml (Scaroni et al. 1984). The MIC against lactobacilli was 0.4 mg/ml, which exceeds the maximum plasma concentration in the human body (about 0.04 mg/ml), indicating that flurbiprofen does not influence *Lactobacillaceae* strains at these doses.

Table II
The summary of activity *in vitro* of tested NSAIDs and analgesics against *Lactobacillaceae* strains.

Active substance	The lowest inhibitory concentration for a minimum 2 strains
Nabumetone	> 3.2 mg/ml
Celecoxib	> 3.2 mg/ml
Nimesulide	> 3.2 mg/ml
Meloxicam	> 3.2 mg/ml
Etoricoxib	> 3.2 mg/ml
Naproxen	> 3.2 mg/ml
Piroxicam	> 3.2 mg/ml
Indomethacin	> 3.2 mg/ml
Tramadol	> 3.2 mg/ml
Paracetamol	> 3.2 mg/ml
Acetylsalicylic acid	3.2 mg/ml
Ketoprofen	0.8 mg/ml
Dexketoprofen	0.8 mg/ml
Ibuprofen	0.4 mg/ml
Flurbiprofen	0.4 mg/ml
Diclofenac	0.05 mg/ml

Regarding acetylsalicylic acid, according to Nagelschmitz et al. (2014), after an oral dose of 500 mg, the C_{max} in blood plasma was 4.84 µg/ml, and after intravenous (iv) administration to 21 volunteers, it was 54.25 µg/ml. In our study, the MIC of acetylsalicylic acid against lactobacilli was 3.2 mg/ml (Table II); it is much higher than in the plasma blood after iv administration (approximately 0.054 mg/ml).

NSAIDs in humans and animals can induce changes in the intestinal microbiome, like dysbiosis (Wang et al. 2021). Potential mechanisms causing NSAIDs-induced dysbiosis can be divided into two groups: NSAIDs-induced changes in the intestinal bacterial environment (such as mucositis, motility, changes in intestinal pH, bile acid metabolome) and direct antibacterial effects of NSAIDs (Maseda and Ricciotti 2020; Zádori et al. 2023). Disturbances in the intestinal microbiome can contribute to the development of many diseases, including irritable bowel syndrome, ulcerative colitis, Crohn's disease, endocrine, cardiovascular, autoimmune and even neuropsychiatric disorders (Zádori et al. 2023). NSAIDs-induced dysbiosis, in most cases, is characterized by a clear transition from Gram-positive to Gram-negative bacteria, which consequently contributes to the development of intestinal damage. Changes can be observed especially in taxa such as Bacteroidetes (*Bacteroides*, *Parabacteroides*, *Prevotella*, *Barnesiella*, *Rikenellaceae*), Proteobacteria (*Enterobacteriaceae*, *Pseudomonadaceae*, *Alphaproteobacteria*), *Enterococcaceae*, and *Akkermansia* (Wang et al. 2021; Zádori et al. 2023). The increase content of Gram-negative bacteria associated with the use of NSAIDs may cause the intestinal damage (Zádori et al. 2023). However, it should be noted that most of the current knowledge comes from animal studies conducted mainly on rats and mice. There are many differences between humans and rodents in both gastrointestinal anatomy and microbiota composition, so the usefulness of these studies is limited (Wang et al. 2021; Zádori et al. 2023).

In several *in vitro* studies, compounds such as acetylsalicylic acid, ibuprofen, naproxen, diclofenac, indomethacin, and celecoxib have been found to inhibit the growth of both Gram-positive and Gram-negative bacterial strains at concentrations ranging from tens to thousands of µg/ml (Jiménez-Serna and Hernández-Sánchez 2011; Laudy et al. 2016; Lázár et al 2021), and all these drugs induce intestinal dysbiosis in animals or humans (Zádori et al. 2023). However, the *in vitro* MICs of NSAIDs found for the tested strains are well above therapeutic plasma concentrations achieved in humans for these compounds. Thus, it is difficult to directly conclude that the antibacterial properties of NSAIDs can induce significant intestinal dysbiosis in humans, unless we consider the long duration of use of these compounds and their pharmacokinetic properties. However, the extent of this phenomenon is still unknown (Maier et al. 2018).

Pharmacological studies on *Lactobacillus*' viability after NSAIDs treatment depends on the substance used and the animal species tested. In rat studies, the relative abundance of *Lactobacillus* strains decreased after treatment with NSAIDs such as diclofenac, indomethacin, celecoxib, on the other hand, the results of other studies indicate no change in the abundance of lactobacilli (Zádori et al. 2023). At present, the reasons for this apparent discrepancy need to be clarified. The loss of lactobacilli likely requires more time, as it has been reported more often after prolonged exposure to the drug (Zádori et al. 2023). In human studies, beneficial effects on *L. casei* (Endo et al. 2011) and *Lactobacillus gasseri* (LG) OLL2716 (Suzuki et al. 2017) have been reported in reducing aspirin-induced small intestine injuries. In addition, studies on small intestine damage induced by indomethacin have demonstrated a cytoprotective effect of *L. casei* (Watanabe et al. 2009). In order to reduce the dysbiosis caused by NSAIDs, introducing the preparations containing probiotic *Lactobacillus* strains to the therapy is very beneficial. Our *in vitro* studies showed that it should not cause disturbances in the bacteria viability.

In summary, the maximum plasma levels of diclofenac, ibuprofen, ketoprofen, dexketoprofen, flurbiprofen, and acetylsalicylic acid during therapy are much lower than the MIC levels inhibiting the growth of the tested lactic acid bacteria. We, therefore, infer that the tested NSAIDs should not inhibit the growth of bacteria from the *Lactobacillaceae* family in the human digestive tract. Moreover, taking the probiotic preparations with lactobacilli strains during NSAID therapy to prevent intestinal injury should not affect the viability of these strains, so that they can be taken simultaneously.

ORCID
Hanna Kruszewska https://orcid.org/0000-0001-9495-9244
Anna Zawistowska-Rojek https://orcid.org/0000-0002-6797-3240
Stefan Tyski https://orcid.org/0000-0003-3352-038X

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Aronson JK. Meyler's side effects of drugs (sixteenth edition). The international encyclopedia of adverse drug reactions and interactions. Dexketoprofen. Oxford (UK): Elsevier; 2016. p. 888–890. https://doi.org/10.1016/B978-0-444-53717-1.01685-1

Bacchi S, Palumbo P, Sponta A, Coppolino MF. Clinical pharmacology of non-steroidal anti-inflammatory drugs: a review. Antiinflamm Antiallergy Agents Med Chem. 2012;11(1):52–64. https://doi.org/10.2174/187152312803476255

Bjarnason I, Scarpignato C, Holmgren E, Olszewski M, Rainsford KD, Lanas A. Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. Gastroenterology. 2018 Feb;154(3):500–514. https://doi.org/10.1053/j.gastro.2017.10.049

Blaabjerg S, Artzi DM, Aabenhus R. Probiotics for the prevention of antibiotic-associated diarrhea in outpatients – A systematic review and meta-analysis. Antibiotics. 2017 Oct;6(4):21. https://doi.org/10.3390/antibiotics6040021

Caillé G, Besner JG, Lacasse Y, Vézina M. Pharmacokinetic characteristics of ketoprofen suppositories. Biopharm Drug Dispos. 1980 Apr–Jun;1(4):195–201. https://doi.org/10.1002/bdd.2510010407

Cheng X, Huang F, Zhang K, Yuan X, Song C. Effects of nonsteroidal anti-inflammatory and antibiotic drugs on the oral immune system and oral microbial composition in rats. Biochem Biophys Res Commun. 2018;507:420–425. https://doi.org/10.1016/j.bbrc.2018.11.054

CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – 10th ed. CLSI document M07-A10. Wayne (USA): Clinical and Laboratory Standards Institute; 2015.

Elshaer SL, Shaldam MA, Shaaban MI. Ketoprofen, piroxicam and indomethacin-suppressed quorum sensing and virulence factors in *Acinetobacter baumannii*. J Appl Microbiol. 2022 Oct;133(4): 2182–2197. https://doi.org/10.1111/jam.15609

EMA. Ipren, suppositories 125 mg [Internet]. Amsterdam (The Netherlands): European Medicines Agency; 2003 [cited 2023 Jun 30]. Available from http://art45-paediatric-studies-docs.ema.europa.eu/GROUP%20I/ibuprofen/ibuprofen%20573-CHC-9127-001.pdf

Endo H, Higurashi T, Hosono K, Sakai E, Sekino Y, Iida H, Sakamoto Y, Koide T, Takahashi H, Yoneda M, et al. Efficacy of *Lactobacillus casei* treatment on small bowel injury in chronic low-dose aspirin users: a pilot randomized controlled study. J Gastroenterol. 2011 Jul;46(7):894–905. https://doi.org/10.1007/s00535-011-0410-1

Gajdács M, Spengler G. The role of drug repurposing in the development of novel antimicrobial drugs: non-antibiotic pharmacological agents as quorum sensing-inhibitors. Antibiotics. 2019;8(4):270. https://doi.org/10.3390/antibiotics8040270

González-Canudas J, García-Aguirre LJ, Medina-Nolasco A, Ruíz-Olmedo MI, Reyes LJM, Zambrano-Tapia L, Romero-Antonio Y, Molina-Pérez A, Amador-Hernández AG. Bioequivalence evaluation of two oral formulations of dexketoprofen-trometamol (solution and tablets) in healthy subjects: Results from a randomized, single-blind, crossover study. Trends Med. 2019;19:1–5. https://doi.org/10.15761/TiM.1000176

Janssen GM, Venema JF. Ibuprofen: Plasma concentrations in man. J Int Med Res. 1985;13(1):68–73. https://doi.org/10.1177/030006058501300110

Jiménez-Serna A, Hernández-Sánchez H. Effect of different antibiotics and non-steroidal anti-inflammatory drugs on the growth of *Lactobacillus casei* Shirota. Curr Microbiol. 2011 Mar;62(3): 1028–1033. https://doi.org/10.1007/s00284-010-9819-7

Kerry GR, Patra JK, Gouda S, Park Y, Shin HS, Das G. Benefaction of probiotics for human health: A review. J Food Drug Anal. 2018 Jul;26(3):927–939. https://doi.org/10.1016/j.jfda.2018.01.002

Klopper KB, Deane SM, Dicks LMT. Aciduric strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus*, isolated from human feces, have strong adhesion and aggregation properties. Probiotics Antimicrob Proteins. 2018 Mar;10(1):89–97. https://doi.org/10.1007/s12602-017-9307-5

Kmieć N, Wełnicka-Jaśkiewicz M, Jassem J. [Nonsteroidal anti-inflammatory drugs in the prevention and treatment of colorectal cancer] (in Polish). J Oncol. 2014;64(2):175–179. https://doi.org/10.5603/NJO.2014.0024

Kruszewska H, Zareba T, Kociszewska A, Tyski S. Activity of selected non-antibiotic medicinal preparations against standard microorganisms including bacterial probiotic strains. Acta Pol Pharm Drug Res. 2021;78(2):179–185. https://doi.org/10.32383/appdr/136439

Kruszewska H, Zaręba T, Tyski S. Examination of antimicrobial activity of selected non-antibiotic medicinal preparations. Acta Pol Pharm Drug Res. 2012;69(6):1368–1371.

Lagadinou M, Onisor MO, Rigas A, Musetescu DV, Gkentzi D, Assimakopoulos SF, Panos G, Marangos M. Antimicrobial properties on non-antibiotic drugs in the era of increased bacterial resistance. Antibiotics. 2020 Mar;9(3):107. https://doi.org/10.3390/antibiotics9030107

Laudy AE, Kulińska E, Tyski S. The impact of efflux pump inhibitors on the activity of selected non-antibiotic medicinal products against Gram-negative bacteria. Molecules. 2017 Jan;22(1):114. https://doi.org/10.3390/molecules22010114

Laudy AE, Mrowka A, Krajewska J, Tyski S. The influence of efflux pump inhibitors on the activity of non-antibiotics NSAIDS against Gram-negative rods. PLoS One. 2016 Jan;11(1):e0147131. https://doi.org/10.1371/journal.pone.0147131

Laudy AE. Non-antibiotics, efflux pumps and drug resistance of Gram-negative rods. Pol J Microbiol. 2018 Jun;67(2):129–135. https://doi.org/10.21307/pjm-2018-017

Lázár B, László SB, Hutka B, Tóth AS, Mohammadzadeh A, Berekméri E, Ágg B, Balogh M, Sajtos V, Király K, et al. A comprehensive time course and correlation analysis of indomethacin-induced inflammation, bile acid alterations and dysbiosis in the rat small intestine. Biochem Pharmacol. 2021 Aug;190:114590. https://doi.org/10.1016/j.bcp.2021.114590

Leão C, Borges A, Simões M. NSAIDs as a drug repurposing strategy for biofilm control. Antibiotics. 2020 Sep;9(9):591. https://doi.org/10.3390/antibiotics9090591

Liang X, Bittinger K, Li X, Abernethy DR, Bushman FD, Fitz Gerald GA. Bidirectional interactions between indomethacin and the murine intestinal microbiota. elife. 2015 Dec;4:e08973. https://doi.org/10.7554/eLife.08973

Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature. 2018 Mar 29;555(7698):623–628. https://doi.org/10.1038/nature25979

Markowiak-Kopeć P, Śliżewska K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients. 2020 Apr;12(4):1107. https://doi.org/10.3390/nu12041107

Maseda D, Ricciotti E. NSAID-gut microbiota interactions. Front Pharmacol. 2020 Aug;11:1153. https://doi.org/10.3389/fphar.2020.01153

Montrose DC, Zhou XK, McNally EM, Sue E, Yantiss RK, Gross SS, Leve ND, Karoly ED, Suen CS, Ling L, et al. Celecoxib alters the intestinal microbiota and metabolome in association with reducing polyp burden. Cancer Prev Res (Phila). 2016 Sep;9(9):721–731. https://doi.org/10.1158/1940-6207.CAPR-16-0095

Nadatani Y, Watanabe T, Suda W, Nakata A, Matsumoto Y, Kosaka S, Higashimori A, Otani K, Hosomi S, Tanakaet F, et al. Gastric acid inhibitor aggravates indomethacin-induced small intestinal injury via reducing *Lactobacillus johnsonii*. Sci Rep. 2019 Nov; 9(1):17490. https://doi.org/10.1038/s41598-019-53559-7

Nagelschmitz J, Blunck M, Kraetzschmar J, Ludwig M, Wensing G, Hohlfeld T. Pharmacokinetics and pharmacodynamics

of acetylsalicylic acid after intravenous and oral administration to healthy volunteers. Clin Pharmacol. 2014 Mar;6:51–59. https://doi.org/10.2147/CPAA.S47895

Nair BM, Cheung KJ Jr, Griffith A, Burns JL. Salicylate induces an antibiotic efflux pump in *Burkholderia cepacia* complex genomovar III (*B. cenocepacia*). J Clin Invest. 2004 Feb;113(3):464–473. https://doi.org/10.1172/JCI19710

Novartis Pharmaceuticals UK Ltd. *Voltarol 12.5 mg Suppositories* summary of product characteristics [Internet]. Electronic Medicines Compendium; 2023 [cited 2023 Jun 30]. Available from https://www.medicines.org.uk/emc/product/1044/smpc

Phillips WJ, Currier BL. Analgesic pharmacology: II. Specific analgesics. J Am Acad Orthop Surg. 2004 Jul–Aug;12(4):221–233. https://doi.org/10.5435/00124635-200407000-00003

Roda A, Sabatini L, Mirasoli M, Baraldini M, Roda E. Bioavailability of a new ketoprofen formulation for once-daily oral administration. Int J Pharm. 2002 Jul;241(1):165–172. https://doi.org/10.1016/s0378-5173(02)00230-2

Sarzi-Puttini P, Atzeni F, Lanata L, Bagnasco M. Efficacy of ketoprofen vs. ibuprofen and diclofenac: a systematic review of the literature and meta-analysis. *Clin Exp Rheumatol. 2013* Sep–Oct; 31(5):731–738.

Scaroni C, Mazzoni PL, D'Amico E, Benvenuti C, Hind ID. Pharmacokinetics of oral and rectal flurbiprofen in children. Eur J Clin Pharmacol. 1984;27(3):367–369. https://doi.org/10.1007/BF00542178
Standards Institute; 2015.

Suzuki T, Masui A, Nakamura J, Shiozawa H, Aoki J, Nakae H, Tsuda S, Imai J, Hideki O, Matsushima M, et al. Yogurt containing *Lactobacillus gasseri* mitigates aspirin-induced small bowel injuries: A prospective, randomized, double-blind, placebo-controlled trial. Digestion. 2017;95(1):49–54. https://doi.org/10.1159/000452361

Syer SD, Blackler RW, Martin R, de Palma G, Rossi L, Verdu E, Bercik P, Surette MG, Aucouturier A, Langella P, et al. NSAID enteropathy and bacteria: A complicated relationship. J Gastroenterol. 2015 Apr;50(4):387–393. https://doi.org/10.1007/s00535-014-1032-1

Tyski S. Non-antibiotics – drugs with additional antimicrobial activity. Acta Pol Pharm Drug Res. 2003;60(5):401–404.

Valentovic M. Flurbiprofen. In: Enna SJ and Bylun DB, editors. xPharm: The comprehensive pharmacology reference. New York (USA): Elsevier; 2007. p. 1–6. https://doi.org/10.1016/B978-008055232-3.61777-5

Wang X, Tang Q, Hou H, Zhang W, Li M, Chen D, Gu Y, Wang B, Hou J, Liu Y, et al. Gut microbiota in NSAID enteropathy: New insights from inside. Front Cell Infect Microbiol. 2021 Jul;11:679396. https://doi.org/10.3389/fcimb.2021.679396

Watanabe T, Nishio H, Tanigawa T, Yamagami H, Okazaki H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Asahara T, et al. Probiotic *Lactobacillus casei* strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. Am J Physiol Gastrointest Liver Physiol. 2009 Sep;297(3):G506–G513. https://doi.org/10.1152/ajpgi.90553.2008

Willis JV, Kendall MJ, Flinn RM, Thornhill DP, Welling PG. The pharmacokinetics of diclofenac sodium following intravenous and oral administration. Eur J Clin Pharmacol. 1979;16(6):405–410. https://doi.org/10.1007/BF00568201

Yilmaz B, Erdem AF. Determination of flurbiprofen in human plasma by high-performance liquid chromatography. J Chromatogr Sci. 2015 Oct;53(9):1443–1448. https://doi.org/10.1093/chromsci/bmv032

Yu T, Lao X, Zheng H. Influencing COX-2 activity by COX related pathways in inflammation and cancer. Mini Rev Med Chem. 2016; 16(15):1230–1243. https://doi.org/10.2174/1389557516666160505115743

Zádori ZS, Király K, Al-Khrasani M, Gyires K. Interactions between NSAIDs, opioids and the gut microbiota – Future perspectives in the management of inflammation and pain. Pharmacol Ther. 2023 Jan;241:108327. https://doi.org/10.1016/j.pharmthera.2022.108327

Zawistowska-Rojek A, Tyski S. How to improve health with biological agents – Narrative review. Nutrients. 2022 Apr;14(9):1700. https://doi.org/10.3390/nu14091700

Zimmermann P, Curtis N. Antimicrobial effects of antipyretics. Antimicrob Agents Chemother. 2017 Mar 24;61(4):e02268-16. https://doi.org/10.1128/AAC.02268-16