Microbiocenosis of the Gastrointestinal Tract in Rheumatological Patients Taking NSAIDs

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ABSTRACT

Modern rheumatology pays special attention to the detection of diseases at the earliest possible stages. To date, there is evidence that the preclinical period of a systemic autoimmune reaction is associated with dysregulation of immune interactions with synanthropic microflora. The article deals with the issues of violation of the microbial landscape in rheumatological patients and the impact on the microbiont of the stomach and intestines of drugs used to treat rheumatological diseases

Keywords: intestinal microbiocenosis, microbiota, rheumatological diseases, non-steroidal anti-inflammatory drugs.

Despite the high level of development of scientific research at the present stage of healthcare development, the etiology and pathogenesis of most rheumatic diseases (RD) are still unknown. However, studies conducted in recent years have shown that the preclinical period of a systemic autoimmune reaction is associated with dysregulation of immune interactions with synanthropic microflora [1]. Over the past decades, special attention of doctors has been focused on the problem of human microbiocenosis disorders and its role in the development of autoimmune diseases, so evidence has been obtained for a theory suggesting that the altered synanthropic microflora is a factor in initiating and maintaining chronic inflammation in rheumatoid arthritis (RA) and spondyloarthritis (SpA) [2-6]. According to modern data, the preclinical stage of RD consists of several phases: a period of genetic risk, exposure to environmental factors, after which an asymptomatic autoimmune reaction occurs with the development of nonspecific clinical manifestations (periodontitis, subclinical inflammation of the intestine, mucostasis, etc.), which leads to further activation systemic immune-inflammatory response and, as a result, to the formation of the final specific clinical signs of RD that meet its diagnostic criteria [8–11]. In this regard, the microbial ecosystem of a macroorganism must be considered as a complex interconnected system formed in the process of ontogenesis and phylogenesis, self-regulating and continuously interacting with the environment [7]. The

microbiont and the ecosystem of the macroorganism caused by it, despite the complex organization, multifunctionality, tendency to variability, is easily vulnerable and very sensitive to the effects of exogenous and endogenous factors. It is known that the most important among the agents that affect the microbiocenosis of the body are "iatrogenic" effects. Numerous studies have shown that significant changes in the microbiocenosis of the body occur after the use of drugs, primarily antimicrobial agents. Along with this, there is evidence that the intestinal microbiota is involved in the biotransformation of drugs, influencing the effectiveness of pharmacotherapy.

Microbiocenosis is easily disturbed under the influence of various environmental factors: diseases of the gastrointestinal tract, intestinal infections, unbalanced nutrition, stressful situations, surgical interventions, hormonal, radiation and antibacterial therapy. At the same time, the nature of the effect of other drug groups on the microbiocenosis of individual biotopes remains not fully understood; these drug groups include non-steroidal anti-inflammatory drugs (NSAIDs), which are the most widely used in everyday practice. These circumstances were the basis for the present study.

Purpose of the study: to study the microbial biocenosis of the intestine in rheumatological patients and the degree of influence of NSAIDs on the normal intestinal microbiota in vitro using the disk diffusion method.

Materials and methods of the study: 40 patients with rheumatological diseases (RD), 11 (27.5%) men and 29 (72.5%) women, were examined, the average age was 38.6 ± 4 years, the average duration of the disease was 5.2 years. RB received non-selective COX-II inhibitors as symptomatic therapy. Studies of the microflora of the gastrointestinal tract were carried out in the biotopes of the stomach and intestines. The comparison was made in relation to the generally accepted indicators of the norm. In order to study the microbial landscape of the stomach and intestines, the total number of asporogenic anaerobes, lactobacilli, bifidobacteria and the total amount of aerobic groups of microbes (streptococci, staphylococci, Escherichia coli, fungi of the genus Candida, etc.).

Inoculations were made in Petri dishes, 5% blood agar medium was used to isolate aerobic microorganisms. Endo and Saburo, yolk-salt agar. Cultivated under normal conditions for 24-48 hours at a temperature of 370C. To isolate asporogenic anaerobes and lactobacilli, the method of "sealed" polyethylene bags [13] filled with main natural gas [14] was used at a temperature of 37C in a thermostat for 2–3 days.

Along with the determination of the microbial landscape of the studied biotopes of the gastrointestinal tract in RB treated with NSAIDs, we also studied in vitro the degree of "lysis" when using nimesil, indomethacin compared with the well-known antibiotic Tarivid - the disk-diffusion method, followed by measuring the zone of growth inhibition in mm.

The results of the study were statistically processed using standard methods of variation statistics using the Student's t-test using the Excel Office 2010 application program on a Pentium IV computer.

Results and discussion.

A comparative analysis of the biotopes of the stomach and intestines in the examined rheumatological patients was carried out and presented against the background of NSAID treatment. The results of the studies of biotopes of the stomach and intestines in the examined rheumatological patients are presented in table No. 1. The results obtained showed that against the background of the use of NSAIDs in the stomach, the total number of anaerobes increases, while in the intestine, on the contrary, it tends to decrease by 20%, respectively, in contrast to anaerobes. The total number of aerobes against the background of the use of NSAIDs increases markedly both in the stomach and in the intestine. Compared with the control group by 35% and 24%, respectively. To the content of bifidobacteria against the background of the use of NSAIDs, if it increases by 1.3 times, then in the intestine, on the contrary, by 1.8 times, respectively, compared with indicators in healthy individuals. Unlike

bifidobacteria, lactobacilli in both the stomach and intestines of rheumatological patients treated with NSAIDs is reduced (Table 1).

Table 1
Characteristics of the microflora of the stomach and intestines in rheumatological patients taking non-steroidal anti-inflammatory drugs

lg M ± m КОЕ/г					
№	Groups Of Microbes	number of microbes in 1g/ml			
		stomach		intestine	
		Norm n-10	больные n-40	норма n-10	больные n-40
1	The Usual Number Of Anaerobes	3,80 ± 0,17	5,30 ± 0,21	10,18 ± 0,21	8,15 ± 0,47
2	Bifidobacteria	1,00 ± 0,11	1,30 ± 0,05	9,60 ± 0,41	5,30 ± 0,31
3	Lactobacillus	3,30 ± 0,15	1,47 ± 0,02	7,47 ± 0,42	6,30 ± 0,32
4	Peptostreptococci	1,11 ± 0,09	4,11 ± 0,19	0,75 ± 0,02	5,15 ± 0,29
5	The Usual Number Of Aerobes	3,60 ± 0,19	5,47 ± 0,17	6,31 ± 0,29	8,30 ± 0,41
6	Stafil. Golden	-	-	-	-
7	Stafil. Saprophytus	1,29 ± 0,01	-	2,31 ± 0,01	-
8	Stafil. Epidermis	2,00 ± 0,10	2,60 ± 0,11	2,47 ± 0,02	4,11 ± 0,21
9	Streptococcus Gr. A	-	-	1,31 ± 0,11	-
10	Streptococcus Gr. D	1,32 ± 0,12	2,30 ± 0,12	5,42 ± 0,32	5,30 ± 0,25
11	Candida Mushrooms	-	4,47 ± 0,15	2,82 ± 0,42	4,30 ± 0,19
12	Escherichia LP	-	2,30 ± 0,10	6,22 ± 0,21	6,11 ± 0,27
13	Escherichia LN	2,11 ± 0,11	3,47 ± 0,16	2,52 ± 0,21	3,47 ± 0,19
14	Proteus	-	-	2,11 ± 0,20	2,30 ± 0,11

n is the number of people examined

The study of the saprophytic flora of the stomach and intestines in the examined patients shows that the content of peptostreptococcus in the stomach increases 3.7 times, then in the intestine - 6.9 times. Staphylococcus epidermidis 1.3 times and 1.7 times, respectively. At the same time, staphylococcus saprophytes does not stand out against the background of the treatment. Microorganisms related to group A streptococci are also not sown in the intestine against the background of ongoing treatment. At the same time, if the microorganisms belonging to group D streptococcus in the biotope of the stomach increase, then in the intestinal biotope it does not change significantly (Table No. 1).

In the gastric biotope, lactose-positive E. Coli appears from gram-negative bacteria, and the titer in this biotope of lactose-negative E. Coli increases 1.6 times. In contrast to the gastric

biotope, in the intestine during the treatment of NSAIDs, only an increase in the titer of lactose negative E. Coli is observed, which exceeds the value of the control (healthy individuals) by 1.4 times. Therefore, in the examined patients, during the treatment with NSAIDs, there are noticeable changes in the content of the main representatives of the microbial landscape in the studied biotopes. Which indicates the development of dysbiotic phenomena, both in the stomach and in the intestines. Indeed, the result of such a dysbiotic change is the appearance of fungi of the genus Candida in the microbial flora of the stomach and an increase in its titer in the intestinal flora by 1.5 times, compared with the norm. Therefore, in RBs receiving NSAIDs, there are distinct dysbiotic changes in the microbiocenosis of the stomach and intestines.

The development of dysbiotic changes in the studied biotopes is undoubtedly reflected in the functional activity of the gastrointestinal tract and, above all, in ensuring its digestive, protective and synthetic functions; these disorders can adversely affect the course of the underlying disease due to increased autoimmune processes. Along with the above, it becomes obvious that it is necessary to clarify the cause of the occurrence of dysbiotic changes in the stomach and intestines.

It is known that the development of RB is based on systemic disorganization of the connective tissue of an autoimmune nature, which can develop in the connective tissue in the stroma of any organs and systems, including the gastrointestinal tract. From this it follows that the examined patients initially have the possibility of disrupting the functional activity of the gastrointestinal tract, which can lead to intestinal dysbiosis, on the one hand, and the NSAID treatments used for them, on the other, can also have a direct effect on the microflora of the stomach and intestines. To confirm this, we studied the degree of "lysis" of known microbial colonies under the influence of nimesil, indomethacin and tarivid under in vitro conditions (Table 2).

Table 2.

Susceptibility of microorganisms to certain drugs - in vitro

lg M±m KOE/г					
№	Groups Of Microbes	Without Drugs	Nimesil	Indomethacin	Tarivd
1	Candida Mushrooms	+	-	-	5,0 ± 0,2
2	Anticolycetes	+	-	-	12,0 ± 0,5
3	Mold Mushrooms	+	-	-	5,0 ± 0,2
4	Stafil. Golden	+	-	6,0 ± 0,2	18,0+0,7
5	Stafil. Epidermis	+	5,0 ± 0,2	12,0 ± 0,5	18,0 ± 0,6
6	Streptococcus Gr. A	+	6,0 ± 0,3	10,0 ± 0,3	20,0 ± 0,6
7	Streptococcus Gr. D	+	8,0 ± 0,2	12,0 ± 0,4	20,0 ± 0,4
8	Escherichia LP	+	6,0 ± 0,3	8,0 ± 0,2	25,0 ± 0,7
9	Escherichia LN	+	6,0 ± 0,3	6,0 ± 0,3	20,0 ± 0,5
10	Pseudomonas	+	-	-	20,0 ± 0,5
11	Diphtheroid	+	10,0 ± 0,3	14,0 ± 0,2	22,0 ± 0,3
12	Klebsiella	+	6,0 ± 0,2	8,0 ± 0,3	25,0 ± 0,8

13	Proteus	+	6,0 ± 0,1	8,0 ± 0,3	20,0 ± 0,4
14	Lactobacillus	+	6,0 ± 0,1	10,0 ± 0,2	21,0 ± 0,5
15	Bifidobacteria	+	5,0 ± 0,1	10,0 ± 0,2	18,0 ± 0,4

Growth retardation zone in mm.

As can be seen from the data presented in the table, under the action of the well-known antimicrobial drug tarivid, the "lysis" of the colonies of almost all used microflora samples occurs. At the same time, indomethacin does not have a significant effect on the flora of Pseudomonas, mold fungi, actinomycetes and fungi of the genus Candida, and nimesil, along with the above, on the colony of Staphylococcus aureus. Both indomethacin and nimesil have a lysing effect on the remaining colonies of microorganisms, but to a much lesser extent than tarivid. So, against the background of the use of indomethacin and nimesil, the diameter of the "lysis" of staphylococcus epidermidis colonies was lower than that of tarivid by 1.5 times and 3.6 times. Streptococcus group "A" 2 times and 3.3 times Streptococcus group "D" 1.7 times and 2.5 times. Lactose positive E. Coli 3.1 times and 4.2 times and others respectively. Consequently, indomethacin has a lysing effect on the colonies of microorganisms to a noticeably lesser extent than tarivid, then nimesil is noticeably less than indomethacin. In general, the data obtained indicate that the presented groups of microbes were highly sensitive to tarivid, sensitive to the action of indomethacin and moderately resistant to the action of nimesil.

Therefore, under in vitro conditions, non-selective NSAIDs, as well as antimicrobial drugs, can have detrimental effects on microorganisms. A distinctive feature of their impact on microorganisms is the predominantly detrimental effect on the normoflora in the absence of a similar effect on the pathogenic flora. If we take into account the results obtained, it becomes clear that NSAIDs also play a role in the change in the microflora of the stomach and intestines in the patients examined by us. However, this does not exclude the role of the pathology itself, the changes we found in the microbiocenosis of the gastrointestinal tract in RB. Apparently, the main pathology creates a condition for influencing the implementation of the negative effect of NSAIDs on the gastrointestinal tract and makes the microflora of the stomach and intestines easily accessible to their destructive effect. Therefore, against the background of the treatment of NSAIDs, it is necessary to develop methods and methods for the prevention of possible dysbiotic disorders from the gastrointestinal tract.

During the study, in the examined patients, against the background of the use of NSAIDs, frequent stool disorders were detected (frequency 2-3 times a month), periodic constipation with stool retention from 48 to 72 hours (with a frequency of 4-5 times a month), in 65% of patients bloating was noted. The results of bacteriological analyzes showed that 70% of patients had II-degree dysbacteriosis, and 30% had III-degree dysbacteriosis. In almost all patients, dysbacteriosis was caused by a decrease in the natural microflora and an increase in mold fungi, Candida and Clebsiel fungi.

Conclusion. To date, an idea is being formed about the parameters that characterize the microbiome of the stomach and intestines in normal conditions, a large number of microorganisms and their role in the human body remain unidentified. In this regard, the study of changes in the microbiome in rheumatological patients and the possibilities of correction, identified violations will contribute to the development of personalized medicine and increase the effectiveness of treatment.

Based on the conducted research, the following conclusions can be drawn:

1. In RB, against the background of NSAID treatment, a change in the microbial flora of the stomach and intestines is observed.

2. A characteristic feature of dysbiosis is a decrease in the proportion of the representative normal flora of the microbial

landscape of the stomach and intestines and an increase in opportunistic and pathogenic microbes.

3. NSAIDs, especially non-selective drugs, are characterized by an antimicrobial effect in vitro against the normal microflora of the gastrointestinal tract.

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