lek. Albert Michał Stec

Rola zaburzeń bariery jelitowej i metabolitów bakteryjnych w twardzinie układowej – implikacje kliniczne i terapeutyczne.

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

Promotor: prof. dr hab. n. med. Lidia Rudnicka

Katedra i Klinika Dermatologiczna

Warszawski Uniwersytet Medyczny

Kierownik Kliniki: prof. dr hab. n. med. Lidia Rudnicka

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych Warszawskiego Uniwersytetu Medycznego

Warszawa 2023

Praca powstała w ramach projektu „TIME 2 MUW doskonałość dydaktyczna szansą rozwoju Warszawskiego Uniwersytetu Medycznego" współfinansowanego z Europejskiego Funduszu Społecznego w ramach Programu Operacyjnego Wiedza Edukacja Rozwój na lata 2014-2020, numer umowy o dofinansowanie: POWR.03.05.00-00-Z040/18-00.

Słowa kluczowe: twardzina układowa, mikrobiota, oś jelito-skóra, dysbioza, zapalenie, choroby autoimmunizacyjne, metabolity mikrobioty jelitowej, SCFA, TMAO, probiotyki, przeszczep mikrobioty kałowej, bariera jelitowa, przepuszczalność jelit

Key words: systemic sclerosis, microbiota, gut–skin axis, dysbiosis, inflammation, immune-mediated inflammatory diseases, intestinal microbial metabolites, SCFA, TMAO, probiotics, fecal microbiota transplantation, intestinal barrier, intestinal permeability

Streszczenie w języku angielskim

Summary

The role of intestinal barrier disruption and bacterial metabolites in systemic sclerosis: clinical and therapeutic implications.

Introduction

The influence of intestinal microbiota on human homeostasis is currently an intensively researched topic. Dysbiosis, i.e. a change in the composition of the intestinal microbiota, is observed in many diseases, including those in the field of dermatology, such as psoriasis, systemic lupus erythematosus, atopic dermatitis, and systemic sclerosis. The impact of dysbiosis on the occurrence and course of dermatological diseases is described by the concept of the gut-skin axis, according to which altered microbiota may affect dermatoses through produced substances (metabolites). This effect may be enhanced by the disruption of the intestinal barrier that often occurs with dysbiosis, which contributes to increased intestinal permeability. This promotes the translocation of metabolites, antigens, and fragments of bacterial cells into the bloodstream, which, by stimulating the immune system, can lead to exacerbation of the symptoms of skin diseases.

Systemic sclerosis (SSc) is an autoimmune connective tissue disease, which course is often severe. The pathogenesis of the disease is not fully understood, but it is believed that impaired connective tissue repair mechanisms in response to cellular damage play a key role. Impaired repair mechanisms are characterized by uncontrolled production of extracellular matrix proteins, mainly type I collagen, by pathologically changed fibroblasts and lead to fibrosis of the skin and internal organs. Involvement of internal organs by the disease process causes specific complications, including interstitial lung disease, pulmonary hypertension, gastrointestinal motility disorders, and heart failure due to myocardial fibrosis.

Changes in the intestinal microbiota in the course of systemic sclerosis are characterized by an increased presence of bacteria of the genera *Fusobacterium*, *Desulfovibrio*, *Ruminococcus,* and *Lactobacillus*, and a decreased presence of bacteria of the genus

Faecalibacterium. In addition, more pronounced dysbiosis was observed in patients with coexisting organ involvement: interstitial lung disease and esophageal motility disorders.

The latest preclinical studies indicate that both harmful metabolites of intestinal bacteria and substances undergoing translocation due to increased intestinal permeability may intensify fibrosis processes by sensitizing fibroblasts to profibrotic factors.

Objective

1. The aim of this study was to determine potential abnormalities in the concentration of the dysbiotic gut microbiota metabolite: trimethylamine N-oxide (TMAO) in systemic sclerosis, as well as its potential association with the occurrence of typical organ-related symptoms of the disease.

2. Evaluation of the intestinal barrier in systemic sclerosis and determination of the relationship between the concentration of gut permeability markers and disease activity and symptoms, particularly with the occurrence of interstitial lung disease and esophageal motility disorders.

3. Analysis of potential differences in the concentration of gut permeability markers among patients with different durations of systemic sclerosis.

Material and methods

The study involved 50 patients with systemic sclerosis who met the ACR/EULAR classification criteria. The control group consisted of 30 volunteers matched for sex, age, and body mass index (BMI). Physical examination and additional tests to assess the severity of organ lesions were performed in accordance with the recommendations of the Polish Society of Dermatology. The severity of skin induration was assessed using the modified Rodnan scale (mRSS). Plasma TMAO levels were determined using high-performance liquid chromatography coupled to mass detection (HPLC-MS). Concentrations of selected markers of the intestinal barrier were determined in the serum by the enzyme immunoassay (ELISA). The p-value of < 0.05 was considered statistically significant.

Results

Compared to the control group, patients with systemic sclerosis showed a statistically significantly higher concentration of trimethylamine-N-oxide (TMAO) (283.0 ng/ml (interquartile range [IQR] 188.5-367.5) vs. 205.5 ng/ml (IQR 101.0-318.0); $p < 0.01$). A significantly higher TMAO concentration was found in the subgroup of patients with interstitial lung disease compared to the subgroup without lung involvement (302.0 ng/ml (IQR 212.0-385.5) vs. 204.0 ng/ml (IQR 135.5-292.0); $p < 0.01$). The subgroup of patients with concomitant esophageal motility disorders (detected by contrast examination of the esophagus) showed significantly higher levels of TMAO compared to the subgroup with normal motility (289.75 ng/ml (IQR 213.75-387.5) vs. 209.5 ng/mL (IQR 141.5-315.0), $p = 0.026$). In addition, TMAO concentration showed a significant negative correlation with the diffusion lung capacity for carbon monoxide (DLCO), a marker of restriction due to interstitial lung disease (rho= -0.53; $p = 0.013$). In addition, Spearman's correlation coefficient showed a statistically significant negative correlation with the left ventricular ejection fraction (LVEF) measured in echocardiography (rho = -0.39; $p < 0.01$) and a statistically significant positive correlation N-terminal B-type natriuretic propeptide (NT-proBNP) concentration (rho = 0.41; $p < 0.001$). There was also a statistically significant positive correlation between TMAO concentration and scleroderma organ damage index (SCTC-DI) (rho = 0.78; $p < 0.001$).

The assessment of the intestinal barrier showed a significantly higher level of bacterial lipopolysaccharides (LPS), which is an indicator of the translocation of bacterial cell elements from the intestinal lumen into the bloodstream, in the serum of patients with systemic sclerosis compared to those in the control group (232.30 pg/mL (IQR 149.00-347.70) vs. 161.00 pg/mL (IQR 83.92-252.20); $p < 0.05$). The subgroup of patients with a shorter duration of disease (less than or equal to 6 years) had significantly higher levels of bacterial lipopolysaccharides and claudin-3 compared to the subgroup of patients with a longer duration of disease (greater than 6 years): LPS: (280.75 pg/mL (IQR 167.30-403.40) vs. 186.00 pg/mL (IQR 98.12-275.90); $p < 0.05$), claudin-3: (16.99 ng/mL (IQR 12.41-39.59) vs. 13.54 ng/mL (IQR 10.29-15.47); $p < 0.05$). In addition, it was observed that in the subgroup with a shorter duration of disease, patients with interstitial lung disease had significantly higher LPS levels compared to patients without involvement of the lungs

(385.55 pg/mL (IQR 266.90-506.50) vs. 217.75 pg/mL (IQR 157.25-280.75); $p < 0.05$). In addition, in the group of patients with systemic sclerosis, the coexistence of esophageal motility disorders was associated with significantly lower serum LPS levels compared to patients with normal esophageal passage (188.05 pg/mL (IQR 102.31-264.40) vs. 283.95 pg/mL (IQR 203.20-356.30); $p < 0.05$).

Conclusions

1. The concentration of the gut microbiota metabolite: trimethylamine N-oxide (TMAO) in the serum is significantly higher in patients with systemic sclerosis compared to the control group. The presence of certain symptoms of the disease, including interstitial lung disease and esophageal motility disorders, is associated with particularly elevated levels of TMAO.

2. Gut microbiota metabolites may serve as a link between gut dysbiosis and organ involvement in systemic sclerosis. Modulating the metabolites derived from gut bacteria may represent a novel therapeutic approach in the treatment of systemic sclerosis.

3. The concentration of the gut permeability marker: lipopolysaccharides (LPS) in the serum is significantly higher in patients with systemic sclerosis compared to the control group. A subgroup of patients with a shorter disease duration (less than or equal to 6 years) is characterized by significantly higher levels of gut permeability markers, LPS and claudin-3, compared to the subgroup with a longer disease duration (above 6 years), suggesting increased gut permeability at an early stage of the disease.

4. Lower levels of intestinal permeability markers (LPS and Claudin-3) in patients with longer disease duration (more than 6 years) compared to patients with shorter disease duration (less than or equal to 6 years) may be due to co-occurring malabsorption resulting from the disease affecting the gastrointestinal tract.