Anti-inflammatory effect of exclusive enteral nutrition in patients with juvenile idiopathic arthritis

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Abstract

Objective

There is extensive evidence for an influence of gut microbiota on the immune system, which has consequences for inflammatory diseases. Exclusive enteral nutrition (EEN), which may change the gut microbiota, is an effective anti-inflammatory treatment for Crohn's disease in children. We wanted to explore the immediate anti-inflammatory effect of EEN in children with juvenile idiopathic arthritis (JIA).

Methods

Thirteen patients with JIA (7–17 years of age), in a disease flare-up, were included in the study. Six children dropped out within 1.5–2.0 weeks of treatment, and seven patients continued, constituting the study cohort. EEN was given for three to eight weeks, with clinical and laboratory status assessed before and after treatment periods. In addition to conventional laboratory tests, 92 inflammatory proteins were analysed with a multiplex system (Proseek Multiplex Inflammation I, Olink Bioscience).

Results

EEN had a significant anti-inflammatory effect on active joints (p=0.031), JADAS27 (p=0.016) and morning stiffness (p=0.031). In the multiplex analysis of inflammatory proteins, MMP-1 (matrix metalloproteinase), involved in the degradation of collagens in chondrocytes, decreased significantly (p=0.047), as did MCP-4 (p=0.031) and 4E-BP1 (p=0.031).

Conclusion

Exclusive enteral nutrition for three to eight weeks had anti-inflammatory effect in all children with JIA that continued with EEN for more than two weeks. The study is only exploratory but the result supports an immunologically important role for the intestinal canal in these patients.

Key words
arthritis, juvenile idiopathic arthritis, exclusive enteral nutrition, child, joints

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Introduction

It has been known for many years that the immunology of the gastrointestinal tract is important for development of arthritis. The commensal bacteria in the intestinal canal have an important role since they interact with and influence the epithelial cells, leading to immunological responses (1).

One of the inflammatory joint diseases in adulthood is ankylosing spondylitis. There is growing evidence for a subclinical intestinal inflammation in the majority of adults with ankylosing spondylitis (2, 3). Children with the corresponding disease, enthesitis-related arthritis (ERA) (4), may also have subclinical inflammation in the gut (5, 6). Patients with other categories of juvenile idiopathic arthritis (JIA) are only scarcely studied. In a Finnish study, a correlation between clinical disease activity and low gene expression of immunologically tolerogenic mediators in the ileum mucosa supports the hypothesis that a link exists between the gut immune system and JIA (7).

An altered and less functional gut microbiota is proposed to have a role in the pathogenesis of for example Crohn's disease. In paediatric Crohn's disease, so called exclusive enteral nutrition (EEN) has been shown to exert an anti-inflammatory and healing effect on the intestinal mucosa, leading to an improved nutritional status and a reduced need for corticosteroids (8). EEN is a whole protein polymeric formula and contains what is needed for complete nutrition. During six to eight weeks, the patients fulfil their nutritional requirements exclusively through a liquid formula taken orally or via a nasogastric tube. They are in close contact with a dietician and a physician, who perform regular check-ups. A majority of children with Crohn's disease reach remission with this treatment, but the immunological factors inducing healing are not known (9, 10). One main theory is that EEN changes the gut microbiota and achieves mucosal healing by suppressing certain bacterial species and changing their metabolism (11). EEN is recommended as first-line therapy for children with Crohn's disease in many countries today (12).

An altered faecal microbiome in JIA has formerly been discussed only in regards to children with the ERA category (13), but was recently shown also in a variety of other JIA categories (14). This supports our theory that EEN could have a possible anti-inflammatory effect in JIA. In this exploratory study we present clinical and laboratory data from one period of EEN treatment in seven children with JIA. Since conventional laboratory tests for inflammation are not very sensitive in JIA (15), we included a multiplex analysis of 92 inflammation-associated proteins before and after treatment.

Materials and methods

At the Paediatric Rheumatology Unit of Uppsala University Children's Hospital, an investigation of the anti-inflammatory effect of EEN in JIA has been running since January 2013, which has also included Sachs' Children's Hospital, Karolinska Institutet at Sodersjukhuset since 2014. Only children and parents who have been strongly motivated have been asked and included in the study. Disease activity and clinical findings have been followed prospectively, using validated variables.

Eligible for inclusion were patients <18 years of age, diagnosed with JIA before 16 years of age, without suspicion of inflammatory bowel disease. Patients were in an active phase of the disease, needing intra-articular corticosteroid injections (IACI) but they were in favour of starting EEN and wait 2 to 2.5 weeks with IACI to see any effect of the EEN. The inflammatory work-up included faecal calprotectin.

EEN was given for three to eight weeks. Enteral nutrition was the sole nutritional source, given as either elemental, semi-elemental or polymeric formula, with total exclusion of a normal diet.

Treatment effects in clinical variables were assessed prospectively using JADAS27 (16), including the number of active joints, global assessment VAS (0–100 mm) of disease activity by the patient/parents and by the doctor, as well as E-SR. Duration of morning stiffness (minutes) and Child Health Assessment Questionnaire CHAQ (17) was also recorded. CHAQ was filled

Table I. Clinical variables in seven patients with juvenile idiopathic arthritis, treated with exclusive enteral nutrition.

Gender /Patient	No. of weeks with EEN*	ILAR** category	Age at onset, years	Age at inclusion, years	HLA-B27***	Heredity****	Clinical features at inclusion	Upper and lower endoscopy	Calprotectin /feces mg/kg Before/after EEN*	IBD°°
F/A	6	Poly RF-	3.2	7.4	–	Father's mother RA°°	Small and large joints	Once	<10/17.2	No
F/B	4	Poly RF-	9.5	16.1	–	None	Knee, acromiclavicular and elbow arthritis	-	<30	–
F/C	4	Poly RF-	8.0	10.3	NA	Mother's father Crohn	Knee arthritis, postinflammatory damage to feet	Gastroscopy	<30	–
M/D	8	ERA°	15.4	16.8	+	Mother's father AS	Previous uveitis Sacroiliitis on MRI TMJ arthritis on MRI	Once	1203/68	Crohn
M/E	4	ERA	10.3	12.7	+	Mother's brother AS	Enthesitis (knee) Wrist arthritis	Once	270/32	No
M/F	4	Oligo persistent	11.1	17.1	–	None joint arthritis	Hip and knee	Twice	131/268	No
M/G	3	ERA	10.5	11.2	+	None	Arthritis in knee, talocrural and subtalar joints	–	45/28	–

*EEN: exclusive enteral nutrition; **ILAR: International League Against Rheumatism; ***HLA-B27: the human leukocyte antigen B27; ****for psoriasis, ankylosing spondylitis, JIA, RA, psoriatic arthritis, psoriasis. NA: not analysed; #RF: rheumatoid factor; ##RA: rheumatoid arthritis; °ERA: enthesitis-related arthritis; °°IBD: inflammatory bowel disease.

out by patients who were older than nine years or by parents for younger patients. In the assessment of clinical variables at follow-up, patients, parents and physicians were blinded to baseline assessments. All joint counts were performed by L.B., except in one patient (I.T.). E-SR, thrombocytes and faecal calprotectin were analysed with conventional clinical methods. The reference value for faecal calprotectin was <10 mg/kg for analyses at Uppsala University Hospital and <30 mg/kg at Sachs' Children's Hospital, Stockholm. Further, a panel of 92 proteins involved in inflammatory processes was analysed in plasma (EDTA) samples, before and after EEN (Proseek Multiplex Inflammation I; Olink Bioscience, Uppsala, Sweden) (18). The method is based on a well-characterised nucleic acid proximity-based assay using antibodies, called Proximity Extension Assay (PEA), with good performance in plasma samples (http://www.olink.com/).

Statistics

The data was analysed with Graph Pad PRISM 6. Wilcoxon signed-rank sum test was used as the non-parametric test to estimate significance of differences in clinical and laboratory variables before and after treatment with EEN. A total of 92 inflammatory proteins were analysed. With only seven paired observations it is impossible to reach statistical significance if correcting for multiple comparisons (*e.g.* Bonferroni). Instead, and since this is an exploratory study, we have reported all comparisons with a p-value <0.10 from the multiplex analysis.

Ethical considerations

The study was approved by the regional ethics committee in Uppsala County (Dnr 2012/378 and 2014/016). Written informed consent was obtained from the parents of children younger than 12 years and directly from the children who were 12 years or older.

Results

A total of thirteen patients were included in the study over the course of 2.5 years. Six patients dropped out during the first two weeks of EEN treatment, all after having made their own decision, and were not included in the final analysis. Five of them stopped at 1.5 weeks of EEN, because of poor compliance, one because of no effect at almost two weeks of EEN treatment and a desire to receive IACI at that time and discontinue the study. Seven patients had a clinical effect starting to be seen after 2-2.5 weeks of EEN and continued treatment for at least four weeks, but one patient needed joint injections after three weeks; thus we have only presented data from his first three weeks of EEN treatment (Table I, Patient G). Four of the seven patients had been investigated with upper and lower endoscopy at some time during the JIA disease course; three because of diffuse gastrointestinal symptoms with normal results and one (patient D) because of high calprotectin in a faecal sample during the work-up for inclusion. We decided to not exclude Patient D from this study despite later findings of intestinal Crohn's disease.

Discussion

In this exploratory study we have shown that treatment with exclusive enteral nutrition (EEN) can have an immediate anti-inflammatory effect,

as shown by reduction in inflammatory proteins, resulting in clinical improvement in patients with JIA.

Experiences of treatment with EEN can mainly be collected through treatment of patients with Crohn's disease. Most paediatric gastroenterologists use a six to eight week period of EEN treatment for induction of remission (12, 19). In our study, we aimed for a treatment duration of at least four weeks. Seven patients in our study continued with EEN for three to eight weeks, six of them gained clinical improvement beginning at two-three weeks, with decreased swelling and morning stiffness in their joints, which motivated patients and parents to continue. Levels of JADAS27, CHAQ and pain VAS decreased. In paediatric Crohn's disease, inflammatory markers have been shown to improve already during the first week of treatment (20), and clinical remission has been reported to occur within 11 days to 2.5 weeks (21). Patients with JIA, responding clinically to EEN, seem to do so after approximately the same duration of EEN as patients with Crohn's disease.

Laboratory analyses partly supported the clinical result. E-SR as well as levels of thrombocytes decreased after treatment, without statistical significance, but the study cohort was small and we know from clinical work and from earlier studies in JIA that E-SR and levels of thrombocytes are not very sensitive to changes in inflammatory activity in JIA (15).

In the multiplex analysis of inflammatory proteins, MMP-1 (matrix metalloproteinase-1) decreased after treatment with EEN. MMP-1 is one of the matrix metalloproteinases, a group of enzymes mainly produced by synovial fibroblasts (22) that have been considered the main enzymes responsible for degradation of collagens in cartilage (23). Studies have shown that the expression of MMP-1 is elevated in synovial tissue and serum of patients with JIA, and upregulated in synovial tissue in relation to disease activity (24). A possible explanation is that microbial products activate the innate immune system through Toll-like receptors (TLR). TLR pathway stimulation by microbial products or endogenous ligands are most likely involved in the production of MMPs in JIA and may contribute to disease pathology (25). Possibly, the change in diet caused a switch to a more beneficial intestinal flora with less stimulation of TLR pathways and decreased formation of MMP-1.

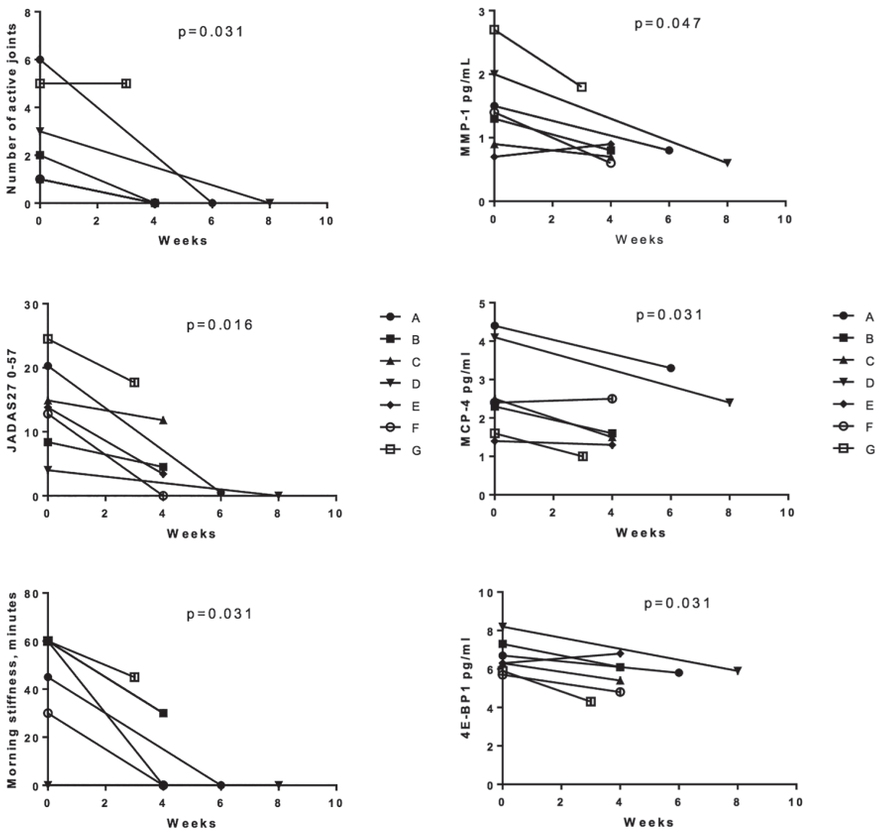

Fig. 1. Plot illustrating the change in the number of active joints, the JADAS-27 score, and the morning stiffness (in minutes) between the first and last observation in individual patients (A-D, see Table I for details). JADAS (juvenile arthritis disease activity score) is a composite score which include global assessment doctor VAS, global assessment patient/parent VAS (visual analogue scale) 0-10 cm, the number of active joints (0-57) and E-SR (0-10).

Fig. 2. Plot illustrating the change in the levels of MMP-1, MCP-4 and 4E-BP1 between the first and last observation in individual patients (A-D, see Table I for details). MMP-1 = matrix metalloproteinase-1, MCP-4 = monocyte chemotactic protein-4, 4E-BP1 = Eukaryotic translation initiation factor 4E-binding protein 1.

The other two proteins that decreased significantly with EEN, MCP-4 and 4E-BP1, are described in inflammatory processes but have not been mentioned earlier in studies of JIA, as far as we know. MCP-4 (monocyte chemoattractant protein 4 or CCL13) can be induced by pro-inflammatory cytokines IL-1 and TNF-alpha and has been described to be involved in several inflammation-related diseases. It is a chemokine that upon binding to its receptor elicits a chemotactic activity in monocytes, eosinophils, T lymphocytes, and basophils (26). The protein 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1) is involved in upregulation of mRNA translation, a common feature of pathological states in which protein synthesis positively correlates with cell proliferation rates (27). Interestingly, three monocyte chemoattractant proteins, MCP-2, MCP-3 and MCP-4, decreased with EEN treatment, while there was an increase of two fibroblast growth factors, FGF-21 and FGF-23, possibly as a sign of less inflammation and increased healing, respectively.

An unexpected finding of our study was that one patient (patient D) was diagnosed with Crohn's disease, at inclusion, revealed by a high calprotectin in faecal sample. The patient was without any symptoms or signs. He was referred after one year of sacroiliac pain, caused

by active sacroiliitis and sclerosis on MRI. Concurrently, he had clinical and MRI findings of an active arthritis in his left temporomandibular joint. A follow-up with MRI of sacroiliac and temporomandibular joints showed no remaining inflammation after EEN treatment. We have not found any study presenting an anti-inflammatory effect of EEN on co-existing arthritis in children with Crohn's disease. On the other hand, the high risk of Crohn's disease in adults with ankylosing spondylitis raises the question if children with the corresponding category, ERA, should be screened for IBD, at least through analysis of faecal calprotectin. As suggested above, a possible explanation for the anti-arthritic response to EEN in this study could be that EEN changed the microbiota to an immunologically more preferable flora, which has been shown in paediatric Crohn's disease (10).

Only seven patients were included in the study, which weakened the results, especially for the laboratory analyses. Also, duration of EEN treatment varied, which may have confounded the interpretation of results. A strength of the study is that it presents a method that alters the condition of the digestive tract in a controlled manner in JIA, opening for possibilities to study the influence of this organ on articular disease. It was not our aim at this stage to study the duration of a possible effect from EEN or to suggest EEN as a new treatment of JIA. However, our conclusion from this exploratory study is that the immune system of the gastrointestinal tract may have a more important role in JIA than we have previously realised, not only in patients with ERA but also in other categories of JIA. However, further studies are warranted.

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