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Effects of oral antibiotics and isotretinoin on the murine gut microbiota

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ABSTRACT

Inflammatory bowel disease (IBD) may develop due to an immunogenic response to commensal gut microbiota triggered by environmental factors in the genetically susceptible host. Isotretinoin, applied in the treatment of severe acne, has been variably associated with IBD, but prior treatment with antibiotics, also associated with IBD development, confounds confirmation of this association. This study investigated the effects of doxycycline, metronidazole (frequently used in the treatment of acne and IBD, respectively) and isotretinoin on murine gut (faecal) microbiota after 2 weeks of treatment and after a 4-week recovery period. Faecal microbiota composition was assessed by 16S rRNA gene sequencing on the GS-FLX 454 platform with primers directed against the variable regions V1–V2. Doxycycline had a modest effect on bacterial richness and evenness, but had pronounced persistent and significant effects on the abundance of certain operational taxonomic units compared with the control group. In contrast, metronidazole induced a pronounced reduction in diversity after treatment, but these effects did not persist after the recovery period. This study demonstrates differential effects of antibiotics on the gut microbiota with doxycycline, unlike metronidazole, mediating long-term changes in the murine gut microbiota. Isotretinoin had no significant effect on the faecal microbiota.

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1. Introduction

The microbiota of the gastrointestinal tract has a profound influence on host physiology and nutrition, including protection of the epithelial cell barrier [1] and regulation of host fat storage [2]. Associations between alterations in gut microbiota composition and a wide variety of pathological conditions including inflammatory bowel disease (IBD), obesity and associated insulin resistance, asthma, allergy, cardiovascular disease and neurological disorders [3] have been shown over the last decade. However, in most cases, it is not clear whether alterations of the gut microbiota are causal or secondary to the disease. An increasing body of evidence rather suggests the former, including IBD-like microbial alterations in healthy siblings [4], as well as an increasing degree of hallmarks of dysbiosis in correlation with the number of genetic alterations [5]. A breakdown of host–microbial mutualism triggered by environmental factors or genetic predisposition leading to dysbiosis and an inappropriate and progressive immune response to the commensal gut microbiota [2] is assumed to be causal for the pathogenesis of IBD [6,7].

The specific pathogenesis of IBD remains unclear, to date, but appears to be multi-factorial. Genome-wide association studies have identified 201 IBD susceptibility loci [8], affecting genes involved in epithelial barrier function, mucosal immune response, autophagy and immune regulation; the majority of these genes participate in the sensing of microbial products or affect defence signalling in response to gut microbes [1]. However, host genotype only explains up to 20–25% of IBD heritability overall, and 30–40% and up to 10% of cases of Crohn's disease (CD) and ulcerative colitis (UC), respectively [9]. Environmental factors potentially contributing to IBD include diet, appendectomy, smoking, breastfeeding, personal hygiene and medication(s) [6].

Evidence is increasing that antibiotics can influence established IBD, as well as IBD flares, and can increase the risk of developing IBD in both children and adults [10–12]. However, remarkably few studies have investigated the effect of individual antibiotics, the underlying

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mechanisms or whether there are any long-term 'persistent' effects of antibiotics on the gut microbiota [13–15]. Furthermore, a number of reports have claimed a potential association between isotretinoin (a non-antimicrobial treatment for severe acne) and development of IBD [16,17], although a causal role has not been established [18,19]. Isotretinoin is typically used in patients unresponsive to antibiotics [11]; as such, any causal relationship with the development of IBD is difficult to confirm due to confounding antibiotic treatment.

This study investigated the effects of doxycycline (used to treat acne but associated with the development of IBD), metronidazole (one of the preferred antibiotic agents for IBD patients) and isotretinoin on murine gut (faecal) microbiota after 2 weeks of treatment (immediate effects) and after a 4-week recovery period (long-term effects). The investigations aimed to identify possible environmental stressors that might have an immediate or persistent impact on gut microbiota composition, which might affect gut homeostasis and contribute to subsequent development of IBD.

2. Methods

2.1. Animals and treatment

In total, 164 female BALB/c mice were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed in individually ventilated cages per treatment in the animal facility of the University Hospital Zurich, with access to rodent chow and water ad libitum (Fig. 1A). Isotretinoin (30 mg/mL, F- Hoffmann-La Roche Ltd, Basel, Switzerland), vehicle (rapeseed oil, *Brassica rapa*, Sigma Aldrich, St. Louis, MO, USA), metronidazole (107 mg/kg, Sigma Aldrich), doxycycline (43 mg/kg, Sigma Aldrich) and water were administered orally for 2 weeks. Fig. 1 shows details of the study design, animals per group and sample collection.

2.2. Sample preparation and 16S rRNA gene sequencing

Total genomic DNA from faecal samples was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Courtaboeuf, France) according to the manufacturer's instructions. Hypervariable regions 1–2 (V1–V2) of the 16S rRNA gene were amplified from isolated genomic DNA using bacterial specific primer Pyro_27F (Adaptor B) and the barcoded reverse primer MIDx_338R (Adaptor A) (Supplementary Table S1). The primer pair had specific eight-base-long identifiers (barcode), a linker sequence and sequencing adaptors as described earlier [20] (Supplementary Table S1).

Amplification reactions were performed in a total volume of 50 µL containing 5x HF buffer (New England Biolabs, Ipswich, MA, USA), 10 mM deoxynucleotide triphosphate (illustra solution dNTP GE

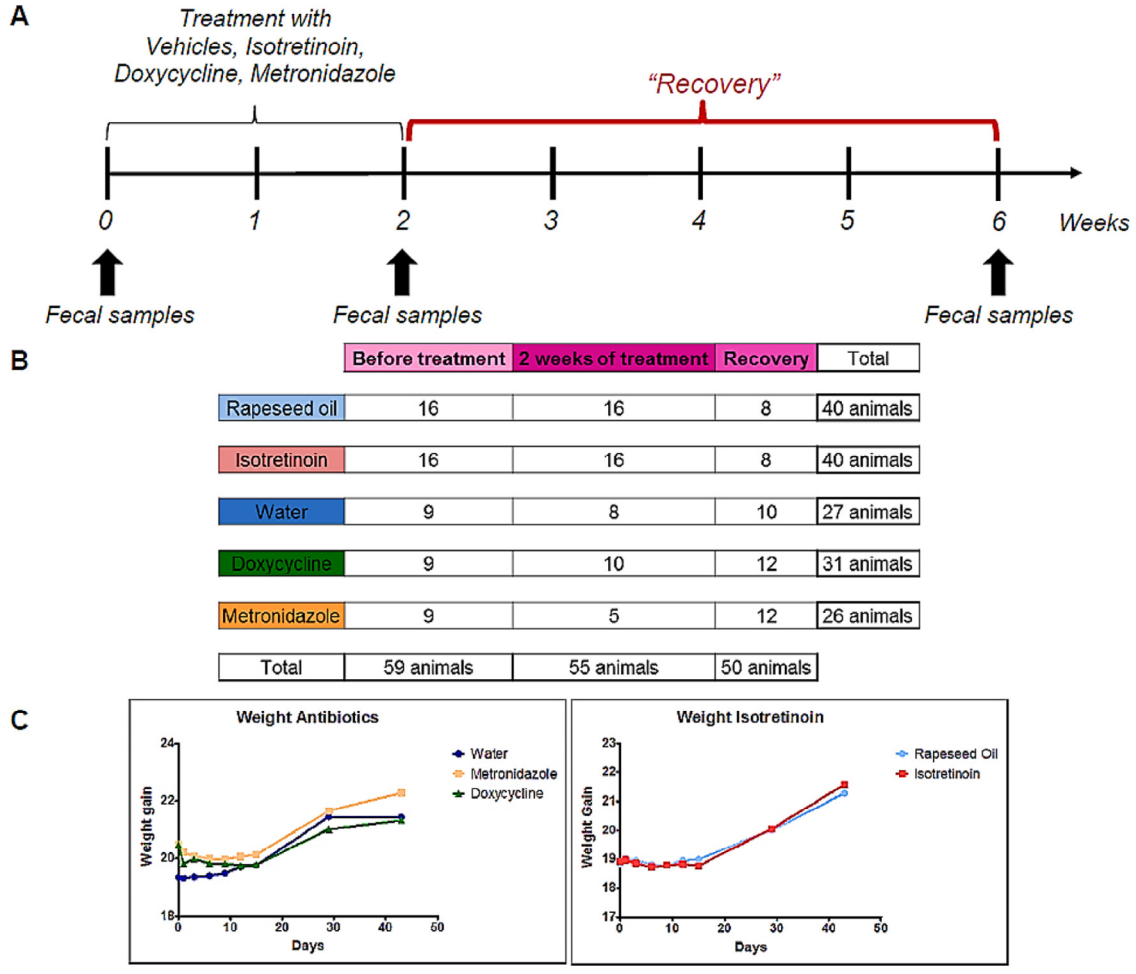

Fig. 1. Experimental design. (A) BALB/c female mice were treated with isotretinoin, rapeseed oil (isotretinoin vehicle), metronidazole, doxycycline or water (antibiotics vehicle) daily by oral gavage for 2 weeks. Faecal samples were collected before treatment, after 2 weeks of treatment (immediate effects) and after a recovery phase of 4 weeks after the cessation of treatment (long-term effects). (B) For isotretinoin and rapeseed oil, 16 animals per group were sampled before and immediately after treatment, and eight animals were sampled after the recovery phase. For metronidazole, doxycycline and water, five to 12 animals were sampled per time point and group. (C) No differences within treatment groups were registered with respect to body weight over all time points.

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