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Effects of senotherapeutics on gut microbiome dysbiosis and intestinal inflammation in Crohn's disease: A pilot study

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ABSTRACT

Inflammatory Bowel Disease (IBD) is characterized by chronic inflammation in the gastrointestinal tract, and is usually accompanied by dysbiosis in the gut microbiome, a factor that contributes to disease progression. Excessive production of reactive oxygen species (ROS) because of gut microbiome dysbiosis—one of the hallmark features of IBD—promotes chronic inflammation and facilitates the transformation of normal cells into senescent cells. Cellular senescence is associated with the development of various chronic and age-related diseases. We hypothesise that senolytic agents, specifically dasatinib (D) and quercetin (Q), could have a beneficial effect on both the gut microbiome and intestinal cells in IBD. The modulatory effects of a combination of $D + Q$ was assessed in the M-SHIME model with faecal microbiota sourced from Crohn's disease patients. $D + Q$ significantly modulated butyrate and lactate levels in the samples from specific patients. In addition, metabolomic analysis showed that $D + Q$ positively impacted the abundance of anti-inflammatory bacteria while also significantly reducing the several species of pathogenic bacteria. Findings from a Caco-2 cell/THP1 co-culture model of IBD demonstrated that $D + Q$ exerted strong immunomodulatory effects on the gut epithelium, evidenced by reduced NF-kB activity, and lower levels of the pro-inflammatory markers TNF-α, CXCL-10, and MCP-1. Furthermore, $D + Q$ induced the secretion of anti-inflammatory cytokines, including IL-6 and IL-10. However, it should be noted that $D + Q$ also led to the secretion of the pro-inflammatory cytokines IL-8. These findings suggest that $D + Q$ could offer a novel therapeutic approach for advanced IBD management by modulating both the gut microbiome and inflammatory pathways. The results support the potential repurposing of senotherapeutic agents as a strategy for addressing the chronic inflammation central to IBD pathogenesis

Introduction

Inflammatory Bowel Disease (IBD) presents a complex and significant challenge in healthcare, encompassing chronic and idiopathic inflammatory conditions such as ulcerative colitis (UC) and Crohn's disease (CD).[1,2] While UC predominantly affects the large intestine, inflammation in CD can occur anywhere along the gastrointestinal tract.[3] Global IBD prevalence has risen sharply, affecting over 6.8 million individuals, with CD accounting for around 60 % of cases.[4,5] Interestingly, incidence rates are stabilizing or declining in Western countries but increasing in Eastern regions.[2,4] The escalating number of overall IBD cases poses a significant socioeconomic burden due to the profound morbidity associated with the disease and the elusive nature of its root cause.[6] Although the precise aetiology of IBD remains elusive, the intricate interplay between gut microbiome factors and oxidative stress is believed to be a potential contributor to immune dysregulation.

The gut microbiome is a complex community of trillions of microorganisms, composed of bacteria, viruses, archaea, and fungi, and plays a vital role in maintaining host health.[7-9] Dysbiosis, defined as an imbalance of gut microbiota composition and characterized by the diminishment of beneficial bacteria, shifting in microbial composition and decreased diversity, is implicated in interrupting immune

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homeostasis in the gut, fostering the development of disease-specific bacterial and fungal pathogens.[10-13] This underscores the profound influence of the microbiome on human health and disease. The significant correlation between IBD and gut microbiome dysbiosis has been highlighted in many previous studies.[14,15] Next-generation sequencing and advanced computational methods have unveiled a notable reduction in both richness and variety of bacteria diversity, especially in anti-inflammatory bacteria such as *Bifidobacterium adolescentis*, compared with healthy controls, and an increase in some virulent microorganisms.[16,17] The disturbance of certain species of gut microbes reduces redox tolerance, leading to production of excessive reactive species[18-20] and altering several metabolic pathways; these changes are strongly associated with IBD pathogenesis.[21]

An excess of radical species leads to chronic inflammation, triggering cellular damage through multiple inflammatory pathways.[22] Oxidants trigger signal transductions such as nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK) cascade, phosphoinositide-3-kinase and nuclear factor erythroid 2–related factor 2 (Nrf2), contributing to endogenous stresses, oncogene activation, telomere dysfunction, and persistent DNA damage.[23,24] These persistent stresses compel the cell to adapt and resist cell apoptosis, eventually transforming into an 'immortal' cell (or senescent cell) — a concept first introduced by Hayflick and Moorhead in the early 1960s.[25-28] Cellular senescence is a hallmark of age-related diseases such as atherosclerosis, cancer, and chronic obstructive pulmonary disease (COPD).[29,30] One of the key features of cellular senescence is 'inflammaging', driven by the progression of cellular senescence.[31] The senescent cell exerts continued inflammatory effects, as this cell has high metabolic activity, secreting a number of proinflammatory cytokines such as TNF-a and IL-6, including the senescence-associated secretory phenotype (SASP).[25,29,32] The latter fuels the vicious cycle of cellular senescent production by inducing biological changes in neighbouring cells.[33,34]

The intricate interplay between cellular senescence, gut microbiome dysbiosis, and IBD is considered a significant burden in IBD management, confirmed in previous studies with in-vitro and in-vivo models.[35-38] In brief, senescent cells downregulate the enteric nervous system, changing intestinal motility, promoting degeneration of the mucosal barrier, and downregulating the innate immune system.[39,40] Meanwhile, cellular senescence promotes gut microbiome dysbiosis by disturbing the composition and abundance of the gut microbiota, further aggravating immune dysregulation and driving disease progression through excessive destruction of intestinal integrity and defaulting the differentiation of CD4+ *T*-cell and CD8+ *T*-cell activation.[41] Cellular senescence results in telomere shortening at the intestinal epithelium and gut microbiome dysbiosis is thought potentially to aggravate telomere shortening.[42-44] These factors drive IBD symptoms and increase the risk of developing age-related comorbidities such as cardiovascular diseases, dementia, and shorter lifetime.[45] Due to its importance, this makes cellular senescence a promising target for IBD.

Senotherapeutic strategies aim to tackle cellular senescence.[33] The two most documented senolytic agents, discovered via a computational-based approach, are quercetin and dasatinib.[33,46-49] These compounds specifically eliminate senescent cells while sparing healthy cells.[33] Quercetin inhibits intracellular phosphoinositide 3-kinase (PI3 K) expression leading to decreased synthesis of mammalian target of rapamycin (mTOR), a regulator of apoptosis.[33,50] Dasatinib inhibits multiple tyrosine kinases that reduce the expression of ephrin ligand B proteins, which trigger cell death via the inactivation of Eph receptors including the p21 protein.[33,49,51] Although each compound exhibits a promising senotherapeutic effect individually, the co-administration of quercetin and dasatinib has shown synergistic senolytic effects in preclinical and clinical studies due to the wider coverage of mechanisms relevant to cellular senescence.[48,49,52-54] Currently, quercetin and dasatinib are FDA-approved for use in humans and appear relatively safe.[55] The potential side effects from suddenly eliminating large amounts of senescent cells are not known, although such effects are unlikely to be significant because of the small number of senescent cells in the body[33]. Recent studies reveal that oral administration of quercetin and dasatinib could alter the gut microbiome composition and function which may be eventually useful for IBD management.[49] Based on the mechanism of action, we hypothesize that senotherapeutic agents may offer superior efficacy compared with current IBD pharmacotherapies, which primarily target specific cytokines. These existing treatments often have limitations in terms of safety, particularly due to potential off-target effects. In contrast, senotherapeutics could provide a sustained therapeutic effect while improving the safety profile by selectively targeting senescent cells, thus minimizing unwanted systemic effects. However, the impact of senotherapeutics on IBD remains largely unexplored, highlighting the need to determine whether repurposing the therapeutic effect of this senolytic combination could be a novel anti-inflammatory therapeutic for IBD.

This study assessed the direct effects of the senolytic combination, dasatinib plus quercetin ($D + Q$), on CD gut health. The composition and functioning of the gut microbiota sourced from three CD patients before and after treatment were observed in short-term colonic simulations with the M-SHIME® model. Additionally, the effect of the senolytic combination on preventing increased epithelial permeability in active CD was investigated with a Caco-2/THP1 co-culture in vitro model. Ultimately, insights from this study contribute towards better understanding of the senotherapeutic effect in the GI tract, paving the way to a new approach to CD management.

Materials and methods

Materials

Quercetin dihydrate was purchased from MP Biomedicals, LLC (California, USA) and dasatinib monohydrate were purchased from Cambridge Biosciences (Cambridge, UK). Caco-2 cells were purchased from American Type Culture Collection (Vurginia, US). Lipopolysaccharide (LPS) sourced from Escherichia coli K112 and THP1-Blue™ cells were purchased from InnivoGen (California, US). Lecithin was ordered from Carl Roth (Karlsruhe, Germany). Sodium chloride (NaCl), pepsin, sodium hydrogen carbonate ($NaHCO_3$), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), di-potassium hydrogen phosphate (K_2HPO_4), and potassium di-hydrogen phosphate (KH_2PO_4) were acquired from Chem-lab analytical BVBA (Zedelgem, Belgium). Pancreatin, gastric lipase, hemin, menadione, mucin type II, l-cysteine HCl, zinc sulfate hepta-hydrate ($ZnSO_4.7H_2O$), iron sulfate hepta-hydrate ($FeSO_4.7H_2O$), sodium dithionite, sodium thioglycolate, and 5-ASA were ordered from Merck Life Science B.V. (Overijse, Belgium). Sodium hydroxide (NaOH), calcium di-chloride di-hydrate ($CaCl_2.2H_2O$), manganese di-chloride tetra-hydrate ($MnCl_2.4H_2O$), methanol (HPLC-gradient grade), acetonitrile (ACN, LC-MS grade), and high purity water for HPLC were all purchased from VWR International Europe BVBA (Leuven, Belgium). Water for media and experimental runs was purified using an Elix Advantage 10 water purification system (Merck Millipore, Darmstadt, Germany).

Methods

Effect of dasatinib and quercetin on CD microbiota

The effect of $D + Q$ on dysbiotic faecal microbiota was investigated using an advanced model of the human colon (M-SHIME® bioreactor, ProDigest, Ghent, Belgium). Faecal samples were collected from three patients diagnosed with remission Crohn's disease under ethical approval from the University Hospital Ghent (reference number: B670201836585). Non has been on antibiotics three months prior to the donation. To ensure the anaerobiosis, the faecal slurry was prepared in an anaerobic chamber by homogenising faecal material with an anaerobic buffer (K_2HPO_4 8.80 g/L; KH_2PO_4 6.80 g/L; sodium thioglycolate 0.10 g/L; sodium dithionite 0.015 g/L) and a cryoprotectant developed

by Hoefman et al.[56] This created 7.50 % w/v faecal suspensions that were stored in aliquots at −80°C prior to experimentation. The incubation study was conducted in the SHIME ® semi-dynamic GII release model.[57] The SHIME ® system is a fully automated computer-controlled system, coupled with the inoculation of gut microbiota sourced from human donors representing the proximal large intestine.[58] The system consists of nine vessels, allowing to test of three conditions in biological triplicate.[59] The system is fully designed and operated in anaerobic conditions and the physiological environment of the large intestine.[60] Thawed faecal suspensions were inoculated at a concentration of 10 % v/v into glass vessels containing 70 mL sterile basal medium and 1.40 g/L mixed fibre. The composition of the basal medium is detailed by Ghyselinck et al..[61] Mucin-coated microcosms were also added to each reaction vessel ($n = 5$ per vessel) to facilitate colonisation of mucosal microbiota. The colonic vessels were incubated under anaerobic conditions and mild shaking (90 rpm) at 37°C for 48 h.

A total of 18 incubations were conducted, consisting of 9 drug-microbiota incubations (0.83 mg/mL dasatinib + 0.083 mg/mL quercetin) and 9 control incubations (Fig. 1). Here, control incubations were drug-free mirrors of the $D + Q$ incubations. Samples were collected at times 0, 6, 24, and 48 h to assess concentrations of lactate, short-chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs), and ammonium. Lactate concentration was determined using a commercial kit (Enzytec™ kit (R-Biopharm AG, Darmstadt, Germany) with a lower limit of quantification (LOQ) of 20.0 mg/L, according to the manufacturer's instructions. SCFA and BCFA concentrations were determined via gas chromatography coupled with flame ionisation detection, as detailed by.[62] Ammonium concentration was measured using the indophenol blue method.[63] In addition, the pH of incubations was assessed with a calibrated pH probe and gas production was measured in the headspace of vessels using a pressure meter.

Microbial community composition analysis

The impact of $D + Q$ on both luminal and mucosal microbiome composition was analysed using 16S rRNA gene sequencing (16S Illumina sequencing) and flow cytometry. The method used to combine these techniques is outlined in reference.[64] In brief, luminal and mucosal samples ($n = 3$ per donor per timepoint) was taken from the incubation vessels at 0 and 48 h. Samples were then separated into two groups for sequencing and cell counting. For sequencing, DNA from samples were extracted and amplified as in references.[61,62,65] Two primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′- GACTACHVGGGTAT CTAAKCC-3′) were used to span 2 hypervariable regions at V3 and V4 of 16S rRNA. 424 bp amplicons were constructed by pair-end sequencing of 2 × 250 bp. A standard operating procedure was then used to clean and assemble reads via the Mothur package (version 1.44.3).[66] Taxonomy (operational taxonomic units, OTUs) were allocated via a naïve Bayesian classifier using 97.0 % sequence similarity. Only bacterial OTUs with ≥ 95 % relative abundance across samples were considered.

The method for cell counting was similar to a previous study.[65] Briefly, samples were diluted with phosphate buffered saline by a factor of ten and cells were stained with propidium iodide and SYTO 24 dye. An Accuri™ C6 Plus Flow Cytometer (BD Biosciences, New Jersey, US) coupled with fluorescent detection was used to count cells, which were passed through the cytometer at a high flow rate. The threshold was set to 700 on the SYTO channel to remove the signal interference from medium debris. The absolute abundances of bacterial OTUs in luminal samples was calculated by multiplying the relative abundances measured during sequencing by the total cell count from cytometry. Mucosal samples were not subject to cell counting, thus OTUs are presented as relative abundances.

Caco2/THP1-blue™ co-culture model

The effect of $D + Q$ on an inflammatory intestinal epithelium was conducted with a co-culture model. The culture method has been described in detail by Daguet et al.[67] Briefly, Caco2-cells were seeded in 24 well semi-permeable plates (density 1×10^5 cells/well) and cultured at 37°C in Dulbecco's Modified Eagle medium supplemented with HEPES (10 mM) and pasteurised foetal bovine serum (HI-FBS, 20 % v/v). Media were changed three times per week. After 14 days of growth the transepithelial electrical resistance (TEER) was confirmed to be over 300 Ω*cm^2, demonstrating a functional monolayer. In tandem, THP1-Blue™ cells were seeded into 24-well plates (density 5×10^5 cells/well). THP1-Blue™ cells were cultured at 37 °C in Roswell Park Memorial Institute 1640 medium, supplemented with HI-FBS (10 % v/v), HEPES (10 mM), and sodium pyruvate (1 mM). THP1-Blue™ cells were treated with 50 ng/mL phorbol 12-myristate 13-acetate for 48 h to facilitate their proper differentiation into macrophage-like cells.

For the co-culture, THP1-Blue™ cells were placed in wells and Caco-2 inserts were placed on top. The initial TEER of the Caco-2 cells was recorded. The apical compartment, which was in contact with the Caco-2 monolayer, was then filled with a 1:5 dilution of samples taken from the M-SHIME® fermentation vessels after 48 h of $D + Q$ incubation. Incubation samples were filtered (0.22 μm) to avoid microbial contamination of the cell cultures. The basolateral compartment, in contact with the THP1-Blue™ monolayer, was filled with Caco-2 culture medium. The TEER of the Caco-2 monolayers was measured after 24 h and the basolateral supernatant was filled with Caco-2 culture medium containing 500 ng/mL LPS to stimulate an inflammatory response. After 6 h immunoregulatory markers within the basolateral supernatant were measured using the Luminex® multiplex (ThermoFisher Scientific, Massachusetts, US). NF-κB activity was determined spectrophotometrically as reported by reference[68]. Controls consisted of: no apical exposure to $D + Q$ fermentation samples; apical exposure to 12 mM sodium butyrate; addition of hydrocortisone (1μM) to the LPS medium.

Statistical analysis

The impact of $D + Q$ on microbial metabolism was evaluated by applying two types of statistical test to find the statistical significance of the treatment effect. Briefly, an unpaired two-sided t-test was used to evaluate the treatment effect with the given donor while paired two-sided t-tests were used to analyse the treatment effect across various

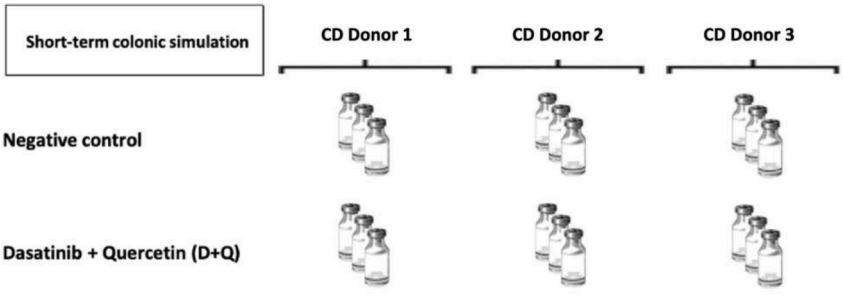

Fig. 1. The experimental set up used to measure the effect of dasatinib + quercetin ($D + Q$, 0.83 mg/mL + 0.083 mg/mL respectively) on the activity and functions of faecal microbiota sourced from patients with Crohn's Disease (CD). The negative control represents drug-free incubations of the CD microbiota.

donors. For beta diversity, the discriminal analysis of principle components (DAPC) was applied to statistically analyse bacteria composition.

Principal component analysis (PCA) was used initially to transform sequence data. The subsequent clusters were identified with discriminant analysis (DA). In parallel, the TreeclimbR analysis was performed to identify the taxa. The outcome was illustrated in a volcano plot which presents the statistical significance (p-value) on the y-axis versus magnitude of change on the x-axis between the control and treatment groups. For the effect of $D + Q$ on gut wall function and immunomodulation, an unpaired, two tailed Student t-test was applied to find statistical significance between two control sets. For the sample set, the one-way Analysis of variance (ANOVA) with Dunnett's multiple comparison test was applied to assess the significant difference between sample set and positive control set (LPS) while two-way ANOVA with Dunnett's multiple comparison test were used to identify significant different in TEER value, immune markers between donors and treatment samples were compared against their non-treated experimental control. All experiments were conducted in triplicate, and significance was determined as a p-value of <0.05. All statistical analyses were performed via GraphPad Prism (Version 10.2.3; GraphPad Software 10.2.3, San diego, CA, USA)

Results and discussion

The effect of D + Q on the gut microbiota sourced from CD patients

Overall pH and gas production

Overall pH is an indicator of the combination of production of short chain fatty acids (SCFA), lactate and ammonium production in the microbial culture. In brief, a decrease in pH is usually a consequence of SCFA and lactate production.[69] However, fluctuation in pH is a normal phenomenon following a pH decrease due to proteolytic fermentation producing ammonium and other ammonium products.[70] Gas production is an indicator of fermentation. In general, this study found $D + Q$ had only a small influence in decreasing the overall colonic suspension pH compared with the initial pH after 48 h of incubation (Fig. 2). The ability of $D + Q$ to lower the pH during different incubation periods was investigated because the growth of bacteria at different time points has a strong influence on overall metabolic activity. The results reveal that the overall pH across the donors in the initial 6 h was slightly higher while $D + Q$ was able to lower the overall pH compared with the control after 24 h and 48 h, but the changes were not significant. There was significant gas production after treatment with $D + Q$ after 48 h in all donors compared with the blank (p-value < 0.05). However, the overall change was not substantial enough to be considered biologically relevant (Fig. 3).

Lactate production

The influence of $D + Q$ on lactate production in CD gut microbiota after 48 h is shown in Fig. 4. Initial lactate production across donors was highly variable and inconsistent, indicating a variability in gut microbiota composition. The results found inhibition of lactate production across the donors, but the highest inhibition was seen in donor 2. The average difference in lactate concentration between the initial time point and the 48-hour end point was not significant when the $D + Q$ treatment group was compared with control. In a healthy gut, lactic acid bacteria (LAB), such as *Lactobacilli* and *Bifidobacteria,* are intestinal flora which utilise and produce lactate through hydrolysis of disaccharides such as lactose, resulting in the production of lactate at the end of glycolytic process[71]. The lactate produced from fermentation decreases the environmental pH, which is not only beneficial for enhancing the viability and diversity of the gut microbiome (lactate can be utilised by Firmicutes to produce butyrate for instance[72]), but also provides an anti-microbial effect to pathogenic bacteria via the disruption of transmembrane potential[73]. In IBD, reduction of lactate may offer a beneficial therapeutic effect, reducing toxicity from acidification of the colonic microbiota when the pH is lower than 5.50, which can lead to disruption in both metabolism function and composition of microbiome[60,74].

Short chain fatty acids (SCFA)

The main products resulting from carbohydrate metabolism within the colon are SCFAs. SCFAs play a vital role in regulating human body function and cross-talk between immunological mechanisms. The SCFAs produced by gut microbiota include acetate, propionate and butyrate.[75] $D + Q$ had no significant effect in altering acetate and propionate production across the different donors. The combination did reduce butyrate production in donors 2 and 3 compared with the experimental control (Fig. 5A). However, the reduction in butyrate level across the donors was not significant. Similarly, there were no significant differences in average acetate (Fig. 5B) and propionate (Fig. 5C) compared with the control.

Interestingly, the average difference in acetate production was positive in the $D + Q$ treatment group compared with the control group after 24 and 48 h. However, the increase in acetate concentration was not statistically significant. It is notable that the development of CD seems strongly linked to dysbiosis of the gut microbiota, a consequence of losing beneficial gut bacteria.[76] Compared with healthy humans, the gut microbiota in CD patients have less biodiversity, a lower abundance of *Firmicutes, Roseburia* and *Faecalibacteria*, and increased prevalence of *Ruminococcus gnavus, Deltaproteobacteria* and *Gammaproteobacteria.*[77] Firmicutes are responsible for butyrate production, while Bacteroides mainly synthesise acetate and propionate.[78] This phenomenon directly affects the steady level of SCFAs in CD patients compared to healthy donors.[79]

SCFA synthesis is extremely important for the body's daily energy

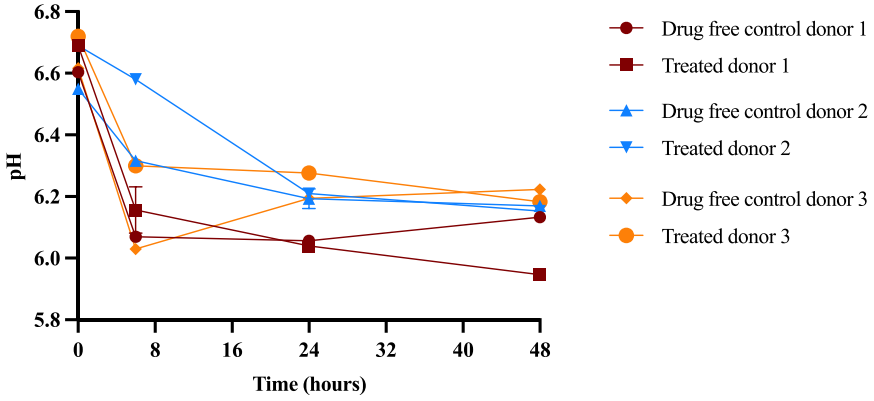

Fig. 2. Change in pH of the incubations compared with the control over 48 h.

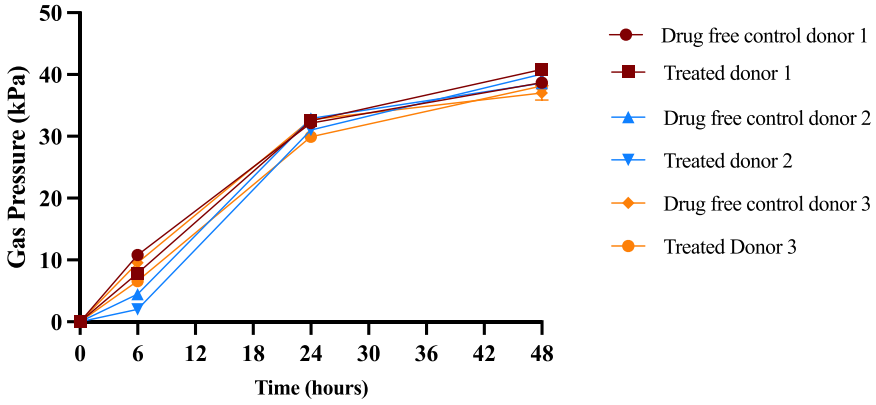

Fig. 3. Change in gas production between the control and $D + Q$ treated group over 48 h.

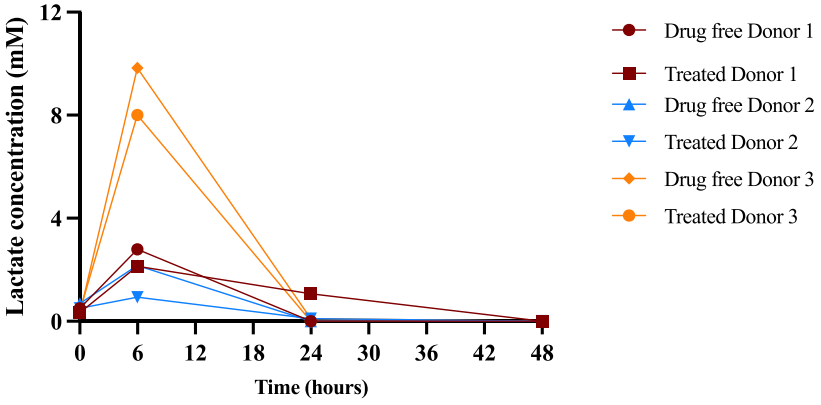

Fig. 4. The effect of $D + Q$ on lactate production in gut microbiota from CD donors.

requirement, affecting epithelial cell transportation and metabolism, cell growth and differentiation, controlling lipid and carbohydrate metabolism of hepatocytes and being a source of energy for muscles, kidneys, heart and brain.[11] Previous studies report the important role of SCFA in IBD pathogenesis through several mechanisms relevant to immune function at the cellular level.[80,81] Loss of butyrate-producing bacteria not only lowers luminal anaerobiosis but also progresses inflammation through increasing Enterobacteriaceae, subsequently decreasing barrier function and impairing mitochondria function in colonic epithelial cells.[82] Additionally, the decrease in butyrate production directly reduces the production of IL-10, an anti-inflammatory cytokine, via the less interaction between butyrate and GPR109A presented on colonic epithelial cells, potentially exacerbating IBD progression.[80,83] Acetate and propionate are the main ligands that activate G protein-coupled receptors, especially GPR41 and GPR43.[80,84] Both receptors play a vital role in recruiting the leukocytes and activating effector T cells in the intestine, including activating the extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways at epithelial cells, resulting in the induction in the production of chemokines and cytokines offering the anti-inflammatory effect.[81,85] The reduction of SCFA alters this regulation, contributing to the effects on colonic and mucosal protection.[86]

Branched chain fatty acid (BCFA) and ammonium (NH_4^+) production

The activity of $D + Q$ on BCFA and NH_4^+ production was investigated. In general, a reduction of in BCFA and NH_4^+ is considered as beneficial for health.[87] $D + Q$ slightly decreased the production of BCFA in donor 1 and 3 while increasing the production of BCFA in donor 2 after 48 h (Fig. 6). $D + Q$ was able to reduce the production of NH_4^+ in all donors compared with the control. However, the reduction in NH_4^+ production across donors was not statistically significant (Fig. 7). An excessive level of BCFA is unfavourable for gut health.[88] BCFA concentration is a strong predictor for protein fermentation in the absence of fibre by BCFA producing bacteria, such as Bacteroides, Clostridium and Propionibacterium.[89] The main by-product of protein fermentation is urea which is the main substrate for ammonia production in the large intestinal tract. Gram negative *Enterobacteriaceae* and some anaerobic gram-positive bacteria are responsible for ammonia production.[90] Increasing ammonia production can alter the intestinal tissue and act as promoter for tumour in gut.[91]

Community composition and shift in microbiome composition

CD causes chronic inflammation inside the GI tract, resulting in gut microbiota dysbiosis, as reported in previous studies.[92] The decrease in abundance of beneficial and anti-inflammatory bacteria, such as *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii,* reduction in specific genera of Bacteroidetes and Firmicutes and increase in the abundance of *Enterobacteriaceae,* especially *E.coli* and *Fusobacterium,* are characteristic of CD gut microbiome dysbiosis.[93]

The luminal gut microbiome compositions of the CD donors were identified by 16S rRNA-targeted Illumina sequencing. The compositions of the microbiota were then compared before and after treatment with $D + Q$. All donors had a unique gut microbiota composition (Fig. 8). Donor 1 had an abundance of *Escherichia-Shigella* and *Bifidobacterium.* Although *Escherichia-Shigella* is considered commensal, some strains are considered pathogenic due to their ability to evade the host immune system and cause infection.[94] Donor 2 had abundances of *Fusobacterium* and *Megamonas*. These genera are microbial markers for Crohn's disease. *Fusobacterium* in particular is associated with the progression of

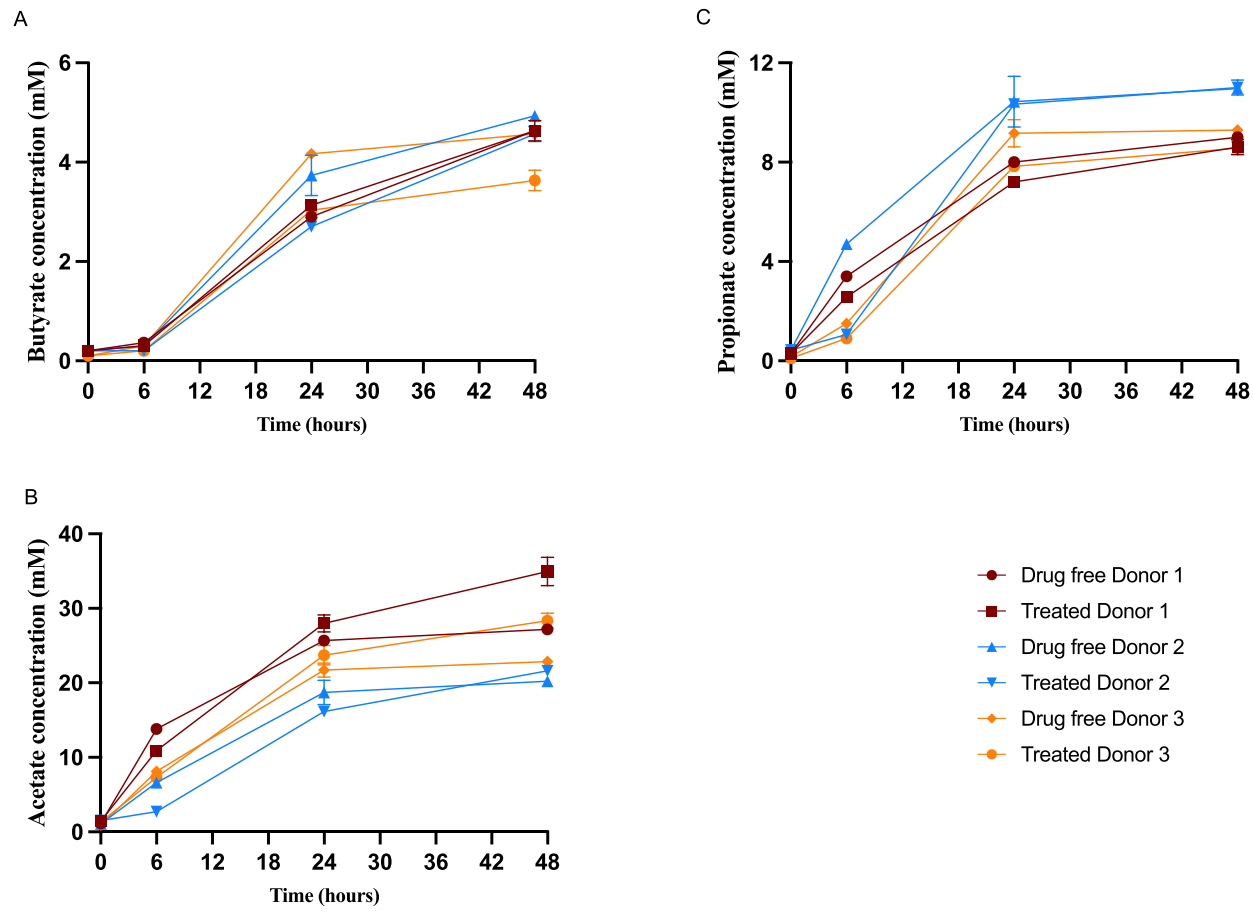

Fig. 5. The effect of $D + Q$ on SCFA production at different time points, shown for: (A) Butyrate, (B) Acetate, and (C) Propionate.

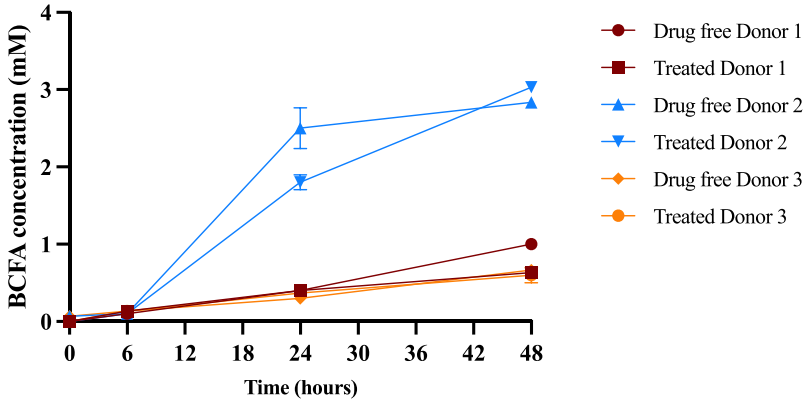

Fig. 6. The effect of $D + Q$ on the BCFA production.

colorectal carcinoma and long-term complications of IBD.[95] Donor 3 had an abundance of *Streptococcus, Eggerthella* and *Enterococcus spp*.

The effect of $D + Q$ on reducing dysbiosis of the luminal community composition was investigated. The data are reported by phylum (supplement Table 1), family (supplement Table 1) and OTU level (Fig. 8). Overall, the findings confirmed $D + Q$ notably increased the abundance of beneficial bacteria, especially *Actinobacteria* (*Eggerthella*) and some Bacteroides (OTU 5 and OTU 4) while decreasing the abundance of pathogenic bacteria, especially *Sutterellacae, Megamonas*. However, $D + Q$ also increased the abundance of *Escherichia-Shigella* and decreased the abundance of several Bacteroides (OTU10, OTU11 and OTU 13), which is not considered beneficial for gut health. The results reveal that treatment with $D + Q$ significantly increased *Peptostreptococcus* (OTU393) and two *Bacteroides sp.* (OTU90 and OTU226) (p-value $<$ 0.05). The abundance of *Peptostreptococcus* changed from 4.7×10^4 cells/CFU to 2.3×10^5 cell/CFU. The change was consistent across three donors. Moreover, $D + Q$ positively stimulated *Bifidobacterium* (OTU93), *Agathobactor* (OTU75), *Collinsella* (OTU24), *Fusicatenibaacter* (OTU45), *Lachnospiraceae* NK4A136 species and several *Bacteroides* sp. However, the stimulative effect across the donors was not significant. Also, $D + Q$ lowered the abundance of *Lachnoclostridium* (OTU117), *Ruminococcis Roseburia* (OTU9) and other *Bacteroides spp.* compared to matched

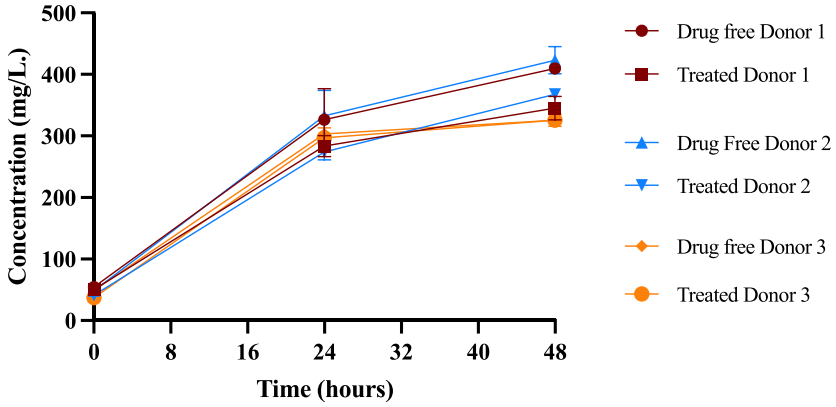

Fig. 7. The effect of $D + Q$ on the ammonia production.

controls. However, the overall reductive effect across donors was not significant.

Unlike the luminal microbiota, the mucosal microbiota showed different dominant genera in IBD donors (Fig. 9). In brief, in donor 1 and 2, there was an abundance of *Roseburia* whilst the major genus in donor 3 was *Streptococcus*. After treatment with $D + Q$ there was a change in the mucosal microbiome compositions, reported by phylum (supplement Table 2), family (supplement Table 2) and OTU level (Fig. 10). $D + Q$ was associated with lowering the abundance of *Bacteroidaceae*, and *Lachnospiraceae* sp. (OTU11 and OTU57) while increasing the abundance of *Actinobacteria*, especially *Bifidobacterium*. Moreover, treatment with $D + Q$ significantly increased the abundance of *Roseburia* (OTU28), especially in donor 1 (p-value < 0.05). The increase in the abundance of *Rosebulia* highlights the beneficial effect of $D + Q$ in modulating CD gut microbiota, which naturally exhibits less abundance in the CD. Genus *Roseburia* is known as a butyrate-producing species. However, the increase in its abundance in this study was not reflected in any significant changes in butyrate concentration in donor 1 compared with the control. Additionally, $D + Q$ tended to increase the abundance of *Fusicatenibacter* (OTU45) and *Bacteroides* (OTU84) whilst decreasing *Parabacteroides* sp. (OTU53) and several genera such as *Sutterellaceae*.

The luminal microbial and mucosal associated microbiota are complex communities that influence a variety of GI functions and can be used as an indicator for health status. However, the sequencing data confirm the distinct composition of the bacterial communities between the two areas which may be relevant to physical implication and surrounding environment such as oxygen concentration.[96] The mucosal associated microbiota has a greater influence on the epithelial cells, involving microbiologic function, metabolic and immunological effects.[97] It's notable that, in a healthy human, 90 % of gut bacteria species belong to four major phyla - Firmicutes, Bacteroides, Proteobacteria and Actinobacteria – and these interact and support a healthy state. The abundance of gut microbiota composition in IBD varies enormously.[98,99] This variability may come from the influence of other microbes in the gut community, like viruses, archaea, and fungal, lifestyle, environmental factors, and heterogeneity in genetic between individuals. Although $D + Q$ offers a positive effect on modulating gut microbiota dysbiosis, the findings do not concur with the results in the previous section, nor indeed with previous studies which report an increase in the abundance of beneficial bacteria that correlate to the increase in SCFA.[65] It is possible that there may be other metabolites, which were not measured in this study, that may contribute to the phenomenon. Therefore, a longer observation period is needed in future work to better understand alterations of bacterial metabolites and modifications in both composition and abundance of gut microbiome dysbiosis.

The potential anti-inflammatory effect of $D + Q$ on an inflammatory gut model

'Leaky gut' is considered one of most important pathologies of IBD, contributing to uncontrollable molecular trafficking in the immune system. Eventually, uncontrollable immune responses lead to local and systemic inflammation.[100] Chronic inflammation caused by cellular senescence increases the severity of a leaky gut.[101] The modulation effect of $D + Q$ on a leaky gut in active CD was studied. The results revealed that $D + Q$ did not show an additional protective effect on the gut barrier integrity, seen by non-significant changes in TEER value across all donors compared with control (Fig. 10A). The finding is inconsistent with previous in-vivo studies.[53,102] However, $D + Q$ showed significant immunomodulatory properties. Treatment with $D + Q$ significantly increased IL-8 (Fig. 10B), pro-inflammatory chemokines, in all donors, including significantly increasing the IL-1β (Fig. 10C) in the majority of CD donors compared to experimental control treated with LPS. On the contrary, $D + Q$ significantly decreased NF-kB activity (Fig. 10D), and increased anti-inflammatory cytokines IL-6 (Fig. 10E), IL-10 (Fig. 10F) and TNF- α (Fig. 10G). Moreover, $D + Q$ showed significant immunosuppressive effects in reducing the secretion of CXCL-10 (Fig. 10H) and MCP-1 (Fig. 10I), the pro-inflammatory chemokines, after co-incubating with LPS in most donors. The findings show that $D + Q$ had a strong effect in terms of immunomodulation, due to the induction of anti-inflammatory cytokines and reduction of NF-kB activity.

Conclusions

This is the first study that explores the potential effect of a senolytic cocktail in order to improve gut health via modulation of CD gut microbiota. The main finding was that $D + Q$ has little to no effect on modulating CD gut microbiota metabolites (SCFA, BCFA and ammonium) production, although a reduction in lactate and butyrate production was seen in most donors. The reduction in lactate accumulation may contribute the beneficial effect for CD patients, as it reduces the risk of acidification which eventually disrupts both the abundance and metabolic function of microbiota.

Metagenomic analysis confirmed the strong modulation effect of $D + Q$ on gut microbiome dysbiosis. There were significant reductions in abundance of several pathogenic bacteria such as *Streptococcus, Sutterellaceae* and *Megamonas* and an increase in abundance of several health-benefiting bacteria such as *Bacteroides*. $D + Q$ positively escalates the abundance of *Peptostreptococcus*, which has a link to increasing the risk of antimicrobial resistance. However, the change of abundance in the community analysis is not noteworthy enough to be considered substantial. Interestingly, $D + Q$ significantly increased the abundance of vital anti-inflammatory bacteria such as Actinobacteria (likely

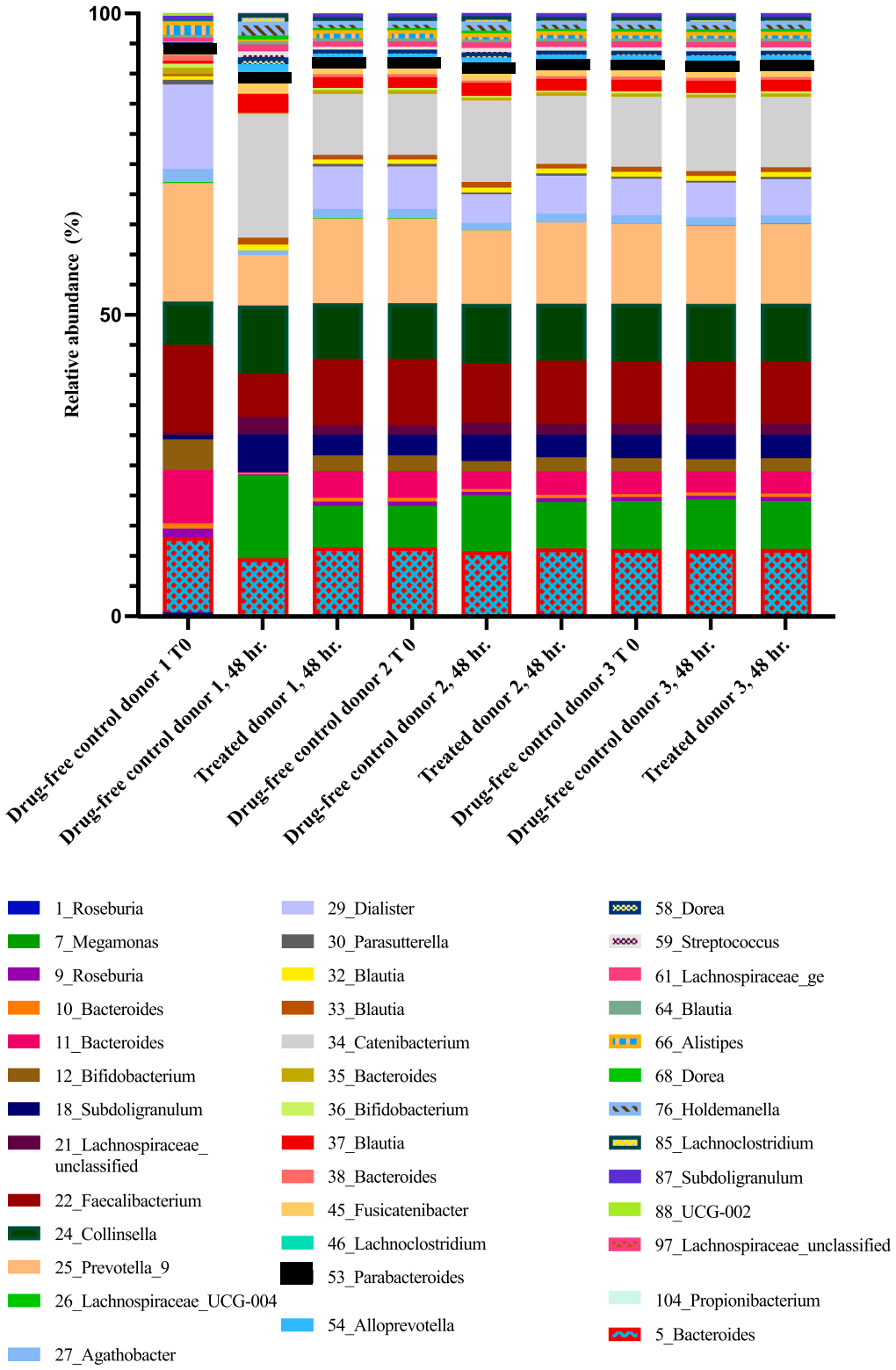

Fig. 8. The relative abundance of the composition of the luminal gut microbiota in each IBD donor before (T0) and after incubation with $D + Q$ for 48 h (T48).

attributed to *Eggerthellaceae*) and *Bifidobacterium*.

Lastly, $D + Q$ exhibited a strong immunomodulatory effect on the gut wall by reducing NF-κB activity, pro-inflammatory cytokines such as TNF-α, and pro-inflammatory chemokines CXCL10 and MCP-1, while inducing the secretion of anti-inflammatory cytokines IL-6 and IL-10. However, $D + Q$ also significantly increased the secretion of pro-inflammatory chemokines IL-8 and IL-1β. Notably, despite these immunomodulatory effects, $D + Q$ did not provide additional protective benefits for gut barrier integrity in the Caco-2/THP-1 co-culture model, contrasting with previous reports.

While this study provides compelling evidence of $D + Q$'s strong modulatory effect on the gut microbiota, its direct role in immune modulation, particularly in inflammation, requires further investigation. To enhance the robustness and generalizability of these findings,

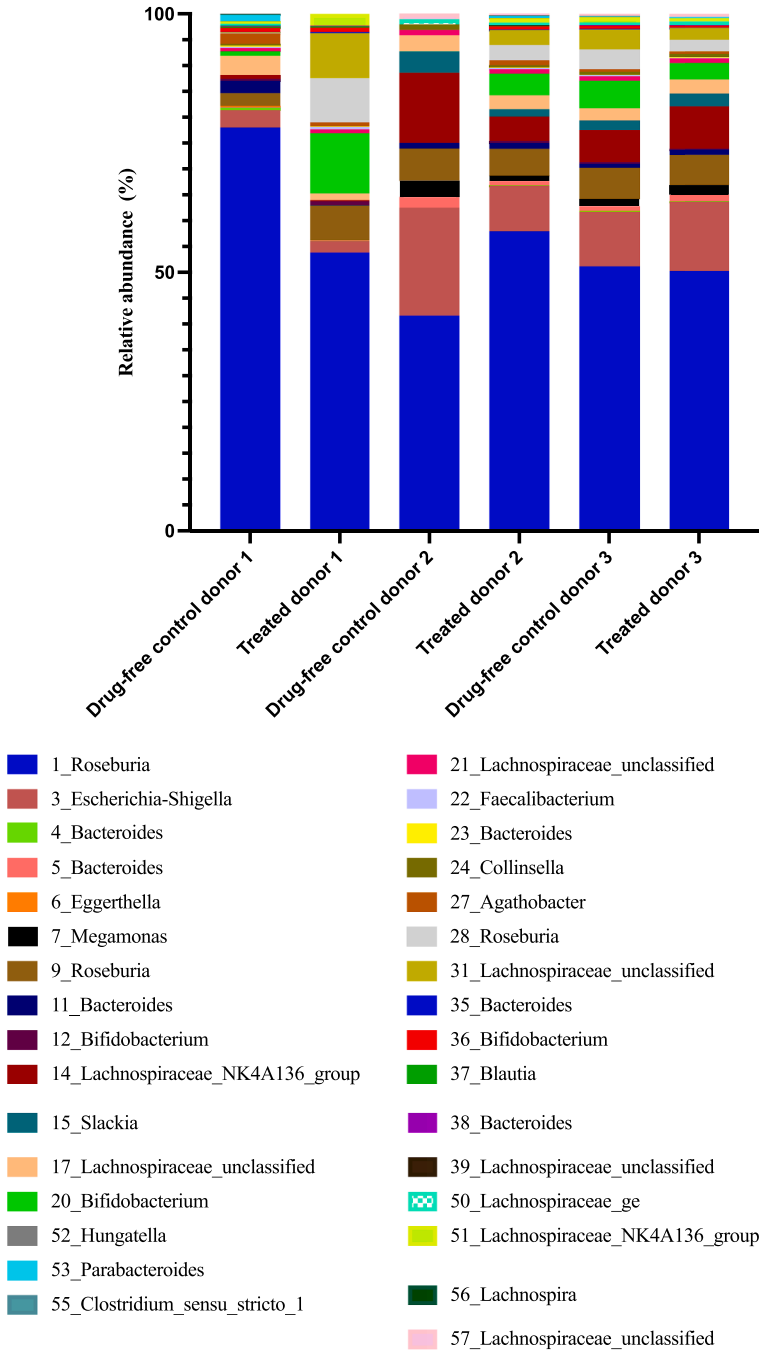

Fig. 9. The bacteria composition of mucusol IBD gut microbiota before and after incubation with $D + Q$ for 48 h.

future studies should replicate these explorations using a larger and more diverse microbiome sample, including individuals with ulcerative colitis (UC) and those not in remission. Additionally, a more comprehensive study design is necessary to validate the therapeutic potential of $D + Q$ across different IBD conditions. Integrating multi-omics techniques will provide deeper insights into the interplay between $D + Q$ and immune modulation. Finally, future research should explore scalable strategies to transition this therapeutic approach from pilot studies to broader clinical applications.

In summary, while the results imply a positive improvements in the gut health of some donors, but overall, the changes are not statistically significant. The findings from this study will improve the understanding of the interaction of senolytic drugs with the gut microbiota and vice versa, with benefits for further clinical translation and repurposing the therapeutic effect for effective CD management.

Data availability

Data will be made available on request.

Brief commentary

Background: New innovative therapeutic approaches are needed to treat inflammatory bowel disease (IBD). Gut microbiome dysbiosis and excessive radical species contribute to chronic inflammation and lead to the transformation of cells into senescent cells. We hypothesise that the senolytic agents, dasatinib and quercetin ($D + Q$), may have a beneficial effect on the gut microbiome and intestinal cells in active IBD

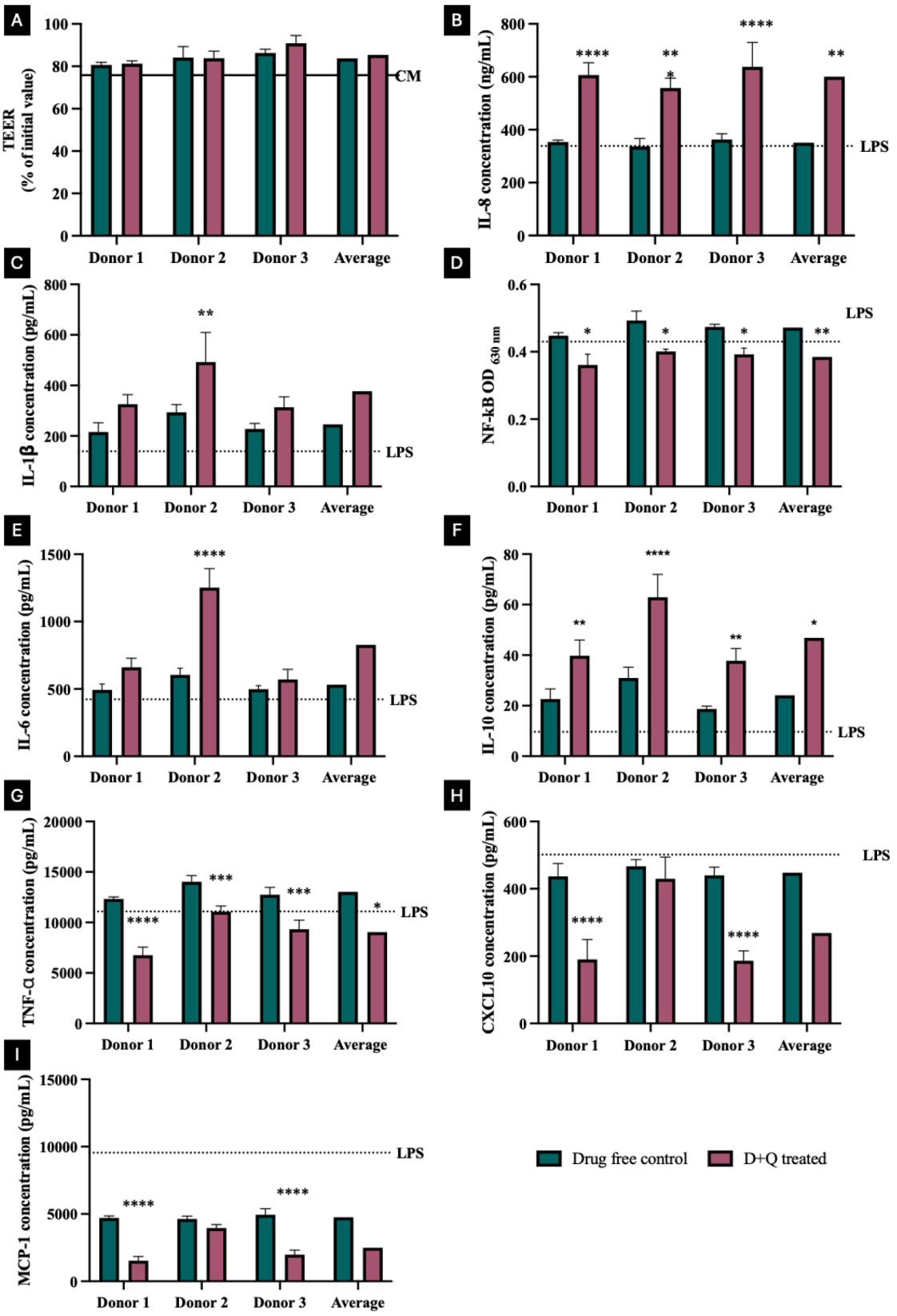

Fig. 10. The effect of $D + Q$ on gut wall immunomodulation as follows: (A) barrier integrity (TEER value), (B) IL-8, (C) IL-1β, (D) NF-kB, (E) IL-6, (F) IL-10, (G) TNF-α, (H) CXCL-10, and (I) MCP-1. The black thick line corresponds to the experimental control (complete media (CM)), and the black dotted line corresponds to the experimental control with LPS. Data is plotted as mean ± SD.

Translational significance: The findings confirm the positive effects of senotherapeutics on improving gut health, particularly in rebalancing the gut microbiome dysbiosis and exerting strong immunomodulatory effects on the intestinal epithelium in Crohn's disease model.

CRediT authorship contribution statement

Nannapat Sangfuang: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Laura E. McCoubrey:** Writing – review & editing, Project administration, Conceptualization. **Atheer Awad:** Writing – review & editing, Project administration, Investigation. **Massimo Marzorati:** Resources, Investigation. **Jonas Ghyselinck:** Methodology, Investigation, Formal analysis, Data curation. **Lynn Verstrepen:** Investigation, Formal analysis, Data curation. **Julie De Munck:** Investigation, Formal analysis, Data curation. **Jelle De Medts:** Investigation, Formal analysis, Data curation. **Simon Gaisford:** Writing – review & editing, Supervision, Resources. **Abdul W. Basit:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors report there are no competing interests to declare. The content of this paper does not reflect the views of GSK.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.trsl.2025.02.004.

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